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HYGIENIC AND TOXICOLOGICAL CRITERIA OF HARMFULNESS IN EVALUATING HAZARDS OF CHEMICAL COMPOUNDS

by I.V. Sanotsky and I.P. Ulanova

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Translated by *A. N. Chabrova* and *O. L. Zherebtsova*

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This book is intended for toxicologists, hygienists and all those responsible for evaluation and control of harmful effects of chemicals to human health and the environment. It could also be useful in postgraduate training of specialists in preventive toxicology.

Translation of a revised text based on the 1975 Russian edition of *Kriterii vrednosti v gigiene i toksicologii pri otsenke khimicheskikh soedinenii* by I. V. Sanotsky and I. P. Ulanova.

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Preface to the English Edition

Given the modern rates of chemicals application in industry and agriculture, the environmental protection and control over the population's health represent a major socio-hygienic issue.

It is well known that to-day some 60,000 chemicals are widely used and hence may enter the environment in a certain state and quantity. The major source of environmental pollution are discharges from industrial enterprises. Therefore, it is very important to assess the safe levels of discharges of such chemicals which would not upset the biological and ecological development of the environment and would not produce a negative impact on man's health.

The development and substantiation of the hygienically prescribed quantities of various chemicals in the air of the working zone forms one of the main tasks of industrial toxicology. This monograph substantiates the hazard criteria in preventive toxicology and hygiene in view of the need to apply correctly the quantitative rates of hazardous substances in the environment. It also lists materials relating to the assessment of potential and real danger as well as to the classification of hazards presented by poisons at different levels of exposure. The suggested classifications helped to obtain the data to study toxicity and the hazards of different groups of chemical compounds which are broadly used in the economy.

It is known that different actions of chemical substances depend, to a large extent, on the peculiarities of the chemical composition of their molecules. Therefore, the results obtained within closely related groups of chemical substances should be most valid. Bearing this in mind, the monograph discusses such chemicals as chlorinated hydrocarbons of the methane and benzene series and their monohalogen derivatives; toluene and its derivatives as well as certain heterocyclic compounds.

The monograph also contains a review of the rapid assessment methods of safe concentrations of industrial poisons in terms of the cumulative properties of substances and the initial stage of chronic poisoning as well as such problems as the age and species sensitivity to poisons and the use of experimental data on animals in hygienic practice.

Besides, the monograph contains an additional chapter on the biological substantiation of the possibility of assessing the average working shift and maximum allowable concentrations.

The authors do hope that the monograph would prove useful for those interested in preventive toxicology and would be grateful for any critical comments.

«Scientific power... of toxicology lies in the indivisible triade of clinic, hygiene and experiment, linking them».

N. S. Pravdin

INTRODUCTION

Scientific and technical progress has called particular attention of preventive medicine to the tasks of hygienic examination of new occupational and communal situations, hygienic classification and standardization of equipment, raw materials and products, and hygienic limitation of the intensity of harmful occupational and domestic factors.

These tasks are covered by preventive[1] and in particular by hygienic toxicology, which is an independent branch in the whole complex of hygienic sciences. In many cases these tasks are reduced to a primary evaluation of the toxicity and hazard posed by new chemical compounds, to a toxicological assessment of manufacturing processes for the purpose of substantiation of recommendations on possible modifications in the synthesis process in order to eliminate undesirable intermediate products and by-products and specify medical and technical requirements to the layout, production and hygienic equipment, including that for purification or dispersal, and to individual means of the personnel protection, if required (I. V. Sanotsky, 1974).

The above-mentioned preventive measures are based on the use of maximum allowable concentrations (MACs) of substances in the environmental media and in biomedia. The MACs are taken into account in designing and serve as the legal basis in hygienic control.

Hygienic control of the air of workplaces, of the atmosphere of residential areas, of water bodies used for public, domestic, fishery and pisciculture purposes, of the soil and food products has become particularly urgent. Rapid transfer of the mineral resourses to the earth surface, an increased production of new synthetic materials, introduction of chemical technology into all branches of economy (including agricultural production), and into everyday life create a real threat of an inadequacy of the environmental conditions of man and ecologically associated species to their hereditary qualities. I. P. Gerasimov and N. G. Fradkin (1973) have raised the question of the existence of metabolism between nature and society.

[1] Preventive toxicology is a science of prevention of immediate and delayed harmful consequences of the effect of substances on all living organisms in general, not only on man (hygienic toxicology).

This circumstance is even more allarming in view of the fact that the rate of environmental changes in some cases exceeds the rate of biological adaptation[1] both in phylogenesis and in ontogenesis. Since the concept of natural selection cannot be applied to man, it is justified to use the concept of «social adaptation», i. e., the organized activities of society aimed at a limitation of further environment pollution with chemical compounds (G. A. Stepansky, 1973; G. F. Helmi, 1973), and at the reduction of the present level of pollution. Social aspect of a successful sollution of the problem of human environmental protection against chemical pollution has been studied by E. K. Fiodorov (1972).

Traditional subdivision of preventive toxicology in industrial, communal, food toxicology, etc. is an analitic stage in the research studies of the environment. Scientific conceptions, formulated in the USSR, have made it possible to achieve a significant limitation of uncontrolled discharge of chemical substances into the biosphere. In particular, hygienic standards (MACs) of the chemical substances content for the air of workplaces (over 750), for water (over 420 medical and over 40 fishing and pisciculture standards), for the atmosphere of residential areas (over 130), for food contaminations (allowable residual quantities of pesticides, etc.), for soil and for cattle-breeding have been substantiated.

Much has been done to implement hygienic limitations in industry, agriculture and everyday life. Thus, for example, the characteristics of toxicity and hazard of substances (including characteristics of the quality of their effect) have been assumed as a basis in designing, in the arrangement of ventilation systems and other means of protection in industry, as a basis for regulations on the use of pesticides in agriculture (instructions on methods for hygienic evaluation of new pesticides), of process additives in food industry, domestic chemical preparations, etc.

At the same time, many problems remain unresolved. Classifications of substances according to their biological effects are not applied extensively enough in early stages of industrial design work and, in particular, for the selection of appropriate equipment, of purifying and dispersing systems, for substantiation of the necessity to create closed cycles of water- and air-supply (non-waste technologies).

The regulations on polymer materials release into the environment are still implemented according to the integral chemical, and not to biological and medical indices (N. I. Shumskaya, 1971). Such an approach brings about gross mistakes, misguide researchers, inducing them to prohibit entirely certain mixtures of

[1] By the term «adaptation» we intend true physiological accomodation of the organism or of a polulation to the environmental changes. Accomodation «at any price», i. e., compensation of a pathologic process (including a temporarily latent one) should not be included in the concept of adaptation.

ingredients. Similar phenomena are registered also in other fields of hygienic standardization. Even minor mistakes in the MACs substantiation cause detriment to human health, involve economic losses, which, in turn, result in secondary detriment to the welfare (including health) of people.

Similar calculations have been cited, for example, for DDT. After spraying DDT in marshes (mosquito control), the marshes' water contained only traces of DDT and was considered harmless for fish. Plankton, however, accumulated DDT rapidly (up to 0.04 ppm). As a result, a depot of poison with its content up to 0.5 ppm was formed in the adipose tissue of small fish feeding on this plankton. The DDT content in the fat of large fish eating the small one increased up to 2 ppm. The sale of this fish to the population turned out impossible. The DDT content in the fat of birds eating this fish was 25 ppm, that is 10 million times higher than its content in the water. In some cases, concentrations of DDT accumulating through the «food chain» caused death of a large number of birds (N. Z. Journal of Agriculture, 1971). According to the data reported at the All—Union Conference on Water Toxicology (1968), as a result of chemical pollution, many rivers in the USSR were insuitable in 1962 for pisciculture.

Another example of the mediate pollution effect is a deterioration of alimentary properties of agricultural plants, registered under certain conditions of the pesticides application.

These examples of secondary detriment to the welfare of people concern almost direct mediation. However, the mediation may be more remote. For example, the industrial discharge of sulfur dioxide into the atmosphere, being non hazardous for human health, resulted desastrous for pine forests; the damage caused by the atmospheric corrosion of materials is enormous, and is several times larger in case of chemical pollution with oxidizers. Similar situation appears in case of water corrosion of materials. According to the data reported by E. I. Ignatyev (1973), an improper and irrational utilization of natural resourses and erroneous impact on the environment causes considerable annual detriment to the USSR.

Economic losses caused by the environment pollution should be estimated by appropriate departments, which would take into account real quantities of pollutants with a special regard to their minimum effective concentrations. A damage estimating procedure has already been developed, for example, in pisciculture.

The problem of estimation of economic losses, connected with the impact of the pollution on the level of public health and on the sickness and disablement rate, is still a pressing matter, in spite of certain proposals (B. A. Katsnelson, 1972; B. A. Urlanis, 1972).

Thus, an isolated study of environment problems results insufficient. The necessity of a complex evaluation of the human en-

vironment conditions has been demonstrated by numerous studies that have been published. A special place belongs here to the collection of works «Introduction to Geohygiene» (1966), edited by N. V. Lazarev.

The urgency of these problems has been proved already by the first results of the research efforts. It turned out that a simultaneous intake of benzene by the organism of the experimental animals at the level of the existed MACs from two media (work places air and drinking water) resulted in the summation of the effects (S. M. Pavlenko, 1972). Chronic inhalation of fluorine by the experimental animals at the MAC level for the atmospheric air of residential areas, taking place simultaneously with the fluorine intake from water with fluorine content at the MAC level for water bodies used for public and domestic purposes caused manifest biological effect (M. S. Sadilova, A. A. Petina, 1970). The conclusion is clear: the existing MACs must be revised.

It is possible that when substantiating the MACs for the mentioned substances (for a given media) some errors have been committed. This fact has already been taken into consideration in revising the MAC for benzene, but nevertheless the results of these investigations are instructive.

These are, in brief, the results of overestimating the MACs, but there is also the probability of their unjustified underestimation, which may lead to unfavourable economic, and thereby to socio-hygienic consequences.

The direct biological substantiation of the hazard of the MACs underestimation for some compounds is based on the so-called returning action, as well on natural deficiency of their content in the medium. The biological effect of some microelements provides such an example (Fig. 1).

As it is known, hygienic standards, as well as the whole modern system of evaluation of the toxicity and hazard, are based on the concept of the threshold of the poison's effect. Many researchers consider the threshold of chronic effect (Lim_{ch}), which is approximated to the MAC, as the main threshold. Therefore, one of the reasons for overestimation or underestimation of a hygienic standard lies in a certain indefinitiveness and variability of the biological effect characteristics, and especially of the poison's effect threshold.

The first reason is the insufficiency of the probabilistic evaluation of a biological phenomenon, the second is insufficient definitiveness of the very notion «harmful effect».

As early as in 1934, N. S. Pravdin wrote: «... The indefinitiveness of the concept 'harmful industrial production' and the absense of scientifically substantiated 'norms' make it difficult to qualify the degree of harmfulness of a given industrial production and often raise arguments between the administration of an enterprise and labour protection institutions».

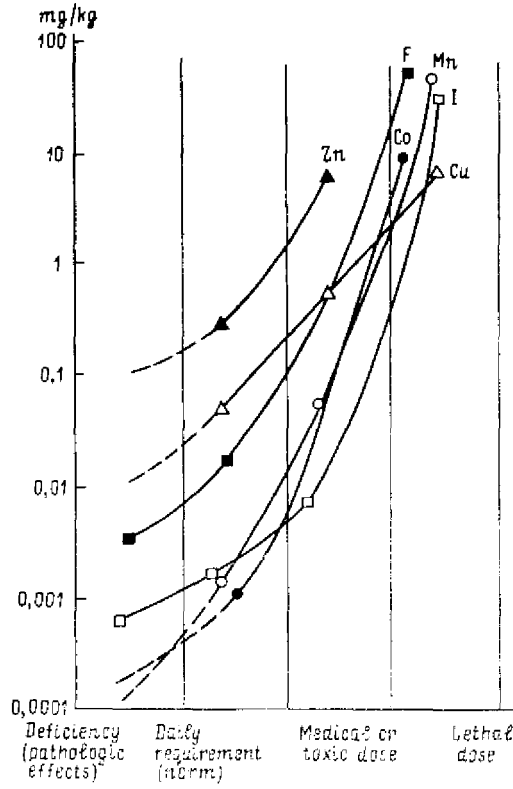

Fig. 1. Biological effect of some microelements.

At the same time, N. S. Pravdin developed the concept of «hygienic significance» of indices of the poison's effect. He emphasized that not all the indices could be used for substantiating preventive measures, but only those which have hygienic significance, i. e., the hazard criterion.

Does it always happen that the «harmful effect» of substaces can be evaluated unambiguously? Under what conditions and for which groups of population? If for the conditions of the residential areas atmosphere any reliable and constant deviations of the index of vital activity of the organism may be, in certain cases, interpreted as a manifestation of a harmful effect of a substance, then for the conditions of the workplaces air not every deviation from the norm (even provided that it is reliable) and not of every index of vital activity is to be considered harmful.

In everyday life conditions, a change in the chemical composition of the environment up to a degree, when it is noticed by the organism, is in many cases unadmissible, since the possibility of a

24-hour effect of substances, in comparison with a 6—8 hour working shift exposure, is more favourable for material cumulation and, evidently, decreases the limits of true adaptation (this latter statement needs additional substantiation). Moreover, the described criterion of harmfulness for the atmosphere of residential areas may be attributed to the fact that among residents of these places there are children, elderly and sick people.

Significant changes in the organism may be allowable under the conditions of the drugs' effect, i. e., the effect which is comparatively rare. In these cases, the criterion of harmfulness is certainly quite different: an effect is to be considered harmful only if it causes changes in the organism which are difficult to control and which are hazardous for health or life under a single or a few applications. It is advisable to mention once more that, in this case, even such doses of relaxants which would have caused death, if artificial respiration is absent, are to be classified as useful.

Thus, the problem of harmfulness criteria lies in the theory and practice of the evaluation of the toxicity and hazard of the chemical pollution of human environments, which serves to substantiate the preventive measures.

Not all the aspects of the mentioned problems have been studied thoroughly enough, and that is why we are far from considering our presentation complete. The authors will welcome constructive discussions and will appreciate any critical remarks.

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Chapter V «Species and age sensitivity» has been written together with G. G. Avilova, Chapter IX «Heterocyclic compounds» together with N. M. Migukina.

Chapter 1. CRITERIA OF HARMFULNESS OF CHEMICAL EXPOSURE IN HYGIENE AND TOXICOLOGY

In the past, by harmfulness of chemical pollution of the environment (mostly of the occupational environment) was intended the capacity of chemicals to harm, i. e., to cause manifest symptoms of poisoning. The concepts of t o x i c i t y and h a r m f u l n e s s were being almost identified. «Toxicity is the capacity of chemical compounds to exert harmful effect in a nonmechanical way», wrote N. V. Lazarev (1964). The same definition of toxicity has been given outside the USSR (Olson, 1961). However, even then some researchers registered the so-called metatoxic effect of such doses and concentrations of substances which did not cause manifest symptoms of intoxication. The investigators understood it as the ability of chemical compounds to reduce the resistance of the organism, mainly to infections.

> On this subject, N. S. Pravdin cites Tsanger, Starkenstein and Kelsh. «There are many poisons which in small doses do not produce any serious disturbances, but which however bring about such slowly evolving changes and tendencies to changes in organs that either transform into morbid symptoms or determine certain peculiar preparedness of the organism for a disease and its liability to the effect of other substances» (N. Tsanger). «We shoud bear in mind as a matter of high importance in what concerns occupational diseases also those conditions which, though not having per se characteristics of a poisoning, lead, because of their chronic effect, to a decrease of the resistance of the organism, and thereby predispose it to other diseases, especially to tuberculosis and, in some way, substantially reduce the limits of endurance of an individual in respect to other harmful factors (E. Starkenstein). «Together with a direct effect of poisons on the organism, together with an acute or chronic poisoning, poisons cause also a general decrease of the resistive capacity of the organism of the affected individual» (F. Kelsh).

N. S. Pravdin gave much consideration to «chemical depression of labour processes», i. e., to a decrease of labour productivity of the working people due to occupationally determined exposure to chemical compounds in such doses and concentra-

tions which do not cause yet manifest pathological processes. Developing this thesis, N. S. Pravdin proposed as methods for determining «the lower parameter of toxicity», i. e., the threshold of the poison's effect, such indices as muscular working capacity, and then psychical working capacity (individual method of conditioned reflexes).

Analysing these terms, N. V. Lazarev (1938) wrote: «Speaking about light chronic harmful effect of poisons, authors often prefer to avoid the term 'chronic poisoning' and use the expressions 'prepathology', 'metatoxic effect', 'preintoxicational depression', 'chemical depression of labour functions' (N. S. Pravdin), etc. Strictly speaking, its not easy to differentiate these notions. The difference lies mainly in the method by which the poison's effect is revealed». We should agree with Lazarev's opinion.

The accelerated development of chemistry in the URSS has called the attention of physiologists and pathophysiologists to toxicology (I. S. Tsitovich, Y. P. Frolov, A. D. Speransky, L. I. Kotlyarevsky, et al.). They assigned to the nervous system the leading role in pathogenesis of intoxications and pointed out the existence of physiological fluctuations in the functions of the integral organism. Considering the question of the influence of substances on the higher nervous activity and on neuro-vegetative responses of man, I. S. Tsitovich emphasized the similiarity in the levels of responses of man and animals under short-term and 24-hour exposures.

At the same time, even then there outlined a tendency to accept any threshold changes in the organs and systems as the «lower parameter of toxicity» or, in other words, as harmfulness. It should be noted that later this tendency turned into tradition in some institutions.

N. S. Pravdin pointed out that the effect of a poison might have numerous thresholds — as many as the number of systems of the organism responding to the administration of a substance. To avoid the absurd statement that any response to external influence is an indication of damage, N. S. Pravdin proposed to lay a special emphasis on the integral indices of the effect, i. e., the responses at the level of the integral organism, having «hygienic significance». This principal thesis could not at that time get a quantitative, probabilistic interpretation, but in a number of cases logical deductions have brought us closer to a realistic approach to the problem of the environment protection against chemical pollution. Several tests substantiating «hygienic significance» are given in Table 1.

The presented data show that the use of direct analogies is not always efficient. We should mention that together with some unequivocal interprepations (like that of the «reduction» of labour productivity), there also appeared some ambiguous interpretations (for example, «changes» — in this or that meaning —, in the

Table 1

Integral indices of harmful effect of the poison which have hugfenic significance (according to N. S. Pravdin, 1934)

Index	Test object	Substantiation of hygienic significance
Decrease of labour productivity	Man	Socially harmful phenomenon
Decrease of attention (even according to a correction test)	Man	Hazardous phenomenon in occupational conditions
Decrease of muscular working capacity	Animal	Phenomenon similar to the decrease of labour productivity if projected on labour conditions of man
Disturbance of the higher nervous activity	Animal	Hazardous phenomenon if projected on labour conditions of man
Changes in the external respiration (oxygen consumption, oxygen utilization, respiration coefficient, etc.)	Man or animal	Changes of the integral index of the energy supply condition, indicating significant effect

external respiration of animals). Later on this fact has put scientists in the dilemma: is it good or bad if some substances, present in the air of the zone of respiration, have caused an increase of labour productivity? To answer this question, it is necessary to study the dynamics of such changes, taking into account phase fluctuations in the physiological functions of the organism and periods of the development of chronic pathological process. The concepts of physiological adaptation and temporary compensation have been later on comprised by preventive medicine.

In connection with an extensive introduction of statistical methods, all satistically reliable ($p<0.05$) indices changes were suggested to be taken as an indication of harmful effect of a substance (histamino-pectic index, alkaline phopshatase activity, content of ascorbic acid in adrenal glands, etc.), without taking into account the biological aspect of the phenomena. Some experimentalists followed the new trend. The others (recently, e. g., G. A. Stepansky, 1971) have somewhat transformed this approach, putting forward the thesis of the existence of substances which are or are not assimilated by the organism (any indications of the effect of the latter are inadmissible). They also claimed that the MACs could not guarantee against harmful after-effects and could be accepted only as a temporary measure: the lower they were the better. There are contradictions in such theses: if a given magnitude of chemical pollution, recommended as MAC, fails to ensure perfect health protection, then it contradicts the concept of the MAC accepted in the USSR.

The maximum allowable concentrations of harmful substances in the air of the working area are those concentrations which, in the case of daily exposure at work for eight hours throughout the period of working life, will not cause any disease or deviations from a normal state of health detectable by current methods of investigation in the workers whether while actually engaged in the work or afterwards (CH 245-71).

According to I. V. Sanotsky (1971), the maximum allowable concentration of a chemical compound in the environment is the concentration which, acting on human organism periodically or throughout the life (directly or mediately through ecological systems, or through possible economic damage), does not cause somatic or mental diseases (including those latent or temporarily compensated) or changes in human health conditions that go beyond the limits of physiological responses of adaptation, detectable with the help of current methods of investigation either immediately or at later periods of life of the present or the following generations.

The definition of the TLV[1], adopted in the USA, is principally different from that of the MAC in the USSR.

The TLV is the level to which nearly all workers can be exposed repeatedly, day after day, without harmful effect. Because of the wide variability in individual sensitivity, a small percentage of workers can feel discomfort under concentrations of some substances at the level or below the limit values; even a smaller percentage can have more serious disturbances which cause deterioration of a pre-existing disease or lead to occupational diseases (TLV, List, 1973).

Conception of the Thresholds of the Effect

The main principle used in substantiating the MACs is the conception of the thresholds of the harmful effect of substances.

> «Beyond certain limits of concentrations, a chemical substance is not poison any more, and it does not exert on the organism the harmful effect which it exerted before» (N. S. Pravdin, 1934). «It is obvious that every poison has a certain limit of the effective concentrations and doses below which no harmful effect will be observed...» (N. V. Lazarev, 1938).

The conception of the thresholds, existing for all types of the effect (including that mutagenic and blastomogenic) is the leading principle of the Soviet hygiene and preventive toxicology.

Legislative and other regulations on the conditions of the environment of man and associated species of plants and ani-

[1] Threshold Limit Value.

mals, toxicological limitations (hygienic standardization) of the pollution of occupational and domestic media, as well as derivative practical measures of technological or other characters depend, in the final analysis, on the solution of the main problem: is there a threshold of the intensity of the harmful action of external factors, or is any intensity, differing from zero, harmful? In other words, may we use objective scientific data as the basis for the projection of the biosphere, or should we utilize more or less subjective solutions (the conception of «admissible risk», on the one hand, or the so-called «zero» pollution, on the other, etc.)? We would like to underline that the matter in question is not that of the threshold of the responses of the living systems to external irritation in general, but that of the responses of the living systems exceeding the limits of usual homeostatic physiological fluctuations.

The feasibility of substantiation of the threshold of harmful effect for the most types of chemical compounds' effects seems indisputable for the majority of authors. However, as to mutagens, blastomogens and radiation lesions, this feasibility has long remained doubted. It was not resolved at the symposium organized by the US Hygiene Association (February of 1970), which specially convened to discuss this issue.

Meanwhile, the existence of the thresholds of the effect of any harmful factors (including radiational, and especially chemical factors) can be proved theoretically on the basis of the main differences between the living organisms and the nonliving objects: permanent metabolism and energy exchange with the environment, continuous restoration of its structure and adaptational changes in the organism determined by environmental conditions, including changes in the process of reproduction of individuals of the same species. Even the simplified formula

$$D_r = D_0 - D_{excr} - D_{met}$$

(where D_r is the dose of poison absorbed by the receptor; D_0 is the dose of poison introduced into the organism; D_{excr} and D_{met} are, respectively, the doses of poison excreted from the organism and detoxicated on route to the receptor) indicates that there are thresholds of the effect, provided that the value $D_{excr} + D_{met}$ does not diminish proportionally to the diminishing of the D_0. The data obtained demonstrate that this condition is observed.

However, even if we study the receptors separately from the integral organism, we shall find out that a simple binding of a poison by a receptor does not mean yet a biologically significant response to the poison. For example, the study of isolated human erythrocytes has demonstrated that the binding of the lead ions by the cell membrane up to a certain limit does not lead to an increase of the removal of potassium (Fig. 2) and hemoglobin into the incubation medium (Grigarzik, Passow, 1958).

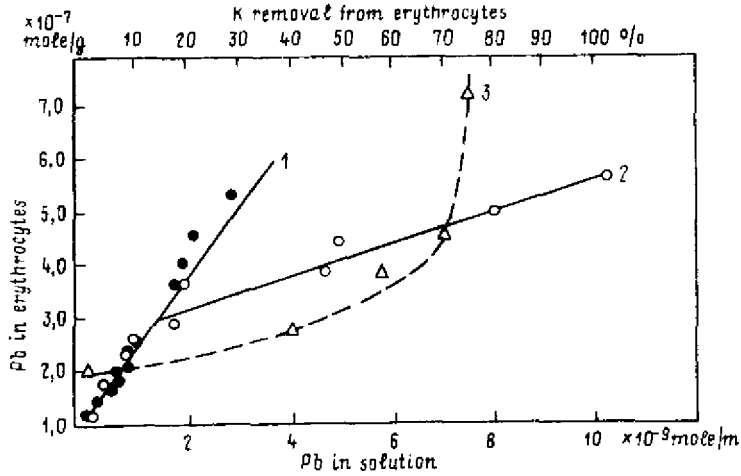

Fig. 2. Dependency of the lead content in erythrocytes (1 in 15 min, 2 in 60 min) and of potassium removal from erythrocytes (3) on the content of lead in the solution.

Thus, the rearrangement of molecules may occur without manifest functional changes in the biosubstrate. Development of a «biologically significant» effect depends on the «importance» of the substrate in the vital activity of the cell or on the existence of a reserve metabolic pathway (Dinman, 1972).

Within the integral organism, this may be supplemented by the processes of adaptation and permanent restoration (activation of detoxication, intensified generation of biological structures linked with the poison, e. g., enzymes' molecules, inclusion of by-pass routes of normal metabolism, activation of macrosystems directed at the adaptation to new living conditions, etc.). The damage occurs only when the rate of the damage development (inactivation, degeneration, etc.) exceeds the rate of the processes of adaptation and restoration. These phenomena have been thoroughly investigated under conditons of fractional application of ionizing irradiation, and exactly these facts have formed the basis of the concept of the thresholds of the radiation effect (G. S. Strelin, 1960). Similar phenomena have been described also for the conditions of the exposure to chemical factors (I. V. Sanotsky, 1974).

The existence of the thresholds of the harmful effect of external factors may be also proved by the analysis of the dose-response curves. Many conceptions which denied the existence of the thresholds were the result of assumptions and extrapolations. The dependency of the effect on the dose is not linear, therefore it is very important to determine the end of the curve, corresponding to the lower level (Brues, 1958; Mantel et al., 1961, 1963;

Bock, 1968; J. Weisburger, E. Weisburger, 1968; Stokinger, 1972; Weil, 1972). The conditions of an experiment (species, sex, age of animals, nutrition, etc.) influence considerably the shape of the curve, therefore the results of the threshold determination must be reproduced many times. As a rule, the intensity of the organism's response decreases together with the decrease of the dose, while the response reaches a zero value before the dose does (Hatch, 1973).

At present, as in the past, the analysis of the dose-response curves, observed under exposure to mutagens and blastomogens, is the most controversial. The blastomogenic effect curve for benzo(a)pyrene, obtained by N. Y. Yanysheva (Fig. 3), has let

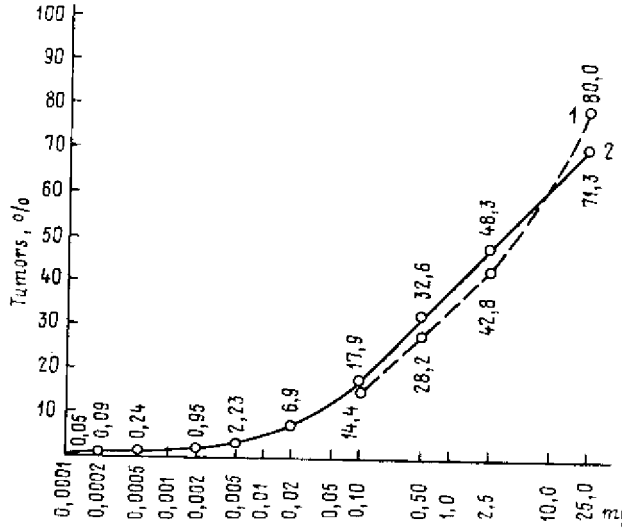

Fig. 3. Dependency of frequency of lungs tumors occurrence under administration of 3,4-benzo(a)pyrene into the respiratory tract of mongrel rats:

(1) experimental curve; (2) calculated curve of logarithmic correlation $Y=[(\ln\frac{1}{x}+1)]10$, where x_n is the dose, mg (for calculated point); x is maximum non-effective dose in the experiment equal to 0.02 mg.

Soviet researchers to be the first in the world to substantiate the MAC for this poison (L. M. Shabad et al., 1973). A similar dependency on the dose under benzo(a)pyrene application to the skin surface (Fig. 4) has been reported by G. N. Zayeva et al. (1972), T. S. Bruyevich and K. I. Panchenko (1969). Nowadays, more and more researchers bome to believe in the feasibility of determination of safety levels for similar substances. Fig. 5 illustrates the curve of the dependency of cytogenic effects in the bone-marrow on the chloroprene concentrations. This curve was obtained in our laboratory by L. D. Katosova (1973). In the investigated range of concentrations, the curve has an exponnential

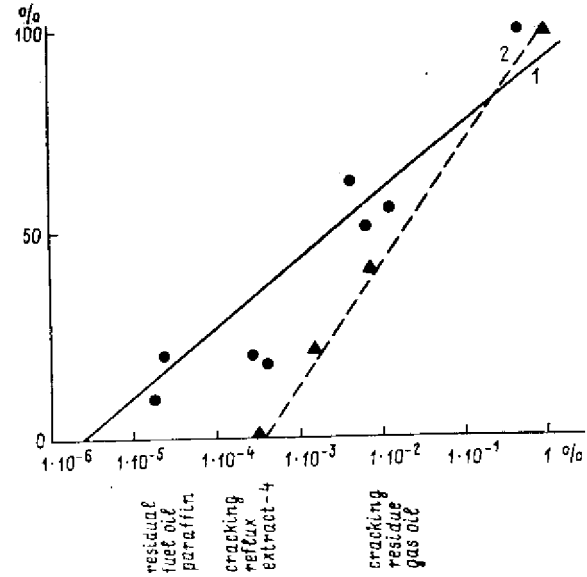

Fig. 4. Dependency of blastomogenic effect on concentration of 3,4-benzo(a) pyrene:

(1) in oil products; (2) in acetone. Y-axis, tumors produced, %; X-axis, concentrations of 3,4-benzo(a)pyrene; %.

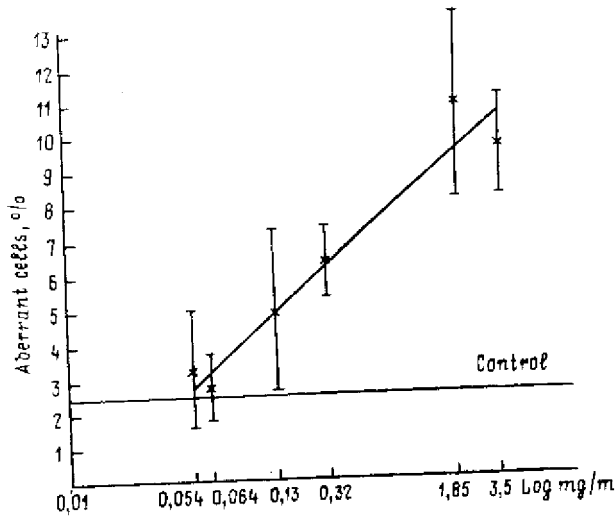

Fig. 5. Dependency of cytogenetic effect in cells of the bone-marrow of animals on concentrations of chloroprene.

shape. However, in relation to this and other similar studies, we should note that an effect is to be considered harmful only if the proportion of the aberrant cells in experimental animals goes beyond the limits of spontaneous fluctuations in the control group, as well as beyond the accepted «norm». In probabilistic evaluation, it might be advisable to take into account some known proposals, such as that of Gaddum (1956), who considered the value of the $DL_{50} - 6\sigma$ as the safe zone, since the probability of lethal outcome in this case is only 10^{-9}.

The threshold of harmful effect of a substance is such its minimum concentration in the media under exposure to which in the organism (under specified conditions of substances absorption) occur changes that go beyond the limits of physiological adaptation responses, or a latent (temporarily compensated) pathology.

Despite these cited facts, the struggle for a real sanitation of the environment still goes on. One of the examples is the combat against the environment pollution with mutagens and blastomogens.

Some authors (L. M. Filippova, 1975; Druckrey, 1973) still assume that the genesis of mutations or modifications cannot, as a matter of fact, have a threshold, since it concerns reactions at the level of molecules, forming chromosomes. At this level, any quantity of «exogenous» or «endogenous» toxic agents is supposed to cause enormous, and sometimes fatal rearrangements. To prove it, the authors often drew the analogy with the effect of ionizing radiation. It is not justified, since, on the one hand, the mechanism of radiation effect differs from that of chemical agents (especially from the direct effect of the latter). On the other hand, the recently obtained data show the reversibility, within certain limits, even of the damage of chromosomes caused by radiation.

The practical importance of these issues can hardly be overestimated: the problem is whether it is possible to establish the MACs for the substances with blastomogenic and mutagenic effect. American authors, followed by some international organisations, try to exclude marked blastomogens completely from occupational environment. Until now only six products have been assigned in the USA to the group of such substances (TLV List, 1972): benzidine, β-naphtylamine, nitrozodimethylamine, β-propiolactone, 4-dimethylaminoazobenzene and dimethyl sulfate (Truhaut, 1964). In the FRG, there are 9 such substances (MAK-Werte List, 1972): 4-aminodiphenyl, arsenic, asbestos, benzidine, benzene, beryllium, chromium, 2-naphtylamine, nickel.

We think that such a point of view is based on a misunderstanding. Certainly, the efforts to exclude completely the exposure of working people to carcinogens in occupational, communal and domestic conditions are well justified.

Various modifications in the manufacturing process, directed at the replacement of harmful substances by that less noxious (i. e., of blastomogenic products by non-blastomogenic), seem to be a right way (L. K. Hotsyanov et al., 1958; G. B. Pliss, 1972).

However, not without reason the authors of the TLV and MAK lists do not include in the lists of substances to be prohibited such active carcinogen as benzo(a)pyrene: it is impossible, as it is impossible to prohibit the extraction and processing of oil and coal.

Meanwhile only hygienic regulations on the benzo(a)pyrene content in the environmental media can provide a sound foundation for current hygienic and epidemiological control.

A categoric denial of the possibility to substantiate the MACs for carcinogenic substances has been recently replaced by a theoretical recognition of the admissibility of an individual approach to the problem of hygienic standardization of separate carcinogenic substances (L. M. Shabad, 1962, 1966; L. M. Shabad, L. A. Andrianov, 1966; L. M. Shabad, A. B. Linnik, 1966).

Without substantiating the MACs for carcinogens (even those very low[1]) a total prohibition of their use (under whatever conditions) will result inefficient, since the detection of their presence or absense in the objects of the environment depends on the sensitivity of the method of analysis which helps to detect substances, but which often reveals itself insufficient (Table 2).

Table 2

Allowable residual quantities (ARQ) of some pesticides in food products

Pesticide	ARQ	Practically determinable quantity (mg per 1 kg of the product)
Hexachloran	0	0.005—0.001
Methaphos	0	0.1—0.2
Polychloropynen	0	0.05
Sevin	0	0.05
2,4D group	0	0.2

This data may be also applied to numerous other substances which can cause modifications or mutations. The problem is complicated by the fact that most of the actually available data have been obtained from experiments on insects. In the lists of mutagens have been included even such alimentary and flavoring substances as alkaloids of tea and ethyl alcohol (V. S. Zhurkov,

[1] In the USSR the MAC for benzo(a)pyrene was established in 1972 as 15 μg/100 m³ for the workplaces air and as 0.1 μg/m³ for the atmosphere of residential areas.

1971; Goldstein, 1962). Traditional genetists have recently come to recognize the existence of such mutagens doses which are eliminated or detoxicated before they reach the «target», or which cause premutational changes restorable later. At the same time, however, these scientists continue automatically to advocate the idea of nonexistence of the thresholds of chemical mutagenesis (L. M. Filippova, 1975).

A similar approach (establishment of appropriate quantitative hygienic limitations) may be recommended also for active sensitizers. However, due to an exceptionally wide range of individual sensitivity (up to 10^5) safe labour conditions, in this case, can be provided by means of isolation of persons with increased sensitivity to these substances from the field of contact with them (A. A. Letavet, A. I. Korbakova, 1967).

Applicability of Economic Criteria

The leading principle for the establishment of hygienic standards is the priority of medical indices.

Principal changes in the organization of chemical technological process (continuity, hermetic sealing), in the design and construction of factories (provision of clean zones, corridors, control panels, etc.), in designing economical and highly efficient hygienic equipment (multimode ventilation, multistage purification plants, etc.) have led to a situation when the principle of «technical feasibility» of hygienic standards (MACs, in particular) has lost its priority and safe labour conditions have been often ensured by the use of individual means of protection, by the reduction of the working day and by other auxiliary measures.

Certainly, individual means of protection are being used also nowadays, especially to protect the respiratory organs and the skin (e. g., in the coal-mining industry). However, general technological progress and the increased amount of information on harmful consequences of the environment pollution for society and individuals have created necessary premises for the priority of medical indices in substantiating the MACs.

In this connection, A. A. Letavet (1962) wrote: «Scientifically substantiated hygienic standards must lead the technical thought forward, to the creation of a more advanced production technology and more advanced industrial equipment. Under the conditions of a rapid technological progress, what appears difficult to be solved today, will appear easy to be overcome tomorrow».

Thus, in the initial phase of introduction of dust suppression and dust collecting techniques in the mines, the MAC value for silicon oxide (2 mg/m^3) seemed to be unrealistically high. The practice has, however, proved that this figure can be achieved. Similar facts may be found in the field of water purification, for

example, of the effluent of the Baikal pulp-and-paper mill. It should be noted that the today's conception of technical feasibility remains at present the leading conception in the USA (M. Pisano, 1974; V. A. Newell, 1974). The progress made by Soviet scientists and the Soviet State in the field of hygienic labour protection and in protection of the welfare of people has caused (or consolidated) a certain underestimation of the importance of the economic aspect of the problem among some scientists. There appear more and more appeals to exclude this or that substance from the productional sphere or to isolate completely some groups of workers (e. g., pregnant or non-pregnant women) from any contact with a harmful substance.

At the same time, the probability of poisons' effect in the earliest periods of pregnancy, as has been proved in experiments with chloroprene (L. S. Salnikova, Z. A. Volkova, 1968), and with ethyleneimine (I. V. Silantyeva, 1973, et al.), makes these measures less effective. Another aspect of the insufficiency of the last measure has been demonstrated in practice. Thus, after there had been registered some cases of new-born children having dysplasia, women were excluded from the process of chloroprene rubber production (G. I. Mirzabekyan, A. O. Akhverdyan, 1964). However, specially arranged clinico-hygienic observations revealed serious abnormalities in the generative function of men engaged in the same process (R. M. Davtyan, 1974).

The thesis that the MACs are not optimal for the environment has already been discussed in the literature (A. A. Letavet et al., 1967). This thesis may be used to justify the underestimated thresholds of harmful effect and the MACs, but this is usually done without taking into consideration two circumstances.

First: if we take any reliably changing indices as the basis for the Lim_{ac} and Lim_{ch}, we shall logically come to the conclusion that statistically significant deviations of physiological indices, for example, leukocytosis of hyperglycemia (after eating a meat breakfast or after a cup of tea with sugar), represent indices of a harmful effect and, therefore, the doses of exogenous substances (in this case, foodstuffs) should be reduced. This conclusion is absurd. The entire organism's activity is directed at adaptation to environmental variations (including those of environmental chemical components), which is impossible without physiological responses to these variations: it is a natural and inherent property of all living organisms. The second circumstance was already considered by the founders of preventive toxicology. Thus, in his «Guide to Industrial Toxicology» (1934) N. S. Pravdin wrote:

> «The most drastic measure to combat the most hazardous industrial poisons is their complete elimination from manufacturing process and their replace-

ment, if required, with other, less hazardous or even absolutely safe substances. But in order to assess the feasibility and reality of implementation of this measure without damages to industry and national economy, it is necessary to be aware of the industrial and economic importance of a given toxic substance for the whole national economy and for specific branches of industry. To evaluate the sanitary and hygienic importance of this measure, it is required to know the scale of production and consumption, the number of workers engaged in the production who contact the poison and run the danger of poisoning, etc.» (pages 15—16). «The more we approach to the total elimination of a given substance from the occupational atmosphere, the higher is the cost of ventilation systems, the more expensive is their operation, since under high levels of capacity every new milligram of the substance removed involves... proportionally growing expenditures» (page 54).

The afore-cited opinions should not, on no account, be interpreted as an appeal of N. S. Pravdin and his followers to ease the struggle for the highest level of public health, for the most rigid (scientifically substantiated) protection of the environment against pollution with chemicals, since the authors emphasize the necessity of a thorough scientific substantiation of the required level of hygienic standards, based on medical indices.

Some toxicologists are trying to justify the lessening of their efforts to ensure healthy labour conditions, referring to economic, technical and other difficulties. Thus, Magnuson (1965) wrote: «Although we recognize extremely low MACs for highly toxic compounds, excessive control for safety of their effect wastes human resourses and prevent the use of chemical substances and processes which by themselves may have enormous importance for progress in social, economic and physical health». It is not difficult to see a contradiction in this statement. One cannot expect any serious progress in health protection unless the principal conditions of its preservation and enhancement are observed and environment pollution with toxic substances is limited.

Speaking at the International exhibition «Chemistry», Smith (1965) asserted that small amounts of toxic substances were, supposedly, necessary to maintain sufficient tonus in living organisms. That is why many US toxicologists propose to take as the threshold of harmful effect such minimum concentrations which cause pathomorphological or narcotic effects (Smith et al., 1962; Stokinger, 1962 et al.), or at least initial symptoms of damage (T. Hatch, 1973; V. A. Newell, 1974).

Abel (1974) has found the «optimal», from his point of view,

levels of expenditures (and thereby, of hygienic standards) by comparing the amount of losses caused to society by the environment pollution (including indirect cost of premature death, calculated from the average lost sallary equal to US $ 240,000) and necessary expenditures for prevention of this pollution. These standards suggest that a considerable number of premature deaths remains.

Practical Recommendations

Thus, there are two tendencies in defining the notion «harmful effect». According to one of them, «harmful effect» is considered injuring. In this case, the following indices of evident pathology are taken as the threshold of the poison's effect: processes of inflammation, degeneration, necrobiosis, metaplasia, etc., or such disturbances of functions as narcotic or spasmodic symptoms. Economic approaches to the determination of the «optimal» pollution concentrations have also been suggested. According to the other tendency, any response to external factors (including, for example, orientation response) is considered to be an index of harmfulness.

In our opinion, both tendencies are unjustified. One of them puts the population in an unfavorable situation: only an outbreak of a disease can make revise the existing hygienic standards. The other trend lead, in some cases, to unjustified underestimation of hygienic standards, and thereby to unjustified expenditures (first of all in industry).

In this connection, we would like to emphasize once again the priority of medical indices in respect to all other approaches to the substantiation of preventive measures connected with the development of the chemical industry and with introduction of chemical technology into all spheres of national economy and into everyday life. In all the cases, we need the most rigid scientific substantiation of necessary preventive measures. For an objective evaluation, we must have appropriate conditions for scientific research: satisfactory statistical groups, a sufficient set of animal species, the possibility to replicate experiments, to detect «informational» noise (S. V. Speransky, 1971). Certainly, there are neded a probabilistic evaluation of the phenomena, extensive sociological investigations on relationships between the conditions of the productional sphere, everyday life and public health conditions. These data — sufficiently formalized in order to be treated by computers — would exclude subjectivism in adopting important decisions. At the same time, these conditions, though providing for an increase of the initial data reliability and for the possibility of the data extrapolation between species (or interpolation), cannot solve per se the problem of objective deter-

mination of the thresholds of harmful effect of poisons. Precise formulations which could be used in practice are urgently needed.

E. B. Kurlyandskaya and I. V. Sanotsky (1965) consider harmful for the organism those responses which, although being still within the limits of physiological variations of the organism, turn into pathology under conditions of long-term continuous exposure to the irritant that has produced them. As the threshold of harmful effect under conditions of chronic exposure to chemical substances should be considered statistically reliable deviations from the control and from the initial values of responses of the complex of the physiological systems which are the most sensitive to a given exposure, i. e., the responses which are on the interface between the physiological variations, physiological means of protection and pathological processes.

