AICE* SURVEY OF USSR AIR POLLUTION LITERATURE

Volume VIII

A COMPILATION OF TECHNICAL REPORTS
ON THE
BIOLOGICAL EFFECTS AND THE PUBLIC HEALTH ASPECTS
OF ATMOSPHERIC POLLUTANTS

Edited By

M. Y. Nuttonson

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USSR literature on air pollution
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*AMERICAN INSTITUTE OF CROP ECOLOGY
809 DALE DRIVE
SILVER SPRING, MARYLAND 20910

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PREFACE

The present volume consists of reports dealing with a number of investigations conducted in the Soviet Union at its various public health institutes and in departments of public health of some universities.

The great strides in the development of industrial chemistry in the country has stimulated studies of the biological effects of chemical air pollutants as well as studies dealing with public health implications of these pollutants. Such studies assume an ever-increasing importance in the Soviet Union.

The results of these investigations provide a basis for the establishment of a new series of maximum permissible concentrations for new toxic substances in the atmospheric air, and constitute the scientific criteria for assessing the degree of pollution. They also form the foundation for a number of ameliorative sanitation measures for control of atmospheric pollution in populated areas.

The material included in this volume deals with the response of the organism:

(1) to low concentrations of chemical air pollutants around major petrochemical complexes that are now under intensive development;

(2) to low concentrations of air pollutants around chemical plants manufacturing as raw material for nylon production the salts of adipic acid and hexamethylenediamine and also salts of sebaic acid and hexamethylenediamine; and

(3) to the various concentrations of air pollutants around organic synthesis plants and other facilities of the new chemical industry.

Methods of determination of low concentrations of various substances in the atmospheric air are also described in some of the papers of this volume.

Much of the background material presented in the prefaces to the preceding volumes of this series is also relevant to the present volume.

Some background information on the distribution of the Soviet industry's production machine may be of interest in connection with that country's present and potential pollution problems and investigations. The planned distribution of production in the Soviet Union favors effective exploitation of the natural resources of the USSR, especially in its eastern areas where enormous

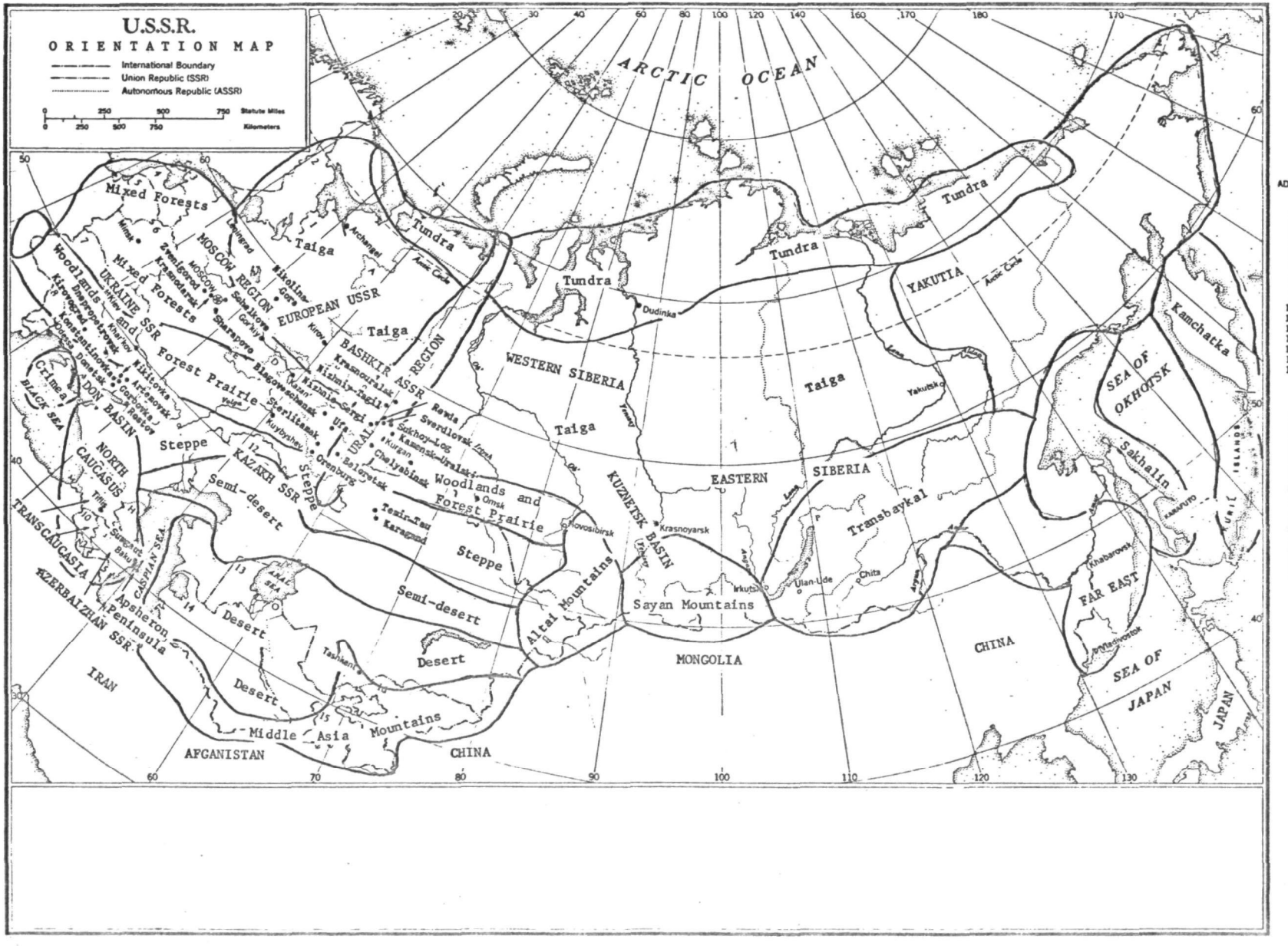
natural resources are concentrated, and has led to the creation of large industrial centers and complexes of heavy industry in many of the country's economic areas (see page ix). The many diverse climatic conditions of the country and its major economic areas as well as the geographical distribution of the Soviet Union's principal industrial and mining centers and of its principal electric power stations and power systems can be seen from the various maps presented as background material in this volume.

Public awareness of the environmental crisis and the pollution problem has been greatly stimulated in the USSR by the description, in the local press, of such phenomena as dirty urban air, polluted rivers, ravaged forests and public parks, and poisoned wildlife as well as by the revealing of the causes of these conditions. In the Soviet Union, like in the West, pollution now poses for the leaders of the country some fundamental choices between the economics of production, on one hand, and the progressively worsening living conditions, on the other. There appears to be, at present, a greater appreciation and a better understanding of the immense problems of air and water pollution on the part of the urban and rural administrative agencies. As a result of a mounting demand for the maintenance of a high quality physical environment, protective measures against the pollution threat are gradually taking shape in the USSR and much relevant air pollution research data are being developed in the various industrial regions of that country.

It is hoped that the papers selected for presentation in this volume will be conducive to a better appreciation of some of the air pollution investigations conducted in the USSR. As the editor of this volume I wish to thank my co-workers in the Air Pollution Section of the Institute for their valuable assistance.

M. Y. Nuttonson

July 1971



ADMINISTRATIVE DIVISIONS

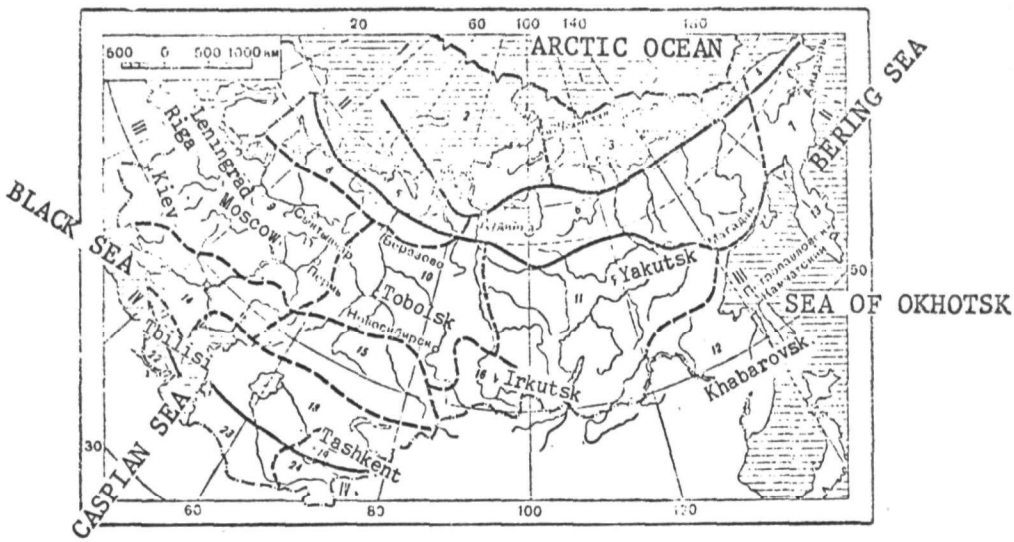
S.S.R.

1. R.S.F.S.R
2. Karelo-Finnish S.S.R.
3. Estonian S.S.R.
4. Latvian S.S.R.
5. Lithuanian S.S.R.
6. White Russian S.S.R.
7. Ukrainian S.S.R.
8. Moldavian S.S.R.
9. Georgian S.S.R.
10. Armenian S.S.R.
11. Azerbaydzhan S.S.R.
12. Kazakh S.S.R.
13. Uzbek S.S.R.
14. Turkmen S.S.R.
15. Tadzhik S.S.R.
16. Kirgiz S.S.R.

A.S.S.R.

A. Komi ASSR
B. Udmurtskaya ASSR
C. Mariyskaya ASSR
D. Chuvashskaya ASSR
E. Mordovskaya ASSR
F. Tatarskaya ASSR
G. Bashkirskaya ASSR
H. Dagestanskaya ASSR
J. Severo-Osetinskaya ASSR
K. Kabardinskaya ASSR
L. Abkhazskaya ASSR
M. Adzharskaya ASSR
N. Nakhichevanskaya ASSR
O. Kara-Kalpakskaya ASSR
P. Buryat-Mongol'skaya ASSR
Q. Yakutskaya ASSR

CLIMATIC ZONES AND REGIONS* OF THE USSR



Zones: I-arctic, II-subarctic, III-temperate, IV-subtropical
Regions: 1-polar, 2-Atlantic, 3-East Siberian, 4-Pacific, 5-Atlantic, 6-Siberian, 7-Pacific, 8-Atlantic-arctic, 9-Atlantic-continental forests, 10-continental forests West Siberian, 11-continental forests East Siberian, 12-monsoon forests, 13-Pacific forests, 14-Atlantic-continental steppe, 15-continental steppe West Siberian, 16-mountainous Altay and Sayan, 17-mountainous Northern Caucasus, 18-continental desert Central Asian, 19-mountainous Tyan-Shan, 20-western Transcaucasian, 21-eastern Transcaucasian, 22-mountainous Transcaucasian highlands, 23-desert south-Turanian, 24-mountainous Pamir-Alay

(After B. P. Alisov, "Climate of The USSR", Moscow 1956)

SOIL AND VEGETATION ZONES IN THE U.S.S.R.

(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

MAJOR ECONOMIC AREAS OF THE U.S.S.R.

PLANNED DISTRIBUTION OF INDUSTRIAL PRODUCTION IN ORDER TO BRING IT CLOSER TO RAW MATERIAL AND FUEL SOURCES

An example of the planned distribution of industrial production in the USSR is the creation of large industrial centers and complexes of heavy industry in many of the country's economic areas: the North-West (Kirovsk, Kandalaksha, Vorkuta), the Urals (Magnitogorsk, Chelyabinsk, Nizhny Tagil), Western and Eastern Siberia (Novosibirsk, Novokuznetsk, Kemerovo, Krasnoyarsk, Irkutsk, Bratsk), Kazakhstan (Karaganda, Rudny, Balkhash, Dzhezkazgan).

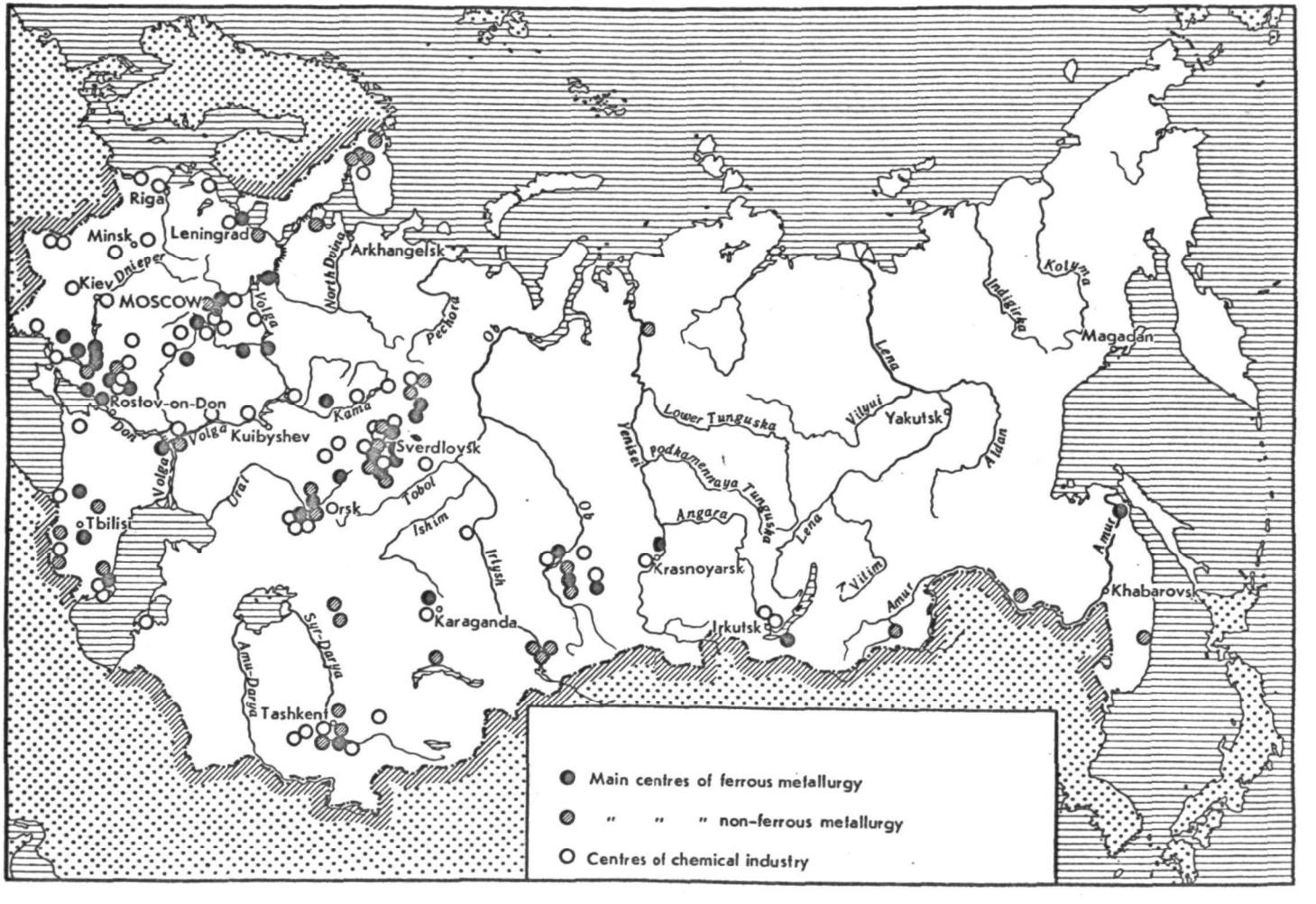
Large industrial systems are being created - Kustanai, Pavlodar-Ekibastuz, Achinsk-Krasnoyarsk, Bratsk-Taishet and a number of others. Ferrous and non-ferrous metallurgy, pulp and paper, hydrolysis and saw-milling industries are being established in the Bratsk-Taishet industrial system. The Achinsk-Krasnoyarsk industrial system is becoming one of the largest centers of aluminum and chemical industries, and production of ferrous metals, cellulose, paper, and oil products.

Construction of the third metallurgical base has been launched in Siberia, and a new base of ferrous metallurgy, using the enormous local iron and coal resources, has been created in Kazakhstan. A high-capacity power system is being organized in the same areas. Non-ferrous metallurgy is being further developed in Kazakhstan, Central Asia and in Transbaikal areas. The pulp and paper, as well as the timber, industries are being developed at a fast rate in the forest areas of Siberia and the Far East.

Ferrous metallurgy is also developing in the European part of the country by utilizing the enormous iron ore resources of the Kursk Magnetic Anomaly and the Ukrainian deposits. Large new production systems are under construction in the North-West, along the Volga, in the Northern Caucasus and the Ukraine.

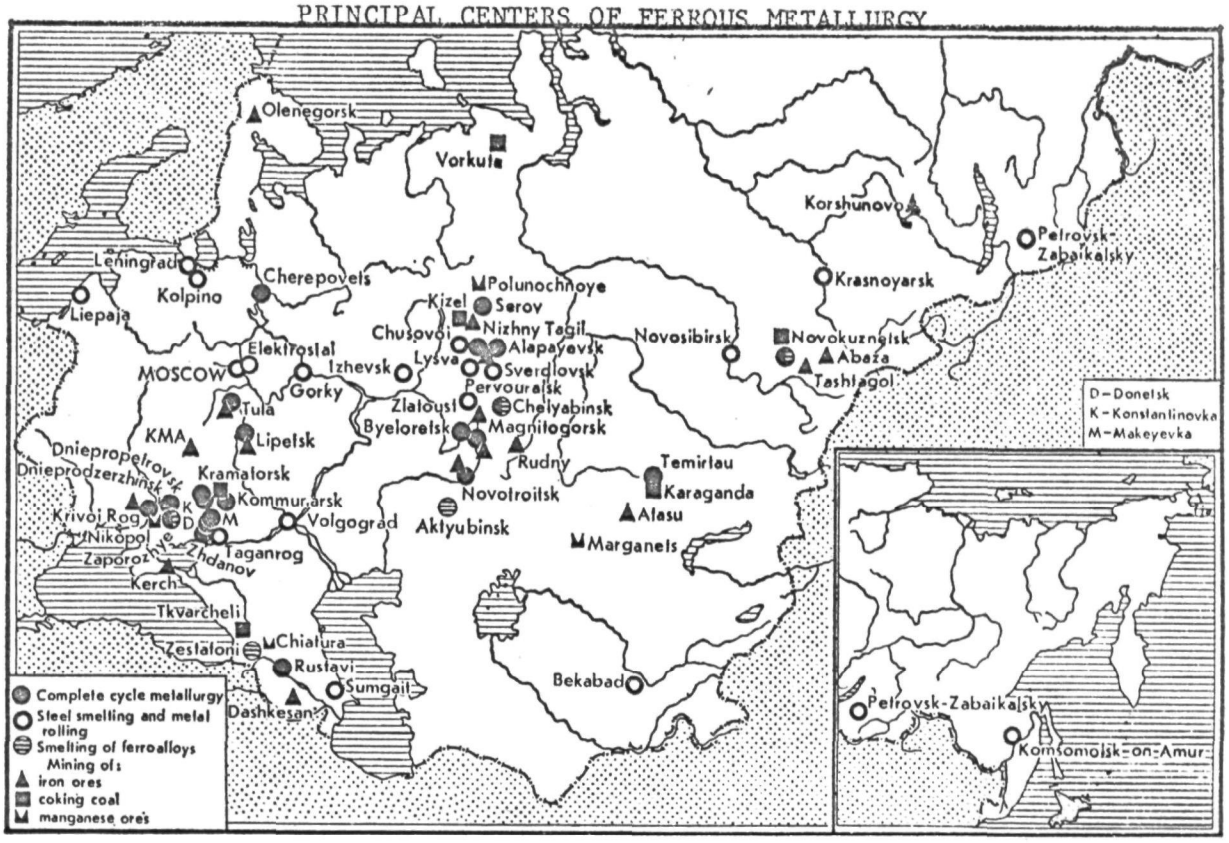
(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

THE MAJOR INDUSTRIAL CENTERS OF THE USSR

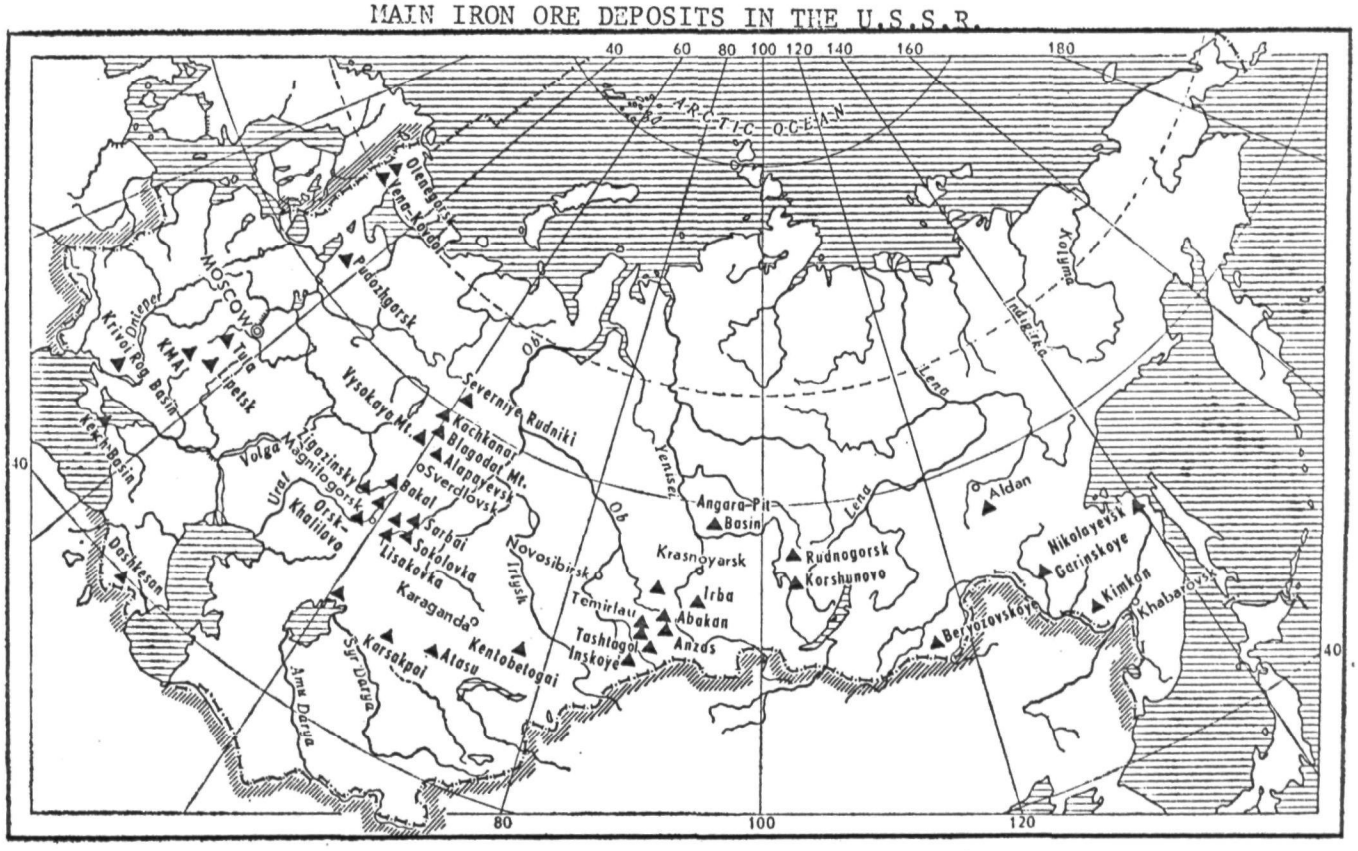


(After A. Efimov, "Soviet Industry", Moscow 1968)

PRINCIPAL CENTERS OF FERROUS METALLURGY

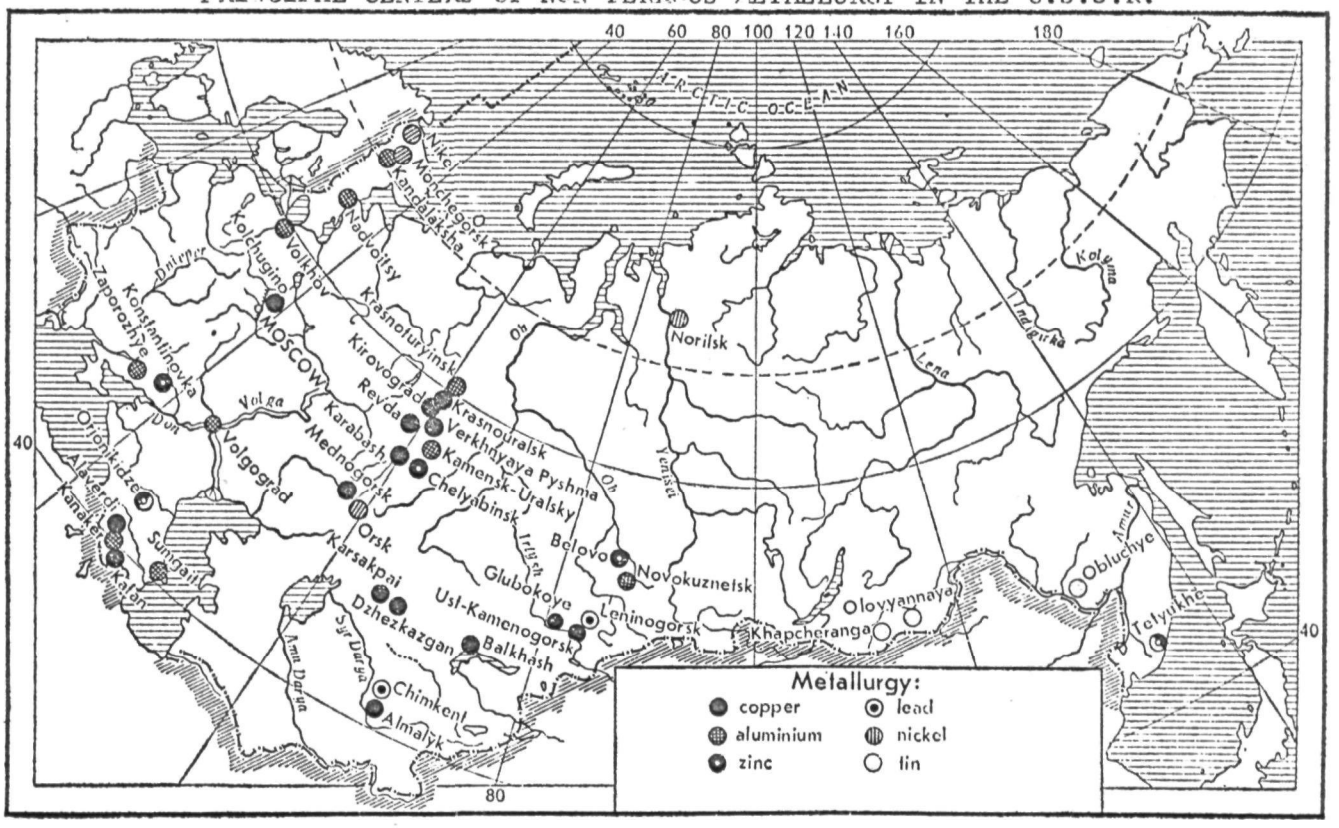


MAIN IRON ORE DEPOSITS IN THE U.S.S.R.

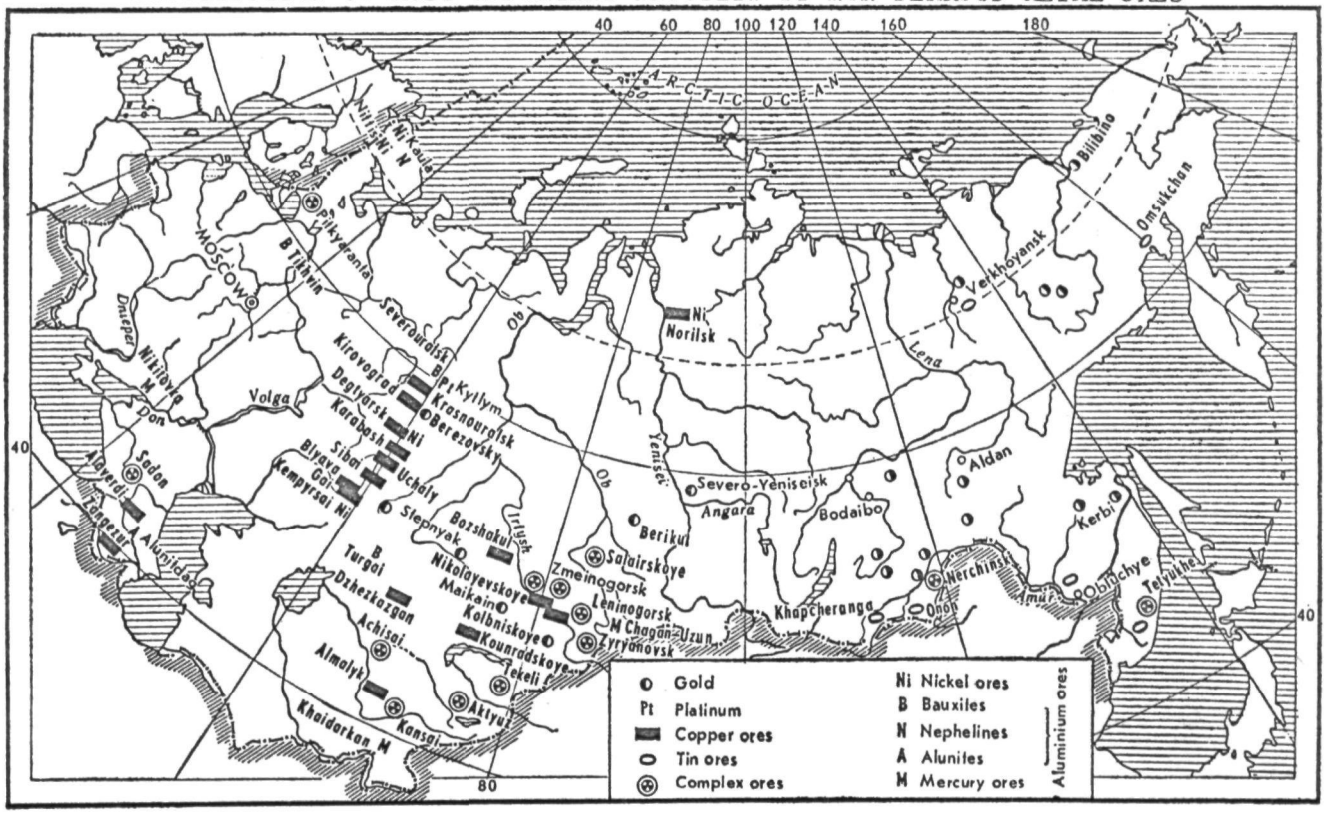


(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

PRINCIPAL CENTERS OF NON-FERROUS METALLURGY IN THE U.S.S.R.

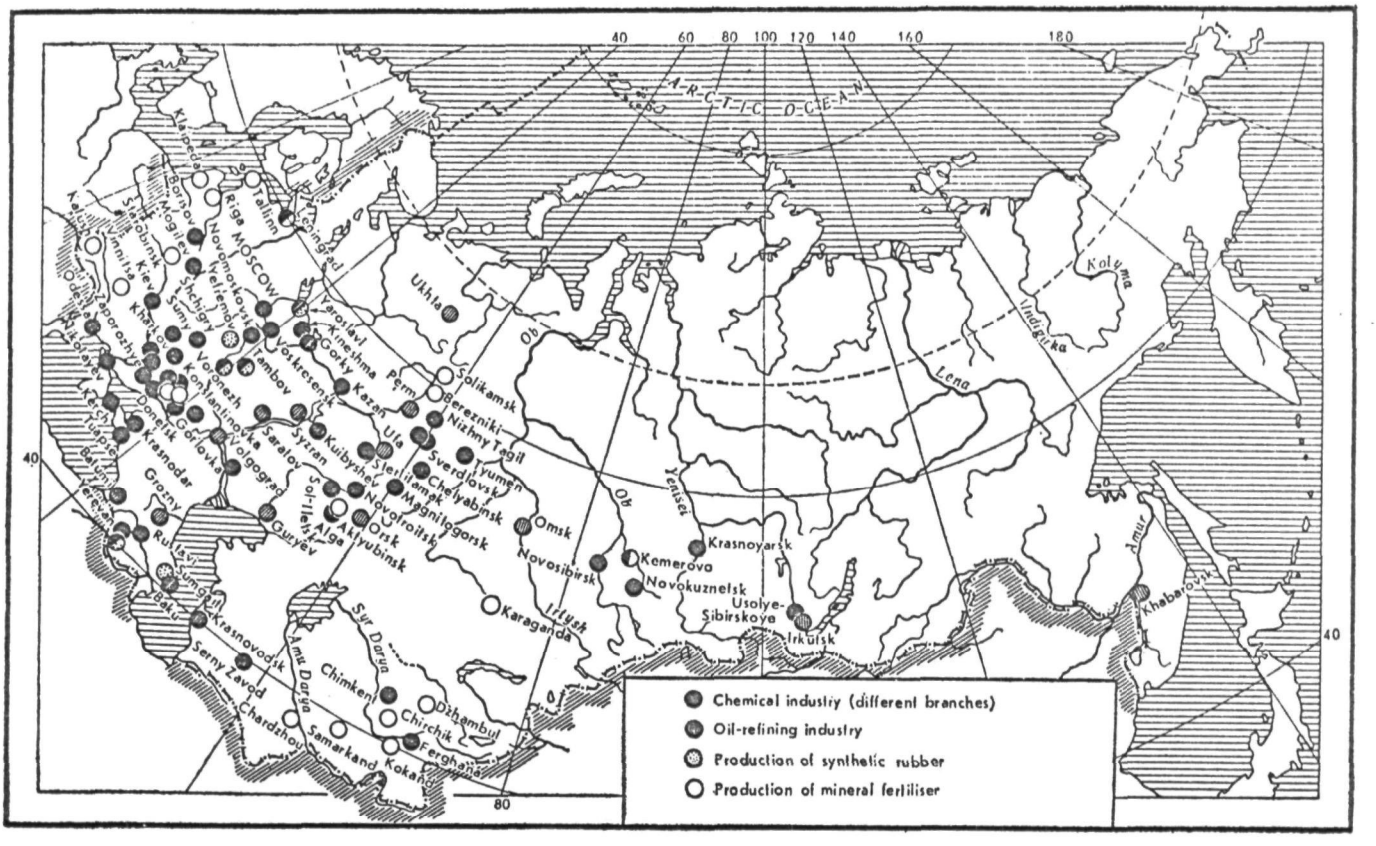


DISTRIBUTION OF MOST IMPORTANT DEPOSITS OF NON-FERROUS METAL ORES

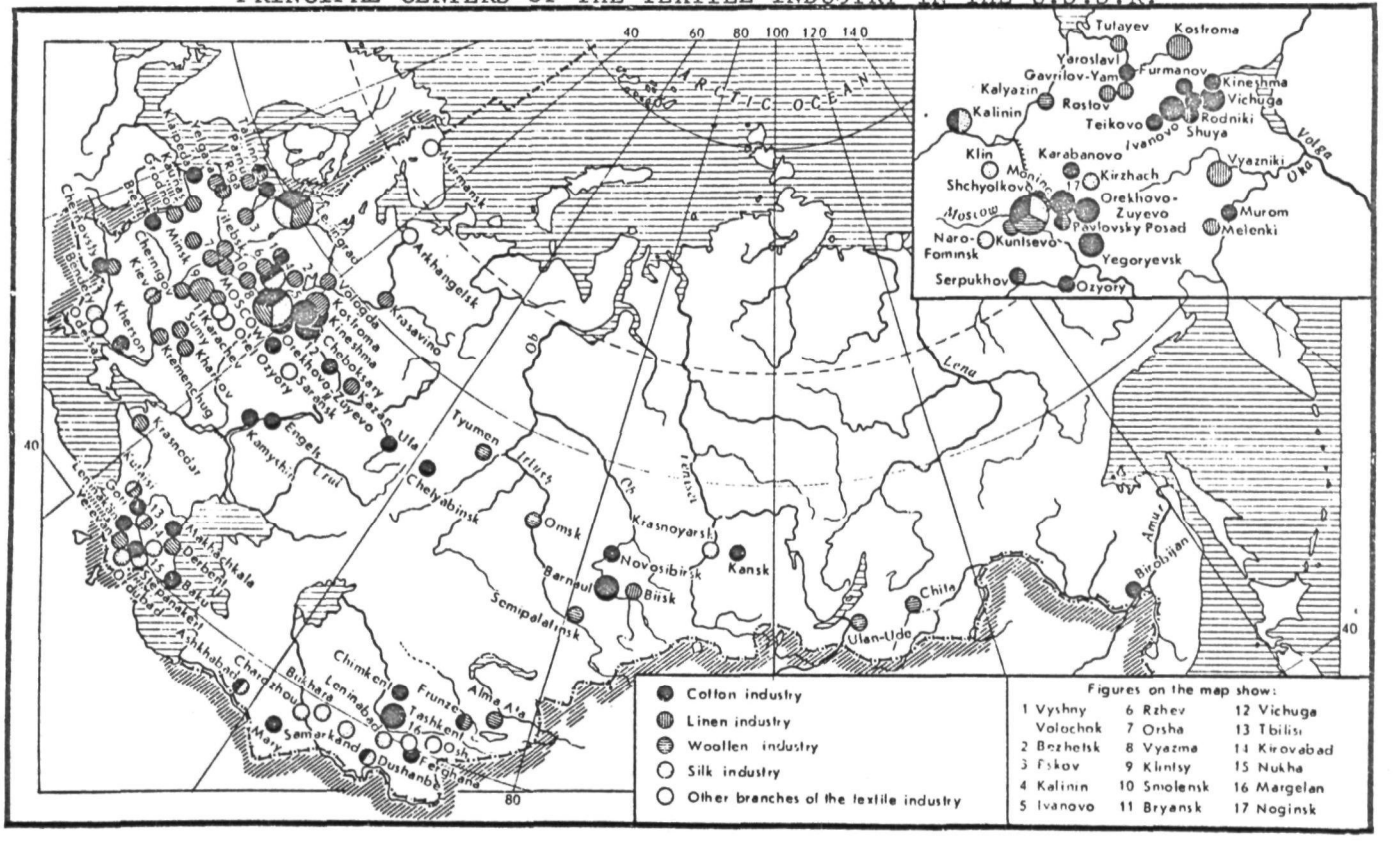


(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

PRINCIPAL CENTERS OF THE CHEMICAL INDUSTRY IN THE U.S.S.R.

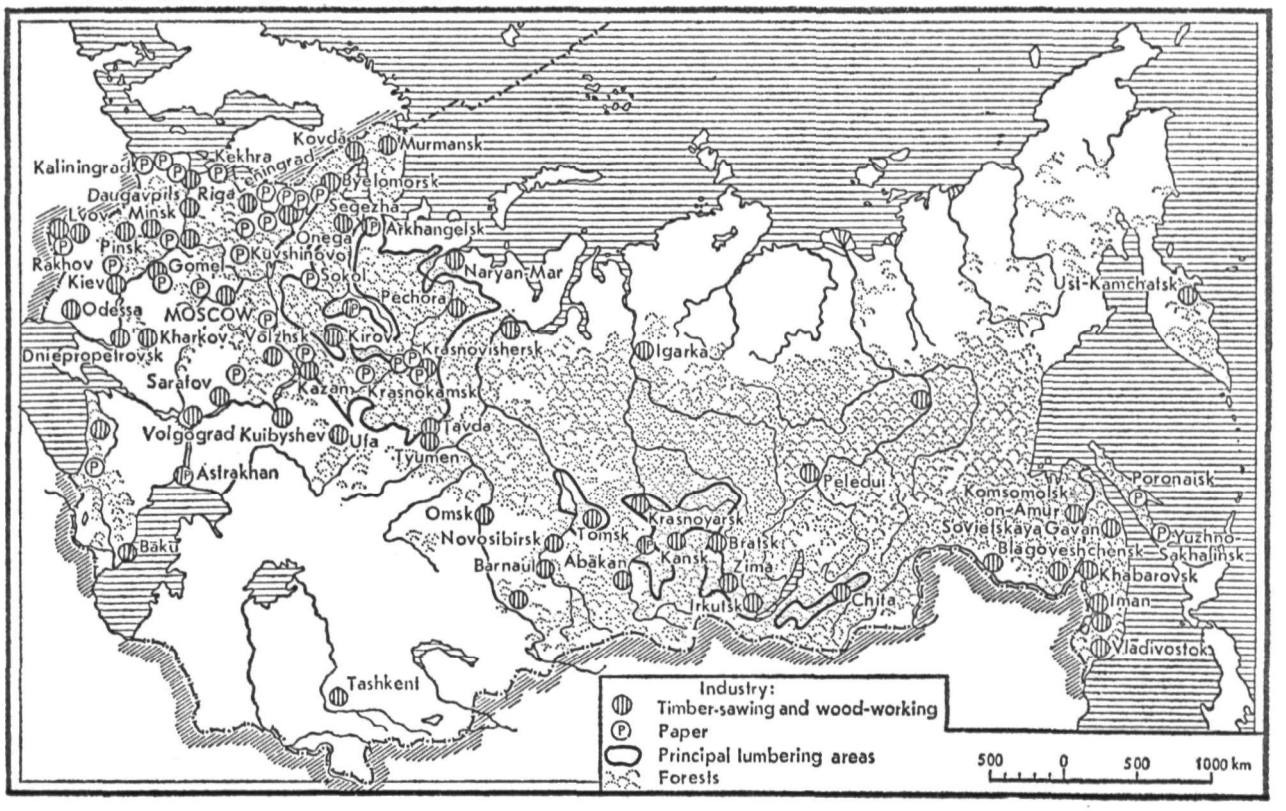


PRINCIPAL CENTERS OF THE TEXTILE INDUSTRY IN THE U.S.S.R.

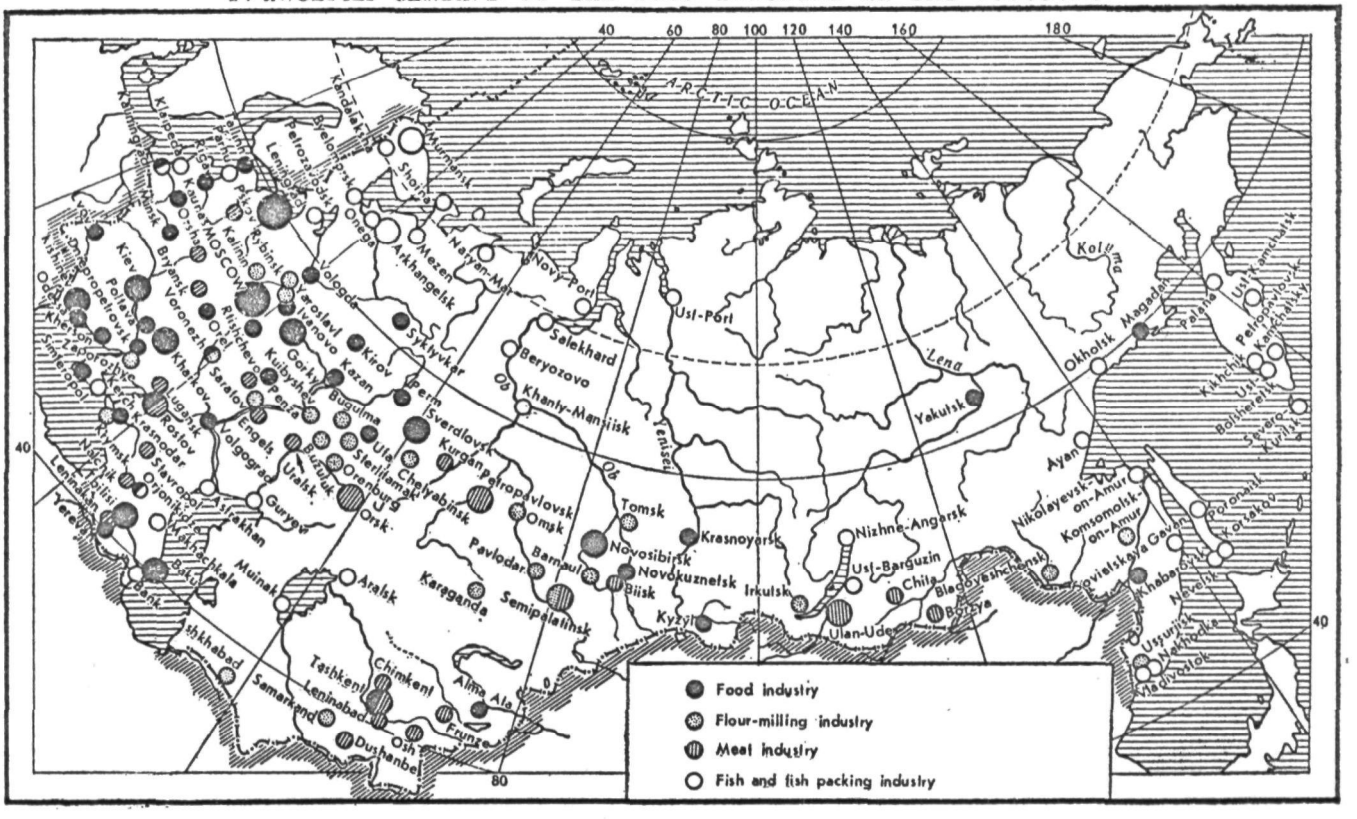


(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

PRINCIPAL CENTERS OF WOOD-WORKING AND PAPER INDUSTRIES IN THE U.S.S.R.