> In his work «20 Years of Experience of Objective Investigation of the Higher Nervous Activity of Animals» I. P. Pavlov (1923) pointed out that «from the biological point of view the functional activity is the result of a many-centuries activity of the animal organisms under the known conditions of the existence on the earth, under the conditions of adaptation to the environment and hereditary reproduction of the acquired morphological and physiological properties». In the process of phylogenesis, the organism has worked out various specific and non-specific adaptive mechanisms. These mechanisms are directed mainly at the adaptation to rapidly changing environmental conditions.

Protective responses have been worked out also to different chemical substances, accumulating in the organism endogenously in the process of metabolism (phenols, amines, alcohols, carbon dioxide, hydrogen sulfide, etc.). To such mechanisms should be assigned detoxication and transformation of poisons as a result of the processes of oxidation, reduction, deamidization, methylation, binding by natural complexones, etc. Substances entering the organism join the cycle of the settled metabolic processes. Though there appeared new categories of substances which the organism has never faced before, these substances, after entering the organism in different ways, join the reactions worked out in the process of phylogenesis. Processes of transformation and detoxication of poisons are protective adaptive responses of the organism, which balance its relationship with environment. The degree of development of this relationship determines the physiological norm worked out in the process of phylogenesis.

In the process of labour activity, however, the organism may be exposed to the effect of chemical irritants, exceeding the

physiological means of protection, which may be conditionally called adaptation. This envolves protective reserves which serve to compensate the disturbed functions. Thus, the threshold of harmful effect is connected with transitional processes lying between the physiological reactions of adaptation and the condition of their «breakdown» (I. P. Pavlov, 1923). I. P. Pavlov pointed out that the pathological condition is «an encounter, a contact of the organism with some extraordinary condition, or rather with an unusual scale of everyday conditions». Determination of the limits of this «extraordinary condition» is, in fact, the task of preventive toxicologists. In practice, this work results difficult. A classification of the main types of the threshold changes, complying with the harmfulness criterion for the conditions of occupational exposure is presented below.

Type of response	Criterion of harmfulness
Evident pathology	Index is not valid for determination of the thresholds of the acute (Lim_{ac}) and chronic (Lim_{ch}) effect
Changes under a single exposure are within the limits of physiological fluctuations, but under chronic exposure they transform into pathology	Application of this index for determination of the Lim_{ac} is difficult
Changes are within the transitional range of phenomena, i. e., between the norm and pathology	Boundary between the norm and pathology is often uncertain
Changes are within the limits of physiological fluctuations, however, functional loads reveal unbalanced condition of the external and internal media of the organism (decrease of adaptive ability)	Detection of changes depends on the magnitude of the applied load

Thus, abstract formulas alone often appear insufficient for practical determination of the threshold of the poisons' effect.

Special investigations (I. V. Sanotsky et al., 1967) have been carried out to clarify which responses, being within the limits of physiological variations, transform into pathological phenomena under conditions of a long-term continuous exposure to the irritants that have produced them. The authors have applied substances with before-established chronic effect thresholds: carbon tetrachloride, furan (poisons affecting mainly the liver), epichlorohydrin (irritates upper respiratory tract and lungs, affects the parenchymatous organs), formaldehyde (mostly irritant), aniline (methemoglobin former), tert-butylperacetate (gonadotropic poison), etc. There have been studied about 30 structural and functional indices, conditionally subdivided into 3 groups according to

the type of responses: at the level of integral organism, at the level of systems and organs, at the level of cellular and subcellular structures (Table 3).

Table 3

The most sensitive responses of white rats under a single 4—hour inhalation exposure to poisons at the level of the threshold of chronic effect

Substance	Indices	
	Immediately after exposure	on the next day
Epichlorohydrin	Diuresis after water load (—), relative density of urine (+), urine chlorides (+) (analysis 18 hr later), relative liver mass (+), bromosulfalein (+), oxygen consumption (—), body temperature (—)	Body temperature (+), oxygen consumption (—)
Carbon tetrachloride	Relative liver mass (+), sorption of neutral red blood cells by the liver during lifetime (+), time of mobility of spermatozoa (—)	No changes
Furan	Frequency of systoles (—), arterial pressure (—), adrenal glands activity (+)	Arterial pressure (—)
Aniline	Oxygen consumption (—), phagocytic number (—), bactericidal properties of plasma (—)	Phagocytic number (—), bactericidal properties of plasma (—)
Formaldehyde	Changes in human spirogram	No changes

Note: (+) increase, (—) decrease.

The data given in Table 3 demonstrate that under a single exposure to poisons at the level of the chronic effect threshold there usually changed the pathogenetic indices: the state of the liver and kidneys (under exposure to epychlorohydrin and carbon tetrachloride); the state of regulation of the cardiovascular system (under exposure to furan[1]); the state of the respiratory organs (under exposure to formaldehyde). However, in a number of cases, at the mentioned level there were also registered changes in the responses closely connected with the neuro-endocrine system (body temperature, activity of the thyroid and adrenal glands, immunologic responses).

In the majority of cases, other integral indices did not change at this level (their changes were usually observed only at the Lim_{ac} level).

Thus, on the one hand, the acute effect threshold is to be considered conditional, since the Lim_{ac}, determined by the pathogene-

[1] The assignment of the latter to hepatotropic poisons becomes therefore doubtful.

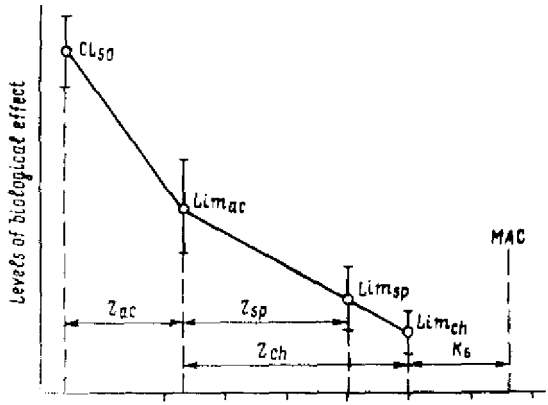

Fig. 6. Parameters of toxicometry (toxicity and hazard).

tic indices is approaching the Lim_{ch} (Fig. 6). On the other hand, specific (pathogenetic) responses to each poison are closely linked with non-specific (integral) neuro-endocrine changes, which are characteristic for minimum external influences of any nature.

As follows from Table 3, under a single exposure to poisons at a concentration equal to the before-established threshold of chronic effect, the following changes of indices appeared in one case stable (i. e., preserved until the moment of the next exposure): the body temperature (its changes were within the limits of physiological variations), the arterial pressure under exposure to furan, and bactericidal properties of the blood serum under exposure to aniline. Under exposure to formaldehyde, the next day after the exposure no residual changes were discovered by means of the applied methods. The endocrine glands were proved to have the largest universality of the condition changes.

Other experiments were conducted in the following way: the substances concentrations, corresponding to the established acute and chronic effect threshold and also (sometimes) to the CL_{50} or MAC, were being produced simulteneously in several chambers. Numerous integral and pathogenetic indices of the condition of the organism, separate organs and systems, tissues and cells were used. During 8—14 days animals were subjected to a daily 4-hour exposure to different poisons (according to some reports, the compensation phase used to set in at a given time).

The functional condition of pituitary body was determined by its relative weight, secretion of ACTH and gonadotropins, and by means of morphological analy-

sis (N. M. Karamzina, N. S. Grodetskaya, 1972). The functional condition of thyroid gland was determined by its relative weight, inclusion of I^{131} and by means of the quantitative morphological analysis (N. S. Grodetskaya, 1971). The functional condition of adrenal glands was determined by their relative weight and ascorbic acid content (investigation by N. M. Karamzina). The condition of testis was assessed by the weight coefficient, using morphological analysis (E. M. Tchirkova, 1970), determination of the content of nucleic acids and of the rate of inclusion of radioactive phosphorous into them (E. Y. Golubovich, 1970).

Table 4

Functional condition of some organs under replicated exposure to substances at the Lim_{ch} level

Substance	Level of exposure, mg/l	Days of exposure					
		2		4		8	
		thyroid gland	adrenal glands	thyroid gland	adrenal glands	thyroid gland	adrenal glands
Ethyleneimine	0.0006	—	+	—	0	+	0
Benzene	0.02	—	0	0	0	0	0
Carbon tetrachloride	0.01	+	0	0	0	0	0
Aminobenzotrifluoride	0.001	+[1]	0	+[2]	0		
Tertbutylperacetate			0	+[3]	0		
Dimethylformamide	0.03	—	0	0	+	0	0
Morpholine	0.006	—	0	0	0	0	0
Mercury vapours	0.00015	—	0	—	—	0	0
Ethylene oxide	0.05	0	0	—	0	0	0
Phosphorous oxychloride	0.00005	+	0	0	0	0	0

Note: (+) increase of activity; (—) decrease of activity; (0) no changes. Investigation was carried out: ([1]) on the 1st day; ([2]) on the 3rd day; ([3]) on the 5th day.

As can be seen from Table 4, the earliest changes at the level of the acute effect threshold were found in the function of the thyroid gland. The condition of adrenal glands, according to the applied orientation tests, usually did not change during the specified period. The results obtained do not fully agree with the data provided by other authors (B. A. Kurlyandsky et al., 1972). In the specified period, however, no changes were registered in the function of adrenal glands. One could have suggested that the activation of hypophysis-adrenal glands system would begin earlier (for example, on the first day). This assumption was not confirmed in course of the investigation of the effect of ethyleneimine and aminobenzotrifluoride. As for furan (exposure at the Lim_{ch}

level), it caused on the first day an activation of the adrenal cortex which disappered on the second day.

Thus, it turned out that not only the acute effect thresholds, determined by pathogenetic indices, could approach the chronic effect threshold (when many structures and functions undergo changes), but also a number of integral (non-specific) responses can be observed under a single exposure to a poison (at this low level of exposure, mainly responses of thyroid gland are registered). It is well known, however, that thyroid gland, as well as

Natural seasonal and average annual data on

Season	Maximum inclusion of I^{131}, %					Epithelium height				
	n	M	±σ	±1.5σ	±2σ	n	M	±σ	±1.5σ	±2σ
Winter	24	28.2	10.2	15.3	20.4	24	3.01	0.3	0.45	0.6
Spring	48	42.4	11.8	17.7	23.6	24	2.3	0.2	0,3	0.4
Summer	24	9.9	3.0	4.5	6.0	—	—	—		
Autumn	24	42.5	11.8	17.7	35.4	48	3.2	0.5	0.75	1.0
Average annual	151	29.8	10.4	15.6	31.2	114	2.78	0.46	0.69	0.92

the marrow of adrenal glands, belongs to the organs of tactic regulation, and there is no guarantee that the acute effect threshold, determined by the indices of the thyroid gland condition, fluctuating within the physiological limits, will appear to be at the Lim_{ch} level, which is being determined later, in case of a new unknown substance. Similar difficulties are encountered regularly also under the conditions of functional and extreme loads application. In this case, the probabilistic evaluation of the considered facts is often insufficiently definite. That is why a new progressive definition of the indices of harmfulness of the changes, occurring under exposure to certain substances, has been elaborated.

The threshold of harmful effect of a substance is its minimum concentration (or dose), exposure to which causes changes in the organism characterized by the following indices:

— changes reliably ($p<0.05$) differ from the control and exceed the limits (over 2σ) of physiological fluctuations of the index for a given animal species for a given season;

— no reliable ($p<0.05$) changes in comparison with the control are observed; latent disturbances of the balance with the environment (decrease of the adaptive ability) revealed, in particular, by means of functional and extreme loads (responses go beyond the limits of $\pm 2\sigma$ of the respective norm);

— changes reliably ($p<0.05$) differ from the control and are

within the limits of physiological norm, but are stably preserved (for over one month in experiments on animals).

There has been introduced the definition of the «physiological norm», i. e., of physiological variations.

The checking of the practical application of the mentioned rules has demonstrated that in many cases the «physiological norm» was unknown. Specially organized experiments have confirmed that this norm is subjected to considerable seasonal and sometimes even daily variations (see Annex, Tables 1—5).

Table 5

the indices of the thyroid gland function

Diameter of nuclei of follicular epithelium					Index of conversion of I^{131}				
n	M	±σ	±1.5σ	±2σ	n	M	±2σ	±1.5σ	±2σ
18	23.5	1.3	1.95	2.6	24	51.5	20.0	30.0	40.0
18	23.4	0.9	1.35	1.8	24	72.3	16.0	24.0	32.0
—	—				—				
—	—				24	48.9	22.3	33.4	44.6
55	23.4	2.01	3.01	4.02	60	70.5	11.6	17.4	23.2

Variations of some indices of the condition of the thyroid gland of rats (Table 5, the data by N. S. Grodetskaya, 1971) can serve as an example. Thus, the investigation of the initial responses of the organism under exposure to 13 toxic substances (metallic mercury, carbon disulfide, benzene, carbon tetrachloride, morpholine, dimethylformamide, ethylene oxide, bromoacetopropylacetate, ethyleneimine, phosphorous oxichloride, sodium fluoride, triphtazine, monoallylamine) in concentrations at the Lim_{ac}, Lim_{ch}, as well as at the MAC level has shown that the functional conditions of thyroid and adrenal glands was, for majority of cases, within the limits of variations of the annual physiological norm of experimental animals. The only exception was the diameter of the nuclei of follicular epithelium under exposure to morpholine and mercury. Those changes were observed under exposure to the poisons in concentrations either at the Lim_{ac} and Lim_{ch}, or at the MAC level.

Although variations of other indices corresponded to annual variations in the control group, in a number of cases they appeared beyond the limits of the seasonal physiological norm. Most of the considered initial responses may, nevertheless, be formally assigned to true accomodation, adaptation responses.

Taking into account the existence of the thresholds of the effects under study, as well as possible differences in the initial

condition of the organism, 2 to 5-fold replications of the experiment under exposure to a number of substances (carbon disulfide, morpholine, triphtazine, carbon tetrachloride) at the same levels and under the same regimens of exposure have been accomplished. Considerable variability of the effects has been revealed under minimum exposures. This is evidently a result of different frequencies of the animal vital rhythms (I. V. Sanotsky, 1973) and it may indicate the presence of the effect, as it has been proved when processing the data by methods of non—parametric statistics (S. V. Speransky, 1970).

Thus, interrelations between processes of true adaptation and the compensation of a latent pathological process in the phase of initial responses of the organism appear rather complicated. A short-term duplicate experiment requires a thorough and many-sided investigation of the regulatory systems.

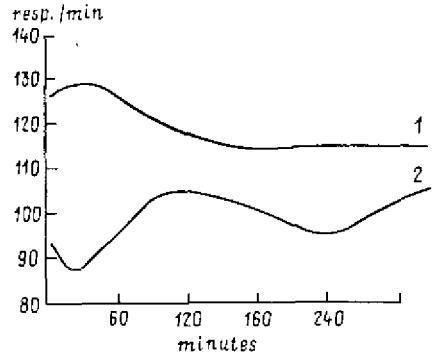

Fig. 7. Changes in the respiration rate of white rats during a 4-hour exposure to an SO_2 concentration at the Lim_{ir} level.
(1) control; (2) experiment. Y-axis, respiration rate; X-axis, duration of exposure.

Similar difficulties have also been encountered when studying the irritant effect thresholds. Thus, it has been found out (A. A. Asmangulyan, A. M. Klyatchkina, 1972) that the respiration rate of animals fluctuates within a wide range during a day or even an hour (Fig. 7). Only a special processing of the experimental data (method of conditional averages, offered by V. N. Bondarenko) has permitted to reveal differences between the test and the control groups of animals. These differencies were, however, within the limits of physiological variations, though it is known that SO_2 produces chronic pathogenetic process when used in the concentrations applied. Determination of the chronic effect threshold by the integral indices of the condition of the cell protoplasm of the pulmonary tissue (intravenous injection of neutral red blood cells, modification by A. L. Germanova, 1970) has shown that the intensity of changes had no direct de-

Table 6

Lifetime coloration of lungs under inhalation exposure to irritant substances (ageing of colorant is not taken into account)

Substance	Concentration, mg/m³	Lifetime coloration (accumulation), extinction units	
		control	test
2-Chloroethane-sulfochloride	4.2	0.51±0.014	0.51±0.028 P>0.05
	12.0	0.60±0.021	0.68±0.022 P<0.05
	34.0	0.69±0.021	0.61±0.02 P<0.05
Trichloroacetic chloride	0.6	0.65±0.013	0.68±0.009 P>0.05
	1.0	0.59±0.014	0.66±0.014 P<0.01
	1.8	0.56±0.015	0.62±0.018 P<0.05
Chloroacetopropylacetate	6.7	0.64±0.017	0.71±0.032 P>0.01
	20	0.63±0.027	0.68±0.025 (P>0.5)
	60	0.69±0.032	0.55±0.045 P<0.05
Bromoacetopropylacetate	4.0	0.65±0.03	0.69±0.032 P>0.25
	12.6	0.6±0.021	0.61±0.031 P>0.5
	18.0	0.69±0.027	0.6±0.019 P<0.05
Morpholine	3.0	0.64±0.016	0.65±0.022 P>0.25
	40.0	0.64±0.016	0.67±0.022 P>0.25
	260.0	0.69±0.016	0.79±0.03 P<0.02
Phosphorous oxychloride	0.8	0.71±0.021	0.75±0.02 P>0.1
	4.0	0.59±0.024	0.5±0.028 P<0.05
	8.0	0.56±0.038	0.72±0.04 P<0.01

pendency on the intensity of exposure (Table 6). The changes did not go beyond the limits of seasonal (to say nothing about annual) physiological variations, while substances in the concentrations applied are known to cause manifest pathological effect under long-term exposures.

Principles of Differentiation between Adaptation and Compensation

The above-presented data show that, though new formal rules have been introduced, it is sometimes difficult for various reasons to decide whether changes occurring in the organism under exposure to chemical compounds are harmful or not.

Sanotsky et al. (1971) have tried to formulate the principles for differentiating the responses of biological adaptation from the responses of compensation under the appeared pathological process, i. e., to reveal the zone lying between health and illness. These principles are based on the following biological laws: preservation of the species population, the unity of the organism and its environment, the integrity of the organism as a biological system.

These principles do not cover all possible approaches to the evaluation of the limits between the physiological adaptation and compensation of the pathological process. Further development of these principles is considered to be one of the major tasks of preventive toxicology. We assume, nevertheless, that the cited formulas complement the regulations established by the Commission on the MACs Substantiation.

According to the definition given by the Statute of the World Health Organization, health is a state of a complete physical, mental and social wellbeing, and not merely the absence of a disease or disablement. Four levels of the health condition are here given: mere survival, absense of disturbances or diseases, safe and effective working capacity, and a fully-fledged life. The first condition, i. e., mere survival is the closest to the disease.

Thus, survival and increase of the species population under specified conditions of existence can be considered as indications of health, which are closely related to the individuals' ability to adapt to their environment. And on the contrary, a reduction of a species population indicates disturbances in the adaptive processes, leading to a disease.

Evaluation of the adequacy of the environmental conditions to hereditary and acquired properties of the organism at the level of population (preservation of the species population). Results of the studies of some indices of generative function and mutagenic effect on somatic tissue under exposure of animals to a number of substances are shown in Table 7. Annual and seasonal variations of the values of these indices are illustrated in Tables 9—11 and in Fig. 8.

As can be seen from the Tables, at certain levels of exposure there have been registered changes in the generative function: in other cases, changes in the number of the offspring (congenital or postnatal). Sometimes, for example when males of white rats were exposed to mercury, the offspring numbers of the first gene-

Table 7

Chromosomal aberrations in somatic tissue of rats exposed to poisons

Substance	Exposure conditions		Goes beyond the limits of			
	concentration, mg/m³	exposure	spontaneous level	±1.5δ	±2.0δ	control
Ethyleneimine	10	1 month	+	+	+	+
	0.6	2 days	—	—	—	—
	0.6	4 days	—	—	—	—
	0.6	8 days	+	+	+	+
	2.4	Single exposure	—	—	—	—
	0.8	Killed on the 3rd day	+	+	+	+
	2.4	On the 8th day	+	+	+	+
	0.8		+	+	+	+
	2.4	On the 14th day	+	+	+	+
	0.8					
	2.4	On the 28th day	+	+	+	+
	0.8		+	+	+	+
Propylene oxide	25	4 months	—	—	—	+
Carbon tetrachloride	40	8 days	+	—	—	—
Dinitryl perfluoradipate	5—8	8 days	+	+	+	+
Di-n-propylamine	20	8 days	+	+	+	+
	2		—	—	—	—
1,3-Chlorobromopropane	3—4	4 months	—	—	—	—
	30—40	4 months	+	—	—	+
Formaldehyde	5	20 days	+	—	—	—
	0.5		+	—	—	+
Chloroprene[1]	3.5	2 months	+	+	+	+
	0.32		—	—	—	—
Mercury	10 and irradiation	Single exposure	+	+	—	+
	0.02		+	—	—	+
	1.0 and irradiation		+	+	—	+

Note: (+) increase; (—) decrease.

[1] Experiments on mice.

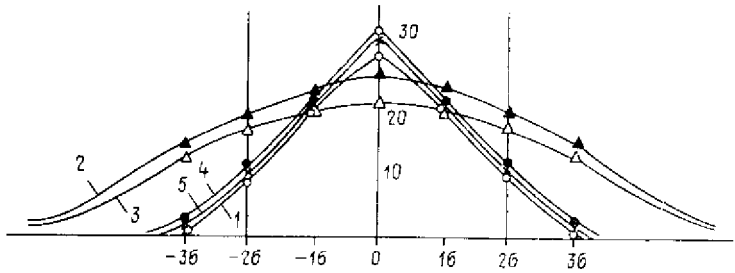

Fig. 8. Physiological fluctuations of the quantity of normal spermatogonia in white rats.

(1) annual; (2) in autumn; (3) in summer; (4) in spring; (5) in winter.

Table 8

Some functional and extreme loads

Type of load	Dose or intensity	Responding system or index	Time of recording
Chemical:			
CCl_4	1600 mg/kg into the stomach (Lim_{ac})	Liver, nervous system	The next day
methylene chloride	16.5 ± 2.7 mg/l ($2Lim_{ac}$), 1-hr inhalation	The same	10 min after
Pharmacological:			
alcohol	2 mg/g of 95° alcohol as 10% solution subcutaneously	Nervous system	The same
hexenal	90 mg/kg of 10% solution intraperitoneally	Nervous system, liver	The same
adrenaline	4 mg/kg of 10% solution intraperitoneally	Cardiovascular system	The same
caffeine	30 mg/kg of 10% solution per os	Nervous and excretory system	The same
	The same		The next 24 hr
bromosulfalein	0.5 g/kg of 0.2% solution subcutaneously	Liver	1 hr and 15 min after
atropine	50 mg/kg of 2% solution subcutaneously	Cardiovascular system	30 min after
neostigmine methylsulfate	1 mg/kg of 0.05% solution subcutaneously	Cardiovascular system	30 and 60 min after
pituitrin	0.15 units/kg intravenously	The same	30 sec after
Physical:			
cold	3—5° for 15 min	Integral index (rectal temperature)	In dynamics 15 min, 30 min, 60 min, 90 min
ionizing irradiation	500—750 R for 1 min	Integral index (death of animals)	Within 2—3 weeks
noise	60—80 dB from 10—20 sec to 1.5 min	ACTH	4 hr after
increased temperature	30—40° for 2 hr	Integral (sweating, rectal temperature)	Immediately after stopping the load
low atmospheric pressure	15 min at a 8000 m height	Integral (respiration rate)	In dynamics before lifting, after lifting, 15 min after reached the height, after descent and 15 min after descent
Mechanical:			
swimming	Water temperature is 37°	Integral (time of swimming)	During the load

Continued

Type of load	Dose or Intensity	Responding index or system	Time of recording
running on moving track	Strip's speed is 25 m/min	Integral (time of the animals' movement)	The same
Biological:			
phlebotomy	1—10% of the body weight	Hemopoetic system (blood count)	1, 3, 7 and 14 days after
hunger	First 24 hr of the experiment	Integral (body weight)	The next day
typho-paratyphoid vaccine	2—5 billion microbic bodies/kg	Integral (rectal temperature)	1—2 hr after
toxin B. perfringens	0.8 of the test unit (mice)	Integral (death of animals)	Within 2—3 days
toxin B. mesentericus	1—2 ml/kg of broth culture	Integral (rectal temperature)	1 hr after
Pregnancy (2 females for 1 male)		Integral (condition of the offspring and of the mother's organism)	21 days after

ration appeared to increase and harmful effect (decrease of the offspring numbers) could be observed only in the second generation.

Assessment of the unity of the organism and its environment, or of the ability to adapt to the environment, is closely related to the first rule considered above. The adaptive reserves have long been evaluated by means of placing the organism under extreme physiological (or pathogenetic) conditions: temperature, nutrition and biologic conditions. There are also various other functional or extreme loads of chemical, physical and biological nature, which usually have integral or pathogenetic (sharply directed) character (some examples are given in Table 8). The loads often help to reveal a latent decrease of the adaptation (to physiological loads) and compensation (to pathogenetic, extreme loads) ability of the organism.

To supplement the publications on this issue (M. N. Rylova, 1964; I. M. Trakhtenberg, 1966; I. P. Ulanova et al., 1971; B. A. Kurlyandsky et al., 1972), we should say that, for example, phlebotomy helped us to reveal latent differences in the rate at which mature and young animals regenerated their peripheral blood after a single intake by inhalation of benzene vapours at the level of the existed MAC (young animals had weaker adaptive abilities). The reverse correlation was registered after exposure to CCl_4 at the Lim_{ac} level, while disturbances of health condition and differences in the summation threshold index seemed to be absent. Since the ethanol load enabled us to reveal that the cent-

Tabl

Deviations of some indices of the function of gonads from the control seasonal physiological norms exceeding 2σ in rats exposed to industrial poisons

Substance	Level and duration of exposure		Concentration, mg/m³	Summated quantity normal spermatogonia		Quantity of tubules				Index of spermatogenesis		Mobility spermat	
						with 12th stage of meiosis		with peeled off germinal epithelium					
				P	2σ	P	2σ	P	2σ	P	2σ	P	
Aminopiramidine	Lim_{ac} integr.,	1 day	61.9	0.001	—	—	—	0.02	+	—	—	—	
	Lim_{ac} spec.,	1 day	22.4	0.02	—	—	—	—	—	—	—	—	
	Lim_{ch},	4 months	11.4	0.01	+	0.002	+	0.002	+	—	—	—	
	1/10 Lim_{ch},	4 months	1.4	—	—	—	—	—	—	—	—	—	
Ethyleneimine	3Lim_{ac} spec.,	1 day	2.4	0.001	+	—	—	—	—	—	—	—	
	Lim_{ac} spec.,	1 day	0.8	0.001	+	—	—	—	—	—	—	—	
	Lim_{ac} integr.,	1 month	12	0.001	+	—	—	—	—	0.001	+	0.001	
	Lim_{ac} spec.,	2 days	0.6	0.01	+	—	—	—	—	—	—	—	
Tert-butylperacetate	10Lim_{ch},	4 months	10	0.02	—	0.001	+	0.05	—	—	—	0	
	7Lim_{ch},	4 months	7	0.02	—	—	—	0.05	+	—	—	0	
	Lim_{ch},	4 months	1	0.05	—	0.002	+	0.05	—	—	—	0	
	1/3Lim_{ch},	4 months	0.3	—	—	—	—	—	—	—	—	0	
Lead	Lim_{ac} spec.,	1 day	40[1]	0.001	—	—	—	—	—	—	—	0	
	1/20Lim_{ac} spec.,	1 day	2[1]	—	—	—	—	—	—	—	—	0	
	1/20Lim_{ac} spec.,	20 days	2[1]	0.01	+	—	—	0.01	+	—	—	0	
1,3-chlorobromopropane	Lim_{ac} integr.,	1 day	410	0.001	+	—	—	—	—	—	—	0.01	
	1/20Lim_{ac} integr.,	1 day	18	—	—	—	—	—	—	—	—	—	
	Lim_{ch},	4 months	45	0.01	—	—	—	—	—	0.01	—	—	
	1/10Lim_{ch}	4 months	5.4	—	—	—	—	—	—	—	—	—	

Note: Statistically insignificant changes (when $p>0.05$) are indicated as O; changes deviating from p siological norms by more than 2σ are indicated as +; those by less than 2σ as —. [1] is dose mg/kg.

ral nervous system inhibition in mature animals was smaller than that in the control group (young animals did not exhibit similar differences), we suggest a possibility of impairment of the hemato-encephalic barrier permeability (G. G. Avilova, 1970).

Latent defects in the offspring of females exposed to metal mixtures (radioactive thorium, thorium, lead) were detected when investigating the death rate of the offspring after total X-ray irradiation (I. V. Sanotsky, M. Y. Savina, 1971). A high percentage of survivals was registered under the extreme load with γ-irradiation under exposure to triphtazine, whereas exposure to carbon disulfide caused depression of the compensatory abilities: the death rate was considerably higher than in the control group (I. V. Sanotsky, G. G. Avilova, G. A. Sheveleva, 1972). Acceleration of restoration of the initial level of rectal temperature after cooling was revealed in animals under a single exposure to mercury and ethylene oxide at the level of the established Lim_{ch} and MAC. Under exposure to the same concentrations of phosphorous oxychloride, the condition of the animal organism, on the contrary, came slowly back to the initial level (N. M. Karamzina, N. S. Grodetskaya, G. I. Pavlenko, 1973). Similar results were obtained also in the field of communal toxicology (water, atmosphere, food).

It is necessary to point out that sharply directed loads allowed to determine the levels of the poisons' effect, connected with the participation in the process of different systems. Thus, the exposure to mercury at the level of the established chronic effect threshold and MAC caused prestress responses in the hypothalamus-pituitary body — thyroid gland system (direct effect on the thyroid gland is not excluded either) without affecting vegetative regulation (as for the indicator, the condition of heart under pharmacologic load was investigated). The vegetative regulation was found to be disturbed (discovered under pharmacologic load with adrenaline) only at a one order higher level.

The problem of determination of the degree of harmfulness of the changes, occurring in the enzymatic systems under exposure to poisons, stands apart. The specific property of poisons to react with the protoplasm components, and thereby to disturb vital functions of cells (including the nervous system cells), the capacity of poisons to disturb the most important function of the organism, i. e., the respiration of organs and tissues, to inactivate enzymes under direct contact and many other properties irrefutably demonstrate that the poison's effect on the organism is directly related to the «chemism of the organism».

Some similar questions have been studied by L. A. Tiunov and V. V. Kustov (1970). The authors drew the attention to the decrease or increase of the enzymatic systems' activity, to changes in the correlation of certain enzymatic systems, occurring under exposure to poisons. If the enzymes activity decrease is followed

Season	Index of spermatogenesis					Summated quantity of normal spermatogonia				Quantity with off epithelium	
	n	M	1σ	1.5σ	2σ	M	1σ	1.5σ	2σ	M	1σ
											For 3
Winter	72	3.75	0.1	0.15	0.2	31.21	9.8	14.7	19.6	1.98	1.7
Spring	55	3.68	0.19	0.285	0.38	31.63	10.2	15.3	20.4	2.16	1.0
Summer	48	3.76	0.03	0.450	0.06	21.7	2.8	4.2	5.6	1.79	0.9
Autumn	30	3.72	0.17	0.265	0.34	25.22	3.0	4.5	6.0	4.25	0.06
Annual	205	3.73	0.15	0.225	0.3	28.15	8.1	12.15	16.2	2.42	1.4
											For 1
Winter	40	3.74	0.1	0.15	0.2	32.1	8.6	12.9	17.2	2.01	1.58
Spring	40	3.69	0.16	0.24	0.32	31.9	9.8	14.7	19.6	2.36	1.2
Summer	32	3.75	0.02	0.03	0.04	20.8	2.2	3.3	4.4	1.9	1.02
Autumn	—	—	—	—	—	—	—	—	—	—	—
Annual	112	3.72	0.12	0.18	0.24	28.9	7.6	11.4	15.2	2.62	1.22

by an increase of the substrate concentration, these changes should not be ignored, since they point out a serious disturbance in homeostasis.

If a significant biological effect of a poison (e. g., an increase of the mortality rate) is discovered under excessive formation in the organism of substrate for those system of the organism which exhibited changes («load tests»), then the degree of changes in the enzymatic system is to be considered as an indication of harmful effect. As an example, the authors cite a 2-fold load with serotonin (30 mg/kg) under exposure to ethylenediamine (300 mg/kg). The joint poisons' effect caused significant mortality among the experimental animals; when the poisons were administered separately, no mortality was observed.

L. A. Tiunov and G. A. Vasilyev (1966) suggested the principle of the «sand-glass» for assessing the significance of the changes in the enzymes. According to this principle, in every system, together with interchangeable enzymes, there are irreplaceable «key» enzymes, any significant change of which is considered by the authors inadmissible.

An increased activity of the enzymatic systems may be a result of the expansion of their compensatory synthesis, which is often observed under conditions of the so-called enzymatic adaptation. If an occupational poison, as a substrate of a given enzymatic system, causes an increase of its activity, this already indicates stress, i. e., the existence of harmful effect.

Table 10

function of spermatozoa in rats

of tubules						Time of mobility of spermatozoa, min				Weight coefficient of testis			
peeling germinal		with 12th stage of meiosis											
1.5σ	2σ	M	1σ	1.5σ	2σ	M	1σ	1.5σ	2σ	M	1σ	1.5σ	2σ
years													
2.55	3.4	3.25	1.3	1.95	2.6	227	42	63	84	0.95	0.06	0.09	0.12
1.5	2.0	2.83	1.2	1.8	2.4	258	22	33	44	1.03	0.05	0.075	0.1
1.35	1.8	3.37	0.3	0.45	0.6	262	23	34.5	46	0.91	0.09	0.135	0.18
3.09	4.12	3.57	1.1	1.65	2.2	282	96	144	192	0.69	0.11	0.165	0.22
2.1	2.8	3.24	1.1	1.65	2.2	259	36.5	86.25	115	0.84	0.12	0.18	0.24
year													
2.37	3.16	3.6	1.28	1.82	2.56	240	52	78	104	0.9	0.05	0.075	0.1
1.8	2.4	2.62	1.3	1.95	2.6	270	20	30	40	1.07	0.06	0.09	0.12
1.53	2.04	3.1	0.2	0.3	0.4	265	25	37.5	50	0.92	0.08	0.02	0.16
—	—	—	—	—	—	—	—	—	—	—	—	—	—
1.81	2.44	3.2	1.5	2.25	3.0	260	40	60	80	0.87	0.1	0.15	0.2

Table 11

Indices of cytogenetic analysis of the bone-marrow of rats

Season	Number of cells with chromosomal disturances, %					Mitotic index, %				
	n	M	1σ	1.5σ	2σ	n	M	1σ	1.5σ	2σ
For 3 years										
Winter	106	3.44	1.4	2.1	2.8	106	1.86	0.57	0.855	1.14
Spring	49	3.67	1.3	1.95	2.6	49	1.58	0.53	0.795	1.06
Summer	62	3.38	1.33	1.95	2.66	62	1.67	0.7	1.05	1.4
Autumn	43	4.7	1.35	2.02	2.7	43	1.85	0.44	0.66	0.88
Annual	260	3.79	1.35	2.02	2.7	260	1.74	0.54	0.81	1.08
For 1 year										
Winter	16	5.3	2·3	3.4	4.6	16	2.1	0.38	0.52	0.76
Spring	17	3.2	0.78	1.17	1.56	17	1.3	0.3	0.45	0.6
Autumn	—					—				
Summer	7	4.4	1.2	1.8	2.4	7	2.85	0.44	0.66	0.88
Annual	40	4.35	1.65	2.47	3.3	40	1.8	0.3	0.4	0.6

N. A. Kachurina and L. A. Tiunov (1965) suggested that a poison's harmfulness should be, in some cases, assessed by changes in the ratio of the activities of several enzymes of the same metabolic cycle. In a number of cases, this approach helped to reveal latent disturbanced caused by a poison.

There are also additional metabolic criteria used for the assessment of harmfulness of poisons. Thus, for poisons non metabolized (practically) — e. g., methylene chloride — the index of the adaptive systems disturbances (harmfulness criterion) is, in our opinion, a reduction of the rate of its excretion from the organism under the increase of the concentration of the latter (original data by N. M. Maltseva), i. e., the increase of the T 1/2 (Fig. 9). In this case, the functional changes ($p<0.05$) at the level, conditionally accepted as the threshold, did not appear beyond the limits of the seasonal physiological norm.

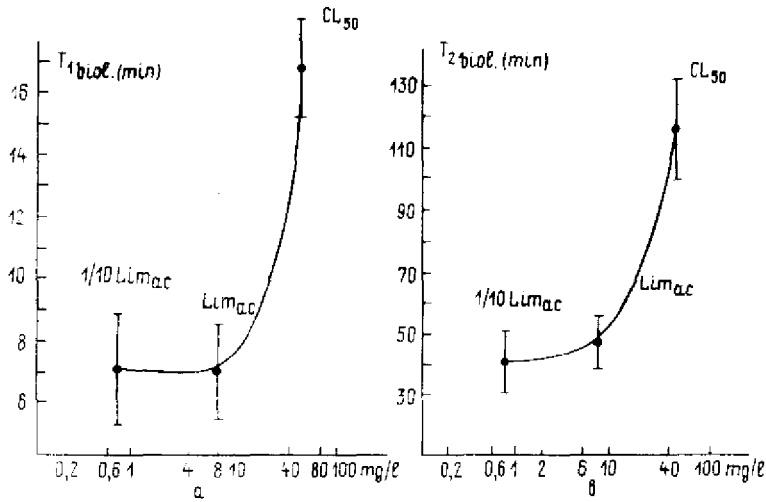

Fig. 9. Half-life of methylene chloride elimination from the blood of animals at different levels of exposure.

(a) for quickly eliminated fraction; (b) for slowly eliminated fraction. X-axis, concentration.

For metabolized poisons, such as ethyleneimine (original data by G. N. Zayeva, S. I. Muravyeva, N. N. Ordynskaya), with the harmfulness criterion may comply the effects produced under such concentrations (doses) of poisons which cause a decrease of metabolism (Fig. 10).

Thus, methods for differentiating the indices of a s i m p l y effect from the indices of a h a r m f u l effect have been proposed. Nevertheless, the problem of assessment of the hygienic significance of biochemical deviations remains: physiological variations

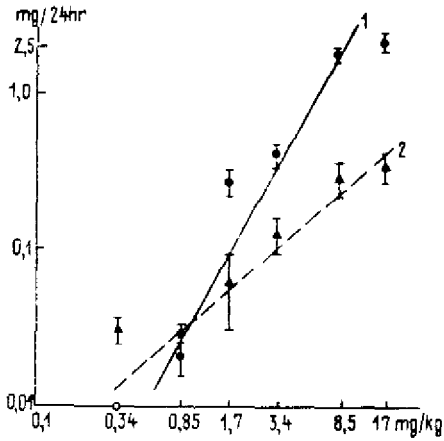

Fig. 10. Intensity of transformation: of ethylenimine (1) into monoethanoleamine (2) depending on the intensity of exposure to ethyleneimine. Y-axis, content of substances in urine, mg/24 hr; X-axis, doses, mg/kg.

of many indices, depending on variuos conditions (seasonal, nutrition conditions, etc.), have not yet been determined.

Investigation of the organism's integrity as a biological system for determining the indices of harmful effect of substances suppose, as we think, that all systems of the organism are studied from many sides and in complex. It has been shown in the literature the «absense» of the effect of a substance on the organism, determined by one or two indices, does not mean yet the absense of the effect (including a harmful effect) actually.

There are many examples of a temporary functional well-being with simultaneously existing serious structural damages. Along with traditional descriptions of the «habituation» to arsenic preparations, including that at the expence of atrophy of the digestive tract and the decrease of absorption, there have been obtained many facts of the decrease of the functional reactivity to irritants as a result of deep atrophic and necrobiotic processes in the mucous membrane of the upper respiratory tract (V. S. Pozdnyakov, 1971; N. A. Gincheva, 1972) (Fig. 11).

Rather significant are serious atrophic processes revealed in the seminal tubules with no disturbances in the fecundation function, in the state of the embryo and offspring (I. V. Sanotsky, 1965) and, on the contrary, there were registered lethal mutations, changes in the condition of the offspring (under exposure of males to such chemical compounds as ethylene oxide) which by themselves did not cause any evident damage in the germinal epithelium. There have been described certain dissonances between the general aggravated condition of the experimental animals and their preserved conditioned reflexes. On the contrary, general physiological well-being could be accompanied by manifest disturbances in embryogenesis. According to the embryotropic index for chloroprene or dimethyl acetamide (L. S. Salnikova, Z. A. Vol-

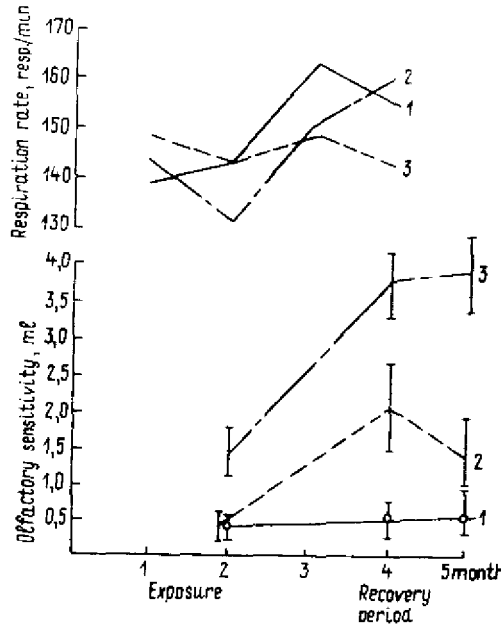

Fig. 11. Respiration rate and olfactory sensitivity (to tar) of rats under long-term exposure by inhalation to bromoacetopropylacetate.
(1) control; (2) Lim_{ir}; (3) Lim_{ch}.

kova, 1968; M. V. Nakoryakova, 1974), their Z_{sp} are 40 and 20, respectively. At the level of the threshold of a single exposure, the Z_{sp}, determined by the mutagenic and gonadotrop ilc effect of butyl trichlorophenoxyacetate, is more than 100 (L. P. Efimenko, 1973). At the same time, the process of the chromosomes reparation, observed in the bone marrow under exposure to prometrine and heptachlor, was accompanied by the increment of chromosomal rearrangements in the liver cells (L. P. Efimenko, A. E. Kulakov, 1969).

Therefore, only a many-sided evaluation of the organism, as an integral biological system (together with the subsystems evaluation), can make reliable our judgements on harmfulness or non-harmfulness of a given level of chemical exposure.

It should be noted that positive changes in the indices of vital activity are being in most cases interpreted as an indication of harmful effect. But is it right?

Studing the problem of «habituation», N. V. Lazarev (1938) pointed out the fact of the existence of true habituation, i. e., true adaptation to environmental changes without pathological «background» and the adaptation systems stresses. Later on there have been developed investigations of the «stage of non-specifi-

cally increased resistance» (SNIR) of the organism under exposure to exogenous substances. Many SNIR responses indicate a higher resistance of the organism to harmful agents and a considerable expansion of the qualitative characteristics of equilibrium between the environment and the organism. In a number of publications, the SNIR has been considered as a true adaptation to the environment. It was discovered, however, that under the prolongation of exposure the SNIR was always displaced by the stage of decompensated pathology, i. e., evident intoxicational conditions. To that phenomenon contributed high levels of the Poisons' effect, which were often chosen for the experiments. At present, most authors consider the SNIR to be an index of the Poisons'effect and a stage of chronic intoxication, though debates on the terms definition continue. E. I. Lyublina et al. (1971) suppose that, to characterize the SNIR, there could be used the term «adaptation». In our opinion, two phenomena are being confused here: some forms of failure to respond to the continuing entry of a foreign substance into the organism might be attributed to true accomodation (adaptation), while other forms indicate stresses of the adaptation systems (and may be even classical stresses) and compensation of the initiated pathological process. Several methods for differentiating these adjacent phenomena have been already considered above.

Recent investigations have enabled us to distinguish several stages of chronic poisoning which reflect the correlation between the processes of adaptation and accumulation of the effect that is at first compensated: primary decompensation («primary responses»); adaptation; compensation; secondary decompensation. The last two stages may repeatedly alternate in the process of chronic exposure («remission» and «exacerbation». The limits for the stage of primary responses, or those for the stage of primary decompensation (I. V. Sanotsky, 1969), as it has been shown by investigations of N. M. Karamzina, N. S. Grodetskaya, G. I. Pavlenko (1963) et al., cannot be determined definitely for all poisons and for all levels of exposure. These limits change according to the type and intensity of exposure, and according to the index chosen to characterize them. Moreover, at low levels of exposure the stage of compensation of the pathological process occurs in the majority of cases within 1—2 weaks (sometimes later).

Physiological adaptation, provided that it exists at the effective level of exposure to substances, is a phenomenon which disappears rapidly. The organism's contact with chemical environmental pollution at even much lower level must result (after possible orientation responses) in a true adaptation of the organism. It is here where should lie the boundary between the adaptation and compensation.

The duration of other stages of chronic poisoning is the same way different under simultaneously occurring processes. For

example, if we compare the rate of the chromosomes regeneration after disturbances with general manifestations of the chronic effect of the poison, certain discrepances will become obvious. The rate of stabilization of the chromosomes aberrations is shown in Fig. 12 (the data by E. E. Strekalova).

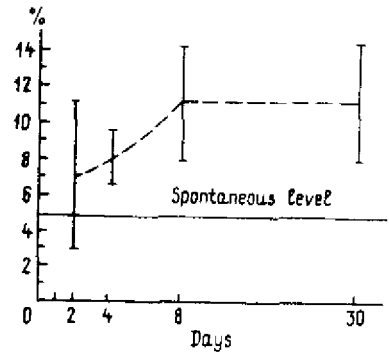

Fig. 12. Stabilization of chromosomal damages under chronic exposure to ethylene oxide.

As can be seen from Fig. 12, despite the continuation of the ethylene oxide effect, even such apparantly stable structures, as the hereditary information carriers, undergo significant, but, at the same time, reversible[1] changes. At the same time, it was determined that the respiration rate did not stabilize by the 30th day («habituation» occurred by the 66th day), although the morphological blood composition, oxygen consumption and spontaneous motor activity began to normalize earlier and by the 30th day they were close to the physiological norm.

Under a 7-month daily exposure to a number of organochlorines DDT, heptachlor, lindane), ethers of chlorophenoxyacetic acid (butyl 2,4-D and 2,4,5-T), thio-derivatives of triazine series (prometrin) and carbamates (zineb), the cytogenetic analysis of the bone marrow cells revealed a maximum number of the chromosomes aberrations (2 times higher than the spontaneous level) during the first 2.5 months of exposure. After 2.5 months, the number of the chromosomes damages began to decrease and it reached the norm by the end of the experiment (Fig. 13) (the data by L. P. Efimenko and A. E. Kulakova, 1969). Thus, we may suppose that the cellular population in response to unfavorable long-term chemical exposure generates mutant forms, among which there appear cells having increased resistance.

It should be remembered, however, that the presence of cytogenetical damages in either somatic or germ cells in the initial phase of intoxication, despite apparent further well-being, later may lead to unfavorable reactions: carcinogenesis, birth of defec-

[1] The possibility of a complete «reversibility» of changes causes justified doubts.

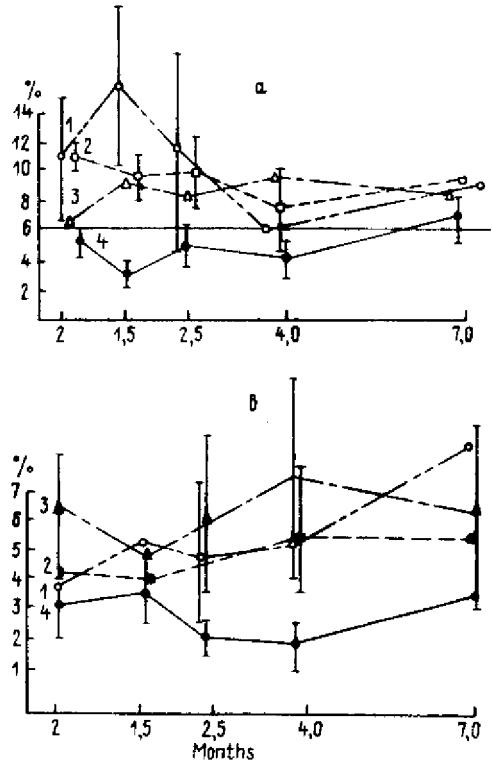

Fig. 13. Comparison of chromosomal damages (Y-axis) in cells

(a) of bone-marrow, (b) of liver under exposure to prometrin. (1) 1/30 DL_{50}; (2) 1/50 DL_{50}; (3) 1/100 DL_{50}; (4) control.

tive offspring, death of embryos at different stages of development, etc. The solution of the problem of the boundary between true adaptation and temporary compensation at the cellular level requires additional investigations and experiments.

L. G. Okhnyanskaya (1973) suggests the following criteria for the true adaptation to the environmental changes: (1) complete reversibility of deviations in case the exposure is intermitted and the organism returns to the initial stationary condition; (2) decrease of the sensitivity to the initial agent with characteristic general minimization of the organism's interaction with the environment, which ensures its vital activity in accordance with the principle of the optimality, and of the maximum efficiency of the energy use in particular; (3) quantitative compliance between the values of the stimulus and responses, as well as their adequacy to the stimulus qualitative characteristics. The limits (in a narrow sense of the word) of the organism's abilities in the process of adaptation may be considered exhausted when there appear da-

mages of irreversible character and changes in the quality of regulation. This definition seems to be complicated for a direct practical use, but nevertheless we think that it substantially fills up the revealed gap. The presented data demonstrate that active research efforts aimed at adopting the optimal formalized definitions of the criterion of harmfulness for different conditions of exposure to poisons, suitable for practical application, are being carried out. Although this work is far from being completed, the main principles of differentiation between common adaptive responses and symptoms of harmful effect (not so different for various trends of preventive toxicology, as it seemed before) have already been established. On the basis of these principles, I. V. Sanotsky (1971) has given a definition of the threshold of harmful effect, which is, evidently, applicable to all media: the threshold of harmful effect of a substance is such its minimum concentration in the environment under exposure to which in the organism (under specified conditions of substances entry) occur changes, going beyond the limits of physiological adaptation responses, or latent (temporarily compensated) pathology.

Chapter 2. CURRENT STATE OF THE PROBLEM OF EVALUATION OF TOXICITY AND HAZARD FROM OCCUPATIONAL POISONS

If before the 1920—30ies the assessment of the hazard posed by various chemical compounds, entering the environment, could be still mainly of qualitative character, then at present the problem of the quantitative criteria which could be used as a basis for the evaluation of the toxicity and hazard from compounds has become of vital importance. Here it is necessary to give a more precise definition of the biological hazard, and first of all for those substances which contact the working people under occupational conditions.

The hazard presented by the poison is a wide concept, since it characterizes the probability of poisong with a substance under real conditions of production and its application (N. S. Pravdin, 1934). A similar definition was given by Goldwater (1961): the hazard of poisoning is the probability of a damage occurrence under conditions of the manufacturing process in which the toxic substance is used. Unfortunately, the hazard from a poison cannot at present be evaluated by one measure, since it has many parameters characterzing it to a smaller or greater extent from the potential and real sides.

The most substantiated analysis is the analysis of two groups of the quantitative hazard indices: the potential probability of the substances entry into the organism and the compensatory properties of the organism in respect to a given poison. The smaller the organism's ability to compensate the damages caused by this or that substance, the more hazardous the substance. The absolute toxicity should be to a certain extent assigned to the first group of indices, since together with an increase of toxicity the amount of substance which is to enter the medium and cause poisoning reduces.

I. V. Sanotsky (1970) has defined toxicity (poisonousness) as a measure of incompatibility between substances and the life of the organism. In this case, it is justified the method used by the vast majority of authors, i. e., to measure the value of toxicity as the inverse value of the lethal dose (concentration).

Critical surveys on the existing concepts of toxicity have been written by N. S. Pravdin (1934),

L. K. Hotsyanov and A. M. Rashevskaya (1958), N. V. Lazarev (1964), I. V. Sanotsky (1970) et al., therefore there is no need to dwell on this subject any more.

Methods for Determination and Classification of Toxicity

The founders of quantitative toxicology (Lehmann, N. S. Pravdin, N. V. Lazarev and their contemporaries) used to determine the degree of toxicity from the data of acute experiments (death of animals, falling on one side, narcosis). There is no doubt that at the lethal level the main regulariries can be registered with a higher precision since, in spite of large individual variations in the time of the death arrival, the moment of death can be nevertheless determined with adequate objectiveness (N. S. Pravdin, 1934).

Very important from the point of view of the harmonization of the conditions of a poison's effect in the comparative evaluation of toxicity and substantiation of the lethal concentrations and dosesof substances is the determination of the time after the exposure during which the mortality rate among the experimental animals should be calculated. Following the recommendation of N. S. Pravdin (1947), a 1-day duration was proposed. Later, however, Pravdin himself extended this period up to 14 days, The same duration was proposed by Deichmann and Leblanc (1943). At present, the period of the animals observation for determining lethal concentrations (doses) of a poison is 2—3 weeks.

The highest limit of the zone of the lethal effect was considered at that time as DL_{100} (the minimum dose causing death of all experimental animals); the lower limit was considered at that time as DL_0 (the maximum tolerable dose which does not cause the death of animals). However, these values do not include probabilistic characteristics, and therefore do not provide sufficient information on the substances toxicity. It is known that with an increase of the number of observations the DL_0 (CL_0) tends to decrease because of individual sensitivity of the experimental animals, while the DL_{100} (CL_{100}) tends to increase. The most statistically significant is the value of the dose which causes death of half of the experimental animals, i. e., the DL_{50} (CL_{50}), since «it makes allowances for particularly resistant or particularly sensitive animals which, in this case, are not taken into consideration» (N. S. Pravdin, 1957).

A variety of statistical methods has been proposed for calculating the DL_{50} (CL_{50}), requiring the observation of certain conditions of the experimental procedure. This fact should be taken into account in the experimental design. Thus, the methods of

Behrens (1929) and Kärber (1931) suggest an equal number of animals in each group; the method of Behrens requires equal intervals between the doses application. At the same time both methods do not allow to calculate the exact value of the standard error and the limits of the reliable range of the DL_{50}. This fact makes these methods significantly less valuable.

When processing the results of experiments by the method of probit analysis, equal intervals between the doses and equal number of the experimental animals in the groups are not necessary, since it is possible to check the correspondence of the drawn straight line to the points on the diagram and compare the toxicity of several substances, as well as to calculate the reliable limits of the unknown effective dose (concentration). Thus, the toxicity, as a measure of the incompatibility between a substance and the life of the organism, can be exactly calculated.

It is advisable to consider classifications of substances, according to their toxicity, which provide to experimentalists primary data on the hazard presented by a compound.

In the USSR and in other countries, a number of classification of substances according to their toxicity has been worked out (E. N. Marchenko, 1962; I. V. Sanotsky, 1967; L. I. Medved et al., 1968; S. D. Zaugolnikov et al., 1970; K. K. Sidorov, 1973; Hodge, Sterner, 1943; Hoyle, McCollister, Rowe, 1957; Burger, 1961; Feierholle, 1961, et al.). These classifications suggest a division of substances into classes according to the magnitude of the median lethal doses (concentrations), to the routes of the poison entry into the organism, etc. It should be noted that these classifications have their deficiences. The boundaries between classes are often established arbitrarily. For example, by the classification of Hodge and Sterner (1943), classes differ by 3, 5, 6, 10 and 20 times according to their effective concentrations. As a rule, the comparison of toxicity is made in most classifications using weight units, which is convenient in practice, though it is known that chemical compounds usually react with biosubstrates at the molecular, ionic and other levels. For experimental studies, this subdivision may result insufficient, since substances with sharply different toxicity may be included in the same class, and, vice versa, toxicologically similar substances may be assigned to different classes. For example, according to the classification of S. D. Zaugolnikov et al. (1970) (Table 12), the first class — extremely toxic substances (the CL_{50} is less than 1 mg/l, the DL_{50} is less than 1 mg/l) — should comprise such substances, as zarin (the CL_{50} is 0.001 mg/l), ethylmercurphosphate (the CL_{50} is 0.008 mg/l) and the compounds having the CL_{50} equal to 0.1 mg/l, i. e., substances differing, according to the degree of toxicity, by 1000 times.

For experimental purposes, it is advisable to use the classification based on the continuous scale of toxicity, proposed by

Categories of absolute toxicity of poisons under inhalation and enteric

Classes of harmful substances	1. Extremely toxic	2. Highly toxic	
	I	II	III
Median lethal dose (enteric), mg/kg	Less than 15	15—50	51—150
Median lethal concentration (inhalation), mg/g	Less than 1	1—4	5—10
Approximate MAC, mg/m³	Less than 1	1—3	5—10
Established MAC for the air of working premises, mg/m³	Less than 1	Up to 10	

I. V. Sanotsky (1967). Fig. 14 shows a diagram for calculating the relative toxicity. As a 100% toxicity there has been taken the inverse value of the lethal concentration of methylethoxyphospholylthiocholine, corresponding to 0.0001 μM/l, and as practically safe substances (0% toxicity) there have been chosen methane and carbondioxide, which lethal concentrations are 10 000 μM/l.

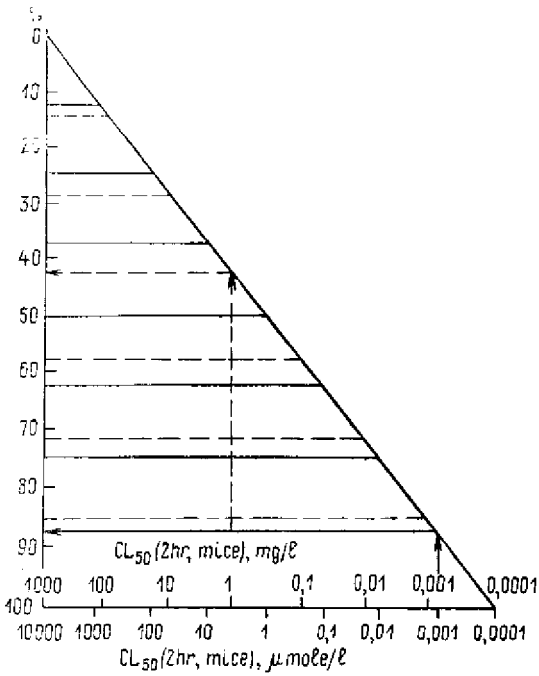

Fig. 14. Diagram for calculation of relative toxicity.

Y-axis, relative toxicity, %; X-axis, CL_{50}, μ mole/litre.

Table 12

administration and degrees of the hazard of inhalation poisoning

3. Moderately toxic		4. Slightly toxic		
IV	V	VI	VII	VIII
151—500	501—1500	1501—5000	5001—15 000	>15 000
11—20	21—40	41—80	81—160	>160
20—30	50—100	200	300—500	>500
Up to 100			More than 100	

Intermediate values of relative toxicity are determined from the diagram. At present, the suggested diagram has been complemented by a second scale (along the X-axis), on which average lethal concentrations in mg/l (for practical calculations) are marked. The mentioned classification gives true idea of the relative toxicity of substances with an accuracy up to 1%. For practical purposes, however, it is more convenient to use enlarged classifications. I. V. Sanotsky (1967) suggested 3 enlarged classes of toxicity: from 51 to 100% of relative toxicity lies the class of highly toxic substances; from 25 to 50%, the class of substances with moderate toxicity; from 0 to 24%, the class of substances with low toxicity (the step of progression is equal to 2).

A more detailed information on the existing classifications is given in surveys by I. V. Sanotsky (1967) and S. D. Zaugolnikov et al. (1970).

For the purpose of establishing a unified classification for the assessment of substances by thier toxicity, there has been worked out the enlarged classification, which has been adopted by the plenary sessions of the MACs Section (Table 13).

Table 13

Classification of harmful substances according to the degree of toxicity

A. Classes of toxicity

Indices	(1) Extremely toxic	(2) Highly toxic	(3) Moderately toxic	(4) Slightly toxic
Median lethal dose (enteric), mg/kg	Lower than 15	15—150	151—1500	More than 1500
Median lethal concentration, mg/l	Lower than 0.5	0.5—5	6—50	More than 50
Median lethal dose under application to skin, mg/kg	Lower than 100	100—500	501—2500	More than 2500

Assessment of the Potential Hazard from Chemical Compounds

The probability of the development of intoxication is to a large extent determined by the capacity of a substance to enter the organism.

Lehmann (1912) proposed to assess the potential «toxicity» of substances not only by the absolute effective doses, but also taking into account the volatility of compounds. This criterion of inhalation hazard (the ratio of toxicity and valatlity) was called by the author «two-phase toxicity». To avoid terminological confusion with the concept of «three-phase toxicity of poisons» (phase of entry, saturation and excretion of the poison) introduced by N. V. Kravkov (1927), N. S. Pravdin (1933) suggested to use instead of the term «two-phase toxicity» the term «effective toxicity». Unfortunately, the absense of standard conditions for determination of the mentioned index limited its application.

Werner (1943) defined the effective toxicity as the ratio of volatility (body temperature was not specified) and the CL_{50}, which was being determined in experiments on mice under a 7-hour exposure. The author called it «hazard index». Later the term «hazard index» was interpreted outside the USSR as the ratio of volatility of substances at 25° C and the CL_{50}, determined in experiments of mice under a 4-hour exposure. I. V. Sanotsky (1962) introduced the term «coefficient of the propability of poisoning by inhalation» (CPPI):

$$\frac{C^{20^\circ}}{CL_{c,0}^{120}m}$$

where C^{20° is the maximum attainable concentration at 20° C; CL_{50}^{120} m^1 is the half-lethal concentration for white mice under a 120-min exposure.

The CPPI is one of the forms to express effective toxicity, which allows to compare the hazards presented by several given substances under specified conditions.

G. N. Zayeva (cited from I. P. Ulanova et al., 1967) suggested a classification of the degree of hazard from occupational substances according to the value of the CPPI (Table 14).

The shortcoming of this classification consists, in our opinion, in including into the class of slightly hazardous compounds such substances which lethal concentrations are equal to tenth parts of the saturated concentration, since this level of concentration is often encountered under occupational conditions, and such exposures may lead to poisoning.

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[1] Symbols proposed by I. V. Sanotsky in 1969.

Table 14

Classification of the degree of hazard from substances according to the value of coefficient of probability of poisoning by inhalation (CPPI)

Degree of hazard	Highly hazardous	Hazardous	Slightly hazardous
Value of CPPI	More than 100	10—100	Less than 10

L. I. Medved et al. (1968) suggested a classification of the hazard from substances, based on the correlation between their volatility and possible biological effect (CL_{ch}, Lim_{ch}) under the concentration of saturation (Table 15). Unfortunately, this classification has limited application, since it is valid only for the assessment of extremely toxic and slightly volatile compounds. Over 100 compounds, studied by us according to the classification given in Table 14, were found to belong to extremely hazardous substances. We did not manage to discover among the compounds used in industry those which, according to this classification, could belong to substances with marked or slightly marked hazard.

Table 15

Degree of hazard	Highly marked	Marked	Slightly marked
Hazard index	$C^{20} \geqslant CL_{ch}$	$C^{20} > Lim_{ch}$	$C^{20} < Lim_{ch}$

In predicting the degree of potential hazard posed by compounds, a very important factor is their solubility in water (or in blood) and, in case of poisoning by inhalation, the solubility in water (in blood) of their gases or vapours (N. V. Lazarev, 1938, 1944; I. D. Gadaskina, 1949; N. A. Tolokontsev, 1960; Henderson, Haggard, 1943). The hazard of acute poisoning is particularly high when the toxic concentration is obtained very quickly. Saturation of the blood with a substance takes place the rapider the lower the solubility of its vapours (N. V. Lazarev, 1938; N. A. Tolokontsev, 1960).

Of no less importance in evaluating the potential hazard posed by occupational poisons is the value of their solubility in fat. Substances with a high solubility in fat (high value of the Overton-Meyer coefficient) can accumulate in fat deposits, then from these deposits they either gradually enter the blood or undergo metabolic transformations in the adipose tissue (I. D. Gadaskina, 1970). Similar substances are potentially hazardous, since they cause the development of chronic poisoning. The system of non-

electrolytes (N. V. Lazarev, 1944), which comprises 9 groups and which is based on physical and chemical properties of substances, allows to predict the speed of saturation and excretion of the poison from the organism, and thereby to determine the degree of its potential hazard.

Besides the above-presented indices, in determining the probability of a substance entry into the organism, an account should be also taken of the «thermodynamic activity» (N. V. Lazarev, V. A. Filov, 1964), of the physical and chemical regularities underlying the absorption through the skin (Y. I. Kundiev, 1965; S. D. Zaugolnikov et al., 1967; V. A. Kondrashov, 1970, et al.), as well as of the principles of the joint action of gases and aerosoles (L. A. Tiunov, N. V. Savateev, 1962; I. V. Sanotsky, 1965, et al.).

The considered criteria of the potential hazard presented by occupational substances do not cover all the approaches to the comparative assessment of the probability of the poison's entry into the organism under real conditions of production and of a given substance application. They only demonstrate the development of ideas in the indicated direction. Let us, however, imagine that two substances have the same probability of entry into the organism. Then for the assessment of their comparative hazard different criteria of the real hazard will become of primary importance.

Evaluation of Real Hazard from Chemical Compounds

Evaluation of the hazard of lethal poisoning. Some authors identify the concept of the hazard from a given compound with the meaning of the median lethal values (Carpenter, Smyth, Pazzani, 1949). However, the comparison of the hazards from substances only by the value of their lethal doses (concentrations) may lead to false conclusions, since the probability of lethal poisoning with substances having equal values of the DL_{50} and CL_{50} may be different.

To characterize the hazard of the development of acute lethal poisoning, V. M. Karasik (1944) proposed to determine the range of the lethal doses (concentrations):

$$\frac{DL_{100}}{DL_0}\left(\frac{CL_{100}}{CL_0}\right),$$

substituted later by the ratio of the probability values

$$\frac{DL_{84}}{DL_{16}} \text{ and } \left(\frac{CL_{84}}{CL_{16}}\right)$$

The smaller this ratio, the weaker the organism's ability of compensation, the higher the probability of lethal outcome under

variation of high concentrations, and therefore the higher the hazard presented by a substance. Several different variants for determining the variability of lethal doses (concentrations) have been suggested: first of all, by determining the angle formed by the straight line of lethal doses (concentrations) with the X-axis, the tangent of this angle (N. A. Tolokontsev, 1964; O. S. Kagan, 1965) and the function of the angle (Litchfield, Wilcoxon, 1949)

$$S=\frac{\frac{DL_{84}}{DL_{50}}+\frac{DL_{50}}{DL_{16}}}{2}$$

The expression of the slope of the lines of the lethal effect through tan a depends on the scale of the co-ordinate system, which makes it difficult to obtain comparable data. More objective criteria are such indices as $\frac{DL_{84}}{DL_{16}}$ and the function S of the angle.

For the same purpose it is advisable to use different temporal characteristics of exposure, for example, the TL_{50}, i. e., the time of exposure during which half of the test animals die. G. N. Zayeva et al. (1968) have determined the TL_{50} for substances under various exposures at the level of absolutely lethal concentrations, which is an additional quantitative criterion of the comparative hazard of poisoning, in particular of the substances capacity to penetrate through the skin. Unfortunately, to the temporal characeristics have not yet been attached that importance which they, as well as qualitative characteristics of the poison's effect, deserve.

Evaluation of the hazard of development of acute non-lethal poisonong. N. S. Pravdin (1934, 1947) more than once pointed out that the preventive tasks of industrial toxicology require the study of the hazard of development of not only severe «classic» poisonings, which result in evident detriment to health, but also of those poisonings which are accompanied by a decrease of the working capacity of the organism and by latent diathesis to general illness. N. S. Pravdin indicated that industrial (chronic) intoxications reveal themselves mostly in forms, symptoms and changes different from those of acute poisonings, which are usually studied by general toxicology.

This problem is particularly urgent now when, as a result of continuous progress in chemical technology, the content of toxic substances in the air of industrial premises is steadiliy decreasing. That is why it is very important to examine the so far developed criteria for the assessment of the hazard of the acute nonlethal, as well as of the chronic poisoning.

N. S. Pravdin (1947) recommended that, to characterize the probability of development of intoxication, besides the DL (CL), there should be also determined the minimum concentrations, being on the threshold of the toxic effect. Stressing the importance

of determining the threshold concentrations, the author drew the attention to the fact that the concept of the threshold concentrations was «even more conditional than the concept of lethal concentrations, since the threshold concentration depends not only on the species and individual sensitivity of the animal and the time of exposure, but also on the method which is used for its determination». According to N. S. Pravdin, for determining the threshold effects, only those indices can be used which have hygienic significance and which correspond to the hazard criterion.

As it is known, the detection of threshold effects requires application of integral and pathogenic indices. Integral indices characterize general condition of the organism and allow one to determine changes occurring in the vital activity of the integral organism, independently of the site of the poison's action. Pathogenic indices are determined by the mechanism of action of a substance, and are used in those cases when pathogenesis or main steps in pathogenic process have been clarified. For new chemical compounds, the mechanisms of action are often unknown, therefore it is difficult to choose corresponding pathogenic indices.

Comparison of the sensitivity of the integral and pathogenic indices, of the stability of their changes under intoxication with compounds having a characteristic type of action[1], carried out in the Laboratory of Toxicology of the Institute of Industrial Hygiene and Occupational Diseases of the USSR Academy of Medical Sciences, has shown that the most sensitive are naturrally pathogenic indices. However, changes in pathogenic indices often appear difficult to be interpreted, since the degree of hazard of the detected changes is difficult to be assessed and the limits of physiological (normal) fluctuations of a particular response are not always clear. Therefore, N. S. Pravdin (1947) considered necessary to use the integral indices for determination of the threshold of the acute effect.

In order not to confuse numerous thresholds of a poison's effect, I. V. Sanotsky (1964) recommended to call the threshold of acute effect, determined by changes in the integral indices, as the integral threshold of the acute effect, so that to differentiate it from pathogenic specific threshold of the acute effect. This makes it possible to compare threshold levels for different poisons. The smaller the threshold value, the greater the sensitivity of the organism to the action of a poison, and therefore the greater the hazard of development of acute non-lethal poisoning.

In experimental pharmacology, quantitative evaluation of the

[1] Carbon tetrachloride, furan (hepatotropic poisons), epicholorohydrin (irritant and kidneys poison), formaldehyde (mainly irritant), aniline (blood poison), etc.

pharmacological activity and toxicity of a preparation requires the determination of the median effective (DE_{50}) and median lethal value (DL_{50}) of the dose. Using these parameters, the range of the therapeutic action, called the therapeutic index $I=\frac{LD_{50}}{ED_{50}}$, is being determined. This index has been first of all used for the assessment of the hazard presented by inhalation narcotics.

For the purposes of industrial toxicology, N. S. Pravdin (1947) proposed a scheme for the experiment comprising the determination of «higher and lower parameters of toxicity» under a single exposure. By these terms there were ment lethal and threshold concentrations of the poison respectively, and their ratio determined the «range of the toxic effect zone». The magnitude of the zone was considered as an index of the hazard of acute poisoning.

Later, because of the necessity to distinguish the zone of chronic effect, the zone of toxic effect was called the zone of acute effect: $Z_{ac}=\frac{CL_{50}}{Lim_{ac}}$ (I. V. Sanotsky, 1962). The zone of acute effect is an integral index of the compensatory abilities of the organism, of its capacity to detoxicate, excrete the poison and to compensate the damaged functions. The same opinion was formulated, independently from N. S. Pravdin, by Spencer et al. (1951), who called attention to the significance of the zone lying between the DL_0 and the DE_0 (according to morphological indices) for characterizing the hazard of a poison.

Similar regularities were also noted by Burger (1961), who, giving a complicated tabulation of «toxicity», marked high significance of the range which included concentrations, producing first symptoms of the effect in man and those including concentrations causing severe effect (Table 16). However, in accordance with the methodological instructions on conduction of experimental studies for substantiating the MACs of harmful substances for workplaces in occupational premices, it is permitted to determine the threshold of harmful effect on people only for irritants under absolute guarantee of safety. There are also used the thresholds of olfactory sensation of the reflex effect.

Concepts like those presented here have been worked out by physiologists independently from toxicologists. Thus, for the comparative characteristic of the toxic effect on animals of different age, V. D. Rozanova (1966) proposed the concept of endurance, which is determined by the ratio of minimum lethal doses to minimum effective doses of a substance. By the «endurance» the author intends the condition of disturbed homeostasis under intoxication conditions which is, however, reversible.

Evaluation of the hazard of chronic poisoning. As noted by N. V. Lazarev (1938) and N. S. Pravdin (1947), when assessing the comparative toxicity (hazard) of poisons, we should never

Tabl

Classification of toxicity and hazard

Class	Part I. Toxicity scale										Part II. Other Informat					
											Recommended safety fa					
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6
Concentration in ppm	0.1	0.1—1.0	1.0—10	10—50	50—100	100—250	250—500	500—1000	1000—3000	3000						
Name of the product and chemical composition																
x				A_1 A_2		B_1	B_2	C	D	F_1 F_2 F_3						
y			A_1 A_2		B_1 B_2	C D E	F_1	F_2 F_3								

Legend: A_1 and A_2, the highest and the lowest MAC according to the literature;
B_1 and B_2, the threshold of the effect on man (the highest and the lowest);
C, early signs and symptoms of poisoning (man); quantitative limits of indications;
D, disease (indications and symptoms);
E, severe disease with the loss of working ability;
F_1, lethal poisoning (under chronic exposure);
F_2, peak concentrations causing severe disease (under acute exposure);
F_3, peak concentrations causing death (under acute exposure);
1, diagram of lethal doses (with indication of the type of animals).
2, recommended safety factor for «normal» people;
3, recommended safety factor for weakened people (with indication of the type of general disease);
4, recommended safety factor in case of increased sensitivity (allergy of combination with other substan
5, some physical data;
6, recommended MAC;
7, special marks and the list of the main literature sources; the substance which position in the Tabl more shifted to the left is more toxic; the hazard from a substance is determined by the distance tween A and B, or between C and D.

proceed only from the results of acute experiments, since under chronic exposure to poisons many intoxications have another pathogenesis, different from that of the acute intoxication. Many industrial poisons with low toxicity result extremely dangerous in acute experiments under conditions of chronic exposure to low concentrations. Classic examples of such poisons are lead, manganese, mercury, and other heavy metals; among organic poisons we should mention benzene, trinitrotoluene and many others.

Chronic poisoning develops either when a poison gradually accumulates in the organism or if there takes place the summation of primary insignificant changes caused by the poison. Attempts to evaluate the hazard of chronic poisoning in terms of manifestation of the cumulative properties of the poison were being undertaken for a long time. By c u m u l a t i o n it is usually intended the summation of the effect under repeated exposure to a poison.

Various methods have been suggested for determining the cumulative properties of substances. For this purpose N. S. Pravdin recommended to use an exposure to concentrations of a poison close to 1/10—1/20 of the CL_{50} during 14—30 days, and then a single exposure at the CL_{50} level. He was of the opinion that an increase of the mortality among the animals in comparison with a single exposure indicated the development of cumulation, and its decrease indicated the development of the so-called habituation. The method proposed by N. S. Pravdin takes a certain account of the real conditions of development of chronic poisoning, but at the same time it does not provide quantitative characteristics of the cumulative processes. Later works have permitted us to make quantitative assessment of the degree of cumulation by means of calculating the coefficient of cumulation under administration of precisely measured doses into the stomach. Although this model is not always adequate to the inhalation administration, it is nevertheless attractive because of its relative simplicity.

By the cumulation coefficient (I_{cum}) it is usually intended the ratio of the summated dose which, being administered fractionally, causes a specified effect to the value of the dose producing the same effect under a single exposure.

Nowadays, the cumulative effect of chemical substances in experiments on animals is in most cases assessed by the lethal outcome.

In modern toxicology, the most widely used methods are those of Y. S. Kagan and V. V. Stankevich (1956), Lim et al. (1961). Y. S. Kagan (1965, 1970) recommended to determine the type of dependency of the cumulative effect on the value of the daily administered dose of a substance, i. e., 1/5, 1/10, 1/20, 1/100, 1/200, which allows one to predict the hazard of occurrence of chronic poisoning. An experiment according to the extended scheme con-

tinues up to 4 months. It is expedient in this case to carry out a chronic inhalation experiment. In case of a substance administration into the stomach, the «sub-chronic toxicity» test proposed by Lim et al. (1961) seems to be more efficient. This test is conducted during 24±4 days, while to reveal the cumulative effect about one week is required. Initial daily dose, equal to 1/10 of the before—established DE_{50} (DL_{50}) for a single exposure, is administered during the first 4 days. On the 5th day the dose is increased by 1.5 times and is administered during the next 4 days, etc. The assessment of the experimental results is made by the ratio of the median effective doses (DE_{50}, LD_{50}) for sub-acute and acute effects. It should be noted that according to the authors of this method a coefficient of less than 1 indicates the existence of cumulative properties, while that more than 1 indicates an increased resistance to the substances under study.

The qualitative assessment of the cumulative effect can be also made by the value of the «filling» and «residual» doses, by the rate of detoxication of a poison, by the time during which the experimental animals die, and by other indices. All these methods are known widely enough (S. N. Tcherkinsky et al., 1964; K. K. Sidorov, 1967; I. P. Ulanova e. al., 1970, et al.). More thorough data can be obtained using two indices as the criteria of cumulation: the DL_{50} and the angle formed by the straight line of the dependency of the observed effect on the effective doses with the positive meaning of the X-axis.

Unfortunately, the gradient of the «lethal» straight line, as a source of additional information on the hoxicity of substances, is used in experimental studies comparatively rarely. According to Horsfall (1948), it is possible to differentiate preparations by the gradients of the straight lines, especially when they cross, having equal DL_{50} values. The author points out that the DL_{50} represents only statistical aspect, while the slope of the curve shows the dynamics of the process. According to V. M. Karasik (1944) and V. B. Prozorovsky (1960), the slope of the effective straight line of an acute experiment may be used to evaluate the mechanism of the intoxication process.

We presume that the magnitude of the straight line slope in sub-acute experiments as well as in acute experiments permits one to assess the degree of a cumulative process. Other factors being equal, the steeper the slope, the greater the cumulative properties of a substance and the weaker the adaptive and compensatory processes in the organism, and vice versa.

An important quantitative characteristic of the cumulative properties of chemical compounds is the half-life period of the substance in the organism. This index, as pointed out by Y. S. Kagan et. al. (1972), permits one to make quantitative assessment of the probability of the substances accumulation under any regimen of the substances administration into the organism. If the

intervals between the administration (Δt) of a certain dose of a substance are smaller than the half-life (T), the substance accumulation will be the rapider, the smaller the value of the ratio $\Delta t/T$. When $T=\Delta t$, the substance will also accumulate, and in this case the maximum level, obtained under a single administration, tends to increase by 2 times already after 5—6 administrations. Then, if we continue to administer a given dose of substance, there is being established a dynamic equilibrium under the maximum content of the substance.

The level of the dynamic equilibrium and the speed at which it is reached can be calculated for each dose. If $\Delta t > T$, the substance will accumulate the slower, the greater the value of $\Delta t/T$; if Δt is substantially greater than T, no material cumulation will occur.

B. M. Shtabsky (1974) suggested a biological method for the determination of the half-life period of the substance (or of the effect), based on the «residual effect» of the substance, which is registered some time after its administration.

One of the methods for determination of the functional cumulation after a single poison administration is that of the double account of lethal outcomes: determination of the DL_{50} by the results of the 1st and 30th days (B. M. Shtabsky, 1974). The ratio of the DL_{50}, calculated from the results of the 30th day, to the DL_{50} of the 1st day represents a qualitative characteristic of cumulative effect.

The higher the time required for the development of the lethal outcome, i. e., the greater the value of this ratio, the more cumulative the substance.

For the quantitative evaluation of the cumulative properties of poisons. L. I. Medved et al. (1968) suggested a classification according to the value of the I_{cum}:

$I_{cum} < 1$, extremely high cumulation,

$I_{cum} = 1 \div 3$, marked cumulation,

$I_{cum} \leqslant 3 \div 5$, moderate cumulation,

$I_{cum} > 5$, low cumulation.

This classification is based on the coefficient of Kagan and Stankevich, determined under fractional (1/20 of the DL_{50}) administration. It should be noted that this coefficient is the inverse value of the cumulation intensity: the smaller it is, the higher the cumulation

$$I_{cum} = \frac{DL_{50}n}{DL_{50}1}.$$

In our opinion, this fact unjustifiably complicates the interpretation of the experimental data.

The most important is, however, the fact that the evaluation of cumulation according to the lethal effect, unfortunately, fails to provide a complete picture of the cumulative processes occurring in the organism under exposure to toxic substances in real

occupational concentrations. Continuous regimens of exposure to substances usually are not encountered under real occupational conditions, there occur intermittent (discontinuous) exposures. The method of Lim et al., described above, simulates to a certain extent the nonuniformity of the poison entry into the organism. We think, however, that the adequacy of this method to the requirements specified above remains not clear, the same way as it is not clear the comparative characteristic of the processes of functional cumulation obtained by different methods. Thus, there is a necessity to develop additional methods for evaluating the cumulative properties of compounds.

Another index of the hazard of occurrence of chronic poisoning is the magnitude of the chronic effect zone (Z_{ch}), determined by the ratio of the Lim_{ac} and the Lim_{ch}, and the magnitude of the biological effect zone, determined by the ratio of single half-lethal concentrations (doses) and the threshold concentrations in chronic experiment:

$$\frac{CL_{50}\,(DL_{50})}{Lim_{ch}}.$$

The term «threshold concentration of chronic effect» was introduced into industrial toxicology by N. V. Lazarev (1938), the zone of chronic and biological effect was distinguished by I. V. Sanotsky (1962). The larger the zone of chronic effect, the higher the hazard of chronic intoxication, and vice versa, since in the first case the effect develops latently. On the one hand, the chronic effect zone reflects cumulative properties of the substance and, on the other hand, it indicates compensatory abilities of the organism at a low threshold level. The Lim_{ac} is being determined in long-term experiments on animals.

It is common knowledge that in different branches of toxicology (industrial, communal, food toxicology, etc.) the duration of chronic experiment is different. For example, the duration of chronic experiment on the MACs substantiation for pesticides in the air of workplaces and in the atmosphere is 4—6 months, for establishing standards for food products and water, it is 10—12 months (Methodological instructions on hygienic evaluation of new pesticides. Kiev. All-Union Institute of Hygiene and Toxicology of Pesticides and Plastics, 1969); in line with recommendations of international organizations, the duration of chronic experiments for setting standards for food additives in alimentary products is up to 2 years for experiments on dogs, and not less than a year for experiments on rats. In industrial toxicology (first by analogy with pharmacological investigations) the exposure was limited by 2—3 months, then its time was increased up to 6—12 months; recently the duration of chronic exposure has been reduced. In accordance with the «Provisional methodological instructions on conducting experimental studies for substantiation of maximum allowable concentrations of harmful substances in the air of

workplaces» (1964), the duration of chronic experiments has been limited by 4—6 months. N. A. Tolokontsev (1963) suggested that the duration of chronic exposure be equal to 1/10 of the average life span of the experimental animal species. The accepted duration (4 months) is close to 1/10 of the life span of the white rat.

For determination of the Lim_{ac}, experimental studies on the effects of substances in different concentrations have been carried out. Prior to 1965, two concentrations were recommended for application: slightly higher than the acute effect threshold and, as a rule, one order of magnitude lower. It was also considered necessary to investigate in chronic experiments the concentrations 5—10 times lower than the Lim_{ac}. The selection of concentrations to be applied in chronic experiments was determined by toxicity and hazard of each substance at a higher level of exposure to it, by the type of action, data available on other substances with similar structure, etc. The concentrations (doses) selected usually differed from each other by one order.

In 1964 the Section on the substantiation of the maximum allowable concentrations in the air of workplaces examined the proposal of I. V. Sanotsky concerning the need to determine the no-effect concentration, which would ensure correct determination of the chronic effect threshold. This proposal is now accepted. It should be noted that the Lim_{ac} value is considered decisive in establishing hygienic standards.

Thus, at present, there have been worked out numerous criteria for assessing the hazard from poisons and specified the indices of hazard. However, we do not have yet models necessary for comparative evaluation of hazards presented by different classes of chemical compounds. Such models can be the corresponding classifications of the criteria of hazard presented by substances under acute lethal, non-lethal and chronic exposures.

The mentioned classifications are necessary for the evaluation of toxicity and hazard from compounds for the following purposes:

— substantiated selection of substances for further studies at the first stage of investigation of toxicity under acute exposure;

— predicting the possibility of chronic intoxication occurrence;

— substantiation of preventive measures;

— further formalization of experimental data aimed at systematic accumulation and generalization of information.

It is clear that these tasks do not cover all the aspects of toxicological and hygienic significance of the classifications.

Chapter 3. SUBSTANTIATION OF CLASSIFICATIONS OF POTENTIAL HAZARD FROM POISONS AT DIFFERENT LEVELS OF EXPOSURE

In accordance with a given task, chemical compounds can be systematized by different indices. Thus, in pharmacology the most widely used classifications are based on the direction of the predominant action and on chemical structure. There are also used mixed classifications (N. V. Lazarev, 1961; V. V. Zakusov, 1966; S. V. Anichkov, M. L. Belenky, 1969). The chemical, biological and mixed classifications are well known. On their basis a number of reference books and manuals on toxicology has been compiled (for example, N. V. Lazarev, 1971). On the relation of poisons to the enzymatic systems there have been based classifications of Pilat (1959), M. Goldblatt and Y. Goldblatt (1960), A. A. Pokrovsky (1962), L. A. Tiunov (1967), E. Y. Golubovich (1970). Classifications according to the degree of toxicity have been already considered above.

Although toxicity is the most important index of the hazard posed by poisons at the lethal level, its characteristics do not provide full evaluation of the hazard. That is why in recent classifications there have been made attempts to evaluate not only toxicity, but also other indices of the hazard presented by chemical compounds, in particular, their volatility, the MACs level (E. N. Marchenko, 1962; S. D. Zaugolnikov et al., 1970), the degree of manifestation of a specific effect (L. M. Medved et al., 1968).

It is important to evaluate the hazard from compounds at different levels of exposure in order to be able to substantiate the preventive measures selectively. To this end, the scattered data on the quantitative characteristics of the hazard presented by occupational poisons have been systematized, different criteria have been substantiated and classifications of the indices of the hazard of poisoning at different levels of exposure (lethal and threshold levels under a single and chronic exposures) have been worked out.

For the purpose of the hazard classification, substances have been divided into 4 classes: (I) extremely hazardous substances; (II) highly hazardous substances; (III) moderately hazardous substances; (IV) slightly hazardous substances. We have tried to keep the difference between classes within one order of mag-

nitude, since the sensitivity of the organism's systems to the exposure to exogenous agents frequently varies proportionally to the logarithm of the intensity of exposure (in case of chemical exposure, it changes proportionally to the logarithm of doses or concentrations). Considerable difficulties have been encountered when determining the bounderies between the classes. Of course, the division into classes in conditional, but nevertheless we considered it important to substantiate the division classes not only evenly in terms of quantity (classes I and IV may be disproportionately large or small), but also taking account of qualitative perculiarities of the poisons' effect. For this purpose, there were used mainly those results of experimental studies which made it possible to evaluate more precisely the quantitative relationship between the dose (concentration) and the effect at the main levels of exposure. When the adequate clinicohygienic data were available, they were used for substantiation of the presented here classifications.

Substantiation of the potential hazard posed by occupational poisons

Classifications of the potential hazard from occupational substances are based on the relationship between the value of the effective concentrations (lethal and threshold) of compounds and the degree of their volatility.

The coefficient of probability of acute lethal inhalation poisoning

$$CPPI_{CL} = \frac{C^{20°}}{CL^{120}_{50M}}.$$

In compiling this classification, we have calculated the CPPI for 66 compounds having different degree of toxicity and volatility (from gases to liquids having the boiling point of 250° C). The data obtained are given in Table 17, in which the substances are arranged according to the decrease of the CPPI value. A possible classification based on the CPPI is presented in Table 18.