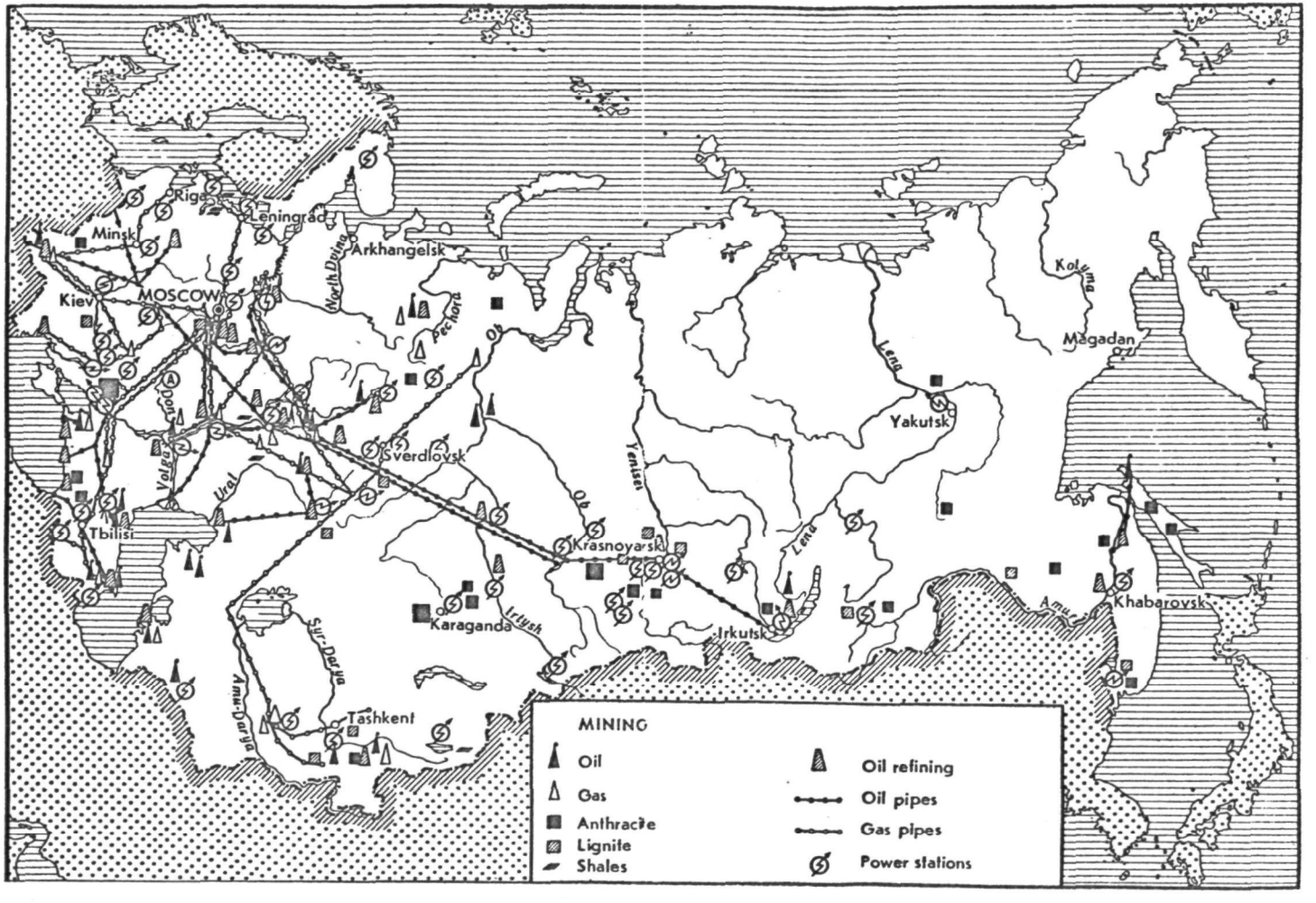


PRINCIPAL CENTERS OF THE FOOD INDUSTRY IN THE U.S.S.R.



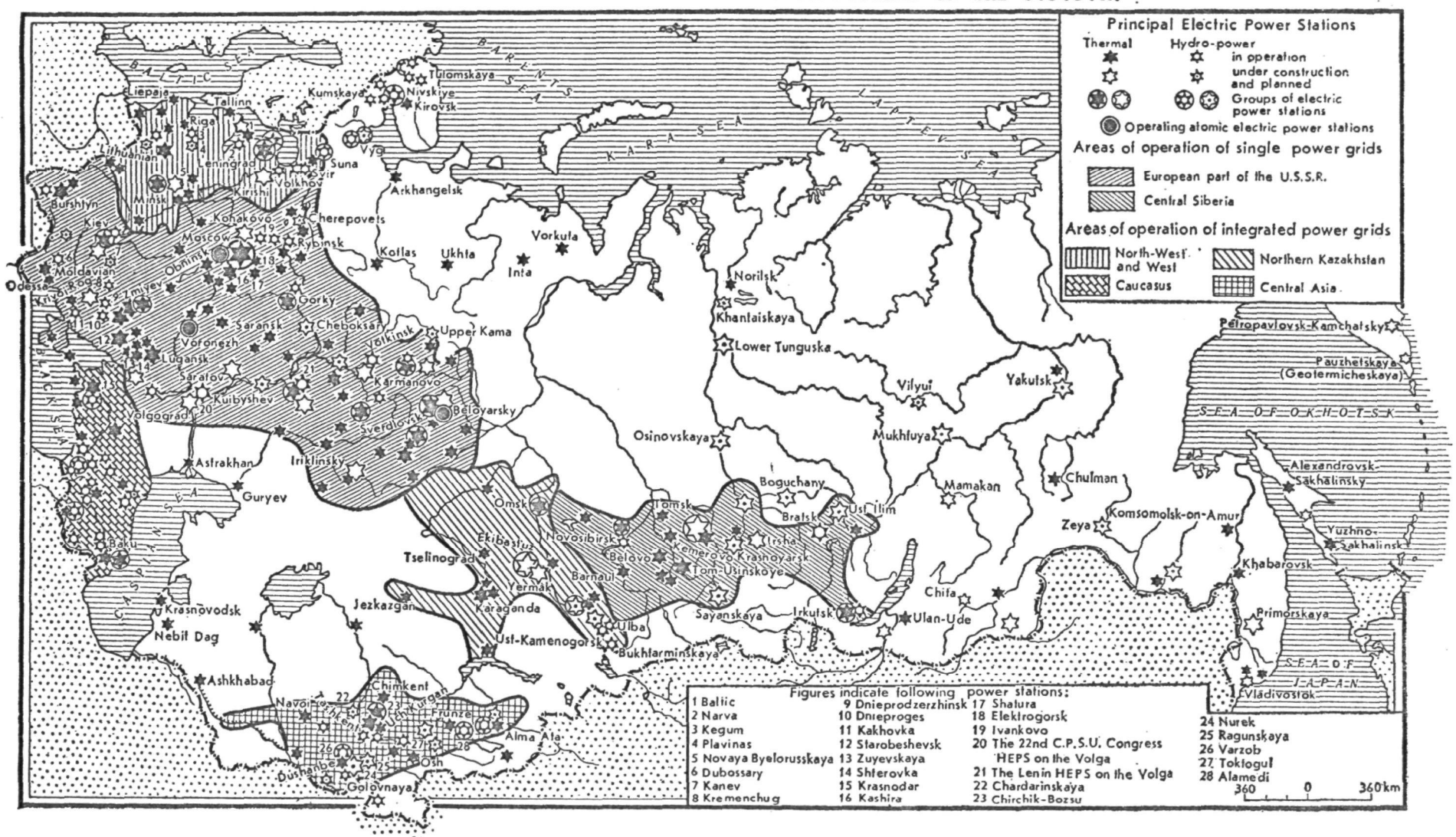
(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

THE MAIN MINING CENTERS OF THE USSR



(After A. Efimov, "Soviet Industry", Moscow 1968)

PRINCIPAL ELECTRIC POWER STATIONS AND POWER SYSTEMS IN THE U.S.S.R.



(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

BIOLOGICAL EFFECT OF LOW CARBON DIOXIDE CONCENTRATIONS*

O. V. Yeliseyeva
Fourth Main Administration, Ministry of Public Health of the USSR and
Communal Hygiene Department, Central Institute for Advanced Training of Physicians

From "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Pod redaktsiey Prof. V. A. Ryazanova i Prof. M. S. Gol'dberga. Izdatel'stvo "Meditsina" Moskva, p. 7-27, (1966).

Studies conducted by Pettenkofer have shown that the amount of CO_2 can be used to estimate the purity of air in dwellings and the degree of their aeration. Pettenkofer proposed a hygienic norm of CO_2 for dwellings of 0.07%, and Flugge, 0.1%. A 0.1% CO_2 content in dwellings is still the hygienic limit at the present time. Although this limit has not been physiologically substantiated, it has played a positive role in practice and is still used for calculating the required air exchange in closed spaces, and serves as the criterion for evaluating the purity of room air and the operation of ventilating systems.

Numerous studies conducted on animals and people have shown that an increase in the CO_2 content of inhaled air does not leave the organism unaffected and may cause substantial shifts in its condition. The central nervous system is particularly sensitive to excess carbon dioxide.

Despite the fact that there has been a considerable number of studies dealing with the influence of CO_2 on the various physiological functions of the organism, they were all made by using large concentrations and are desultory in character. Of greatest interest in this respect are the studies of V. P. Zagryadskiy, Z. K. Sulimo-Samuylo, P. F. Vokhmyaninov and O. Yu. Sidorov (1961). The authors studied the influence of relatively low CO_2 concentrations on man in short- and long-term exposure. The CO_2 content in the inhaled air ranged from 1 to 2%. Breathing with the gas mixture lasted from five hours to ten days. The authors note that an increase in the CO_2 content causes first of all a change in the activity of the respiratory and cardiovascular systems. When the stay in the chamber was brief, the respiration rate increased or remained unchanged, and after ten days there was a tendency for the respiration rate to slow down. An increase in pulmonary ventilation due to a considerable deepening of the respiratory movements was noted. There was also observed a lasting spasm of the peripheral vessels, a considerable slowing down of the frequency of cardiac contractions, and an increase of the minimum pressure by 10-15 mm Hg. After a prolonged exposure, changes in the electrocardiogram were observed, i.e., an increase of the P-Q, QRST intervals, indicating a certain slowing down of the conduction functions.

* The physiological studies were directed by Candidate of Medical Sciences A. D. Semenko.

Short-term exposure to CO_2 impaired the trace conditioned reflexes to time, the percentage of erroneous reactions in the study of motor conditioned reflexes increased, and so did the number of erroneous answers. The subjects could not sustain an active attention for six minutes and began to doze off. A long exposure to carbon dioxide in a concentration of 1.5-2% was associated with lability of the functions of the motor and visual systems, impairment of motor coordination, high emotional excitability, restless sleep, irritability, and marked drowsiness in the morning hours. There were up-and-down fluctuations of working capacity and a considerable increase in the number of errors in the course of work. Changes in the electroencephalogram were very slight. It is important to note that the change of the physiological functions of the organism was observed not only during exposure to atmosphere with a higher CO_2 content, but also during the period of the aftereffect on passing to the conditions of a normal gaseous medium. The longer the organism was exposed to conditions of higher CO_2 content, the higher was the carbon dioxide concentration, and the longer was the duration of the aftereffect period.

In the course of the last few decades, the CO_2 content of atmospheric air in large cities has been increasing during the winter months. Thus, in Moscow, the mean daily concentration at that time may reach 0.07%, and the single concentrations are still higher (K. A. Bushtuyeva, V. A. Ryazanov, Ye. F. Popova, 1956), this being explained by a markedly increased amount of burned fuel. Under these conditions, it becomes impossible to maintain the CO_2 concentration in the air of dwellings at the level of Pettenkofer and Flügge's norm. The question arises, to what extent is this norm valid under changing living conditions? Actually, Pettenkofer's suggestion was based on the idea of using carbon dioxide as an indirect index for the pollution of air of residential and public buildings by volatile products of human metabolism contained in exhaled air, respiration, and malodorous gases from body surface and clothing. In a modern city, where CO_2 is generated chiefly by the combustion of fuel, it loses the meaning of an indirect sanitary index. For this reason, the observance of this norm for the air of residential and public buildings is not justified.

To insist on the observance of this norm is to require a decrease in the concentration of carbon dioxide in the surrounding atmosphere, which would involve extremely costly measures aimed at decreasing the discharge of CO_2. This could be done only if carbon dioxide as such were proven to have undesirable hygienic effects on man in concentrations such as 0.1%. However, as we have seen above, data on the physiological action of CO_2 concentrations below 1% are lacking in the literature.

For these reasons, we undertook a study of the influence of CO_2 in concentrations of 0.5% and below on the respiratory function, the cardiovascular system, and the electrical activity of the human cortex. The methods of pneumography, rheovasography and electroencephalography were used for this purpose.

Our studies were conducted on healthy persons 19 to 45 years of age. A constant, predetermined CO_2 concentration was maintained during the entire investigation.

The source of gas was a cylinder of liquid carbon dioxide at a pressure of 36-46 atm, from which carbon dioxide flowed through a reducing valve, a float-type flowmeter, and a capillary rheometer into a mixing tank. Pure air was also fed into the latter at a constant rate of 20 l/min. The gas mixture was then fed into a cylinder whose contents the subject breathed.

The lines carrying the air consisted of glass and Duralumin tubes. The air supply was purified with silica gel, activated carbon, and a cotton filter.

The actual concentrations of carbon dioxide in the cylinder were determined by a spectrophotometric method that we developed (M. D. Manita, O. V. Yeliseyeva, T. V. Dyshko, 1964), both before and after the experiment.

The apparatus that we used insured an adequate constancy of the concentrations. During the entire observations, the subject remained in an electrically and acoustically insulated room, sitting in a comfortable position in a soft chair. A cylinder continuously supplied with pure air or with air of a known carbon dioxide concentration was placed at the height of his face. The apparatus for dispensing the gas fnr for recording the functions studied and the experimenter were located in an adjoining room.

The examinations were conducted with a background of rhythmic photic stimulations of varying frequency, duration and intensity, generated by a photostimulator built by the Kaiser Co. The lamp was placed before the subject's eyes at an optimum distance determined for each person individually. This distance was kept constant during the period of all the experiments. The intensity of the rhythmic photic stimulation was changed every five seconds from 0.1 to 0.6 Joule. The entire series of stimulations lasted 40 sec in the following sequence (eight 5-second stimulations in Joules): 0.2, 0.6, 0.2, 0.1, 0.2, 0.6, 0.2, 0.1. The subject sat quitely in the room and signaled the change in light intensity by slightly depressing a button. This movement was recorded on a myogram. The method made it possible to control and maintain the subject's wakefulness, otherwise they usually sank into a drowsy state toward the end of the experiment. The conditions were identical in all the tests. The main tests were conducted after repeated training. After the subjects had become accustomed to the experimental situation, we recorded the curves during inhalation of pure air. This served as the background against which the influence of corresponding carbon dioxide concentrations was studied. Because we studied low concentrations (from 0.1 to 0.5%), the pure air was regularly checked

for its carbon dioxide content. The CO_2 concentration did not exceed 0.05% and amounted to an average of 0.04%. During the entire experiment we alternated the recordings of the inhalation of different concentrations of CO_2 and pure air.

Numerous investigations, particularly those of Holden and co-workers, established that the physiological action of carbon dioxide depends on the partial pressure of this gas. A 0.23% increase of its content in alveolar air when the body is at rest leads to a 100% increase in the ventilation volume. A 0.01% decrease or increase of CO_2 content in alveolar air causes a corresponding 5% average decrease or increase of the ventilation volume (Holden and Priestley*, 1937). Thus there is a very significant dependence of the change of pulmonary ventilation on the CO_2 pressure in alveolar air. An increase in pulmonary ventilation is a compensatory reaction of the organism designed to decrease the carbon dioxide content of alveolar air when it increases in the inhaled air.

Ye. M. Berkovich (1939) has shown that the inhalation of CO_2 starting at a concentration of 1% increases the respiratory capacity, extends the duration of both breathing phases, and slightly decreases the respiration rate.

In the light of modern physiological concepts (Heymans and Cordier**, 1940; M. V. Sergiyevskiy, 1950, 1961; M. Ye. Marshak, 1961, and others), carbon dioxide alters normal breathing by acting not on the breathing center directly, but primarily on the vascular receptor zones and the cortex of the cerebral hemispheres. Of particularly great importance in the stimulation of breathing, along with the receptors of the lungs, are the chemoreceptors located at the point of branching of the common carotid. As the CO_2 content increases, stimulation of the chemoreceptors of the aortal-carotid zone takes place in the blood. By reflex action, this causes a deepening and a certain acceleration of breathing.

In addition, it has been shown (Holden and Priestley, 1937) that the nerve cells of the respiratory center can be stimulated by carbon dioxide present in the tissue fluid bathing them, into which it penetrates from the blood. Thus, carbon dioxide acts reflexly on the respiratory center by stimulating the chemoreceptors, and also automatically through the blood.

To study the influence of CO_2 on the function of external respiration, we selected seven persons aged 19 to 45, nonsmokers, without a change in the nasopharynx or upper respiratory tract. The respiratory movements were recorded by using the method of pneumography with a cuff attached to the frontal surface of the chest.

The duration of a single examination of different persons varied from 20 to 24 minutes. During the first five minutes the subject breathed pure

* [Translator's note: Kholden and Pristli, according to the transliteration of Russian reference.]
** [" " Geymans and Kord'ye, according to the transliteration of Russian reference.]

air, then, without his noticing it (by turning a three-way stopcock) a mixture with a given CO_2 concentration was supplied to the cylinder, which he also breathed for five minutes. Pure air was then fed into the cylinder again. The instant of start and finish of the gas supply was marked on the tape by means of an electric marker connected to a switch.

The data obtained were treated according to the rules of variation statistics with calculation of the confidence factor. Changes with a confidence factor of no less than 95% were considered statistically significant.

The treatment was used in two periods: the first two recordings during inhalation of pure air for five minutes were taken as the "background", and the next three recordings during inhalation of a given CO_2 concentration for five minutes as the "effect". The period of the "aftereffect" during inhalation of pure air was divided into the "close aftereffect" (3 recordings) and "distant aftereffect". In each curve, the depth and frequency of respiration were calculated. Results of the determinations were expressed in percent. The average level of the first two ("background") determinations were taken as 100%.

The action of each concentration studied on the respiration was repeated two to three times on the same subject.

Two CO_2 concentrations were studied, 0.5 and 0.1%. The 0.5% concentration was found to be active for all seven subjects. In all of them, a decrease in the amplitude of the respiratory movements was noted. However, in different persons the statistically reliable changes were not the same and were manifested in different periods: in subjects S. and Ch. the changes took place during the "effect" period and were preserved during the "aftereffect" period. The same results during the "aftereffect" period were obtained in subjects O., R., Z. and M. In subject A, changes were observed only during the "aftereffect" period (Table 1).

Table 1

Changes in the Amplitude of Respiratory Movements (in %) During Inhalation of CO_2 in 0.5% Concentration

Subject	Periods of Observation			
	Background	Exposure	Aftereffect	
			Close	Distant
S.	100	91,6 (a)	88,3	70,1 (c)
O.	100	87,0	58,9 (b)	74,7 (c)
R.	100	92,5	74,35 (a)	72,5 (c)
Z.	100	94,4	74,8 (a)	71,1 (c)
A.	100	89,0	84,5 (a)	99,4
M.	100	98,2	83,1 (a)	—
Ch.	100	77,1 (b)	60,6 (c)	—

Note. Confidence factor: a - 95%; b - 99%; c - 99.9%

At a CO_2 concentration of 0.1%, statistically reliable changes (also in the decreasing direction) in respiration amplitude were obtained in five out of six subjects (Table 2).

As an illustration, we shall cite the dynamics of changes in the respiration amplitude in percent for subject O. during inhalation of different CO_2 concentrations (Fig. 1).

Table 2

Changes in the Amplitude of Respiratory Movements (in percent) During Inhalation of CO_2 in 0.1% Concentration

Subjects	Periods of Observations			
	Background	Exposure	Aftereffect	
			Close	Distant
G.	100	96,7	96,3	96,0
O.	100	88,7	66,9 (c)	75,2 (b)
R.	100	94,4	78,8 (a)	78,6 (b)
Z.	99,9	95,2	90,5	85,7 (c)
Zh.	99,9	79,4 (c)	71,6 (b)	88,6
M.	100	91,2	80,2(c)	—

Note: a, b, c - see Table 1.

As far as the respiration rate is concerned, and also the inspiration-expiration ratios, they did not undergo any noticeable changes in any of the subjects. Thus, carbon dioxide in concentrations of 0.5 and 0.1% against a background of inhalation of pure air causes changes only in the depth of breathing.

The data which we obtained disagre with the literature data to some extent. However, such discrepancies in the results are readily explained by the fact that our examinations involved the use of considerably lower carbon dioxide concentrations than those used by the authors of the above studies.

Both the excitability of the respiratory center and the sensitivity of the receptors accepting carbon dioxide as an adequate stimulus display substantial differences in the effect of different concentrations. This is necessarily reflected in the characteristics of external respiration responses to the action of carbon dioxide.

In order to study the influence of carbon dioxide on the state of the peripheral circulation in man, we made use of the rheovasography method.

As we know, high frequency curves for studying the fluctuations of the heart and blood vessels were first used in 1932 by Atzler and Lehmann. In the Soviet Union, the first investigators to develop a method of study

of blood circulation by means of high frequency currents were A. A. Kedrov and A. I. Naumenko (1941, 1949, 1954), who validated it theoretically and applied it in experiments on animals and in observations on people in the study of intracranial blood circulation.

The method of rheovasography was used for several years at the Institute of Therapy of the USSR Academy of Medical Sciences in the study of cardiovascular diseases. The rheovasography method consisted essentially of the fact that an electrical current of high frequency encounters a certain resistance on passing through a portion of live tissue. The magnitude of this resistance is affected by the degree of blood filling and the rate of blood flow in the given portion of tissue (A. A. Kedrov and A. I. Naumenko, 1949). The more the blood circulation rate changes per unit time (positive and negative acceleration of the blood flow), the wider the range over which the resistance of the portion of live tissue to the electric current changes. After a cardiac systole, the rate of

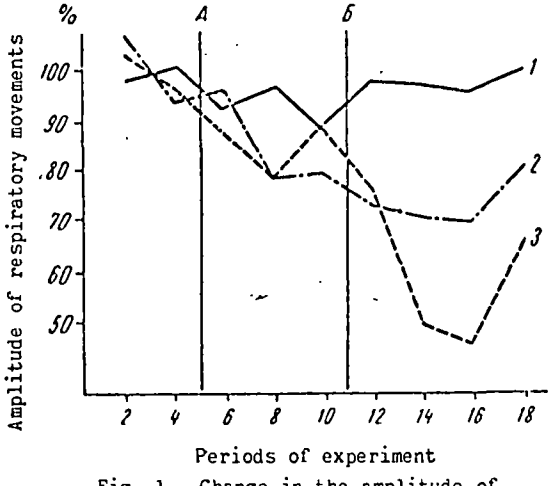


Fig. 1. Change in the amplitude of respiratory movements in subject O. during inhalation of different CO_2 concentrations.
1 - pure air; 2 - 0.1%; 3 - 0.5%;
AB - period of gas supply.

blood flow always increases (positive, pulse acceleration) during a certain period of time. The rate then slows down. After a certain time interval following the systole, under the influence of the elastic force of the vessels, a new increase of the rate of blood flow takes place (positive vascular acceleration). The rate then slows down again and reaches the level it had before the start of the systole. The blood filling also changes correspondingly. Such changes in any portion of live tissue arise regularly during the period of each cardiac cycle and are recorded by means of rheovasography. This method is marked by a high sensitivity. In serious disorders of the peripheral blood circulation, the data of rheovasography always reflect the existing hemodynamic disturbances in a more differentiated manner (V. A. Karelin, 1957). The same

complexes are repeated on a rheovasogram. Each complex consists of one large and two small spikes. The large spike has a steep rising edge and a gentler descending edge. It is generally recognized (V. A. Karelin, 1957; A. S. Loginov and Yu. T. Pushkar', 1962) that the large spike is a reflection of the systolic acceleration of the blood flow. In time, it almost coincides with the ventricular complex of the electrocardiogram. The small spikes appear after the systole in time and may correspond to an acceleration of the blood flow as a result of the elastic force of the vessels (positive vascular acceleration).

Our studies were made by means of an RG1-0.1 type rheograph whose output was connected to the recorder of a Kaiser 8-channel electroencephalograph. Seven people aged 18 to 45 were selected for the experiment. The rheovasogram was recorded from the right arm, the electrode being placed on the skin of the shoulder and forearm. The recording was made in the morning hours, always at the same time.

Each experiment lasted 20-24 minutes. The rheovasogram was recorded 10-12 times every two minutes. The first two recordings were made during inhalation of pure air and were taken as the background; the next three recordings, during the period of exposure, which lasted five minutes; and the remaining recordings, during the period of the aftereffect, when the subject breathed pure air again. During treatment of the data, the aftereffect was divided into two periods: three recordings pertained to the "close aftereffect", and the remaining ones, to the period of the "distant aftereffect".

To analyze the rheovasography data, we measured the sizes of the rising and falling edges of the large spike and second spike. The results of the determinations were expressed in percent of the average of the first two "background" determinations.

Data from the study of the influence of 0.5% carbon dioxide on the rheovasographic indices are listed in Table 3.

The data of Table 3 show that carbon dioxide in 0.5% concentration caused distinct and significant changes in blood filling of the vessels in all seven subjects. The index of the influence of carbon dioxide was an increase in the size of the rising and falling edges of the large spike and also of the second spike. Only subject S. responded to carbon dioxide inhalation with a decrease in the size of the descending edge of the large spike of the rheovasogram.

In the majority of subjects, the action of carbon dioxide manifested itself already during the period of exposure. Changes in the rheovasogram were stable in character and were preserved during the entire period of

the aftereffect.

Table 3

Changes of Complexes (in %) of the Rheovasogram Under the Influence of Carbon Dioxide in 0.5% Concentration.

Subject	Complexes of Rheovasogram	Periods of Observations			
		Background	Exposure	Aftereffect	
				Close	Distant
R.	Rising edge	100,0	117,9(b)	131,1(c)	135,0(c)
	Descending edge	100,0	113,3(a)	120,4(b)	116,2(a)
	Second spike	100,2	138,3	179,0(a)	203,2(c)
Z.	Rising edge	99,9	102,4	112,8(b)	119,5(a)
	Descending edge	100,0	95,8	104,1(a)	109,5
	Second spike	99,9	100,2	112,2	111,2
O.	Rising edge	100,3	114,7	142,3(c)	151,5(c)
	Descending edge	100,0	102,4	111,5(c)	120,1(c)
	Second spike	100,1	103,7	133,6(a)	158,5(c)
A.	Rising edge	99,9	98,3	109,8	98,5
	Descending edge	99,9	104,3	105,5	100,7
	Second spike	100,0	96,2(c)	114,7(a)	99,4
G.	Rising edge	99,6	120,6(c)	123,3(c)	124,5(c)
	Descending edge	100,0	117,2(a)	121,5(c)	118,4(b)
	Second spike	100,0	133,8(a)	134,8(a)	129,7(a)
B.	Rising edge	100,0	112,6(a)	126,0(b)	125,8(c)
	Descending edge	100,0	109,4(a)	119,7(b)	126,2(b)
	Second spike	100,2	100,0	129,1	109,1(a)
C.	Rising edge	100,0	110,9	113,9(c)	112,1(c)
	Descending edge	100,0	93,8(a)	80,0(c)	75,4(c)
	Second spike	100,0	98,9	108,9	103,8

Note: a, b, c - see Table 1.

Similar but less distinct results were obtained in a study of carbon dioxide in 0.1% concentration. The changes were statistically reliable in 5 out of 7 subjects (Table 4).

As is evident from Table 4, the inhalation of carbon dioxide in 0.1% concentration caused an increase of all three indices of the rheovasogram. These data show beyond any doubt that even a carbon dioxide concentration as low as 0.1% is capable of affecting the state of the

blood flow in healthy people. Fig. 2 shows graphs of the changes in the size of rheovasogram complexes for subject O.

Table 4

Changes in Rheovasogram Complexes (in percent)
Under the Influence of Carbon Dioxide in 0.1% Concentration.

Subject	Rheovasogram Complexes	Periods of Observations			
		Back-ground	Exposure	Aftereffect	
				Close	Distant
R.	Rising edge	100,0	104,8	111,7(a)	115,7(c)
	Falling edge	100,0	104,9(b)	111,1(a)	114,7(b)
	Second spike	100,0	103,0	115,3	126,8(c)
Zh.	Rising edge	98,5	114,3	127,5(c)	120,2(c)
	Falling edge	100,0	109,0(a)	110,5(b)	106,1(b)
	Second spike	100,6	115,8	137,1(c)	132,3(c)
Z.	Rising edge	100,0	99,6	96,5	95,3
	Falling edge	100,0	102,0	97,9	97,7
	Second spike	100,5	95,4	75,5(c)	79,3(b)
O.	Rising edge	100,0	109,6	108,3	120,5(a)
	Falling edge	100,0	103,3(a)	101,0	108,2(c)
	Second spike	100,0	98,7	84,0	76,0
A.	Rising edge	99,9	107,6	109,3	96,4
	Falling edge	97,0	101,1	103,1	89,8
	Second spike	99,9	131,4	117,5(a)	123,3

Note: a, b, c - see Table 1.

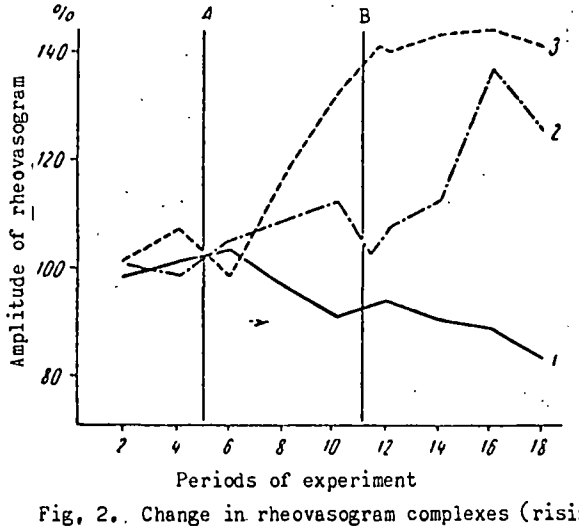


Fig. 2. Change in rheovasogram complexes (rising edge) during inhalation of different CO_2 concentrations in subject O. (Reliability of changes 95%).
Notation same as in Fig. 1.

The responses of the electrical activity of the brain to the inhalation of increased carbon dioxide concentrations were studied by Bremer and Thomas (1936), S. I. Subbotnik and P. I. Shpil'berg (1946), and others. They established that the inhalation of increased carbon dioxide concentrations leads to the development of the desynchronization response. I. S. Repin (1960) observed desynchronization in rabbits in all parts of the central nervous system during their inhalation of up to 5% CO_2. Similar data were obtained by Yu. N. Ivanov (1962).

The effect of carbon dioxide concentration below 1% was not studied.

In recent years, practical sanitary investigations have made increasingly broader use of methods based on the recording of changes in the electrical activity of the brain under the influence of low concentrations of noxious substances. These methods have found wide applications in the studies performed in the Department of Communal Hygiene of the Central Institute for Advanced Training of Physicians.

We undertook the task of following the changes in the electrical activity of the human brain during inhalation of carbon dioxide in concentrations below 1%.

Three electroencephalographic methods were used for this purpose: method of development of the electrocortical conditioned reflex, method of rhythm assimilation, and method of reinforcement of the intrinsic potentials of the brain.

The experimental conditions were identical in all the tests.

The tests were made on an 8-channel encephalograph with a Kaiser photostimulator. The electroencephalograms were taken by the unipolar method (in combination with the bipolar method) off the occipital, parietal, and temporal areas of the head.

At the same time, the time mark, frequency and intensity of rhythmic photic stimulations and the time of the gas supply were recorded.

The recording of biocurrents was made on the same channels with the same degree of sensitivity, and the electrodes were placed on the same areas. The subjects were asked to participate in the experiment only during the morning hours.

For each subject, training recordings of electroencephalographic curves were first made for one month. Afterward, the curves were recorded during inhalation of pure air, then with a given carbon dioxide concentration.

The method of development of the electrocortical conditioned reflex was first used in hygienic studies by K. A. Bushtuyeva, Ye. F. Polezhayev and A. D. Semenenko (1960). This method makes it possible to reveal fine functional changes in the cerebral cortex arising under the influence of various external factors. We studied the development of the electrocortical conditioned reflex in five healthy persons with a well-defined α rhythm. The inhalation of the gas lasted 15 seconds, and during the next five seconds was reinforced with intermittent light. The conditioned reflex changes in electroencephalography with reinforcement with rhythmic light have been described by R. A. Pavlygina and V. S. Rusinov (1960) and also by Pavlygina (1960). In order to exclude the development of the conditioned reflex to time, each new pairing of the action of the gas and light was given at different time intervals.

We expected that in the case of formation of a temporal relationship to the inhalation of definite carbon dioxide concentrations, the imposed rhythm should appear before the rhythmic light was turned on.

However, the 0.5% carbon dioxide concentration did not cause the formation of an electrocortical conditioned reflex in any of the 5 subjects. The number of pairings reached 23-25. Concentrations of 2-2.5% also failed to cause the appearance of an imposable rhythm before the light was turned on.

The second electroencephalographic method used for the determination of thresholds of the reflex action of carbon dioxide - rhythm assimilation - was proposed by A. G. Kopylov (1956).

The method as modified by us has been described by A. D. Semenenko (1963) and was used in studies conducted at the communal hygiene department of the Central Institute for the Advanced Training of Physicians by V. I. Filatova (1962), B. Mukhitov (1962), N. B. Imasheva (1963), V. A. Chizhikov (1963), and P. G. Tkachev (1963).

Two subjects with a well-defined α rhythm and a satisfactory assimilation of the rhythm in the range of usual frequencies were selected for the experiments. For each, a frequency of flickering light was chosen which differed (was greater or smaller) from "his" α rhythm. An optimum distance for each subject was then established between the subject and the photostimulator lamp; the maximum total amplitude of rhythm assimilation for a large intensity of flashes (0.6 Joule) and the minimum amplitude for the lower intensity of flashes (0.1 Joule) were then observed at this distance. The frequency of the Flickering light and the distance of the lamp were kept unchanged during all the experiments. The entire series of light stimulations lasted 40 seconds. The intensity of the rhythmic photic stimulus was changed every 5 seconds from 0.1 to 0.6 Joule.

The recording of the assimilation curves was repeated every 2 minutes for 20 minutes. The subjects breathed pure air during this entire period.

In studies with gas, the first two recordings of the curves were made during inhalation of pure air, the next three recordings during the period of inhalation of the gas, and the following ones, 5 seconds after cessation of the supply of carbon dioxide, then every 2 minutes until the reestablishment of the initial level of the assimilated rhythm or the appearance of a tendency toward its reestablishment.

The electroencephalograms obtained were analyzed from the change in the amplitude of the assimilated rhythm, expressed in microvolts. To calculate the amplitude, oscillations exactly correspodning to the frequency of the applied light pulses were recorded first, then the amplitude of the recorded assimilated rhythm was measured in millimeters for each intensity separately and the total amplitude was measured for every 40 seconds of rhythmic photic stimulations. The total amplitude of the assimilated rhythm based on all the intensities for the entire period of the experiment was calculated in percent of the average total amplitude obtained from the results of the first two curves of rhythm assimilation recorded in the second and fourth minutes of the examination, and taken as 100%. The results of the examinations are given in Fig. 3 and 4.

A carbon dioxide concentration of 0.5% was found to be the active concentration for both subjects.

In subject R. T., there was noted a well-defined tendency toward an increase in the amplitude of the assimilated rhythm, although statistically reliable changes appeared only during the period of the aftereffect (the reliability of the changes was 99%).

Somewhat different results were obtained in subject I. A., in whom the action of the gas was manifested in the second minute of inhalation (reliability of changes 95%) and was also characterized by an increase in the total amplitude of the assimilated rhythm. When using the third method of study of the electrical activity of the brain, we employed the frequency of the rhythmic light stimulus corresponding to the frequency of the intrinsic potentials of the subjects' brain. The calculation of the total amplitude of the reinforced rhythm was made by means of a special electronic device, an integrator built by engineer B. N. Balashov.

The study of the reinforcement of potentials of the intrinsic rhythm during inhalation of low carbon dioxide concentration was made on seven practically healthy people aged 19-45. The experimental conditions and the treatment of the results were analogous to the ones described above.

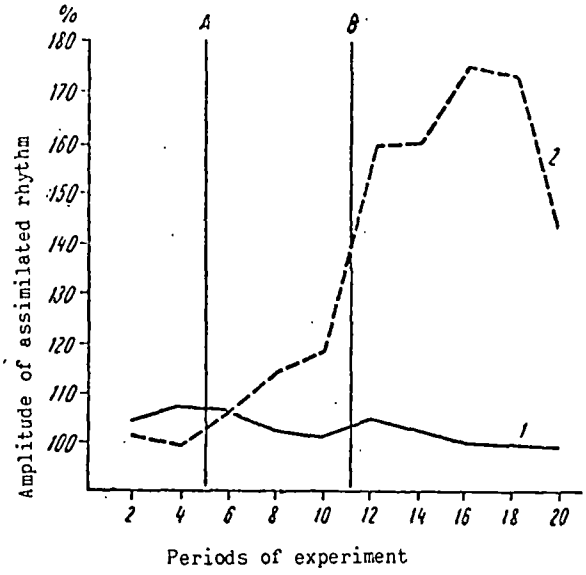


Fig. 3. Change in the total amplitude of assimilated rhythm in subject R. under the influence of CO_2 inhalation (reliability of changes 99%).
1 - pure air; 2 - 0.5%; AB - period of gas supply.

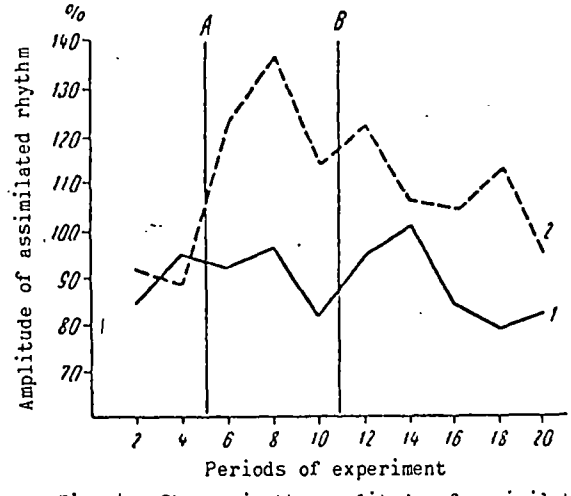


Fig. 4. Change in the amplitude of assimilated rhythm in subject I. under the influence of CO_2 inhalation (reliability of changes 95%).

Notation same as in Fig. 3.

The total amplitude was expressed in percent of the three initial changes taken as the background. Statistical treatment of the results was carried out in comparison with data for inhalation of pure air in the following periods of observations: "background", "exposure", "close aftereffect" and "distant aftereffect" (Table 5).

Table 5

Changes in the Amplitude of Reinforced Rhythm (in percent) Under the Influence of Inhalation of the Carbon Dioxide Concentrations Studied

Subject	CO_2 concentrations studied	Periods of Observations			
		Background	Exposure	Aftereffect	
				Close	Distant
Zh.	Менее 0,05	101,4	102,4	95,9	100,8
	0,1	98,8	96,2	97,7	92,0 (a)
	0,5	102,7	95,0	87,7	89,7 (a)
V.	Менее 0,05	99,4	99,3	104,5	104,3
	0,1	100,0	104,9 (b)	105,6	110,0
	0,5	98,5	89,6 (b)	91,5 (c)	96,8 (a)
O.	Менее 0,05	99,2	100,7	104,1	109,0
	0,5	99,4	96,5	103,3	103,9
A.	Менее 0,05	102,3	105,1	102,5	104,4
	0,1	100,0	104,0	107,7	108,7
	0,5	101,3	97,7	99,5	97,3
R.	Менее 0,05	99,1	96,9	91,6	94,3
	0,1	100,0	108,2	106,6	124,5 (a)
	0,5	98,0	83,2 (b)	74,5 (b)	79,5 (b)
N.	Менее 0,05	102,5	101,9	107,9	102,9
	0,1	100,1	108,3	116,0	108,4
	0,5	101,0	94,7	93,5 (a)	82,9 (c)
E.	Менее 0,05	100,5	100,1	102,1	99,0
	0,1	99,2	103,7	106,4	106,9 (a)
	0,5	100,0	92,6 (b)	89,1 (c)	87,8

Note: a, b, c - see Table 1.

From Table 5 it is apparent that carbon dioxide in 0.5% concentration affected five of the seven subjects tested. This effect consisted in a decrease of the amplitude of reinforced rhythm (Fig. 5). The 0.1% concentration caused an increase in the amplitude of decreased rhythm in four out of seven subjects (in 3 during the period of "distant aftereffect" and in 1 during the period of exposure).

Thus, it may be concluded that carbon dioxide in 0.5 and 0.1% concentrations is capable of influencing the functional state of the cerebral cortex.

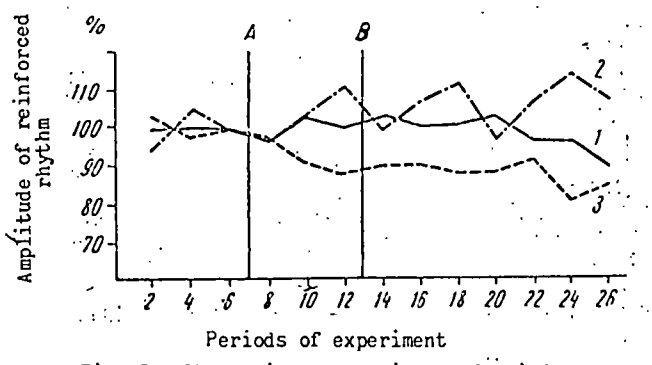


Fig. 5. Change in the magnitude of reinforced potentials of the intrinsic rhythm in subject N. under the influence of inhalation of different CO_2 concentrations.
1 - pure air; 2 - 0.1%; 3 - 0.5% AB - period of gas supply

Conclusions

1. Brief inhalation of carbon dioxide in 0.5 and 0.1% concentrations by healthy people causes distinct shifts in the function of external respiration, blood circulation and electrical activity of the brain.

2. Changes in these functions are pronounced during the action of CO_2 in 0.5% concentration.

3. The data obtained strongly suggest that single CO_2 concentrations in the air of residential and public buildings should not exceed 0.1%, and average daily concentrations 0.05%, independent of the source of CO_2.