As follows from the data in Table 18, substances are distributed between classes of hazard relatively uniformly. Glass I has the lower limit of the CPPI equal to 300, which corresponds to the value of 0.003 of the saturating concentration, accepted as the limit of this class according to the classification of S. D. Zaugolnikov et al. (1970). Similar concentrations of substances are often encountered in the air of workplaces. This class comprises gases or highly volatile substances which, according to the clas-

Table 17

The CPPI values for some chemical compounds

No	Substance	CPPI
	Extremely hazardous compounds	
1	Methyl chloride	2 156 603
2	Ozone	685 710
3	Dimethylamine	685 710
4	Chloromethyltrichlorosilane	1 766
5	Hydrogen sulfide	1 056
6	Monoisopropylamine	1 039
7	Ethyleneimine	935
8	Ethylene sulfide	900
9	Furan	560
10	Trichloroacetic chloride	525
11	Isopropylnitrite	521
12	N-propylamine	465
	Highly hazardous compounds	
13	Carbon monoxide	280
14	Triethylamine	192
15	Ammonia	170
16	Carbon disulfide	126
17	Ethylenediamine	117
18	Methylfuran	109
19	Diethylamine	102.8
20	Vinyl acetate	90.0
21	Diisopropylamine	84.4
22	Nitroform	60.0
23	Cyclopentadiene	57.5
24	Chloroform	49.5
25	Pentachloroacetone	42.2
26	Benzotrichloride	39.2
	Moderately toxic substances	
27	Thiophene	26.5
28	Methylene chloride	25.7
29	Hydrazine	25.0
30	Carbon tetrachloride	22.1
31	Benzoyl chloride	20.1
32	Benzyl chloride	17.7
33	Hexachloroacetone	16.0
34	Piperidine	15.0
35	Fluorobenzene	14.9
36	Isoprene	14.5
37	Methylpiperazine	13.7
38	Benzyl cyanide	13.0
39	Benzal chloride	12.9
40	m-Amino-benzotrifluoride	11.9
41	Benzene	8.8
42	Methylethylketone	7.6

Continued

No	Substance	CPPI
43	m-Nitrobenzotrifluoride	6.4
44	Bromoacetopropylacetate	5.4
45	Trichloropropane	5.0
46	1,3-Chlorobromopropane	4.1
47	Acetone	3.9
48	Methyl alcohol	3.2
49	Dioxane	3.1
50	Toluene	3.0
51	Cumene	3.0
	Slightly hazardous compounds	
52	Chlorobenzene	2.6
53	Hexamethyleneimine	2.5
54	Methacrylic anhydride	2.4
55	Benzotrifluoride	2.38
56	Dimethylethanolamine	2.2
57	Ethylmorpholine	2.1
58	Dimethylformamide	1.5
59	Ditolylmethane	1.5
60	Diethylchlorothiophosphate	1.4
61	Bromobenzene	1.2
62	Diethylethanolamine	1.0
63	Cyclohexanone	0.9
64	Furforol	0.9
65	Mesityl oxide	0.82
66	Iodobenzene	0.5

Table 18

Classification of potential hazard of lethal poisoning posed by chemical compounds (according to the value of $CPPI_{CL}$)

Class	CPPI	Distributions of substances between classes		CL_{50} in fractions of the saturating concentration (C^{20})
		Absolute number	%	
I	More than 300	12	18.2	Less than 0.003
II	299—30	14	21.2	0.03
III	29—3	25	37.8	0,3
IV	Less than 3	15	22.8	More than 0.3

sification of the MACs Section, belong to the group of extremely toxic substances (ozone, ethyleneimine, chloromethyltrichlorosilane, dimethylamine, etc.) or highly toxic substances (methyl chloride, hydrogen sulfide, monoisopropylamine, etc.).

The mentioned substances are also characterized by an insignificant variability of lethal concentrations ($S<2.0$), which indicates high hazard of lethal poisoning. Most of the substances (dimethylamine, chloromethyltrichlorosilane, monoisopropylamine, ethyleneimine, trichloroacetic chloride) are irritants, and only 3 of them (methyl chloride, hydrogen sulfide, ozone) — all gases — are neurotoxic poisons. The lower boundary of this class, in accordance with the considered division, is occupied by N-propylamine. The most dangerous is methyl chloride, which has been used in industry for over 100 years and which has a detailed description of toxicologic characteristics.

Class IV (potentially slightly hazardous substances) is restricted by the higher limit of the class III, i. e., the CPPI equal to 3, which is a 0.33 fraction of the saturating concentration, and corresponds to the category of moderately toxic compounds according to the classification of S. D. Zaugolnikov (IVB). Under the current organization of the manufacturing process, such concentrations are seldom encountered in practice in the air of workplaces. Substances, belonging to this class, are mostly either slightly toxic, though relatively volatile (ethyl alcohol, benzotrifluoride, butylacetate, etc.), or slightly volatile, though relatively (slightly) toxic (ditolylmethane, mesithyl oxide, hexanon, etc.). In occupational conditions, these compounds usually do not cause acute inhalation poisonings. The boundaries of the two middle classes (II and III) have been set so that to differentiate them from the neighbouring classes (I, IV) by approximately one order.

It should be emphasized that the evaluation of the hazard of acute poisoning based on the CPPI (taking into account the substance's volatility) supplements the characteristics of the real toxicity of a substance at a high level of exposure.

The potential hazard of poisoning presented by compounds at the threshold (Lim_{ac}, Lim_{ch}) levels of exposure may be assessed by the values characterizing the probability of formation of effective concentrations based on the rations: $\frac{C^{20}}{Lim_{ac}}$; $\frac{C^{20}}{Lim_{ch}}$ (according to the CPPI at the lethal level). The higher these ratios, naturally the greater the potential hazard from a substance. For what purpose have these indices been introduced? Is the knowledge of the potential hazard from substances, based on the CPPI, sufficient for us? We presume that together with the changes of the real hazard from compounds, occurring at the period of transition from high to low exposure intensities, there changes also the degree of their potential hazard, which should be taken into account. Therefore, if we take into consideration the effectivity of compounds at low levels of exposure, the evaluation of the potential hazard posed by compounds will be significantly more complete.

Table 19

The ratio of volatility of substances at 20° C to the value of the acute effect threshold ($CPPI_{ac}$)

No	Substance	$\frac{C^{20}}{Lim_{ac}}$
	Extremely hazardous compounds	
1	Methyl chloride	49 695 652
2	Ozone	1 923 077
3	Perfluoroglutaric dinitrile	500 000
4	Dimethylamine	357 143
5	Perfluoroadipic dinitrile	212 000
6	Monoisopropylamine	200 000
7	N-propylamine	77 000
8	Hydrogen sulfide	66 666
9	Ethyleneimine	37 400
10	Trichloroacetic chloride	24 390
11	Carbon monoxide	22 222
12	Furan	15 625
13	Diisopropylamine	13 513
14	Methylfuran	12 048
15	Isopropylchlorocarbonate	11 231
16	Triethylamine	11 111
17	Chloromethyltrichlorosilane	10 617
	Highly hazardous compounds	
18	Isopropylnitrite	4 762
19	Piperidine	4 650
20	Hydrazine	1 668
21	Methylene chloride	1 620
22	Methyl piperazine	1 515
23	Chloroform	1 500
24	Carbon disulfide	1 265
25	Diethylamine	1 205
26	Nitroform	1 205
27	Isoprene	1 044
	Moderately hazardous compounds	
28	Pentachloroacetone	910
29	Hexachloroacetone	713
30	Carbon tetrachloride	637
31	Freon-11	557
32	m-Amino-benzotrifluoride	410
33	Benzene	362
34	Dioxane	263
35	Freon-141	213
36	Chlorobenzene	201
37	Morpholine	189
38	m-Nitrobenzotrifluoride	188
39	Benzyl cyanide	185
40	Thiophene	167
41	Cyclopentadiene	163
42	Toluene	148
43	Bromobenzene	104
44	Benzotrifluoride	100

Continued

No	Substance	$\frac{C^{20}}{Lim_{ac}}$
	Slightly hazardous compounds	
45	Fluorobenzene	82
46	1,3-Chlorobromopropane	73
47	Isopropylbenzene	72
48	Bromoacetopropylacetate	72
49	Benzyl chloride	69
50	Freon-113 (trichlorotrifluoroethane)	59
51	Diethylchlorothiophosphate	45
52	Furfurol	28
53	Chloroacetopropylacetate	24
54	β-Ethoxy-propionitrile	18
55	Freon-112 (tetrachlorodifluoroethane)	14
56	Dimethyl formamide	12
57	Methacrylic anhydride	11
58	Ditolylmethane	10
59	Acetopropylacetate	8.3
60	Dimethylacetamide	7.7
61	Acetopropyl alcohol	6.7
62	Dimethylethanolamine	6.3
63	Diethylchlorothiophosphate	5.0
64	Diethylethanolamine	4.5
65	Cyclohexanone	3.0

Coefficient of probability of acute non-lethal inhalation poisoning ($CPPI_{ac} = \frac{C}{Lim_{ac}}$). Table 19 presents the data on 65 compounds, arranged according to the decrease of the CPPI values. At this level, the potential activity of substances is the same way significantly different as at the lethal level. The classification based on this index is shown in Table 20.

Table 20

Classification of the hazard of acute non-lethal poisoning posed by chemical compounds[1] (by $CPPI_{ac} = \left(\frac{C^{20}}{Lim_{ac}}\right)$

Class	$CPPI_{ac}$	Distribution of substances between classes		In parts of C^{20}
		absolute number	%	
I	More or equal to 10 000	17	25.8	Less or equal to 0.0001
II	9999—1000	10	15.2	0.0002—0.001
III	999—100	17	25.8	0.002—0.01
IV	Less than 100	22	33.2	>0.01

[1] It is evident that substances may be compared only by the comparable indices of the Limac, in this particular case by the integral indices (at the level of the integral organism).

Table 21

The ratio of volatility of substances at 20° C to the value of chronic effect threshold ($CPPI_{ch}$)

No	Substance	$CPPI_{ch}$
	Extremely hazardous compounds	
1	Methyl chloride	571 500 000
2	Ozone	2 399 875
3	Ethyleneimine	935 500
4	Acetyl chloride	822 333
5	Dimethylamine	450 000
6	Furan	314 600
7	Isopropylnitrite	291 980
8	Hydrogen sulfide	94 285
9	Methylfuran	89 900
10	Isopropylchlorocarbonate	39 800
11	Carbon tetrachloride	38 200
12	Carbon monoxide	37 333
13	Hydrazine	25 000
14	Benzene	19 900
15	Chloromethyltrichlorosilane	151 28
	Highly hazardous compounds	
16	m-Aminobenzotrifluoride	8 200
17	Nitroform	6 857
18	Benzyl chloride	6 900
19	Methylene chloride	6 480
20	Pentachloroacetone	6 330
21	Hexachloroacetone	2 940
22	Diethylamine	2 398
23	Toluene	1 920
24	Thiophene	1 398
25	Dioxane	1 333
26	Bromobenzene	1 305
	Moderately hazardous compounds	
27	1,3-Chlorobromopropane	622
28	Metacrylic anhydride	535
29	Benzyl cyanide	434
30	Bromoacetopropylacetate	307
31	Furfurol	220
32	Dimethylethanolamine	150
	Slightly hazardous compounds	
33	2-Tretbutylpara-cresol	81
34	Acetopropyl alcohol	50
35	Diethylchlorothiophosphate	49
36	β-Ethoxypropionitrile	48
37	Dimethylacetamide	28
38	Ditolylmethane	12
39	Diethylethanolamine	8
40	Acetophenol	0.093

The division into classes has been done in geometrical progression with 10 as the denominator. According to this classification, substances are distributed between classes of hazard rather uniformly.

Class I comprises the substances which are extremely hazardous according to the $CPPI_{CL}$ (ozone, dimethylamine, ethyleneimine, trichloroacetic chloride, chloromethyltrichlorosilane) and those biologically active according to the value of the Lim_{ac} (methyl furan). As a rule, these substances have irritant properties (dimethylamine, trichloroacetic chloride, ethyleneimine, chloromethyltrichlorosilane) and also cause changes in the functional condition of the nervous system (methyl chloride, carbon monoxide, ozone, etc.). It should be noted that cases of acute (nonlethal) poisoning of workers with carbon monoxide, methyl chloride, ethyleneimine have been many times described in the literature. This proves that the chosen division of compounds into classes is justified.

Class IV (potentially slightly hazardous compounds, according to the $CPPI_{ac}$) comprises a vast majority of compounds of the same class as according to the $CPPI_{CL}$. **Coefficient of the probability of chronic inhalation poisoning** ($CPPI_{ch} = \frac{C^{20}}{Lim_{ch}}$). The data on the $CPPI_{ch}$ are systematized in Table 21. Substances are arranged according to the decrease of the coefficient value. In compiling this classification, to characterize the potential hazard of chronic poisoning (Table 22), boundaries between classes have been chosen in accordance with the classification by the ratio $\frac{C^{20}}{Lim_{ac}}$.

Table 22

Classification of the hazard of chronic poisoning posed by chemical compounds (according to $CPPI_{ch}$)

Class	$CPPI_{ch}$	Distribution of substances between classes		In parts of C^{20}
		absolute number	%	
I	More or equal to 10 000	15	36.5	Less or equal to 0.0001
II	9999—1000	12	29.4	0.0002—0.001
III	999—100	6	14.6	0.002—0.01
IV	Less than 100	8	19.5	More than 0.01

The distribution of substances between the classes of hazard, according to the index under consideration, mainly coincides with their distribution according to the $CPPI_{CL}$ and $CPPI_{ac}$. Thus, out of 15 extremely hazardous substances under chronic exposure, 11 compounds belong to the same class of hazard under a single

exposure. For 4 substances, however, (carbon tetrachloride, benzene, hydrazine, isopropylnitrite), the hazard classes have risen: for the first two compounds, by 2 classes, and for the last two, by 1 class. This fact is surely related to the increase in the activity of compounds at the Lim_{ac} level. Similar relationship has been observed also in other classes.

Evaluation of the hazard of acute lethal poisoning

The main index of the hazard at a high level of exposure is the value of the CL_{50} (DL_{50}). The classification of toxicity based on this index has been considered above. At the same time, there appears a need (for example, for experimental therapy) for a more detailed characteristic of the hazard of the lethal poisoning occurrence. In such cases it is advisable to use together with median lethal concentrations the index of inclination of the straight line, expressing the dependency of the effect on the effective concentration, to the X-axis.

Out of the methods for expressing the slope of the dose—effect straight line, we should recommend to use the value S (or the ratio $\frac{CL_{84}}{CL_{16}}$). The last two indices do not depend on the scales on the Y-axis, and they may be easily calculated with the help of statistical treatment of the experimental results. The toxicity indices, i. e., the value of the CL_{50m}^{120} (a 2-hour inhalation exposure of mice), and the indices of the hazard of lethal poisoning, calculated by the formula $\frac{1}{CL_{50}S}$ for 56 substances, are given in Table 23. The substances are arranged according to the decrease of their median lethal values. From the data in Table 23 follows that the hazard of occurrence of lethal poisoning with compounds (the coefficient S being taken into consideration) in some cases changes disproportionately to the changes of toxicity. Thus, the substances under nn. 10—11 have almost equal values of the CL_{50}, but relatively different meanings of S, therefore the hazard of the lethal poisoning occurrence, posed by these compounds, differs by more than 2 times.

Because of a small variability of the lethal concentrations (from 2.75 to 1.03) these changes are not so significant, but nevertheless they may appear decisive for the comparison of the compounds with similar chemical structure (or for the evaluation of the effect of chemical preparations, etc.). Thus, according to the data given in Table 23, the degree of toxicity of metachlorophenylisocianate (n. 51) and parachlorophenylisocyanate (n. 53) is the same, but the degree of hazard, taking account of the coefficient S, differs by more than 2 times (this kind of difference may

Table 23

Indices of the hazard of lethal poisoning posed by some chemical compounds (2-hour exposure by inhalation, experiments on mice)

No	Substance	CL_{50}, mg/l	S	$\frac{i}{CL_{50} \cdot S}$
1	Freon-142 (chlorodifluoroethane)	1758	1.24	0.0004
2	Freon-113 (trichlorotrifluoroethane)	543.3	1.37	0.001
3	Freon-141 (dichlorofluoroethane)	151.4	1.31	0.005
4	Ethyl chloride	145	1.035	0.006
5	Freon-112 (tetrachlorodifluoroethane)	123	1.26	0.006
6	Benzotrifluoride	92.24	1.55	0.007
7	Bottoms of freon-142	89.1	1.87	0.006
8	Methylene chloride	63.0	1.19	0.0133
9	Benzene	45.0	1.46	0.0152
10	Methylcyclohexane	41.5	1.155	0.02
11	ß-Diethylamino-ethylmercaptan	42.5	2.47	0.009
12	Bottoms of freon-141	39.8	1.25	0.02
13	Carbon tetrachloride	34.5	1.48	0.0196
14	Toluene	32.0	1.52	0.0205
15	Fluorobenzene	27.5	1.35	0.019
16	Chloroform	21.2	1.34	0.035
17	Bromobenzene	21.0	1.64	0.029
18	Chlorobenzene	19.0	1.85	0.0284
19	Trimethylamine	19	1.31	0.04
20	1,2-Dichloropropane	17.6	1.325	0.04
21	Jiodobenzene	17.5	1.64	0.035
22	Cyclopentadiene	14	1.365	0.05
23	Ethylmercaptan	13.8	1.175	0.06
24	Fumigant-93	11.8	1.67	0.06
25	Hexamethyleneimine	10.8	1.9	0.05
26	Allyl chloride	10.7	1.155	0.08
27	Thiophene	9.4	1.64	0.06
28	Piperidine	6.5	2.0	0.08
29	Diethylamine	5.6	2.22	0.08
30	Methyl chloride	5.3	1.37	1.138
31	1,3-Dichloropropylene	4.6	1.25	0.17
32	Isopropylnitrite	2.5	1.45	0.27
33	Methyl bromide	1.54	1.33	0.49
34	Ethylene sulfide	1.4	1.93	0.37
35	Hexachloracetone	0.92	1.27	0.86
36	m-Nitrobenzotrifluoride	0.88	1.25	0.926
37	Dicyclopentadiene	0.74	1.76	0.77
38	m-Amino-benzotrifluoride	0.69	1.27	1.136
39	4-Vinylpyridine	0.53	1.585	1.19
40	2-Vinylpyridine	0.46	1.89	1.15
41	Pentachloroacetone	0.45	1.28	1.74
42	Ethyleneimine	0.4	1.63	1.53
43	Benzyl chloride	0.39	1.64	1.55
44	Mesidine	0.29	1.36	2.56
45	Dimethylchlorothiophosphite	0.29	1.445	2.38
46	Pentadecylamine	0.24	2.75	1.51
47	Sodium pentadecylamine	0.24	1.60	2.60
48	Isopropylchlorocarbonate	0.23	1.365	3.19

Continued

No	Substance	CL_{50}, mg/l	S	$\frac{1}{CL_{50} \cdot S}$
49	Benzal chloride	0.21	2.62	1.90
50	6-Methyl-2-vinylpyridine	0.15	1.225	5.46
51	Methachlorophenylisocyanate	0.07	2.34	6.09
52	Benzotrichloride	0.05	2.0	8.34
53	Parachlorophenylisocyanate	0.053	1.45	13.0
54	Trichloroacetochloride	0.043	1.7	14.3
55	Germanium tetrachloride	0.044	1.60	14.28
56	Cyanuric chloride	0.01	2.30	43.47

be significant only in the experiments conducted simultaneously on animals of the same batch, as it has been in this given case).

Thus, a simultaneous use of two indices, of the CL_{50} and of the index of variability of the lethal concentrations, gives a more complete («dynamic»), according to D. G. Horsfall (1948), characteristic of the hazard of lethal poisoning.

Substantiation of the classification of the hazard of acute non-lethal poisoning

Before turning to the substantiation of classifications, serving to evaluate the hazard of acute non-lethal poisoning, it is advisable to consider once more the indices which have been chosen for this purpose.

Just as toxicity is the major index of the hazard posed by compounds at the lethal level, the value of the Lim_{ac} indicates the biological activity of a substance (the term «toxicity» is not applicable, in our opinion, at this level). The smaller the value of the Lim_{ac}, the more active the substance, and vice versa. The evaluation of the hazard of acute non-lethal poisoning by the value of the Lim_{ac} only is certainly insufficiently. If for the evaluation of the hazard posed by compounds at the lethal level it is necessary to determine — in addition to the value of the CL_{50} — also the variability of the lethal concentrations (doses), then at low level we should determine the acute effect zone ($Z_{ac} = \frac{CL_{50}}{Lim_{ac}}$). The narrower the zone, the greater the hazard of poisoning.

Table 24

Acute effect threshold (Lim_{ac}) of some chemical compounds

No	Substance	Lim_{ac}, mg/l
	Extremely hazardous compounds	
1	Cyanuric chloride	0.0006
2	Ozone	0.001
3	Triphtazine	0.001
4	Aminazine	0.003
5	Dimethylamine	0.005
6	Ditolylmethane	0.006
7	Benzyl cyanide	0.007
8	Metacrylic anhydride	0.0098
	Highly hazardous compounds	
9	Chloromethyltrichlorosilane	0.01
10	Perfluoroadipinic dinitrile	0.01
11	Perfluoroglutaric dinitrile	0.01
12	Trichloroacetochloride	0.01
13	Ethyleneimine	0.01
14	Primary aliphatic amines C_7—C_9	0.01
15	Monoisopropylamine	0.01
16	N-Propylamine	0.01
17	Pentadecylamine	0.01
18	Bromoacetopropylacetate	0.013
19	Hydrazine	0.015
20	Piperidine	0.02
21	Cyanamide	0.02
22	Hexachloroacetone	0.02
23	Pentachloroacetone	0.02
24	Hydrogen sulfide	0.02
25	m-Aminobenzotrifluoride	0.02
26	Methylpiperazine	0.025
27	Diisopropylamine	0.03
28	m-Nitrobenzotrifluoride	0.03
29	Isopropylchlorocarbonate	0.032
30	Sodium pentachlorophenolate	0.035
31	Nitroform	0.04
32	Carbon monoxide	0.05
33	Chloroacetopropylacetate	0.06
34	Aminopirimidine	0.05
35	Methylfuran	0.075
36	Formamide	0.075
37	Calcium cyanamide	0.09
38	Triethylamine	0.1
39	Furan	0.1
40	Benzyl chloride	0.1
	Moderately hazardous compounds	
41	Acetophenone	0.12
42	Acetopropylacetate	0.15
43	Trifluoroethanol	0.20
44	1,3-Dichloroisobutylene	0.20

Continued

No	Substance	Lim_{ac}, mg/l
45	2,3-Dichloropropylene	0.20
46	Methyl chloride	0.23
47	3,3-Dichloroisobutylene	0.25
48	Bromobenzene	0.25
49	Chlorobenzene	0.25
50	Dioxypentadiene dioxide	0.28
51	Isopropylnitrite	0.3
52	Acetopropyl alcohol	0.3
53	Morpholine	0.3
54	Allyl chloride	0.34
55	Trifluoropropylamine	0.35
56	Styrene	0.35
57	Furfurol	0.4
58	1,3-Chlorobromopropane	0.41
59	β-Ethoxypropylnitrite	0.47
60	Dioxane	0.5
61	Diethylamine	0.6
62	Toluene	0.7
63	Chloroform	0.7
64	Hexafluoropropylene	0.92
65	Isopropylbenzene	1.0
66	Carbon disulfide	1.0
67	Methylene chloride	1.0
68	Vinylbutyl ether	1.0
	Slightly hazardous compounds	
69	Diethylethanolamine	1.1
70	Benzene	1.1
71	Dimethylethanolamine	1.2
72	Dimethylformamide	1.2
73	Carbon tetrachloride	1.2
74	Dymethylacetamide	1.23
75	Thiophene	1.5
76	Isoprene	2.0
77	Fluoroethane	2.0
78	Benzotrifluoride	2.2
79	1,2-Dichlorisobutane	3.0
80	Trifluoroacetic acid	3.0
81	Trifluoroethylamine	3.0
82	Cyclohexanone	3.0
83	Cyclopentadiene	5.0
84	Fluorobenzene	5.0
85	Freon-11 (trichlorofluoromethane)	12.0
86	Freon-114 (dichlorotetrafluoroethane)	14.0
87	Freon-114 (dichlorofluoroethane)	15.0
88	Tetrahydrofuran	20.0
89	Freon-112 (tetrachlorodifluoroethane)	30.0
90	Freon-113 (trichlorotrifluoroethane)	60.0

In Table 24 there are presented indices of the acute effect threshold for 90 substances. The substances are arranged in order

of the increase of the Lim_{ac} value, i. e., according to the decrease of their biological activity.

It is appropriate to cite once again the statement of N. S. Pravdin on the possibility of the existence of as many thresholds of the effect of a substance as the number of systems affected by the poison. Therefore, different substances may be compared only by homogenous indices, like, for example, the integral indices. We have considered mainly those substances for which the threshold values have been determined by the integral indices. However, we should at once make a reservation for some possible errors committed in determining the acute effect threshold. Unfortunately, in spite of the known proposals, the threshold value has not got yet the probabilistic evaluation, and one is not always sure of the complete comparability of the Lim_{ac} values.

The analysis of the obtained data has revealed a significant variability in the values of the threshold of a single exposure: from 0.0006 mg/litre for cyanuric chloride, to 60 mg/litre for freon-113 (Table 24). Such a wide range of the Lim_{ac} values has made it possible to divide substances into classes in the geometrical progression (10 as the denominator) (Table 25).

Table 25

Classification of the hazard of acute non-lethal poisoning posed by chemical compounds at the Lim_{ac} level

Class	Lim_{ac}	Distribution of substances between classes	
		Absolute number	%
I	0.01	8	9
II	0.01—0.1	32	35.5
III	0.11—1.0	29	32.2
IV	1.0	21	23.3

Class I (extremely hazardous compounds) comprises 8 substances which, according to the value of the CL_{50} (classification of the MACs Section) have been assigned to categories I and II, i. e., to extremely and highly toxic compounds. It should be noted that none of the substances having a lower activity, according to the CL_{50} value, has passed into this class. Class IV comprises 13 slightly hazardous compounds, which according to the same classification belong to the category of either moderately toxic (diethylethanolamine, dimethylformamide, dimethylacetamide, thiophene, isoprene, fluorobenzene), or slightly toxic compounds (benzene, CCl_4, tetrahydrofuran, benzotrifluoride, etc.), and only one compound, dimethylethanolamine, belongs to highly toxic substances.

Table 26

Acute effect zone (Z_{ac}) of some chemical compounds

No	Substance	Z_{ac}
	Extremely hazardous compounds	
1	Dimethylethanolamine	2.7
2	Cyclopentadiene	2.8
3	Chlorothene	3.3
4	Tetrahydrofuran	3.4
5	Benzyl chloride	3.9
6	Calcium cyanamide	4.1
7	Diethylethanolamine	4.5
8	Fluorobenzene	5.5
	Highly hazardous compounds	
9	3,3-Dichloroisobutylene	6.0
10	Chloromethyltrichlorosilane	6.0
11	Thiophene	6.3
12	Ditolylmethane	6.6
13	Sodium pentachlorophenolate	6.8
14	Dimethylformamide	7.8
15	Isopropylnitrite	9.3
16	Hexafluoropropylene	10.0
17	Carbon sulfide	10.0
18	Diethylamine	11.6
19	1,2-Dichlorobutane	13.0
20	Bromoacetopropylacetate	13.0
21	Dimethylacetamide	14.1
22	2,3-Dichloropropylene	15.5
23	Acrylic acid	17.6
24	Dinitryl of perfluoroadipinic acid	17.7
25	1,3-Chlorobromopropane	17.7
	Moderately hazardous compounds	
26	Perfluoroglutaric dinitrile	19.0
27	Dimethylamine	20.0
28	Nitroform	20.0
29	Cyanuric chloride	20.0
30	1,3-Dichlorobutylene	22.0
31	Freon-142 (chlorodifluoroethane)	22.0
32	Pentachloroacetone	22.5
33	Methyl chloride	23.0
34	Furan	23.0
35	Isopropylbenzene	25.0
36	Ozone	28.0
37	Primary aliphatic amines C_7—C_9	28.0
38	Carbon tetrachloride	29.0

Continued

No	Substance	Z_{ac}
39	m-Nitrobenzotrifluoride	29.0
40	Allyl chloride	30.0
41	Chloroform	30.0
42	Furfurol	30.5
43	m-Aminobenzotrifluoride	34.0
44	Ethyleneimine	40.0
45	Benzene	40.9
46	Benzotrifluoride	42.0
47	Metacrylic anhydride	45.0
48	Hexachloroacetone	46.0
49	Trichloroacetic chloride	47.0
50	Toluene	49.0
	Slightly hazardous compounds	
51	Triethylamine	60.0
52	Cyanobenzyl	61.4
53	Vinylbutyl ether	62.0
54	Hydrogen sulfide	62.5
55	Methylene chloride	63.0
56	Hydrazine	66.0
57	Isopropylchlorocarbonate	67.0
58	Isoprene	72.0
59	Chlorobenzene	76.0
60	Carbon monoxide	80.0
61	Bromobenzene	84.0
62	Dioxane	86.0
63	Pentadecylamine	90.0
64	Methylpiperazine	109.0
65	Methylfuran	111.0
66	Monoisopropylamine	140.0
67	Diisopropylamine	160.0
68	N-Propylamine	170.0
69	Piperidine	300

Thus, with the decrease of the intensity of the substances' effect (from lethal to threshold effect), general regularity remained unchanged: highly toxic substances, as a rule, turned out to be biologically highly active under a single exposure.

Another index of the hazard of the substance effect at the threshold level is, as has been stated above, the acute effect zone. The acute effect zones, calculated for 69 substances, are given in increasing order in Table 26.

As can be seen from Table 26, the Z_{ac} of the first and the last substances differ by 111 times (from 2.7 to 300.0). The magnitude of the acute effect zones for 66% (2/3) of substances does not

go beyond 40.0; 75% (3/4) beyond 60. It should be noted that the value of the Lim_{ac} smaller than 1/10 of the CL_{50} have 20 substances (28%), and the Lim_{ac} below 1/20 of the CL_{50} have 35 substances (about 50%). On the basis of the comparison of the data given in Table 26, there has been proposed a classification of the hazard of acute poisoning according to the magnitude of the acute effect zone (Table 27).

Table 27

Classification of the hazard of acute poisoning with occupational poisons based on the value of the acute effect zone

Class	Z_{ac}	Distribution of substances between classes	
		absolute number	%
I	Less than 6	8	11.6
II	6—18	17	24.6
III	18.1—54	25	36.0
IV	More than 54	19	27.8

The division into classes has been made in the geometrical progression with 3[1] as the denominator. This division does not comply with the considerations presented above, however, we presume that it can be accepted conditionally. According to this division, the substances have been distributed between classes relatively uniformly.

Class I (extremely hazardous compounds with the $Z_{ac}<6$) comprises 8 substances. Most of them (tetrahydrofuran, dimethylethanolamine, etc.) have low biological activity at the Lim_{ac} level (class IV of hazard). It should be mentioned that the Lim_{ac} of these substances are equal only to 1/3—1/4 of the CL_{50}, hence a possibility of an error in the calculated magnitude of the Z_{ac}, resulting from approximate values of the indices chosen for the determination of the Lim_{ac}, is not excluded.

A vast majority of substances belonging to class IV, i. e., to slightly hazardous substances, have low biological activity (classes IV and III of the hazard by the Lim_{ac} level): bromobenzene, dioxan, vinylbutyl ether, etc. However, some biologically active substances (classes I and II) as, for example, methyl-piperazine, hydrazine, pentadecylamine, benzyl cyanide, etc. are slightly hazardous, according to this index.

In this way, the comparison of the hazard from substances by the value of their biological activity (Lim_{ac}) and the magnitude

[1] The logarithmic scale is not suitable in this case because of relatively narrow limits of the acute effect zone (the mentioned values differ only by 111 times).

of the acute effect zone (Z_{ac}) fails to reveal any definite regularity: substances with a low value of the Lim_{ac} may have a narrow zone of the acute effect, and vice versa. This depends, as already pointed out, on the ability of the organism to compensate the damage caused by a given poison.

Substantiation of classifications of the hazard of chronic poisoning

It is known that the qualitative characteristics of the poisons' effect and the quantitative regularities observed under acute and chronic exposures often do not coincide. Hence, for evaluating the real hazard presented by substances under chronic exposure, special indices have been established: the absolute values of the Lim_{ch} and Z_{ch}.

The value of the chronic effect threshold (Lim_{ch}). In order to distribute substances between the classes of hazard, taking into account the absolute value of the Lim_{ac}, 66 compounds have been considered (Table 28).

As can be seen from Table 28, the extreme meanings of the absolute values of the chronic effect threshold of the compounds differ by more than 3,333 times (0.0003 mg/l for trichloroacetic chloride, and 1.0 mg/l for tetrahydrofuran). In comparison with the variability of the absolute values of the acute effect threshold, the variability of the chronic effect thresholds has decreased approximately by 3 times. The division of substances according to the absolute value of the Lim_{ac} has been done in the geometrical progression with 10 as the denominator (Table 29).

According to the proposed division, the substances have been distributed between the classes of hazard rather uniformly, although classes I and IV comprise somewhat less substances than the medium classes II and III.

Class I comprises only 3 extremely hazardous, according to the Lim_{ch}, substances: cyanuric chloride, triphtazine, and ozone which are extremely hazardous by the Lim_{ac} value; and 5 compounds (trichloroacetic chloride, perfluoradipinic dinitrile, etc.) which belong to the II boundary class of hazard, according to the Lim_{ac} value. The substances included in this class have either selective system activity (triphtazine is poison affecting the nervous system, hydrazine is vascular poison etc.), or a narrowly directed antienzymatic action (mercaptophos, methyl mercaptophos, cyanuric chloride, etc.). All the substances of class IV are included in the category of slighthly hazardous compounds (5 substances) or moderately hazardous compounds (5 substances) of the classification based on the Lim_{ac} value. The only exception is carbonic oxide (class II of hazard according to the Lim_{ac}).

Table 28

Threshold of chronic effect (Lim_{ch}) of some chemical compounds

No	Substance	Lim_{ch}, mg/l
	Extremely hazardous compounds	
1	Trichloroacetyl chloride	0.0003
2	Metaphos	0.0004
3	Ethyleneimine	0.0004
4	Cyanuric chloride	0.0005
5	Triphtazine	0.0005
6	Perfluoroadipinic dinitrile	0.0006
7	Ozone	0.0008
8	Methylmercaptophos	0.001
9	Mercaptophos	0.001
10	m-Aminobenzotrifluoride	0.001
11	Hydrazine	0.001
12	Benzyl chloride	0.001
	Highly hazardous compounds	
13	Piperidine	0.002
14	Metacrylic anhydride	0.002
15	Primary aliphatic amines C_7—C_9	0.002
16	Chlorothene	0.002
17	Monoisopropylamine	0.003
18	Pentachloroacetone	0.003
19	Benzyl cyanide	0.003
20	Allyl chloride	0.003
21	Bromoacetopropylacetate	0.003
22	Dimethylamine	0.004
23	Phosphamide	0.005
24	Furan	0.005
25	Hexachloroacetone	0.005
26	3,3-Dicloroisobutylene	0.005
27	Ditolylmethane	0.005
28	Pentadecylamine	0.005
29	Isopropylnitrite	0.005
30	Cyanamide	0.006
31	Sodium pentachlorophenolate	0.007
32	Chloromethyltrichlorosilane	0.007
33	Nitroform	0.007
34	Formamide	0.008
35	Morpholine	0.008
36	DDT	0.008
37	Aminopirimidine	0.01
38	Butyphos	0.01
39	Methylfuran	0.01
	Moderately hazardous compounds	
40	Calcium cyanamide	0.011
41	Sevin	0.016
42	Benzene	0.02

Continued

No	Substance	Lim_{ch}, mg/l
43	Bromobenzene	0.02
44	Methyl chloride	0.02
45	Diethylchlorothiophosphate	0.02
46	Hexafluoropropylene	0.028
47	Acetopropylic alcohol	0.04
48	1,3-Chlorobromopropane	0.045
49	Carbon tetrachloride	0.05
50	Triethylamine	0.05
51	Toluene	0.05
52	Furfurol	0.05
53	Dimethylethanolamine	0.05
54	Dimethylformamide	0.08
55	Dioxane	0.1
56	β-Ethoxypropionitrile	0.18
57	Thiophene	0.18
	Slightly hazardous compounds	
58	Methylene chloride	0.25
59	Diethylamine	0.3
60	Carbon monoxide	0.3
61	Dimethylacetamide	0.34
62	Cyclopentadiene	0.35
63	Formalglycole	0.4
64	Diethylethanolamine	0.61
65	Vinylbutyl ether	0.64
66	Tetrahydrofuran	1.0

Table 29

Classification of the hazard of chronic poisoning with occupational poisons based on the Lim_{ch} value

Class	Lim_{ch}	Distribution of substances between classes	
		absolute number	%
I	More or equal to 0.001	12	18.2
II	0.001—0.01	27	39.6
III	0.011—0.1	16	24.2
IV	More than 0.1	11	18.0

The chronic effect zone. The chronic effect zone is determined, as is generally known, by the ratio of the acute and chronic effect thresholds. The wider the zone, the more hazardous the poisons, since chronic poisoning can develop under exposure to low (far below the Lim_{ac}) concentrations of substances. Therefore, chronic poisoning may develop in such cases gradually or latently. It should be noted that in determining the Z_{ch} errors may be committed as a result of an inaccuracy in determination of the threshold values, especially as the thresholds usually do not have probabilistic characteristics and up to now the no-effect concentrations are being determined in experiments relatively seldom.

Data on the chronic effect zones of different occupational poisons are given in Table 30. From the Table follows that the highest Z_{ch} is 113 (allyl chloride), and the lowest is 1.2 (ozone, cyanuric chloride).

The chronic effect zones of the majority of the compounds (81%) do exceed 15.0; 73% of the compounds have the Z_{ch} smaller or equal to 10; below or equal to 5 have 44%; 6 substances (9%) have the Z_{ch} larger or equal to 50.

Analysis of the data given in Table 30 has permitted us to classify the hazard posed by chemical compounds according to the absolute value of the Z_{ch} (Table 31). The division has been done in geometrical progression with 2 as the denominator. It was impossible to choose a higher denominator since the differences in the Z_{ch} values of the considered compounds were insignificant. It is advisable to compare the Z_{ch} with the Z_{ac}.

Class I (extremely hazardous compounds) includes only 8 substances which are extremely or highly hazardous according to the Z_{ac}. The other 10 substances of this class of hazard, according to the mentioned index, under a single exposure appear to be either moderately hazardous (8 substances), or even slightly hazardous (2 substances). To the latter belong bromobenzene and hydrazine. The substances of this class are polytropic poisons, though separate system poisons are also found here (benzene, trichloroacetic chloride, etc.). Clinico-hygienic observations in the industries where carbon tetrachloride and hydrazine are used prove that it was wright to assign these poisons to extremely hazardous substances from the point of view of occurrence of chronic poisoning.

Class IV (slightly hazardous substances from the point of view of development of chronic poisoning) comprises 15 substances, 5 of which are slightly and 3 moderately hazardous under a single exposure (according to the Z_{ac}). However, this class comprises also compounds which are extremely hazardous (diethylethanolamine) and highly hazardous compounds (diethylamine, chloromethyl, trichlorosilane, ditolylmethane, etc.) under a

Table 30

Chronic effect zones (Z_{ch}) of some chemical compounds

No	Substance	Z_{ch}
	Extremely hazardous compounds	
1	Allyl chloride	113
2	Benzyl chloride	100
3	Isopropylnitrite	60
4	Carbon tetrachloride	60
5	Benzene	55
6	3,3-Dichloroisobutylene	50
7	Trichloroacetyl chloride	33
8	Hexafluoropropylene	33
9	Dimethylethanolamine	24
10	Furan	20
11	Tetrahydrofuran	20
12	m-Aminobenzotrifluoride	20
13	Hydrazine	15
14	Dimethylformamide	15
15	Cyclopentadiene	14.2
16	Toluene	13
17	Bromobenzene	12.5
18	Methyl chloride	11
	Highly hazardous compounds	
19	Diethylchlorothiophosphate	10
20	Piperidine	10
21	Methylmercaptophos	10
22	Formamide	9.4
23	1,3-Chlorobromopropane	9.1
24	Metaphos	8.7
25	M-81 (0,0-dimethyl-β-ethylmercaptoethyldithiophosphate)	8.3
26	Thiophene	8.3
27	Calcium cyanamid	8.1
28	Butyphos	8
29	Acetophenone	8
30	Furfurol	8
31	Methylfuran	7.5
32	Acetopropylic alcohol	7.3
33	Pentachloroacetone	6.6
34	Perfluoroadipinic dinitrile	6
35	Nitroform	5.7
36	Aminopirimidine	5.4
37	Dioxane	5
38	Primary aliphatic amines C_7—C_9	5
39	Metacrylic anhydride	5
40	Sodium pentachlorophenolate	5

Cont inued

No	Substance	Z_{ch}
	Moderately toxic compounds	
41	Bromoacetopropylacetate	4.3
42	Hexachloroacetone	4
43	Methylene chloride	4
44	Dimethylacetamide	3.9
45	Isopropylchlorocarbonate	3.5
46	Monoisopropylamine	3.3
47	Chloroprene	3
48	β-Ethoxypropionitrile	2.7
49	Mercaptophos	2.5
50	DDT	2.5
	Slightly hazardous comp ounds	
51	Benzyl chloride	2.3
52	Freon-141 (dichlorofluoroethane)	2.2
53	Diethylamine	2
54	Pentadecylamine	2
55	Triphtazine	2
56	Phosphamide	2
57	Triethylamine	2
58	Diethylethanolamine	1.8
59	Carbon monoxide	1.7
60	Vinylbutyl ether	1.5
61	Chloromethyltrichlorosilane	1.4
62	Dimethylamine	1.25
63	Ditolylmethane	1.2
64	Ozone	1.2
65	Cyanuric chloride	1.2

Table 31

Classification of the hazard of chronic poisoning based on the magnitude of the zone of biological effect

Class	Z_{ch}	Distribution of substances between classes	
		Absolute number	%
I	10	18	27.2
II	10—5	22	33.4
III	4.9—2.5	11	16.8
IV	2.5	15	22.6

single exposure. It should be mentioned that this class includes many irritants (8 out of 17): diethylamine, dimethylamine, etc. Pesticides which are extremely toxic under a single exposure are also included in class II (M-81; methyl mercaptophos, metaphos, calcium cyanamid).

The zone of biological effect. The determination of the magnitude of this zone is of particular importance, since the use of the Z_{ch} is confined to the field of industrial toxicology only, since other fields of toxicology (e. g., communal toxocology) do not have determined acute effect thresholds.

K. K. Sidorov (I. P. Ulanova, K. K. Sidorov, A. I. Halepo, 1971) has suggested a classification of the degree of manifestation of cumulative properties of chemical compounds, based on the magnitude of the biological effect zone. This classification can be used for the evaluation of the hazard of chronic poisoning (Table 32).

Table 32

Degree of manifestation of cumulative properties of compounds according to the magnitude of the chronic effect zone

Degree of cumulation	Magnitude of the zone		
	Exposure by inhalation		Enteric administration
	daily 4-hour	24-hour	
Weak	10	500	100
Moderate	11—100	501—5000	101—1000
Marked	101—1000	5001—50 000	1001—10 000
High	1000	50 000	10 001—100 000
Extreme	—	—	100 000

According to the magnitude of the biological effect zone, there have been distinguished 4 hazard classes under inhalation, and 5 classes under enteric administration of substances. Sufficiently large (within one order) intervals, characterizing the degree of the cumulative effect, have been chosen in compiling this classification, since the values of the chronic effect thresholds are subjected to fluctuations, depending on the sensitivity of the applied methods of investigation.

The biological effect zone appeared to be wider under continuous 24-hr exposure by inhalation during 60—90 days than that of the same compounds under periodical 4-hr exposure during 120 days. The zone expansion under 24-hr exposures may be explained by the increase of the cumulative effect of the inhaled substances as a result of the increase of the total exposure time.

The analysis of the magnitudes of the zones of biological effect of substances which have established standards for the air of workplaces, for the atmosphere of residential areas and for water basins, carried out according to the suggested classification, allowed us to reveal, in the majority of cases, the correspondence between the degrees of cumulation in different media. The discovered correlation gives one an opportunity to predict orientational degrees of cumulation (and thereby, also the hazard of chronic poisoning) for the substances entering the organism from different media, using the data on the magnitude of the biological effect zone of the compounds the standards of which have been established for one of these media.

Thus, the presented data confirm the statement above on principal differences existing in the course of the pathological process occurring in the organism under acute and chronic exposure to chemical compounds. It should be noted that in evaluating the hazard from a compound by a number of indices it is possible to obtain different hazard classes, but, in the last analysis, the decisive index must be that revealing the highest degree of hazard, according to a specific task. The indices of chronic effect of poisons (Lim_{ac}, Z_{ch}), provided that they have been obtained, have a decisive role in determining the class of hazard when substantiating the MACs for chemical compounds. A different approach to the evaluation of the hazard posed by a poison is to be used under single exposures, e. g., in fire fighting. The determining index in this case is that revealing the highest hazard under a single exposure to a poison (CL_{50}, Lim_{ac}, Z_{ac}). Anyway, the index of the highest hazard from a poison (i. e., a limiting index) indicates the most hazardous link, which should be taken into a particular consideration.

The proposed 4-stage classification allows one to adopt a differentiated approach to the substantiation of preventive measures. At the same time, in experimental investigations it is expedient to use the continuous scale classification, which allows one to determine precisily (not only the class) the degree of differences in the biological activity of poisons.

S. D. Zaugolnikov et al. (1973) have recently worked out a classification of the hazard degree of substances for water bodies (Table 33) according to the hazard classes of occupational poisons considered above. The hazard index of substances in this case (per os administration) is represented by the logarithm of the ratio of their solubility in water ($S_{20}°$) and their MACs.

Since the indices of water solubility of substances are oftennot given in official reference books, the authors have investigated the three-dimentional raws by the multiple regression method: the boiling point, the MAC and the hazard index. The obtained correlation may be expressed by the following regression equation:

Table 33

Scale of the hazard from substances in water of reservoirs

Hazard index	Hazard class			
	extremely hazardous I	highly hazardous II	moderately hazardous III	slightly hazardous IV
$\lg \frac{S_{20°}\text{ (g in 100 g of } H_2O)}{\text{MAC in water (mg/l)}}$	More than 2	2—1	0.9—0	0—(—2)

$$\lg \frac{S_{20°}\text{ (g in 100 g } H_2O)}{\text{MAC in water (mg/litre)}} = 1 - 0.007 \cdot t_{boil} - \lg \text{MAC in water (mg/litre)}$$

The authors are of the opinion that the evaluation of the real hazard according to the considered principle can be also applied for determining the MACs for water bodies used for pisciculture, and for assessing the impact on the biocenosis as a whole.

In conclusion, it should be mentioned that in presenting the data on the substantiation of the classifications of the potential and real hazard posed by poisons at different levels of exposure the main attention has been given to the characteristics of the hazard degree according to the quantitative criteria. It does not mean, however, that the qualitative aspect of the effect of substances should be disregarded. The mentioned problem is rather complex. The available data on this issue are presented in Chapter X.

Chapter 4. PREDICTION OF THE HAZARD OF CHRONIC POISONING BY THE INDICES OF CUMULATIVE PROPERTIES OF CHEMICAL COMPOUNDS

As has already been shown, the hazard of chronic poisoning, posed by a given substance, is evaluated by the magnitude of the chronic and biological effect zone calculated from the results of a chronic experiment (its duration is not less than 4 months in industrial toxicology, 4 and 6 months in communal toxicology, and up to 2 years in food toxicology). It is understood that chronic poisoning is possible only in case of accumulation of a substance, and in case latent residual changes occurring in the organism after the intake of each portion of a substance. It is therefore possible to predict the development of chronic poisoning using quantitative evaluation of the cumulative properties of a poison.

The prediction of chronic poisoning with pesticides has been studied in the most thorough and many-sided way. The correlation between the cumulation coefficient (I_{cum}) and the safety factor, the dependency of the cumulation coefficient on the chemical structure of a compound and on the magnitude of fractions of the administered dose, the relationship between cumulation and adaptation, the criteria and methods for the evaluation of cumulation — these problems have investigated by Y. S. Kagan (1965, 1970 et al.). At the same time, it should be remembered that the evaluation of the cumulative properties of a compound by the method of Y. S. Kagan and V. V. Stankevich (1964), including the calculation of the I_{cum}, requires a long-term experiment (more than 3 months, which approximates to the usual duration of chronic exposure). That explains why there have been carried out investigations on the possibility to determine the cumulative ability in shorter periods of time. Using the method of Lim et al., there have been determined the I_{cum} at the lethal level, as well as under exposure to poisons at the threshold levels, which is of particular importance for the purposes of hygienic standardization of the content of substances in the environment (I. P. Ulanova et. al., 1966; K. K. Sidorov, 1967; Y. S. Kagan, 1968; G. N. Krasovsky et al., 1970; G. N. Krasovsky, 1970; N. S. Gizatullina, 1970).

G. N. Krasovsky emphasizes the fact that during the investi-

gation of the cumulative properties of different substances there has been always registered the development of the adaptation condition determined by the exposure level. According to G. N. Krasovsky, large doses can paralyze the adaptive systems of the organism, and in this case the effect of the repeated exposure will have nothing in common with the adaptive processes occuring under exposure to low concentrations (doses) of substances which take place in real conditions.

Indeed, since the cumulative effect is determined by the combination of the material and functional cumulation, the material cumulation may prevail under application of large doses (a substance, being applied in large doses, does not have time to be excreted from the organism). For many compounds, the material cumulation will be of a limited significance or completely absent if smaller doses, approximating to the levels studied in chronic experiments, are introduced into the organism (B. M. Shtabsky, 1974). G. N. Krasovsky and S. A. Shigan (1970), G. N. Krasovsky et al. (1971) suggest a method for assessing the cumulative properties of compounds based on calculation of effective doses using functional indices. After calculating the DE_{50} for each hour of each chosen day of observation, the cumulation coefficients are calculated (the DE_{50} for the lst day is taken as 1).

This method has been successfully applied in investigating the cumulative properties of 4 organophosphorous compounds and some other substances having specific action.

Investigation of the dependency of the cumulative properties of compounds on the exposure level

Investigation of the cumulative properties of compounds, belonging to different classes, at high level of exposure has been carried out by the method of Lim et al. The data obtained are given in Table 34.

Judging by the cumulation coefficients, the substances have weak cumulative properties and, therefore, present slight hazard of chronic poisoning. However, in chronic experiments bromobenzene, m-aminobenzotrifluoride, carbon tetrachloride and benzene are very hazardous poisons. As to benzene and carbon tetrachloride, their hazard has been assessed in appropriate clinico-hygienic observations.

In order to characterize the relationship between the I_{cum}, determined by the lethal effect, and the chronic effect zone (Z_{ch}), a special correlation coefficient has been calculated. The data and materials of the Laboratory of Toxicology of the Institute of Industrial Hygiene and Occupational Diseases of the USSR Academy of Medical Sciences have been used for this purpose. There

Table 34

Some parameters of the toxicity of chemical compounds under a single and repeated administration into the stomach of white rats

Substance	DL_{50}, mg/kg		$I_{cum}=\frac{DL_{50}\ n}{DL_{50}\ 1}$	Time of administration, days
	single experiment	repeated experiment		
Carbon tetrachloride	3 650	2 400	6.57	30
Benzene	6 400	82 688	6.67	20
Bromobenzene	3 200	11 500	3.6	18
Iodobenzene	2 650	6 100	2.3	16
Chlorobenzene	3 300	9 200	2.8	17
Benzotrifluoride	15 000	96 000	6.13	26
m-Aminobenzotrifluoride	480	5 000	10.4	32
m-Nitrobenzotrifluoride	610	6 500	10.65	31

Table 35

Calculation of the coefficient of correlation between the chronic effect zone (Z_{ch}) and the cumulation coefficient (I_{cum})

Substance	Z_{ch}	I_{cum}	$x_1-\bar{x}$	$y_1-\bar{y}$	$(x_1-\bar{x})(y_1-\bar{y})$	$(x_1-\bar{x})^2$	$(y_1-\bar{y})^2$
Carbon tetrachloride	60	6.57	—43.64	—2.89	126.11	1904.44	8.35
m-Aminobenzotrifluoride	20	10.4	—3.64	—6.72	24.46	13.24	43.15
Formamide	12.5	0.86	3.86	2.82	10.88	14.89	7.95
Dimethylformamide	30	1.3	—13.64	2.38	—32.46	186.04	5.66
Bromobenzene	20	3.6	—3.64	0.98	—0.29	13.24	0.0064
Benzene	55	6.87	—38.64	—3.19	123.26	1493.04	10.17
Trifluoroethanol	3.6	2.4	12.76	1.28	16.33	162.81	1.63
Trifluoroethylamine	6	3.0	10.36	0.68	7.04	107.32	0.46
Chlorothene	3	3.8	13.36	—0.12	—1.6	178.48	0.01
Aminopirimidine	6	4.5	10.36	—0.82	—8.49	107.32	0.67
Piperidine	10	0.96	6.36	2.72	17.29	40.44	7.39
Bromoacetopropyl acetate	4.5	2.6	11.86	1.08	12.8	140.65	1.16
Acetopropylic alcohol	7.3	4.3	9.06	—0.62	—5.61	82.08	0.38
Diaminophenylsulfone	4	1.5	12.36	2.18	26.94	152.76	4.75
Pyromel dianhydride	3.5	2.5	12.86	1.18	15.17	165.37	1.39

$\bar{x}=16.36$; $\bar{y}=3.68$; $\Sigma=331.83$; $\Sigma=4762.12$; $\Sigma=95.126$;

$$r=\frac{331.83}{\sqrt{453\,001.43}}=\frac{331.83}{673}=+0.49$$

have been selected cases when the I_{cum} was determined by the method of Lim et al. (initial fraction equal to 1/10 of the LD_{50}), and the chronic effect zone was assessed using the adequate investigation methods.

The calculation of the correlation coefficient for 15 compounds, which appeared to be equal to +0.49, is given in Table 35. Hence, between the magnitude of the chronic effect zone and the cumulation coefficient there is a weak positive relationship. The diagram describing the scatter of the treated results is given in Fig. 15.

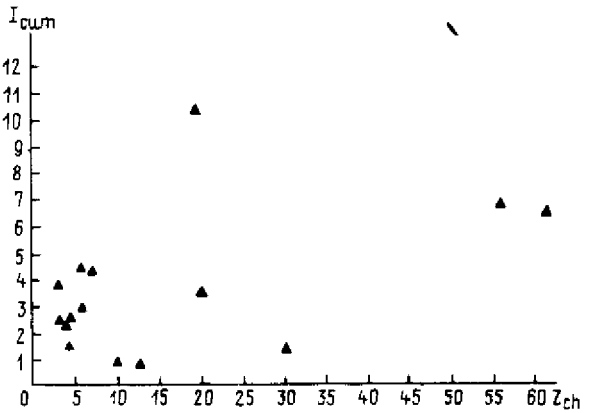

Fig. 15. Diagram of scatter of the values of I_{cum} and Z_{ch} for a number of chemical compounds.

Thus, the determination of the cumulative properties of compounds by lethal outcome fails to provide preventive medicine with the true idea of the hazard of chronic poisoning. The discrepancy between the I_{cum} (by lethal outcome) and the Z_{ch} may be related to different routes by which poisons enter the organism or to different exposure levels. It is known that the processes of absorption, transformation and excretion of poisons from the organism are not the same under acute and chronic intoxications. It is therefore recommended (I. P. Ulanova et al., 1966) to investigate the cumulative properties of compounds by the changes of indices occurring under poisons administration in fractions of the Lim_{ac} and not of the DL_{50}.

For this purpose, the acute effect threshold under administration of substances into the stomach was the first to be determined. The indices of the experimental animals' vital activity were investigated the day after, which made it possible to reveal only stable changes, determining the development of chronic poisoning. The cumulation coefficient was calculated by the formula

$I_{cum} = \frac{Lim_{ac1}}{Lim_{acn}}$. In this calculation, the cumulative activity of compounds is directly proportional to the value of cumulation coefficient. The subacute experiment was started by the administration of 1/10 of the determined Lim_{ac} value, according to the scheme of Lim et al., i. e., increasing the administered dose by 1.5 times every 5th day. To illustrate this fact, we would like to cite some data on the assessment of cumulative properties of benzene and carbon tetrachloride at the threshold level, i. e., using exactly those substances for which the cumulation, being determined by the lethal effect, does not reflect the possibility of a chronic intoxication occurrence.

Benzene. The acute effect threshold of benzene under a single administration into the stomach of white rats was assessed by changes in the body weight (integral index), the functional state of nervous system (STI) and in the blood system (leukocytes quantity), i. e., in the systems which appeared to be the first to respond to the repeated exposure to this compound by inhalation.

Table 36

Summated threshold index and quantity of leukocytes in peripheral blood of rats after a single administration of benzene into the stomach

Index	Dose, mg/kg			Control
	1000	1600	2200	
Summated threshold index, cond. units	2.33±0.41 p>0.05	2.83±0.3 p<0.02	2.66±0.21 p<0.01	1.66±0.21
Quantity of leukocytes, thousand	14.88±0.56 p>0.05	15.86±0.97 p>0.05	16.23±1.17 p>0.05	14.15±0.94

As can be seen from Table 36, the threshold dose of benzene, according to the STI changes, is 1,600 mg/kg. Under the replicated experiment, benzene was administered in doses equal to 1/10 of the Lim_{ac}, i. e., 160 mg/kg. After administering the summated dose equal to 0.4 of the Lim_{ac} and to 1.0 of the Lim_{ac} (i. e., on the 4th day), the animals were examined. The results are given in Fig. 16.

It can be seen from Fig. 16 that no changes in the indices under study were registered in test animals after 4 days of the experiment. After administration of the summated dose (1.0 of the Lim_{ac}), system changes were neither revealed, but a certain reduction in the body weight increase was, however, registered. The cumulation coefficient of benzene, calculated from the data obtained, is equal to 1.

Table 37

Summated threshold index and rectal temperature of rats after a single administration of CCl_4 into the stomach

Index	Dose, mg/kg					
	200		400		1600	
	test	control	test	control	test	control
Summated threshold index, cond. units	4.3±0.49	4.5±0.43	5.8±0.3	5.3±0.3	4.3±0.28	3.1±0.3
	p>0.05		p>0.5		p<0.02	
Rectal temperature, °C	38.1±0.21	37.9±0.12	38.6±0.2	38.5±0.2	38.4±1.14	38.3±0.14
	p>0.5		p>0.5		p<0.5	

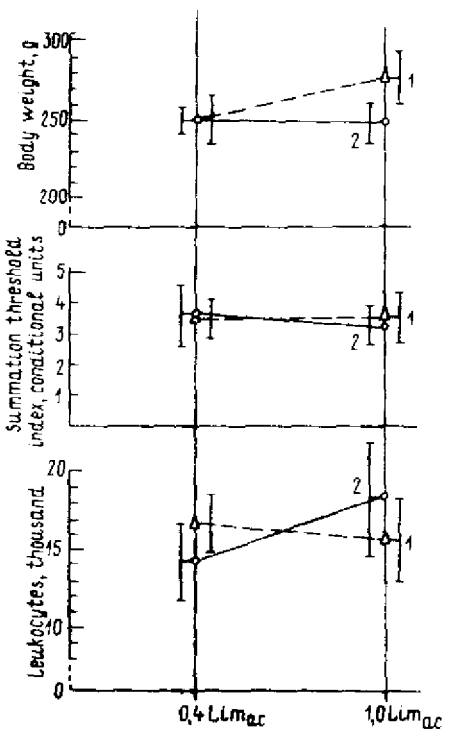

Fig. 16. Some indices of the state of white rats under replicated administration of benzene into the stomach.
(1) control; (2) test.

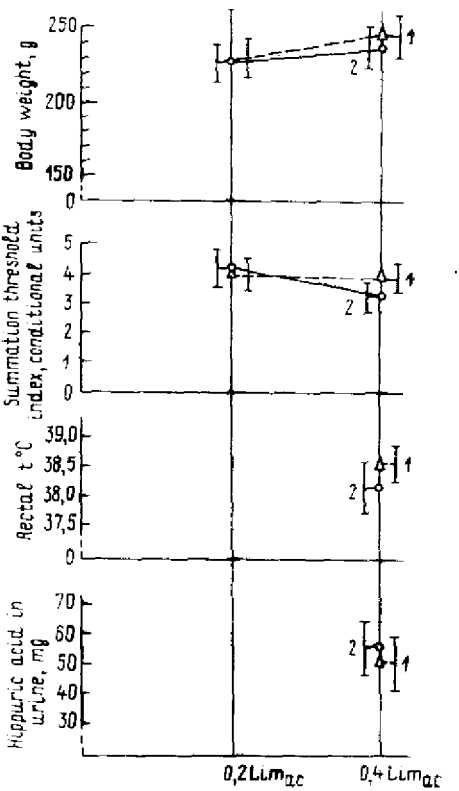

Fig. 17. Some indices of the state of white rats under replicated administration of CCl_4 into the stomach.
(1) control; (2) test.

Carbon tetrachloride. Results of the doses test and the indices used for determining the Lim_{ac} for CCl_4 under administration of the poison into the stomach are given in Table 37.

The first changes in the STI, preserved until a day after the poison administration, were obtained under administration of carbon tetrachloride in a dose equal to 1,600 mg/kg. This dose was accepted as conditional threshold of the acute effect (indices of the liver condition were omitted in that given experiment because of technical conditions).

In the replicated experiment, 2 days after its beginning, functional changes in animals were not registered (Fig. 17). However, already 4 days after the administration of the summated dose (0.4 of the Lim_{ac}), the summation-threshold index of the experimental animals was changed. Thus, the cumulation coefficient for carbon tetrachloride in the investigation at the threshold level was equal to 2.5.

It should be emphasized that investigations at the threshold level make it possible to characterize the processes of cumulation of compounds in much shorter periods of time.

Dependency of the cumulative effect on the regimen of the substances administration

One of the most important conditions, determining the poisons' effect on the organism, is the amount of the poison in the inhaled air with due consideration to the time of exposure.

It is known that concentrations of toxic substances may considerably vary under occupational conditions. It is not a monotonous exposure (when the poison concentration is practically constant) that the working people are usually subjected to, but that intermittent or interrupted. Under intermittent exposure, the poison concentration in the air usually vary; under interrupted exposure, inhalation of a poison alternates with inhalation of clean air.

Z. A. Volkova (1965), N. F. Izmerov et al. (1973) have shown that fluctuations of the poisons concentrations within one-two orders are registered in the air of workplaces even under a constant technological regimen. Even a wider variation in the content of toxic substances is registered under periodical processes (loading and unloading of products, sampling, opening of machinery hatches, etc.). Izraelson (1937) was the first to pay attention to this fact. He suggested to take into consideration the variability of the poisons concentrations in evaluating the labour conditions. The movement of working people about the working premises and the absence of fixed workplaces give rise to the possibility of the intermittent effect of harmful substances on the organism.

Similar fluctuations of concentrations of toxic substances have also been observed in the atmosphere of residential areas (V. A. Ryazanov, 1954; G. I. Sidorenko, M. A. Pinigin, 1969). According to G. I. Sidorenko and M. A. Pinigin (1969), the ratio of the maximum single concentration to the daily average in the atmospheric air varies within the limits of 1.9—1.8; according to V. A. Ryazanov (1954), it varies up to 7 or more times; the maximum concentration at a given point may be ten to several hundred times as high as the minimum concentration during the same 24 hours (G. I. Sidorenko, M. A. Pinigin, 1969).

These distinguishing features of the poison effect on the organism should be kept in mind when investigating complicated relatioships between the organism and the poison. Meanwhile, in conducting a chronic experiment on substantiation of the MACs of substances for the air of workplaces and for the atmosphere of residential areas, the researchers tend to produce in the exposure chambers permanent concentrations, and they calculate the average concentration for the whole experiment.

At present, in connection with a decrease of the level of exposure to occupational factors, the attention of researches is atracted to the investigation of the organism's ability to adapt chemical factors of minor intensity. In this respect the investigation of the processes of the organism adaptation to the monotonous and intermittent exposures is rather urgent.

Comparison of the organism's responses to the monotonous and intermittent exposure to poisons. There is an opinion that the character and the degree of changes, occurring in the organism under constant and intermittent exposure to poisone, depend to a large extent on physical and chemical properties of substances, mainly on their water and fat solubility (N. A. Tolokontsev, 1957, 1960; E. I. Lyublina, I. V. Olyunin, 1957).

Analysis of the data from the literature on the comparison of the effect of constant and intermittent exposure to substances on the organism has shown that for almost all considered poisons and, in particular, for narcotics of the 2nd type of action[1], the intermittent regimen of exposure produces a more marked biological effect than the monotonous exposure. This conclusion seems to contradict the results of studies on the comparison of the intermittent exposure with the twenty-four-hour exposure, i. e., the experiments with the exposure to the same concentrations of a poison, but under different regimens. In our opinion, this is probably connected with the fact that in similar investigations the organism's responses should be compared only in definite iso-

[1] According to the type of the effect of organic solvents on the nervous system, E. I. Lyublina divided them into 2 groups: to the first group belong hydrophilic narcotics, to the second group belong hydrophobic narcotics.

effective periods, since the summated dose of a substance introduced into the organism under intermittent regimen was smaller than under monotonous exposure (G. P. Tikhonova et al. 1970; Y. P. Bizin et al., 1971; M. A. Pinigin, 1972; H. S. Markaryan, 1972; Y. E. Yakushevich, 1973, et al.). Almost in all experimental studies the accumulation of substances in the blood under the two types of exposure was not taken into account, though the assessment of a poison's content in the blood helps not only to choose appropriate concentrations for comparing the regimens, but also to explain the essence of the revealed differences.

Intermittent effect of substances under occupational conditions and in everyday life can manifest itself in alternation of periods in which compounds affect man in constant and varying concentrations and the «clear» intervals, when a substance is completely absent in the environment. The comparison of the manifested cumulative properties under different regimens has been carried out under continuous administration of substances (daily enteric) and under administration of substances with a 2-day interval each week, which is of particular importance at present because of the introduction of a 5-day working week.

Under such regimens substances were administered in constant doses (by the method of Y. S. Kagan and V. V. Stankevich, 1964), and in increasing doses (by the method of Lim et al., 1961). The intermittent and continuous exposure to a number of substances during 1—1.5 months was the subject of special investigations.

The cumulative activity was assessed by the death of experimental animals, summated administered dose, causing death, and by changes of individual indices of the functional condition of the animals. White rats and mice were used in this experiment. The investigation was conducted with benzene, CCl_4 and m-aminobenzotrifluoride (m-ABTF), i. e., the compounds having good solubility in fats and practically no solubility in water. Piperidine was chosen as a compound having good solubility in water.

Enteric administration of substances in increasing doses. Results of the experiment are shown in Table 38. In the majority of experiments, under administration of substances with a 2-day interval each week, the I_{cum} turned out to be lower than the I_{cum} obtained under daily administration of the product (i. e., the cumulative properties were more significant). The difference in the I_{cum} is significant only for benzene (1/10 and 1/20 fractions) and for carbon tetrachloride (1/10) (Fig. 18).

In all experiments, under administration for 5 days a week the minimum dose causing the death of animals appeares to be noticeably lower than under each day administration. However, by the end of the experiment (100% death of animals) the summated doses, administered under such regimens, are equal or

Table 38

Some indices of cumulative properties of compounds under daily administration and under administration with a 2-day interval each week

Index	Fractions in parts of the DL_{50}	Benzene		CCl_4		m-ABTF		Piperidine	
		7 days	5 days	7 days	5 days	7 days	5 days	7 days	5 days
Summated dose causing initial death	1/5	4.0	5.52	2.69	1.46	28.87	35.61	0.6	0.4
	1/10	4.75	1.44	5.92	1.9	33.41	—	0.2	0.3
	1/20	5.27	2.65	0.15	0.1	—	—	0.15	0.1
causing 100 % death	1/5	9.51	9.51	15.0	6.5	44.03	44.03	2.0	1.4
	1/10	7.5	7.5	12.8	12.8	61.76	22.07	0.7	1.0
	1/20	11.03	8.08	25.2	11.02	37.99	11.03	0.5	0.64
Duration of experiment, days	1/5	19	29	21	21	33	45	8	9
	1/10	23	36	28	38	43	45	6	10
	1/20	33	43	41	45	45	45	8	11
$I_{cum}=\frac{DL_{50n}}{DL_{50}^{1}}$	1/5	6.5	6.8	6.0	3.7	36.0	40.0	0.97	0.68
	1/10	5.73	3.4	7.33	4.11	44.0	—	0.39	0.58
	1/20	7.0	4.0	5.6	—	—	—	0.39	0.23

nearly equal. Therefore, the total duration of experiment under 5-days a week administration is naturally longer. In other cases, when the differences in the I_{cum} under each day administration and administration with a 2-day interval each week are not registered, the minimum and absolute lethal doses under both regimens are equal or differ insignificantly.

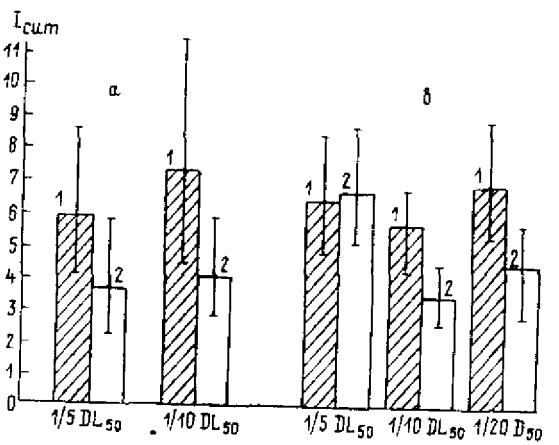

Fig. 18. Cumulation coefficient for CCl_4 (a), for benzene (b) for mice (method of Lim et al.).

(1) seven days; (2) five days.

Results of investigation of the functional condition of animals after administration of the same poisons are presented in Tables 39 and 40. As can be seen from these Tables, the indices changes were revealed already during the first examination after the administration of CCl_4 and benzene to animals in a summated dose equal to 1/5 of the DL_{50}.

As the administration of substances proceeded, manifestation of functional changes increased. Fractional method of the poisons administration had no significant importance in that case. No differences in the degree of the changes manifestation were revealed under continuous and intermittent regimens of administration.

Thus, considering the results of the investigation of the functional condition of the organism of rats under administration of substances by the method of Lim et al., it is difficult to give preference to the continuous or intermittent regimens of administration. At the same time, by the indices of mortality among experimental animals it is possible to draw the conclusion that a 5-days a week administration of a substance is quite acceptable, since it does not disguise the cumulative properties of substances, which are revealed earlier under continuous administration. On

Table 39

Some indices of the condition of white rats under repeated administration of benzene into the stomach

Index	Administered fractions of the DL_{50}				
	1/5		1/10	1/20	
Summated dose	2.9	$1DL_{50}$	3.3	$1DL_{50}$	$3DL_{50}$
Daily administration $p \leqslant 0.05$					
	DL_{50}		DL_{50}		
Body weight	—	—	—	—	—
STI	—	—	—	—	—
Rectal temperature	—	0	0		0
Hemoglobin quantity	0		0		0
Erythrocytes quantity	0		0		0
Leukocytes quantity	0		0		0
5 times a week administration					
Body weight	—		—	0	—
STI	—		—	—	—
Rectal temperature	0		0	0	0
Hemoglobin quantity	0		0	0	0
Erythrocytes quantity	0		0	0	0
Leukocytes quantity	0		—	0	—

Note: (+) increase; (—) decrease; (0) no changes.

Table 40

Some indices of the condition of white rats under repeated administration of CCl_4 ($p \leq 0.05$)

Index	Administered fraction of the DL_{50}						
	1/5		1/10		1/20		
Summated dose	1.0	3.8	5.14	1.0	2.9	4.8	1.0
Daily administration							
	DL_{50}	DL_{50}	DL_{50}	DL_{50}	DL_{50}	DL_{50}	DL_{50}
Body weight	0	—	—	—	—	—	—
STI	0	0	0	—	0	—	—
Rectal temperature	0	0	0	+	0	—	+
Amount of hippuric acid	—	0	—	0	0	0	0
5 times a week administration							
Body weight	0	—	—	—	—	—	—
STI	0	0	0	0	0	0	0
Rectal temperature	0	0	0	0	0	0	0
Amount of hippuric acid	0	—	0	0	0	—	—

Note: (+) increase; (—) decrease; (0) no changes.

Table 41

Some indices of cumulative properties of compounds in experiments on mice by the method of Y. S. Kagan under daily administration and administration with a 2-day interval each week

Index	Fractions of the DL_{50}	Benzene		CCl_4		m-ABTF		Piperidine	
		7 days	5 days	7 days	5 days	7 days	5 days	7 days	5 days
Summated dose:									
causing primary mortality	1/5	3.4	3.4	0.2	0.2	2.6	—	—	—
	1/10	—	—	—	—	3.8	—	—	—
	1/20	0.5	0.25	0.25	—	—	—	—	—
By the end of the experiment	1/5	5.6	5.6	6.0	4.4	9.0	6.6	9.0	7.0
	1/10	3.2	2.8	1.9	1.4	4.5	3.3	4.5	3.5
	1/20	2.25	1.65	2.25	1.65	2.25	1.65	2.25	1.75
Duration of the experiment, days	1/5	28	36	30	30	45	45	45	49
	1/10	69	32	18	18	45	45	45	49
	1/20	45	45	45	45	45	45	45	49
Animals' mortality, %	1/5	30	20	22.2	44.4	12.5	0	30	50
	1/10	0	0	0	0	10	0	0	10
	1/20	11.1	30	10	0	0	0	10	20

the contratry, one can get an impression that cumulation in the organism is manifested more under a 5-day regimen of administration. However, the number of substances used in experiments is limited, though applied in different fractions of the DL_{50}. Therefore, we cannot draw a final conclusion, since we do not have enough evidence.

Administration of substances in constant doses. In none of those experiments did we manage to calculate the I_{cum}, though the experiments duration varied from 18 to 49 days (mostly 1.5 months). In many cases death among the animals did not occur at all.

As can be seen from Table 41, differences in the cumulative properties of compounds under 5- and 7-day regimens of exposure, according to the chosen indices, are insignificant and do not exceed the limits of the standard error of a biological experiment. The state of individual systems and functions of the rat's organism after administration of poisons is illustrated in Tables 42 and 43.

From the data in Table 42 folloys that under administration of benzene in 1/5 and 1/10 fractions of the DL_{50} under 5-day regimens of exposure the changes are slightly more significant than

Table 42

Some indices of the condition of white rats under repeated administration of benzene by the method of Y. S. Kagan ($p \leqslant 0.05$)

Index	Administered fraction of the DL_{50}			
	1/5		1/10	
	summated dose			
	3.0 DL_{50}	4.6 DL_{50}	2.9 DL_{50}	4.3 DL_{50}
Daily administration				
Body weight	—	—	—	0
STI	0	—	0	0
Rectal temperature	0	0	0	0
Hemoglobin	0	0	0	0
Erythrocytes	0	0	0	0
Leukocytes	0	0	—	0
5 times a week administration				
Body weight	0	—	—	—
STI	—	—	+	+
Rectal temperature	0	0	0	0
Hemoglobin	0	0	0	0
Erythrocytes	0	0	0	0
Leukocytes	0	—	0	0

Note: (+) increase; (+) decrease; (0) no changes.

Table 43

Some indices of the condition of white rats under repeated administration of CCl_4 by the method of Y. S. Kagan ($p \leq 0.05$)

Index	Administered fraction of the DL_{50}				
	1/5		1/10	1/20	
	summated dose				
	1.0 DL_{50}	3.0 DL_{50}	5.2 DL_{50}	1.0 DL_{50}	1.0 DL_{50}
Daily administration					
Body weight	—	—	—	—	0
STI	0	0	0	0	0
Rectal temperature	0	0	0	+	—
Amount of hippuric acid	0	0	0	0	0
5 times a week administration					
Body weight	0	—	—	—	—
STI	0	0	0	+	—
Rectal temperature	0	+	+	—	0
Amount of hippuric acid	0	0	—	0	+

Note: (+) increase; (—) decrease; (0) no changes.

under continuous administration. However, definite regularities in the changes of the effect, under an increase of the summated dose, were not registered.

The data obtained under introduction of CCl_4 (see Table 43) demonstrate even more clearly the advantage of the 5-day regimen of substances administration for revealing the cumulation by the functional indices. It was only under 5-day regimen of CCl_4 administration (1/5 fraction of the DL_{50}) that the functional changes increased with the increase of the summated dose of the substance.

Monotonous and intermittent inhalation exposure has been studied using as an example certain substances with low water solubility, i. e., narcotice of the II type: CCl_4 and methylene chloride.

It should be emphasized that by the monotonous regimen was conditionally intended the regimen of a 4-hour daily exposure to the poison at a relatively constant concentration. Intermittent exposure occurred under intermittent regimens, consisting of short 15-min exposures («peaks») and periods of the clean air inhalation («intervals»). Under exposure to CCl_4, regimens with 2, 3, 4 and 5 exposure peaks were produced in the chambers during 4 hours of the animal experiment. The peak concentration values and the duration of intervals under different regimens varied,

while the average suspended concentrations under all intermittent regimens and under monotonous regimen were similar, being at the level of 1/10 of the threshold value for a single exposure. In all the cases, peak concentrations did not exceed the value of the threshold of a single exposure (Fig. 19).

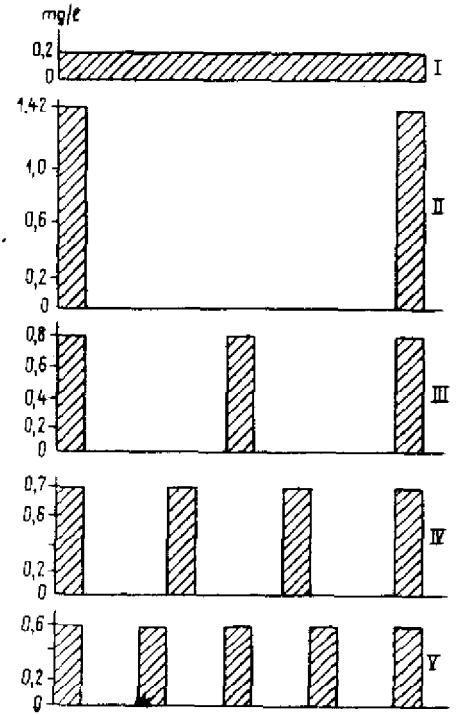

Fig. 19. Different variants of intermittent exposure to CCl_4.
I, conditionally monotonous regimen (median suspended concentration of 0.2 mg/l); II, regimen with 2 peaks (0.17 mg/l); III, regimen with 3 peaks (0.15 mg/l); IV, regimen with 4 peaks (0.18 mg/l); regimen with 5 peaks (0.18 mg/l).

Concentrations of substances in the air were determined with the help of fast and highly sensitive method of gas chromatography. Under monotonous regimen, the concentrations were registered 5—6 times; under intermittent regimen, they were registered during every peak exposure. The poison was practically absent in the air of the chambers already 5 minutes after the end of exposure (N. M. Maltseva, 1974).

The duration of the experiment was 1—1.5 months. The time of examination was 1, 4, 8, 15, 27 and 42 days of exposure, which corresponded to the scheme of investigation accepted in the Laboratory of Toxicology for revealing primary responses and the dynamics of the processes in the short-term experiment (I. V. Sanotsky, 1972; N. M. Karamzina et al., 1973). White mongrel male rats were used as experimental animals. Nearly 800 animals were used for each experiment. The experiment with CCl_4 was replicated twice; with CH_2Cl_2 three times.

The data from publications on the predominant action of the

indicated poisons on the nervous system and on parenchymatous organs was used as the basis for selecting methods for the evaluation of the state of the organism (N. V. Lazarev, 1971; I. P. Ulanova et al., 1971, etc.). The subject of investigation was the general state of the organism (body weight, orientation reflex, rectal temperature, working capacity) and the state of the nervous system (STI). The liver functions were assessed by the amount of synthesized hippuric acid, the rate of the bromosulfaleine elimination from the blood, the activity of a number of enzymes, specific for the liver, in the serum (sorbite-dehydrogenase, fructose-monophosphataldolase, urokaninase, ornithine-carbomoiltransferase, alanine-amintransferase, butyrile-cholinesterase), the duration of hexenal sleep (N. P. Kazmina). The evaluation of the functional condition of kidneys comprised the assessment of diuresis, the content of protein and chlorides in urine.

It is known that a significant role in forming the adaptive responses and in maintaining the stability in the internal medium has, besides the nervous system, also the endocrine system. The relative weight of the pituitary body and adrenal glands, as well as the content of ascorbic acid in the adrenal glands and of 17-ketosteroids in urine, have served as the indices of the function of the pituitary body — adrenal glands system. Another criteria of non-specific adaptation has been the state of the thymic-lymphatic system: the weight of thymus, the mitiotic index of thymus, the weight of peribronchiolar lymph node and the lymph nodes of the inguinar group (primary data by E. K. Redkina).

The pathomorphological investigations of different organs and the determination of their relative weight were also carried out. In order to reveal possible latent changes, occuring in the process of exposure to a poison, and the organism's capacity to adapt, the load with the same poison, in a dose of 1,600 mg/kg (Lim_{ac}) was applied. To differentiate the adaptation and temporary compensation conditions of the pathological process, besides the load application, there were taken into account the natural physiological fluctuations of the average values in the control animals, there were compared the conditions of different organs and systems, as well as the functional changes caused by a poison in the organism.

The statistical group consisted of 10 animals. The data were treated statistically using the Student's t-test. The comparison of the organism's responses to the exposure to CCl_4 under the regimens with 2, 3, 4 and 5 peaks (I. P. Ulanova et al., 1973) allowed us to consider a 5-fold exposure as one of the unfavorable variants, which we have used and thoroughly investigated in further experiments.

The results of the experiment conducted under monotonous and 5-fold exposure to CCl_4 are given in Figs. 20 and 21. As can

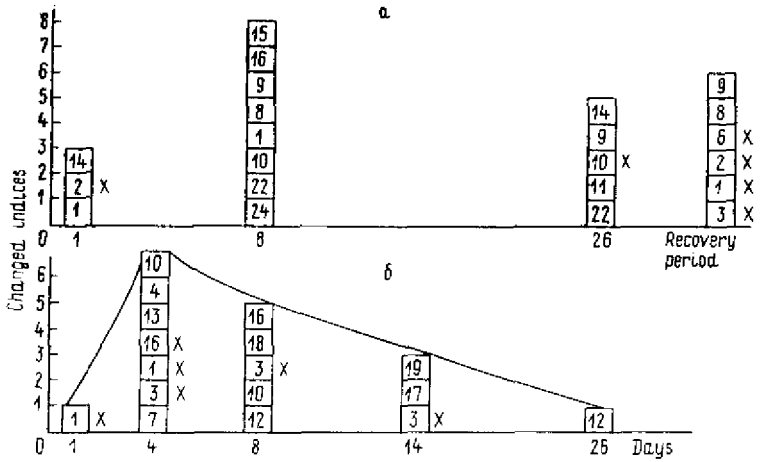

Fig. 20. Results of the experiments under monotonous exposure to CCl_4: (a) with load; (b) without load.

(1) fructosomonophosphataldolase; (2) ornithine carbomoyltransferase; (3) sorbitol dehydrogenase; (4) butyrilcholinesterase; (5) alanine aminotransferase; (6) urokaninase; (7) amount of hippuric acid; (8) elimination of bromosulfalein from blood; (9)duration of hexenal sleep; (10) relative mass of liver; (11) summation threshold index; (12) orientation reflex; (13) ascorbic acid in adrenal gland; (14) relative mass of adrenal gland; (15) quantity of of 17-ketosteroids in urine; (16) relative mass of thymus; (17) quantity of lymphocytes in peripheral blood; (18) mitotic index of thymus; (19) total number of cells of inguinal lymph node; (20) mass of peribronchial lymph node; (21) mass of inguinal lymph node; (22) amount of protein in urine; (23) quantity of chlorides in urine; (24) diuresis; (25) fluctuations exceeding the limits of 2 .

be seen from Fig. 20, no changes in the weight of animals, rectal temperature, condition of the nervous system and in the function of kidneys were registered under monotonous exposure. On the 8th and 27th day, the orientation responses of the animals appeared to decrease, but insignificantly in comparison with the natural fluctuations. Changes in the state of lymphoid tissue were revealed. The most marked changes were discovered in the liver. Already on the first day the activity of fructose-monophosphataldolase increased.

By the 4th day the functional changes in liver appeared to grow: there added an increase of the relative weight of the liver and of the amount of sorbite-dehydrogenase, while the quantity of hippuric acid after a load with benzoic acid sodium reduced. Later the changes in liver began to decrease and disappeared completely by the end of the experiment.

Much more changes, in comparison with the monotonous exposure, were registered under the intermittent regimen already after a single exposure (see Fig. 21). Besides an increase in the activity of the mentioned enzymes, reflecting, to a certain extent, the state of hepatocytes, there were observed changes in the lymphoid tissue. All the changes revealed showed a tendency to intensification by the 4th day. From the same day there used to

begin undulating changes in the function of the nervous system, by the 27th day they completely disappeared: only changes in the lymphoid tissue, a decrease of the orientation reflex and an increase of the chlorides content in the urine still remained.

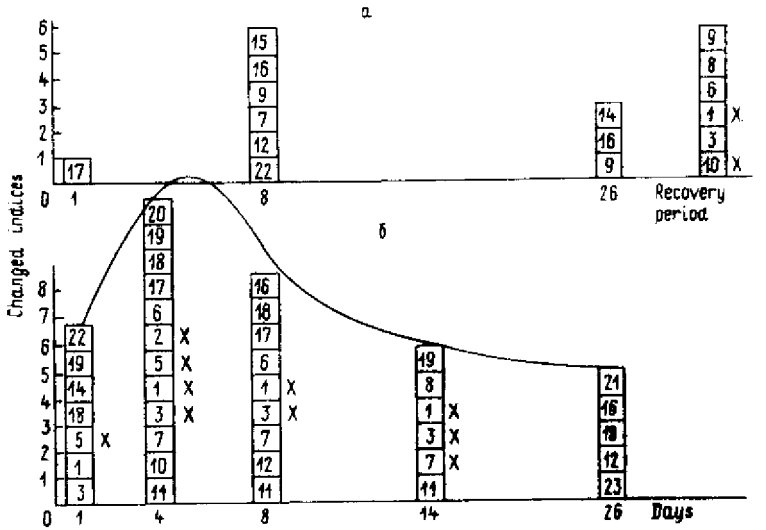

Fig. 21. Results of the experiment under intermittent exposure to CCl_4.

Symbols are the same as in Fig. 20.

Thus, from the number of the changed indices it can be seen that the intermittent exposure to CCl_4 is more hazardous for the organism. Under monotonous regimen, there appeared an increase in the activity of only two enzymes specific for the liver: fructose-monophosphataldolase and sorbite-dehydrogenase; under intermittent exposure, there occurred a simultaneous increase in the activity of almost all the enzymes under investigation. The degree of the changes manifestation turned out to be higher under intermittent regimen.

The load with the same substance in a dose of 1,600 mg/kg showed that a reconstruction and activation of the adaptive mechanisms occurred in the liver (the experimental animals responded to this load less than the control group).

The poison's content in the blood was determined in the experiment under exposure to methylene chloride. The poison concentration in the blood was found to be higher under monotonous than under intermittent regimen ($p<0.05$) (Fig. 22). Nevertheless, the animals' response to the intermittent regimen of exposure was more expressed. Thus, all indices (except an increase of the body weight and the retention of BSF from the blood) (Fig. 23) underwent changes at different time, while under mo-

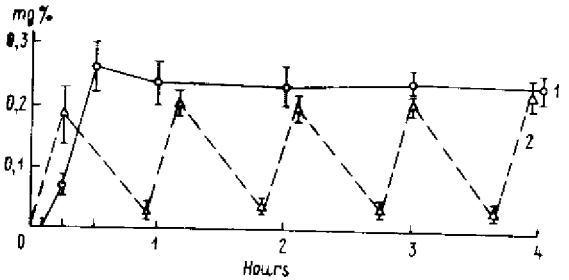

Fig. 22. Accumulation of methylene chloride in the blood of animals under monotonous (1) and intermittent (2) exposures.

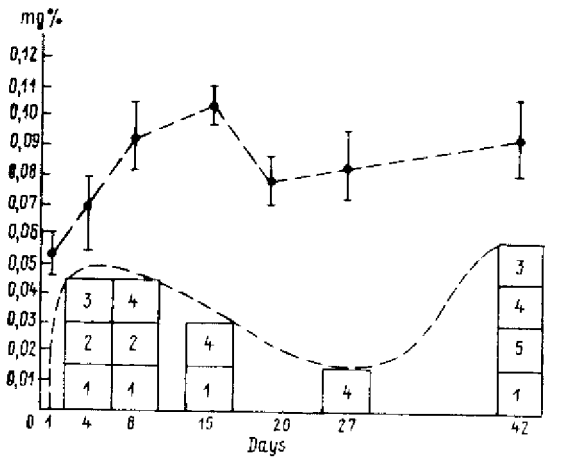

Fig. 23. Number of changed indices ($p<0.05$) of the functional condition of the organism and content of methylene chloride in blood under intermittent regimen of exposure.

(1) STI, (2) content of hippuric acid in urine; (3) diuresis; (4) content of chlorides in urine; (5) body weight.

notonous regimen there changed only the STI and the chlorides content in urine (Fig. 24). The degree of the changes manifestation was more significant under intermittent regimen and, in a number of cases, it exeeded the limits of physiological fluctuations.