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HYGIENIC EVALUATION OF POLLUTION OF THE ATMOSPHERE WITH HEXAMETHYLENEDIAMINE

A. Ye. Kulakov
Institute of General and Commmal Hygieme im. A. N. Sysin,
Academy of Medical Sciences, USSR

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Hexamethylenediamine was first prepared by V. Solonin in 1896. It is used in modern industry chiefly as an intermediate in the production of HA or HS salts (hexamethylenediamine adipate or sebacate) from which the polyamide resin nylon, used as the material for a synthetic fiber of the same name and for various plastic products, is prepared by polycondensation. As an intermediate, hexamethylenediamine is used in the production of polyurethanes, from which a special rubber and plastics are obtained. As a hardener, it finds applications in the production of epoxy resins and certain pigments. Hexamethylenediamine is also used in the production of a chemical antifoaming agent. In industry, hexamethylenediamine is obtained from adipic acid. According to the data of A. P. Martynova (1957) and V. S. Filatova et al. (1958), hexamethylenediamine is observed in substantial concentrations in the air of plants producing nylon and HA salts. Our attempts to find data on atmospheric pollution with hexamethylenediamine in the literature have been unsuccessful.

Hexamethylenediamine, $H_2N(CH_2)_6NH_2$, is a colorless crystalline substance with an unpleasant odor. The melting point is 42° (41-39°C.), and the boiling point, 204-205°C. It volatilizes readily. The volatility at 65°C. is 22.2 mg/l, and amounts to 1980 mg/l at 186°C. Hexamethlenediamine dissolves very well in water, alcochols, acetone, more sparingly in benzene, and poorly in ether. It is a strong organic base (stronger than ammonia). From air, hexamethylenediamine strongly absorbs water and carbon dioxide, forming with the latter a carbonate quite soluble in water. When stored in contact with air, hexamethylenediamine oxidizes and darkens. It keeps well in aqueous and alcohol solutions.

In chemical structure, hexamethylenediamine is similar to such poisons as cadaverine and putrescine, which are formed during putrefaction of proteins. Its toxic effect has been little studied, and the available data pertain chiefly to the action of high concentrations. The studies of Hindelbrandt [cited by Curtius, Clemm (1900), Ceresa, Blasiis (1950)], V. S. Filatova et al. (1951), and A. P. Martynova (1957) have shown that hexamethylenediamine has a general resorptive action which is manifested in

an impairment of the permeability of fine and medium blood vessels, various dystrophic and degenerative changes in internal organs (lungs, liver, kidneys, adrenals, heart muscle, brain, bone marrow, stomach, and intestine). It has a depressing effect on the central nervous system, lowers the arterial pressure, and changes the morphological composition of the blood. The inhalation of hexamethylenediamine causes strong headache (S. I. Vol'fkovich et al., 1959). When deposited on the skin even in small concentrations, it causes dry and wet necroses. During storage in open air, the toxic action of hexamethylenediamine gradually disappears. If humidity reaches the carbonate formed with air, the toxic action on the skin is reestablished (V. S. Filatova et al., 1951). In low concentrations, hexamethylenediamine has a sensitizing effect on human skin, causing dermatitides and eczemas (Duvernevil, Buisson, 1952; F. P. Odintsova, 1961; L. P. Tsirkunov, 1963, M. D. Bagnova, 1962). We observed four cases of such sensitizing action on sensitive persons, three of whom were in contact with hexamethylenediamine for two to four weeks, and one for two days.

Gipstein (1941), Anderson (1950), Rein, Rogin (1950), Themine (1955), Zawahry (1956), Morris (1960), and M. I. Leonenko (1962) indicate the ability of certain nylon products to sensitize susceptible people and cause such dermatitides and eczemas.

However, Fanburg (1940), Dobkevitch, Baer (1947), Sibney, Jennes (1951), Calnon, Wilson (1956), and Janson (1959) do not share this opinion. In their view, dermatitides and eczemas are not caused by nylon, but by the dyes used in the nylon products, and properly treated nylon does not have any sensitizing action.

Last et al. (1950), Schweisheimer (1948) and others successfully used certain nylon products in surgical practice (catheters, suture material) and hold that nylon products have no toxic effects. However, Openheimer et al. (1953) observed a reticulum cell sarcoma around a nylon film which had been sutured into rats.

The basis of the mechanism of the toxic action of hexamethylenediamine is the ability of nono- and diamines to "free" bound histamine and possibly also other biologically active substances (Paton, 1957). This apparently explains the similarity of the toxic action of hexamethylenediamine and histamine, as well as the ability of the former to exert a sensitizing influence on susceptible people.

Taking into account the industrial importance of hexamethylenediamine and the possibility of contamination of atmospheric air with this compound, we undertook a study of certain aspects of the biological action of hexamethylenediamine, and on this basis attempted to propose its maximum permissible concentration in atmospheric air.

We determined hexamethylenediamine in air by means of a colorimetric method based on its reaction with dinitrochlorobenzene, proposed in 1957 by V. I. Kuznetsov, Z. M. Pimenova and A. P. Martynova. The reaction forms 1.6-bis-aminohexane, which dissolves in chloroform and gives a yellow color. Hexamethylenediamine is absorbed in cotton wetted with distilled water. Up to 15 liters of air was collected at the rate of 3 liters per minute. The sensitivity of the method was 0.0025 mg per ml of solution; ammonia does not interfere when present in amounts up to 5 mg. This method, suitable for the determination of hexamethylenediamine in industrial quarters, was modified by us under the supervision of N. G. Polezhayev in order to increase its sensitivity. In the new modification, the air was passed through two absorbers with a porous plate No. 1, 20-25 mm in diameter. The absorbing solution used was a 0.1 N solution of sulfuric acid (10 ml). For the analysis, 300 liters of air was collected at a rate of 1-2 liters per minute.

The reaction of hexamethylenediamine with dinitrochlorobenzene took place in alkaline medium with heating for 30 min. in a water bath. After cooling, the compound formed (1.6-bis-aminohexane) was extracted by agitating in acid medium with 0.5 ml of chloroform for 20 minutes. Using the intensity of the yellow color of chloroform, the sample was compared with a standard scale. The sensitivity of the method was 0.0003 mg in the sample. 0.5 ml of ammonia gives a yellow coloration corresponding to 0.0003 mg of hexamethylenediamine.

In order to establish the threshold of the reflex effect of hexamethylenediamine on people, we carried out the determination of the threshold of olfactory sensation by using a method recommended by the Committee on Sanitary Protection of Atmospheric Air (V. A. Ryazanov, K. A. Bushtuyeva, Yu. V. Novikov, 1957).

The study was made on 19 people whom an otorhinolaryngologist pronounced healthy for this type of test. A total of 273 determinations of odor with concentrations of 0.0110, 0.0074, 0.0041, 0.0032 and 0.0027 mg/m^3 were made.

Table 1

Threshold of Olfactory Sensation of Hexamethylenediamine.

Number of Subjects	Quantity of Observations	Concentration of Hexamethylenediamine, mg/m^3	
		Threshold	Subthreshold
5	69	0,0074	0,0041
12	169	0,0011	0,0032
2	35	0,0032	0,0027

As is evident from Table 1, the threshold of olfactory sensation for the most sensitive persons of this group was a concentration of 0.0032 mg/m^3. The concentration of 0.0027 mg/m^3 was not perceptible to any of the subjects tested.

As we know, studies made by G. V. Gershuni et al. have shown that a low intensity factor may give rise in the organism to responses whose strength does not reach the capacity to involve mechanisms causing conscious perception, i.e., sensation, but which can be objectively recorded by means of highly sensitive methods of investigation. One such method, which we used, consists in studying the light sensitivity of the eye in the course of dark adaptation during simultaneous inhalation of low concentrations of chemical substances. This method has been validated by the studies of several authors. The light sensitivity was determined by means of an ADM adaptometer in three persons ranging in age from 21 to 26 years and having a smell threshold of 0.0041 mg/m^3.

In subject S. (Table 2, Fig. 1), concentrations of 0.0041 and 0.0032 mg/m^3 caused a marked depression of the light sensitivity of the eye over the entire course of the experiment after the gas mixture was supplied. The 0.0027 mg/m^3 concentration caused a much slighter depression, statistically reliable only in the 20th minute. The 0.0017 mg/m^3 concentration was found to be inactive: the curve representing the change of the light sensitivity of the eye almost coincided with the curve obtained during inhalation of pure air.

Table 2

Change in the Light Sensitivity of the Eye in the Course of Dark Adaptation During Inhalation of Different Hexamethylenediamine Concentrations.
(Average values in percent at 15th minute of experiment).

Subject	Period of Experiment, Minutes	Pure Air	Concentrations, mg/m^3			
			0,0041	0,0032	0,0027	0,0017
T.	20	198	337(b)	319(b)	293(a)	201(0)
	25	261	381(a)	405(0)	335(0)	277(0)
	30	301	442(b)	409(0)	359(0)	316(0)
	40	320	445(a)	496(a)	389(0)	353(0)
S.	20	119	85(c)	94(c)	105(a)	119(0)
	25	129	99(b)	96(a)	114(0)	125(0)
	30	142	103(b)	103(b)	127(0)	134(0)
	40	150	105(c)	107(c)	143(0)	142(0)
N.	20	174	101(a)	168(0)	—	—
	25	201	174(0)	197(0)	—	—
	30	212	178(0)	21(0)	—	—
	40	219	206(0)	214(0)	—	—

Note. Confidence factor: a - 95%, b - 99%, c - 99%, 0 - unreliable

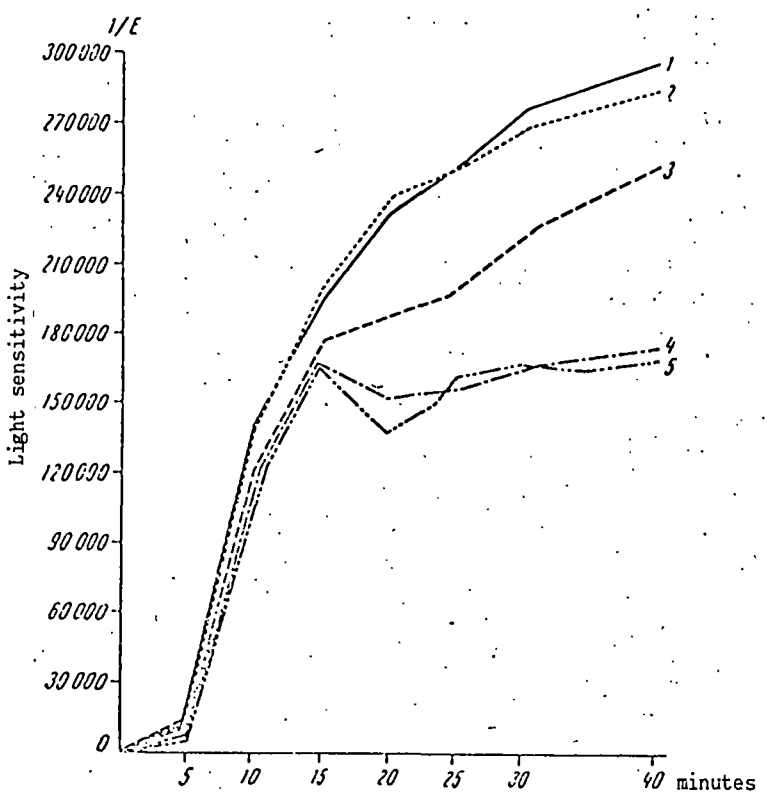

Fig. 1. Change of the light sensitivity of the eye in subject T. during inhalation of hexamethylenediamine.
1 - pure air; 2 - concentration of 0.0017 mg/m^3;
3 - 0.0027 mg/m^3; 4 - 0.0032 mg/m^3; 5 - 0.0041 mg/m^3.

In subject T., on the contrary, the 0.0041 and 0.0032 mg/m^3 concentrations caused a marked reliable increase of the light sensitivity of the eye at the 20th minute of the experiment. A reliable but slighter increase in the light sensitivity of the eye at the 20th minute was also noted at the 0.0027 mg/m^3 concentration. The 0.0017 mg/m^3 concentration was found to be inactive.

In the third subject, N., the 0.0041 mg/m^3 concentration at the 20th minute caused a reliable depression of the light sensitivity of the eye, while the 0.0032 mg/m^3 concentration proved inactive.

Thus, the threshold of the reflex effect of hexamethylenediamine on the light sensitivity of the eye for the two subjects corresponded to the 0.0027 mg/m^3 concentration, and the subthreshold concentration was 0.0017 mg/m^3.

In order to establish the lowest subsensorial threshold of the reflex effect of hexamethylenediamine, we also used the method of study of the electrical activity of the brain. A modification of this method was used in the form of reinforcement of the intrinsic rhythm with a flashing light. This method was first employed by A. D. Semenenko and B. N. Balashev in hygienic studies (1963).

The subject was placed in a weakly illuminated (1.5 lx) screened electroencephalographic chamber. At a distance of 0.7-2 m from his eyes was placed the flash lamp of a photostimulator that was turned on for 20 seconds every minute, the light intensity being changed every 5 seconds. The flicker frequency of the light of the flash lamp was close to the subject's α-rhythm frequency. In the intervals separating the switching on of the flickering light, the subject limbered up for 20-25 seconds (movement in the chair), to keep his general muscle tone, then a sound generator whose frequency varied from 200 to 2000 Hz was turned on for 10 seconds. During the test, the subject closely followed the variation of the intensity of the light flashes and sound pitch, which he noted by squeezing a pressure-sensitive detector held in his hand. The subject could be seen by means of a mirror through a window in the chamber. The examination lasted 13-14 minutes. During the examination, an electroencephalogram was recorded; for each lead of the latter, the integrated energy of the brain potentials was recorded every 5 seconds on separate channels by means of a multichannel integrator of Balashev's design. The pulses of the pressure-sensitive gauge, which the experimenter used to follow the responses of the subject to changes in the photic and acoustic stimuli, were recorded on a separate channel. An electroencephalogram segment obtained while the flash lamp was on was subjected to analysis. Each subject underwent a preliminary training.

The physiological fluctuations over the course of the examination were insignificant. The cylinder whose contents were breathed by the subject was continually supplied with pure air. The gas mixture of known concentration was connected to pure air at the 4th minute of the test and was cut off after 6 minutes. All the switching operations were performed in another room out of the subject's sight. Data obtained during inhalation of pure air and a certain hexamethylenediamine concentration were compared in relative units and subjected to variational statistical treatment. The examination was made with a 16-channel electroencephalograph of the Galileo Co. The recording was made from both hemispheres with uni- and bipolar leads, and statistical treatment was applied to data obtained with occipito-temporal leads. Four subjects having a smell threshold of 0.0041 mg/m^3 participated in the experiments. A total of 64 examinations were performed.

Three hexamethylenediamine concentrations were studied: 0.0027, 0.0017 and 0.0011 mg/m^3. The 0.0027 mg/m^3 concentration caused a decrease in the energy of the brain potentials in all four subjects (Table 3 and Figs. 2 and

3). In subject T. the decrease in the energy of brain biocurrents was noted in both hemispheres, but the most statistically significant changes were observed in the left hemisphere. In the remaining subjects significant changes were observed in one hemisphere only.

The 0.0017 mg/m^3 concentration caused a decrease in the energy of brain potentials in only three subjects, and in subject T. reliable changes also occurred in both hemispheres. On the whole, the decrease in the energy of brain potentials for the 0.0017 mg/m^3 concentration was less pronounced than for the 0.0027 mg/m^3 concentration. The 0.0011 mg/m^3 concentration did not cause significant changes in the electrical activity of the brain in any of the subjects, although in T. and S. there was noted a tendency toward a decrease in the energy of biocurrents in the right cerebral hemisphere at the 7th-9th minute of the test. However, this decrease did not reach a statistically significant value.

In our investigations, we were able to subject the data obtained from the two hemispheres to separate statistical treatments. The experiments showed that low hexamethylenediamine concentrations caused unequal changes in the electrical activity in different cerebral hemispheres of one and the same subject. The 0.0027 mg/m^3 concentration caused the largest decrease in the energy of potentials in the left cerebral hemisphere, and the 0.0017 mg/m^3 concentration, in the right hemisphere. The unequal effect of hexamethylenediamine on the electrical activity of the cerebral hemisphere was also observed in other subjects.

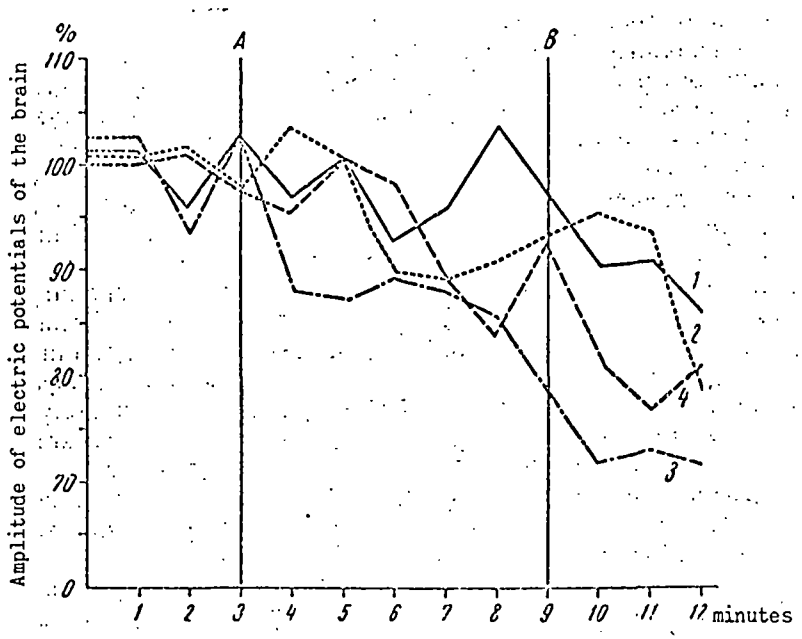

Fig. 2. Change in the electrical activity of the brain in subject T. during inhalation of hexamethylenediamine (right hemisphere). 1 - pure air; 2 - concentration, 0.0011 mg/m^3; 3 - 0.0017 mg/m^3; 4 - 0.0027 mg/m^3; AB - period of gas supply.

The literature contains indications of the presence of a temporary interhemispheric asymmetry in some healthy persons under normal conditions (Ye. A. Zhirmunskaya, 1963). It is possible that the interhemispheric asymmetries manifest themselves more markedly under the influence of low-intensity factors.

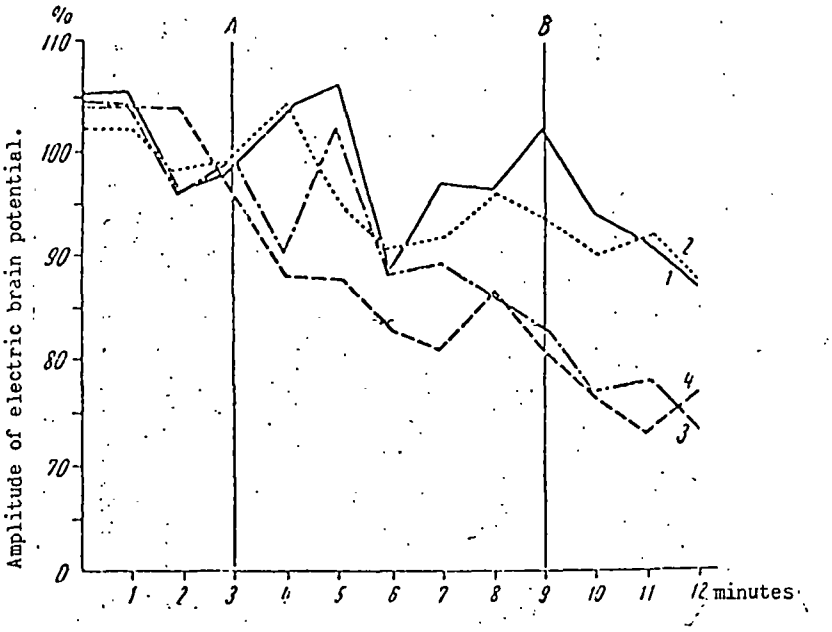

Fig. 3. Change in the electrical activity of the brain in subject T. during inhalation of hexamethylenediamine (left hemisphere). Notation same as in Fig. 2.

Thus, the threshold of change in the electrical activity of the brain in the most sensitive persons during inhalation of hexamethylenediamine corresponds to a concentration level of 0.0071 mg/m^3.

On the basis of the data obtained, we recommend as the highest single maximum permissible concentration of hexamethylenediamine in atmospheric air a level of 0.001 mg/m^3, which is below the threshold of action according to the most sensitive method of investigation (Table 4).

We studied the contamination of atmospheric air with hexamethylenediamine around a plant producing HA salt. In addition to hexamethylenediamine, the discharges of the plant also contained ammonia, which in high concentrations interferes with the determination of hexamethylenediamine, and for this reason we determined both of these substances simultaneously. Ammonia was considered in the calculation of the hexamethylenediamine concentration.

Table 3

Change in the Energy of Brain Potentials During Inhalation of Different Hexamethylenediamine Concentrations (Average Values in Percent Relative to the 1st, 2nd and 3rd Minutes of the Experiment).

Subject	Period of Experiment, Minutes	Right Hemisphere					Left Hemisphere				
		Pure Air	0.0027 mg/m³	Pure Air	0.0017 mg/m³	0.0011 mg/m³	Pure Air	0.0027 mg/m³	Pure Air	0.0017 mg/m³	0.0011 mg/m³
T.	4—6	99	98(o)	94	88(o)	99(o)	104	86(a)	94	93(o)	97(o)
	7—9	96	89(o)	98	84(c)	91(o)	98	78(a)	99	86(a)	94(o)
	10—12	93	80(a)	85	72(o)	90(o)	95	76(a)	87	76(o)	90(o)
S.	4—6	95	100(o)	93	92(o)	96(o)	107	100(o)	98	101(o)	98(o)
	7—9	97	98(o)	106	89(a)	98(o)	112	93(c)	97	92(o)	95(o)
	10—12	95	84(o)	92	84(o)	95(o)	107	89(a)	85	86(o)	92(o)
M.	4—6	102	102(o)	105	100(o)	102(o)	106	97(a)	103	98(o)	98(o)
	7—9	102	103(o)	108	100(o)	106(o)	114	100(b)	100	95(o)	96(o)
	10—12	100	97(o)	107	95(a)	101(o)	105	96(o)	104	94(o)	96(o)
R.	4—6	101	88(a)	101	97(o)	93(o)	104	99(o)	104	106(o)	102(o)
	7—9	99	82(c)	94	95(o)	90(o)	102	95(o)	101	104(o)	101(o)
	10—12	95	80(b)	93	94(o)	85(o)	99	94(o)	102	99(o)	100(o)

Note. Confidence factor: a - 95%, b - 99%, c - 99%, o - unreliable.

Table 4

Thresholds of the reflex effect of hexamethylenediamine on the human organism during inhalation

Threshold	Concentration in mg/m³	
	Minimum Active	Maximum Inactive
Olfactory sensitivity	0,0032	0,0027
Effect on the light sensitivity of the eye	0,0027	0,0017
Effect on the electrical activity of the brain	0,0017	0,0011

The collection of samples was started at a distance of 300 m on the leeward side of the plant, since at a closer distance the odor of hexamethylenediamine was clearly perceptible.

The results of the study of pollution of atmospheric air are shown in Table 5.

Table 5

Contamination of Atmospheric Air with Hexamethylenediamine around an HA Plant

Distance From Source, m	Number of Samples		Concentration, mg/m³		Distribution of Concentrations, mg/m³			
	Total	Positive	Maximum	Average	Above 0,01	0.01—0,006	0,005—0.002	0,001 and below
300	24	15	0,012	0,0011	1	3	7	4
500	56	1	—	0,0010	—	—	—	1

The atmospheric air around the plant at a distance of 300 m is contaminated with hexamethylenediamine in concentrations several times greater than the highest single maximum permissible concentration that we are proposing. At a distance of 500 m, of the 56 collected samples, hexamethylenediamine was observed in only one, and only in the amount of 0.001 mg/m³.

Since other chemical compounds are also discharged by the HA plant into the atmospheric air, the problem of the width of the sanitary protective zone can be solved only on the basis of a hygienic evaluation of the entire group of chemical substances discharged into the atmosphere.

Conclusions

1. The threshold of olfactory perception of hexamethylenediamine in the most sensitive persons is 0.0032 mg/m^3. The 0.0027 mg/m^3 concentration is imperceptible.

2. The threshold of the reflex effect of hexamethylenediamine on the light sensitivity of the eye is 0.0027 mg/m^3. The 0.0017 mg/m^3 concentration did not cause any significant changes.

3. The threshold of the reflex effect of hexamethylenediamine on the electrical activity of the brain corresponds to 0.0017 mg/m^3. The 0.0011 mg/m^3 concentration is the subthreshold level.

4. On the basis of the data obtained, a highest single maximum permissible concentration of hexamethylenediamine of 0.001 mg/m^3 is proposed.

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CONTAMINATION OF ATMOSPHERIC AIR WITH MALEIC ANHYDRIDE AND ITS HYGIENIC EVALUATION

K. V. Grigor'yeva
Ukrainian Scientific Research Institute of Communal Hygiene

From "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Pod redaktsiey Prof. V. A. Ryazanova i Prof. M. S. Gol'dberga. Izdatel'stvo "Meditsina" Moskva, p. 42-57, (1966).

Maleic anhydride, $C_4H_2O_3$, is a white crystalline powder with a fairly pungent odor, boils at 202°C. and sublimes. It is used as the raw material for the preparation of valuable lacquer-paints, and plasticizers in the production of artificial resins.

Maleic anhydride is obtained from the oxidation of benzene, furfural, or any other hydrocarbons of the paraffin and olefin series with more than four carbon atoms. The method of preparation of maleic anhydride from waste products of phthalic anhydride formed by catalytic oxidation of naphthalene by atmospheric oxygen at high temperature has come into widespread use. In this process, 7-15% of maleic anhydride is formed. The mixture of vapors of phthalic and maleic anhydrides, naphthoquinone, carbon monoxide and water is fed into special units for condensation of phthalic anhydride on cooling. The uncondensed vapor of all these substances form the tail gases, which are then fed into a scrubber. Maleic anhydride is obtained from the wash waters by evaporation.

The residual gases after the scrubber, consisting of vapors and aerosols of maleic and phthalic anhydrides and naphthoquinone, are discharged into the atmosphere.

The toxic properties of maleic anhydride have been described in very few papers. Acute poisoning is characterized by an irritating effect on the mucous membranes of the eye and upper respiratory tract (N. V. Lazarev, 1954).

Menschick (1955) studied the health of workers over the course of 20 months since the start of operation of equipment for synthesizing phthalic anhydride. The air of the plant was contaminated with a number of substances including phthalic and maleic anhydrides, naphthoquinone, and carbon monoxide. The author found a significant decline in the health of the workers, manifested in disorders of the nervous system (fits of excitation, tetanic state, sensitivity impairments in the area of the trigeminal nerve), hypersecretory excitation of the stomach, increased permeability of the stomach wall and

vessel walls, anginal pains, decrease of systolic and diastolic pressures (of central origin), irritation of the mucous membrane of the eye (conjunctivitis), blood effusion in the nasal cellular tissue up to and including nasal hemorrhages, and ulcerations of the mucosa of the larynx, pharynx, trachea, and bronchi, blood changes (normochromic anemias, leucocytoses, thrombopenia), a sharp decrease in the content of vitamin C in the organism, and impairment of the phosphorus and calcium metabolism.

Marsico and Rozero (1957) cite nine cases of acute poisoning with maleic anhydride in loaders during its loading. The symptoms of poisoning are characterized primarily by the irritant action of the poison: an acute irritation of the mucous membranes of the eye, nose, and upper respiratory tract.

Angielski and Rogulski (1960) describe the aminoaciduria and decrease of protein and protein-free SH groups in the kidneys of animals which had received maleic anhydride in subcutaneous injections in doses of 250-300 mg/kg.

M. P. Slyusar' and I. A. Cherkasov (1961), while administering maleic anhydride to experimental animals by inhalation, found a considerable weight decrease, a drop in vitamin C content of the tissues, and an increase of total protein in the blood serum. The authors observed the earliest and most substantial changes in a study of the phagocytic activity of neutrophiles.

These few studies show that maleic anhydride is a toxic substance. However, there are no data on its content in atmospheric air, the effect of low concentrations on man has not been studied, and no maximum permissible concentration has been established for the atmospheric air of populated areas.

Considering these facts and the necessity of expanding the production of maleic and phthalic anhydride for the national economy, the Committee on Sanitary Protection of Atmospheric Air charged the Ukrainian Institute of Communal Hygiene with the problem of investigating the contamination of the atmosphere with maleic anhydride in the surroundings of a chemical plant and with submitting a hygienic evaluation of the values obtained.

The study of the atmosphere was made by using a polarographic method developed by I. B. Kogan (1958) and slightly modified by us. Maleic anhydride was trapped from air on an FPP-15 filter, then in two sequentially connected absorbing containers filled with 10 ml of a 0.1 N solution of hydrochloric acid. The polarography was carried out after dissolving the maleic anhydride collected on the filter in the liquid from the first absorber. The half-wave potential of maleic anhydride is 0.69. The sensitivity of the method is 1 mg per ml. The accuracy of the method is ±10-15%.

Phthalic anhydride, benzoic acid and α-naphthoquinone do not interfere with the determination. The atmospheric air around the plant complex was collected at distances of 500, 1000, and 2000 m from it. Two series of samplings were carried out. The results of the study are listed in Table 1.

The highest maleic anhydride concentrations were found at a distance of 500 m from the plant, while at the distance of 2000 m the maleic anhydride content in all 28 samples was below the sensitivity limit of the method.

The second stage of the study consisted in an experimental validation of the maximum permissible concentration of maleic anhydride in atmospheric air. This was done by using the methods recommended by the Committee on Sanitary Protection of Atmospheric Air (V. A. Ryazanov, K. A. Bushtuyeva, and Yu. V. Novikov, 1957).

An experimental mixture of vapors and aerosols of maleic anhydride was obtained by heating it in distillation flasks on a water bath at 37°C.

To solve the problem of the highest single maximum permissible concentration of maleic anhydride, the threshold of smell and its irritating effect on the upper respiratory tract as well as the threshold of the reflex effect on the functional state of the cerebral cortex were determined. To determine the threshold of olfactory sensation and irritating effect, 753 determinations were made with the 12 subjects. The results obtained are given in Table 2.

Table 1

Content of Maleic Anhydride in Air Samples Collected in the Area of the Chemical Complex

Distance From Source of Discharges, m	Number of Tests	Number of Positive Samples	Concentration, mg/m³	
			Maximum	Average
First Series				
1 000	16	7	2,6	0,29
2 000	9	—		
Second Series				
500	78	29	0,06	0,007
1 000	37	11	0,015	0,001
2 000	19	—		

During the inhalation of maleic anhydride in concentrations from 8 to 11 mg/m³, a strong reflex cough, buring in the laryngeal part of the pharynx, and lachrymation in the majority of subjects, were observed.

A concentration from 5 to 7 mg/m^3 causes the perception of odor and irritation of the upper respiratory tract.

Maleic anhydride in concentrations from 2 to 3 mg/m^3 has an insignificant odor and a slight irritating effect.

Table 2

Thresholds of Perception of Odor and Irritating Effect of Maleic Anhydride.

Number of Subjects	Concentration, mg/m^3			
	Smell		Irritating Effect	
	Minimum Perceptible	Imperceptible	Minimum Perceptible	Imperceptible
1	1,7	1,6	1,5	1,4
2	1,6	1,5	1,2	1,1
1	1,5	1,4	1,1	1,0
1	1,4	1,3	1,4	1,2
1	1,4	1,3	1,1	1,0
1	1,4	1,3	1,0	0,9
5	1,3	1,2	1,0	0,9

A concentration of 1.3 mg/m^3 is the odor threshold for the most sensitive persons. Symptoms of the irritating action are of major significance here. The subjects note dryness and tickling in the lower part of the pharynx. The threshold concentration for the irritating effect was found to be 1 mg/m^3. It gives a sensation of freshness and a barely perceptible tickling.

The next stage of the study was the determination of the influence of low maleic anhydride concentrations on the reflex change of the light sensitivity of the eye.

A number of authors (F. I. Dubrovskaya, K. A. Bushtuyeva, M. T. Takhirov, N. F. Izmerov, G. I. Solomin, and others) have demonstrated that this method permits the recording of the change in the cerebral cortex caused by stimulation of the reflexogenic zones of respiratory organs by slight concentrations of chemical substances. The light sensitivity of the eye was studied with the aid of an ADM adaptometer. The threshold of the irritating effect of maleic anhydride was first determined in three subjects. The course of dark adaptation was studied while supplying pure air and a gaseous mixture containing 6.2, 1.65 and 0.85 mg/m^3 of maleic anhydride. Each concentration was studied no fewer than three times. A total of 78 determinations were made. The results of the examination are given in Table 3.

The 6.2 and 1.65 mg/m^3 concentrations caused a reliable change of the light sensitivity of the eye in all the subjects during the 20th minute in comparison with pure air. The 0.85 mg/m^3 concentration was found to be inactive.

Table 3

Average Values of the Light Sensitivity of the Eye in Relative Units During Inhalation of Different Maleic Anhydride Concentrations.

Subject	15th Minute				20th Minute				25th Minute			
	Pure Air	Maleic Anhydride			Pure Air	Maleic Anhydride			Pure Air	Maleic Anhydride		
		0.85	1.65	6.2		0.85	1.65	6.2		0.85	1.65	6.2
M.	58 500	64 700	54 766	59 200	73 380	75 433	53 500 (a)	55 900 (a)	82 220	83 800	63 566	81 100
K.	81 250	66 466	62 760	59 550	94 550	60 633	67 620 (a)	72 850 (c)	104450	95 166	75 380 (a)	74 350 (c)
T.	51 020	48 400	57 966	—	61 360	60 433	113 333(a)	—	77 180	73 333	120 566	—

Note. Confidence factor: a - 95%, b - 99%, c - 99.9%.

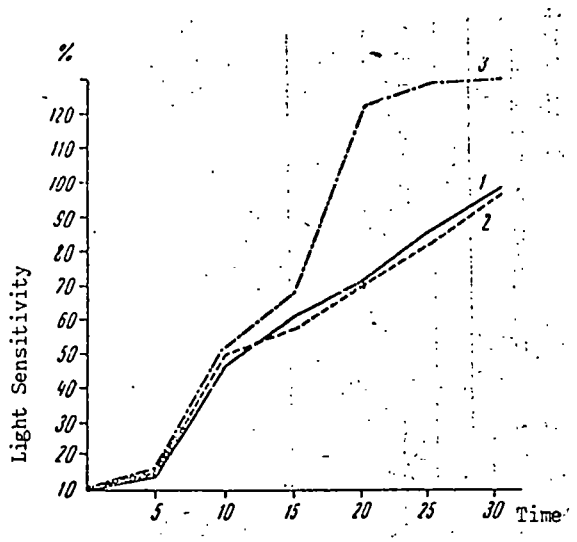

Fig. 1. Effect of maleic anhydride on the light sensitivity of the eye in subject T.
1 - pure air; 2 - concentration, 0.85 mg/m^3;
3 - 1.65 mg/m^3;

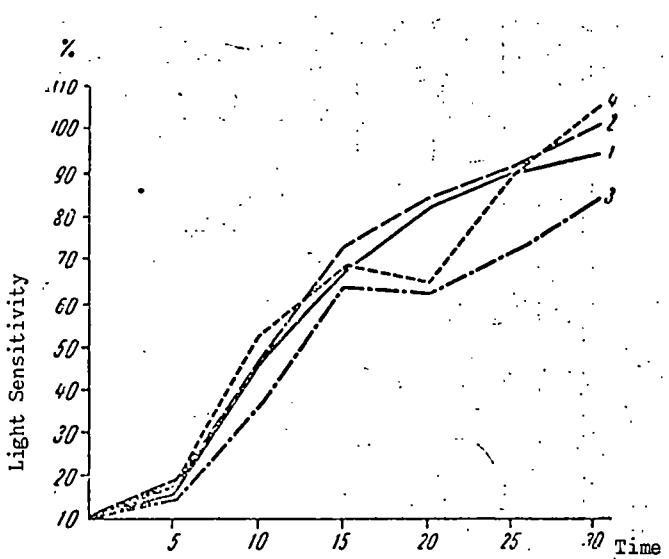

Fig. 2. Effect of maleic anhydride on the light sensitivity of the eye in subject N.
1 - pure air; 2 - concentration, 0.85 mg/m^3;
3 - 1.65 mg/m^3; 4 - 6.2 mg/m^3

Dark adaptation curves for two subjects illustrate the influence of maleic anhydride on the light sensitivity of the eye (Figs. 1, 2).

Thus, the threshold of the effect of maleic anhydride on the light sensitivity of the eye is 1.65 mg/m^3, and 0.85 mg/m^3 is a subthreshold concentration. Assuming a certain storage coefficient, one can recommend 0.5 mg/m^3 as the highest single maximum permissible concentration of maleic anhydride.

To study the effect of low maleic anhydride concentrations under conditions of an extended, continuous experiment on laboratory animals, a round-the-clock exposure of white rats was conducted for 70 days.

The experiments involved 50 male rats weighing 90 to 120 g. The exposure was carried out in 5 chambers containing 10 rats each.

The first group of rats was subjected to the action of maleic anhydride in 0.03 mg/m^3 concentration, the second to 0.08 mg/m^3, the third to 0.34 mg/m^3, the fourth to 0.8 mg/m^3, and the fifth was the control group. The exposure was made in two series: 1) with 0.08, 0.34, and 0.8 mg/m^3 concentrations; 2) with 0.03 mg/m^3 concentration. Each series had its own control. The effect of maleic anhydride in different concentrations is described without separation into series.

During the course of the exposure, air samples were taken from the chambers almost daily. The concentrations in the chambers varied between the following limits: in the first group, from traces to 0.042 mg/m^3, in the second from traces to 0.2 mg/m^3, in the third from traces to 0.78 mg/m^3, and in the fourth from 0.3 to 1.56 mg/m^3.

The fluctuations of the concentrations are shown in Fig. 3.

Table 4

Weight of Animals Subjected to Chronic Action of Maleic Anhydride.

Average Weight of Animal	Group and Concentration in mg/m^3	$M \pm m$	t	Confidence Factor
Before the start of exposure	Control	158,2±12,8		
	First (0.03)	160,6±5,6	0,1	
	Control	229,4±8		
	Second (0.08)	212,8±9,7	1,3	
	Third (0.34)	216,7±8,8	1,06	
	Fourth (0.8)	211,5±10,6	1,3	
During exposure	Control	212±8,0		
	First (0.03)	213±5,8	0,1	
	Control	250,0±3,74		
	Second (0.08)	245,2±4,4	1,04	
	Third (0.34)	237,7±3,87	2,4	98,0%
	Fourth (0.08)	226,6±5,38	3,7	99,9%

To evaluate the toxic effect of maleic anhydride, the general condition of the animals, their weight, morphological composition of the blood, content of SH groups in the blood serum and phagocytic activity of neutrophiles were followed during the experiment. At the end of the exposure, anatomic pathological analyses of the animals' organs were made.

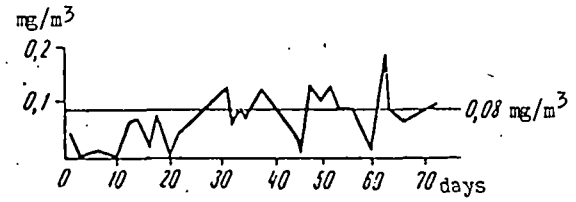

Fig. 3. Fluctuations of maleic anhydride concentrations in the second group.

In the course of the experiment, maleic anhydride showed a substantial influence on the overall condition and weight dynamics of animals of the third group (0.34 mg/m^3) and particularly the fourth group (0.8 mg/m^3). In the second (0.08 mg/m^3) and first (0.03 mg/m^3) groups, no changes were observed. Since the first days of contact with the substance, the animals of the fourth group were agitated, constantly sniffed, sneezed, and rubbed their noses with their paws. A few days later the rats calmed down and were sluggish, failing to become animated even during feeding. They ate less than the animals in the other groups.

The weight change in animals of the third and fourth groups was particularly pronounced during the period of experimental starving. After 5 days of underfeeding (the rats received 1/5 of the usual ration during this period) they lost more weight than the others, and the restoration of their weight was subsequently less rapid (Figs. 4, 5, Table 4).

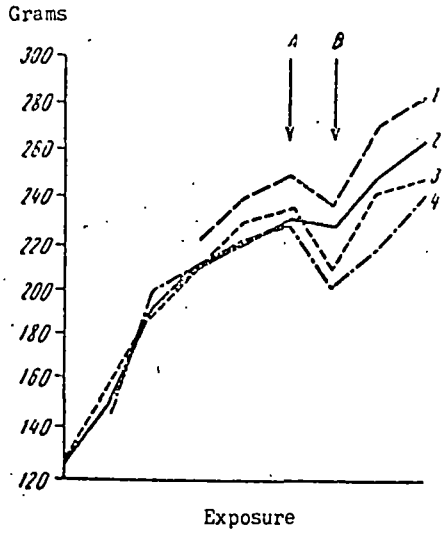

Fig. 4. Average weight of rats of the second, third, fourth and control groups of the experiment during the period of exposure.

1 - control group; 2 - second group (0.08 mg/m^3); 3 - third group (0.34 mg/m^3); 4 - fourth group (0.8 mg/m^3); AB - experimental starving.

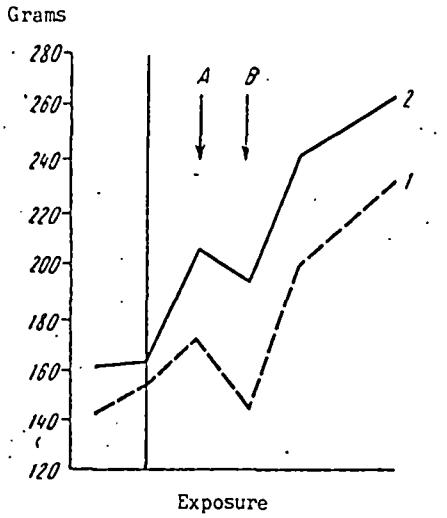

Fig. 5. Average weight of rats of the first and control groups during the period of exposure.
1 - control group; 2 - first group (0.03 mg/m^3); AB - experimental starving.

Observation of the state of the morphological composition of the blood did not show any significant changes in the amount of hemoglobin in animals of any groups. The number of erythrocytes decreased significantly in animals of the fourth group (0.8 mg/m^3).

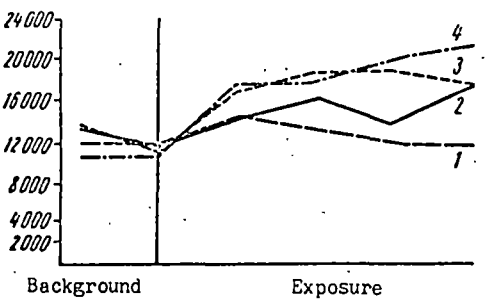

Fig. 6. Average number of leucocytes in rats of the second, third, fourth and control groups during the period of exposure.
Notation same as in Fig. 4.

As far as the number of leucocytes is concerned, stable leucocytosis was observed in animals of the third and fourth groups during the period of exposure (Fig. 7).

No such effect was observed in rats of the second group (concentration 0.08 mg/m^3), but they showed an unstable leucocytic reaction. This was particularly apparent after ten days' starving.

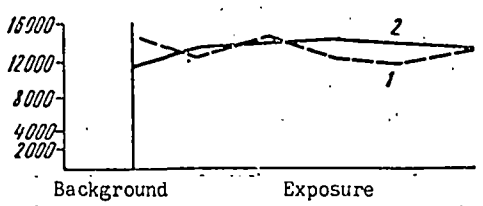

Fig. 7. Average number of leucocytes in rats of the first and control groups during the period of exposure.
Notation same as in Fig. 5.

In rats of the first (0.03 mg/m^3) and control groups, the number of leucocytes remained unchanged over the course of the experiment (Table 5, Figs. 6, 7).

One of the indices characterizing the influence of external factors on the organism is the change of its immunobiological reactivity, in particular, the phagocytic activity of the neutrophiles. Recently, this test has begun to be used for the validation of maximum permissible concentrations of noxious chemicals (M. P. Slyusar' and I. A. Cherkasov, 1961).