Under monotonous regimen, the dynamics of the poison content in the blood during the experiment reflected changes in the functional indices. Thus, the largest number of changes was registered in the period of the maximum poison content in the blood (on the 15th day): when the poison concentration in the blood decreased, the general state of the organism improved. Under intermittent regimen, such correlation was not discovered.

When the concentration of the poison in the organism was maximum, the changes were smaller than under low concentra-

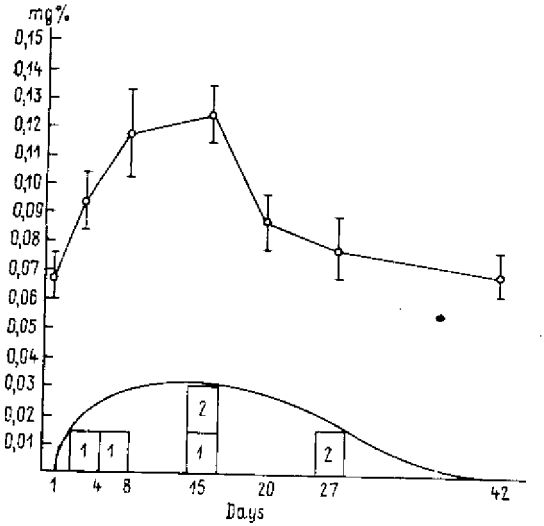

Fig. 24. Number of changed indices ($p<0.05$) of the functional condition of the organism and content of methylene chloride in blood under monotonous regimen of exposure.

(1) STI; (2) content of chlorides in urine.

tions. Significant deterioration of the general state was observed by the end of the experiment.

The existence of the functional changes was confirmed by a pathomorphological investigation of the organs. Under monotonous regimen, the morphological changes did not differ from the control, while under intermittent regimen they were characterized by moderately manifested circulatory and dystrophic changes in the liver, kidneys and brain.

So we may assume that under monotonous regimen in animals develops the state of adaptation, while under intermittent regimen prevail the processes of cumulation, which at first lead to a latently developing process (habituation), and then to evident intoxication. It is known that the processes of adaptation and cumulation are closely interrelated and, under exposure to chemical factors, are directed at reduction of the poison content in the organism. It may occur as a result of changes in the permeability of the membranes, of the inclusion of additional routes of elimination, an increase of the speed of elimination, changes in the rate of metabolism, etc. Some of the indicated mechanisms were investigated when determining the concentration of methylene chloride in the inhaled air, urine and bile, taking into account possible routes of its elimination from the organism.

It was assessed that the methylene chloride content in the exhaled air is correlated with its changes in the blood, and on the 27th day it also dropped. Probably, it is connected with the

increase of the speed of the poison elimination from the organism. N. M. Maltseva (1974) made a comparison between the dynamics of the methylene chloride elimination from the blood on the 27th day of exposure and that in the period of its maximum concentration in the blood, i. e., on the 15th day, 10, 30 minutes, 1 and 2 hours after the end of exposure.

In accordance with the revealed peculiarity of the methylene chloride elimination in two biological half-life periods, the comparison of the dynamics of the product elimination was carried out separately for each half-life period (Table 44).

Table 44

Biological half-life elimination periods of methylene chloride under monotonous and intermittent exposures

T_{biol}, min	Monotonous regimen		Intermittent regimen	
	15th day	27th day	15th day	27th day
T_1	8.0±0.8	8.6±0.9	7.8±1.2	9.1±0.9
	$p>0.5$		$p>0.5$	
T_2	43.3±5.8	24.7±2	46.2±4.6	27.7±1.4
	$p<0.05$		$p<0.05$	

It was determined that the first periods, characterized by a fast elimination of a poison from the organism, were practically equal under both regimens, either on the 15th or on the 27th day of exposure. At the same time, the excretion of the slowly eliminated fraction of the product under the two regimens occurred faster on the 27th day.

The level of the methylene chloride content in urine and bile was relatively low and rather stable throughout the experiment. However, taking into account the fact that the poison concentration drops by the 27th day, but remains approximately at the same level in urine and bile, the importance of these elimination routes also somewhat increases.

Hence, the conducted investigations made it possible to suggest that one of the mechanisms leading to a decrease of the poison concentration in the blood was the change in the speed of its elimination from the organism.

Thus, the investigation of the cumulative properties of chlorinated hydrocarbons of methane series, of benzene and its monohalogenated derivatives, halogenides of toluene and nitro-amino-derivatives of fluorotoluene, based on the data on the death of the experimental animals, does not reflect the true cumulative activity of compounds, and therefore does not allow one to make correct assessment of the probability of chronic poisoning. An investigation of cumulation by changes in the functional indices

under repeated administration of poisons in 1/10 fractions of the DL_{50} characterizes the processes of cumulation more precisely, making the time of the experiment noticeably shorter.

The cumulation coefficients of benzene, CCl_4, ABTF, determined by the formula Lim^1_{ac}/Lim^n_{ac}, i. e., the ratio of the minimum effective dose under a single administration and the minimum effective summated dose under repeated exposure, turned out to be higher: the cumulation was more expressed than when assessed by the death of animals (Fig. 25).

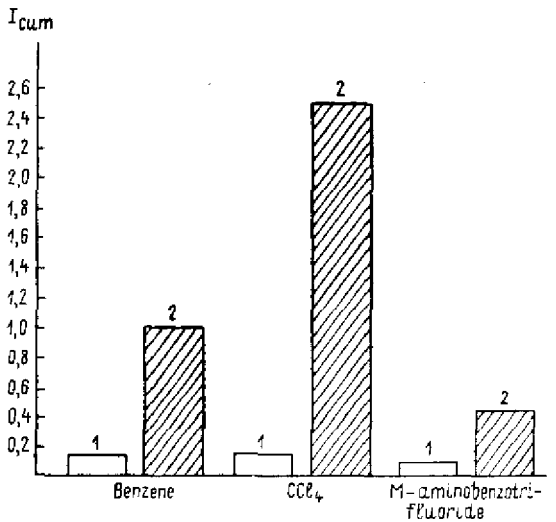

Fig. 25. Cumulation coefficients for some chemical compounds, obtained at lethal (1) and threshold (2) levels.

In should be noted that in the Soviet literature there have recently appeared certain works (G. N. Krasovsky et al., 1970; N. S. Gizatullina, 1970) in which the authors emphasize high significance and methodological correctness of the investigation of the cumulative properties of poisons exactly at the threshold level, based on the indices of sensitivity to the poison's effect.

G. N. Krasovsky et al (1970), using the example of methemoglobin former (dinitroethyleneglycol), suggest a method of an each hour analysis of the most sensitive index (in this case, methemoglobin; administration of 1/5 — 1/25 and 1/125 fractions of the DL_{50}). The authors recommend this method for evaluating the cumulative effect of substances having a known (specific) type of action (organophosphorous compounds, heavy metals, methemoglobin formers, inhibitors of monoamine oxidase, poisons inhibiting the respiratory enzymes). By the example of triphenylphosphate, N. S. Gizatullina has proved the possibility of assessing the cu-

mulative properties at the threshold level (the substance was administered in a dose of 1/5 and 1/2 of the Lim_{ac}) by the inhibition of the blood cholinesterase, representing a specific index for this group of substances. However, none of the mentioned works has given quantitative characteristics of the cumulative properties of the considered substances.

The investigations of the cumulative properties of chemical compounds at the threshold level which we have carried out make it possible to give quantitative characteristics of the poisons' cumulative properties according to the integral indices (body weight, STI), and to reveal the cumulative properties of compounds in a short-term experiment (during 30 days).

The study of cumulation under different regimens of exposure, continuous (7 days a week) and intermittent (5 days of administration, followed by a 2-day interval), permits one to draw the conclusion that in order to reveal the cumulative activity of compounds it is expedient to use the second type of regimen. The limited character of the data does not allow one to conclude that under different regimens the cumulation depends on physico-chemical properties of the considered compounds.

We would like to mention one peculiarity discovered when investigating the development of acute poisoning: the death of animals, often registred at the beginning of the experiment, used to stop suddenly, and the survived animals preserved their resistance in spite of the continuing exposure and died much later. Similar phenomena have been registered by Y. S. Kagan (1970), G. N. Krasovsky, S. A. Shigan (1970).

The appearance of the stage of resistance to different poisons is dose-dependent. The diagram of the death of animals under exposure to CCl_4 in different fractions (1/5, 1/10 and 1/20 of the DL_{50}) is shown in Fig. 26. The stage of resistance is being discovered when CCl_4 is administered in 1/20 fractions of the DL_{50}. Under exposure to m-ABTF and morpholine, the stage of resistance appears at other levels. It has been noticed that with the increase of the cumulative properties of substances there increases the fraction of the administered dose at which this stage is being registered. The analysis of chemicals (CCl_4, benzene, m-ABTF, morpholine, methyl- and ethylmorpholine) allows one to make a hypothesis that the fraction of the administered poison under which the stage of resistance is being discovered may, to a certain extent, characterize the degree of manifestation of the cumulative properties of compounds.

It has been shown that the exposure regimen may significantly influence the cumulative processes. This is particularly important in carring out experiments on the substantiation of preventive measures. The tendency to produce in the experiment the monotonous regimen of exposure is, apparently, not always justified; although this regimen could serve as a standard for the determi-

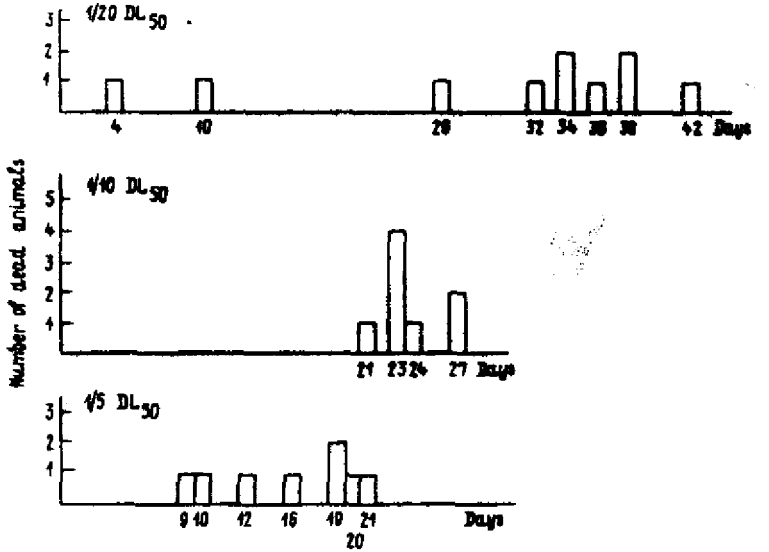

Fig. 26. Diagram of the death of mice under exposure to CCl_4 in different fractions of the DL_{50}.

nation of the quantitative relationships between the comparative sensitivity of the living organisms under continuous and intermittent regimens of exposure.

M. A. Pinigin et al. (1973) have made such attempts. It has been demonstrated that a continuous 23-day exposure by inhalation to aniline results in the «derangement» of the «adaptation» mechanisms (a 12% decrease of the capacity to compensate the methemoglobin formation) of the system of blood enzymes, while the intervals between 24-hour periods of continuous exposure lead to working out of protective mechanisms. These mechanisms reduced by 50% the methemoglobin formation, produced by common for all the groups load dose of the same poison. It is understood that M. A. Pinigin et al. have considered a specific given case. The weak side of the authors' position lies in the assumption that the physiological condition of animals is common for all experimental groups (common gaseous metabolism, in particular), on which basis the presupposed absorbed dose of aniline is being calculated. An inadequacy of the periods of investigation of the absorbed dose under different regimens of exposure is also a source of uncertainty. Nevertheless, the data obtained are interesting.

One should bear in mind that the investigation of the processes of cumulation and adaptation to substances, having marked specific action (wide Z_{sp}), is rather complicated, since the specific changes under a long-term administration of a poison may be determined only against the background of preserved equilibrium

between the integral organism and the environment. This has been shown by E. Y. Golubovich when studying changes in the testis RNA under exposure to small doses of lead.

Some hygienists have raised a question of a necessity to immitate completely in the experiment concrete conditions of the chemical pollution variations in the environment. Such an approach appears not well enough justified, since it does not allow one to discover general regularities.

Thus, a relatively new problem of the relationship between the organism's responses and the regimen of chemical exposure, in spite of all its urgency, is still in the stage of the data accumulation. However, the data obtained up to the present time may already be used for the elaboration of practical guidelines for investigations in experimental and natural conditions. Further studies of the mechanisms of this phenomenon would be of great importance. Attention is now concentrated on assessing the role of physical and chemical properties of substances (mainly their relative water and lipids solubility), and on closely related to them peculiarities of absorption and elimination of substances from the organism in conditions of intensification of the biological effect under intermittent regimen. Minor importance is being attributed to the metabolism of compounds. On the other hand, mainly specific changes are being studied. The principles underlying the changes in the general regulatory responses are being studied not widely enough. The appearance of the new data, expected in the near future, will certainly clarify many unsolved problems.

Chapter 5. SPECIES AND AGE SENSITIVITY TO POISONS AND USE OF THE DATA FROM ANIMAL EXPERIMENTS IN HYGIENIC PRACTICE

Differences in the sensitivity of man and animals to chemical exposure may be, to a great extent, ascribed to the rates of absorption, distribution and elimination of substances from the organism, to the species metabolic peculiarities (the metabolic rate, in particular), and to the differences in the detoxicating ability of the enzymatic systems. According to Williams (1956), aromatic amines are subjected to acetylization in man as well as in rabbits and rats. In dogs, this process has not been found. In man, dog, rabbit and rat poisons are detoxicated by means of formation of conjugated glucuronic acids, which is not registered, for example, in cat.

The degree of the methemoglobin formation, in case of poisoning with sodium nitrite, is relatively similar in man and in white rats, but gunea pigs and rabbits form less methemoglobin (S. N. Tcherkinsky et al., 1966). By the degree of responses of sulfhydryl groups of the blood to the administration of silver, the organism of man is slightly more sensitive (not more than by 5 times) than the organism of guinea pigs and rabbits, and is 25 times as sensitive as the organism of white rats (G. K. Krasovsky, 1973).

The rate of the poison transformation in different species of laboratory animals and man differs significantly. Thus, hexabarbital sodium, N-isopropylmethoxamine and phenylbutazone are subjected to a faster metabolic transformation in the organism of small rodents than in man; trimexane, on the contrary, is metabolized faster in man (Brodie, 1962; Burns et al., 1965).

Differences in absorption, distribution, transformation and elimination of poisons in man and animals cause, in a number of cases, qualitative inadequacy in the organism's responses, which is to be taken into consideration when selecting species of animals for an experimental investigation. It happens, however, relatively seldom. Pathogenesis of poisonings with a vast majority of substances is of the same type for man and animals, therefore, by exposing laboratory animals to toxic substances, it is possible to reproduce poisoning similar to that of man (G. N. Krasovsky, 1973).

Of major importance is the quantitative aspect of the problem. In a number of cases, the organism of man appears to be more sensitive to poisons, as compared to the organism of experimental animals, which is particularly characteristic of the exposure to alkaloids. Thus, man, as it is known, is the most sensitive to atropine, while cats are slightly less sensitive to it. Dogs and rabbits can endure atropine in a dose exceeding the lethal dose for man by 100 times. In other cases, different species of animals appear to be more sensitive to poisons than man.

For example, the sensitivity of dogs to hydrocyanic acid is higher than that of man (O. N. Elizarova, 1962).

The differences in the species sensitivity at the nonlethal levels of exposure may seem even more manifested and it may form an impression that the data obtained from animal experiments cannot be used in hygienic practice. Fortunately, that is not so. The sensitivity of man appears, in most cases, close to that of animals.

Several methods for substantiating the selection of experimental animals for modelling processes of interaction of poisons with the organism in view of further data extrapolation to man have been proposed. One of these methodes is based on the assumption that man has approximately equal sensitivity in case of an equal sensitivity of several species of animals (N. S. Pravdin, 1947; Noordwijk, 1964). O. N. Elizarova (1962) recommends to use for the analysis 2 animal species; Blas (1964), 3 species; Williams (1967), 12 species. The second method consists in choosing an animal species which would be close to man as to metabolizing a given poison (L. A. Tiunov, 1967). The third method suggests the use of the most sensitive animal species (G. N. Krasovsky, 1970), and the last method is based on the use of the «rule of the body weight» (G. N. Krasovsky, 1973). According to this rule, the toxicity closely correlates with the body weight of animals of a given species.

Only the comparison of experimental data can bring one close to formulating practical recommendations in this field. The determination of the comparative species sensitivity of experimental animals, as well as that of experimental animals and man, is, apparently, one of the approaches to the solution of this problem.

Determination of differences in the sensitivity of animal species by the value of the median lethal doses

In order to determine the animal species sensitivity, the authors of this book together with K. K. Sidorov and A. I. Halepo have analysed the lethal dose values for 52 chemical compounds, obtained in experiments on 4 species of rodents (mice, rats, guinea pigs and rabbits) under a single administration of substances

into the stomach. These species of animals are the most frequently used in toxicological investigations. There were used only those compounds for which the lethal doses had been determined. The coefficient of differences in the species sensitivity (CSS), which is the ratio of the lethal dose for the least sensitive species to that for the most sensitive animal species, was calculated for each compound. A similar «coefficient of selectivity»

$$\frac{(DL_{50} \text{ for warm-blooded animals})}{DL_{50} \text{ for insects}}$$

was recommended by Y. S. Kagan (1965) for the purpose of finding insecticides which would be highly toxic for insects and slightly toxic for man. The coefficients of the species sensitivity for a number of substances are given in Table 45, and are summarized in Table 46.

From the presented data follows that the CSS of animals under exposure to over 50% of the included in the Tables substances is smaller or equal to 3, and for 75% of the compounds is smaller or equal to 4.

What difference in the lethal dose values is to be considered an index of significance of the species differences in sensitivity?

The data from publications indicate that the values of the median lethal dose for one substance may differ for each animal species by 2—3 times, depending on a number of factors. These factors include the type of solvent, volume of the administered solution, sex and age of experimental animals, season, diet, the fact whether the animals are kept separately or in groups, etc.

Depending on the season, the value of the DL_{50} differ by almost 2 times (Selisko et al., 1963; Bekemeier, 1965); depending on the body weight and of experimental animals, by 2—5 times (Weil et al., 1966); depending on the degree of the substances dilution, by 2—3 times (Griffith, 1964); according to the type of solvent and the administered volume, the DL_{50} value also changes within the specified limits (E. G. Sharina, 1964; Ferguson, 1962). Significant differences in the values of the DL_{50} (males, up to 9.5; females, up to 3.5 times) between the mice kept in groups and those kept separately were registered by I. P. Lapin and M. P. Samsonova (1964). Even larger fluctuations were observed under exposure to specific spasmodic poisons.

Thus, depending on the incoming factors (not taking into account the factor of isolation, since animals are usually kept in cages in groups, except special investigations), the toxicity of substances in most cases changes within the limits of 2—3 times. This fact has been emphasized also by S. D. Zaugolnikov et at. (1970). Therefore, it is not justified to consider significat those species differences when the CSS values vary from 2 to 3. Analysis of the coefficients of the species sensitivity for 52 chemical com-

Table 45

Lethal doses of chemical compounds (mg/kg) for animals of different species under administration into the stomach and the CSS values

No	Substance	Mouse	Rat	Guinea pig	Rabbit	CSS—$\frac{DL_{50max}}{DL_{50min}}$
1	Acetocyanhydrine	2.9	13	9	13	4.4
2	Acetophos	210	45	27	45	7.7
3	Ammonium perchlorate	1 900	4 200	3 310	1 900	2.2
4	Acetonenitrile	48	105	50	19	5.5
5	Butylacetate	7 700	13 100	4 700	3 200	4.0
6	1,4-Butanediol	2 062	1 525	1 200	2 531	2.1
7	1,4-Butinediol	104	104	130	150	1.5
8	Butyphos	179	217	146	242	1.6
9	N-Butylpyrocatechol	3 000	4 700	1 400	2 800	3.3
10	Gramoxon	37	188	38.6	49.8	3.2
11	Hydrazine hydrate	83	129	40	55	3.2
12	Hexachlorobutane	2 000	1 413	940	1071	2.1
13	Hexachlorobutadiene	87	350	90	90	4.0
14	3,4-Dichloroaniline	500—700	500—700	500—700	500—700	1.0
15	2,5-Dichloroaniline	2 500—3 000	2 500—3 000	2 500—3 000	2 500—3 000	1.0
16	Dichlorobutyl-tin	35	112	190	125	5.4
17	DDT	180	400	400	300	2.2
18	α-2,4-Dinitrophenol	46	31	81	30	2.7
19	1,2-Dibrom-3-chloropropane	410	300	210	180	2.2
20	Diethanolamine	3 300	3 460	2 200	2 200	1.5
21	Ammonium dimethyldithiocarbonate	592	1 458	1 680	450	3.7
22	1,2-Dibromoethane	420	117	110	55	7.6
23	Diphenylpropane	2 400	12 000	4 000	4 000	5.0
24	Indalon	11.6	7.4	3.2	5.4	3.6
25	Carbathione	266	700	815	320	3.0
26	Monoethanolamine	1 476	2 050	620	1 000	3.3
27	Monoethanolethylenediamide	3 550	3 600	1 500	2 000	2.4
28	Methylacetophos	322	380	214	420	1.9
29	Murbetol	50	250	250	200	5.0
30	Melprex	266	1 118	176	535	6.3
31	Nicotine (base)	24	50	220	30	9.1
32	n-Nitrotoluene	330	2 400	3 600	2 400	10.9
33	Sodium fluoride	80	200	250	100	3.1
34	Parachlorobenzene	3 220	2 512	7 593	2 812	3.0
35	Pentachlorobutane	2 500	2 108	1 410	1 560	1.7
36	Regione	79.7	281.9	123.6	227.8	3.5
37	Ethyl alcohol	9 488	13 660	1 600	6 300	8.5
38	Carbon disulfide	2 780	3 188	2 125	2 550	1.5
39	Titanium	150	472	100	100	4.7
40	Tetraethyl-tin	40	15	37	7	5.7
41	1,1,1-Trichloroethane	17 200	12 300	9 470	5 660	3.0
42	Phenylhydrazine	175	188	80	80	2.1

Continued

No	Substance	Mouse	Rat	Guinea pig	Rabbit	CSS = DL_{50max}/DL_{50min}
43	Triethanolamine	7 750	8 400	5 160	5 300	1.6
44	2-Phenylcyclohexenal	5.4	3.5	1.6	2.7	3.3
45	3-Chloro-4-methylcoumarinyl	28	38	57	75	2.6
46	Chlorobenzene	1 445	2 390	5 060	2 250	3.5
47	Chloroindane	1 000	700	1 000	500	2.0
48	Carbon tetrachloride	9 066	6 200	5 760	5 760	1.5
49	Calcium cyanamide	415	513	415	353	1.4
50	Ethyleneglycol	8 348	6 122	8 213	9 000	1.4
51	Epichlorohydrine	194	141	280	345	2.4
52	Ethoxyphos	375	190	300	375	1.9

Table 46

Distribution of the variability coefficients of the species sensitivity of mice, rats, guinea pigs and rabbits under administration of chemical compounds into the stomach

Index	CSS									
	2	2.1—3.0	3.1—4.0	4.1—5.0	5.1—6.0	6.1—7.0	7.1—8.0	8.1—9.0	9.1—10.0	10
Absolute number of substances,	14	13	12	4	3	1	2	1	1	1
%	26.9	25.0	23.1	7.7	5.8	1.9	3.8	1.9	1.9	1.9

pounds has made it possible to assess differences in the animal species sensitivity in the following way:

CSS	Assessment of differences in the animal species sensitivity
Smaller or equal to 3	Not expressed
3.1—9	Expressed
More than 9	Sharply expressed

Out of the listed substances a CSS smaller than 3 have 27 compounds; from 3.1 to 9—23 compounds, and only 2 compounds are exeeding 9. It is however understood that any enlarged tabulations are, to this or that extent, conditional.

Comparison of the coefficients of the species differences in sensitivity of animals and man

In order to reveal regularities in the sensitivity of animals and man, the analysis of the species differences in sensitivity has been carried out in accordance with the classification suggested by the authors of the present study. The man's lethal doses of 34 chemical compounds have been borrowed from the book by M. D. Shvaikova «Forsenic Chemistry» (1965) and from periodicals. Since the data from the literature on the median lethal doses for animals usually vary by 2—3 times for the same substance, in order to obtain homogenous material to be used in the investigation, the average values of the lethal doses were used. When the literature gave several man's lethal doses of the same substance, there was also used the average dose, since the publications adduced, besides minimum lethal doses of substances (e. g., poisoning of a sick person), also evidently lethal doses (an intake for suicide). The data on distribution of variability of the $CSS=\frac{DL_{50max}}{DL_{50min}}$ and, on the ratio of the lethal dose for the least resistant animal species to the lethal dose for man ($K_2=\frac{DL_{50min}}{DL_{man}}$) are given in Table 47, and are summarized in Table 48.

As can be seen from Tables 47 and 48, the absence of differences in species sensitivity to a poison among rodents ($K_1 \leqslant 3$) in 2/3 of cases may serve as the ground for the assumption of the existence of a similar sensitivity in man ($K_2 \leqslant 3$). The following group comprises the compounds to which human organism is slightly more resistant in comparison with the organism of the most sensitive animal species (within the limits of $K_2 \leqslant 3$, however):

calcium cyanamide, 0.5
dichloromethane, 0.8
ethyl alcohol, 0.5
butyl alcohol, 0.6
aldrin, 0.7
dieldrin, 0.9
sodium fluoride, 0.9

To the following compounds man is, on the contrary, slightly more sensitive than the most sensitive animal species:

dichloroethane, 1.1
chloroform, 2.0
formaldehyde, 1.8
octamethyl, 1.3
DDT, 1.8
salicylic acid, 2.2

It should be noted that in the first case the average sensitivity of rodents is almost equal to the sensitivity of man, but in

Table 47

Lethal doses (mg/kg) and their ratio for different species of laboratory animals and for man

No	Substance	Mouse	Rat	Guinea pig	Rabbit	Man	$\frac{DL_{50}\ max}{DL_{50}\ min}$	$\frac{DL_{50}\ min}{DL_{50}\ man}$
1	Barium carbonate	200	125	235	—	12.1	1.9	10.3
2	Barium chloride	350	350	—	170	5.0	2.1	34.0
3	Sodium chloride	—	12 000	—	10 000	4285	1.2	2.3
4	Mercury dichloride	17.5	80	—	30	4.2	4.6	4.2
5	Zinc sulfite	—	2 200	—	2 057	107.1	1.1	19.2
6	Calcium cyanamide	415	513	415	353	642.5	1.4	0.5
7	Dichloromethane	5 600	—	—	1896	2 385	3.0	0.8
8	Chloroform	1 750	2 180	1 750	—	856.5	1.2	2.0
9	Carbon tetrachloride	9 066	6 200	5 760	5 760	428	1.6	13.4
10	Dichlorethane	910	770	—	910	671	1.2	1.1
11	Phenol	—	415	—	510	140	1.2	3.0
12	Methyl alcohol	8 712	12 880	—	9 029	338.5	1.5	25.7
13	Ethyl alcohol	9 488	13 660	2 400	7 900	4 514.2	5.7	0.5
14	Butyl alcohol	2 835	4 360	—	1 750	2 892.8	2.5	0.6
15	Ethyleneglycol	8 348	7 331	8 213	9 000	1 667.5	1.2	4.4
16	Chloralhydrate	—	650	—	1 300	142	2.0	4.6
17	Formaldehyde	—	800	260	—	142	3.1	1.8
18	Paraldehyde	1 790	1 650	—	5 000	1715	3.0	0.96
19	Malathione (carbophos)	1 187.5	3 000	570	—	17.8	5.3	32.0
20	Octamethyl	22.5	16	—	—	12.5	1.4	1.3
21	Parathione (thiophos)	17	10.5	18.5	50	1.7	4.8	6.2
22	Tricresylphosphate	—	—	400	100	7.1	4.0	14.1
23	Chlorophos	377.5	525	—	—	40	1.4	9.4
24	Luminal	325	660	—	150	71	4.4	2.1
25	Barbital (veronal)	600	200	—	262.5	100	3.0	2.0
26	Mephenizine	150	110	—	50	17.5	3.0	2.8
27	DDT	190	500	400	300	107.1	2.6	1.8
28	Aldrin	20	49	—	—	27.5	2.4	0.7
29	Dieldrin	25	68.5	—	—	27.5	2.7	0.9
30	Salicylic acid	430	900	—	1 100	214	2.3	2.2
31	Aniline	1 075	—	2 500	1 000	285	2.5	3.5
32	Nitrobenzene	—	640	—	660	21.4	1.0	29.9
33	Dinitroorthocresol	47	28	—	—	11.4	1.7	2.4
34	Sodium fluoride	80	200	250	150	87.5	3.1	0.9

the second case the difference slightly increases, although remaining insignificant. From the investigation had been excluded alkaloids, to which man has a higher sensitivity in comparison with the sensitivity of animals, which depends on the adaptation mechanisms linked with the natural way of their life.

Consequently, the before-mentioned opinion (N. S. Pravdin, 1947; Noordwijk, 1964), concerning equal responses ($K_s \leqslant 3$) of

Table 48

Coefficients of variability of differences in the animal species sensitivity and the ratio of the lethal doses for the most sensitive animal species and for man

$K_1=\frac{DL_{50max}}{DL_{50min}}$	Number of substances	$K_2=\frac{DL_{50min}}{DL_{50man}}$	Number of substances
Less or equal to 3	28	Less or equal to 3	18
		3.1—9	3
		More than 9	7
3.1—9	6	Less or equal to 3	2
		3.1—9	2
		More than 9	2

man and animals in case of sligth differences in the species sensitivity of rodents (CSS≤3), has been partially (in 2/3 of cases) confirmed. Thus, for extrapolating the data from animals to man, in case of non marked differences in the species sensistivity, special amendments, as a rule, are not required. In this case, the experiment may be carried out on any species of experimental animals.

When differences in the species sensitivity are manifested or strongly manifested, the extrapolation of the experimental data to man is to be done with caution. Experiments, in these cases, are to be conducted on not less than 2 species of animals (resistant and sensitive), using carnivora (dogs, cats) and omnivora (dwarfish breeds of pigs). An obligatory use in investigations of 12 species of animals including monkeys (Williams, 1967) seems to be unjustified. Laboratory species of monkeys (rhesus monkeys, barboons, etc.) are by no means «closest relatives» of man and often differ from him significantly in toxicokinetics, metabolism and sensitivity to harmful environmental factors.

The regularities which we have revealed in the species sensitivity of animals and man have been registeret at a high, lethal level of exposure. Therefore, it is advisable to mention the work of B. Macnamara (1967) in which the author demonstrates a good coincidence (the difference is not more than by 3 times) in the threshold doses of a number of medical substances (sernil, pentabarbital, chloropromazine, amphetine, etc.) for different species of animals (mouse, rat, dog, monkey) and man.

A similar thesis on the absence of the species differences in sensitivity to chemical compounds at a low level of exposure, if the species sensitivity at a high level is not revealed, has been put forward by G. N. Krasovsky (1970).

Analysing the data on hygienic standardization of water for 106 different chemical compounds, G. N. Krasovsky (1973) de-

monstrated that, according to the values of the DL_{50} and CL_{50}, as well as according to the threshold doses in subacute and chronic experiments, the species differences are approximately equal. As to other recommendations, their practical use, from our point of view, faces serious difficulties. The modelling of the diagrams of extrapolation of the DE[1] (Fig. 27) or the CE[2] from animals to man, in accordance with their body weight, often lead to errors, exceeding one order.

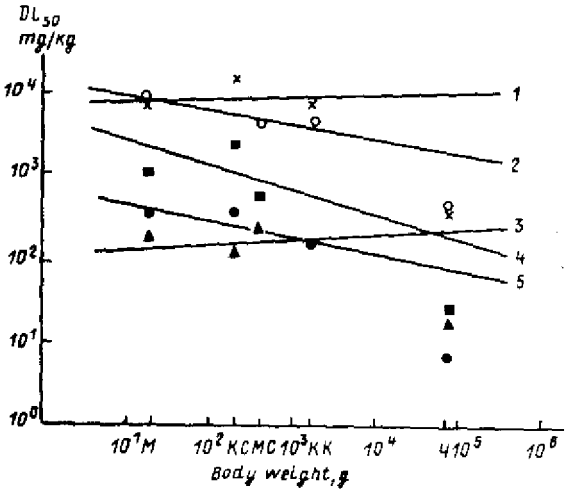

Fig. 27. Extrapolation of the lethal doses effect from animals to man in accordance with their body weight.

(1) methanol; (2) CCl_4; (3) $BaCO_3$; (4) carbophos; (5) $BaCl_2$.

Evaluation of the age sensitivity to poisons

Intensive development of the chemical industry leads to an increase of the number of people of different age who may be exposed to occupational poisons. There increases not only the number of young workers, as a result of the expansion of the system of vocational technical training, but also the number of elderly workers, which happens because of a general demographic growth of the number of people of this age, and because of the use of the pensioneers' labour. To this leads also the «chemization» of the everyday life. There appear more and more data in the literature, showing that the responses of the young and the old organism to the exposure to substances may differ from the responses of the mature organism.

[1] DE — effective doses
[2] CE — effective concentrations

Certain idea of the age reactivity may be given by the works dedicated to the age pharmacology and physiology. Many data on this subject have been accumulated by the school of I. A. Arshavsky. Pharmacologic investigations demonstrate that the tolerance of animals of different age to the exposure to poisons is not equal (V. B. Prozorovsky, 1962; P. N. Sirotinin, 1966; V. D. Rozanova, 1966, et al.).

It should be emphasized that as a rule in these investigations there have been used rather significant doses of poisons (often lethal), while hygienists are naturally interested first of all in minimum levels of exposure, corresponding to possible occupational and communal exposures. Pharmacologists have not usually determined the thresholds of general and specific effects of poisons, but searched for the qualitative differences in responses of animals of different age. If we add to this the absence of uniformed conditions of experiments («adolescents» are usually called 2 to 3-month old rats, while in some works the age and body weight of animals are not mentioned at all), then the difficulties arising in the analysis of pharmacologic data would become understandable. We should mention that also hygienists-experimentalists do not provide the reader with sufficient information for comparing the obtained data, since the conditions of experiments are far from being standardized. That is why the use of the experimental data (even leaving aside the problem of species sensitivity) presents serious difficulties.

Provided certain requirements were fulfilled, the observations over working adolescents could have been more effective (the data accumulation). Such observations have revealed that the young organism has an increased sensitivity to the action of a number of substances: quartz dust, lead, carbon disulfide, benzene, acetone and other solvents, — as well as to a complex of substances contained in the discharges of the plunts, producing synthetic divinyl rubber.

The mechanisms, underlying the differences in resistance of the mature and young organism, have not been sufficiently analysed. The authors explain these differences by the degree of development of the nervous and endocrine systems, by different permeability of the hematoencephalic barrier, pecularities of the cardiovascular system, etc., which depend on the environmental agent and the age of experimental animals. Of certain interest for toxicologists is the investigation of the adolescent organism's reactivity or that of the organism in the period of puberty.

Analysing the obtained data, one cannot help noticing a universality in the approach of researchers to the evaluation of their own material, which, in a number of cases, contradicts the experimental data.

Despite the known physiological peculiarities of the adolescent organism, it is hard to suppose that adolescents have in all cases

a higher sensitivity to harmful environmental factors than the mature organism. Errors in the judgements may result from unproperly selected control groups of working people (or their absence), from underestimation of the complex character of occupational and social conditions, inadequate methods of investigation, etc. The replication of experiment, conducted on laboratory animals under strictly standardized conditions, serves as the guarantee against these sources of artefacts.

Unfortunately, the investigations in the field of age toxicology are very few. For comparing the age endurance, many authors used the lethal doses of poisons, determined by the value of the CL_{50}, DL_{50}, or by the time of the death of animals. Meanwhile, the criteria for differentiation of the values of doses and concentrations are not yet universally recognized. Thus, Z. A. Druchevskaya (1967), assessing the age sensitivity of white rats to granozan by the DL_{50} value under administration of the poison into the stomach (the DL_{50} for the mature animals is 3.16 g/kg; for the young, 3.25 g/kg), has come to the conclusion that mature rats are more sensitive to the poison than the young. Analogous conclusions have been drawn by M. F. Savchenkov (1969), who used the DL_{50} of dichlorethane (the DL_{50} for the mature animals is 1.12 g/kg; for the young, 1.3 g/kg). I. I. Kondratyeva (1968), on the contrary, thinks that young animals in the period of puberty are more sensitive to carbon disulfide, while the values of the CL_{50} differ by 1.3 times. We think that in similar cases one should draw the conclusion that young and mature animals have equal resistance, since the values of the DL_{50} and CL_{50} are practically equal. According to I. P. Ulanova et al. (1969), a 3-fold difference in the data from separate experiments is possibly a result of additional influences, which is a common phenomenon and should not be considered significant.

The experiments of G. G. Avilova (1970) on mature male rats (body weight 250—300 g) and on young animals (1—1.5 months, body weight 120—150 g) exposed to benzene and carbon tetrachloride (at the lethal level) have shown the same type of clinical picture of intoxication; the animals died approximately at the same time. As can be seen from Table 49, the toxicity of the indicated poisons (administration into the stomach) is the same for mature and young rats. Judging from other indices of the hazard of lethal poisoning, these poisons appear to be slightly more hazardous for mature rats. Under inhalation of the mentioned products (Table 50), the hazard of acute lethal poisoning of young animals is slightly higher than that of the mature animals, while the difference between the values of the CL_{50} for carbon tetrachloride is significant.

It is possible that this difference results from a large amount of poison that has entered the organism of young rats in connec-

Table 49

Some indices of the hazard of acute lethal poisoning of rats under administration of poisons into the stomach

Substance	Group of animals	DL_{50}, g/kg	$\frac{DL_{84}}{DL_{16}}$	S	$\frac{2}{DL_{50} \cdot S}$	Slope of the mortality straght line to X-axis
C_6H_6	Mature	6.4 (7.74÷5.30)	1.8	1.33	0.118	63
	Young	6.2 (8.25÷4.66)	2.6	1.60	0.101	50
CCl_4	Mature	3.65 (5.18÷2.58)	2.4	1.55	0.175	52
	Young	3.90 (5.73÷2.65)	3.3	1.81	0.141	44

Table 50

Some indices of the hazard of acute lethal inhalation poisoning of rats of different age

Substance	Group of animals	DL_{50}, mg/l	$\frac{CL_{84}}{CL_{16}}$	$\frac{1}{CL_{50} \cdot S}$	Slope of the mortality straight line to X-axis	CPPI
C_6H_6	Mature	65 (83.2÷50.8)	2.17	1.47	0.010455	4.9
	Young	54.0 (60.7÷48.0)	1.43	1.20	0.015473	6.0
CCl_4	Mature	82.0 (93.5÷71.5)	1.62	1.27	0.009568	9.3
	Young	56.0 (60.2÷52.1)	1.25	1.12	0.015080	13.6

tion with the differences in the gas exchange and pulmonary ventilation. According to D. U. Ermatova (1965), S. I. Enikeeva, I. I. Oganesyan (1951), the indices of activity of the respiratory and cardiovascular systems of the 2-month old rats still exceeds the level typical for mature animals. However, the knowledge of the responses at the lethal level is evidently insufficient for drawing substantiated conclusions. The evaluation of the age differences at the threshold and maximum allowable concentrations levels only by the lethal or similar exposure may lead to erroneous deductions. As a criterion for assessing the age sensitivity to different exposures, V. D. Rozanova (1950, 1966), I. A. Arshavsky (1959), A. N. Kudrin (1967) suggest to use the threshold, and not the lethal value. The smaller the threshold dose value, the more sensitive the organism.

A number of indices has been investigated in order to establish the threshold of a single exposure (Lim_{ac}) for benzene and carbon tetrachloride. The choice of the indices has been determined by the data from the literature on the character of the effect of benzene and carbon tetrachloride on the organism. For the effect of benzene, the following indices have been selected: the STI, spontaneous motor activity (SMA), rectal temperature, relative weight of the liver, as well as the content of hemoglobin, erythrocytes, leukocytes, thrombocytes, reticulocytes in the blood and the ratio of elements in the leukocytic formula. Under exposure to carbon tetrachloride, there have been determined the SMA, rectal temperature, relative weight of the liver and kidneys.

The Lim_{ac} for benzene, according to changes in the peripheral blood, is 1.1 mg/litre for mature rats (the quantity of leukocytes in 1 μl is 16.5 ± 1.22 thousand in test rats; $12{,}6 \pm 1.08$ thousand in control rats; $p < 0.05$); the Lim_{ac} for young rats is 0.1 mg/litre (the quantity of thrombocytes in 1 μl is 950 ± 63 thousand in test rats; 371 ± 74 thousand in control group; $p < 0.05$), i. e., young rats appear more sensitive to exposure to benzene than mature animals (by one order).

The threshold values have been determined by different indices, since the character of changes, revealed under exposure to benzene, is not similar in mature and young animals. The changes in young rats appeared to be more manifested (the indices fluctuations exceeded the limits of physiological variations).

According to M. F. Savchenkov (1969), the value of the DL_{50} for benzene, under administration into the stomach, is more than 4 times smaller for young animals than for mature ones. By the end of a 2-week observation, young rats which survived after the administration of the half-lethal dose had a more marked reduction in the body weight and more marked changes in the relative weight of internal organs. In replicated experiments with subcutaneous administration of benzene to rabbits, young animals appeared to be less sensitive (according to the dynamics of the body weight, changes in the relative weight of organs, condition of the peripheral blood and bone-marrow).

Thus, chronic experiment at the minimum levels of exposure with the use of adequate methods of investigation would be the most substantiated. Similar experiments have been described in the literature. Unfortunately, these experiments are rather laborious of prolonged.

Searches for ways to accelarate the evaluation of the age differences in sensitivity to poisons are well justified (I. V. Sanotsky, 1969). Our laboratory together with the Department of Hygiene of Children and Adolescents of the I Moscow Sechenov Medical Institute have made an attempt to assess the significance of differences in the age sensitivity of animals according to the I_{cum} and the rates of the development of adaptation (rate of ap-

pearance and disappearance of primary responses). There has been used the exposure by inhalation to several poisons at the level of the Lim_{ac}, established before for mature animals in a complete chronic experiment.

When assessing primary responses (according to numerous indices) of young and mature animals under a 8-day 4-hour daily exposure to benzene at the Lim_{ac} level, it was found that young animals immediately responded by changes in their endocrine system (primary inhibition and then normalization of the thyroid gland activity by the inclusion of I^{131} (Table 51) and by changes in the blood (primary increase, then reduction of the quantity of agranulocytes). The mature animals also exhibited changes in the activity of the thyroid gland (according to this

Table 51

Indices of the condition of mature rats after exposure to benzene by inhalation

Index	Concentration, mg/l													
	0.585			0.023			0.031				0.0034			
	Days of exposure													
	2	4	8	2	4	8	2	4	8	15	2	4	8	15
Body weight	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STI	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STI (after alcohol load)							—	0	0	0	0	0	0	0
SMA	0	—	0	0	0	0	0	0	0	—	0	0	0	0
Rectal temperature							0	0	0		0	0	0	
Relative mass of:														
liver	0	0	0	0	0	—	0	0	0	0	0	0	0	0
kidneys	0	0	0	0	0	—	0	—	0	0	0	—	0	0
spleen	0	0	0	0	0	0								
pituitary body	0	0	0	0	0	+	0	0	0	0	0	0	0	0
thyroid gland	0	0	0	0	0	0	+	0	0	0	+	0	0	0
adrenal glands	0	0	0	0	0	0	0	0	0	0	0	0	0	0
testis	0	0	+	0	0	0								
Absorption of I^{131} by thyroid gland:														
after 4 hr	—	0	0	0	0	0	0	0	0	0	0	0	0	
after 24 hr	—	0	0	0	0	0	0	0	+		0	0	0	
after 48 hr	—	0		0		0	0		0		0		0	
Morphometry of thyroid gland (height and diameter of follicular epithelium)			0	0	0									
Amount of ascorbic acid in adrenal glands	0	0	0	0	0	0	0	0	0	+	0	0	0	0
Morphological assessment of germinal epithelium	0	0	0	0	0	0								

Note: (+) increase; (—) decrease; (0) no changes.

index, adaptation had developed already by the 4th day), however, changes in the blood appeared only on the 8th day.