Table 5

Number of Leucocytes in White Rats Subjected to Chronic Action of Maleic Anhydride.

Average Number of Leucocytes	Group and Concentration in mg/m^3	$M \pm m$	t	Confidence Factor
Prior to the start of exposure	Control	13 150 ± 1000		
	First (0.03)	11 727 ± 518	1,26	
	Control	12 320 ± 616		
	Second (0.08)	12 150 ± 541	0,1	
	Third (0.34)	12 090 ± 346	0,3	
	Fourth (0.8)	10 690 ± 844	1,5	
During Exposure	Control	14 049 ± 803		
	First (0.03)	14 204 ± 335	0,1	
	Control	12 960 ± 516		
	Second (0.08)	16 120 ± 935	2,9	99%
	Third (0.34)	18 465 ± 873	5,4	99,9%
	Fourth (0.8)	20 033 ± 988	6,2	99,9%

We determined the opsono-phagocytic numbers (number of microbes absorbed by one leucocyte) and the opsono-phagocytic activity of leucocytes (percent ratio of active neutrophiles to the total number of counted neotrophiles) (Table 6).

As is evident from Table 6, the phagocytic activity of leucocytes in animals of the third and fourth groups (0.34 and 0.8 mg/m^3) decreased.

In rats of the first and second group (0.03 and 0.08 mg/m^3), the phagocytic activity of leucocytes did not differ in any way from the control.

According to our data, the opsono-phagocytic numbers in animals of all the groups remained unchanged during the period of the chronic experiment.

Protein macromolecules carry on their surface a large number of polar groups including mercapto groups. When toxic substances enter the organism, they react with these groups. Blocking of an SH group decreases the protein activity. At the same time, the SH groups have a considerable influence on the activity of the enzymes participating in protein, fat, and carbohydrate metabolism. This in turn indicates the great influence of a given functional group of proteins on the generation, intensity and direction of the various physiological processes (B. N. Gol'dshteyn, 1955).

Table 6

Opsono-phagocytic Activity of Leucocytes in White Rats Subjected to Chronic Action of Maleic Anhydride.

Average Activity of Leucocytes	Group and Concentration in mg/m^3	$M \pm m$	t	Confidence Factor
Before the start of exposure	Control	75,6±4,3		
	First (0.03)	73,5±1,9	0,4	
	Control	82,0±4,3		
	Second (0.08)	79,4±2,8	0,5	
	Third (0.34)	84,4±3,2	0,4	
	Fourth (0.8)	82,6±2,3	0,1	
During exposure	Control	70,0±1,7		
	First (0.03)	72,5±1,09	0,8	
	Control	73,8±2,4		
	Second (0.08)	72,1±1,5	0,8	
	Third (0.34)	61,0±2,8	2,5	95%
	Fourth (0.8)	64,5±3,7	2,2	95%

There is a report in the literature that maleic acid decreases the content of protein and protein-free mercapto groups in experimental animals (Angielski and Rogulski, 1960).

Taking the above into account, we used this test in our investigations. A polarographic method for determining mercapto groups in a protein-free filtrate of the blood serum was employed. The method is based on the ability of substances containing SH groups to cause catalytic hydrogen waves on a dropping mercury electrode with an ammonia solution of cobalt as the base electrolyte. A decrease in the amount of proteins containing mercapto groups lowers the catalytic wave. Results of these experiments are shown in Table 7. In animals of the second and third groups (0.08, 0.34 mg/m^3),

no appreciable changes of any kind could be demonstrated in the content of active SH groups as compared to the control group, so that in a repeat experiment with the lowest concentration, 0.03 mg/m^3 (first group), this test was not employed. Blocking of the SH groups occurred only in animals of the fourth group (0.8 mg/m^3).

Table 7

Change in the Height of Polarographic Waves of Protein-Free Filtrate of Blood Serum in Rats Subjected to the Chronic Action of Maleic Anhydride.

Average height of polarographic waves	Group and Concentration in mg/m^3	$M \pm m$	t	Confidence Factor
During exposure	Control	7,2±0,16	1,2	
	First (0.08)	7,5±0,19	1,2	
	Third (0.34)	7,4±0,14	1,9	
	Fourth (0.8)	5,3±0,18	7,9	99,9%

In animals of the fourth group (0.8 mg/m^3), histomorphological analyses made after completion of the exposure showed a vague atrophy of the nasal mucosa and inflammatory changes in the lungs, primarily around the medium and fine bronchi. In the remaining groups as compared to the control, no appreciable changes could be established.

Thus, continuous chronic exposure of white rats to maleic anhydride for 70 days has shown that the 0.08 mg/m^3 concentration has a marked effect on the experimental animals, causing a weight lag, a decrease in the number of erythrocytes and neutrophile activity, an increase in the number of leucocytes, a decrease in the activity of SH groups of blood serum proteins, and inflammatory changes in the lungs. The 0.34 mg/m^3 concentration showed a lesser effect on the animals during the same period of time. Only a weight lag, a decrease in the phagocytic activity of neutrophiles and an increase in the number of leucocytes were observed. The 0.08 mg/m^3 concentration caused only an unstable leucocytic reaction in rats of this group. Maleic anhydride in 0.03 mg/m^3 concentration caused no changes of any kind in the experimental animals during the course of exposure.

Comparison of the experimental data with indices of the content of the substance, detected in the surroundings of the chemical complex under consideration during the first series of studies, showed maleic anhydride to be present in amounts substantially exceeding the single maximum permissible concentration of maleic anhydride which we have proposed. This was noted during a period when for all practical purposes the purification equipment was not operating. High-efficiency scrubbers with a degree of purification of 98-99% (M. Kh. Tsipenyuk and Yu. Ye. Tsipenyuk, 1963) have now been installed, which purify the discharge gases of the phthalic production, and the highest maximum single concentrations of maleic anhydride

at a distance of 500 m from the source of discharge do not exceed our recommended maximum permissible value of 0.5 mg/m^3.

Conclusions

1. The threshold of smell of maleic anhydride in the most sensitive persons is 1.3 mg/m^3, and the threshold of irritating action is 1 mg/m^3. The imperceptible concentration for these studies was determined at 0.9 mg/m^3.

2. The subthreshold concentration for the reflex effect on the cerebral cortex was found to be 0.85 mg/m^3.

3. The highest single maximum permissible concentration of maleic anhydride in atmospheric air which can be recommended is 0.5 mg/m^3.

4. In a round-the-clock exposure for 70 days, maleic anhydride in 0.8 mg/m^3 concentration causes the following changes in white rats: decrease of the number of erythrocytes and activity of the neutrophiles, increase in the number of leucocytes, decrease in the activity of SH groups of blood serum proteins, weight lag, and inflammatory changes in the lungs. A 0.34 mg/m^3 concentration causes a decrease in the activity of the neutrophiles, an increase in the number of leucocytes, and a weight lag in the animals. A concentration of 0.08 mg/m^3 of maleic anhydride is the threshold value for its toxic effect on the organism of warm blooded animals, and a concentration of 0.03 mg/m^3 is the subthreshold value.

5. The average daily maximum permissible concentration of maleic anhydride in atmospheric air which can be recommended is 0.05 mg/m^3.

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BIOLOGICAL EFFECT OF LOW NITROBENZENE CONCENTRATIONS IN ATMOSPHERIC AIR

N. G. Andreyeshcheva

Communal Hygiene Department, Central Institute for Advanced Training of Physicians

From "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Pod redaktsiey Prof. V. A. Ryazanova i Prof. M. S. Gol'dberga. Izdatel'stvo "Meditsina" Moskva, p. 58-73, (1966).

Nitrobenzene, $C_6H_5NO_2$, is the simplest nitro compound of the aromatic series. It is a pale yellow liquid with a bitter almond odor. The boiling point is 210.8°C., the specific gravity 1.21, and the melting point 5.7°C. Nitrobenzene dissolves readily in fats, alcohol, and ether and is insoluble in acids and alkalis. It is prepared by nitrating benzene.

Nitrobenzene is widely employed in aniline dye plants in the production of artificial aniline dyes, explosives, drugs, etc. During the current seven-year plan (1959-1965), the output of aniline dyes will double, thereby sharply increasing the production and consumption of nitrobenzene.

The toxic properties of nitrobenzene as a result of the action of large doses on the organism are marked (V. K. Navrotskiy, 1955; K. I. Morozova, 1934; Laubender, Schwamb, 1951; Goldstein, Popovici, 1958; P. Sulamit, 1960, 1962).

The authors administered nitrobenzene chiefly subcutaneously and perorally, most frequently once. Its effect on the blood hemoglobin is the most pronounced. It involves the formation of methemoglobinemia followed by sulfhemoglobinemia; the activity of the blood catalase and peroxidase decreases, Heinz bodies are formed intensively, and the number of erythrocytes and reticulocytes decreases.

The chronic effect of nitrobenzene in small doses, chiefly on the secretory function of the stomach and partly on the blood, was studied only with peroral introduction by M. I. Kazakova (1954) and G. A. Kalashnikova (1955).

The lack of data on the toxicity of small doses of nitrobenzene during long-term inhalational penetration into the organixm or data on its contamination of atmospheric air has led to a study of the biological effect of low nitrobenzene concentrations and of its hygienic importance as an air pollutant.

The experimental part of the study was carried out by using a method of determination of low nitrobenzene concentrations in atmospheric air proposed by M. V. Alekseyeva. The method is based on the absorption of nitrobenzene by a nitrating mixture. This forms dinitrobenzene, which is determined from the purple color formed by its reaction with a ketone or alkali. The sensitivity of the method is 0.25 mg in a volume of 2 ml.

We selected the optimum conditions for complete nitration of low nitrobenzene concentrations (50 minutes in a boiling water bath). The nitrobenzene concentrations were measured in a photoelectrocolorimeter with a green filter or a spectrophotometer at a wavelength of 560-570 mμ after first recording the calibration curves.

We started the validation of the highest single maximum permissible concentration of nitrobenzene in atmospheric air, in accordance with the recommendation of the Committee on Sanitary Protection of Atmospheric Air, from the determination of the threshold of olfactory sensation. The experiment involved the participation of 29 practically healthy people aged 17-35.

Table 1

Results of Determination of Olfactory Sensation Threshold of Nitrobenzene.

Number of Subjects	Nitrobenzene Concentration, mg/m^3	
	Threshold	Subthreshold
4	0,0182	0,0169
2	0,023	0,018
7	0,027	0,020
8	0,031	0,024
4	0,037	0,028
1	0,045	0,032
1	0,057	0,011
1	0,070	0,055
1	0,094	0,069

The olfactory sensation was tested once every 24 hours. A total of 1252 determinations were made, and it was found that in the most sensitive persons, the minimum perceptible concentration of nitrobenzene was 0.0182 mg/m^3 (Table 1).

In the works of several authors (K. A. Bushtuyeva, 1957; F. I. Dubrovskaya, 1957; M. M. Plotnikova, 1957, and others), the dark adaptation method was used for studying the threshold of reflex action. We used this method in three cases. The smell of subthreshold concentrations of nitrobenzene affected the light sensitivity of the visual system in all three subjects. Results of the experiment are listed in Table 2.

Table 2

Results of Determination of the Change in the Light Sensitivity of the Eye During Inhalation of Nitrobenzene.

Subject	Concentration, mg/m^3			
	Light Sensitivity		Olfactory Sensation	
	Threshold	Subthreshold	Threshold	Subthreshold
S.	0,0157	0,0118	0,0182	0,0169
G.	0,0167	0,0144	0,0182	0,0169
V.	0,0168	0,0144	0,0230	0,0190

In one of the three subjects, a nitrobenzene concentration of 0.0157 mg/m^3 caused a reliable decrease of the light sensitivity of the eye which may be considered the threshold sensitivity in this method (Fig. 1). The method of dark adaptation made it possible to reveal the effect of subthreshold nitrobenzene concentrations for olfactory sensation on the functional activity of the central nervous system. Electroencephalographic studies were also made to determine the reflex effect of nitrobenzene.

In hygienic practice, the study of brain biocurrents was started by using the method of development of the electrocortical conditioned reflex (K. A. Bushtuyeva, Ye. F. Polezhayev, and A. D. Semenenko, 1960; V. A. Gofmekler, 1961, and others).

In subsequent studies use was also made of the method of functional loading with calculation of the amplitude of the assimilated rhythm to light stimulation (A. D. Semenenko, 1963; N. B. Imasheva, 1963; V. I. Filatova, 1962, and others).

We used the electroencephalographic method of reinforcement of intrinsic brain potentials under conditions of functional loading with flickering light of varying intensity, proposed by A. D. Semenenko. The method is based on the ability of the central nervous system to produce a reinforcement of the intrinsic potentials in response to the action of a rhythmic stimulus.

The observations were made on six practically healthy people 20-35 years of age who had not previously been in contact with the chemical substances.

The electrical activity of the brain was recorded on a Kaiser 8-channel electroencephalograph with a photostimulator. The biocurrents were taken off the temporal and occipital areas uni- and bipolarly. Simultaneously, the respiration and a myogram were recorded.

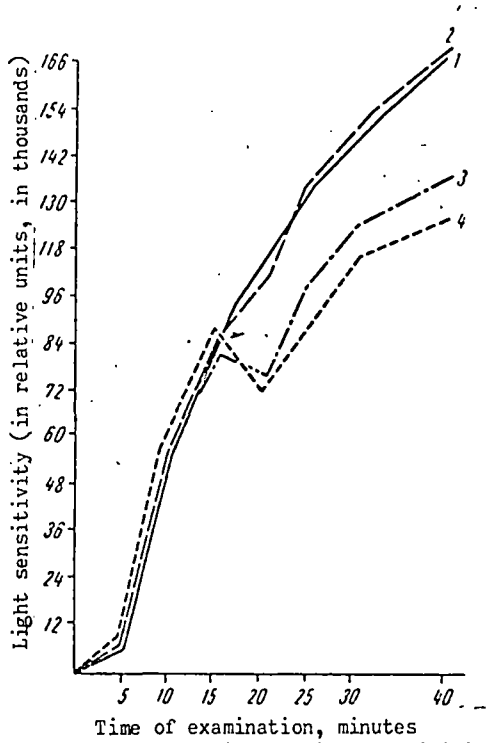


Fig. 1. Change in the light sensitivity of the eye during inhalation of nitrobenzene in subject S.

1 - pure air; 2 - concentration, 0.0118 mg/m^3; 3 - 0.0157 mg/m^3; 4 - 0.0169 mg/m^3

Stimulation by rhythmic light with a frequency equal to that of the intrinsic potentials of the brain (10-12 seconds) and with different intensities was carried out every 2 minutes for 45 seconds. During this period, the intensity of the flash of the photostimulator lamp was changed 9 times.

One of the channels of the instrument was connected to a Balashov integrator, which made it possible to obtain the total energy output in the form of 9 spikes measured in millimeters during processing of the curve.

After establishing the background (obtaining curves during inhalation of pure air), nitrobenzene was supplied in concentrations below the value of the threshold of smell. The inhalation of the gas lasted 6 minutes and was carried out from the 8-th to the 14-th minute of the experiment. The first 6 minutes of the recording on the day of gas inhalation constituted the background for the given day of the examination. After disconnecting the gas, the recording of the curves was continued in the same sequence up

to the 24-26th minute of the experiment, until a tendency to restore the initial level of intrinsic rhythm appeared. The experiments with inhalation of nitrobenzene were alternated with the inhalation of pure air. Concentrations of 0.0129 and of 0.008 mg/m^3 were investigated. The 0.0129 mg/m^3 nitrobenzene concentration was found to be active in five subjects, while the 0.008 mg/m^3 concentration was the subthreshold value for them. One subject was found to be less sensitive (Table 3).

Table 3

Results of Study of Reflex Effect of Nitrobenzene by Functional Electroencephalography.

Subject	Concentration, mg/m^3			
	Electroencephalography		Olfactory Sensation	
	Threshold	Sub-threshold	Threshold	Sub-threshold
Kh.	0,0129	0,008	0,0177	0,0160
G.	0,0129	0,008	0,0182	0,0169
I.	0,0129	0,008	0,027	0,02
L.	0,0129	0,008	0,027	0,02
A.	—	0,0129	0,0182	0,0169
Ya.	0,0129	0,008	—	—

Analysis of the electroencephalographic curves established that in one subject (Kh.) after a 6-minute inhalation of nitrobenzene, no restoration of the rhythm and energy occurred, not only during the 24 minutes of the experiment, but also 40-50 minutes after the gas was disconnected during a 20-30 minute rest. Changes in the curve of this subject (Fig. 2) took place in the 10th minute of the experiment, 2 minutes after the gas was supplied, were then reinforced in the 14th minute, when the gas was disconnected, and during the following 2 minutes. The confidence factor of the changes reached 99.9%.

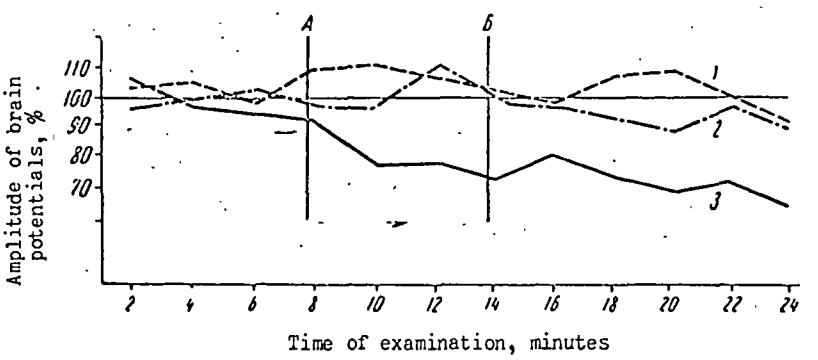


Fig. 2. Changes in the amplitude of reinforced intrinsic potentials of the brain in subject L.

1 - pure air; 2 - concentration, 0.008 mg/m^3; 3 - 0.0129 mg/m^3; AB - period of gas inhalation.

As a result of the analysis of the curves, we came to the conclusion that the method employed in the study of the reflex effect of atmospheric pollutants is highly sensitive.

On the basis of studies of the threshold of olfactory sensation of nitrobenzene, its influence on the light sensitivity of the visual system, and a study of the reflex effect of nitrobenzene on the electrical activity of the human brain, we recommend the highest single maximum permissible concentration of nitrobenzene in atmospheric air of populated areas as 0.008 mg/m^3, the subthreshold concentration according to the most sensitive method (Table 4).

Table 4

Results of Study of the Reflex Effect of Nitrobenzene.

Threshold	Concentration, mg/m^3	
	Minimum Active	Maximum Inactive
Olfactory sensation	0,0182	0,0169
Effect on light sensitivity of the eye	0,0157	0,0118
Effect on electrical activity of cerebral cortex	0,0129	0,008

To substantiate the average daily maximum permissible concentration of nitrobenzene, a round-the-clock dynamic exposure of white rats was carried out for 73 days.

Four groups of male white rats with 18 in each group were exposed. In the first group, the nitrobenzene concentration was 0.8 ± 0.022 mg/m^3, in the second 0.08 ± 0.01 mg/m^3, in the third 0.008 ± 0.0008 mg/m^3, and the fourth group was the control.

It is known from the literature that nitrobenzene affects chiefly the blood. However, the clinical picture of poisoning with nitrobenzene indicates the possibility of selective injury to the central nervous system (Ya. I. Kukhta et al., 1934).

This enabled us to select the following tests characterizing the influence on the central nervous system as the chief indicators of the effect of nitrobenzene on the organism: behavior and weight of the animals, motor chronaxy of the antagonist muscles of the shin, cholinesterase activity, and on the blood - total hemoglobin, methemoglobin, sulfhemoglobin and oxyhemoglobin, Heinz bodies, and histopathological data.

The investigation lasted 101 days. During the first week of exposure, irritation of the upper respiratory tract (head cold, sneezing) and the appearance of a serous crust on the eyelids were observed in rats of the first group (0.8 mg/m^3). A few days later, these symptoms passed. The animals were active in all groups except the first, in which a periodic sluggishness of the rats was noted.

No statistically reliable changes in the weight of the animals of all three groups were observed as compared with the control group.

The central nervous system under normal conditions has a subordinate influence on the neuromuscular system, as expressed in a correct ratio of the chronaxias of the extensors and flexors. When the process of inhibition arises in the cerebral cortex, this ratio changes and even becomes inverted (Chao Cheng-ch'i, 1961; V. A. Gofmekler, 1961, and others).

We studied the chronaxy and rheobase of antagonist muscles of the hind leg of the rat by means of an ISE-01 electronic pulse chronaximeter once every ten days in six rats of each group during the first half of exposure and in five rats during the second half.

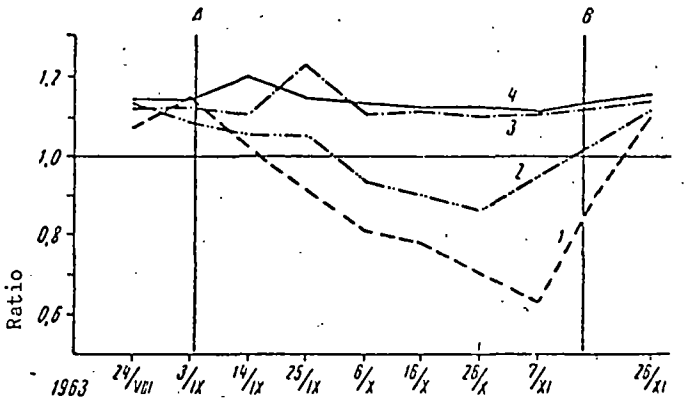
An inverse ratio of the chronaxies of antagonist muscles in rats of the first group (0.8 mg/m^3) occurred on the 11th day and was preserved during the entire exposure. In rats of the second group (0.08 mg/m^3), the inversion of the ratio was observed on the 21st day and was also preserved during the entire period of the experiment. A disturbance of the correct ratio of the chronaxies of antagonist muscles occurred as a result of an increase in the chronaxy of the flexors (the confidence factor of the changes reached 95-99%). The reestablishment of the correct ratio of chronaxies was observed after ten days of the recovery period. No change of the ratios occurred in the third group (Fig. 3).

During the development of inhibitory processes in the centers, an accumulation of acetylcholine takes place in the tissues. An increase in the activity of cholinesterase is one of the mechanisms of compensation for this disturbance (D. Ye. Al'pern, 1958, and others).

The study of cholinesterase activity of whole blood was made every fortnight by using A. A. Pokrovskiy's chemical method (1953) modified by A. P. Martynova (1957).

The nitrobenzene concentration of 0.8 mg/m^3 (first group) caused in the rats a statistically reliable increase of cholinesterase activity (99.9%) on the 12th day of exposure, and the 0.08 mg/m^3 concentration (second group), starting on the 39th day of exposure. During the recovery period, the cholinesterase activity returned to the initial level in both the first and second groups. The cholinesterase activity in rats of the

third group (0.008 mg/m^3) was similar to that of the control group over the course of the entire study (Fig. 4).



Dates of investigation

Fig. 3. Ratio of chronaxies of antagonist muscles during chronic exposure to nitrobenzene.

1 - first group (0.08 mg/m^3); 2 - second group (0.08 mg/m^3); 3 - third group (0.008 mg/m^3); 4 - fourth group, control; AB - period of exposure.

The disturbance of the correct ratio of chronaxies of antagonist muscles and the increase in the cholinesterase activity of the blood indicates the presence of inhibitory processes in the central nervous system, arising from the influence of nitrobenzene vapors.

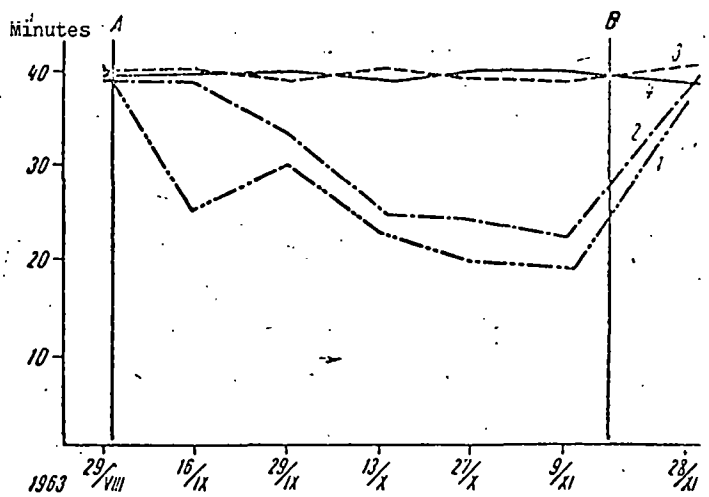


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Fig. 4. Activity of cholinesterase of whole blood in rats during chronic exposure to nitrobenzene.
Notation same as in Fig. 3.

Of late, the literature increasingly emphasizes the view that the basis for the various disturbances during poisoning with aromatic nitroamino derivatives are changes in respiratory enzymes. The formation of methemoglobin itself is attributed to the depression of certain intraerythrocytic oxidation-reduction enzyme systems whose normal activity consists in reducing methemoglobin to active hemoglobin (P. Sulamit et al., 1960).

Sulfhemoglobin may be formed as an intermediate in the formation of bile pigments from hemoglobin.

To measure the total hemoglobin, methemoglobin, sulfhemoglobin and oxyhemoglobin (every 10 days), we chose the method of determination of hemoglobin with a photoelectrocolorimeter (Evelyn, Malloy, 1938). The quantity of blood employed was decreased to 0.05 ml. We did the determination with an SF-5 spectrophotometer.

In rats of all groups, the total hemoglobin before exposure ranged from 10.307 to 13.453 g per 100 ml of blood; there was up to 1.63% methemoglobin of the total hemoglobin, up to 0.28% sulfhemoglobin, and up to 98.0-98.95% oxyhemoglobin of the total hemoglobin.

During exposure in rats of the first group (0.8 mg/m^3), on the 19th day of exposure, as the methemoglobin increased from 4.20 to 12.65%, there was a sharp decrease in the amount of total hemoglobin (7.565-9.252 g per 100 ml of blood) and oxyhemoglobin (87.01-95.27%). In rats of the second group (0.08 mg/m^3), the changes were similar in nature but somewhat less pronounced.

Starting with the 48th day of exposure, an increase of sulfhemoglobin to 1.91% with a sharp decrease of methemoglobin were noted in animals of the first group. At the same time, the total hemoglobin slightly increased. In the second group on the 58th day of exposure, sulfhemoglobin increased up to 1.40% in some rats. These changes were preserved until the end of the exposure (Fig. 5).

Restoration of the amount of hemoglobins to the original level occurred on the 12th day after completion of the exposure. In rats of the third group, the content of hemoglobins did not exceed the limits of fluctuations of these indicators in animals of the control group (see Fig. 5).

Isolated Heinz bodies appeared on the 48th day of exposure in rats of the second group only (0.8 mg/m^3).

Thus, nitrobenzene in concentrations of 0.8 and 0.08 mg/m^3 during long-term round-the-clock penetration of the organism of white rats decreases the quantity of total hemoglobin and oxyhemoglobin, causes methemoglobinemia, and subsequently sulfhemoglobinemia. A nitrobenzene concentration of

0.008 mg/m^3 was shown to be inactive by all the tests, and we therefore propose it as the average daily maximum permissible concentration in atmospheric air.

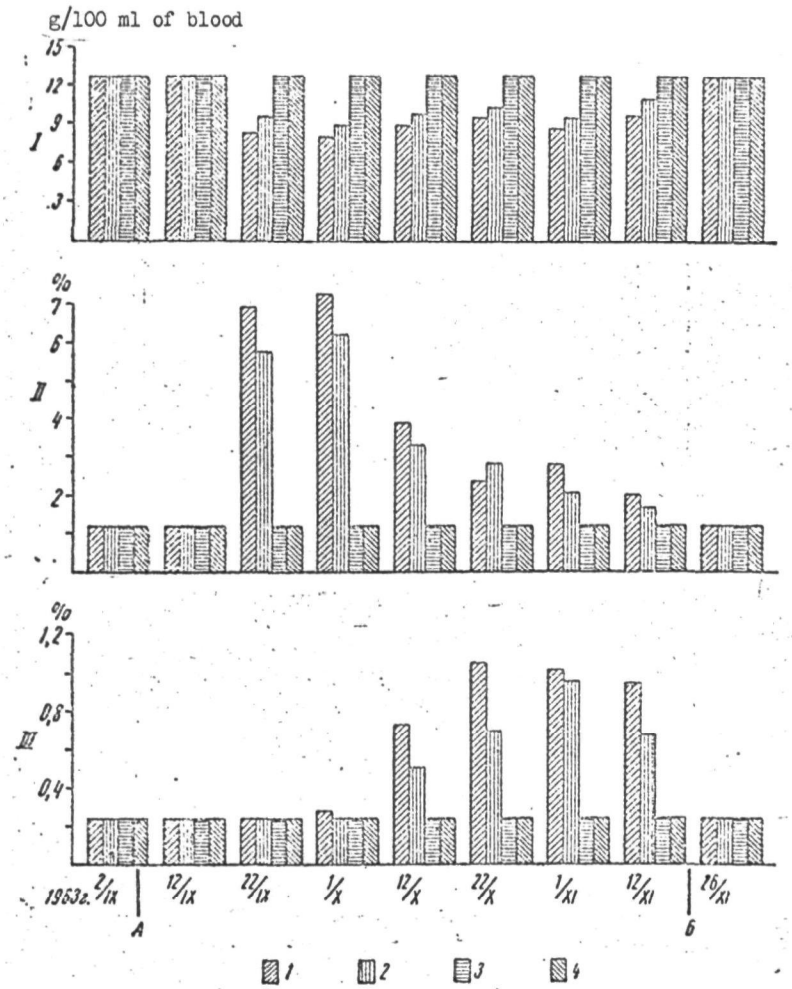

Fig. 5. Content of total hemoglobin (I), methemoglobin (II) and sulfhemoglobin (III) of the blood in rats during chronic exposure to nitrobenzene.
Notation same as in Fig. 3.

On the basis of the experimental studies, we attempted to follow the process of action of nitrobenzene on the organism, in particular, on the hemoglobin system, during long-term inhalation of low concentrations. The extent of the action is indicated by sulfhemoglobin, whose quantity increased not only in percent ratio to the total hemoglobin but also in absolute terms, in milligrams per 100 ml of blood.

Methemoglobinemia and sulfhemoglobinemia constitute one of the characteristic biochemical manifestations of nitrobenzene intoxication. However, before the appearance of changes in the blood, there are earlier changes in the central nervous system, as indicated by data obtained from the study of the motor chronaxy of antagonist muscles, and of cholinesterase of whole blood.

Apparently, by indirectly altering the processes of cellular metabolism, nitrobenzene impairs the tissue respiration of the brain and of other internal organs, subsequently causing blood hypoxia as a result of the transformation of part of the hemoglobin into methemoglobin and sulfhemoglobin and of hemolysis of the erythrocytes.

The ability of nitrobenzene during long-term penetration into the organism in concentrations of 0.8 and 0.08 mg/m^3 to impair the vital functions of cells of the central nervous system and the respiration of organs and tissues, and to alter the state of the enzyme system in the blood of rats indicates that the action of the poison directly affects the "body chemistry" I. P. Pavlov always kept in mind, particularly when dealing with the activity of the central nervous system, which he viewed as a function of the biochemical processes of the organism, while at the same time distinguishing its coordinating role in all the processes taking place in the organism.

In a hygienic evaluation of the pollution of the atmosphere with nitrobenzene around an experimental plant that used this chemical as the raw material, we collected samples of atmospheric air during the spring-summer period of 1963. The discharges of the plant studied contained vapors of nitrobenzene, nitrochlorobenzene and benzene. In order to carry out a separate determination of these substances, we developed a method of determination of nitrochlorobenzene with measurement on a spectrophotometer.

The peak for the measurement of nitrochlorobenzene absorbed in distilled water (0.5 l/min) was at a wavelength of 207-208 mμ with a slit opening of about 1.9. The sensitivity of the method was 0.25 μg per ml.

Benzene was also measured on the spectrophotometer by using the method of Alekseyeva et al. (1963). Nitrobenzene was determined colorimetrically by Alekseyeva's method, as indicated above.

We determined nitrobenzene in concentrations exceeding our proposed highest single maximum permissible concentration (0.008 mg/m^3) at a distance of up to 200 m, and in lower concentrations at a distance of up to 300 m (Table 5).

Table 5

Pollution of Atmospheric Air with Nitrobenzene Around One of the Plants.

Distance From Source of Discharge	Number of Samples Collected	Maximum Nitrobenzene Concentration, mg/m³	Distribution of Concentrations (mg/m³) of nitrobenzene in the samples		
			0.14—0.01	0.011—0.008	Below 0.008
150	27	0,0957	27	—	—
200	34	0,0439	28	1	1
250	37	0,0081	—	1	11
270	34	0,0060	—	—	6
300	25	—	—	—	—

Nitrochlorobenzene was determined at a distance of 100-300 m in concentrations of 0.4-0.08 mg/m^3. Benzene was not observed in any of the samples.

Results of the laboratory studies were used by the plant for the introduction of changes in the flowsheet of the planned operations.

Conclusions

1. Nitrobenzene has a high toxic activity which injures first the central nervous system, then the blood.

2. On the basis of a study of the threshold of olfactory sensation of nitrobenzene, the reflex effect of its vapors on the light sensitivity of the visual system and on the electrical activity of the brain, we recommend that 0.008 mg/m^3 be established as the highest single maximum permissible concentration of nitrobenzene in the atmospheric air of populated areas.

3. A chronic action of nitrobenzene vapors in concentrations of 0.8 and 0.08 mg/m^3 caused an impairment in the correct ratio of chronaxies of antagonist muscles, an increase in the cholinesterase activity of whole blood, a decrease in total hemoglobin and oxyhemoglobin, and an increase of methemoglobin and sulfhemoglobin.

4. As the average daily maximum permissible concentration of nitrobenzene in atmospheric air of populated areas, we propose the concentration of 0.008 mg/m^3, which did not cause any significant changes during chronic round-the-clock exposure.

5. Atmospheric air is contaminated with nitrobenzene vapors up to a distance of 250 m around one of the plants that use it as the raw material.

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LOW CONCENTRATIONS OF UNSATURATED HYDROCARBONS OF THE ETHYLENE SERIES IN AIR AROUND PETROCHEMICAL COMPLEXES

Candidate of Medical Sciences M. L. Krasovitskaya and L. K. Malyarova
Ufa Institute of Hygiene and Professional Diseases

From "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Pod redaktsiey Prof. V. A. Ryazanova i Prof. M. S. Gol'dberga. Izdatel'stvo "Meditsina" Moskva, p. 74-100, (1966).

Thus far, hygienists have been working with the concept of the "sum" of hydrocarbons, which includes saturated, unsaturated, aromatic and chlorinated hydrocarbons. Because of the great variety of hydrocarbons in atmospheric air, the variability of their composition and their diverse toxicity, this overall definition excludes the possibility of a hygienic evaluation of their contents in the atmosphere.

The object of the present study was to separate from the "sum" the hydrocarbons of the ethylene series, to establish their content in atmospheric air, and to study the effect of low concentrations of ethylene, propylene, and butylenes on man. A hygienic evaluation of hydrocarbons of the ethylene series is of particular interest in areas with a large petroleum refining and petrochemical industry polluting atmospheric air with unsaturated hydrocarbons (cracking gas contains approximately 20-25% propylene, up to 12-15% butylenes, 10-12% ethylene, 5-6% C_5 hydrocarbons).

Unsaturated hydrocarbons of the ethylene series up to C_4 inclusive are gaseous substances, and those beginning with C_5, liquids. All these hydrocarbons have a narcotic effect, which, however, is observed only at high concentrations, on the order of several tens of volume percent. The strength of the effect increases with the number of carbon atoms. In addition to the narcotic effect, olefins, starting with butylenes, cause an irritation of the respiratory tract. The threshold of smell for butylene (N. V. Lazarev, 1954) is 0.59 mg/m^3. Compounds with branched chains had a weaker effect than normal olefins. According to the Gor'kiy Institute of Labor Hygiene and Professional Diseases, the maximum permissible concentration of olefin vapors in the plants is 50 mg/m^3, and for butylenes, the maximum permissible concentration is 100 mg/m^3.

In the literature available to us, there are no data on the effect of low concentrations of individual hydrocarbons on the human organism and its higher nervous activity. Studies dealing with the action of hydrocarbons on the human organism pertain to the sum of saturated and unsaturated hydrocarbons and refer to concentrations existing in the air of industrial quarters (R. S. Ostrovskaya, 1961; S. Kh. Nikolov, 1961; S. Kh. Karimova, 1961;

R. F. Gabitova, 1960; B. A. Shekhtman, 1929, 1961, and others). No studies have been found dealing with the influence of microconcentrations of ethylene, propylene, and butylenes on the human or animal organism during administration by inhalation.

The hygienic substantiation of the maximum permissible concentrations of ethylenic hydrocarbons in the atmosphere consists in the experimental investigation of the effect of low concentrations of ethylene, propylene, and butylenes on the physiological reactions of the human organism.

To perform the experiment, propylene and butylenes were obtained in the laboratory by dehydration of the corresponding alcohols - isopropyl and n-butyl alcohols - over active aluminum oxide at 375-400°C. (for propylene) and 350°C. (for butylenes). The alcohols were passed at a rate of 800-900 ml per liter of catalyst per hour. The purity of the gases obtained was continually checked with a KhT-2M chromathermograph. The content of pure gases varied between 95.5 and 99.5%. The petroleum refinery supplied ethylene of 99.9% purity.

To substantiate the highest single maximum permissible concentration, the olfactory threshold of these substances and the influence of their low concentrations on the optical chronaxy and dark adaptation were determined, and the threshold concentrations causing the formation of the electrocortical conditioned reflex during inhalation were established.

To obtain constant concentrations of the hydrocarbons studied, use was made of a method recommended by the Committee on the Sanitary Protection of Atmospheric Air. The gaseous mixture for the experiment was obtained by mixing a known concentration of the gas studied with pure air in certain ratios.* The two air streams were controlled by stopcocks and checked with flow meters. The actual gas content prior to the experiment was determined with a titrimetric gas analyzer. The constancy of the concentration was checked for three days for each substance in the course of 4-6 hours (Table 1).

In eleven persons who showed no changes in the olfactory organs, the threshold of smell for butylene, propylene, and ethylene was determined. A total of 536 observations were made. Each concentration was checked an average of three times. The thresholds of olfactory sensation are given in Table 2.

* The gas was dispensed with a stopcock. A hermetically sealed cylinder was evacuated with a vacuum pump to a certain reduced pressure. The stopcock was rinsed with the substance being dispensed and was then connected to the evacuated cylinder. A certain volume of gas contained in the stopcock was washed out with pure air until the pressure was equalized. The gas-air mixture obtained was agitated by means of glass rods on a shaker.

Table 1

Constancy of the Concentration in the Experimental Air Mixture.

Substance	Days of Observation	Concentration in mg/m³ after 2 hours			
Butylenes	1	3,12	3,12	3,18	3,18
	2	2,16	1,99	1,92	
	3	3,71	3,18	3,64	3,64
Propylene	1	5,0	4,75	4,68	
	2	2,08	2,6	2,15	
	3	7,02	6,75	7,25	
Ethylene	1	2,0	2,0	2,5	
	2	11,3	11,6	11,3	
	3	3,24	3,24	3,18	

As is evident from Table 2, the thresholds of olfactory sensation for butylenes, propylene, and ethylene are similar: in the most sensitive persons they amount to 15.4, 17.3 and 20 mg/m³ respectively.

Table 2

Results of Determination of the Threshold of Olfactory Sensation for Unsaturated Hydrocarbons (C_2-C_4)

Number of Subjects	Minimum Perceptible Concentration, mg/m³	Imperceptible Concentration, mg/m³
	Ethylene	
2	20,0	19,0
7	22,0	20,0
2	26,3	22,0
	Propylene	
1	17,3	15,1
2	18,6	17,3
6	22,7	18,6
2	24,0	22,7
	Butylenes	
2	15,4	14,3
7	17,3	15,4
2	21,4	17,3

The optical chronaxy was determined in 3-4 subjects. During the first 3-4 days, training of the subjects was carried out for each substance. The rheobase and chronaxy were established six times with 3-minute intervals during the course of the experiment. After the third measurement, the subject was given a gas of known concentration or pure air to breathe.

Three examinations were made with each concentration. The threshold for the determination of the rheobase and chronaxy was the first sensation of phosphene.

The results of the study are listed in Tables 3, 4 and 5.

Table 3

Change of Optical Chronaxy Before and After Inhalation of Ethylene (in percent at 0 minute).

Concentration, mg/m³	Before Inhalation	After Inhalation				
	Time of Experiment, minutes					
	0	3	6	9	12	15
			Subject Ye.			
Pure Air	100	101	101	102	101	101
9,6	100	101	101	102	103	100
13,9	100	101	101	67(c)	100	100
19,0	100	100	101	66(c)	68(c)	100
			Subject T.			
Pure Air	100	100	98	98	98	98
9,6	100	98	98	98	98	98
13,9	100	100	101	101	101	101
19,0	100	102	99	129(b)	100	100
			Subject R.			
Pure Air	100	99	99	90	100	99
9,6	100	100	100	99	99	99
13,9	100	99	100	40(c)	80(c)	100
19,0	100	101	100	134(a)	125	94

Note. Confidence factor a - 95%, b - 99%, c - 99.9%.

The change of the rheobase was noted in a study of the effect of ethylene in a concentration of 19 mg/m^3 in subject Ye. In the remaining cases, the rheobase remained without changes.

Thus, the thresholds of the reflex effect of ethylene, propylene and butylenes on the optical chronaxy are at the same level and amount to 13.9-14.3 mg/m^3 (Fig. 1 and 2). A trace response during inhalation of threshold concentrations occurred in the 12th minute during the action of propylene (subject G.) and ethylene (subject T.).

The method of dark adaptation was also used to establish the threshold of the reflex effect of the hydrocarbons studied.

The examinations were conducted with an ADM adaptometer. The dark adaptation curves were obtained with three subjects for each substance

Table 4

Change of Optical Chronaxy Before and After Inhalation of Propylene (in percent at 0 minute).

Concentration, mg/m³	Before Inhalation			After Inhalation		
	Time of Experiment, minutes					
	0	3	6	9	12	15
Subject Ye.						
Pure Air	100	101	101	102	101	101
9,6	100	100	100	100	101	100
14,3	100	100	100	95(a)	100	100
20,7	100	100	100	76(c)	89(b)	96
Subject G.						
Pure Air	100	100	99	99	99	99
9,6	100	100	100	100	101	100
14,3	100	100	100	163(c)	118(a)	100
20,7	106	100	100	192(c)	101	100
Subject O.						
Pure Air	100	100	101	101	101	100
9,6	100	100	100	100	100	100
14,2	100	100	100	66,5(b)	93	100
20,7	100	100	100	139(c)	100	100

Note: Confidence factor: a - 95%, b - 99%, c - 99.9%.

studied. During the first few days, the subjects were trained, then the initial curve of dark adaptation was recorded during inhalation of pure air. Subsequently, a definite hydrocarbon concentration was studied every three days. Results of the action of the substances studied on the light sensitivity of the eye are shown in Table 6.

Thus, a concentration of 14 mg/m^3 of ethylene, propylene, and butylenes caused a change of the light sensitivity in all the subjects. In two cases, the action of this concentration caused a decrease of the light sensitivity (in subject G. during inhalation of propylene and in subject M. under the influence of butylenes). In the remaining cases, a rise of the dark adaptation curve was noted (Fig. 3). The 11 mg/m^3 concentration did not act on all of the subjects either. Thus, the threshold of the reflex effect of the hydrocarbons studied on the course of the dark adaptation curve lies below the threshold of smell and amounts to 11 mg/m^3. The 8 mg/m^3 concentration was found inactive for all the hydrocarbons studied. The next stage of our study was the determination of the action of ethylene, propylene, and butylenes on the development of the electrocortical conditioned reflex.*

* The electrical activity of the brain was studied in collaboration with Yu. A. Terekhov and K. I. Sukhotina, members of the scientific staff of the physiology laboratory.

Table 5

Change of Optical Chronaxy Before and After Inhalation of Butylenes (in percent at 0 minute).

Concentration, mg/m³	Before Inhalation		After Inhalation			
	Time of Experiment, minutes					
	0	3	6	9	12	15
Subject Ye.						
Pure Air	100	101	101	102	101	101
10,4	100	100	99	99	100	100
14,0	100	99	100	71(a)	99	100
20,0	100	100	100	52(c)	98	99
Subject G.						
Pure Air	100	100	99	99	99	99
10,4	100	100	100	100	100	102
14,0	100	100	100	110(c)	100	100
20,0	100	100	100	121(b)	105	100
Subject O.						
Pure Air	100	100	101	101	101	100
10,4	100	101	102	102	102	99
14,0	100	99	100	164(c)	100	100
20,0	100	100	100	165(c)	100	100
Subject R.						
Pure Air	100	100	100	103	100	100
10,4	100	100	101	100	100	101
14,0	100	100	100	150(a)	100	100
20,0	100	100	100	147(b)	100	100

Note. Confidence Factor: a - 95%, b - 99%, c - 99.9%.

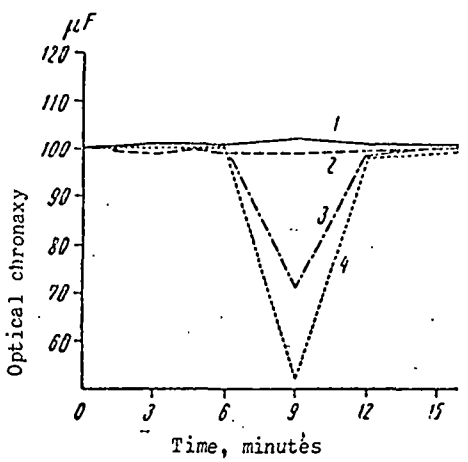

Fig. 1. Change of optical chronaxy during inhalation of butylenes in subject Ye.

1 - pure air; 2 - concentration of butylenes, 10.4 mg/m³; 3 - 14 mg/m³; 4 - 20 mg/m³.

The examinations were made on a 2-channel encephalograph of domestic manufacture using the method of Bushtuyeva, Polezhayev and Semenenko (1960). Results of the determination of the threshold of influence of the hydrocarbons on the electrical activity of the brain are given in Table 7.

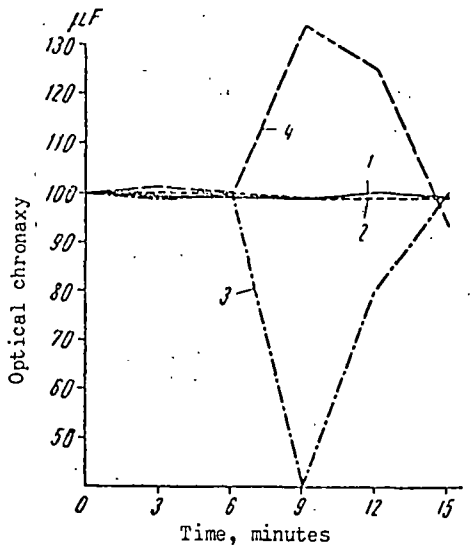

Fig. 2. Change of optical chronaxy during inhalation of ethylene in subject T.
1 - pure air; 2 - ethylene concentration 9.6 mg/m³; 3 - 13.9 mg/m³; 4 - 19 mg/m³.

Table 6

Average Values of Light Sensitivity of the Eye During Inhalation of Different Concentrations of Hydrocarbons of the Ethylene Series (C_2-C_4) in Percent of 15th Minute.

Subject	20th Minute				25th Minute			
	Pure Air	Concentration, mg/m³			Pure Air	Concentration, mg/m³		
		14.0	11.0	8.0		14.0	11.0	8.0
Ethylene								
G.	161	252(a)	153	156	191	296(c)	210(a)	201
M.	146	288(a)	182(b)	148	221	239	244	238
A.	129	194(a)	126	129	149	214(b)	155	140
Propylene								
G.	161	104(c)	191(b)	153	192	100(b)	258(c)	189
M.	146	234(c)	156	152	221	190(a)	230	213
A.	129	215(c)	199(b)	135	149	193(a)	220(a)	154
Butylenes								
G.	161	234(c)	187(b)	158	192	265(b)	237(c)	205
M.	146	120(b)	175(b)	152	221	165(c)	234(c)	204
D.	124	150(b)	121	129	177	130(c)	166	177

Note. Confidence Factor: a - 95%; b - 99%; c - 99.9%.

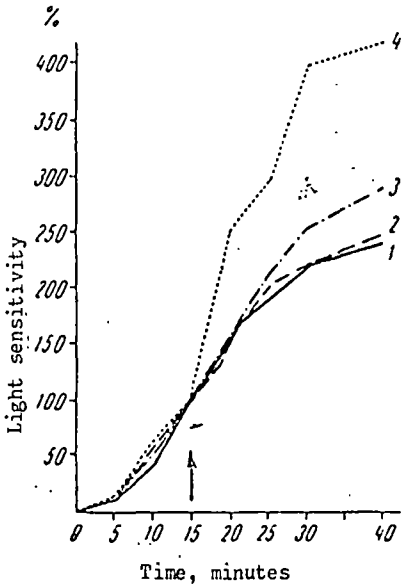

Fig. 3. Change in light sensitivity of the eye under the influence of inhalation of ethylene in subject G. (in percent of 15th minute).
1 - pure air; 2 - ethylene concentration, 8 mg/m³; 3 - 11 mg/m³; 4 - 14 mg/m³

Table 7

Formation of Conditioned Electrocortical Reflex During Inhalation of Unsaturated Hydrocarbons of the Ethylene Series (C_2-C_4).

Subject	Concentration, mg/m⁴				Threshold Concentration, mg/m³
	9,5	5,9	4,4	3,3	
			Ethylene		
M.	+	+	+	—	4,4
A.	+	+	+	—	
N.	+	+	+	—	
R.	+	—	—		
			Propylene		
M.	+	+	+	—	4,4
A.	+	+	+	—	
		+	+	—	
			Butylenes		
M.	+	+	+	—	4,4
A.	+	+	+	—	
	+	+	—	—	

Arbitrary notation: + active concentration; - inactive concentration.

The conditioned reflex was usually developed at the 4-9th pairing, more seldom at the 11-14th. The threshold of reflex change of the electrical activity of the brain lies at the level of 4.4 mg/m^3 for all the gases (Fig. 4, 5). Combined data from the study of the reflex effect of ethylene, propylene, and butylene on the receptors of the respiratory organs are listed in Table 8.

Table 8

Threshold Concentrations of Ethylene, Propylene and Butylenes Acting on Receptors of Respiratory Organs.

Threshold	Threshold Concentrations, mg/m^3		
	Ethylene	Propylene	Butylenes
Sensation of smell	20,0	17,3	15,4
Effects on electrical excitability (optical chronaxy)	13,9	14,2	14,0
Effects on light sensitivity	11,0	11,0	11,0
Effects on electrical activity of the brain.	4,4	4,4	4,4

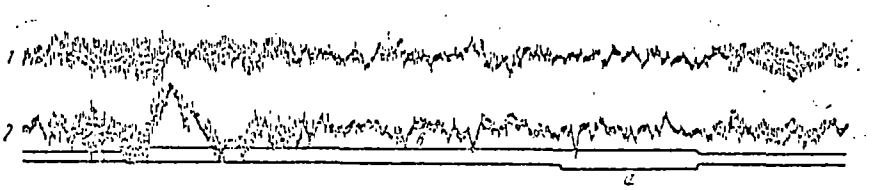

Fig. 4. Electroencephalogram of subject M. Conditioned-reflex desynchronization occurred during inhalation of propylene in 4.4 mg/m^3 concentration (at 7th pairing).

1 - electroencephalogram of the left occipital area; 2 - right occipital area; a - mark indicating when light was turned on; B - mark indicating supply of gas.

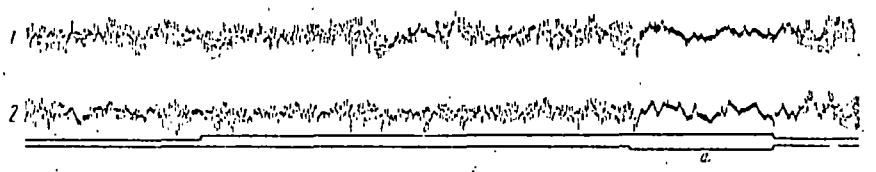

Fig. 5. Electroencephalogram for Subject M. No conditioned-reflex desynchronization occurred during inhalation of propylene in 3.3 mg/m^3 concentration (19th pairing).

Notation same as in Fig. 4.

The results obtained made it possible to propose 3 mg/m^3 as the highest single maximum permissible concentration of ethylene, propylenes, and butylenes.

In view of the fact that the hydrocarbons which we studied are constantly present in the atmosphere, the threshold of their effect on the electrical activity of the brain was determined for their combined presence (Table 9).

Table 9

Combined Action of Ethylene, Propylene, and Butylenes on the Development of the Electrocortical Conditioned Reflex.

Concentrations of ethylene + propylene + butylenes, mg/m^3	Total concentration in fractions of maximum permissible value	Subject		
		M.	A.	K.
1,1+1,1+1,1	1,0	—	—	—
2,1+1,1+1,1	1,3	+	+	+
1,1+2,1+1,3	1,3	+	+	+
1,1+1,1+2,1	1,3	+	+	+
3,2+3,3+3,2	3,0	+	+	+

Arbitrary notation: + active concentration, - inactive concentration.

Thus, desynchronization of the *a* rhythm takes place during inhalation of ethylene, propylene, and butylenes in amounts exceeding in sum the maximum permissible concentration we have proposed (Fig. 6 and 7).

The next section of the paper is devoted to a study of the influence of low concentrations of ethylenic hydrocarbons in a chronic experiment. The results of the study are used to substantiate the average daily maximum permissible concentration of each of the substances obtained during their isolated action and combined presence. Two series of round-the-clock exposures were carried out with ethylene, propylene, butylenes, and their

mixture. Each series involved five groups of 15 white male rats each (Table 10).

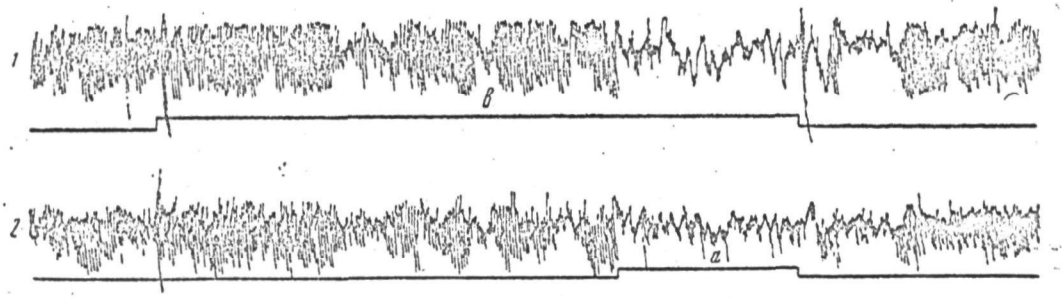

Fig. 6. Electroencephalogram of subject M. No conditioned reflex desynchronization of the rhythm occurred during inhalation of ethylene, propylene, and butylenes (total concentration in fractions of maximum permissible value equal to unity) (15th pairing). Notation same as in Fig. 4.

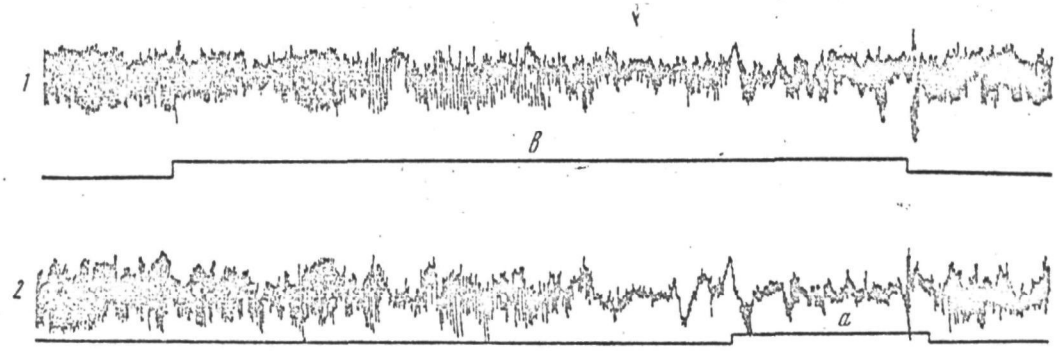

Fig. 7. Electroencephalogram of subject M. Conditioned-reflex desynchronization of the rhythm during inhalation of ethylene, propylene and butylenes (total concentration in fractions of maximum permissible value equal to 1.3) (11th pairing). Notation same as in Fig. 4.

The 3 mg/m^3 concentration is the highest single maximum permissible concentration that we are proposing, and 100 mg/m^3 is the maximum permissible concentration of butylene in the air of industrial quarters.

The exposure of each series of animals lasted 70 days.

The gases for the experiment were prepared and their purity was checked in the same manner as in the first part of the study. The observations were made by using the following tests: weight and behavior of the animal, ratio of the chronaxies of antagonist muscles, blood pressure, number of blood leucocytes and their phagocytic activity, whole blood

cholinesterase, and fluorescent* microscopy of leucocytes.

Table 10

Concentrations of Substances Studied in the Chambers,

	Series	Concentration, mg/m³				
		First Group (Butylenes)	Second Group (Propylene)	Third Group (Ethylene)	Fourth Group (mixture of ethylene, propylene and butylenes)	Fifth Group (control)
I	Concentration Assumed	100,0	100,0	100,0	33.3% of each (total of 100.0)	0
	Actual	104,3± 1,0	103,1± 1,3	104,1± 0,7	103.1 ± 0.5	0,24±0,1
II	Concentration Assumed	3,0	3,0	3,0	1.0 of each (total of 3.0)	0
	Actual	3,12± 0,02	3,13± 0,02	3,18± 0,03	3.12 ± 0.02	0

The animals in each chamber were divided into two subgroups: the ratio of chronaxies of antagonist muscles and the blood pressure were determined in the first group (8 rats), whereas in the second (7 rats), the number of leucocytes, their phagocytic activity, and whole blood cholinesterase were determined and fluorescent analysis of the leucocytes was performed. At the end of the exposures, some of the animals of each group were given the "swimming test", and the remaining ones were sacrificed. The organs of the sacrificed animals were then subjected to histopathological analysis. The choice of the above methods was determined by the nature of the action of the substances studied (narcotics).

The ratio of chronaxies of antagonist muscles is the most sensitive test reflecting the functional shifts in the central nervous system. The present day understanding of the physiological essence of chronaxy has permitted the use of chronaximetry as a method for the investigation of the functional state of the nervous system in the study of the chronic action of low concentrations for the standardization of atmospheric pollutants (V. P. Melekhina, 1962; V. A. Gofmekler, 1961, and others).

*Editor's note: For the Russian use of the terms "luminescent" and "luminescence" in this paper, we have substituted "fluorescent" and "fluorescence", on the basis of the definitions of these terms.

Cholinesterase. The physiological role of cholinesterase is closely related to the activity of the central nervous system. The mechanism of action of narcotics on the central nervous system may be attributed to an impairment of the processes of chemical transfer of stimulation in the brain synapses. Judging from the investigations of M. Ya. Mikhel'son, the inhibition of cholinesterase by the narcotic plays an important part in the mechanism of nerve-type narcosis in the broadly accepted cholinergic mechanism of synaptic transfer of nerve impulses. According to this author's data, with certain ratios of the enzyme to acetylcholine, narcotic concentrations of various substances decrease the cholinesterase activity by 50-70%. A parallelism has been established between the strength of action of 16 different narcotics and their concentrations, which depress cholinesterase to the same extent (N. V. Lazarev, 1961).

Blood Pressure. The hypotensive action of hydrocarbons has been established by numerous authors in experiments on animals and clinical observations of people in contact with these substances under industrial conditions (R. F. Gabitova, 1960; Z. Sh. Zagidullin, 1960; R. S. Ostrovskaya, 1961; L. I. Geller et al., 1963, and others).

Our observations of animals (white rats) exposed to natural conditions in the immediate vicinity of petroleum refineries (1963) established a statistically reliable lower level of blood pressure in rats of this group as compared with the control (in the 13-17th week of the experiment).

A considerably larger (by a factor of 2-1/2 - 6) number of cases of hypotonia occurs in children who have lived for 5 years in the vicinity of petroleum refineries, as compared with the control group.

Phagocytic activity. The literature contains indications of a decrease of immunobiological activity under the influence of the gas factor (A. N. Volkova, 1959; P. A. Zhilova, 1959; D. G. Pel'ts, 1960).

A decrease in the phagocytic activity of the blood under the influence of atmospheric pollutants has been noted by E. A. Sarkisyants (1959), M. L. Krasovitskaya, L. K. Malyarova, and T. S. Zaporozhets (1963).

Fluorescent microscopy is one of the methods for determining early quantitative and qualitative changes of the formed elements of white blood corpuscles. The use of this method is based on the ability of nucleic acid to exhibit fluorescence on combining with the fluorochromes of the acridine series: the nuclear nucleoproteins of degenerating cells combine with acridine dyes differently than do the nucleoproteins of uninjured cells, as is reflected in the character of their fluorescence (M. P. Meysel' and V. A. Sondak, 1956).

Normally, the nuclei of white cells fluoresce with an emerald-green color. When the synthesis is disturbed, and there is depolymerization of

the nuclear deoxyribonucleic acid, the nuclei begin to fluoresce with a red or orange color. The method of fluorescent microscopy of white blood corpuscles for the evaluation of the effect of low concentrations of chemical substances was first used by A. D. Semenenko at the department of communal hygiene of the Central Institute for Advanced Training of Physicians.

Fluorescent microscopy was successfully used by M. I. Gusev and K. N. Chelikanov (1963) in the study of amylene in chronic experiments on animals.

The "swimming" test for endurance (adapted for toxicology by M. I. Rylova, 1959) was used in our studies because it involved the mobilization of all the resources of the organism. This in our view should be an integral indicator. During our previous investigations on animals under natural conditions, this test yielded demonstrative results.

During the entire course of the two series of exposures, all the rats were healthy and active, and their behavior did not differ from that of the controls. Starting on the 48th day of exposure, all the animals received a scorbutigenic diet for ten days. A statistically reliable weight decrease was then observed in rats of the first and second groups in the first series of experiments. At the end of the exposures, the weight of all the animals was at the same level.

Measurements of the chronaxies of antagonist muscles were made by means of an ISE-01 electronic pulse stimualtor. The chronaxy was determined in eight rats of each group under identical conditions and at the same time. The character of the change in the ratio of the chronaxies of antagonist muscles over the course of the exposures is shown in Tables 11 and 12.

Table 11

Ratio of Chronaxies of Antagonist Muscles in Rats of Series I.

Group	Before Exposure	Day of Experiment			
		17th	35th	52nd	68th
First	1,7±0,15	1,5±0,1(b)	0,9±0,22(b)	1,0±0,17(b)	0,7±0,13(c)
Second	1,9±0,14	1,6±0,23	1,3±0,31(a)	1,0±0,24(a)	1,0±0,09(c)
Third	1,6±0,14	2,0±0,25	1,2±0,28(a)	1,1±0,25	0,9±0,13(c)
Fourth	1,7±0,15	2,0±0,28	1,4±0,12(a)	1,1±0,26	0,9±0,11(c)
Fifth	1,6±0,15	2,0±1,2	2,3±0,28	1,6±0,1	1,6±0,02

Note. Confidence factor: a - 95%; b - 99%; c - 99.9%.

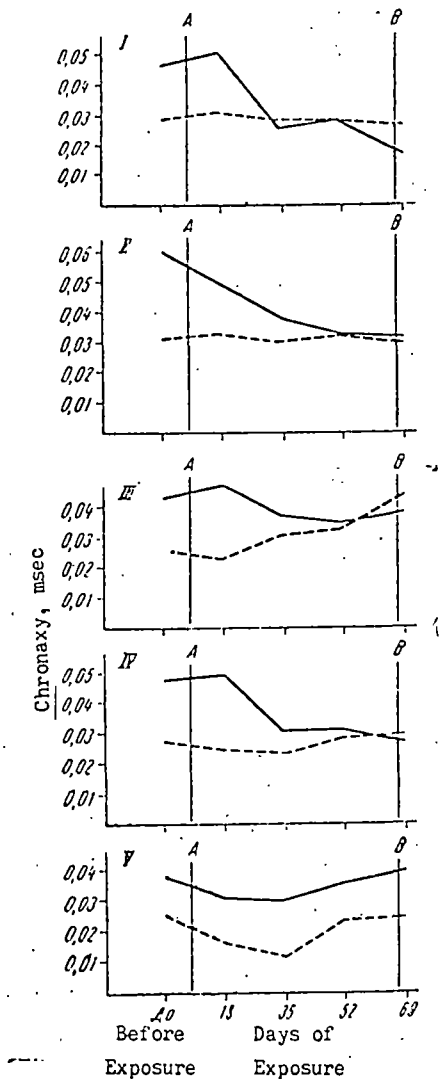

Fig. 8. Motor chronaxy of antagonist muscles in rats of series I. I, II, III, IV, V - exposure groups; solid line denotes the chronaxy of extensors, dashed line, that of flexors; AB - period of exposure.

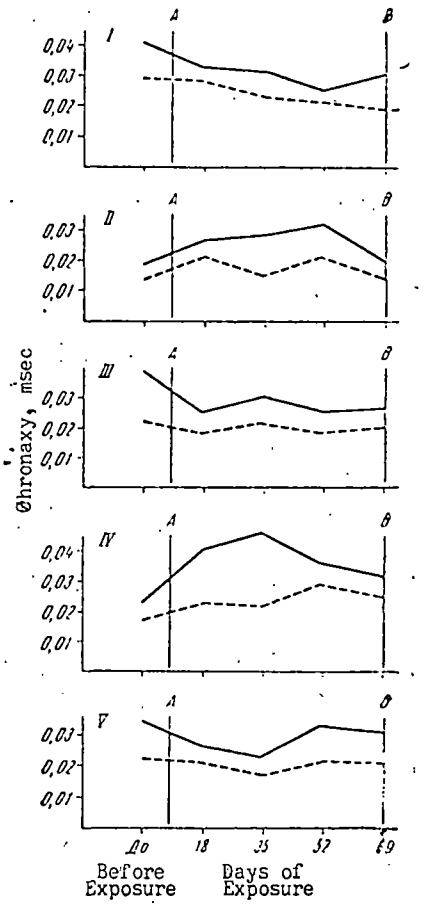

Fig. 9. Motor chronaxy of antagonist muscles in rats of series II.
Notation same as in Fig. 8.

In the course of the exposure, the ratios of the chronaxies of flexors and extensors in experimental rats of series I underwent a change: over the course of the experiment, these indicators were found to converge, and toward the end of the exposure their inverse ratio was established in the first, third, and fourth groups. In rats of the second group, the chronaxies of the flexors and extensors were found to be equal toward the end of the experiment.

Table 12

Ratios of Chronaxies of Antagonist Muscles in Rats of Series II.

Group	Before Exposure	Day of Experiment			
		17th	35th	52nd	68th
First	1,4±0,3	1,2±0,2	1,4±0,15	1,2±0,18	1,6±0,1
Second	1,35±0,15	1,3±0,18	1,9±0,11	1,5±0,15	1,4±0,18
Third	1,6±0,3	1,4±0,1	1,4±0,18	1,4±0,13	1,4±0,15
Pourth	1,3±0,29	1,8±0,11	1,6±0,11	1,3±0,1	1,3±0,1
Fifth	1,5±0,22	1,3±0,14	1,3±0,15	1,6±0,08	1,6±0,1

In the control rats, the ratio of chronaxies of antagonist muscles did not change substantially during the course of the experiment and ranged from 1.6 to 2.3. In rats of all the groups of series II, the ratios of chronaxies of antagonist muscles remained within the limits of the physiological norm.

The difference in the changes of the rheobase of the extensors and flexors was insignificant, i.e., statistically unreliable in all the groups. The chronaxy of antagonist muscles in rats of series I according to groups is shown in Fig. 8.

The blood pressure was determined in eight rats of each group before and during exposure under identical experimental conditions, using the method of Martynova (1956). Results of the investigations are given in Tables 13 and 14.

Table 13

Level of Blood Pressure in Rats of Series I.

Group	Before Exposure	Day of Experiment			
		18th	35th	52nd	69th
First	100±3,0	98±1,7	97±3,4	85±3,1(b)	76±3,5(c)
Second	104±4,2	99±1,2	99±14,0	90±2,1(a)	80±3,5(b)
Third	104±3,0	90±2,1	88±4,2(a)	88±2,1(b)	76±2,1(b)
Fourth	105±1,4	96±3,0	88±3,6(a)	84±2,3(c)	79±3,3(b)
Fifth	102±2,1	104±5,9	103±4,4	98±0,6	98±2,9

Note. Confidence factor: a - 95%; b - 99%; c - 99.9%.

Table 14

Blood Pressure Level in Rats of Series II.

Group	Before Exposure	Day of Experiment			
		18th	35th	52nd	68th
First	103±1,2	107±1,9	109±1,8	106±2,8	106±1,1
Second	105±1,2	104±1,3	106±0,9	109±2,1	104±1,5
Third	104±1,2	107±1,4	105±0,8	106±0,9	106±1,4
Fourth	106±0,9	105±1,3	110±2,0	109±1,7	106±1,4
Fifth	106±1,3	107±1,5	103±1,5	108±3,4	108±1,1

The blood pressure in rats of series I before exposure and in the first two weeks did not appreciably differ from that of the controls. From the middle of the exposure time, a statistically reliable decrease was noted in the third and fourth groups. From the 52nd day of the experiment, the change of this indicator was already established in all the animals of this series. The blood pressure level in the control was within the limits of physiological norm over the entire course of the experiment.

In rats of series II, the blood pressure level in all the groups (both experimental and control) was the same and corresponded to the physiological norm during the entire period of observations.

To study the activity of whole blood cholinesterase, we used a potentiometric method of determination (M. I. Goshev, 1958) modified by Dmitriyeva. The results obtained are given in Tables 15 and 16.

Table 15

Activity of Cholinesterase of Whole Blood in Rats of Series I (in Micromoles of CH_3COOH).

Group	Before Exposure	Day of Experiment			
		19th	36th	53rd	70th
First	2,19±0,21	2,81±0,22	1,84±0,25	1,51±0,09(c)	1,32±0,04(c)
Second	2,30±0,37	2,35±0,23	2,02±0,13	2,01±0,1	2,01±0,09
Third	2,01±0,19	1,92±0,21	1,82±0,09	1,70±0,09(c)	1,5±0,1(c)
Fourth	2,63±0,3	2,56±0,27	2,09±0,19	2,0±0,15	1,77±0,07(c)
Fifth	2,35±0,35	2,51±0,34	2,2 ±0,13	2,32±0,3	2,1±0,01

Note. Confidence factor: a - 95%, b - 99%, c - 99.9%.

Thus, in series I of the experiment, the enzymatic activity of the blood before exposure and during the first half of the experiment was maintained at the same level in the animals of the five groups. A statistically reliable depression of cholinesterase activity was observed on the 53rd day of exposure in rats of the first and third groups. At the end of the experiment, a statistically reliable decrease of cholinesterase activity as compared with the control was observed in rats of the first, third and fourth groups.

Table 16

Activity of Whole Blood Cholinesterase in Rats of Series II (in Micromoles of CH_3COOH).

Group	Before Exposure	Day of Experiment			
		19th	37th	53rd	70th
First	2,3±0,15	2,20±0,20	2,28±0,18	2,15±0,18	2,08±0,17
Second	1,8±0,007	1,72±0,08	1,66±0,10	1,75±0,11	1,82±0,08
Third	1,86±0,007	1,83±0,10	1,73±0,10	1,77±0,19	1,83±0,09
Fourth	1,92±0,007	1,96±0,07	1,97±0,11	1,99±0,10	2,0 ±0,09
Fifth	1,98±0,15	1,97±0,20	2,03±0,12	2,09±0,13	2,11±0,16

No statistically reliable difference in cholinesterase activity was established in experiments of series II as compared with the control.

The leucocytes were counted by the standard method. No statistically reliable difference in the number of leucocytes was established during the course of the experiments in any of the rats of series I and II as compared with the control.

In the determination of the phagocytic activity of the blood (D. G. Pel'ts, 1954), the percentage of phagocytizing neutrophiles and the phagocytic number were taken into account. In series I of the experiments, two reliable results were obtained in the study of phagocytic activity: in rats of the second group (growth of the phagocytic number on the 19th day of exposure) and of the first, second and third groups (on the 36th day). No changes of the phagocytic number and percentage of phagocytizing neutrophiles were observed in animals of series II.

In the fluorescent microscopy of white blood cells, no differences in the content of leucocytes whose nuclei had an orange color were observed between rats of the experimental and control groups of both series.

The "swimming" test was conducted after completion of the exposure.

No reliable differences were established in the duration of swimming in rats of all the groups. The indicator of this test within each group was marked by a great variability.

No pathologic changes in the internal organs were observed in the autopsy of animals of series I and II.

Thus, the studied exposure to ethylene, propylene, butylenes, and a combination of these substances showed that a concentration of 3 mg/m^3 of each of these gases and their mixture is inactive under chronic experimental conditions and can be recommended as the average daily maximum permissible value.

To determine the hydrocarbons in atmospheric air, a method of hydrocarbon analysis developed in the laboratory and involving the use of a KhT-2M chromathermograph was employed.

The developed method made it possible to establish the actual content of unsaturated hydrocarbons in atmospheric air. The samples were collected within a 2.5 km radius of petroleum refineries. Single and average daily concentrations were determined. A total of 140 analyses were performed. The results are shown in Table 17.

Table 17

Pollution of Atmospheric Air with Unsaturated Hydrocarbons Within a 2.5 km Radius from Petroleum Refineries.

Hydrocarbons	Concentrations, mg/m^3		
	Single		Average Daily
	Maximum	Average	Maximum
Ethylene	4,97	2,31	2,37
Propylene	3,5	1,37	1,80
Butylene	4,15	2,43	1,08
Amylenes	3,68	2,13	1,97

Thus, the highest single concentrations of each of the hydrocarbons which we determined at the indicated distance are slightly higher than the maximum permissible concentrations. The highest content of hydrocarbons when present together exceeds the proposed maximum permissible value severalfold.

Conclusions

1. The hydrocarbon gases ethylene, propylene, and butylene have similar thresholds of olfactory sensation and thresholds of reflex effect on the human organism.

The threshold of smell for butylenes, propylene, and ethylene for the most sensitive persons amounts to 15.4, 17.3 and 20 mg/m^3 respectively.

The threshold of reflex effect determined by the method of chronaximetry was 13.9-14.2 mg/m^3.

The threshold of the effect on the reflex change of the light sensitivity of the eye was 11 mg/m^3.

The threshold concentration causing the formation of the electrocortical conditioned reflex was found to be 4.4 mg/m^3. The maximum inactive concentration was 3.3 mg/m^3.

2. The maximum permissible concentrations of unsaturated hydrocarbons of the ethylene series should lie below the threshold of olfactory sensation, the thresholds of their action on the electrical excitability and light sensitivity of the eye, and also below the threshold of formation of the electrocortical conditioned reflex.

In this connection, the highest single maximum permissible concentration in atmospheric air which can be recommended is 3 mg/m^3 for ethylene, propylene, and butylene.

3. During the combined action of ethylene, propylene and butylenes, a simple summation of the effect of these substances is observed. The combined action of these hydrocarbons in concentrations equal to the proposed maximum single values causes a change in the electrical activity of the brain. Similar changes were obtained in all combinations of these substances at which their sum exceeded the proposed maximum permissible concentration (3 mg/m^3).

The highest single maximum permissible concentration for hydrocarbon gases of the ethylene series when present together should be 3 mg/m^3.

4. The hydrocarbon gases - ethylene, propylene, and butylenes - in a concentration of 100 mg/m^3 during chronic, round-the-clock action on animals cause changes in the functional state of the central nervous system, i.e., an impairment of the subordinate influence on the chronaxy of antagonist muscles, depress the cholinesterase activity, and have a marked hypotensive effect.

During the exposure, the number of leucocytes did not change, and there were no differences in the content of leucocytes, whose nuclei were orange in color. Changes in the phagocytic activity of the blood were not regular in character and cannot therefore be correlated with the action of the hydrocarbons.

The duration of swimming of the rats in all the experimental groups was much shorter than in the control, but the great scatter of this indicator within the group has led to a statistical unreliability of these results.

5. Ethylene, propylene, and butylenes in concentration of 100 mg/m^3 under conditions of a chronic experiment have the same strength of action on the organism. The existing fluctuations in the strength of action of these substances are not distinctly expressed in the various tests and are

therefore attributed to the individual sensitivity of the experimental animals.

6. During the combined action of ethylene, propylene, and butylenes, a simple summation of the action of these substances is observed in a chronic experiment. Changes in the animals during the isolated action of ethylene, propylene, and butylenes in concentration of 100 mg/m^3 of each substance were analogous to the changes taking place during exposure to a combination of these substances with the same total concentration.

7. Exposure of the animals to the hydrocarbons in concentration of 3 mg/m^3 has no effect on the behavior and weight of the animals, on the blood pressure level and ratio of the chronaxies of the antagonist muscles, on the cholinesterase activity, or on the number and phagocytic activity of leucocytes, does not injure the nuclear nucleoproteins (according to the data of fluorescent microscopy), and is not reflected in the results of the "swimming" test.

8. The highest single maximum permissible concentration of these hydrocarbon gases which we are proposing, 3 mg/m^3, is inactive in a chronic experiment and can also be recommended as the average daily concentration.

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THRESHOLD CONCENTRATIONS OF ACETOPHENONE

DURING SHORT- AND LONG-TERM INHALATION

Candidate of Medical Sciences
N. B. Imasheva
Department of Communal Hygiene, Central Institute for Advanced Training of Physicians, and Department of General Hygiene, Kuybyshev Medical Institute

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A rapid industrial development is associated with the discharge into atmospheric air of new chemical substances whose biological effects on man are unknown and whose maximum permissible concentrations have not been established. One such chemical is acetophenone, which is the subject of our study.

Acetophenone is used in organic synthesis in the chemico-pharmaceutical industry, the perfume industry, etc. However, the consumption of acetophenone in these industries is not large, and no high acetophenone contents in the discharges should therefore be expected. The chief source of contamination of atmospheric air with acetophenone is the production of acetone and phenol by the cumene process. A product of side reactions, acetophenone is present in atmospheric air in equal amounts with the starting materials and end products, and frequently surpasses them in quantity (M. I. Fongauz, 1958; V. P. Melekhina et al., 1961). However, no data on the effect of low concentrations of acetophenone on the human organism have been reported thus far in the Soviet or foreign literature. We therefore undertook the task of making a hygienic evaluation of acetophenone as an atmospheric contaminant.

Acetophenone C_8H_8O (methyl phenyl ketone) is an organic compound, an aliphatic-aromatic ketone. It has a characteristic pungent odor resembling that of the bird cherry. Its melting point is 19.6°C. and boiling point 202.9°C. Acetophenone is sparingly soluble in water, but dissolves well in ethyl alcohol, diethyl ether and other organic solvents. Methyl phenyl ketone is quite reactive: it can be nitrated, sulfonated, gives all the reactions with mercuric chloride, ammonium chloride, and phosphoric and picric acids. The chlorination product of acetophenone, chloroacetophenone, is a very strong lachrymator.

To determine low acetophenone concentrations in atmospheric air, we initially used Khrustaleva's method, based on the property of acetophenone of giving with metadinitrobenzene a pink color in an alcohol medium in the presence of an alkali. However, the sensitivity of this method, 1 μg/2 ml, proved inadequate. For this reason, a spectrophotometric method of determination of acetophenone was developed under the supervision of M. D. Manita. The method is based on a selective absorption of certain rays of the light source passed through a layer of the liquid under study. In addition to a simple qualitative method in which photographs of spectra of the solution

of the absorbing substance and pure solvent are compared, use may be made of a photometer to measure the fractions of the light flux which are absorbed at different wavelengths, and the data obtained can be used to plot a curve, characteristic of the given substance, of the dependence of the absorption intensity on the wavelength.

In a quantitative determination by the spectral method, one must check to see whether the substance obeys Beer's law, according to which the absorption is proportional to the number of molecules in the path of the beam, i.e., to the concentration of the substance. Studies made with acetophenone along these lines have shown that Beer's law is obeyed in work with low concentrations of an alcohol solution of acetophenone. Its absorption spectra in ethyl alcohol are characterized by a rather high optical density. Thus, at a maximum λ of 244 mμ, the log of the molar extinction coefficient is 4.2, which corresponds to the number 15,850. Such a coefficient permits the observation of small amounts of acetophenone in alcohol solution (Gilem and Shtern, 1957). On the basis of theoretical calculations, a solution of acetophenone in ethyl alcohol in 1 mg/l (1 μg/ml) concentration is characterized by an optical density of 0.130 (quotient obtained by dividing the molar coefficient 15,850 by a millimole of acetophenone 120,144). The optical density of an alcohol solution of acetophenone, determined by means of an SF-4 spectrophotometer, was found to be 0.128 in our case. Commercial acetophenone contains only 97% of pure acetophenone, and therefore the optical density of our standard solution was 0.130.

To determine acetophenone vapors, the air was collected in a modified Zaytsev absorber containing AMS brand silica gel, at a rate of 4 l/min. Five ml of ethyl alcohol was then poured over the adsorbent, decanted after 30 min, and examined in the spectrophotometer. The standard used was ethyl alcohol which had been in contact with 2 g of silica gel in order to create identical conditions for the sample and the standard. The optical density of the solution thus obtained was used to determine the amount of acetophenone by means of a calibration graph, then its concentration was calculated from the formula

$$\frac{A \times 5 \times 1000}{V} \text{ mg/m}^3,$$

where A is the optical density and V is the volume of air in liters drawn through the absorber. This method made it possible to raise the sensitivity of the determination of acetophenone to 0.25 μg/ml.

In the medical literature, acetophenone is mentioned in the 1870-1880's, when Dujardin-Beaumetz* and Barde proposed it under the name "hypnone" as a soporific. However, studies of several authors (Hirt, 1886; Rohfenbiller, 1887; Seifert, 1887) showed that it is not always possible to achieve the soporific effect of hypnone, and that the use of the compound in high doses involves a certain risk: the blood pressure of the patients drops, the

* [Translator's note: Dyuzharde-Bomets, according to the transliteration of Russian reference.]

breathing rhythm is altered, the pulse weakens and fatigue increases (Laborde, 1885, S. S. Kamenskiy, 1889). A study of the literature dealing with the effect of hypnone reveals contradictions in the interpretation of the problems of action of acetophenone. We have been unable to find any indications of a study of the threshold of action of acetophenone on the human and animal organism either in the domestic or in foreign literature.

The presence of the characteristic pungent odor of acetophenone enabled us to start our investigations from a certain threshold of olfactory sensation. To produce specific concentrations of acetophenone vapors, we used an apparatus recommended by the Committee on the Sanitary Protection of Atmospheric Air.

We determined the threshold of olfactory sensation of acetophenone in 18 practically healthy people 18 to 60 years old. The effect of a single concentration was observed during the day; pauses between the experiments lasted over four hours. An individual sensation threshold was established for each subject, and the threshold concentration was considered to be that which the subject could correctly identify no fewer than twice out of three determinations. A total of 455 determinations were made.

Table 1

Threshold of Olfactory Sensation of Acetophenone Vapors.

Number of Subjects	Concentration, mg/m^3	
	Minimum Perceptible	Imperceptible
1	0,08	0,05
2	0,027	0,02
14	0,02	0,01
1	0,01	0,008

As can be seen from Table 1, the threshold of olfactory sensation in the most sensitive persons is 0.01 mg/m^3. The 0.008 mg/m^3 concentration was found to be the subthreshold value.

The stimulation of one perception system inevitably results in a change in the functions of the other perception systems. This is explained by the fact that after stimulating the receptors of one system, the impulses reach the cortex, where they produce an excitation center which radiates over the cortex and thus encompasses other areas. This situation also applies to the olfactory system. Several authors have shown that the stimulation of the olfactory system by ammonium hydroxide, bergamot oil, and pyridine in toluene causes an increase in the light sensitivity of the eye

(K. Kh. Kekcheyev, O. L. Matyushenko, 1936; P. O. Makarov, 1958).

In hygienic practice, researchers have observed the action of subthreshold odor concentrations of various substances on the light sensitivity of the eye (F. I. Dubrovskaya, 1957; Yu. G. Fel'dman, 1960, and others).

We also studied this phenomenon in three subjects by means of an ADM adaptometer in a darkened room. Pure air or acetophenone of known concentration was supplied to a cylinder for 10 minutes until the first determination of the light sensitivity. The observation lasted no fewer than three days with each concentration. Acetophenone in 0.02 mg/m^3 concentration was found to cause a decrease in the light sensitivity of the eye in all three subjects. The 0.015 mg/m^3 concentration led to a decrease in light sensitivity in two subjects, but it was less pronounced than during the action of a higher concentration, and in one subject, to an increase of the sensitivity. Acetophenone in 0.01 mg/m^3 concentration caused a change in the light sensitivity of only one subject; the 0.007 mg/m^3 concentration was inactive. Table 2 lists data on the light sensitivity of the eye during the course of the study.

Fig. 1 shows data on one of the subjects. The changes occurring under the influence of inhalation of acetophenone vapors in the brain itself were then investigated. This was done by using the method of functional loading, which is based on the property of living tissue of reproducing a certain number of electric oscillations (N. Ye. Vvedenskiy, 1952).

Under natural conditions, afferent impulses from various exteroceptors and interoceptors continually reach the central nervous system. Consequently, the use of rhythmic stimuli in the study of the functional capacity of the central nervous system is natural and expedient.

"In the lability parameter we had a perfectly independent characteristic of the functional capacity of the tissue; this parameter reaches deeper into the intimate essence of the processes taking place and predicts the functional shifts taking place more accurately than temporal parameters (chronaxy, etc.) aimed at characterizing a single excitation wave" (L. V. Latmazanova, 1938).

Recently, many studies have appeared dealing with the ability of cortical elements to rearrange their rhythmics to correspond to a given frequency (V. S. Rusinov, 1957; N. V. Golikov, 1956, and others). To evaluate the functional state of the brain, the electroencephalographic method was made to include functional tests allowing for changes in the electroencephalogram in response to afferent stimuli. They are based on the following electrophysiological law: any excitation is associated with an electrical effect, which constitutes one of the aspects of the complex process of

excitation. The presence of the constant activity of the cortex interferes with the study of the biocurrents at rest. However, the use of a load, for example, a rhythmic photic stimulus, may give rise to processes taking place in latent form in the organism. The rearrangement

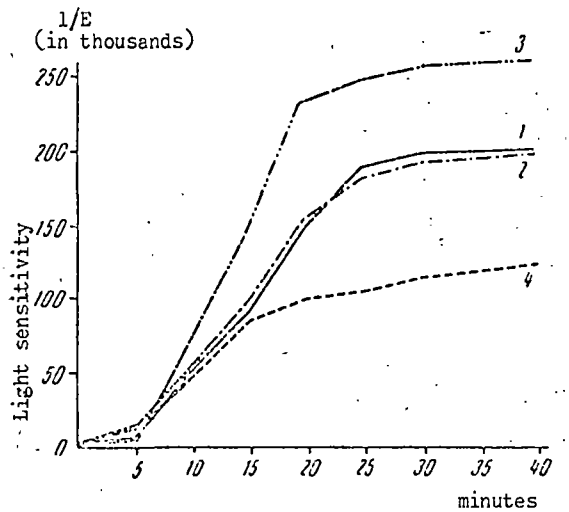

Fig. 1. Change in the course of dark adaptation curve after the action of acetophenone vapors in subject P.

1 - pure air; 2 - acetophenone concentration, 0.01 mg/m³; 3 - 0.015 mg/m³; 4 - 0.02 mg/m³.

of the rhythm depends on the intensity of the rhythm itself, its stability, the lability of the nervous system, and the initial functional state (V. Ye. Mayorchik, B. G. Spirin, 1951; V. I. Klimova, 1958; N. A. Aladzhalova, 1962, and others). The method of functional loading was successfully used by Soviet researchers: L. N. Smolin (1959), with application of the stimulus against the recording background of the electroencephalogram; G. N. Speranskiy, and Yu. M. Pratusevich (1961), while administering barbamyl.