Changes in some indices of the state of the endocrine system of young animals at the Lim_{ac} level were revealed only by the end of the experiment (an increase in the activity of the thyroid gland) (Table 52). The changes in the blood, mainly returning

Table 52

Indices of the condition of young rats after inhalation exposure to benzene ($p \leqslant 0.05$)

Index	Concentration, mg/l													
	0.585			0.023			0.031				0.0034			
	Days of exposure													
	2	4	8	2	4	8	2	4	8	15	2	4	8	15
Body weight	0	0	—	0	0	0	0	0	0	0	0	0	0	0
STI	0	0	—	0	0	0	0	0	0	0	0	0	+	0
STI (after alcohol load)							—	0	0	0	0	0	0	—
SMA	0	0	0	0	0	0	0	0	—	0	0	0	0	0
Rectal temperature							0	0	0		0	0	0	
Relative mass of:														
liver	0	0	0	0	0	0	0	0	—	0	0	0	0	0
kidneys	0	0	0	0	0	0	0	0	0	0	0	0	0	0
spleen	0	0	0	0	0	0								
pituitary body	0	0	0	0	0	0	0	0	0	0	0	0	0	0
thyroid gland	0	+	0	0	0	0	0	0	0	0	0	0	0	0
adrenal glands	0	+	0	0	0	0	—	0	0	0	—	0	0	0
testis	0	0	0	0	—	0								
Absorption of I^{131} by thyroid gland:														
after 4 hr	—	0	0	0	0		0	0	0		—	0	0	
after 24 hr	—	0	0	0	0	0	0	0	0		—	0	0	
after 48 hr	—			0	0	0	0		0		—		0	
Morphometry of thyroid gland (height and diameter of follicular epithelium)				—	0	0								
Amount of ascorbic acid in adrenal glands	0	—	0	0	0	0	0	0	0	0	0	0	0	0
Morphological assessment of germinal epithelium	0	0	0	0	0	0	0							

Note: (+) increase; (—) decrease; (0) no changes.

to the norm by the end of the experiment, were registered early (on the second day). Changes in the endocrine system of mature animals were not registered, changes in the blood appeared for the first time only on the 4th day of exposure.

Thus, young animals proved to be more sensitive to benzene exposure at the Lim_{ac} and Lim_{ch} levels, according to the ade-

quate indices, which was confirmed under the functional load with blood-letting from the heart (1% of the body weight). Adaptive capacity of young animals turned out to be lower than that of the mature animals.

Under the same scheme of exposure to carbon tetrachloride at the Lim_{ac} level, the functional condition of the endocrine glands changed on the 2nd day both in young and mature animals (decrease of the I^{131} inclusion), and on the 4th day (changes in plethora of the pituitary body and adrenal glands). Later the indices were normalized. The reflex vascular response of parechymatous organs changed earlier in young than in mature animals (Tables 53 and 54).

Table 53

Indices of the condition of mature rats after exposure to CCl_4 by inhalation ($p<0.05$)

Index	Concentration, mg/l						
	0.041		0.046		0.0014		
	Days of exposure						
	2	4	8	2	4	2	4
Body weight	0	0	0	0	0	0	0
STI				0	0	0	0
STI (after alcohol load)				—	0	0	0
SMA	0	0	0				
Rectal temperature	0	0	0	0	0	0	0
Relative mass of:							
liver	0	0	+	0	0	0	0
adrenal glands	0	0	0	0	+	0	0
testis	0	0	0	0	0	0	0
kidneys	0	0	0	0	0	0	0
pituitary body	0	—	0	0	0	0	0
thyroid gland	0	0	0	0	0	0	0
Elimination of bromosulfalein from blood	0	0	0				
Absorption of I^{131} by thyroid gland:							
after 4 hr	—	0	0		0		0
after 24 hr	—	0	0		0		0
after 48 hr	0	0	0		0		0
Morphometry of thyroid gland (karyometry, height of follicular epithelium)	0	0	—	—	0	—	0
Amount of ascorbic acid in adrenal glands	0	0	0	0	0	0	0
Morphological assessment of germinal epithelium	0	0	0				

Note: (+) increase; (—) decrease; (0) no changes.

Table 54

Indices of the condition of young rats after inhalation exposure to CCl_4 ($p<0.05$)

Index	Concentration, mg/l						
	0.041			0.046		0.014	
	Days of exposure						
	2	4	8	2	4	2	4
Body weight	0	0	0	0	0	0	0
STI				0	0	0	0
STI (after alcohol load)				—	0	0	0
SMA	0	0	0				
Rectal temperature	0	0	0	0	0	0	0
Relative mass of:							
liver	0	+	0	0	0	+	0
adrenal glands	0	—	0	0	0	0	0
testis	0	+	0	0	0	0	0
kidneys	0	+	—	0	0	0	0
pituitary body	0	+	0	0	0	0	0
thyroid gland	0	0	0	0	0	0	0
Elimination of bromosulfalein from blood	0	0	0	0	0	0	0
Absorption of I^{131} by thyroid gland:	—	0	0	0	0	0	0
after 4 hr	—	0	0	0	0	0	0
after 24 hr	—	0	0	0	0	0	0
after 48 hr	0	0	0	0	0	0	0
Morphometry of thyroid gland (karyometry, heignt of follicular epithelium)	0	0	0	0	0	0	0
Amount of ascorbic acid in adrenal glands	0	0	0	0	0	0	0
Morphological assessment of germinal epithelium	0	0	0				

Note: (+) increase; (—) decrease; (0) no changes.

Thus, there have been revealed tendencies to more manifested primary responses in young animals. The condition of the central nervous system (or may be of the hematoencephalic barrier) in young animals, under exposure to carbon tetrachloride at the Lim_{ac} level, did not differ from its condition in mature animals. The load with spirit caused a paradoxical response in young animals (an increase of the excitability of the central nervous system), while mature animals did not exhibit changes in the CNS excitability (in the control group inhibition of the CNS function was registered).

Hygienic significance of primary nonspecific responses (in particular, responses of the endocrine system) has not yet been completely investigated.

Though the conduction of chronic experiments for the comparison of the age responses of animals to the exposure to poisons is rather laborious, it is very necessary, especially since such investigations are rare.

G. G. Avilova from the Institute of Industrial Hygiene and Occupational Diseases of the USSR Academy of Medical Sciences together with scientific workers of the I Moscow Sechenov Medical Institute conducted a chronic experiment on old, mature and young rats under exposure to benzene.

> Mongrel white female rats were used in the experiment young animals with a body weight of 110—120 g; 1.5-month old mature animals with a body weight of 220—240 g; 4-month old and old animals (11 months) with a body weight of 310—330 g. The inhalation exposure of the animals was carried out in 800-litre chambers under the dynamic regimen for 4 hours every day during 4 months at two levels of exposure ($0.03\pm\pm0.003$ mg/l) and the MAC (0.005 ± 0.003 mg/l). To determine the concentration in the chamber, N. M. Maltseva used chromatograph «Chrom-2».

The animals were being examined every month. The considered indices made in possible to characterize the general condition of the organism and the condition of the most sensitive systems: the nervous system and the blood. The condition of animals was assessed by the body weight, the STI values, reflecting the functions of the CNS, by the picture of the peripheral blood (amount of hemoglobin, erythrocytes, leukocytes, thrombocytes, reticulocytes, the phagocytic activity of neutrophiles).

The relative weight of the organs (liver, kidneys, pituitary body, thyroid gland, adrenal glands), the albuminous fractions of the serum, the thyroid gland activity (according to the ability to absorb I^{131}), the morphologic changes in the internal organs were determined at the end of the experiment. Loads on the nervous system with ethanol (the dose of 2 mg 96% per 1 g of the body weight) and on hemopoetic system with blood-letting (1.5% of the animals' body weight) were applied. Further investigations on the condition of the blood were carried out on the 3rd, 10th and 14th day. The static working capacity, the permeability and steadiness of the skin capillaries were assessed during the recovery period.

It is known that a poison can exhibit its toxic effect not only immediately in the process of exposure, but also in a remote period after the end of the contact. Thus, when investigating the condition of the peripheral blood in a remote period of chronic intoxication with benzene, I. A. Gribova and L. A. Zorina (1971) registered stable changes in blood, especially in persons who had

Tabl

Indices of the state of rats under chronic inhalation exposure to benzene

Index	Concentration, mg/litre										
	0.03±0.003						0.005±0.0003				
	young		mature		old		young		mature		o
	test	control	test	control	test	control	test	control	test	control	test
Body weight, g	0		0		0		(2 months) 235.2±3.5 | 249.5±3.7 p<0.01		(3 months) 271.3±3.2 | 284.5±3.5 p<0.01		0
STI, cond. units	0		0		(4 months) 4.3±0.18 | 3.7±0.2 p<0.05		(4 months) 4.9±0.18 | 4.0±0.18 p<0.02		0		0
STI after alcohol load, cond. units	0		0		0		0		0		0
Hemoglobin, g/litre	0		0		0		0		0		0
Erythrocytes, million	0		0		0		0		(4 months) 5.65±0.25 | 4.76±0.19 p<0.01		0
Leukocytes, thousand	0		0		0		0		0		0
Thrombocytes, million	0		0		0		0		0		0
Reticulocytes, %	0		0		0		0		0		0
State of the blood after phlebotomy	0		0		0		(3rd day) 17.6±1.29 | 13.1±0.73 p<0.02		0		0

Phagocytic activity of neutrophiles	0		0		0		0		0		0	
Relative mass of organs	0		0		Spleen 0.42±0.02 | 0.35±0.03 p<0.05		Liver 3.30±0.05 | 3.82±0.16 p<0.01		0		0	
Protein fractions of blood serum, %	0		0		0		0		0		0	
Absorption of I^{131} by thyroid gland	0		0		0		0		0		0	
Morphology of internal organs	0		0		0		0		0		0	
Working capacity	8.9±1.0	18.2±2.5 p<0.02	0	Recovery period	0		0		0		0	
Permeability of vascular wall	0		150±9.7	214±25.7 p<0.05	0		0		0		0	
Steadiness of capillaries (quantity of petechiae)	0		0		0		0		0		0	
Mass, g	0		0		0		0		0		0	
Reticulocytes, %	0		0		0		0		0		0	

Note: (0) no changes; time of exposure is given in brackets.

started their work with benzene in adolescence. Chemical exposure may affect the general vital capacity of the organism and its reproductive capacity. The effect of benzene on the reproductive function of animals has been studied by V. R. Tchevpetsov, A. A. Lyapkalo (1970), but only at high levels of exposure and on mature animals.

To assess the functional condition of the ovary, the duration of the estral cycle and of its single phases was being determined every month throughout the experiment, taking account of the number of animals having rhythmical cycle. After the end of the chronic experiment, the female animals were mated with intact males, then the reproductive function was evaluated by the methods proposed by our laboratory («Methods of Experimental Research on Determining the Thresholds of the Effect of Industrial Poisons of the Generative Function», 1969). Another group of pregnant animals was kept until the natural delivery with the purpose of assessing whether the offspring would be normal or not. This study comprised registration of the number of offspring, their body weight, postnatal deaths (before they reached a 2-month age), the condition of the CNS (according to the STI) and the condition of the peripheral blood.

The results of the investigation of the general toxic effect of benzene are given in Table 55. As can be seen from the Table, under exposure to benzene at the Lim_{ac} level, young animals exhibited a decrease of their working capacity during the recovery period, in mature animals there was registered an increase of the vascular permeability, while old animals exhibited a decrease of the excitability of the nervous system and an increase of the relative weight of the spleen in the process of the exposure.

Under exposure to benzene at the MAC level, in young animals there were registered a decrease of the body weight, a decrease of the excitability of the nervous system, a decrease of the relative mass of liver, a decrease of the rate of restoration of the quantity of leukocytes after blood-letting. A decrease of the body weight in mature animals occurred later, there was also revealed an increase of the quantity of erythrocytes. Old animals did not exhibit any changes in the indices.

Pathomorphologic changes in the organs of all experimental animals, according to R. S. Vorontsov, cannot be attributed to the effect of benzene.

Thus, the sensitivity of animals of different age at the Lim_{ch} level is approximately equal, at the MAC level, however, young animals appeared to be less resistant. Almost all the changes in the functional indices lay within the range of fluctuations of average values in $1\,\delta$, i. e., they did not exceed the limits of the physiological norm for rats (according to the data of the Laboratory of Toxicology of the Institute of Industrial Hygiene and Occupational Diseases of the USSR Academy of Medical Sci-

ences), and therefore we consider them insignificant. Attention should be paid to the inadequacy between the intensity of the effect and the value of the effective dose. The effect was stronger at the MAC level than at a higher level of the Lim_{ch}.

When analysing the data, it turned out that the air temperature in the chamber, where the animals were exposed to benzene at the MAC level, sometimes exceeded the air temperature in the other (test and control) chambers by 5° C. In spite of this fact, we consider it possible to compare the responses of mature and young animals, since they were kept in the same chamber and were together exposed to benzene under the increased temperature. It is generally known that under similar minimum exposures the role of additional factors increases. According to T. A. Kozlova (1957), high temperature (30—40° C) augmented the toxic effect of benzene (in experiment on rabbits).

Age differences in the sensitivity of rats to the exposure to benzene, on the one hand, may be related to changes in the processes of absorption, distribution and elimination of benzene under increased temperature and, on the other hand, to different metabolism of benzene in the organism, unequal permeability of the hematoencephalic barrier, the state of the enzymatic systems, and so on.

I. D. Gadaskina et al. (1973) demonstrated that under administration of benzene to rabbits the combination of phenolproduct of the oxidation of benzene in the organism — with sulfuric and glucuronic acids is practically absent in young animals (in contrast with the mature ones). Free circulation of phenol seems to be one of the reasons of high sensitivity of young animals.

Thus, when exposure to benzene begins at a young age, it leads to a decrease of the organism's tolerance, in comparison with when the contact with the poison begins in maturity. An additional factor — increased temperature as a functional load made it possible to reveal this fact. Therefore, to determine possible latent changes and the degree of tension of the adaptive responses, it is advisable to use different types of loads.

The question of how benzene influences the reproductive function of the animals the exposure of which to the poison began not in maturity, but in a «young» age has long remained unclear.

The study of the estral cycle has shown that during the 4th month of exposure at the Lim_{ch} level mature animals exhibited an increased duration of the estrus (1.46 ± 0.13 in experiment; 1.15 ± 0.08 in control; $p < 0.05$), and at the MAC level the duration of the entire cycle decreased (4.80 ± 0.16 in experiment; 5.32 ± 0.18 in control; $p < 0.05$). Among young animals, exposed to benzene at the MAC level for 3 months, the number of females having rhythmical cycle decreased. The ability to conceive was damage neither in mature, nor in young animals. Results of the investigation of the killed pregnant animals are shown in Table 56.

Tab

Indices of reproductive function of rats after chronic inhalation exposure to benzene

Index	Concentration, mg/litre							
	young		mature		young		mature	
	test	control	test	control	test	control	test	cor
Absence of pregnancy	4/28 (14.3%)	5/30 (16.5%)	4/28 (14.3%)	8/27 (29.5%)	2/28 (7.15%)	5/30 (16.5%)	2/22 (9.1%)	8/ (29.
Fertility (1 female)	9.7±1.05	10.7±1.19	10.5±1.19	11.5±0.91	11.0±1.95	10.7±1.19	9.0±1.24	11.1
Quantity of yellow bodies (per 1 female)	14.4±1.08	14.8±0.65	15.0±0.61	17.0±0.79	14.7±1.67	14.8±0.65	17.3±1.29	17.0
Death of ovum before implantation	5.1±1.84	4.0±1.7	6.9±1.86	4.25±1.86	6.0±2.7	4.0±1.7	10.2±1.95 $p<0.05$	4.25
Death of embryo after implantation	2.5±0.97	1.2±0.54	1.6±0.69	3.0±1.19	1.33±0.59	1.2±0.54	1.7±0.54	3.0
General embryos' mortality, %	50.5±10.8	34.3±10.8	54.7±8.6	42.6±11.5	49.9±11.9	34.3±10.8	66.8±9.38	42.6
Length of embryos, mm	27.6±0.72	27.2±0.62	27.9±0.25	27.1±0.54	27.5±0.74	27.2±0.62	26.4±0.57	27.1
Embryos' weight, g	2.17±0.08	2.26±0.09	2.38±0.08 $p<0.01$	2.09±0.06	2.31±0.09	2.26±0.09	2.04±0.07	2.09
Number of embryos with hemorrhages	5/68 (7,3%)	11/95(11,6%)	12/84(14.3%) $p<0.05$	4/78(5.1%)	7/65(10.7%)	11/95(1.16%)	1/54(1.9%)	4/78(

It can be seen from Table 56 that no changes were registered in young pregnant animals at the Lim_{ch} and MAC levels; mature animals at the Lim_{ch} level had an increase in the embryo weight and in the relative number of fetuses with hemorrhages. This is, possibly, related to the changes in vascular permeability. An increase of preimplantation deaths of the ova was revealed in mature animals at the MAC level.

When observing the development of the first generation offspring of the animals which had been exposed to benzene at the Lim_{ch} level, there was revealed an increase of postnatal deaths (44/115—38.3% in test; 24/118—16.2% in control; $p<0.001$) and a decrease of the quantity of erythrocytes in the females ($4.87\pm$ ±0.16 million in 1 μl in test; 5.39 ± 0.17 million in 1 μl in control; $p<0.05$); at the MAC level there was observed an increase of the body weight of the 1 to 2-week old offsprings.

1-month old offsprings of mature animals, exposed to higher concentrations of benzene, had a decrease of the body weight (52.7 ± 1.4 g in test; 67.4 ± 2.2 g in control; $p<0.05$), and an increase of the excitability of the nervous system (3.8 ± 0.1 in test; 4.5 ± 0.3 in control; $p<0.05$) of the males; under exposure to lower concentrations of the poison, 1-month old animals had a decrease of the body weight (58.9 ± 1.8 g in test; 67.4 ± 2.2 g in control; $p<0.01$).

Thus, benzene affects the reproductive function of the animals the exposure of which to the poison started either in maturity or in puberty period. When contact with the poison began in puberty, there did not occur any more serious changes than those developed as a result of the contact beginning in maturity. One could have assumed that this is connected with a more «physiological» age of young animals, since by the time of mating the age of young animals was already 5 and a half months, and 8 months of mature animals.

A similar effect of benzene at a concentration of 1 mg/l under chronic exposure has been revealed by V. P. Tchevpetsov and A. A. Lyapkalo. The offspring of the animals which had been exposed to the poison exhibited some changes in the quantity of leukocytes, in the leukocytic formula and an increase in the sensitivity to γ-irradiation. V. A. Gofmekler (1968) demonstrated that benzene exhibited embriotropic effect under a 24-hour exposure in concentrations of 1.0 mg/m³ and higher.

The described experiment does not mean that the revealed effect of benzene has a pure gonadotropic character, since benzene may be deposited and circulate in the organism for a long time, especially under chronic exposure (E. S. Mironos, 1969), therefore it is possible that the poison could have affected the fertilized ovum or have penetrated the placenta. Benzene penetrates the milk, affect the lactation and this, possibly, causes a decrease in

the body weight of young animals and an increase of the postnatal mortality.

Probably, such effect of benzene is not specific, since the investigated levels of exposure revealed an increase of the vascular permeability and of the working capacity, a reduction of the body weight, changes in the function of the nervous system, in the blood, etc. This agree with the data of K. A. Kormilitsin, V. I. Vashakidze (1961), N. P. Mikhailova (1960). The authors did not reveal among working women specific women's diseases, caused by exposure to benzene in concentrations from 0.05 to 1.6 mg/litre.

Thus, the substantiation of preventive measures in experiments on animals is a complicated problem. Currently, it does not appear advisable to propose separate MACs for different age groups.

Relatively modest data on toxicological evaluation of the age sensitivity enable one to draw only preliminary conclusions. Practical tasks require a fast accumulation of new data on this issue. Very few investigations have been carried out in order to study the rate of aging in case the work with poisons began in different age periods. There is a demand for investigation on the sensitivity of old animals, which results from difficulties connected with their long-term maintenance.

In order to obtain comparable results, it is necessary to harmonize the conditions of experiments. The existing provisonal guidelines on methods for substantiating the maximum allowable concentrations of harmful substances in the air of workplaces, with necessary changes and amendments, may serve as the basis for such work.

Chapter 6. CHLORINATED HYDROCARBONS OF METHANE SERIES. EVALUATION OF TOXICITY AND HAZARD

Chlorinated hydrocarbons of methane series are widely used as solvents of fats, oils, lacquers, rubber, waxes, cellulose acetate, in synthesis of silicons, etc.

It has been important to follow changes in the physical, chemical and biological properties related to the degree of the methane chlorination. The physical and chemical properties of the chlorinated hydrocarbons which we have investigated are shown in Table 57. An increase of the inclusion of chlorine atoms into the molecule of methane is accompanied by an increase of the molecular weight, an increase of the boiling point and a decrease in the volatility, by an increase of the solubility in oil, while the water solubility increases from the first series member to the second and decreases from the third to the fourth.

Characteristics of the hazard of lethal poisoning

As has already been shown, the primary values for evaluating the hazard of lethal outcome under exposure to poisons at high levels of doses and concentrations are parameters of distribution of the individual sensitivity of animals. The smaller the individual differences, the more hazardous the poison (all other factors being equal), since the ability of compensation systems is considerably restricted in this given case. Under administration of the poisons into the stomach, the lethal doses have been determined only for $CHCl_3$ and CCl_4 (Table 58).

From Table 58 follows that chloroform is more toxic than carbon tetrachloride by approximately one order. Therefore, according to different classifications of toxicity, chloroform belongs to a higher (boundary) class of toxicity (Table 59).

The toxicity of chlorinated hydrocarbons of methane series under exposure by inhalation is shown in Table 60. As can be seen from Table 60, the toxicity of compounds augments in the following sequence: methylene chloride, carbon tetrachloride, chloroform, methyl chloride. However, the absolute difference in toxicity of the 3 last members of the series, which has been deter-

Physico-chemical properties of methane

Substance	Physical state at 20°C	Molecular weight	Relative density	Boiling point, °C	Vapour pressure, mm of Hg
CH_4	Gas	16.04	0.55	—161.5	—
CH_3Cl	Gas	50.49	1.785	—24.73	3510
CH_2Cl_2	Liquid	84.94	1.336	42	348.9
$CHCl_3$	Liquid	119.37	1.489	61.2	160.5
CCl_4	Liquid	153.81	1.595	76.8	90.6

mined at different periods and on different groups of animals, cannot be recognized as significant. When the lethal concentrations are expressed in molecular units, the difference in the toxicity of chloroform and methylene chloride becomes relevant.

The relative difference in the toxicity of separate chlorinated members of methane series is shown in Table 61. According to other classifications, only methylene chloride belongs to a lower (boundary) class of toxicity. As to methyl chloride, chloroform and carbon tetrachloride, they belong to the same class of toxicity, according to most classifications.

For a general comparative toxicological characteristic of the series, it is possible to introduce the conditional notion of the «toxicological gradient» of the series. It means a relative degree of the increase (or decrease) of toxicity from one member to another. In the series CH_2Cl_2—$CHCl_3$—CCl_4 the toxicological gradient is not so high (about 2.0). At the same time, at a high level of exposure, under administration of substances into the stomach and under exposure by inhalation, chloroform is slightly more toxic than carbon tetrachloride.

Distribution parameters of individual sensitivity of animals, characterizing the probability of lethal outcome (i. e., the hazard of lethal poisoning by inhalation) under respiratory application of the poisons, are given in Table 62. This Table also gives the absolute toxicity and the toxicity, determined by the volatility of the poisons (CPPI).

From Table 62 follows that methylene chloride is the most hazardous, and carbon tetrachloride is the least hazardous, according to the indices of variability of the individual sensitivity. Just because the concentrations causing narcotic and lethal outcomes were very close, it was impossible to use methylene chloride for narcosis. The integral hazard index $\left(\frac{1}{CL_{50}S}\right)$, which takes into account the toxicity and variability of the lethal concentrations

Table 57

and its chloro-derivatives

Volatility, mg/l, (20°C)	Water solubility, mmole/l, (20°C)	Oil/water partition coefficient (20°C)	Refractive index, n	Energy of C-Cl bond rupture, kcal/mole
—	1.5	19—20	—	101
11 430 000	0.012	25.3	—	80.5
1 620.0	0.024	26	1.4237	73.4
1 050.0	0.007	154	1.4455	66.5
784.0	0.0005	1 000	1.4603	76.0

Table 58

Lethal doses of chlorinated hydrocarbons of methane series

Substance	Animals	Lethal doses, g/kg		
		DL_{16}	DL_{50}	DL_{84}
$CHCl_3$	Rats	—	2180 (Raventos, 1956)	—
	Mice	—	1750 (1550÷2020) (V. E. Miklashevsky et al., 1966)	—
	Rats	800	1250 (1580÷985)	2200
	Mice	620	1000÷260 (I. P. Ulanova, 1971)	1650
CCl_4	Rats	—	6000 (E. N. Kutepov, 1967)	—
	Mice	—	9067 (E. N. Kutepov, 1967)	—
	Rats	2350	3650 (7570÷1750)	5 700
	Mice	6200	9600±2250	15 000
	Rabbits	—	4600 (4970±4260)	—
	Guinea pigs	—	5760 (I. P. Ulanova, 1971)	—

Table 59

Evaluation of the degree of toxicity of chloroform and carbon tetrachloride under administration into the stomach

Authors of classification	$CHCl_3$	CCl_4
S. D. Zaugolnikov et al.	IV-A	V
Hodge and Sterner	IV	V
MACs Section	III	IV

Table 60

Lethal concentrations of chlorinated hydrocarbons of methane series

Substance	Animals	Lethal concentrations, mg/litre		
		CL_{16}	CL_{120}	CL_{240}
CH_3Cl	Rats		6—8 (CL) (N.V. Lazarev, 1971)	
	Mice	3.9	5.3(6.30÷4.45) (G. Y. Evtushenko, 1966)	7.4
CH_2Cl_2	Mice		50 (CL) (N. V. Lazarev, 1971)	75.0
	Mice	53.0	63.0(73.0÷54.5) (I. P. Ulanova, 1971)	
$CHCl_3$	Mice		30—40 (CL) (N.V. Lazarev, 1971)	
	Mice	16.0	21.2 (26.0÷17.2) (I. P. Ulanova, 1971)	29.0
CCl_4	Rats		65.0 (B. A. Kurlyandsky, 1970)	
	Rats		25.0 (Carpenter, 1949)	
	Mice	23.0	34.5±7.1 (I. P. Ulanova, 1971)	51.0
	Mice		67.5 (CL)(N.V. Lazarev, 1971)	

Table 61

Classification of toxicity of chlorohydrocarbons of methane series under inhalation exposure

Authors of classification	CH_3Cl	CH_2Cl_2	$CHCl_3$	CCl_4
S.D. Zaugolnikov et al.	III—A	IV—B	IV—A	IV—A
Hodge and Sterner	IV	V	IV	IV
MACs Section	III	IV	III	III
I. V. Sanotsky				
according to mg/litre scale	32%	12%	25%	21%
according to mm/litre scale	27%	14%	22%	21%

for the last 3 members of the series, indicates insignificance of the general hazard gradient.

The first member of the series — methyl chloride — is the most hazardous compound at this level of exposure because of its high absolute toxicity, which is explained by its specific metabolism. The CPPI for methyl chloride (gas) is rather high, while for the other members of the series it is practically the same.

Methane (CH_4) is usually assigned to narcotics, but under normal pressure it is physiologically inactive. Acute poisonings with this compound are possible only under considerable concentrations which can be produced only under increased pressure (N. V. Lazarev, 1940). Numerous descripted «poisonings» with

Table 62

Some indices of the hazard of lethal poisoning of rats under inhalation exposure to hydrocarbons of methane series

Index	CH_3Cl[1]	CH_2Cl_2	$CHCl_3$	CCl_4
$\frac{1}{CL_{50}}$	0.19 (0.013)[2]	0.016 (0.00134)	0.047 (0.0056)	0.029 (0.0044)
$\frac{CL_{84}}{CL_{16}}$	1.89	1.41	1.81	2.21
S	1.37	1.19	1.33	1.48
$\frac{1}{CL_{50} \cdot S}$	0.138 (0.0094)	0.0134 (0.0011)	0.0353 (0.0042)	0.0195 (0.0029)
$CPPI_{CL}$	2 156 603	25.7	49.5	22.1

[1] Experiments on rats.
[2] Values of the CL_{50} expressed in mm/l are given in brackets.

methane in mines resulted from asphyxia caused by the displacement of oxygen from the atmospheric air.

Substitution of a hydrogen atom by chlorine in the molecule of methane intensifies the narcotic effect (methyl chloride makes an exception) and causes lesion of the parenchymatous organs, of the liver and kidneys in particular (N. V. Lazarev, 1938, 1971; Oettingen et al., 1949). The clinical picture of acute poisoning with chloro-derivatives of methane is characterized by loss of coordination of movements, dyspnea, hyperreflection, hematuria, irritation of the mucous membrane of eyes and of the upper respiratory tract. Death comes as a result of the respiratory center paralysis.

The morphological changes under lethal oxidation consist in the development of degenerative changes in the nervous system and in the parenchymatous organs.

The intensity of the narcotic effect of chlorinated hydrocarbons increases with the increase of the number of chlorine atoms in the molecule, but up to a certain limit (N. V. Lazarev, 1938; Ferguson, 1939): in methane series it increases up to chloroform, having a stronger effect than carbon tetrachloride, which results from lower solubility of CCl_4 in water and, consequently, in blood (N. V. Lazarev, 1938). Methyl chloride falls out of this pattern, since it was found in blood in smaller concentrations than could be expected in view of a high pressure of saturating vapours, proper to this compound (3,510 mm of Hg), and comparatively high solubility in water (higher than that of chloroform and only half that of dichloromethane). Antiseptic and hemolytic effect of chloro-derivatives of methane grows with the increase of the molecular weight, relative density, boiling point and oil/water partition coefficient (N. V. Lazarev, 1938; Lehmann, Schmidt-Kehl, 1936).

As was justly noted by Oettingen (1953), the toxicity of the considered compounds is inadequate to the intensity of their narcotic effect. Thus, methyl chloride — the weakest narcotic of the series — is at the same time the most toxic substance. Oettingen et al. (1949) proved in experiments on dogs that the most hazardous was carbon tetrachloride, the least hazardous was methylene chloride, methyl chloride and chloroform hold intermediate position.

Narcotic properties of methyl chloride do not correspond to the quantitative characteristics, in particular, to the time of survival of animals (Table 63). It may be explained by a more intensive

Table 63

Toxic effect of chlorinated hydrocarbons of methane series at the CL_{50} level (G. Y. Evtushenko, 1966; I. P. Ulanova, 1971)

Substance	Time of beginning of narcosis, min	End of narcosis, min	Mean life span
CH_3Cl	Narcosis did not occur		1.2 days
CH_2Cl_2	15	10—15	1.7 hr
$CHCl_3$	30—40	30—50	4.7 days
CCl_4	50—60	30—50	3.2 days

hydrolysis of methyl chloride and by formation of more toxic products of metabolism. Thus, investigating the rate of the reaction of these substances with water, Oettingen et al. (1949) revealed that this rate was the highest in methyl chloride and the lowest in methylene chloride.

The clinical picture of poisoning with methyl chloride is also rather peculiar. According to G. Y. Evtushenko (1966), symptoms of irritation of the mucous membrane of eyes and of the upper respiratory tract, as well as falling on one side were not observed in animals exposed to methyl chloride. In case the death came 2—6 hours immediately after the exposure, a foamy liquid oozed out from the nose and mouth of animals. Macroscopic examination of lungs during autopsy revealed that in some cases the lungs were enlarged and a foamy liquid was oozing out from the trachea, while in other cases complete or lobar hepatization was registered. When the animals died at later periods (2-6th day), mucosanguineous liquid oozed out from the nose and mouth in large number of the animals and pneumonia was certified by the autopsy. According to the rate of development of symptoms of poisoning in animals exposed to isoeffective concentrations of chlorinated hydrocarbons, methylene chloride ranks the first, then follow chloroform and carbon tetrachloride (see Table 63).

After the end of exposure, inhibitory phenomena were the fastest to disappear in animals exposed to methylene chloride, and the slowest to disappear in those exposed to carbon tetrachloride.

As can be seen from Table 63, most animals exposed to methyl chloride and methylene chloride died at early periods. Under exposure to chloroform and carbon tetrachloride, the period of dying was prolonged, which was explained by their more manifested narcotic properties, preventing the development of acute vascular disturbances in lungs and brain, and thereby giving time for development of degenerative changes in the parenchymatous organs.

Characteristics of the hazard of acute non-lethal poisoning

As has been stated above, the hazard of acute non-lethal poisoning may be characterized either by the absolute value of the acute effect thresholds[1], or by the ratio $CL_{50}/Lim_{ac}=Z_{ac}$ (real hazard). The potential hazard of the acute effect (harmful effect) can be characterized by the ratio of volatility to the acute effect threshold:

$$CPPI_{ac}=\frac{C^{20}}{Lim_{ac}}.$$

For this purpose, the thresholds of the single effect of substances have been determined (Table 64). It is known that the Lim_{ac} of carbon tetrachloride, according to changes in the flexor reflex time, is 1.5 mg/l (E. I. Lyublina, 1951). A concentration of chloroform which is close to the threshold value is 0.5—0.6 mg/l (O. Masahiro et al., 1965).

As can be seen from the data in Table 64, the lowest thresholds of acute effect, according to the integral and spesific indices, have been registered under exposure to methyl chloride, then, according to increase of the Lim_{ac} values, follow chloroform, carbon tetrachloride, methylene chloride. Other indices of the hazard are given in Table 65.

The data in Table 65 indicate that the highest potential hazard under exposure by inhalation at this level is presented, naturally, by methyl chloride (gas), the lowest is that of carbon tetrachloride. The degree of biological activity of chlorinated hydrocarbons of methane series, according to the absolute value of the Lim_{ac}, is approximately equal for all compounds, except methyl chloride, which is the most active. According to the Z_{ac}, the most hazardous compound is methyl chloride, the least hazardous is methylene chloride. The differences in the hazard degree are not to be considered significant (by less than 3 times).

[1] We mean the Lim_{ac} integral, i. e., changes at the level of the integral organism.

Table 64

Acute effect thresholds (mg/litre) for chlorinated hydrocarbons according to the integral indices, indices of the state of nervous system and liver (non-effective concentrations are given in brackets)

Index	CH_3Cl	CH_2Cl_2	$CHCl_3$	CCl_4
Rectal temperature			1	4
Oxygen consumption			1	
Number of respirations per min	0.65			
STI	0.65	6—8	0.7	1.7
	(0.23)		(0.12)	(4)
SMA (number of rushes per 15 min)	0.65		1	4
			(0.7)	
Time of working out of conditioned reflexes	0.23			
Changes in motor food conditioned reflexes			1	1.2
			(0.05)	(0.05)
Elimination of bromosulfalein per 15 min				1.7
Vital coloration of the liver tissue with neutral red blood cells			1	1.7
				(1.2)
Quick-Pytel test				1.7
				(1.2)
Relative mass of liver			1	4
				(1.2)
Fat content in liver				4

Table 65

Some indices of the hazard of acute non-lethal poisoning under inhalation exposure

Index	CH_3Cl	CH_2Cl_2	$CHCl_3$	CCl_4
Lim_{ac}, mg/litre	0.23	1.0	0.7	1.2
$\frac{Lim_{ac}}{C^{20}}$	23	63	30	29
$\frac{C^{20}}{Lim_{ac}}$	$2 \cdot 10^{-8}$	$62 \cdot 10^{-5}$	$66 \cdot 10^{-5}$	157.10^{-5}
Z_{ac}	49 695 652	1620	1500	637

Characteristics of the hazard of chronic poisoning

The basis for evaluation of the hazard of chronic poisoning is the chronic effect threshold (Lim_{ch}). The Lim_{ch} may be determined with a sufficient accurancy only provided that the non-effec-

tive concentration is substantiated. In other cases, elements of subjectivity are being involved in the evaluation of the Lim_{ch} value.

Chronic intoxication with chlorinated hydrocarbons of methane series under exposure to low concentrations often occurs without marked clinical manifestations. Sometimes a decrease in the body weight, the untidiness of hair and an increase of aggressiveness are observed in animals.

The morphological changes in organs under chronic intoxication caused by these compounds consist, as in case of acute intoxication, in the degenerative changes in liver, kidneys, adrenal glands, nerve stems; there have also been registered symptoms of inflammation in bronchi and in lungs. The few data on chronic intoxication[1] which are available in the literature have been obtained by foreign, mostly American researchers at obviously effective levels, which cannot be considered complying with the concept of harmful effect considered above (Table 66).

In order to obtain comparable data, special investigations have been carried out (Table 67). As follows from Table 67, the most biologically active appeared to be methyl chloride; the least biologically active, methylene chloride. The threshold concentration of CCl_4 is below 0.05 mg/l; after the end of exposure to CCl_4 in concentrations at the level of 0.05 mg/l, the animals did not recover completely from morphological changes in the central nervous system and in the liver (one of the criteria of harmfulness). According to B. A. Kurlyandsky (1970), the Lim_{ch} for CCl_4 is equal to 0.02 mg/l. In this case, the biological activity of CCl_4 and methyl chloride are expressed in the same degree.

Characteristic qualitative features have been revealed under chronic intoxication caused by each chlorinated hydrocarbon of methane series.

Chronic intoxication with methylene chloride and carbon tetrachloride usually disturbed the equilibrium between the excitation and inhibitory processes in the nervous system. Compensation periods alternated with periods of disturbances of the conditioned reflexes. In the first period of chronic intoxication (approximately 2 months from the beginning of exposure), a weakening of the inhibitory processes and a relative increase of the excitation processes were observed. Stable disturbances of the conditioned reflexes followed by a complete falling out of positive conditioned reflexes (protective inhibition) and development of phase conditions were registered in the second period. By the end of exposure, the animals often exhibited disturbances also in their natural reflex to the sight and smell of food, and some of

[1] Here are not mentioned the works on mechanisms of the CCl_4 action, which appeared mainly of a model character. These works do not provide additional information on qualitative characteristics of poisoning at different, mainly low, levels of inhalation exposure.

Table 66

Dependency of the magnitude of biological effect of chlorinated hydrocarbons of methane series on the concentration (inhalation, chronic exposure for not less than 4 months)

Substance	Concentration, mg/l (animals)	Biological effect
CH_3Cl	2.0 (rats)	Evident manifestations, morphological changes in organs
	1.0 (rabbits)	Fatty dystrophy in liver, kidneys, heart, degenerative changes in optic and sciatic nerves, in adrenal glands
	34.0[1] (monkeys, dogs, rabbits, guinea pigs, rats)	Narcosis, fatty dystrophy in liver
CH_2Cl_2	25.0[2] (cats)	Sharp disturbances in coordination of movements, fatty dystrophy in liver and kidneys
	17.0[1] (monkeys, dogs, rabbits, guinea pigs, rats)	No visible morphological changes
	6.0—4.0 (rats)	Slight atrophy, center—lobar fatty dystrophy in liver
$CHCl_3$	0.3—0.5 (rats)	Evident morphological changes in liver and kidneys
	0.15 (rats)	Slight morphological changes in liver and kidneys
CCl_4	2.0[2] (rabbits)	Adiposis of liver, of straight and intercalary renal tubules
	1.25—0.3 (rats)	Cirrhotic changes in liver, degenerative changes in sciatic nerve, necrotic nephrosis
	0.16 (rats)	Mederate fatty infiltration of liver, cirrhosis
	0.32 (rats)	Toxic effect not revealed

Note: The Table has been compiled from the data obtained by the authors cited in the work of I. P. Ulanova (1971).
[1]2—month exposure.
[2]1—month exposure.

them had disturbances in the unconditioned reflex to food, which indicated that inhibition from the cerebral cortex was extended to the subcortical sphere.

The recovery of the disturbed conditioned reflexes proceeded gradually and was completed only by the end of the second month after the end of exposure, which was in line with the third criterion of harmfulness, according to the MACs Section. Those data were not assessed statistically, though in qualitative terms the described changes did not occur in the control animals. Therefore, it is reasonable to consider those changes unusual, going beyond the limits of physiological variations (the first criterion of harmfulness, according to the MACs Section).

Table 67

Indices characterizing the state of white rats under chronic exposure to chlorinated hydrocarbons of methane series ($p \leq 0.05$)

Substance	Concentration, mg/litre	Changed indices	Lim_{ch}, mg/litre
CH_3Cl	0.24	Body weight (—), Hb in blood (—), erythrocytes (—), neutrophils (+), lymphocytes (—), morphological changes (+), STI (+), working out of conditioned reflex to the bell (—)	At the level of 0.02
	0.02	Body weight (—), Hb in blood (—), erythrocytes (—), STI (—), working out of conditioned reflex to the bell (—)	
	Recovery period	Complete recovery from the revealed changes caused by exposure to a low concentration	
CH_2Cl_2	0.25	Disturbances in the conditioned reflexes, falling out of natural reflex to the sight and smell of food, disturbance of interneuronal connections in the cerebral cortex	0.25
	0.06	Non-effective concentration	
CCl_4	0.05—0.1	Hb (—), erythrocytes (—), reticulocytes (+), neutrophils (+), lymphocytes (—), STI (—), disturbances in the conditioned reflexes, hippuric acid in urine (—), protein in urine (+), aminoacids in urine (+), content of SH-groups in blood serum (+), morphological changes (+)	0.05
	Recovery period	Incomplete recovery from morphological changes in the central nervous system and in liver	

Note: (+) increase, (—) decrease.

Changes in the speed of working out and fixation of the conditioned reflex to a strong stimulus in animals was evaluated under exposure to methyl chloride in the concentrations of 0.24—0.17 and 0.04—0.02 mg/l. By the 6th month of exposure, there were registered disturbances in the natural reflex to the sight and smell of food. Recovery from those changes did not occur 1 and a half month after the end of exposure. This enables us to suggest the existence of significant disturbances in the higher nervous activity of animals under chronic exposure to methyl chloride which went beyond the limits of physiological fluctuations and were steadily preserved.

In view of the fact that the considered chlorinated hydrocarbons cause changes in the functional condition of the nervous system, and especially in the higher nervous system, I. P. Ulanova et al. (1961) made an attempt to reveal the relation between the functional and morphological changes in interneuronal (axo-

dendral) connections of the cerebral cortex. It is known that, as a result of the development of associative connections, axodendral (indirect) connections acquire primary importance in the cerebral cortex.

S. A. Sarkisov (1949), A. D. Zurabashvili (1951) et al. found that these connections are possible because of the existence of numerous tiny appendages-spines located on dendrites. These peculiar formations represent a receptor apparatus of the nerve cells of the cerebral cortex, which has an extremely high sensitivity to different pathological exposures (S. A. Sarkisov, 1949; A. D. Zurabashvili, 1951; M. S. Tolgskaya, 1955; M. S. Tolgskaya et al., 1961).

Pieces of the cerebrum, taken from the regions of motor and sensory analyzers of control and test animals, were impregnated with silver by the method of Goldgi. It was found that apical and basal dendrites of the control animals were abundantly covered with small protoplasmic formations (Fig. 28).

Fig. 28. Normal structure of dendrites of 1—2 layers of cortex. All dendrites are covered with a mass of spinous appendages.

In the test animals killed after the discovery of evident disturbances in the conditioned reflexes, the protoplasmic formations on the dendrites of efferent neurons disappeared, while irregular swellings appeared instead of them, and the dendrites acquired the form of beads (Fig. 29). The apical dendrites leading to the

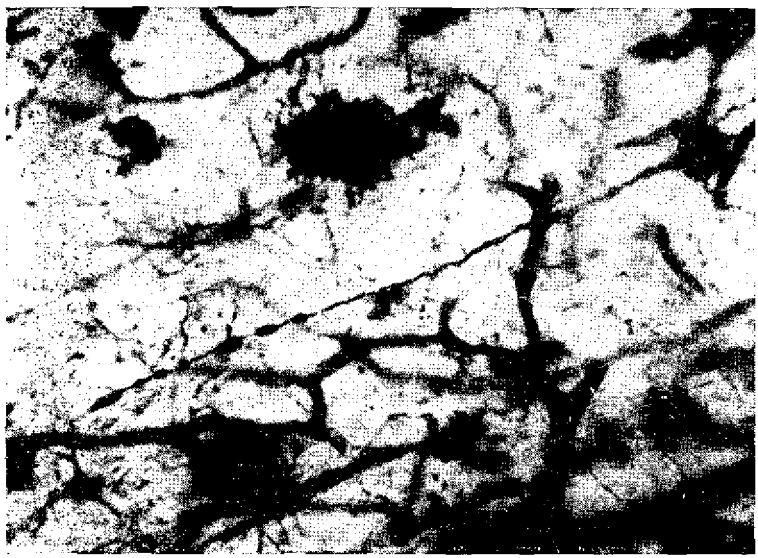

Fig. 29. Changes in the apical dendrite of efferent neuron of cortex. Reduction of the quantity of spinous appendages and appearance of swellings in the form of beads on the distal end of apical dendrite (rat; chronic exposure to methylene chloride, X400).

higher associative layers of the cerebral cortex were especially changed. By colorating the brain tissue by the method of Nissle, certain changes in the form of turbid swellings of protoplasm of separate cortex nerve cells were found, which, according to P. E. Snesarev (1949), is an initial reversible response of the nerve cells to the exposure to different irritants. During the recovery of the conditioned reflexes in animals (after 2 months), no changes in the interneuronal connections of the cerebral cortex were detected by morphological investigation. All the dendrites of the cortex neurons had even contours and were covered with a large number of protoplasmic formations (Fig. 30).