In the course of our investigations, the electrical activity of the brain was recorded by means of a Kaiser 8-channel electroencephalograph in five practically healthy persons 18 to 35 years of age. During the study, the subject lay in a sound- and lightproof chamber. The apparatus for dispensing the gas and the electroencephalograph were located in another room. During the entire experiment, the subject breathed air at a rate of 20 l/min through a glass cylinder. At a certain moment, unbeknownst to him, a mixture of air with acetophenone vapors of a given concentration was fed into the cylinder. The subject was exposed to a flickering rhythmic light of rising and falling intensity (0.1, 0.2, 0.6, 0.2, 0.1, etc.). The stimulations were applied every three minutes in

Table 2

Change in the Light Sensitivity of the Eye After Inhalation of Acetophenone Vapors.

Acetophenone Concentration, mg/m³	Light Sensitivity of the Eye During Observation							
	½ minute	5 minutes	10 minutes	15 minutes	20 minutes	25 minutes	30 minutes	40 minutes
Subject N.								
Pure Air	26,8	987	7 835	14 650	16 200	19 600	25 000	22 900
0,02	12,3	376	4 350	4 583	5 857	6 037	6 180	6 323
0,015	12,2	359	3 590	6 083	6 726	7 262	7 426	7 604
0,010	20,1	1 170	6 520	8 080	10 740	11 333	11 600	11 900
0,007	25,6	448	8 273	15 167	17 166	21 100	23 933	24 433
Subject T.								
Pure Air	22,8	2 930	13 167	16 900	21 567	27 600	29 133	29 867
0,02	8,0	2 113	6 530	9 427	10 687	11 267	11 533	11 867
0,015	29,9	2 930	8 533	10 740	15 400	22 300	22 800	2 300
0,010	22,3	2 680	14 967	18 367	20 667	25 633	27 600	28 467
Subject P.								
Pure Air	90,7	7 543	56 200	92 275	148 250	187 000	192 500	199 250
0,02	24,2	10 113	48 700	89 366	97 200	104 000	114 000	117 000
0,015	14,3	4 910	72 000	147 333	231 666	248 000	254 000	258 000
0,010	86,0	1 186	58 600	99 666	150 666	182 333	189 666	192 000

the fourth minute. Prior to the administration of the experimental mixture, the background was recorded two to three times, then the mixture of pure air and acetophenone was supplied for five minutes. In the fourth minute the light stimulus was turned on, and further determinations were made as in the case of supply of pure air. The entire experiment lasted 40-50 minutes. To achieve a better objectivity of the results of the electroencephalographic study, we recorded the action potentials by using a method proposed by the British scientists Lovell and Dosset*(cited by G. N. Speranskiy, Yu. M. Pratusevich and N. M. Korzh, 1960). The synchronization factor, expressed as the ratio of the quantity of synchronized (coinciding) oscillations to the quantity of light stimuli supplied during 5 seconds, was calculated in percent from the formula

$$K_s = \frac{P_s}{P_n} \times 100,$$

where K_s is the synchronization factor, P_s is the quantity of synchronized oscillations, and P_n is the quantity of light pulses supplied.

At the same time, the energy of each synchronized potential (either in millimeters of the height reached by the pen of the electroencephalograph, or in microvolts) and the height of the synchronized spike multiplied by the calibration factor with which the recording of the electroencephalogram was made were calculated. The total energy of assimilated rhythms during the action of the light stimulus in the form of rhythmic light flickerings lasting 45 seconds was also determined.

Studies of the acetophenone concentrations (0.007 and 0.003 mg/m^3) did not reveal any distinct change in the synchronization factor in any of the subjects. As far as the total energy of the rhythm assimilated by the brain during inhalation of acetophenone vapors in 0.007 mg/m^3 concentration, there occurred a 35-40% statistically reliable decrease of this energy in all the subjects in the 2nd-6th minute. The 0.003 mg/m^3 concentration did not cause any changes. Such phenomena were also observed in healthy people (V. V. Artem'yev, 1962; Ye. N. Sokolov, 1962). Fig. 2 graphically shows the dynamics of change in the amplitude of the assimilated rhythm (energy) under the influence of inhalation of acetophenone vapors in two subjects in whom changes were observed in the 2nd minute after the gas mixture was supplied.

Thus, the threshold of action of acetophenone on the electrical activity of the brain is 0.007 mg/m^3, whereas the thresholds of smell and change of the light sensitivity of the eye are 0.01 mg/m^3. The 0.003 mg/m^3 concentration was found to be inactive, as indicated by all the tests, and can be recommended as the highest single maximum permissible value.

Experimental studies of the effect of acetophenone on the organisms of animals were made in the 1870's - 1880's simultaneously with clinical

* [Translator's note: Lovel' and Doset, according to the transliteration of Russian reference.]

observations of the effects of hypnone. The experiments were conducted on frogs, cats, guinea pigs and monkeys. Acetophenone was injected subcutaneously and intravenously, and also introduced into the stomach. However, all these studies were made with relatively high concentrations and had the character of acute experiments (S. S. Kamenskiy et al.). Mairet and Combemalle (1885) attempted to carry out a dynamic exposure of the animals, and for this purpose placed two cats, a guinea pig, and a rabbit in a wooden box with a volume of 175 dm^3 with slits and kept them there for 1 hour and 10 minutes. An atomizer was used to introduce a mixture of 3 g of acetophenone, 10 ml of alcohol and 200 ml of water into the box during this entire period. Muscular weakness and paretic symptoms were noted in all the animals. Unfortunately, it is impossible to use the authors' data for an accurate calculation of the concentration to which the animals were exposed. There is no doubt that this concentration was high. No data are available on the influence of low acetophenone concentrations during a long-term exposure.

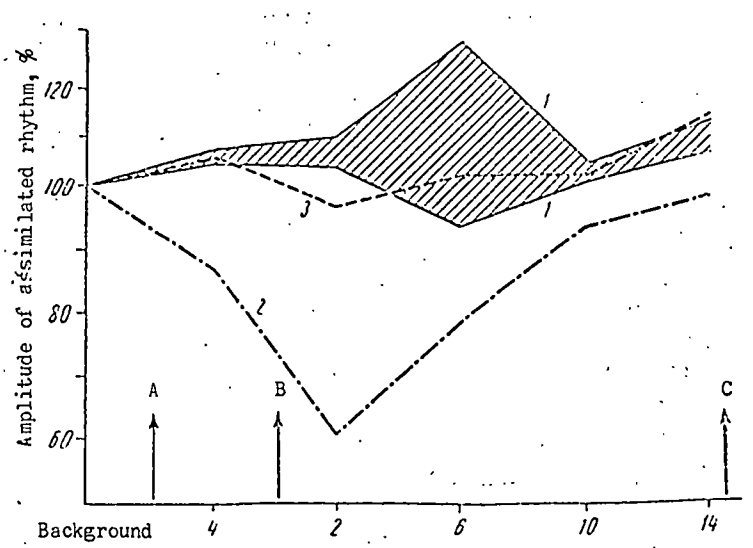

Fig. 2. Dynamics of change in the amplitude of assimilated rhythm during the action of acetophenone vapors. Average data for subjects V. and A.

AB - 4 minutes of inhalation of gas; BC - period in minutes after inhalation of gas. 1 - inhalation of pure air; 2 - acetophenone concentration, 0.003 mg/m^3; 3 - acetophenone concentration, 0.007 mg/m^3.

To study the chronic action of acetophenone, a round-the-clock exposure of white rats to acetophenone in concentrations of 0.007 and 0.07 mg/m^3 was carried out for 70 days. The 45 males selected, weighing 60-70 g, were divided into three groups. The first group was exposed to acetophenone in 0.007 mg/m^3 concentration, the second to 0.07 mg/m^3, and the third was the control. The acetophenone content in the first chamber

was an average of 0.007 $\pm$ 0.0008 mg/m^3, and in the second chamber, 0.076 $\pm$ 0.002 mg/m^3.

The influence of acetophenone on the organism was evaluated by observing the behavior and weight change of the animals and studying the chronaxy of antagonist muscles, the cholinesterase activity, and protein fractions of the blood serum.

No peculiarities of any kind were observed in the behavior of the animals during the entire course of the chronic experiment. The rats were active and gained weight equally in all groups. The weight was measured every ten days.

Numerous authors who have studied the ratios of chronaxies of antagonist muscles have shown that this parameter may be used as a criterion for the effect of noxious substances on the central nervous system, as expressed in a decrease of subordination (Yu. M. Uflyand, 1941; O. V. Verzilova, 1947; F. S. Trop, 1958).

The hygienic literature contains a report of a decrease in the influence of the cerebral cortex on the level of motor chronaxy of antagonist muscles during long-term exposure to vapors of noxious substances in low concentrations (V. A. Gofmekler, 1961; Yu. G. Fel'dman, 1960; G. I. Solomin, 1961, and others).

We determined the motor chronaxy by means of an electronic pulse stimulator, model ISE-01, once every 10 days. Fig. 3 shows that the curve of the chronaxy of extensors in rats of the first (0.007 mg/m^3) and control groups is higher than the curve of chronaxy of flexors during the entire course of observation, i.e., a normal ratio of chronaxies of the antagonist muscles exists. In the second group (0.07 mg/m^3), however, by the end of the second month of exposure, an inverse ratio of the chronaxies of flexors and extensors occurred.

In our study, we decided to follow the change of cholinesterase activity as well. A sharp change of this activity is observed in many diseases, and also when the organism is acted upon by chemical substances. Investigators note that chemical substances (hydrogen sulfide, tetraethyl lead, Dowtherm, phenol, etc.) in high concentrations cause an inactivation of the enzyme, which may be reversible or irreversible, depending on the degree to which the activity of the enzyme has been depressed. The hygienic literature also contains studies indicating a change of cholinesterase activity even during exposure to noxious substances in low concentrations (G. I. Solomin, 1961, R. Ubaydullayev, 1962, and others).

To determine the cholinesterase activity, we used the chemical method of Pokrovskiy (1953) modified by A. P. Martynova (1957). The method is

based on a determination of the time required for a change in the color of the indicator to occur as a result of the pH shift during hydrolysis of acetylcholine.

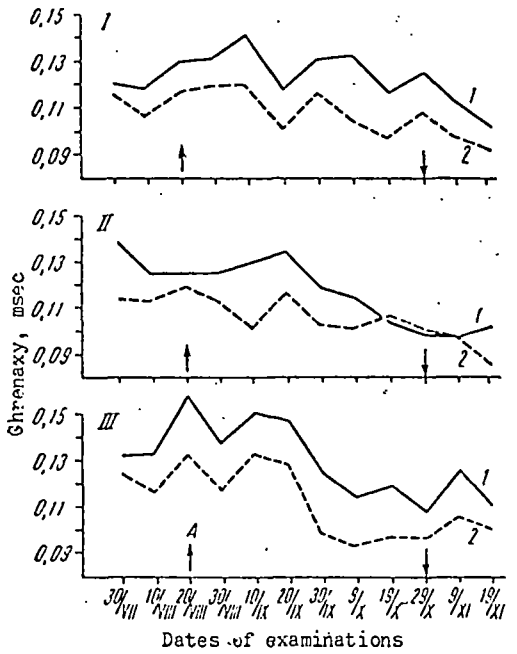

Fig. 3. Average data on the chronaxy of antagonist muscles in rats during exposure to acetophenone.
1 - first group (0.007 mg/m^3); II - second group (0.07 mg/m^3); III - control group; 1 - extensors; 2 - flexors; AB - period of exposure.

The change of cholinesterase activity was observed on five rats of each group, once every two weeks. During exposure, a change in the activity of the enzyme was observed in rats of the second group (0.07 mg/m^3) toward the second month of exposure, but these changes were not the same. Thus, in three rats of this group (Fig. 4), there was a 22% depression of cholinesterase activity (as compared with the background of this group and the control group). A reverse reaction was observed in one rat, i.e., a 45% increase in cholinesterase activity.

Acetophenone in 0.007 mg/m^3 concentration (first group) did not cause any statistically reliable changes of cholinesterase activity under these conditions.

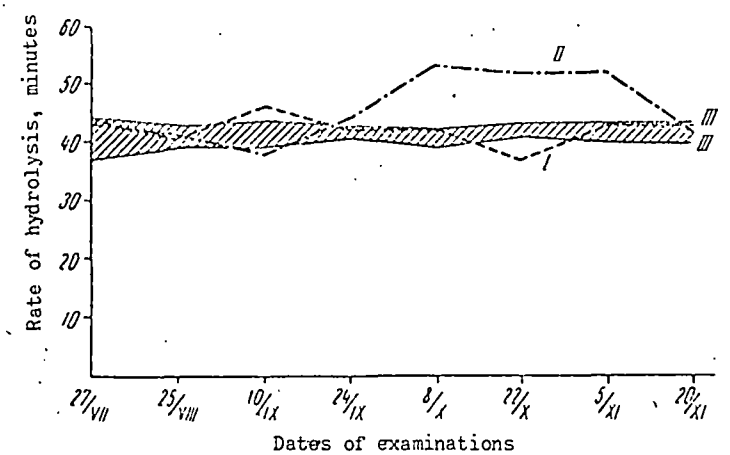

Fig. 4. Influence of acetophenone on the cholinesterase activity of whole blood of white rats.
Notation same as in Fig. 3.

Recent literature contains abundant material pertaining to the study of blood proteins. As we know, serum proteins possessing the same mobility in an electric field can be fractionated into albumins and α, β and γ globulins. In various diseases, and also under the influence of unfavorable external factors, both the total amount of protein and the ratio of the protein fractions in the blood undergo a change.

In the practice of hygienic standardization of noxious substances in atmospheric air, the method of study of the ratio of protein fractions in the blood was first used by R. Ubaydullayev (1961). The author explained that furfural in 10 mg/m^3 concentration in long-term action causes a marked change in the ratio of the protein fractions of the blood: the content of albumins decreases by 33-40% when compared with the initial level. When furfural was present in 0.33 mg/m^3 concentration, the changes were less definite in character. Only a concentration of 0.052 mg/m^3 was found to be inactive under the same conditions.

We determined the total amount of protein by means of an RPL-2 refractometer, and the protein fractions by paper electrophoresis. The phoregrams obtained, colored with an acid blue-black dye, were cut into fractions and eluted with a 0.1 N solution of sodium hydroxide, then subjected to colorimetric analysis on FEK-N-57. The sum of the figures obtained from the colorimetric analysis of each individual fraction was taken as 100, then the percent content of each fraction was correspondingly calculated.

To check the investigation, five rats of each group were selected. The blood sample was taken prior to feeding, once every 20 days. No

changes of total protein were observed, but the ratios of the protein fractions underwent a change. In rats of the second group (0.07 mg/m^3), a decrease in albumins to 24-35% was observed by the end of the first month of exposure as compared with the initial level (38.7-48.9%) and with rats of the control group. The amount of globulins increased cor respondingly. At the start of the second month of exposure, a sharp decrease of the albumin - α globulin ratio was noted. Only on the 20th day after the end of exposure had the protein indicators of rats of this group reverted to their initial values.

In rats of the first group (0.007 mg/m^3), no changes in the protein fractions were observed as compared with the control group (Fig. 5).

Some of the rats from each group were sacrificed after the completion of exposure for the purpose of histopathological analysis. Changes in the form of congestion of the cardiac vessels and a pronounced dystrophy of the liver were observed only in rats of the second group (0.07 mg/m^3).

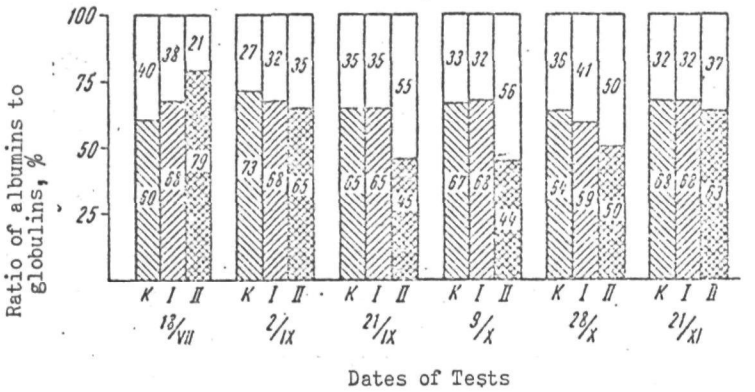

Fig. 5. Change in the ratio of albumins and globulins in the blood of white rats during exposure to acetophenone. I - first group (0.007 mg/m^3); II - second group (0.07 mg/m^3); K - control group

On the basis of the results of the studies, we can recommend an average daily maximum permissible concentration of acetophenone in atmospheric air at the level of the highest single concentration, i.e., 0.003 mg/m^3.

We also determined acetophenone in air around the plant section producing acetone and phenol by the cumene process at a synthetic alcohol plant. Acetophenone was formed as the product of a side reaction. The sources of its discharge into the atmosphere are sections for the production and decomposition of isopropylbenzene hydroperoxide and for processing of the by-products. The discharges seep through leaks in the equipment, packing glands, flanged joints, during the collection of samples, and through the system of local ventilation. In addition to acetophenone, many other substances reach the atmospheric air.

In June-July 1962, we collected single samples of atmospheric air around this plant at a distance of 100 to 2000 m. Acetophenone in the samples was determined colorimetrically. Results of analyses of the air samples are given in Table 3.

Table 3

Single Concentrations of Acetophenone in Atmospheric Air Around a Synthetic Alcohol Plant.

Distance from Source of Discharges, m	Number of Samples		Maximum Concentration, mg/m^3
	Total	Of these, above the sensitivity of the method	
100	3	3	0,023
400	26	25	0,028
800	28	18	0,016
1,200	25	11	0,007
1,600	26	6	0,007
2,000	25	0	--

On the basis of the laboratory data obtained for the contamination of atmospheric air with acetophenone, the administration of the plant was given specific recommendations for improving the surrounding air reservoir. We believe, however, that this is only a temporary measure.

The enterprise constitutes a large source of contamination of atmospheric air with various chemical substances. The only radical means of purifying the surrounding medium would be the development of new technological processes.

Conclusions

1. The threshold of olfactory sensation of acetophenone and its reflex effect on the light sensitivity of the eye lie at a level of 0.01 mg/m^3.

2. The threshold of the reflex effect on the electrical activity of the brain in the most sensitive persons is 0.007 mg/m^3.

3. Acetophenone in 0.07 mg/m^3 concentration during chronic exposure of experimental animals causes changes that are both functional (disturbance of the ratio of chronaxies of antagonist muscles, and of protein fractions of the blood, depression or activation of the enzyme cholinesterase) and organic (pathological changes in internal organs).

4. Under the same conditions, acetophenone in 0.007 mg/m^3 concentration does not cause any changes in the organism.

5. On the basis of the study, we are proposing a highest single and average daily maximum permissible concentration of acetophenone of 0.003 mg/m^3.

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CYCLOHEXANOL AND CYCLOHEXANONE IN ATMOSPHERIC AIR AND THEIR HYGIENIC SIGNIFICANCE

A. A. Dobrinskiy
Department of Communal Hygiene, Central Institute for Advanced Training of Physicians

From "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Pod redaktsiey Prof. V. A. Ryazanova i Prof. M. S. Gol'dberga. Izdatel'stvo "Meditsina" Moskva, p. 119-132, (1966).

The main object of our study was to establish the maximum permissible concentrations in atmospheric air for two substances: cyclohexanol and cyclohexanone. The present report presents data for the validation of the highest single maximum permissible concentrations of these substances.

Both substances are good solvents and find broad applications in such branches of industry as the production of leather, lacquers, dyes, and other synthetic materials. Cyclohexanol and cyclohexanone are mainly used as starting materials and intermediate products for syntheses. In particular, they are widely employed as intermediates in the production of caprolactam and adipic acid, which are the monomers in the production of the synthetic fibers caprone and nylon.

In 1958, 40% of all the polyamide resins were obtained from caprolactam. The preparation of this product is one of the large-tonnage processes in present-day industrial organic synthesis.

When cyclohexanol and cyclohexanone are used in industry, they may escape into atmospheric air. Thus, in the production of caprolactam, the operational cycles are carried out at high temperatures, which in the processes of synthesis of intermediate products promotes the escape of cyclohexanol and cyclohexanone into the ventilation air, etc.

Both substances studied belong to the group of aliphatic hydrocarbons of the cyclohexane series. One of them (cyclohexanol) is an alcohol and the other a ketone. Both are colorless liquids with similar physicochemical constants (Table 1).

These indices demonstrate to some extent the similarity of the toxicological properties, since a definite relationship has been established between the structure of many organic compounds and their biological effect (N. V. Lazarev, 1944; A. A. Golubev, Ye. I. Lyublina, 1962).

Table 1

Physicochemical Constants of Cyclohexanol and Cyclohexanone.

No.	Constants	Cyclohexanone	Cyclohexanol
1	Molecular weight	100.1	98.1
2	Specific gravity	0.962	0.947
3	Boiling point	160.1°	157°
4	Ignition point	67.8°	64°
5	Temperature coefficient of vapor pressure in deg/mm at 760 mm Hg	0.045	0.046
	Vapor pressure in mm Hg, 141.4°/132.5°	400	400
6	Refractive index for the wavelength of sodium D line 30°/15°	1.461	1.451

The methods of preparation of cyclohexanol and cyclohexanone have been worked out on a large industrial scale and are carried out in a single production cycle. The product thus obtained contains both substances. Various means are used to separate the mixture and isolate cyclohexanol and cyclohexanone. The starting product in the different methods of preparation may be phenol, cyclohexane, nitrocyclohexane, cyclohexylamine, nitrobenzene, or benzene (M. M. Shumilina et al., 1959; Pölhar, Sugino Kiichiro et al., 1957; D. R. Cova, 1961).

Data on the toxicological characteristics of these substances are scarce. Cyclohexanol and cyclohexanone are capable of irritating the upper respiratory tract and of having a narcotic effect in high concentrations. Inhalation of their vapors leads to disorders of the central nervous system and changes in the picture of the peripheral blood. Both substances or products of their transformation are excreted with the urine in the form of compounds with sulfates, causing the excretion of bound sulfites. Cyclohexanone has a marked damaging effect on the blood vessels and liver, a depressing effect on the function of the adrenal cortex, and an effect on the thyroid gland (G. V. Lobovikova and A. A. Preobrazhenskaya, 1950; G. V. Lobovikova, 1954; A. P. Voronin, 1958; Treon, Crutchfield, Kitzmiller, 1943; Nelson, Ege, 1943, 1945).

Cyclohexanone has a pronounced chronoconcentration effect (A. P. Voronin, 1958). This is confirmed by a decrease in the threshold concentrations of the gas when the exposure is extended.

In practice, the main routes by which these substances reach the organism are the upper respiratory tract and the skin. The majority of the authors who have studied the influence of cyclohexanol and cyclohexanone on

the organism have tested high concentrations of these substances.

In short-term experiments with penetration into the organism of experimental animals by inhalation, G. V. Lobovikova tested cyclohexanol concentrations above 250 mg/m^3, and cyclohexanone concentrations above 160 mg/m^3. In A. P. Voronin's experiments, the cyclohexanone concentrations exceeded 500 mg/m^3.

Under chronic action conditions, the concentrations were also high: for cyclohexanol, 400 mg/m^3, and for cyclohexanone, 100 mg/m^3 (G. V. Lobovikova) and 2000 mg/m^3 (A. P. Voronin).

The influence of cyclohexanol and cyclohexanone on the higher nervous activity of animals has been the subject of several studies. Lobovikova established that cyclohexanone vapors in 100-400 mg/m^3 concentration under conditions of chronic administration and with an exposure of 3-6 hours by inhalation caused changes in the conditioned reflex activity of white rats.

Voronin describes disturbances of the conditioned reflex activity of rabbits (the study was made by using Ye. I. Lyublina's method) with an average cyclohexanone concentration of 4000 mg/m^3.

V. A. Savelova, A. S. Bruk and N. V. Klimkina (1962) observed that the threshold dose in the study by the conditioned reflex method (rats) was 0.04 mg/kg of cyclohexanol.

Foreign authors (Treon et al.) believe cyclohexanol to be more toxic than cyclohexanone.

Lobovikova studied the toxicity of these substances when administered via the gastrointestinal tract. She established that cyclohexanone causes intoxication symptoms in lower doses.

The maximum permissible concentration for plant sections was determined to be 10 mg/m^3 for cyclohexanone and was not determined for cyclohexanol.

We have been unable to find any data on the contamination of atmospheric air by these substances in the domestic or foreign literature.

O. M. Gavruseyko and A. S. Maslennikov (1950) in a survey of production department of a caprolactam plant observed concentrations of cyclohexanone from 1.5 to 10 mg/m^3 and cyclohexanol from 0.4 to 2.8 mg/m^3 in working areas in the dehydrogenation section, and up to 14 mg/m^3 of cyclohexanone and up to 16 mg/m^3 of cyclohexanol in the fractionation section. These concentrations caused intoxication symptoms in the workers. Headache was the most frequent complaint, and various autonomic disturbances were noted. The

prime objective symptom was a tendency to develop hypochromic anemia (cited by O. M. Gavruseyko and A. S. Maslennikov). The authors ascribe the principal effect to cyclohexanone.

Thus, there are data on the toxic effect of relatively high concentrations of cyclohexanol and cyclohexanone in both the domestic and foreign literature. The investigations were carried out only in acute experiments or under conditions of chronic action with exposure lasting up to 8 hours.

We have not found in the literature any data on the thresholds of action of these substances, which could have been used for validating their maximum permissible concentrations. Concentrations of the substances at the level of those studied are lacking not only for atmospheric air but also for the air of industrial buildings.

All of this prompted us to carry out studies that would permit the validation of the maximum permissible concentrations of cyclohexanol and cyclohexanone in atmospheric air. Colorimetric methods proposed by A. S. Maslennikov (1960) were used in our studies for determining cyclohexanol and cyclohexanone in air.

The method of determination of cyclohexanone is based on its condensation with furfural and the formation of furfurylidenecyclohexanone. In an acid medium, the compound turns a crimson color. The sensitivity of the method is 2 μg/ml.

The method of determination of cyclohexanol is based on its reaction with p-dimethylaminobenzaldehyde. In the presence of sulfuric acid, the compound colors the solution a cherry red. The sensitivity of the method is 5 μg/ml. The methods are nonspecific, designed for the determination of these substances when the latter are separately present in air. However, in the experimental validation of the maximum permissible concentrations, the work was done with each substance individually.

In view of the inadequate sensitivity of the methods described, they were modified under the direction of M. V. Alekseyeva. A spectrophotometric determination was worked out in the visible region of the spectrum with an absorption maximum for cyclohexanol at a wavelength of 510 mμ, and for cyclohexanone at 550 mμ, which made it possible to increase the sensitivity of the methods of determination substantially (to 0.05 μg/ml for cyclohexanone and to 0.4 μg/ml for cyclohexanol).

This increase in sensitivity was achieved by measuring the optical density of the solutions in special glass cells with a length of 42 mm and by modifying the method of collection of air samples.

In the collection of air samples for cyclohexanone, the absorbing solution used consisted of purified distilled ethyl alcohol mixed with distilled water in the proportion of 1:2 in the amount of 3 ml. Absorbers with porous plate No. 2 placed in melting ice were employed. At a rate of sample collection of 0.5 l/min, no breakthrough into the second absorber occurred within the sensitivity limits of the method.

During the collection of air samples for cyclohexanol, the conditions were the same, but the amount of the absorbing solution was reduced to 3 ml.

In substantiating the highest single maximum permissible concentrations of cyclohexanol and cyclohexanone, we used the methods recommended by the Committee on Sanitary Protection of Atmospheric Air. The thresholds of olfactory sensation of the two substances, and the reflex change of the light sensitivity of the eyes under the influence of low cyclohexanone concentrations were determined, and the influence of low concentrations of both substances on the electrical activity of the brain was studied by the method of reinforcement of the intrinsic brain potentials and by the method of development of the electrocortical conditioned reflex.

The threshold of olfactory sensation was determined for cyclohexanol in 22, and for cyclohexanone in 15 practically healthy persons. Results of the determination are listed in Table 2.

Table 2

Thresholds of Olfactory Sensation for Cyclohexanol and Cyclohexanone.

Number of Subjects	Concentration, mg/m³	
	Minimum Perceptible	Imperceptible
	Cyclohexanol	
1	3,26	1,71
9	0,66	0,54
8	0,52	0,35
3	0,35	0,25
1	0,24	0,20
	Cyclohexanone	
2	3,0	1,21
3	1,19	0,80
1	0,77	0,41
6	0,37	0,23
3	0,21	0,14

It is evident from Table 2 that the threshold for the most sensitive persons is a vapor concentration of 0.24 mg/m^3 for cyclohexanol and 0.21 mg/m^3 for cyclohexanone. Concentrations whose odor was imperceptible were 0.20 mg/m^3 for cyclohexanol and 0.14 mg/m^3 for cyclohexanone. In order to substantiate the thresholds of the reflex effect on the functional state of the nervous system by the method of dark adaptation, considering the similarity of the thresholds of olfactory sensation of the two substances, we decided to experiment with cyclohexanone only, which has the lower threshold of olfactory sensation.

Three people participated in the study. The threshold of olfactory sensation in one of them was 0.21 mg/m^3, and in the other two, 0.37 mg/m^3. The investigations were carried out with an ADM adaptometer by using a standard procedure. The active concentrations were considered to be those at which the difference of the average values of the light sensitivity during inhalation of pure air and of the gas concentration studied was statistically reliable (Table 3).

Table 3

Light Sensitivity of the Eyes in the 20th and 25th minutes of Adaptation During Inhalation of Cyclohexanone Vapors in Percent of 15th Minute.

Subject	Minute of Examination	Pure Air	Cyclohexanone Concentration, mg/m^3			
			0.11	t	0.06	t
L.	20-я	119,3	132,0	4,57	116,0	0,7
	25-я	163,1	151,0	1,2	152,3	1,1
B.	20-я	128,5	148,3	3,24	131,3	0,6
	25-я	197,0	196,0	0,2	197,6	0,3
K.	20-я	125,0	143,0	4,3	126,3	0,6
	25-я	148,4	167,0	0,4	145,3	1,3

Table 3 shows that statistically reliable changes in the light sensitivity of the eyes occurred only in the 20th minute of dark adaptation, i.e., immediately following the inhalation of the gas, at a cyclohexanone concentration of 0.11 mg/m^3. This cyclohexanone concentration was found to be the threshold value according to the method employed. A concentration of 0.06 mg/m^3 was found to be inactive.

In order to substantiate the highest single maximum permissible concentrations of the various chemical substances in atmospheric air, a number of researchers (K. A. Bushtuyeva, Ye. F. Polezhayev, A. D. Semenenko, 1960; V. A. Gofmekler, 1960; Yu. G. Fel'dman, 1960; Li Sheng, 1961; G. I. Solomin, 1961; R. U. Ubaydullayev, 1962; B. M. Mukhitov, 1962; D. G. Odoshashvili, 1963, and others) successfully used the method of development of the

electrocortical conditioned reflex. Low concentrations of the chemical substances which had no subjective influence on the olfactory system caused distinct changes in the electrical activity of the brain.

Of late, there has been an increasing emphasis in electrophysiology on a trend dealing with the bioelectric indicators under conditions where a functional load is applied to the brain. The methods used are those of rhythmic stimulation (V. Ye. Mayorchik and B. G. Spirin, 1951; N. I. Zislina, 1955; A. G. Kopylov, 1956, 1960, and others), and also the method of change in the electrical activity of the brain during the action of a rhythmic photic stimulus of increasing brightness ("responsiveness curves," M. N. Livanov, 1944). In these tests, the characteristics of the encephalographic responses to stimuli are used to evaluate the indexes of the condition of the brain.

The method of electroencephalography with functional loading as applied to the determination of permissible concentrations of atmospheric pollutants was developed by A. D. Semenenko (1963) and used by a number of researchers (N. B. Imasheva, 1963; T. G. Tkachev, 1963; V. A. Chizhikov, 1963).

To elucidate the influence of low concentrations of cyclohexanol and cyclohexanone on the electrical activity of the brain, we used two methods: the method of development of the electrocortical conditioned reflex, and the method of electroencephalography with functional loading. The electroencephalographic studies were made with a Kaiser 8-channel electroencephalograph with an electrophotostimulator.

During the examination, the subject was placed in a state of relative rest in a special electrically shielded room equipped for these purposes. During the devebopment of the electrocortical conditioned reflex, the unconditioned stimulus was light causing desynchronization of the α rhythm, and the conditioned stimulus was the inhalation of various concentrations of cyclohexanol and cyclohexanone. The gas was supplied to the subject for 15 seconds, and the last five seconds were reinforced with light. The conditioned reflex was considered formed and the concentration of the gas active if desynchronization of the α rhythm occurred after a series of pairings before the light was turned on.

In the second method, the electrical activity of the subject's brain was recorded during exposure to intermittant light of changing intensity. The intensity of the flickering light changed every five seconds within a range from 0.1 to 0.6 Joule over the course of 25 seconds of recording of the reinforced rhythm curve. The reinforced rhythm curves were recorded every minute. After the third minute of the examination, a given concentration of the gas studied was mixed in with the pure air fed into the cylinder and breathed by the subject. After the sixth minute, the gas supply was discontinued, and a period of close and distant aftereffect was

observed. Usually, 11-12 reinforced rhythm curves were recorded. Thus, the entire time of the study was divided into four periods: I - background, II - action of the gas, III - close aftereffect, IV - distant aftereffect. Each period lasted 3 minutes.

Each curve was analyzed from the change of the amplitude of the intrinsic rhythm. For convenience in carrying out the comparative analysis, the total amplitude for all the intensities was expressed in percent. The average amplitude of the first three reinforced rhythm curves was taken as 100%.

The amplitude of reinforced rhythm in the gas experiment was compared with the amplitude of assimilated rhythm in experiments with pure air. Data for different periods of the study were also compared. The results were subjected to statistical treatment. The tests with cyclohexanone involved six people and those with cyclohexanol, five people. The results are given in Tables 4 and 5.

Table 4

Effect of Cyclohexanol on the Electrical Activity of the Brain.

Subject	Method	Concentration, mg/m^3			
		0.2	0.14	0.11	0.06
V.	Rhythm Reinforcement	+	—	—	0
T.	» »	+	—	—	0
S.	» »	+	0	—	0
L.	Conditioned Reflex	0	+	+	—
M.	» »	0	+	+	—

Notation: + action; - absence of action; 0 - not studied.

Table 5

Effect of Cyclohexanone on the Electrical Activity of the Brain.

Subject	Method	Concentration, mg/m^3		
		0.09	0.06	0.04
[illegible]	Rhythm Reinforcement	+	—	0
N.	» »	—	0	0
V.	» »	+	—	0
R.	» »	+	—	0
L.	Conditioned Reflex	0	+	—
M.	» »	0	+	—

Notation same as in Table 4.

In the study of the influence of cyclohexanol vapor inhalation on the change of the electrical activity of the brain, concentrations of 0.2, 0.14, 0.11 and 0.06 mg/m^3 were investigated.

In the study by the method of reinforcement of intrinsic brain potentials, a gas concentration of 0.2 mg/m^3 was found to be the threshold value for three people. In two out of three subjects, the gas in this concentration caused a decrease (Fig. 1) and in one person, an increase in the amplitude of the imposed rhythm. In 0.11 mg/m^3 concentration, the gas did not cause any change in the electrical activity of the brain in any of the subjects.

In the study with the method of development of the electrocortical conditioned reflex, a cyclohexanol concentration of 0.11 mg/m^3 was found to be the threshold value in both subjects. A concentration of 0.06 mg/m^3 was inactive.

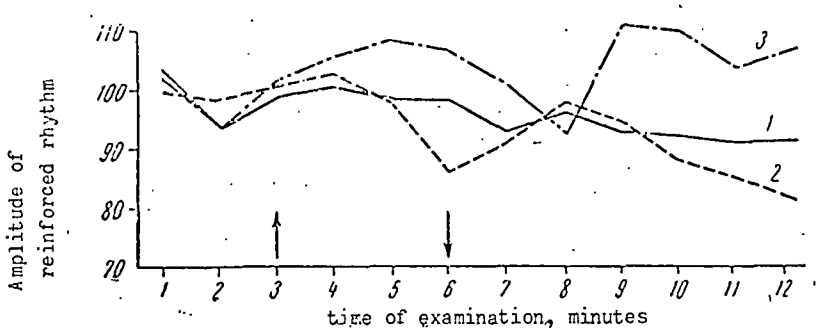

Fig. 1. Effect of cyclohexanol on the electrical activity of the brain in subject V.
1 - pure air; 2 - concentration, 0.11 mg/m^3; 3 - 0.14 mg/m^3; 4 - 0.20 mg/m^3.

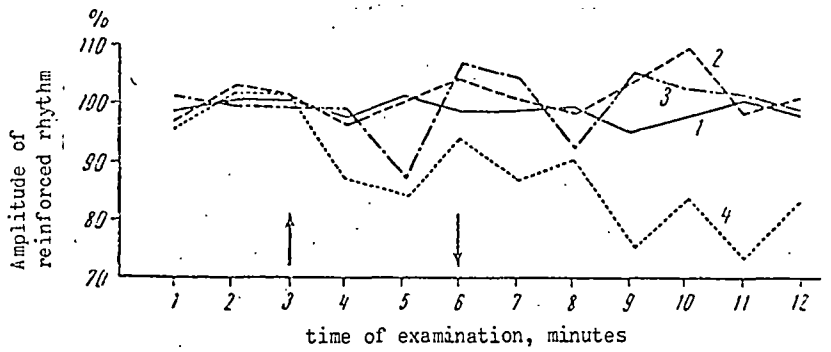

Fig. 2. Effect of cyclohexanone on the electrical activity of the brain in subject I.
1 - pure air; 2 - concentration, 0.06 mg/m^3; 3 - 0.09 mg/m^3.

In the study of the influence of inhalation of cyclohexanone vapors on the electrical activity of the brain, three concentrations were investigated: 0.09, 0.06 and 0.04 mg/m^3. In the investigation by the method of reinforcement of intrinsic brain potentials, a concentration of 0.09 mg/m^3 was found to be the threshold value for three people. In two persons this concentration caused a decrease and in one, an increase (Fig. 2) in the amplitude of the imposed rhythm. The 0.09 mg/m^3 gas concentration was found to be inactive for one subject.

Cyclohexanone in 0.06 mg/m^3 concentration caused no change in the electrical activity of the brain in any of the subjects. The electrocortical conditioned reflex was formed with the 0.06 mg/m^3 concentration in both subjects. In this method, a gas concentration of 0.04 mg/m^3 was found to be inactive.

Table 6

Thresholds of Action of Cyclohexanol and Cyclohexanone on Man.

Substance	Olfactory Sensation		Light Sensitivity of the Eye		Electroencephalography				Highest Single Maximum Permissible Concentration, mg/m^3
					Rhythm Reinforcement		Conditioned Reflex		
	Threshold	Subthreshold	Threshold	Subthreshold	Threshold	Subthreshold	Threshold	Subthreshold	
Cyclohexanol	0.24	0.20	Not Determined	Not Determined	0.20	0.14	0.11	0.06	0.06
Cyclohexanone	0.21	0.14	0.11	0.06	0.09	0.06	0.06	0.04	0.04

Thus, the concentrations of 0.06 mg/m^3 of cyclohexanol and 0.04 mg/m^3 of cyclohexanone did not cause a change in the electrical activity of the brain in any of the subjects. Comparative data on the thresholds of action of the two substances are listed in Table 6.

Conclusions

1. The threshold of olfactory sensation in the most sensitive persons is 0.24 mg/m^3 for cyclohexanol and 0.21 mg/m^3 for cyclohexanone.

2. The threshold of change in the light sensitivity of the eyes during inhalation of cyclohexanone vapors lies at a level of 0.11 mg/m^3.

3. The threshold of the reflex effect on the electrical activity of the brain for cyclohexanol lies at a level of 0.11 mg/m^3 and for cyclohexanone

at a level of 0.06 mg/m^3.

4. Both substances caused a change in the electrical activity of the brain in lower concentrations when studied by the method of development of the electrocortical conditioned reflex than when studied by the method of reinforcement of intrinsic brain potentials.

5. Cyclohexanone has lower thresholds of olfactory sensation and of effect on the electrical activity of the brain.

6. The highest single maximum permissible concentrations in atmospheric air which we recommend are 0.04 mg/m^3 for cyclohexanone and 0.06 mg/m^3 for cyclohexanol.

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ON THE COMBINED ACTION OF THREE MINERAL ACIDS

Candidate of Medical Sciences V. P. Melekhina
Department of Communal Hygiene, Central Institute for Advanced Training of Physicians

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The study of the combined action of various environmental factors has attracted the attention of many hygienists. The necessity of studies of this type has been emphasized in resolutions of the 13th and 14th congresses of hygienists.

In recent years, in connection with the rapid growth of the chemical industry in the Soviet Union, studies in the area of hygienic standardization of a series of combinations of atmospheric pollutants have been considerably expanded. The combinations of sulfur dioxide and sulfuric acid aerosol (K. A. Bushtuyeva, 1961), chlorine and hydrogen chloride (V. M. Styazhkin, 1962), and carbon disulfide and hydrogen sulfide (B. K. Baykov, 1963) have been studied. The authors showed that in the range of low concentrations which may occur in the air of populated areas, the character of the action of two atmospheric pollutants is determined as a total or partial summation. However, the insufficient number of studies in this area does not yet permit one to state that any combination of atmospheric pollutants will be characterized by an additive synergism.

The object of the present study was to establish the nature of the combined action of three mineral acids - sulfuric, nitric and hydrochloric. The study of this combination is of practical importance, since the source of their joint discharge into the atmosphere are chemical complexes and inorganic fertilizer plants whose number and capacity (particularly the fertilizer plants) are increasing considerably in the Soviet Union. The combination under consideration is also of major scientific interest, since all three acids are strong electrolytes and in dilute solutions dissociate completely into hydrogen ions and the corresponding acid residue. The more dilute the acid, the less its specific properties are manifested and the more manifest are the properties common to all the acids, due to the hydrogen ions. In atmospheric air at low concentrations of these acids, we encountered those properties of the acids which are characteristic of dilute solutions. In this connection, the question arises whether mineral acids have an effect due solely to the hydrogen ion and whether they should therefore be standardized on the basis of this indicator alone. For example, can the concentration of acids be expressed in milligrams of hydrogen ion per m^3 of air? If one could obtain a confirmation of this hypothesis, the control of the purity of atmospheric air in populated areas would be considerably simplified.

To achieve the stated objective, we used methods widely employed in the hygienic standardization of maximum permissible concentrations of atmospheric pollutants. These were the methods of determination of the threshold of olfactory sensation and the very sensitive method of dark adaptation.

The experiments were conducted by using a procedure recommended by the Committee on the Sanitary Protection of Atmospheric Air.

In determining the precise concentrations of the volatile acids in the experiment, we could not use their determination from the corresponding anions, since in addition to the anions of these acids, anions of their corresponding salts are also present in air and are therefore determined. On the other hand, we found it impossible to use the microtitrimetric method of determination of hydrochloric acid (M. V. Alekseyeva, 1963) because of its inadequate sensitivity. For this reason, in collaboration with Candidate of Biological Sciences M. D. Manita, we worked out a spectrophotometric method of determination of mineral acids in the presence of their salts. The method is based on the measurement of the optical density of colored aqueous solutions of the acids in the presence of a 0.01% alcohol solution of methyl red. The color depends on the pH of these solutions in the range from 5.3 to 4.3, which corresponds to a concentration of hydrochloric acid from 0.18 to 1.83 μg/ml, nitric acid from 0.31 to 3.15 μg/ml, and sulfuric acid from 0.24 to 2.4 μg/ml. The sensitivity of the determination of hydrochloric acid was 0.18 μg/ml, sulfuric acid 0.24 μg/ml, and nitric acid 0.31 μg/ml.

Other acids and alkalis interfere with the determination. Sulfuric acid in amounts up to 1 mg/m^3 and carbon dioxide in amounts up to 0.05 vol. % do not interfere.

Optimum conditions were selected for the spectrophotometric analysis: the aqueous solutions of the acid were determined in the visible region of the spectrum at γ_{max} = 530 mμ in a cell with a thickness of 1 cm. A calibration graph was plotted for each acid.

The samples were collected by using a procedure recommended by M. V. Alekseyeva, i.e., into double-distilled water in a V-shaped absorber with a porous plate at a rate of 0.5-1 l/min.

In starting the work we considered the fact that sulfuric and hydrochloric acids had been standardized for the atmosphere of populated areas (0.3 and 0.05 mg/m^3). We had to check the data obtained earlier and in the case of discrepancies, to introduce corrections. The influence of low concentrations of nitric acid had not been studied and its maximum permissible concentration for atmospheric air had not been established.

We began the study of the effect of low concentrations of the acids on the human organism with the determination of their thresholds of olfactory sensation. The observations were made on 16 people 17 to 36 years of age. A total of over 800 tests were conducted (including 415 for the determination of the threshold of olfactory sensation of hydrochloric acid, 243 for nitric acid, and 148 for sulfuric acid). Results of the determination are listed in Table 1.

Thus, the thresholds of olfactory sensation for the most sensitive persons were found to be 0.70 mg/m^3 for nitric acid, 0.39 mg/m^3 for hydrochloric acid, and 0.60 mg/m^3 for sulfuric acid.

Table 1

Thresholds of Olfactory Sensation of Mineral Acids.

Number of Subjects	Threshold Concentrations		Subthreshold Concentrations	
	mg/m^3	H^+, mg/m^3	mg/m^3	H^+, mg/m^3
		Nitric Acid		
1	0,70	0,01099	0,60	0,00942
3	0,80	0,01256	0,70	0,01099
7	0,85	0,01334	0,70	0,01099
1	0,90	0,01413	0,85	0,01334
1	1,07	0,01679	0,85	0,01334
1	1,25	0,01962	1,07	0,01679
1	1,50	0,02355	1,40	0,02198
		Hydrochloric Acid		
3	0,39	0,01053	0,30	0,0081
7	0,40	0,01080	0,32	0,0086
3	0,45	0,01215	0,40	0,0108
		Sulfuric Acid		
2	0,60	0,0120	0,43	0,0096
8	0,74	0,0148	0,60	0,0120
1	0,81	0,0162	0,74	0,0148

The thresholds were equal in hydrogen ion concentrations: for nitric acid - 0.01099 mg/m^3, for hydrochloric acid - 0.01053 mg/m^3, and for sulfuric acid - 0.0120 mg/m^3. As is evident from the data cited, the figures defining the thresholds of olfactory sensation in hydrogen ion concentrations are rather close to each other.

The subthreshold concentrations of the acids were found to be as follows: for nitric acid 0.60 mg/m^3, for hydrochloric acid 0.30 mg/m^3, and for sulfuric acid 0.43 mg/m^3; in hydrogen ion concentrations this corresponds to 0.00942, 0.0081, and 0.0096 mg/m^3. Essentially, it may be said that these figures are of the same order.

The results of our studies differ from the data on the threshold of olfactory sensation of hydrochloric acid obtained earlier by Ye. V. Yelfimova. Unfortunately, we do not know the reason for this, but we can surmise that a factor is our use of a more sensitive method of determination of the acids.

The threshold concentrations of olfactory sensation of sulfuric acid are essentially no different from the existing literature data (K. A. Bushtuyeva, 1961). Having determined the thresholds of olfactory sensation separately for each acid, we undertook the determination of the thresholds in different combinations. Initially, we studied the threshold of olfactory sensation of a combination of the two acids - nitric and hydrochloric. Twelve people participated in the experiments. A total of 86 determinations were made. Nine combinations were studied. The results are given in Table 2. The concentrations of the acids are expressed in fractions of the threshold concentrations for each acid observed and are summed up.

All the perceptible concentrations in terms of the hydrogen ions were above 0.01 mg/m^3. All the imperceptible combinations in terms of the hydrogen ion were found to be below 0.01 mg/m^3. Analyzing the data from the standpoint of the combined action of the acids we can state that we are dealing with a simple summation.

Table 2

Thresholds of Olfactory Sensation of the Sum of the Two Acids (Nitric and Hydrochloric).

Subject	Threshold Concentrations		Subthreshold Concentrations	
	Sum of Fractions	Sum of Acids in H^+(mg/m^3)	Sum of Fractions	Sum of Acids in H^+(mg/m^3)
N.	1,17	0,01764	0,86	0,008363
M.	1,06	0,01204	0,76	0,008363
S.	1,06	0,01283	0,98	0,012040
G.	0,97	0,01283	0,69	0,008363
E.	0,99	0,01283	0,76	0,008363
T.	0,99	0,01204	0,76	0,008363
B.	1,0	0,01283	0,93	0,008363
K.	1,14	0,01332	0,71	0,008465
L.	1,10	0,01283	0,71	0,008465
Yu.	0,99	0,01204	0,76	0,008363
B.	1,0	0,01204	0,77	0,008363
A.	0,99	0,01282	0,69	0,008363

The olfactory sensation threshold of the sum of the three acids was determined on nine people. A total of 83 observations were made and ten pairings of different combinations of the three acids were studied. The results are given in Table 3.

Table 3

Olfactory Sensation Threshold of the Sum of the Three Acids.

Subject	Threshold Combinations		Subthreshold Combinations	
	Sum of Fractions	Sum of Acids in H^+ (mg/m^3)	Sum of Fractions	Sum of Acids in H^+ (mg/m^3)
N.	0,98	0,01103	0,96	0,01101
M.	1,14	0,01555	0,93	0,01187
S.	1,14	0,01555	0,93	0,01187
Ch.	1,10	0,01565	0,78	0,01103
E.	1,06	0,01555	0,88	0,01187
T.	1,13	0,01555	0,81	0,01103
B.	1,01	0,01268	0,88	0,01103
K.	1,16	0,01573	0,96	0,01187
L.	0,99	0,01268	0,94	0,00870

It is clear from Table 3 that a perceptible combination of acids was that in which the sum of the fractions of threshold concentrations exceeded unity. All the imperceptible combinations were those in which the sum was less than unity. On the basis of the data obtained it may be concluded that the threshold concentration of the sum of the three acids for the most sensitive persons in terms of the hydrogen ion is 0.01103 mg/m^3.

Thus, the threshold concentrations obtained, expressed in hydrogen ions of the individual acids, as well as of combinations of two and three acids, are almost at the same level. This is to say, the principal physiological effect in terms of the threshold of olfactory sensation is due to the concentration of hydrogen ions in the solutions of these acids.

The next stage of the present study consisted in investigating the reflex effect of the mineral acids on the light sensitivity of the eye. The tests were conducted on three people, 17, 22 and 32 years old.

The thresholds of olfactory sensation of all three acids were determined for two subjects, and that of sulfuric acid only was determined for the third subject (Table 4).

The investigations of dark adaptation were started with the determination of the individual thresholds for each acid separately, and then for their mixture. The estimate of the influence of the total acids was also expressed in fractions of the threshold values. The gas was inhaled starting with the 15th minute of adaptation for 4 1/2 minutes. As a result of the study, the thresholds listed in Table 6 were established.

Table 4

Thresholds of Olfactory Sensation of Acids in Persons Participating in Adaptometric Tests.

Subject	Threshold Concentrations of Acids					
	Nitric		Hydrochloric		Sulfuric	
	mg/m³	H^+, mg/m³	mg/m³	H^+, mg/m³	mg/m³	H^+, mg/m³
T.	0.85	0.01334	0.40	0.0108	0.74	0.0148
K.	0.80	0.01256	0.40	0.0108	0.74	0.0148
R.	-	--	-	--	0.73	0.0146

Comparing the results of the determination of the thresholds of olfactory sensation and thresholds of the reflex effect (Tables 4 and 5), one can observe that they are essentially quite similar. In this respect our results are in complete agreement with the data obtained earlier in a study of the acids by other authors (K. A. Bushtuyeva, 1961; Ye. V. Yelfimova, 1962; V. M. Styazhkin, 1962).

Table 5

Threshold Concentrations of Acids Determined by the Dark Adaptation Method.

Subject	Concentrations of Acids					
	Nitric		Hydrochloric		Sulfuric	
	mg/m³	H^+, mg/m³	mg/m³	H^+, mg/m³	mg/m³	H^+, mg/m³
T.	0,86 (c)	0,0135	0,40 (b)	0,0108	0,80 (c)	0,0160
K.	0,85 (a)	0,0133	0,40 (a)	0,0108	0,81 (b)	0,0161
R.	0,84 (b)	0,0131	0,42 (b)	0,0113	0,73 (b)	0,0146

Note. Confidence factor: a - 95%, b - 99%, c - 99.9%.

We then studied the influence of inhalation of different combinations of the three acids on the dark adaptation. The effect was estimated in the same manner as was indicated in the determination of the threshold of olfactory sensation. The data obtained are listed in Table 6.

The active combination of acids was found to be the one in which the sum of the fractions of the threshold concentrations exceeded unity.

Table 6

Effect of Inhalation of the Sum of the Three Acids on Dark Adaptation in the 20th Minute of the Experiment.

Subject	Sum of Acid Fractions of Threshold Values > 1		Sum of Acid Fractions of Threshold Values < 1	
	Light Sensitivity in Percent of Background	Concentration of Sum of Acids in H^+, mg/m^3	Light Sensitivity in Percent of Background	Concentration of Sum of Acids in H^+, mg/m^3
T.	130 (a)	0.02070	117.5	0.01057
R.	78 (c)	0.02018	94.4	0.01158
K.	140.4 (p)	0.01908	97	0.00625

Note. Confidence factor: a - 95%, b - 99%, c - 99.9%.

Conclusions

1. The established thresholds of olfactory sensation of nitric, sulfuric and hydrochloric acids are respectively equal to 0.70, 0.60 and 0.40 mg/m^3, which in terms of the hydrogen ion per cubic meter corresponds to 0.01099, 0.01203 and 0.01053 mg/m^3.

2. A mixture of two and three acids is perceived when the sum of the fractions of their threshold values exceeds unity. The perceptible concentrations expressed in hydrogen ions per cubic meter are above 0.01 mg/m^3.

3. The threshold concentrations of the reflex effect, studied by the dark adaptation method, lie at the level of the threshold values, or slightly above, for olfactory sensation. The combined action manifests itself as a type of additive synergism.

4. Our data are in good agreement with the assumption that the reflex effect of mineral acids (nitric, sulfuric and hydrochloric) in the atmospheric air of populated areas is determined by the concentration of the hydrogen ions.

5. The results of the studies make it possible to propose that the contamination of atmospheric air by the mineral acids which we studied be evaluated on the basis of the concentration of hydrogen ions alone, independently of the anionic residue of the acid.

6. The highest single maximum permissible concentration of the sum of these acids and separately for each acid in terms of the hydrogen ion should not exceed 0.010 mg/m^3.

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TOXICITY OF SULFUR OXIDES UNDER CONDITIONS OF LONG-TERM CONTINUOUS EXPOSURE

Docent K. A. Bushtuyeva
Department of Communal Hygiene,
Central Institute for Advanced Training of Physicians

From "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Pod redaktsiey Prof. V. A. Ryazanova i Prof. M. S. Gol'dberga. Izdatel'stvo "Meditsina" Moskva, p. 142-172, (1966).

This paper presents materials and results of studies with various concentrations of sulfur dioxide, sulfuric acid aerosol, and their combinations under conditions of extended continuous exposure. The studies were made on white rats in seven series of experiments. A total of 120 animals, with 15 in each series and the control group, were used. The sulfuric acid aerosol used in our experiments had a particle size of less than 2 μ. The following tests were utilized to study the resorptive effect of sulfur oxides: behavior and weight of the animals, ratio of chronaxias of antagonistic muscles, whole blood cholinesterase, serum protein fractions, content of coproporphyrins in the animals' urine, and pathomorphological studies following the completion of exposure.

Tests with sulfur dioxide. The toxicity of sulfur dioxide has been dealt with in many studies, but the majority of the latter cannot be discussed in this present paper, since they deal with very high concentrations. They will therefore be omitted here.

We studied the resorptive effect of sulfur dioxide on white rats in two series of experiments under conditions of continuous exposure in the course of 65 days. The actual concentrations for each series were ($M \pm \sigma$) in series I, 4.86 ± 1.26 mg/m^3, and in series II, 8.53 ± 2.1 mg/m^3. The air samples were taken from the chambers and analyzed daily. The indicated sulfur dioxide concentrations did not affect the behavior or weight of the animals.

The chronaxia of the antagonist muscles of the right hind limb was examined every ten days according to the usual procedure with the aid of an ISE-01 electronic stimualtor. This method has been widely adopted in the practice of hygienic studies for the substantiation of maximum permissible concentrations (R. V. Borisenkova, 1954; Chzhao Chzhen-tsi, 1959; V. A. Gofmekler, 1961; Yu. G. Fel'dman, 1962; V. A. Chizhikov, 1963, and others) as the method for studying the state of the central nervous system.

Data on the ratio of chronaxias of extensors to flexors are presented

in Fig. 1. As we know, the normal ratio, which is a relatively constant value ranging from 1.15 to 1.30, does not always remain greater than unity. Under the influence of various factors, these normal ratios may change, and in the presence of deep shifts, become inverted and fall below unity.

As is evident from Fig. 1, sulfur dioxide in the concentrations studied affects the central nervous system. The changes in the presence of the 8.53 mg/m^3 sulfur dioxide concentration were more pronounced, more constant, and statistically more reliable. Recovery of the initial ratio was observed only 17 days after the completion of exposure. At a lower concentration (4.86 mg/m^3), the changes were less constant and rapidly returned to the initial values after the exposure was discontinued.

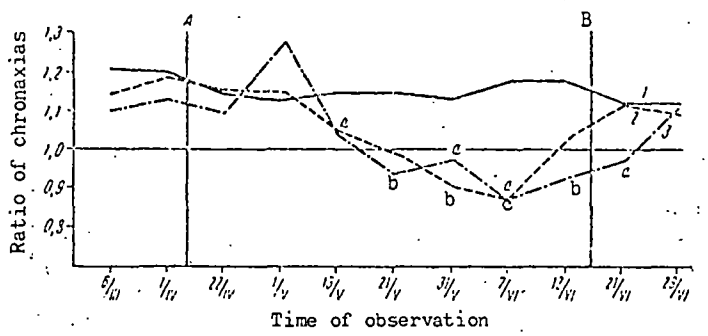


Fig. 1. Ratio of chronaxias of antagonist muscles in animals exposed to sulfur dioxide.
AB - period of exposure; 1 - control group, 2 - series I (4.86 mg/m^3); 3 - series II (8.53 mg/m^3); a, b, c - confidence factor of the changes (respectively 95, 99 and 99.9%).

We studied the activity of whole blood cholinesterase by Pokrovskiy's method (1953) modified by Martynova (1957). Recently, this method has also been widely adopted in studies of the influence of chemical substances, and has proven highly sensitive and reliable (G. I. Solomin, 1961; R. Ubaydullayev, 1961, and others). The cholinesterase activity was determined in five rats of each series and control group at 15-day intervals (Table 1).

A decrease in the time of decomposition of acetylcholine chloride and hence, an increase in cholinesterase activity was observed in both series as early as the first determination following the start of exposure, i.e., on the 13th day. This increase grew during the exposure and reached its highest values toward its end. The recovery of cholinesterase activity was gradual and reached the background values only on the 17th day following the end of exposure.

Table 1

Time of Decomposition of Acetylcholine Chloride of Whole Blood of Rats Exposed to Sulfur Dioxide.

Date of Investigation		Concentration, mg/m³ series I 4.86	series II 8.53
Background on the basis of 17 determinations		41,1	41,4
Exposure 23/IV		35,6 (a)	32,0 (c)
7/V		35,4 (c)	32,6 (c)
21/V		31,8 (c)	25,7 (c)
4/VI		25,8 (c)	24,2 (c)
Recovery period	18/VI	38,8 (a)	36,2 (c)
	29/VI	40,4	40,6

Note. In Table 1 and ff., the confidence factor of the changes is: a - 95%, b - 99%, c - 99.9% as compared with the background.

The protein fractions of the blood serum were determined by paper electrophoresis followed by recording of the phoregrams on photographic paper and weighing of the sections of individual fractions. The percent content of each fraction was determined from the weight. The determination was made every 20 days in five rats of each group (Table 2).

Table 2

Protein Fractions of the Blood Serum in Percent During Exposure to Sulfur Dioxide.

Period of Observation	Fraction	Concentration, mg/m³ Control	Series I 4.86	Series II 8.53
Background	Albumins	40,26	40,26	40,26
	α - Globulins	18,1	18,1	18,1
	β - Globulins	24,1	24,1	24,1
	γ - Globulins	17,6	17,6	17,6
Exposure	Albumins	40,4	35,8 (b)	37,8
	α - Globulins	17,7	17,5	14,8 (b)
	β - Globulins	25,5	27,0 (b)	28,6 (c)
	γ - Globulins	16,9	19,6 (a)	18,7
Recovery	Albumins	38,2	35,3 (c)	32,2 (b)
	α - Globulins	18,7	19,5	19,4
	β - Globulins	25,6	24,6	26,8
	γ - Globulins	18,4	20,5	21,6

In the control group of animals, there were no reliable changes during any of the periods as compared with the background, whereas under the influence of sulfur dioxide, a decrease of the percent content of albumins and a corresponding increase of the globulin fractions, particularly β globulins, took place.

To evaluate the degree of influence of different sulfur dioxide concentrations on the protein fractions, Table 3 lists data during the exposure and recovery periods in percent of the background concentrations. The table shows that at the 4.86 mg/m^3 concentration changes are observed in the percent content of albumin, β globulin, and γ globulin fractions, whereas at the 8.53 mg/m^3 concentration there is a shift in all the fractions.

Table 3

Change in Protein Fractions Under the Influence of Sulfur Dioxide in Percent of Original Values.

Series	Exposure				Recovery Period			
	Albumins	Globulins			Albumins	Globulins		
		α	β	γ		α	β	γ
I(4,86 mg/m^3)	11,1	0	12,0	11,3	12,4	0	0	0
II(8,53 mg/m^3)	6,2	18,3	18,6	6,2	20,0	0	0	22,7

The last index, which we used to evaluate the effect of sulfur dioxide, was the excretion of coproporphyrins in the urine. As an indicator of the general biological shift in the organism, this method has lately begun to be extensively applied to the substantiation of maximum permissible concentrations of atmospheric pollutants (G. I. Solomin, 1962; B. G. Odoshashyili, 1963; V. A. Chizhikov, 1963; P. G. Tkachev, 1963).

The coproporphyrins were determined in a 24-hour portion of urine collected from five rats of each series every ten days. A quantitative determination was carried out by using a spectrophotometric method (Yu. K. Smirnov, M. I. Gusev, 1956).

The data obtained are shown in Fig. 2, where data on the excretion of coproporphyrins by animals of the control group ($M \pm 3\sigma$) are crosshatched. As can be seen from Fig. 2, the 8.53 mg/m^3 concentration of sulfur dioxide causes an increase in the content of coproporphyrins excreted in the urine as early as four weeks after the start of exposure. At the 4.86 mg/m^3 sulfur dioxide concentration, this increase is already observed 1 1/2 months from the start of exposure. The change in the content of coproporphyrins in both series was found to be unstable, and disappeared on the 9th day after the exposure was discontinued.

Autopsy of animals subjected to the action of sulfur dioxide in 4.86 mg/m^3 concentration immediately after exposure did not show any pathologico-anatomical changes. The internal organs and brain of the rats were subjected to histopathological analysis. Microscopic examination of the lungs of all the rats of this series showed the presence of small foci of interstitial pneumonia and of emphysema in the adjoining areas (Fig. 3), a slight swelling of the collagenous fibers in the adventitia of the blood vessels

and bronchi, and in some cases a catarrhal-desquamative bronchitis. The elastic fibers in the stroma of the lung were evenly distributed over the entire cross section. Substantial changes were observed in the trachea of the animals: desquamation of the mucous membrane, and lumps of mucopurulent content in the lumen of the trachea. Examination of the cerebral cortex revealed a subdued hyperchromatism of individual pyramidal neurons of layer V of the cortex, turbidity of the cytoplasm, and small foci of gliosis. The cerebellum showed angularity and wrinkling of the Purkinje cells. Golgi staining in the top layers of the cortex, primarily in layer III, showed the apical dendrites of certain pyramidal neurons to be devoid of spinelike processes and to have swellings and beaded thickenings. Neurons arranged in rows were unchanged. Such changes were absent in animals sacrificed at the end of the recovery period.

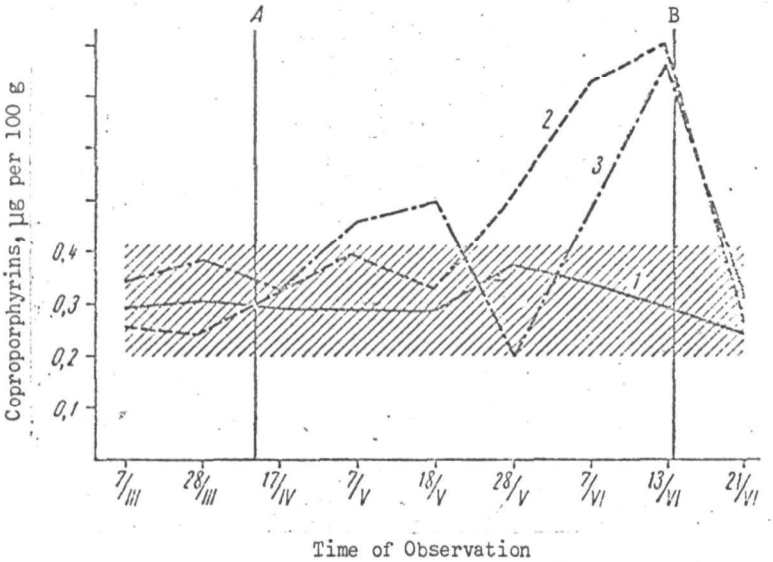


Fig. 2. Content of coproporphyrins in the urine of experimental animals exposed to the action of sulfur dioxide.
Notation same as in Fig. 1.

Autopsy of animals subjected to the action of sulfur dioxide in 8.53 mg/m^3 concentration, sacrificed immediately after the exposure was completed, did not show any macroscopic changes. Small foci of pneumonia and hyperemia of the spleen were found in rats killed at the end of the recovery period.

Microscopic examination of the lungs showed areas of interstitial pneumonia, emphysema in the adjoining areas, and catarrhal-desquamative bronchitis. The elastic fibers in the injured areas of the lung frequently appeared broken, coiled and spiral. The collagenous fibers in the adventitia of the blood vessels and bronchi were swollen. Toward the end of the recovery period, the number of histiocytes filled with the pigment increased. In the

trachea, there was necrosis and desquamation of the mucosa over a large area (Fig. 4), and hyperplasia of the mucosa with papillomatosis. The lumen of the trachea had a purulent content and blood clots. There was hyperplasia of the lymphatic follicles of the submucosa.

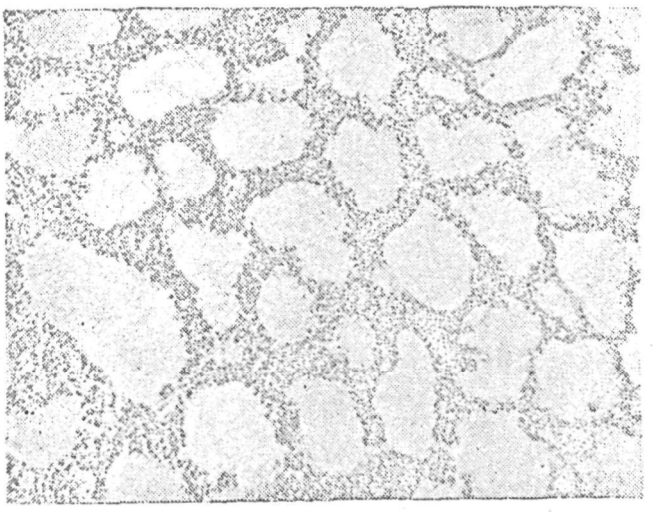


Fig. 3. Lung of white rat No. 5 of series I. Diffuse thickening of interalveolar septa caused by infiltration of cellular elements into them. Staining with hematoxylin-eosin. Magnification 20x5.

The liver had areas of dystrophy, and the heart, fine and isolated blood extravasations in the myocardium. No changes were established in the kidneys or other organs. In the cerebral cortex, the cloudiness of the cytoplasm and hyperchromatism of certain pyramidal neurons of layers II and V of the cortex were noted. There were isolated and small foci of gliosis, and, in the cerebellum, areas of fallout and hyperchromatism of the Purkinje cells. The medulla oblongata had no special characteristics.

When Golgi staining was used, individual neurons whose apical dendrites were devoid of spines and had beaded thickenings and swellings (Fig. 5) were found among the cells of different layers more often than in animals of series I. In animals sacrificed at the end of the recovery period, the changes were considerably evened out.

Studies with sulfuric acid aerosol. The high toxicity of sulfuric acid aerosol as compared with that of sulfur dioxide has been demonstrated by several investigators (Treon et al., 1949; Amdur, 1953, and others).

Our early work (K. A. Bushtuyeva, 1956, 1961) indicates that changes in the pulmonary tissue such as interstitial pneumonia with a change in the content of histamine are observed at a sulfuric acid aerosol concentration of

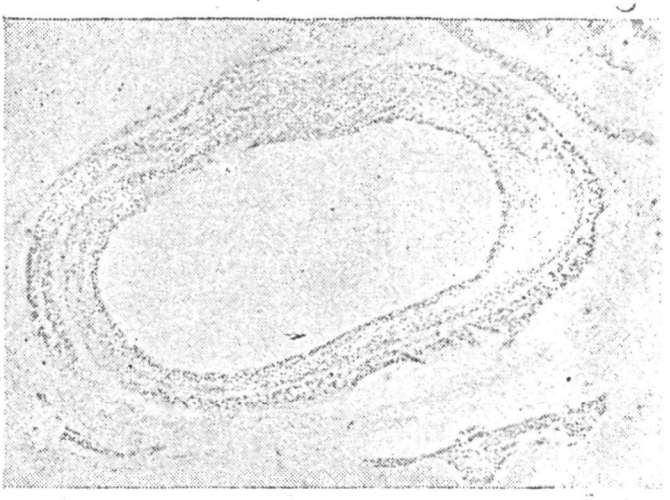


Fig. 4. Trachea of white rat No. 24 from series II. Necrosis and desquamation of mucosa over a large area. Staining with hematoxylin-eosin. Magnification - - magnifying glass.

1 mg/m^3 and after a continuous exposure of 120 hours. When the exposure lasted one month, the initial symptoms of irritation of the pulmonary tissue are also observed at a concentration as low as 0.1 mg/m^3.

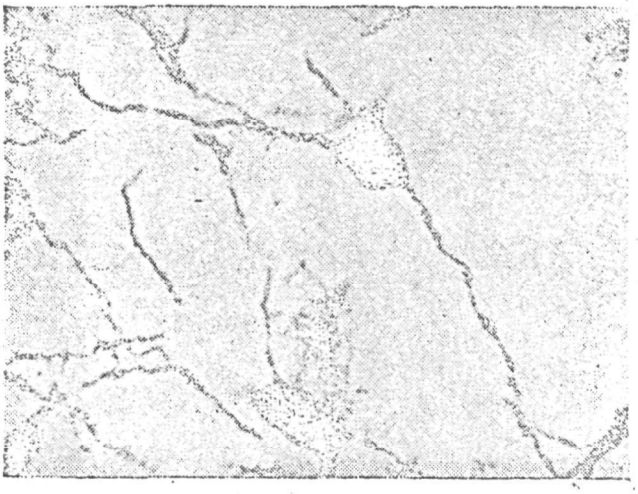


Fig. 5. Brain of rat No. 17 of series II. Apical dendrites of neurons are devoid of spines and have beaded thickenings and swellings. Golgi staining. Magnification 40x10.

The present investigations were conducted in two series on white rats. The actual concentrations of sulfuric acid aerosol in the chambers were 1.04 $\pm$ 0.057 mg/m^3 (series III) and 1.8 $\pm$ 0.162 mg/m^3 (series IV). The same tests as in the preceding series I and II were employed.

Data on the weight of the animals did not show any substantial changes.

Results of the study of the ratio of chronaxies of antagonist muscles for animals of series III and IV are shown in Fig. 6. A disturbance of the normal ratio under the influence of sulfuric acid aerosol occurred in animals of both series only a month after the start of exposure. In animals of series III, it is preferable to speak of a convergence of the chronaxies, and in those of series IV, of an inversion of the normal ratio. Results of the determination of cholinesterase activity in animals of series III and IV are presented in Table 4.

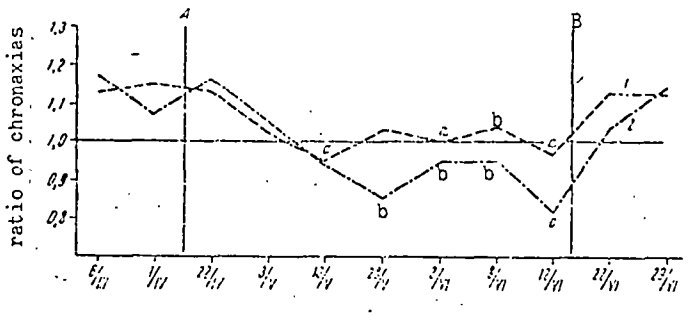


Fig. 6. Ratio of chronaxias of antagonist muscles in animals exposed to sulfuric acid aerosol.
AB - period of exposure; 1 - series III (1.04 mg/m^3); 2 - series IV (1.8 mg/m^3); a, b, c - confidence factor of the changes (95, 99 and 99.9% respectively).

Thus, sulfuric acid aerosol in the concentrations studied, like sulfur dioxide, causes a decrease in the time of decomposition of acetylcholine chloride, i.e., an increase in cholinesterase activity. The recovery of the activity took place only six days after the completion of exposure.

Table 4

Time of Decomposition of Acetylcholine Chloride of Whole Blood of Rats Exposed to Sulfuric Acid Aerosol.

Date of Investigation		Concentrations, mg/m^3	
		Series III 1.0	Series IV 1.8
Background on the basis of 4 determinations	23/IV	41,8	40,5
		40,6	37,8 (c)
	7/V	37,4 (c)	33,6 (a)
Exposure	21/V	36,8 (c)	30,6 (c)
	4/VI	34,0 (c)	30,0 (c)
Recovery period	18/VI	42,0	41,0
	29/VI	40,0	40,0

Results of a study of the total blood serum protein in animals of series III and IV showed no statistically reliable changes when compared with the background data. The ratio of the protein fractions changed significantly, however. At the same time, there was a decrease in the percent content of albumins and an increase in β globulins without a change in the other fractions (Table 5).

Table 5

Protein Fractions of the Blood Serum (in Percent) During Exposure to Sulfuric Acid Aerosol.

Period of Observation	Fractions	Concentrations, mg/m^3	
		Series III 1.0	Series IV 1.3
Background	Albumins	40,26	40,26
	α - Globulins	18,1	18,1
	β - Globulins	24,1	24,1
	γ - Globulins	17,6	17,6
Exposure	Albumins	34,2 (c)	33,6 (c)
	α - Globulins	18,6	17,7
	β - Globulins	27,3 (a)	29,1 (c)
	γ - Globulins	18,7	19,6
Recovery period	Albumins	36,5	33,8 (c)
	α - Globulins	17,5	20,05
	β - Globulins	27,1	26,2
	γ - Globulins	18,9	19,95

Table 6

Change in the Albumin-β-Globulin Ratio During Exposure to Sulfuric Acid Aerosol.

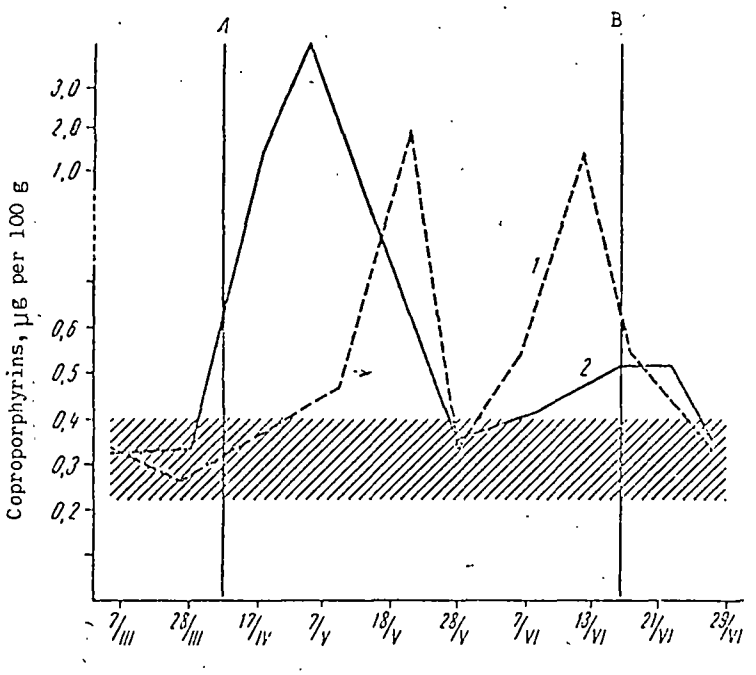
Series	Background	Exposure	Recovery Period
III	1,67	1,184 (c)	1,346
IV		1,181 (c)	1,380

As is evident from Table 6, a considerable decrease of the albumin-β-globulin ratio takes place under the influence of sulfuric acid aerosol.

Interesting results were obtained in experiments on animals of series III and IV with administration of coproporphyrins (Fig. 7). The amount of coproporphyrins is expressed in micrograms per 100 g of animal weight. The interval within the range of the physiological norm has been crosshatched.

As is evident from the data cited, sulfuric acid aerosol in 1.8 mg/m^3 concentration (series IV) caused a marked increase in the amount of coproporphyrins only seven days after the start of exposure. At the end of the first month, the amount of coproporphyrins reached 3.139 μg per 100 g for an average norm of 0.305 μg per 100 g. However, this index gradually

decreased, and 1 1/2 months later corresponded to the physiological norm. A fresh increase was observed toward the end of the exposure. This course was approximately repeated by the curve of excretion of coproporphyrins for series III as well, but a sharp increase was first observed only one month and eight days after the start of exposure.



Time of observation

Fig. 7. Content of coproporphyrins in the urine of rats exposed to sulfuric acid aerosol. Notation same as in Fig. 6.

A pathomorphological analysis of animals of series III, sacrificed both immediately after exposure and at the end of the recovery period, showed the presence of focal pneumonia in the lungs and congestion of the spleen.

Microscopic analysis of the lungs revealed interstitial pneumonia with swelling of collagenous fibers of interstitial tissue, bronchi, and adventitia of the blood vessels, catarrhal bronchitis, and emphysema of the lungs. The elastic fibers were severely loosened and, in areas of emphysema, fragmented. No changes were noted in the trachea.

In the kidneys, there were dystrophic changes in the epithelium of the convoluted tubules and wrinkling of the glomeruli. The liver had small areas of dystrophy and degeneration with signs of venous congestion. There were fine myocardial extravasations of blood. The spleen showed a hyperemia of the pulp and a marked hemosiderosis.

The cerebral cortex had focal hyperchromatism of some pyramidal neurons of layers II and V. Fine foci of gliosis and fallout of cells of Ammon's horn and their replacement by microglia were observed. In the cerebellum, there was fallout and wrinkling of the Purkinje cells.

Golgi staining showed, among pyramidal neurons of layers III and V, certain neurons having beaded enlargements and swellings with a lack of spines, but they were few in number. Unchanged neurons with even dendrite contours covered with spines were observed more frequently.

A pathomorphological examination of animals of series IV, sacrificed both immediately after exposure and at the end of the recovery period revealed large hemorrhagic foci in the lungs and hyperemia of the spleen.

Microscopic examinations of organs of animals sacrificed both immediately after exposure and at the end of the recovery period gave approximately the same results.

In the lungs, there was observed a large-focus and interstitial pneumonia consisting in a diffuse enlargement of the interalveolar septa as a result of accumulation of histiocytes, lymphocytes, and a small number of polymorphonuclear leucocytes. The lumina of the alveoli were empty, the collagenous fibers of the interstitial tissue were swollen, the elastic tissue was markedly loosened and consisted of a tangled network with breaks of the fibers in areas of emphysema (Fig. 8). The obliterated alveoli were threaded with elastic and argyrophil fibers (Fig. 9). There was venous congestion and catarrhal-desquamative bronchitis. In the kidneys, liver and heart, the changes were similar to those in animals of series III.

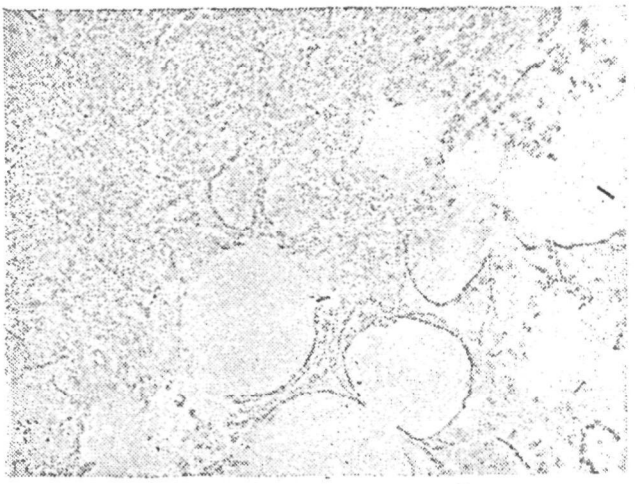


Fig. 8. Lung of white rat No. 60 from series IV. Elastic tissue is loosened and consists of a matted network with rupture of the fibers in areas of emphysema. Weigert staining. Magnification 40 x 10.

Examination of the cerebral cortex showed hyperchromatism of neurons of layers II and V of the cortex. There was focalgliosis, wrinkling and serious damage to neurons shown by Nissl staining. The cerebellum showed wrinkling and fallout of Purkinje cells. There was focalgliosis in the medulla oblongata. Golgi staining showed some neurons whose apical dendrites were devoid of spines and had beaded enlargements and swellings (Fig. 10). The main mass of neurons contained spines on the dendrites.

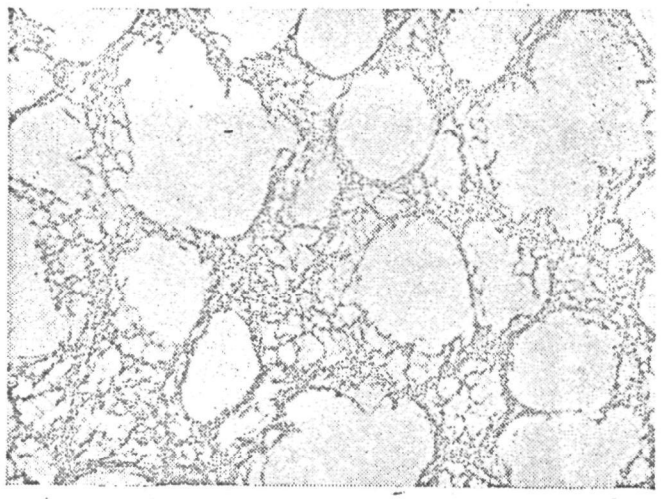


Fig. 9. Lung of rat No. 58 from series IV. Obliterated alveoli are threaded with argyrophil fibers. Tibor - Papp staining. Magnification 40 x 10.

Fig. 10. Brain of rat No. 60 from series IV. Neuron whose apical dendrite has beaded enlargements and swellings. No spines are present. Golgi staining. Magnification 40 x 10.

Studies of the combined action of sulfur oxides. We studied three combinations of sulfur oxides. In the first combination (series V), a sulfur dioxide concentration of about 5 mg/m^3 was used (4.79 $\pm$ 1.51 mg/m^3) with a sulfuric acid aerosol concentration of 1 mg/m^3 (0.92 $\pm$ 0.211 mg/m^3). This combination should have been helpful in evaluating the nature of the combined resorptive action of sulfur oxides. The other two combinations (series VI and VII) made it possible to substantiate and evaluate the existing maximum permissible concentrations (average daily values) of sulfur oxide present together. Therefore, the nature of the combined action will be evaluated first on the basis of materials of series V, and then the results of the study in series VI and VII will be assessed.

The first combination of sulfur oxides had no effect on the general state and weight of the experimental animals, either in absolute indices of weight or as expressed in percent. Data on the ratio of chronaxies of antagonist muscles are shown in Table 7.

Table 7

Ratio of Chronaxies of Antagonist Muscles in Animals of Series V.

Dates of Investigation		Ratio of Chronaxies
Background	6/III	1,194
	1/IV	1,104
Exposure	22/IV	1,115
	1/V	1,114
	13/V	1,108
	21/V	1,013 (a)
	31/V	0,948 (a)
	7/VI	0,865 (c)
	12/VI	0,876 (c)
Recovery period	21/IV	0,894 (c)
	29/VI	1,110

The data cited show that the combined action of sulfur dioxide after one month and 10 days causes the chronaxies to become similar, and then the ratio of the chronaxies becomes reversed. Comparison of the data cited with the effect of each individual sulfur oxide (Fig. 1 and 6) shows that the combined action leads to a greater inversion of the chronaxies, as shown by lower values of the ratio and also the presence of an inverse ratio in the first week of the recovery period. This is characteristic of all five rats of series V. A normal ratio prevailed during the isolated action of each oxide during this period.

To compare the action of sulfur oxides, we calculated the percent decrease of the ratio of chronaxies during the periods of exposure and the recovery period for each oxide from double concentrations and their combination (Fig. 11). The ratio of chronaxies in the control group was taken as

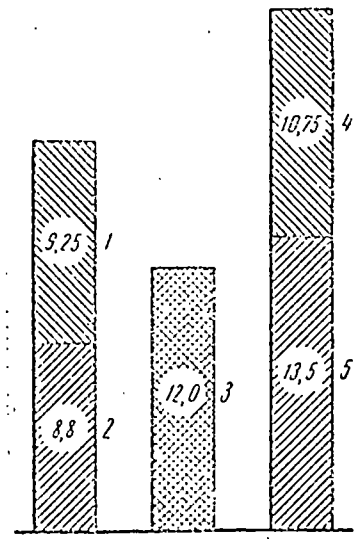


Fig. 11. Comparative evaluation of the action of sulfur oxides on the ratio of chronaxies of antagonist muscles in percent in animals of series I, II, III, IV and V.

1, 4 - sulfur dioxide in concentrations of 4.86 and 8.53 mg/m^3 respectively; 2, 5 - sulfuric acid aerosol in concentrations of 1 and 1.8 mg/m^3 respectively; 3 - combination of sulfur oxides in concentrations of 4.79 mg/m^3 of sulfur dioxide and 0.92 mg/m^3 of sulfuric acid aerosol.

100%. It is evident from a comparison of these data that the total decrease of this index when each oxide is used separately (9.25 $\pm$ 8.8 = 18.05) exceeds the decrease in the ratio of chronaxies during inhalation of the mixture of the same concentrations (12%) by slightly less than a factor of 2. We compared the change of the ratio during inhalation of the combination (series V) with 1/2 the sum of the changes in the ratio of double concentrations of sulfur oxides (series II and IV). In such a comparison, one-half the sum of the percent decrease of the chronaxies for double concentrations of each oxide ($\frac{24.25\%}{2}$) is approximately equal to the change in the ratio of chronaxies during the combined action of sulfur oxide (12%) in series V of the study. These data show that the inhalation of a mixture of sulfur oxides causes a change in the ratio of chronaxies corresponding to a simple summation.

Results of the investigation of cholinesterase activity of the blood of rats in all five series of the exposure are shown in Fig. 12.

It is obvious from the data obtained that during inhalation of the mixture of sulfur oxides, the decomposition time of acetylcholine chloride is shortest, i.e., the increase in cholinesterase activity is most pronounced At the same time, during the two weeks of the recovery period, there was a statistically reliable although slight increase in activity.

Fig. 13 gives comparative data on the increase of cholinesterase activity (in percent of the initial value) in the period of exposure and recovery, i.e., those periods during which the changes were observed. These data indicate a significant increase of the effect during inhalation of the mixture of sulfur oxides as compared with the sum of the effects during the

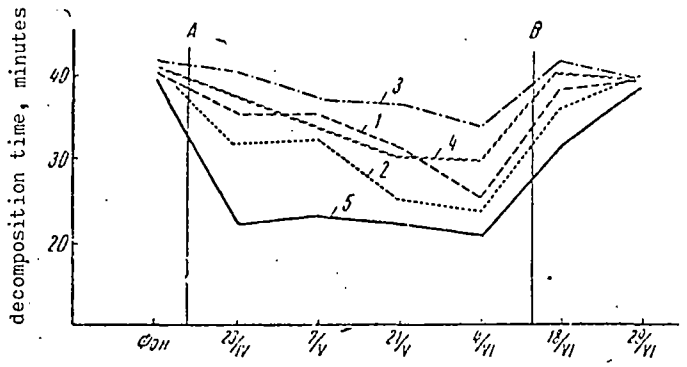


Fig. 12. Time of decomposition of acetylcholine chloride during different exposures to the action of sulfur oxides. 1 - series I (sulfur dioxide) - 4.86 mg/m³; 2 - series II (sulfur dioxide) - 8.53 mg/m³; 3 - series III (sulfuric acid aerosol - 1.04 mg/m³); 4 - series IV (sulfuric acid aerosol - 1.8 mg/m³); 5 - series V (sulfur dioxide - 4.79 mg/m³, sulfuric acid aerosol - 0.92 mg/m³); AB - period of exposure.

action of each of them individually (33.3% for series V versus 15.8% + 6.8% = 22.6%). The effect of increase of cholinesterase activity in the first case substantially exceeds the effect for the double concentrations of each oxide and is very close to their sum.

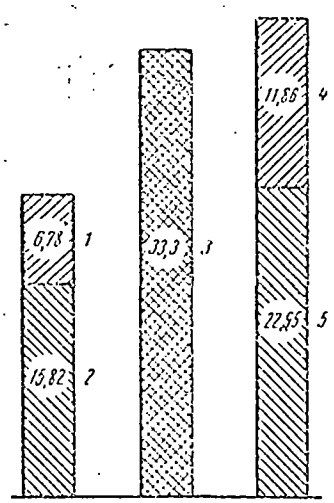


Fig. 13. Comparative evaluation of the effect of sulfur oxides on the increase of cholinesterase activity in average percentages for the period of exposure and the recovery period.

1, 4 - sulfuric acid aerosol in concentrations of 1.04 and 1.8 mg/m³ respectively; 2, 5 - sulfur dioxide in concentrations of 4.86 and 8.53 mg/m³ respectively; 3 - combination of sulfur oxides in concentrations of 4.79 mg/m³ of sulfur dioxide and 0.92 mg/m³ of sulfuric acid aerosol.

In a study of the total blood serum protein, we did not obtain any reliable changes during the period of exposure to the combination of sulfur oxides. However, during the recovery period, the increase in the content of total protein was statistically significant. Thus, whereas during the period of background measurements the total protein content was 7.67 mg %, during the exposure period it increased to 7.85 mg %, and during the recovery period climbed to 8.10 mg %.

Comparison of the results obtained with data for sulfur dioxide (series I) and sulfuric acid aerosol (series III) in animals of these series did not show any reliable changes in the content of total protein of the blood serum.

Thus, the combination of sulfur oxides causes greater changes in this index than does each oxide individually. The protein fractions of the blood serum for animals of series V are listed in Table 8.

Table 8

Protein Fractions of the Blood Serum (in percent) During Inhalation of Mixture of Sulfur Oxides (Series V).

Protein Fraction	Periods of Observations		
	Background	Exposure	Recovery Period
Albumins	40,26	35,6 (c)	32,6 (c)
α - Globulins	18,1	16,2 (b)	19,1
β - Globulins	24,1	29,0 (c)	28,5 (b)
γ - Globulins	17,6	19,2 (b)	19,7 (a)

As can be seen from Table 8, a shift takes place in all the protein fractions under the influence of the mixture of sulfur oxides. At the same time, there is a decrease in the percentage of albumins and α globulins with a sharp increase of β and γ globulins.

An evaluation of the character of the reaction to sulfur oxides when the latter are present together is shown in Table 9. Statistically significant changes in the various fractions in all the series are indicated as a percent change relative to the background data. The plus sign marks an increase of the content, and the minus sign marks a decrease.

Table 9

Comparative Evaluation of Changes in the Protein Fractions in Percent Under the Influence of Sulfur Oxides.

Series	Concentrations mg/m³		Albumins	Globulins		
	SO_2	H_2SO_4		α	β	γ
I	4,86	—	—11,1	0	+13,6	+11,3
III	—	1,04	—15,1	0	+13,2	0
Sum			—26,2	0	+26,8	+11,3
V	4,79	0,92	—11,6	—10,5	+19,8	+9,1
II	8,53	—	— 6,2	—18,3	+18,6	0
IV	—	1,8	—16,6	0	+20,7	0
Sum			—22,8	—18,3	+39,3	0

From Table 9 it may be concluded that each concentration of sulfur dioxide causes a particular reaction: a low concentration (4.86 mg/m^3) decreases the percent content of albumins during the period of exposure and increases it for β globulins; a double concentration causes a smaller decrease of the percent content of albumins, but decreases it for α globulins as well, without changing the percentage content of γ globulins. A change in the latter in the presence of a double concentration of sulfur dioxide is manifested only during the recovery period. Sulfuric acid aerosol causes more regular shifts in the protein fractions, decreasing the percent content of albumins and increasing the content of β globulins when it is present in both lower and higher concentration.

In a comparative evaluation of the combined action of sulfur oxides, attention is drawn to the great similarity of the response reaction with double concentrations of each oxide. Thus, one-half the sum of double concentrations for each oxide is nearly equal in effect to the combination of their half-concentrations. This is particularly apparent in the case of α and β globulins: for the combination during the period of exposure, there was a 10.5% decrease in α globulins versus $\frac{18.3\%}{2} = 9.15\%$ for double concentrations. The same is observed in the ratio of β globulins: 19.8% versus 19.6% respectively. A good agreement is given by this calculation for albumins and β globulins during the recovery period. This analysis of the material on the protein fractions leads to the conclusion that there is a simple summation of the effects during inhalation of a mixture of sulfur oxides. It would be difficult to expect a higher degree of agreement between the results when the experimentation is performed on a living organism.

Results of the determination of coproporphyrins in the urine of animals of series V are shown in Fig. 14. An increase in the amount of coproporphyrins in the urine was already observed seven days after the start of exposure. No such rapid reaction was noted in animals of series I (sulfur dioxide - 4.86 mg/m^3) and series III (sulfuric acid aerosol - 1 mg/m^3). When each oxide was used separately, the first reaction was manifested 1 1/2 months after the start of exposure in animals of series I and one month and eight days in animals of series III. In series with double concentrations of sulfur oxides, a reaction was already observed one week after the start of exposure in animals of series IV (sulfuric acid aerosol) - 1.8 mg/m^3).

After comparing the data on coproporphyrins in all 5 series, we came to the conclusion that their excretion in the urine during the action of the mixture of sulfur oxides is approximately the same as for isolated action, but the first reaction appears earlier.

Focal pneumonia and hyperemia of the spleen were established by pathologico-anatomical autopsy of animals of series V both after the exposure and at the end of the recovery period.

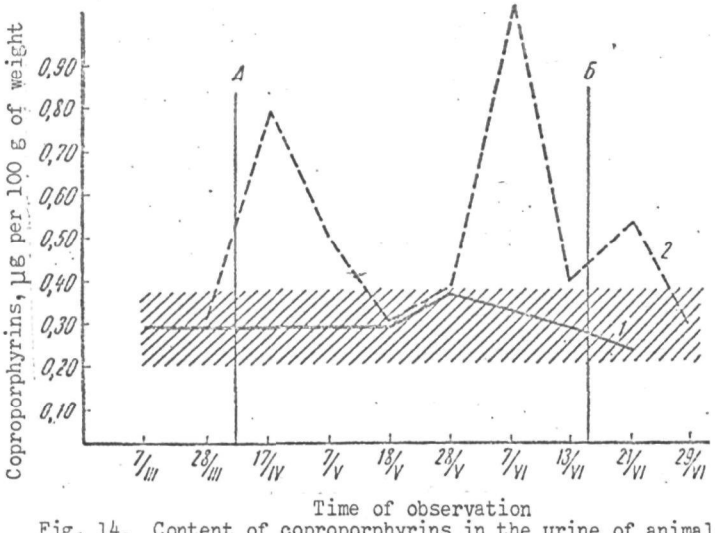


Fig. 14. Content of coproporphyrins in the urine of animals of series V.
1 - control group; 2 - combination of sulfur oxides; AB - period of exposure.

Microscopic examination of organs of the animals, sacrificed immediately after exposure and at the end of the recovery period, revealed approximately identical changes. Large-focus and diffuse interstitial pneumonia was discovered in the lungs. There was catarrhal-desquamative bronchitis and catarrhal-purulent tracheitis with hyperplasia of the mucosa of the trachea (Fig. 15). There were peribronchial and perivascular edema and sclerosis, and an irregular thickening of the vessel wall. The elastic framework was diffuse, and the fibers were frequently broken (Figs. 16, 17). The pneumonic segments were threaded with a tightly woven elastic network. The histiocytes in the lungs of rats of this series had short, teratic processes, larded with tiny lumps and coarse grains of pigment (Fig. 18).

Fig. 15. Trachea of rat No. 68 from series V. Catarrhal-purulent tracheitis with hyperplasia of the mucosa. Staining with hematoxylin-eosin. Magnification 10 x 10.

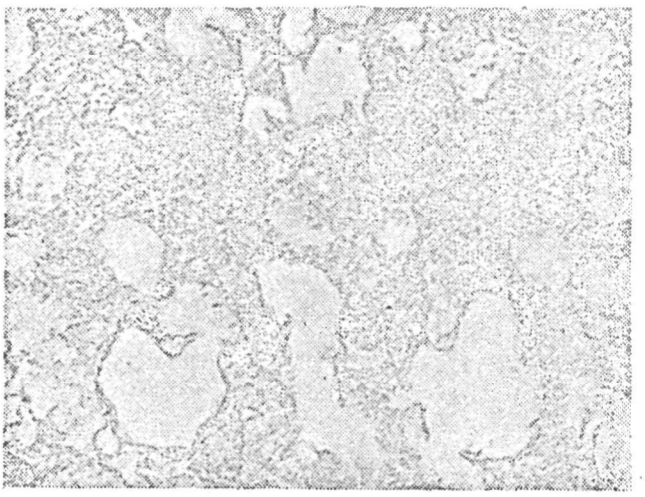


Fig. 16. Lung of rat No. 66 from series V. Diffuse elastic framework, frequently broken and coiled fibers. Weigert staining. Magnification 40 x 10.

Examination of the cerebral cortex revealed areas of wrinkling and hyperchromatism of pyramidal neurons of layers II and V of the cortex. There was fallout of cells of Ammon's horn and their replacement with microglia. There were perivascular cuffs, consisting of round cells, edema in the lower parts of the brain, hyperchromatism in the cerebellum, and angularity of Purkinje cells (Fig. 19). In the medulla oblongata, the contours of large neurons were smooth. There was gliosis and neuronophagia.

In Golgi staining, among normal neurons with well-defined spines on their dendrites, there are fairly frequent neurons whose apical dendrites are devoid of spines and have beaded enlargements and swellings (Fig. 20). Such changes disappear toward the end of the recovery period.

Two other combinations of sulfur oxides (series VI and VII) were studied in order to check the existing maximum permissible (average daily) concentrations of sulfur oxides when present together. These two series of studies were carried out in different periods. The actual concentrations were ($M \pm \sigma$), in series VI: sulfur dioxide 0.182 ± 0.067 mg/m^3, and sulfuric acid aerosol 0.104 ± 0.042 mg/m^3. In series VII, the concentrations were: sulfur dioxide 0.101 ± 0.25 mg/m^3, and sulfuric acid aerosol 0.099 ± 0.008 mg/m^3 (versus the given concentrations of 0.1 mg/m^3 for each oxide).

We did not observe any influence of the combinations studied on the behavior and weight of the animals. The weight gain of the animals was the same in both the experimental and control groups.

Results of the study of the ratio of chronaxies of antagonist muscles for animals of series VI are given in Fig. 21. A normal ratio of chronaxies in animals of series VI was preserved during the first month and half of exposure. However, at the end of the second month of exposure, a convergence began which proved to be statistically reliable on 7/VI. The week after the completion of exposure, the ratio of chronaxies reached the background values. Thus, the combined action of sulfur oxides in concentrations close to the maximum average daily values for each oxide manifests itself after two months of continuous round-the-clock exposure, causing a convergence of the chronaxies. Despite the fact that statistically significant changes were obtained during only one period of observation, we are forced to acknowledge this combination as being active on the central nervous system. The conclusion drawn from group data is supported by analysis of material on the individual rats of this series.

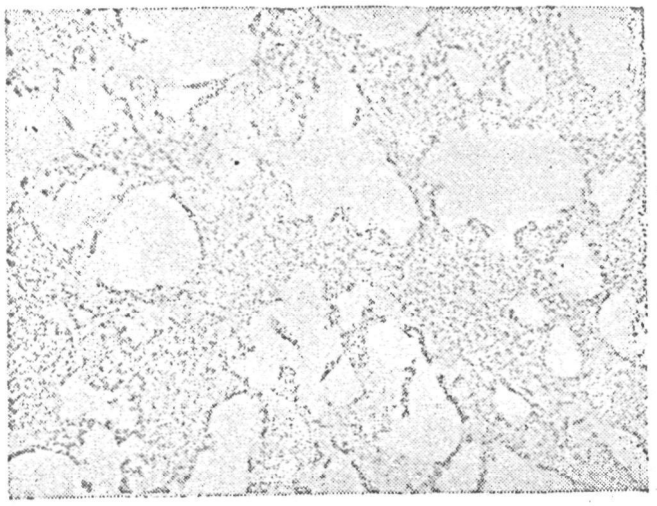


Fig. 17. Lung of rat No. 71 from series V. Ruptures of argyrophil fibers. Tibor-Papp staining. Magnification 40 x 10.

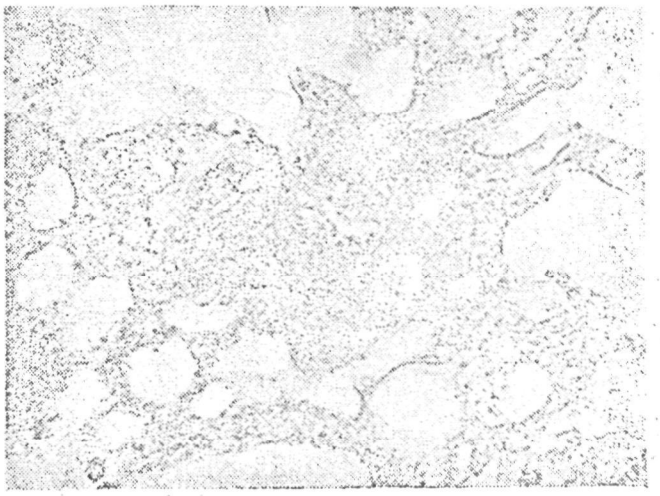


Fig. 18. Lung of rat No. 64 from series V. Histiocytes with short teratic processes filled with small lumps and coarse grains of pigment. Beletskiy staining. Magnification 20 x 10.

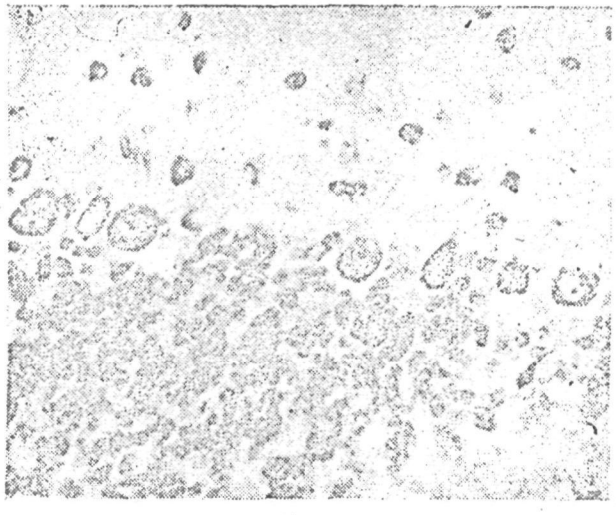


Fig. 19. Cerebellum of rat No. 74 from series V. Fallout, hyperchromatism and angularity of Purkinje cells. Staining with hexatoxylin-eosin. Magnification 40 x 10.

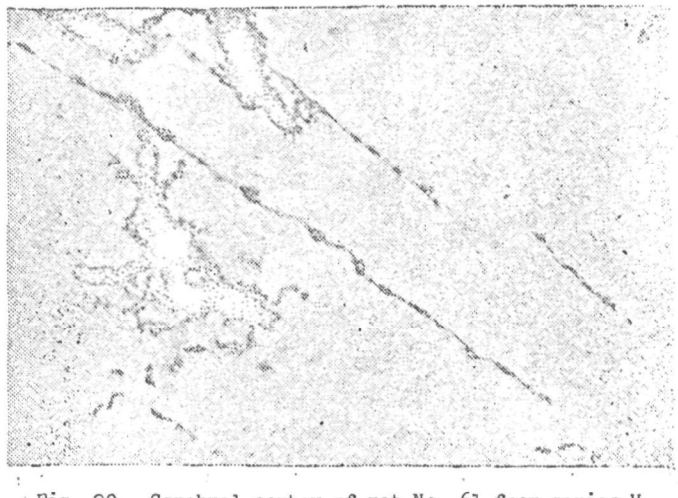


Fig. 20. Cerebral cortex of rat No. 61 from series V. Neuron whose apical dendrites are devoid of spines and have beaded enlargements and swellings. Golgi staining. Magnification 40 x 10.

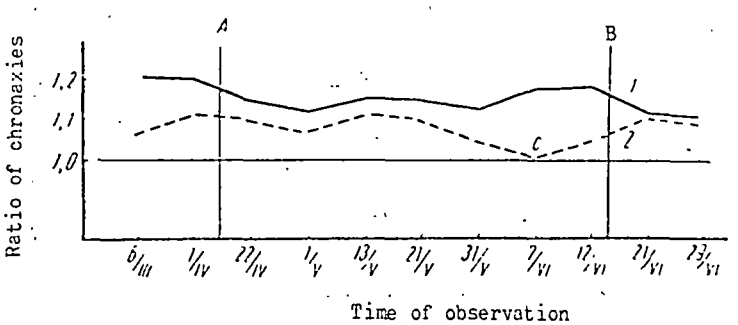


Fig. 21. Ratio of chronaxies of antagonist muscles in animals of series VI.
AB - period of exposure; 1 - control group; 2 - series VI; c - confidence factor of the change, 99.9%.

In animals of series VII, the normal ratio of chronaxies was preserved during the entire course of the observation. There was not a single case of convergence or crossing of the chronaxies in the individual animals, despite the fact that the exposure was extended to 75 days.

Results of determination of cholinesterase activity in animals of series VI showed that the second combination of sulfur oxides causes a statistically reliable decrease in the decomposition time of acetylcholine chloride, i.e., an increase in cholinesterase activity. This increase in cholinesterase activity also manifests itself only at the end of the second month of exposure. Analysis of the material for individual rats shows a decrease in decomposition time to 30 minutes (or 40 minutes in background measurements).

No changes in cholinesterase activity in animals of series VII were observed, either in average indices or in the analysis of the material for the individual animals.

No statistically reliable changes in the content of total protein were observed in animals of series VI or VII.

Results of the study of the ratio of protein fractions of the blood serum in animals of series VI are presented in Table 10. Data for the period of exposure are divided into two groups: the first includes materials for 4 and 22/V, i.e., the first 1 1/2 months of exposure, and the second for 6/VI; i.e., the end of the second month of exposure.

From Table 10 it is evident that reliable changes in the ratio of protein fractions were observed only at the end of the second month in a single fraction, the β globulins. The percent content of albumins also decreased, but not in all the rats, so that the average value of the difference proved to be statistically insignificant. During the recovery period,

the decrease of the percent content of albumins was reliable, without appreciable deviations in any of the globulin fractions.

Table 10

Protein Fractions of Blood Serum in Percent in Animals of Series VI.

Protein Fractions	Back-ground	Exposure		Recovery Period
		4 and 22/V	6/VI	
Albumins	40,26	39,7	35,0	35,0 (a)
α - Globulins	18,1	17,9	18,7	18,8
β - Globulins	24,1	24,9	29,4 (c)	24,7
γ - Globulins	17,6	17,5	16,9	21,4

The combined action of sulfur oxides in concentrations of 0.1 mg/m^3 each did not cause any changes in the ratio of the protein fractions (Table 11).

The last index which we used for the evaluation of the effects of the investigated concentrations of sulfur oxides was the content of coproporphyrins in the urine of the experimental animals. The sulfur oxides in combinations of series VI and VII did not influence the excretion of coproporphyrins. Nor were any changes observed in the histopathological examinination of the internal organs and brain of animals of these series.

Summing up the results of the study with the last two combinations of sulfur oxides (series VI and VII), one can conclude that the inhalation of a mixture of sulfur oxides in concentrations close to the average daily maximum permissible ones for atmospheric air is not without effect under conditions of prolonged continuous action. This is demonstrated by a decrease in the subordinational influence of the central nervous system, manifested by a convergence of the chronaxies of the antagonist muscles, and also by an increase in cholinesterase activity and a change in the protein fractions of the blood serum, chiefly as a result of an increase in the percent content of β globulins. All these disturbances occurred at the end of the second month of exposure. The above suggests that the combined presence of sulfur dioxide and sulfuric acid aerosol in the atmospheric air of populated areas is not permissible in concentrations at the level of the existing maximum permissible average daily concentrations for each oxide. At the same time, the combined presence of sulfur dioxide and sulfuric acid aerosol in concentrations of 0.1 mg/m^3 each, when the period of exposure is extended to 75 days, turns out to have no effect on the animals. If this concentration is expressed in fractions of the maximum permissible average daily values for each oxide, the sum is 1.66 (0.1:0.15 + 0.1:0.1).

Table 11

Protein Fractions of the Blood Serum in Percent in Animals of Series VII.

Group of Animals	Background				Exposure			
	Albumins	Globulins			Albumins	Globulins		
		α	β	γ		α	β	γ
Control	41,5	19,9	24,1	14,5	39,4	19,0	26,0	15,3
Series VII.	41,0	19,2	24,9	14,9	40,2	17,7	25,8	16,2

The results of the last series of the experiment on the combined action of sulfur oxides indicate that the joint presence of sulfur dioxide and sulfuric acid serosol in the atmospheric air of populated areas (average daily concentrations) may be evaluated by using a formula derived from the principle of simple summation:

$$\frac{a}{m} + \frac{b}{n} \geqslant 1$$

where a is the observed sulfur dioxide concentration; m is its maximum permissible (average daily) concentration; b is the observed concentration of sulfuric acid aerosol, and n is the maximum permissible average daily concentration.

Conclusions

On the basis of the above, it may be concluded that a long-term continuous inhalation of a mixture of sulfur oxides enhances the effect of the influence of each individual oxide according to additive synergism. Therefore, the joint presence of sulfur dioxide and sulfuric acid aerosol in the atmospheric of populated areas (average daily concentrations) should be estimated by using a formula derived from the principle of simple summation.

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BIOLOGICAL ACTION OF LOW MANGANESE CONCENTRATIONS DURING INHALATIONAL EXPOSURE

Candidates of Medical Sciences
F. V. Dokuchayeva and N. N. Skvortsova
A. N. Sysin Institute of General and Communal Hygiene,
Academy of Medical Sciences, USSR

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The preceding paper* reported on the contamination of atmospheric air with industrial discharges of manganese and their influence on the health of the population, and also on the intratracheal administration to white rats of low doses of manganese approximately corresponding to the maximum permissible value for plant buildings.

The present paper discusses the results of chronic inhalational exposure of white rats to low manganese concentrations.

The experimental validation of maximum permissible concentrations of dust materials involves certain difficulties because of the lack of the means for providing stable and low concentrations in the exposure chambers. In addition, the dust frequently has neither an odor nor an irritating effect. For this reason, the establishment of its maximum permissible content is possibly only on the basis of a general toxic effect during an extended inhalational exposure.

We used a dust chamber with a volume of ~ 0.8 m^3, patterned after a model chamber built by the Kiev Institute of Labor Hygiene and Professional Diseases. A modification of the Erman dust generator made it possible to produce in the chamber a manganese dust concentration of 0.3 and 0.03 mg/m^3, i.e., 275 and 2750 times lower than in the author's experiments.

The experiments were conducted on white rats of pure Wistar strain in two series: in the first (nine males and 16 females) with the 0.3 mg/m^3 concentration (maximum permissible value for plant buildings) and in the second (12 males and 16 females) with the 0.03 mg/m^3 concentration (highest single maximum permissible value for atmospheric air). Each series had its own group of control rats. Because of the presence of only one exposure

*V. F. Dokuchayeva and N. N. Skvortsova. Contamination of atmospheric air with manganese compounds and their influence on the organism. In: Maximum permissible concentrations of atmospheric contaminants. Prof. V. A. Ryazanov, ed., 1962, Vol. VI.

chamber (characterized by a large bulk), the exposure of the two series of animals was carried out in sequence (Table 1).

Table 1

Summary Table of Experiments

Series and Date of Exposure	Experiment			Control		
	No. of rats		MnO_2 concentration, mg/m^3, $M \pm m$	No. of rats		In the course of the experiment, total no. of animals sacrificed
	Males	Females		Males	Females	
I 15/VII 1960 r.—20/I 1961 r.	9	16	0,302±0,054	9	16	13
II 27/III—1/XI 1961 r.	12	16	0,033±0,002	15	15	8
III. 8/IX 1961 r. —7/II 1962 r.	8	—	4,12±0,181	6	—	1

The tests employed included a dynamic observation of the weight of the animals, a study of the red blood, histopathological examination of the lungs, and certain biochemical tests (porphyrin metabolism, cholinesterase activity of the blood, the ratio of blood serum protein fractions, and ascorbic acid content of the liver and adrenal glands).

To check the adequacy of the biochemical tests chosen, another series of experiments (III) was carried out which included eight experimental and six control male rats - a positive control group exposed in an adapted gas chamber to higher manganese concentrations, on the order of 3-6 mg/m^3 (average concentration 4.12 mg/m^3), i.e., 100 to 200 times higher than the maximum permissible value for atmospheric air.

Manganese dioxide powder (95% MnO_2) predried at 100°C. and ground, containing an average of 82% of particles measuring less than 5 μ, was used for the exposure of animals.

The manganese concentrations in the chambers were checked by taking samples every hour (the collection of each sample lasted one hour).

The manganese content was determined by using a method modified by the air hygiene section of the A. N. Sysin Institute of General and Communal Hygiene, Academy of Medical Sciences, USSR.*

*Instructions for the Organization of the Study of Atmospheric Pollution. Medgiz, 1963, p. 123.

The exposure was carried out for 5-6 hours daily on working days only. The total duration of the exposure for series I (0.3 mg/m^3) was six months, for series II (0.03 mg/m^3) seven months, and for series III (3-6 mg/m^3) five months.

The analyses showed that samples with concentrations of 0.15 to 0.45 mg/m^3 of series I comprised 89.4%, with more than half of the total number of samples (53.5%) consisting of MnO_2 concentrations in the range of 0.25-0.35 mg/m^3. Samples with higher concentrations were generally observed only in the course of 61 hours out of 872, i.e., in 7% of the cases. The average of all 872 samples was 0.302 ± 0.054 mg/m^3 MnO_2.

The scatter of the concentrations in magnitude for the seven months of exposure of series II animals was approximately the same. The largest number of hourly samples (915, or 89.1%) ranged from 0.015 to 0.045 mg/m^3. The average hourly concentration of MnO_2 in these groups of concentrations was 0.027 mg/m^3, and the average of all 1027 samples was 0.033 ± 0.002 mg/m^3. Manganese concentrations in the 100-liter gas chamber also corresponded to the specified conditions of the experiment, the average of all 545 samples being 4.12 ± 0.181 mg/m^3.

As we know, two MnO_2 concentrations - the highest single value (0.03 mg/m^3) and the average daily value (0.01 mg/m^3) - have been confirmed as tentative maximum permissible values for atmospheric air. These concentrations were established by calculations on the basis of the maximum permissible concentration of manganese for the air of plant buildings, a value also determined without experimental verification.

It was not possible to establish the highest maximum permissible concentration of manganese experimentally, since manganese compounds have no odor and no irritating effect. Cases of acute poisoning with manganese under industrial conditions are not known either. However, even very slight amounts of manganese, as will be shown below, are capable of causing certain reactions in the organism. In intensity of toxic action, manganese may be classified with a certain approximation among such industrial poisons as lead and mercury (A. F. Makarchenko).

For this reason, the noxiousness of manganese concentrations which may be present in atmospheric air should be evaluated only on the basis of their general toxic effects, checked by experiments on animals, and the value of the maximum permissible concentration of manganese should be determined in the form of a daily average, as was recommended by V. A. Ryazanov for substances having a general toxic effect.

We did not have the technical facilities available for round-the-clock exposure, and therefore conducted the exposure for 5-6 hours a day. However, since our experiments were chronic in character, they can be used as

material for substantiating the average daily maximum permissible concentration.

Occupational pathology data indicate a high toxicity of manganese compounds, which are capable of accumulating in the organism and causing ill effects. The toxic influence of manganese does not manifest itself immediately, but only as a result of more or less lasting contact with it (V. S. Surat, A. P. Sapozhnikov and A. P. Shilova, 1936; E. A. Drogichina, 1948; G. N. Cherepanova, 1946; V. A. Mikhaylov and F. S. Trop, 1953; Couper, 1937, and others).

The most frequent manifestations of manganese intoxication under industrial conditions are lesions of the striopallidal system of the brain and lesions of the respiratory organs (pneumonia, pneumonoconioses, chronic bronchitis, laryngitis, etc.) (Z. I. Bakradze, 1923; Büttner, Lenz, 1937; M. E. Machabeli, 1957, and others).

In a medical survey of children in the vicinity of industrial discharges we also observed a high disease rate, particularly pneumonias and diseases of the upper respiratory tract, i.e., the demonstrated shifts in the state of health of the child population are similar to the changes developing in workers under industrial conditions.

For this reason, the chief criterion in series I of the animals was the histopathological examination of the lungs.* The results of the examination after six months of exposure showed that the histopathological changes in the lungs of males of series I could not be evaluated as being the consequence of exposure, since some changes were frequently observed in the majority of the control animals.

In contrast, examinations of the female rats showed an adverse effect of manganese in 0.3 mg/m^3 concentration on the respiratory tract. A consequence of this effect was chiefly the appearance of inflammatory changes in the pulmonary tissue and also the appearance of circulatory disorders in the vascular system of the lungs and the development of moderate symptoms of peribronchial and perivascular sclerosis of the pulmonary tissue. Changes of this kind were noted in the experimental group in all 15 female rats, whereas they occurred in only four out of 13 control rats.

The statistically reliable difference between the number of lesions of the lungs in female rats in the experimental and control groups suggests that the manganese concentration of the order of the maximum permissible value for plant buildings is active on the organism of female rats during chronic inhalational exposure.

*The histopathological examination was made by Candidate of Medical Sciences Yu. N. Solov'yev.

No changes of any kind were observed in the weight (the rats were weighed twice a month) and in the state of red blood (the number of erythrocytes and the hemoglobin were determined at the start, in the middle and at the end of exposure).

Number of determinations

Excretion of coproporphyrin with urine during inhalational exposure of rats to manganese.
1 - concentration, 4.12 mg/m^3; 2 - control; AB - period of exposure.

According to the data of biochemical investigations, exposure to the 4.12 mg/m^3 concentration of MnO_2 (series III) increased the cholinesterase activity of the blood and caused the effect of porphyrinuria in the experimental animals (Table 2 and Figure).

In series II of experiments, no changes were observed in the porphyrin metabolism. The same applies to the determination of ascorbic acid in the liver and adrenals (using the method of the Vitaminology Institute), in the content of which no shifts were observed, in contrast to the data of Rudra (1944), N. V. Tatarinova (1951), and A. M. Birenbaum (1957), who observed such shifts when manganese was administered with food.

Table 2

Activity of Blood Cholinesterase in Rats Exposed to the 4.12 mg/m^3 Concentration of MnO_2 (series III), Minutes.

Males	Date of Determination					
	25/VII 1961 г.	6/IX	9/X	17/XI	30/I 1962 г.	6/II
Experimental	30	35	35	30	17	21
Control	30	35	35	30	22	27

In a study of the composition of protein fractions of the blood*

*The determination of the protein fractions was carried out by Doctor of Medical Sciences A. A. Tyurina and Candidate of Medical Sciences L. I. Kulikova.

in experimental female rats of series II, changes were observed in the ratio of the content of protein fractions, i.e., an increase in the amount of globulins and a decrease in the amount of albumins. In the males, such changes were not detected even when concentrations such as 4.12 mg/m^3 of MnO_2 were employed (Table 3).

At the end of the experiment, a histopathological examination of the pulmonary tissue was also performed on animals of series II. Preparations of the lungs were stained with hematoxylin-eosin and hematoxylin picrofuchsin according to Van Gieson* and argyrophil fibers were developed by Bielschowsky's** method. In addition, the preparations were stained with toluidine blue, and the reaction was carried out according to Hotchkiss*** (the MacManus reaction).

The examination of the experimental group of males of series II in two out of 12 animals showed definite inflammatory phenomena in the lungs (purulent bronchitis, chronic abscessing bronchopneumonia). No signs of gross pathology were observed in the remaining animals of this group. However, accumulations of macrophages containing lumps of brown dust pigment were found in the peribronchial lymphoid tissue and under the pleura in the majority of animals of this group. In some cases, vague signs of desquamation of the alveoli and bronchial epithelium were observed along with manifestations of a certain increase in the number of collagenous fibers in the peribronchial zones and in the stroma of lymphatic nodes of the hilum of the lung.

In the experimental group of females, gross inflammatory changes of the lungs were observed in six rats out of 14. The remaining changes were analogous to those found in the male rats of this group. In almost all the cases, minor accumulations of dust macrophages were also observed without any sings of reaction among the surrounding tissue components.

Among the 14 male rats of the control group, inflammatory changes were noted in four cases, but they were less definite than in the experimental group. In the control group of females, inflammation signs in the respiratory organs were observed in only two rats out of 13.

In Hotchkiss staining and staining with toluidine blue, as well as in the development of argyophil fibers according to Bielschowsky, no fundamental structural changes were detected in the lungs of either the experimental or the control animals.

Comparing these materials of the histological examination with similar data obtained from the exposure of animals of series I (0.3 mg/m^3), one can note that in the groups of males being compared, the number of animals with inflammatory phenomena in the lungs in the experimental and control groups was approximately the same (Table 4), and in any case does not permit one to consider any kind of relationship between the appearance of inflammatory

*, **, *** [Translator's note: Van Gizon, Bilishov, Khochkis, according to the transliteration of Russian reference.]

Table 3

Protein Composition of Blood Serum (in percentages) in Animals of Series II and III During Exposure to Manganese Dioxide.

MnO_2 Concentration, mg/m^3	Group of Animals	Total Protein	Globulins			Total Amount of Globulins	Total Amount of Albumins	Albumin-Globulin Ratio
			γ	β	α			
0.033 (Series II)	Experimental Females	8,53	12,70	20,86	11,86	45,49	54,24	1,19
	Control Females	8,34	12,44	19,39	10,39	42,57	57,46	1,35
	Experimental Males	7,49	13,98	25,08	13,07	52,56	47,84	0,91
	Control Males	6,86	14,24	25,74	11,52	51,91	48,15	0,93
4.12	Experimental Males	8,46	15,20	19,42	15,03	49,66	50,59	1,02
(Series III)	Control Males	8,52	14,12	21,15	13,84	49,11	50,98	1,04

processes in the experimental males and the penetration of manganese into the respiratory tract.

Table 4

Number of Animals with Changes in the Lungs.

Series	MnO_2 Concentration, mg/m^3	Males		Females	
		Experimental	Control	Experimental	Control
I	0 302	4 out of 8	7 out of 9	15 out of 15	4 out of 13
II	0 033	2 out of 12	4 out of 14	6 out of 14	2 out of 13
III	4 12	5 out of 8	3 out of 6	—	—

In experimental females of series II, inflammatory changes were observed in six rats out of 14 (somewhat more frequently than in the control), but this difference proved to be statistically unreliable, and therefore a manganese concentration of 0.03 mg/m^3 cannot be considered to be active on the respiratory organs of female rats.

However, during inhalational exposure to the 0.03 mg/m^3 manganese concentration for seven months, the pulmonary tissue of the animals, independently of the sex, showed a deposition of particles of manganese dioxide aerosol, which was absorbed more slowly than other oxide compounds of manganese, but was also excreted more slowly (E. N. Levina, 1962). This fact is alarming if one takes into account the cumulative properties of manganese. It is possible that during a longer exposure, even such low manganese concentrations may prove to be active on the pulmonary tissue when the lability of the organism is greater.

Moreover, inhalational exposure of animals of both sexes to 0.3 and 0.03 mg/m^3 concentrations also showed that the organism of female rats is more sensitive to the action of low doses of manganese than that of male rats. The high sensitivity of females to manganese has also been observed in intratracheal administration.

A study of the red blood in series II of the experiment (0.03 mg/m^3) did not reveal any changes in the hemoglobin content or in the number of erythrocytes, either in the males or in the females. In series III (4.12 mg/m^3) did not reveal any changes in the hemoglobin content or in the number of erythrocytes, either in the males or in the females. In series III (4.12 mg/m^3), toward the end of the fifth month of exposure, a statistically reliable decrease in the number of erythrocytes was observed in the experimental rats as compared with the controls. Toward the end of exposure, the amount of erythrocytes in the experimental group increased to 106.5% of the initial value, and in the control, to 113.4%. The difference in the hemoglobin

content and magnitude of the color index was not significant.

Dynamic observation of the weight of the animals showed that in series III there was no substantial difference in the weight of the experimental and control animals, exposed to the 0.03 mg/m^3 manganese concentration as in the group of females of series II. In the group of males (0.03 mg/m^3), for the same average weight at the start of the experiment, the curve of the average weight of experimental males starting from the end of the first month of exposure runs all the way to the end of the experiment above the curve of the control animals. At the same time, the difference in the average weight in the fifth month and at the end of the exposure becomes statistically reliable.

Conclusions

1. The inhalational exposure of white rats to an average concentration of 0.302 mg/m^3 of MnO_2 for 5-6 hours a day for six months causes changes in the lungs of female rats manifested in inflammatory phenomena in the pulmonary tissue, the onset of circulatory disorders in the vascular system of the lungs, and the development of moderate symptoms of peribronchial and perivascular sclerosis of the pulmonary tissue.

2. An average concentration of 0.033 mg/m^3 of MnO_2 during inhalational exposure for 5-6 hours a day for seven months proves to be active on the composition of the protein fractions of the blood serum in female rats, causing a change in the ratio of the content of albumins and globulins.

At the same concentration, the lungs of animals of both sexes show dust particles of manganese dioxide, and in male rats there is observed a tendency toward a gradual and more rapid weight increase as compared with the control animals.

3. In the inhalation exposure of male rats to an average concentration of 4.12 mg/m^3 of MnO_2 in the course of five months (group of positive control), the phenomena of prophyrinuria, increase of cholinesterase activity of the blood and decrease of the number of erythrocytes at the end of the exposure were observed.

4. Our data suggest that the reaction of white rats to the action of manganese may be different depending on the sex of the animals. For this reason, the experiments should be conducted on animals of both sexes, since the reactivity of the females to the action of toxic substances may be greater than that of males.

5. A further experimental study of even lower doses of manganese administered in round-the-clock inhalational exposure is necessary.

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MAXIMUM PERMISSIBLE CONCENTRATIONS OF NOXIOUS SUBSTANCES

IN THE ATMOSPHERIC AIR OF POPULATED AREAS*

From "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Pod redaktsiey Prof. V. A. Ryazanova i Prof. M. S. Gol'dberga. Izdatel'stvo "Meditsina" Moskva, p. 185-187, (1966).

No.	Contaminant	Maximum Permissible Concentrations mg/m^3	
		Maximum Single	Daily Average
1	Acrolein	0.30	0.10
2	Amyl acetate	0.10	0.10
3	Amylene	1.5	1.5
4	Aniline	0.05	0.03
5	Acetone	0.35	0.35
6	Acetophenone	0.003	0.003
7	Benzene	2.4	0.80
8	Low-sulfur gasoline in terms of C	5.0	1.5
9	Butyl acetate	0.1	0.1
10	Butylene	3.0	3.0
11	Vinyl acetate	0.2	0.2
12	Hexamethylenediamine	0.001	-
13	Isopropylbenzene hydroperoxide	0.007	-
14	Dichloroethane	3.0	1.0
15	Dimethyl	0.03	0.03

* Approved by the Assistant Chief Physician of Public Health of the USSR on May 26, 1964.

(Cont'd)

No.	Contaminant	Maximum Permissible Concentrations mg/m^3	
		Maximum Single	Daily Average
16	Dowtherm	0.01	0.01
17	Isopropylbenzene	0.014	-
18	Methanol	1.5	0.5
19	Methyl acetate	0.07	0.07
20	Maleic anhydride	0.5	0.05
21	Methyl methacrylate	0.1	0.1
22	Manganese and its compounds (in terms of MnO_2)	-	0.01
23	Arsenic [inorganic compounds except hydrogen arsenide (in terms of As)]	-	0.03
24	Nitrobenzene	0.008	0.008
25	Carbon monoxide	6.0	1.0
26	Nitrogen oxides (in terms of N_2O_5)	0.3	0.1
27	Propylene	3.0	3.0
28	Nontoxic dust	0.5	0.15
29	Metallic mercury	-	0.0003
30	Soot (carbon black)	0.15	0.05
31	Lead and its compounds (except tetraethyl-lead)	-	0.0007
32	Lead sulfide	-	0.0017
33	Hydrogen sulfide	0.008	0.008

(Cont'd)

No.	Contaminant	Maximum Permissible Concentrations mg/m^3	
		Maximum Single	Daily Average
34	Sulfur dioxide	0.5	0.15
35	Sulfuric acid	0.3	0.1
36	Carbon disulfide	0.03	0.01
37	Sulfuric (in concentration of H ions)	0.01	-
38	Hydrochloric	0.01	-
39	Nitric	0.01	-
40	Styrene	0.003	0.003
41	Formaldehyde	0.035	0.012
42	Toluylene diisocyanate	0.05	0.02
43	Phosphoric anhydride	0.15	0.05
44	Fluorine compounds (in terms of F)	0.03	0.01
45	Phenol	0.01	0.01
46	Furfural	0.05	0.05
47	Chlorine	0.10	0.03
48	Chlorobenzene	0.1	0.1
49	Hydrogen chloride	0.05	0.015
50	Chloroprene (2-chloro-1, 3-butadiene)	0.25	0.08
51	Hexavalent chromium (in terms of CrO_3)	0.0015	0.0015

(Cont'd)

No.	Contaminant	Maximum Permissible Concentrations mg/m^3	
		Maximum Single	Daily Average
52	Cyclohexanol	0.06	-
53	Cyclohexanone	0.04	-
54	Ethyl acetate	0.1	0.1
55	Ethylene	3.0	3.0

Remarks. 1. When several substances characterized by a complete summation of action are present together in atmospheric air, the sum of their concentrations as calculated by the formula below should not exceed 1.

$$x = \frac{a}{m} + \frac{b}{m_2} + \frac{c}{m_3} \text{ etc.}$$

where x is the unknown total concentration; $\frac{a}{m}$, $\frac{b}{m_2}$, $\frac{c}{m_3}$ are the concentrations of the substance being determined divided by the corresponding maximum permissible concentration for isolated action.

Substances characterized by such action include the following groups:

a) sulfur dioxide and sulfuric acid aerosol;
b) hydrogen sulfide, carbon disulfide and Dowtherm;
c) isopropylbenzene and isopropylbenzene hydroperoxide;
d) ethylene, propylene, butylene and amylene;
e) strong mineral acids (sulfuric, hydrochloric and nitric) in terms of the concentration of H ions.

2. When hydrogen sulfide and carbon disulfide are present together in atmospheric air, the maximum permissible concentrations for each of them individually are preserved.

3. Lists of indexes of maximum permissible concentrations of noxious substances in the atmospheric air of populated areas, approved on 14 February 1961 for Nos. 221-61 on 13 April 1962, and since 20 June 1963 for Nos. 442-63 should be considered as no longer in force.