Thus, the morphological changes in the interneuronal connections of the cortex appear to be functional. It should be noted that they are not specific for the exposure to methylene chloride. Similar changes were found by M. S. Tolgskaya (1955) under exposure of animals to ammonia, arsenic, lead.

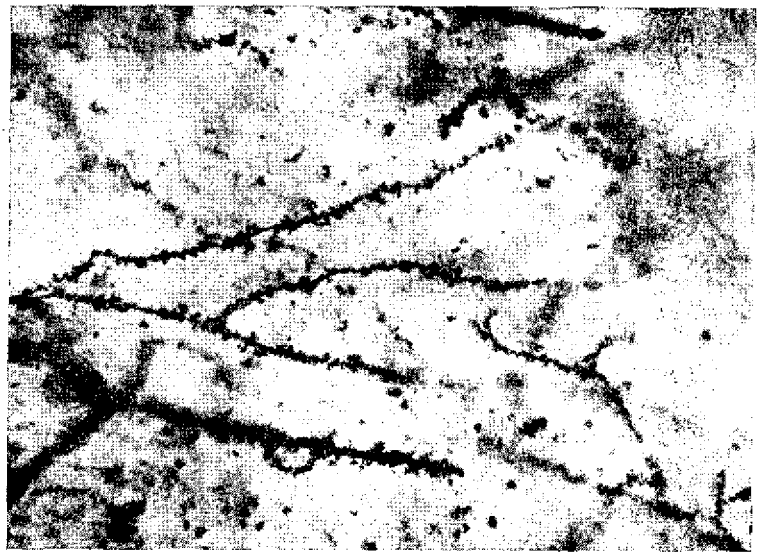

Fig. 30. Restoration of normal configuration of apical dendrites of efferent neurons of cortex. Spinous appendages can be seen on dendrites.

Of the same type are qualitative changes in the picture of the peripheral blood of the experimental animals under chronic intoxication with methylene chloride and carbon tetrachloride. A decrease of the quantity of erythrocytes and hemoglobin was registered in the peripheral blood of white rats, rabbits and guinea pigs. Leukocytes content in the blood of the experimental animals remained within the physiological norm. Disturbances in the peripheral blood composition were not revealed under exposure to methylene chloride.

The qualitative peculiarities of chronic intoxication caused by separate chlorinated hydrocarbons at the Lim_{ch} level concern mainly functional condition of the liver and kidneys.

Under chronic intoxication with methyl chloride, which had caused changes in the nervous activity, no disturbances in the functional condition of the liver (according to the results of evaluation of the hippuric acid content in urine under the load with sodium benzoate and the assessment of the bromosulfalein content in blood) were revealed. The morphological changes (in comparison with the exposure to CCl_4) were less manifested. Albuminous dystrophy in the liver cells and proliferation of reticuloendothelial elements were the only changes detected.

The functional and morphological changes in the liver and kidneys were revealed under exposure to CCl_4. I. P. Ulanova et al. (1970) showed that in chronic experiment the first changes were

registered in the Quick-Pytel test and in the excretory function of the liver.

Under chronic intoxication with CCl_4, changes registered by the Quick-Pytel test were characterized by the existence of phases. A sharp (up to 80%, as compared to the control) increase of the hippuric acid content in the urine of the test animals after the load with sodium benzoate was revealed during the first (1—2 months) period of exposure. No morphological changes in the liver of white rats, killed in this period of intoxication, were found. During the 5th month of chronic intoxication with carbon tetrachloride, the amount of hippuric acid excreted with urine by experimental animals was 40% less than that of the controls. The microscopic investigation undertaken in this period revealed a small-drops fatty dystrophy of the cells of the peripheral sections of lobules in the liver of experimental animals. The excretory function of the liver, according to the data from the bromosulfalein test conducted in this period, remained however unchanged.

The distinguishing feature of chronic intoxication with methyl chloride is the lesion of the optic apparatus[1]. Already 2 months after the exposure to methyl chloride at the Lim_{ac} level, rabbits had hyperemia of the mucous membrane of eyelids and eyeball, particularly manifested in the region of the lid slit. The picture of the eye fundus was characterized by hyperemia, edema of the optic nerve disk located at the site of the vessels' outlet, moderate hyperemia of pupilla. Pathomorphological investigation of the eye tissue of rabbits revealed plasmorrhagias of different proportions. Vessels of the ciliary type were plethoric. The layer of bacilli and cones was rarified. Structural changes in the optic nerve were not detected.

Thus, under chronic exposure to chlorinated hydrocarbons at the Lim_{ch} level there are being registered the phenomena common for all the mentioned substances, which are characterized mainly by disturbances in the functional condition of the nervous system and which have their own peculiar features. At the same time, there are being revealed specific features of the effect of separate compounds. For example, exposure to methyl chloride at the Lim_{ch} level causes disturbances in the optic apparatus; carbon tetrachloride causes functional and morphological changes in the liver. The changes discovered under exposure to methylene chloride were connected only with the conditioned reflexes and did not concern the internal organs. Quantitative characteristics of the hazard of chronic poisoning are given in Table 68.

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[1] Ophthalmoscopic investigation of the optic apparatus was undertaken by S. F. Belova, candidate of medical sciences, oculist of the Clinic of the Institute of Industrial Hygiene and Occupational Diseases, USSR Academy of Medical Sciences. Histologic investigations were conducted by the Histologic Laboratory of the Ophthalmic Clinic, I Moscow Sechenov Institute.

Table 68

Indices of the hazard of chronic poisoning under inhalation exposure to chlorinated hydrocarbons of methane series

Index	CH_3Cl	CH_2Cl_2	CCl_4
Lim_{ch}, mg/litre	0.02	0.25	Less than 0.05[1]
Z_{ch}	11	4	60
$\frac{Lim_{ch}}{C^{20}}$	$1 \cdot 10^{-9}$	$1.55 \cdot 10^{-4}$	$2.6 \cdot 10^{-5}$
$\frac{C^{20}}{Lim_{ch}}$	571 500 000	6480	38 200

[1] According to the data by B. A. Kurlyandsky, 0.02 mg/litre.

As can be seen from Table 68, the potential hazard of the development of poisoning (taking account of volatility) is the highest under exposure to methyl chloride (gas), the smallest is that of methylene chloride. Differences in the degree of biological activity (according to the Lim_{ch}) of methyl chloride and carbon tetrachloride have not been revealed; methylene chloride at this level is the least active. According to the magnitude of the chronic effect zone, the most hazardous is CCl_4, the comes CH_3Cl, and the least hazardous is CH_2Cl_2.

Some changes in the relative hazard indices resulting from metabolic peculiarities

Comparison of the qualitative characteristics of the effect of chlorinated hydrocarbons of methane series on the organism at different quntitative levels (CL_{50}, Lim_{ac}, Lim_{ch}) has shown that methylene chloride, chloroform and carbon tetrachloride have much in common in the type of their action, while the action of methyl chloride has qualitative peculiarities.

The biological activity (by CL_{50}) of these compounds (except methyl chloride) is closely related to their physico-chemical properties, and to the energy produced by the rupture of carbon-halogen bonds in molecules in particular. The correlation between the lethal concentrations of substances and the energy of rupture of these bonds in molecules appeared to be almost absolute (+0.99).

Ferguson (1939) demonstrated that the magnitude of the narcotic effect of substances, irrespective of their chemical structure, is proportional to the thermodynamic activity of their solutions in the medium, where the action takes place (in our experiment, in

the blood of animals). The thermodynamic activity is inversely proportional to the solubility of a narcotic substance. For methylene chloride, chloroform, carbon tetrachloride solubilities in water (in blood) are 0.024—0.007—0.0005 mmole/litre, respectively. Therefore, the thermodynamic activity is the highest in carbon tetrachloride, which should have the highest biological and also narcotic activity. However, the biological activity of $CHCl_3$ appears to be slightly more manifested than that of CCl_4. These correlations are, possibly, related to the effect of the products of metabolism of these substances. Thus, the least toxic product — methylene chloride — does not undergo transformations in the organism and is almost entirely (98—100%) eliminated unchanged through the lungs (N. M. Maltseva, 1974; Fassett et al., 1967). According to the data by different authors (Mc. Collister et al., 1951; Souček, 1961), from 44 to 80% of carbon tetrachloride is eliminated through the lungs with the exhaled air, and an insignificant amount is excreted with urine and feces (Mc. Collister et al., 1951; Benoy, Rubinstein, 1963).

It has been found out that the blood of animals exposed to CH_2Cl_2 contains an insignificant amount of HBCO, which in the opinion of the authors (Boudéne et al., 1978; H. P. Giuchta et al., 1979, and others) is the metabolite of the given substance.

N. M. Maltseva (1974) has shown that the main elimination of methylene chloride at both lethal and threshold level (Lim_{ac}) occurs with the exhaled air. A much smaller amount of methylene chloride is excreted with bile and urine.

It has been shown that, irrespective of the value of the effective concentrations, the elimination of methylene chloride from the organism occurs in two phases (fast and slow), which may be described by the exponential curve, expressing a first order process.

It should be emphasized that the values characterizing the rate of elimination of methylene chloride from the organism (T_{biol}) are lower at the lethal than at the threshold level ($p>0.05$), and at the threshold and sub-threshold levels are practically equal ($p>0.05$).

A decrease in the rate of methylene chloride elimination at the lethal level is, in the author's opinion, connected with the inhibition of processes of elimination of the poison from the organism under high intensity of exposure.

We presume that the biological elimination half-life or, to be precise, its dependence on the effective concentration of non-metabolized poisons may serve as a basis for substantiating the criterion of harmfulness.

According to Y. Srbova (1962), in persons and animals exposed to CCl_4 there forms trichlorethanol, which the author recommends to use as the exposure test under exposure to carbon tetrachloride. It is possible that chloroform is an intermediate

metabolite of CCl_4 (Butler, 1961; Fowler, 1969). Theories, explaining the toxic effect of CCl_4, have been examined by A. E. Grebennikova (1967), and more thoroughly by Recknagel (1967).

The toxicity of CCl_4 has been recently explained by its transformation into free radicals under the action of a number of intracellular enzymes. Free radicals, which are formed in the reaction $CCl_4+e \rightarrow CCl_3+Cl^-$, attack methylene arches of non-saturated fatty acids, the side chain of microsome structure lipoids and form peroxides, which results in their oxidizing decomposition (Butler, 1961; Reynolds, 1967; Fowler, 1969).

It is not yet clear how much effective this process is at low levels of exposure and what metabolic criteria could be proposed in this case.

The qualitative pecularities of poisoning with methyl chloride are linked with the effect of the products of its transformation in the organism. This supposition was put forward by Flury (1928), F. Flury, F. Zernik (1938). Methanol, formaldehyde and formic acid were cited as probable products of methyl chloride transformation. Later this hypothesis was confirmed. Sperling et al. (1950) and Souček (1961) demonstrated that about 80% of methyl chloride, administered to rabbits intravenously, disappeared from the blood immediately, another 10% within the next hour, and after 25 minutes the substance disappeared from the blood completely. Sperling found that in this case about 5% of the administered methyl chloride (according to Souček, 27%) was eliminated with the exhaled air, and a very insignificant amount was excreted with urine, feces and bile. Baker (1930) et al. revealed an increased content of formic acids salts in urine. However, the quantity of formic acid and its salts does not exceed the usual physiological level in cases of poisonings with methyl chloride, therefore it cannot be considered as the index of the hazard (see Chapter I).

In experiments on rabbits and dogs, Smith (1947) did not discover the expected increase of the amount of formic acid and methanol in the organism after poisoning with methyl chloride.

Table 69

Formaldehyde content in the blood of white rats after a single 4-hour exposure to methyl chloride by inhalation

Rated concentration of methyl chloride, mg/m³	Number of animals	Formaldehyde content, mg %		
		before test	1—2 hours after	18—20 hours after
16 000	5	0.05	0.65±0.35	—
6 000	7	0.075	1.32±0.41	0.075
2 000	6	Traces	1.00±0.25	—

Our investigations, conducted using gas chromatography, have shown that, under exposure to lethal concentrations of methyl chloride, this substance completely disappeared from the blood after 20—30 minutes. At the same time, the amount of formaldehyde[1] in the blood increased sharply (Table 69).

However, also in this case we do not have data on the dependency of the intensity of metabolism on the effective poison's concentration, therefore the metabolic criteria of harmfulness which we propose cannot be proved.

Conclusions

The main parameters of toxicometry of chlorinated hydrocarbons of methane series are given in Table 70. The most toxic and hazardous compound (potentially and really, class I of the ha-

Table 70

Major parameters of toxicometry and hazard classes of chlorinated hydrocarbons of methane series

Indices	Methyl chloride	Hazard class	Methylene chloride	Hazard class	Chloroform	Hazard class	Cabon tetrachloride	Hazard class
DL_{50}, mg/kg	—		—		1000	III	3 650	IV
S	—		—		1.63		1.55	
$\frac{1}{DL_{50} \cdot S}$	—		—		0.60		0.064	
CL_{50}, mg/l	5.3 1.37	III	63.0 1.19	IV	21.2 1.33	III	34.58 1.48	III
$\frac{1}{CL_{50} \cdot S}$	0.138		0.0134		0.0353		0.0195	
$CPPI_{CL}$	2 156 603	I	25.7	III	49.5	II	22.1	III
Lim_{ac}, mg/l	0.23	III	1.0	III	0.7	III	1.2	IV
$CPPI_{ac} = \frac{C^{20}}{Lim_{ac}}$	49 695 652	I	1620	II	1500	II	637	II
Z_{ac}	23	III	63	IV	30	III	29	III
Lim_{ch}	0.02	III	0.25	IV	—		0.05	III
$CPP_{Ich} = \frac{C^{20}}{Lim_{ch}}$	571 500 000	I	6480	II	—	—	38 200	I
Z_{ch}	11	I	4	III	—	—	60	I
I_{cum}	—	—	—		—		6.57	
MAC, mg/m³	5.0		50.0		—		20	

[1] The method for detecting formaldehyde, as applied to biomedia, has been worked out by L. A. Duyeva (1966).

zard) under a single exposure is methyl chloride. Under chronic exposure, the biological activity of carbon tetrachloride is approximately the same as that of methyl chloride, but, according to the Z_{ch}, it is 5 times higher. Methylene chloride is moderately hazardous from the point of view of chronic poisoning. Differences in the character of the biological effect of separate chloro-derivatives of methane are the most manifested under acute lethal exposure (all compounds, except methyl chloride, are narcotics), and much less marked under threshold exposures. The data obtained make it possible to substantiate the MAC values for chlorinated hydrocarbons of methane series.

Attention should be paid to the fact that the difference in the MAC values between the most toxic methyl chloride and the least toxic methylene chloride is one order. Approximately the same difference exists between the biological activity of these compounds at the threshold levels of exposure. At the same time, the MAC for carbon tetrachloride — 20 mg/m^3 — exeeds the MAC for methyl chloride by 4 times, although the biological activity of these compounds at the Lim_{ac} level is practically equal. According to B. A. Kurlyandsky, the threshold of the chronic effect of CCl_4 is at the level of 0.02 mg/l, i. e., at the level of the existing MAC. Data from clinico-hygienic observations have been used for the correction fo the MAC values of chlorinated hydrocarbons.

Methyl chloride. When investigating the occupational conditions of workers engaged in the production of butyl rubber (Yaroslavl Scientific Research Institute of Monomers for Synthetic Rubber), G. Y. Evtushenko (1966) has shown that the determined concentrations of methyl chloride usually exceeded the experimentally substantiated MAC (5 mg/m^3) by 4—6 times. In a number of cases (10% of analysis) these concentrations exceeded the MAC by hundreds of times (workers had to use gas masks). The concentrations of isobutylene, isoprene, methanole, hydrogen chloride, etc. did not exceed their MACs. The clinical examination showed that 46 out of 99 investigated persons had functional disturbances in the nervous system (neurotic and astheno-neurotic reactions, vegeto-vascular and vegeto-endocrine instability, asthenic reactions); 10% of persons exhibited disturbances in the function of the liver (mainly of pigmentary function); 8% of people had vascular disturbances (hypertonic reactions), and 14% of persons had changes in the function of the gastro-intestinal tract.

The longer the working record, the more persons had functional disturbances of the liver. The examination of the people with a 2 to 3-year length of service revealed that approximately 1/3 of them had a decrease of the cornea sensitivity. 1/3 of the persons who had worked at this plant for 5—8 years had an abasement of the acuity of sight, and 67% had an impairment of the cornea sensitivity. An objective investigation of the fundus of the eye revealed

bilateral pallidness of the optic nerve disks in 50% of workers, accompanied by angiopathy of retina in half of the cases. Thus, the poison in concentrations of approximately 20—40 mg/m^3 is not harmless for the organism. Unfortunately, we have no data from clinico-hygienic observations for lower concentrations of methyl chloride. However, the results of this investigation permit one to think that at present the experimentally substantiated value of the MAC does not need revision.

Methylene chloride. Clinico-hygienic observations, carried out by I. P. Ulanova et al. at a plant producing noninflammable film pellicle, where methylene chloride was used as solvent, have shown that methylene chloride in concentrations of the order of whole units and tenth parts (mg/litre) caused disturbances in health condition. Out of 64 investigated persons with a length of service of 1—3.5 years, over 50% had functional disturbances of the nervous system. An enlarged liver had 1/3 of the examined persons; hepatitis was revealed in 5 persons; in 50% were detected changes in the function of the gastro-intestinal tract; 10 persons had disturbances of the cardiovascular system.

After implementation of sanitary measures, concentrations of methylene chloride in the air of workplaces decreased considerably and did not exceed tenth parts of mg/litre. The examination, conducted after 1 year and a half of work under these conditions, revealed a considerable decrease of subjective and objective indices of health disturbance. It is important to mention that in 5 persons with diagnosed hepatitis who had been isolated from the workshop and who had worked for a year and a half out of contact with methylene chloride no disturbances in the function of the liver were revealed, i. e., the changes appeared to be reversible. The cited clinico-hygienic observations allow one to think that a concentration of methylene chloride of 0.05 mg/litre (50 mg/m^3) meets the current recuirements of the MACs for occupational poisons.

Carbon tetrachloride. There has been a number of reports on the course of acute and subacute poisonings with CCl_4. At the same time, the data on the effect of CCl_4 in minimum concentrations on working people are rather limited. We have only one work by N. E. Golubovsky and K. V. Malysheva (1960), where results of the examination of 23 persons, working in contact with CCl_4, are given. Concentrations of the substance were assessed mainly at the MAC level, and they exceeded this level only in separate cases (by how much, unfortunately, is not known). The length of service of the workers was 1—3 years, 7 persons had worked for over 3 years. Persons of young and middle age were examined, and only 4 persons were 45 years old. Almost all the persons had complaints of headache, poor appetite, bitter taste in the mouth. Pains in the right hypochondria were revealed in 7 persons, i. e., in 1/3 of those examined. The conducted biochemi-

cal investigation showed the following: increased activity of alkaline phosphatase in 15 persons; a decrease of the antitoxic function of the liver in 12; a decrease of the prothrombin index in 9; disturbances of the sugar curve in 14 persons. The bilirubin level in blood appeared to be normal in most of the examined persons.

Sanitary measures had been undertaken at this chemical plant (the CCl_4 concentrations are not given), and a year later repeated examinations of the working people showed that the health condition of the working people improved.

According to the data provided by N. E. Golubovsky and K. V. Malysheva, a concentration of CCl_4 equal to 20 mg/m^3 cannot be considered harmless for the organism, therefore it must be decreased.

It does not seem possible to predict from the results of clinico-hygienic observations by how much the value of the MAC should be decreased, while the available experimental data allow one to decrease it considerably (more than by one order). However, one should take into account that changes in the function of the liver, which mainly determine chronic intoxication with CCl_4, appear to be reversible, according to the clinical observations (A. S. Mukhin, M. A. Potekayeva, 1964). The presented information permits one to lower the MAC for CCl_4 down to 5 mg/m^3.

Chloroform. The maximum allowable concentration for chloroform has not yet been established. On the basis of the comparison of the parameters of its toxicometry under a single exposure with other chloro-derivatives of methane series, and taking into account the similiarity between qualitative manifestations of the effect of chloroform and that of carbon tetrachloride, we think it possible to recommend the MAC for chloroform at the level of 5 mg/m^3.

Considering the results of clinico-hygienic comparisons in general, one should pay attention to the insufficiency of the data on the everyday life farctors, acting on working people. Only an analysis which covers many factors of the formalized primary material can make clinico-hygienic comparisons more significant.

Chapter 7. BENZENE AND ITS MONOHALIDES. EVALUATION OF TOXICITY AND HAZARD

It is advisable to consider the regularities, underlying the characteristics of toxicity and hazard of substances, in homologous or substitutional series. The comparative characteristics of physico-chemical properties of separate representatives of monohalide derivatives of benzene provide the material for preliminary toxicological evaluation.

Compounds of this series have poor watersolubility. When a halogen (from F to I) substitutes hydrogen in the benzene ring, the molecular weight increases, the volatility decreases considerably, while the stability of the carbon-halogen bond (except the carbon-fluorine bond[1], which is more tight than the carbon-hydrogen bond) reduces. Thus, according to volatility, iodobenzene should have been 40 times less hazardous than benzene, if not for the supposed growth of toxicity as a result of the decrease of stability of the halogen-benzene ring bond. Results of experiments prove the latter hypothesis.

Characteristics of the hazard of lethal poisoning

As can be seen from Table 71, in the considered series of substances, halogenation of benzene produces a slight increase of the toxicity of the compounds in comparison with the initial product, though the difference in the degree of toxicity appears insignificant.

When comparing the substances in molar units, the difference between monohalides of benzene and benzene itself becomes more evident. The toxicity of compounds of this series increased in the following sequence: benzene, F-benzene, Cl-benzene, Br-benzene, I-benzene. The degree of toxicity of benzene is 7 times higher than that of iodobenzene.

According to the classification of S. D. Zaugolnikov et al. (1970), all the mentioned compounds (by the DL_{50} value) belong to the same category of moderately toxic compounds (IV-B); according to the classification of Hodge and Sterner (1943), to slightly toxic compounds (4th degree of toxicity); according to

[1] Fluorination of benzene had no significant impact on its toxicity.

Table 71

Physico-chemical properties of benzene and its halides

Substance	Molecular weight	Relative density	Boiling point (t°C)	Vapour pressure, mm of Hg (20° C)	Volatility, mg/l (20° C)	Water solubility (g per 100 ml) (3)° C)	Oil/water partition coefficient	Refractive index	Energy of the carbon-halogen bond rupture (kcal/mole)
C_6H_6	78.12	0.879	80.0	93.2	398	0.08	10.96	$1.50165^{20°}$	102[1]
C_6H_5F	96.11	1.024	84—85	77.8	409	0.154	19.50	$1.46773^{20°}$	115
C_6H_5Cl	112.56	1.106	132.0	8.17	50.3	0.049	50.12	$1.52479^{20°}$	80
C_6H_5Br	157.02	1.495	156.1	3.03	26.1	0.045	77.62	$1.5625^{15°}$	71
C_6H_5I	204.01	1.832	188.6	0.81	9.0	0.034	131.8	$1.6217^{17.8°}$	61

[1] Except the carbon-hydrogen bond.

the classification of the MACs Section, to slightly toxic compounds (class IV).

The lethal concentrations of compounds of this series are given in Tables 72 and 73.

Table 72

Lethal doses of benzene and its monohalides for white mice (administration into the stomach)

Substance	Lethal doses, mg/kg		
	DL_{16}	DL_{50}	DL_{84}
C_6H_6	3500	460±685 (G. G. Avilova, 1970)[1] 8 175 (M. F. Savchenkov, 1959) 10 260 (Smith, Carpenter, 1962)	6200
C_6H_5F	2900	4400 (518÷3300) (I. P. Ulanova, 1971)	6800
C_6H_5Cl	1450	2300 (2898÷1825) (I. P. Ulanova, 1971) 2830÷1445 (Patty, 1965; S. P. Varshavskaya, 1968) 2910÷2399 (Patty, 1965; S. P. Varshavskaya, 1968)	3800
C_6H_5Br	1600	2700 (3537÷2051) (T. A. Shamilov, 1969)	4500
C_6H_5I	1150	1800 (2394÷1353) (T. A. Shamilov, 1969)	3000

[1] Experiments on rats.

Table 73

Lethal concentrations of benzene and its monohalides for mice

Substance	Lethal concentration, mg/litre		
	CL_{16}	CL_{50}	CL_{84}
C_6H_6	32.0	45.0±9.15 34.0 (2 hr) (M. A. Akhmatova, 1967) 51.0 (4 hr) (Carpenter et al., 1949) 66.7 (2 hr) M. F. Savchenkov, 1969) 45.0 (B. A. Kurlyandsky, 1970)	68.0
C_6H_5F	20.2	27.5 (33.5÷22.5)	37.0
C_6H_5Cl	10.2	19.0 (28.5÷12.6) (I. P. Ulanova, 1971) 37÷17 (F. Flury, F. Zernik, 1938) 20÷5 (N. D. Rosenbaum et al., 1947, E. N. Levina, 1950)	35.5
C_6H_5Br	13.0	21.10 (27.5÷16.0) (T. A. Shamilov, 1969)	35.0
C_6H_5I	10.7	17.5 (24.2÷12.7) (I. P. Ulanova, 1971)	29.0

Thus, under inhalation exposure to monohalides of benzene, the degree of their toxicity somewhat increased in comparison with benzene, which cannot be considered significant when toxicity is being expressed in mass units. Although according to the classifications of S. D. Zaugolnikov et al., Hodge and Sterner, the compounds belong to different (boundary) classes of toxicity, this division is conditional. It is proved by almost equal degree of toxicity of the compounds, calculated using the continuous scale of toxicity proposed by I. V. Sanotsky (Table 74).

Table 74

Evaluation of the toxicity degree of benzene and its monohalides (exposure by inhalation)

Authors of classification	Benzene	Fluorobenzene	Chlorobenzene	Bromobenzene	Iodobenzene
S. D. Zaugolnikov et al.	IV—B	IV—A	III—B	IV—A	III—B
Hodge and Sterner	5	4	4	4	4
MACs Section	3	3	3	3	3
I. V. Sanotsky, mg/l	19.5	22.5	25.0	24.0	26
mM/l	15.2	20.0	22.5	23.5	26.0

The difference in the degree of toxicity of the compounds is more significant when toxicity is being expressed in molar units (toxicity increases with the increase of the molecular weight), especially in comparison with the first and the last members of

the series. Benzene appeared to be more toxic than iodobenzene by one order.

Some indices of the hazard from benzene and from its monohalides under administration into the stomach are presented in Table 75. From Table 75 follows that the degree of the hazard of

Table 75

Indices of the hazard of acute lethal poisoning of mice under exposure to benzene and its halides (administration into the stomach)

Index	Benzene	Fluorobenzene	Chlorobenzene	Bromobenzene	Iodobenzene
$\frac{1}{DL_{50}}$	0.217 (0.0169)	0.22 (0.0218)	0.43 (0.0489)	0.37 (0.0582)	0.55 (0.113)
$\frac{DL_{84}}{DL_{16}}$	1.77	2.34	2.62	2.81	2.61
S	1.33	1.53	1.62	1.68	1.62
$\frac{1}{DL_{50} \cdot S}$	0.16 (0.0127)	0.14 (0.0142)	0.27 (0.0302)	0.22 (0.0346)	0.34 (0.0697)

Note: Values of the DL_{50} expressed in μ M/kg are given in brackets.

benzene and its monohalides, according to the variability of lethal doses $\left(\frac{DL_{84}}{DL_{16}} \cdot S\right)$ increases, in general, inversely to the increase of toxicity. The hazard presented by the considered compounds, according to the summation hazard index $\left(\frac{1}{DL_{50} \cdot S}\right)$ differs less significantly (in mass units, by 2 times; in molar units, by 5 times).

As follows from Table 76, the highest potential hazard of lethal poisoning by inhalation (according to the value of the CPPI) is presented by fluorobenzene, which has much higher volatility than the other monohalides of benzene.

The relative hazard degree of the compounds of the considered series under exposure by inhalation appeared to be the same as under administration into the stomach.

Qualitative characteristics of the hazard. A detailed description of acute and chronic intoxication with benzene is given in the monograph by A. M. Rashevskaya and L. A. Zorina (1968). The lethal concentrations of benzene produce narcotic and spasmodic effects. Death is usually caused by the paralysis of the respiratory center.

According to the data by E. N. Levina (1950), Oettingen (1955), N. V. Lazarev (1971), chlorobenzene, under acute exposure, belongs to the group of narcotics which have at the same

Table 76

Some indices of the hazard of acute lethal poisoning of mice under inhalation exposure to benzene and its monohalides

Index	Benzene	Fluorobenzene	Chlorobenzene	Bromobenzene	Iodobenzene
$\frac{1}{CL_{50}}$	0.022 (0.0017)	0.036 (0.0036)	0.0525 (0.00625)	0.0476 (0.00769)	0.057 (0.0125)
$\frac{CL_{84}}{CL_{16}}$	2.12	1.83	3.46	2.70	2.71
S	1.46	1.35	1.85	1.64	1.64
$\frac{1}{CL_{50} \cdot S}$	0.015 (0.0011)	0.019 (0.00266)	0.0284 (0.00338)	0.029 (0.00468)	0.035 (0.00762)
CPPI	8.8		2.65	1.24	0.51

Note: Values of the CL_{50} expressed in μM/litre are given in brackets.

time irritant action. The data on the changes in the morphological composition, according to these authors, are contradicting.

There are many studies of the effect of bromobenzene on the liver (Heim et al., 1956; Severi, Fonnesi, 1956; Rizzoli et al., 1959; Varga et al., 1960). The authors have come to the conclusion that administration of bromobenzene in a dose of 0.5 g/kg causes in rats acute center-lobar necrosis of the liver, which gravity depends on the degree of preliminary albuminous deficiency.

In many cases of the liver lesion it is not clear, whether it is caused by a direct effect of bromobenzene on the liver, or the intoxication develops as a result of the relative deficiency of sulfur-containing aminoacids.

Close interrelation between the cellular necrosis and sulfurous metabolism is not limited by the liver only. Thus, according to Sos, Kemeny, Schnell (1953), in animals, having a diet poor with methionine, regions of necrosis appear in pancreas, kidneys and other organs. There is an opinion that the liver necrosis is a result of the combined effect of bromobenzene and the protein deficiency in liver (Varga et al., 1960).

Thus, the data concerning the character of the effect of monohalides of benzene, depending on the intensity of the exposure, are given only for benzene and chlorobenzene. There are no practically data for F-, Br- and I-derivatives of benzene. We have obtained such data (Table 77).

As follows from the data in Table 77, manifestations of the narcotic effect are typical for benzene and its monohalides at the isoeffective level. The irritant properties at this level were the most manifested under exposure to chlorobenzene. Mean life span of the animals is directly proportional to the degree of ma-

Table 77

Some indices of toxic effect of benzene and its monohalides on mice under a 2-hour exposure (at the CL_{50} level)

Substance	Irritant properties	Appearance of tremor, min	Beginning of falling on one side, min	Beginning of spasmodic twitchings, min	Beginning of narcosis, min	Life span, hr
C_6H_6	—	1	15	30	60	2
C_6H_5F	—	10	30	30	40	2
C_6H_5Cl	Strong	30	60	60	—	24
C_6H_5Br	Weak	30	30	—	—	32
C_6H_5I	Weak	40	120	—	—	72

nifestation of the narcotic effect of the substanses, and is inversely related to the hazard degree of compounds (see summation hazard index expressed in molar units in Table 76). Because of this, benzene and fluorobenzene, although having marked narcotic properties at the lethal level, appear to be the least hazardous members of the series, and iodobenzene — the weakest narcotic — is the most hazardous in view of the development of lethal poisoning. Therefore, in the series of monohalides of benzene, as in the series of chloro-derivatives of methane, the magnitude of the narcotic effect does not correspond to the degree of toxicity of the compounds.

Characteristics of the hazard of acute non-lethal poisoning

According to E. I. Lyublina (1951), the threshold of the effect of benzene, determined by the changes in the time of the flexor reflex in rabbits under a single 40-min inhalation, is 0.3—1.0 mg/litre.

The data on the thresholds of the effect of benzene, obtained by G. G. Avilova (1970) using some integral and pathogenetic indices, are presented in Table 78. As can be seen from Table 78, the minimum concentration of benzene, causing changes in the peripheral blood of rats, is 1.1 mg/litre. This concentration may be considered as the threshold concentration, since leukocytosis exceeded the limits of natural physiological fluctuations. A decrease of rectal temperature and an increase in the excitability of animals were observed under a more intensive exposure (3.1 mg/litre).

Due consideration should be given to the fact that under our standard conditions benzene, used in the mentioned concentration (3.1 mg/l), did not cause leukocytosis, which was registered at

Indices characterizing the state of white rats und a single 4-hour inhalation exposure to benzene

Index	3.1 mg/l		1.10 mg/l		0.5 mg/l		0.10 mg/l	
	test	control	test	control	test	control	test	contr
STI, cond. units	3.5±0.22	4.3±0.21 p<0.5	4.2±0.44	4.7±0.42 p>0.5	4.2±0.31	4.7±0.42 p>0.5	3.8±0.3	4.3± p>0.25
SMA (number of rushes per 15 min)	98±39.8	95±57.6 p>0.5	149±72.6	183±56.6 p>0.5	48±25.1	183±56.6 p>0.05	155±92.8	95± p>0.05
Rectal temperature, °C	37.7±0.125	38.1±0.068 p<0.02	37.8±0.17	38.1±0.2 p>0.25	38.2±0.09	38.1±0.2 p>0.5	38.1±0.09	38.1± p>0.5
Relative mass of liver	2.63±0.08	2.85±0.089 p>0.25	2.78±0.075	2.75±0.07 p>0.5	2.71±0.088	2.75±0.07 p>0.5	2.63±0.33	2.85± p>0.5
Quantity of:								
hemoglobin, mg/l	119±3.8	129±5.2 p>0.25	123±3.9	122±7.7 p>0.5	134±8.9	122±7.7 p>0.5	183±8.5	129± p>0.25
erythrocytes, million in 1µl	6.79±0.17	6.79±0.25 p>0.5	6.24±0.5	6.07±0.46 p>0.5	7.27±0.46	6.07±0.46 p=0.1	6.17±0.62	6.79± p>0.5
leukocytes, thousand in 1µl	12.4±1.22	11.1±0.97 p>0.5	16.5±1.22	12.6±1.08 p<0.05	17.5±2.20	12.6±1.08 p>0.05	11.5±1.15	11.1± p>0.5
reticulocytes, %	22.2±4.12	13.0±1.95 p>0.05	17.5±0.94	23.6±6.34 p>0.5	18.3±1.81	23.6±6.34 p>0.5	22.5±5.72	13.0± p>0.25
thrombocytes, thousand in 1µl	715±199	472±85 p>0.25	512±103	728±202 p>0.5	595±29	728±202 p>0.5	505±124	472± p>0.05
agranolocytes, %	55.3±4.27	62.7±3.91 p=0.25	52.2±4.66	57.3±3.16 p>0.5	58.6±2.46	57.3±3.16 p>0.5	58.3±3.16	62.7± p>0.5

a 3 times lower concentration. Similar conditions are frequent phenomena, which are explained either by differences in the time characteristics of the process, or by the very essence of the reactions (quantitative boundary of instability, when excitation is being replaced by inhibition).

The acute effect thresholds of bromobenzene, according to the complex of integral and pathogenetic indices, are given in Table 79 (the data by T. A. Shamilova, 1969). As follows from Table 79, the threshold of the bromobenzene effect is a concentration

Table 79

Indices characterizing the state of white rats und a single 4-hour exposure to bromobenzene by inhalation

Index	0.02 mg/l		0.25 mg/l	
	test	control	test	control
STI, cond. units	6.7±0.48 p>0.5	6.3±0.50	6.78±0.21 p<0.02	5.8±0.16
Respiration rate, min	115.0±3.0 p>0.5	112.0±5.0	110±3.2 p>0.5	107.5±6.0
Consumption of O_2, ml/100g per 1hr	116±5.8 p>0.5	112±6.6	129±3.4 p>0.25	125±3.4
Quantity of:				
hemoglobin, g/l	15.2±0.2 p>0.5	15±4	144±5 p>0.5	143±5
erythrocytes, million in 1μl	6.16±0.26 p>0.5	6.53±0.39	5.62±0.39 p>0.5	5.72±0.10
reticulocytes, %	17.3±1.2 p>0.5	16.0±0.7	15.0±1.0 p>0.25	14.0±0.6
leukocytes, thousand in 1μl	9.34±0.79 p>0.5	9.74±0.84	9.30±1.04 p>0.25	10.55±0.81
neutrophils, thousand in 1μl	2.5±0.2	2.5±0.3	2.2±0.2	2.1±0.2
lymphocytes, thousand in 1μl	6.3±0.3 p=0.05	7.1±0.2	6.0±0.3 p<0.001	8.1±0.3
thrombocytes, thousand in 1μl	445.3±34.2 p>0.5	439.2±21.3	405.6±23.3 p>0.5	416.0±25.0
Activity (μM/ml/min) of:				
alanine-aminotransferase	0.044±0.015 p>0.5	0.041±0.019	0.039±0.013 p>0.5	0.038±0.017
alkaline phosphatase	0.120±0.021 p>0.5	0.112±0.017	0.117±0.016 p>0.5	0.124±0.013
Common protein of blood serum, g/l	73.8±2.2 p>0.5	75.2±2.0	72.3±2.7 p>0.4	70.7±1.2
Content of SH—groups, μM/100:				
in blood serum	114±5.8 p>0.5	109±4.1	116±2.9 p>0.5	118±6.5
in liver tissue	2.51±0.09 p>0.5	2.42±0.07	2.21±0.06 p>0.5	2.17±0.05

of 0.25 mg/l. Only changes in the functional condition of the nervous system (increase of the STI) and in the morphological composition of the peripheral blood were revealed in this case. A concentration of bromobenzene of 0.02 mg/l appeared to be non-effective.

Using the lethal and threshold values of compounds under single exposures, the indices for evaluating the hazard of acute non-lethal poisoning were calculated (Table 80). One can see from Table 80 that the most manifested biological activity at the Lim_{ac} level in the considered series is that under exposure to bromo- and chlorobenzene; then comes benzene, and the least active appeared to be fluorobenzene.

Table 80

Some indices of the hazard of acute non-lethal poisoning with benzene and its monohalides

Index	Benzene	Fluoroben-zene	Chloroben-zene	Bromoben-zene
Lim_{ac}	1.1	5.0	0.25	0.25
$Z_{ac}=\frac{CL_{50}}{Lim_{ac}}$	40.9	5.5	76.0	84.0
$\frac{Lim_{ac}}{C^{20}}$	0.0027	0.0122	0.005	0.0096
$\frac{C^{20}}{Lim_{ac}}$	361.8	81.8	201.2	104.4

The hazard of acute non-lethal poisoning is, on the contrary, high under exposure to fluorobenzene ($Z_{ac}=5.5$). Then, according to the decrease of the hazard degree, the substances are arranged in the following order: benzene, chlorobenzene, bromobenzene.

Qualitative features of the effect of benzene and its monohalides under a single low level exposure. We would like to present the data which we have obtained for comparing the qualitative peculiarities of the effect of benzene and its monohalides under administration into the stomach in a dose of 1/10 of the DL_{50} value. According to the data from the literature, doses of 1/10, 1/20 DL_{50} are close to the threshold values for this route of administration. When these substances are administered into the stomach, the most marked changes appear during the next 24 hours (Table 81, Fig. 31).

Changes in the leukocytic formula (neutrophilia with relative lymphopenia) have been revealed under exposure to chloro- and bromobenzene; changes in the morphological composition of the peripheral blood in the mentioned experiment with benzene

Table 81

Indices of morphological composition of the peripheral blood of white rats after a single administration into the stomach of benzene and its monohalides in a dose of 1/10 DL_{50}

Index	Benzene	Chlorobenzene	Bromobenzene	Iodobenzene	Control
Hemoglobin, g/l	13.0±1.5 p>0.25	126±5.3 p>0.25	123±3.5 p>0.25	121±1.3 p>0.5	126±4.4
Erythrocytes, million in 1 μl	6.13±0.17 p>0.5	6.32±0.25 p>0.5	6.40±0.19 p>0.25	6.18±0.17 p>0.5	6.10±0.25
Reticulocytes, %	20.0±2.3 p>0.5	20.7±2.7 p>0.5	16.0±2.1 p>0.1	17.2±2.8 p>0.25	20.0±1.8
Leukocytes, thousand in 1 μl	11.25±0.74 p>0.5	10.04±0.67 p=0.5	10.41±0.80 p>0.25	9.72±0.91 p>0.5	9.41±0.59
Thrombocytes, thousand in 1 μl	427.4+25.0 p>0.5	416.0+0.38 p<0.5	398.5+42.7 p>0.25	424.8+33.0 p>0.5	419.6+25.0
Neutrophils, %	28.2±2.6 p>0.5	35.2±2.1 p<0.05	34.0±2.0 p>0.05	30.0±2.2 p=0.25	26.0±2.4
Lymphocytes, %	70.3±2.6 p>0.5	63.3±2.3 p<0.05	64.2±2.8 p<0.05	68.5±2.4 p>0.25	72.5±2.6

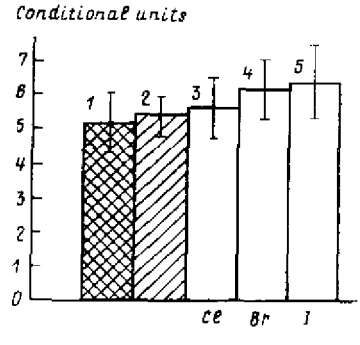

Fig. 31. Summation threshold index (STI) for rats on the next day after administration of benzene and its monohalides into the stomach in a dose of 1/10 of the DL_{50}.
(1) control; (2) benzene; (3—5) benzene derivatives.

and iodobenzene have not been observed. Excitability of the nervous system (according to the STI, see Fig. 31) was found reduced under exposure to bromo- and iodobenzene. A considerably smaller decrease of the excitability has been discovered under exposure to benzene and chlorobenzene.

Characteristics of the hazard of chronic poisoning with benzene and bromobenzene

Early stages of chronic intoxication with benzene are characterized by disturbances in the functional condition of the central nervous system with symptoms of vegetative dystonia (I. S. Gusev, 1967; Horiuchi et al., 1967), as well as by changes in the parenchymatous organs, mainly in liver (L. A. Zorina, 1960; A. M. Monayenkova, K. V. Glotova, 1968). The most important symptom of chronic intoxication is constituted by changes in the initial period of intoxication there appears leukocytosis, followed later by leukopenia, which develops as a rule at the expence of reduction of the quantity of neutrophils (A. P. Rusinova, 1967; S. M. Gerasimov, 1967).

Particularly important is the comparison of the sequence of changes appearing in the organism of animals under exposure to the «standard» product, i. e., to benzene (Table 82)[1]. The expe-

Table 82

The sequence of development of changes in the functional condition of nervous system and peripheral blood under chronic exposure to benzene at concentrations of 0.02—0.05 mg/litre

Index	Observation period, months												
	1	2	3	4	5	6	7	8	9	10	11	12	13
State of the higher nervous activity (method of conditioned reflexes)	0	+	+	+	+	+	+	—	—	—	—	—	—
State of nervous system (method of summation of irritants)	0	+	+	+	+	+	+	+	—	—	—	—	—
Excitation of sciatic nerve (rheobase)	0	—	—	—	+	+	+	+	+	+	+	+	+
State of peripheral blood (content of thrombocytes)	0	0	0	0	0	0	—	—	—	—	—	—	—

Note: (+) increase, (—) decrease, (0) no changes.

riment was conducted on different species of animals: rabbits (to investigate the excitability of the sciatic nerve), cats (conditioned reflexes, blood), rats (conditioned reflexes, summation of irritations). The concentration of benzene was numerically equal to the Lim_{ac} of bromobenzene.

From the data in Table 82 follows that the initial changes were registered in the nervous system. Those changes developed

[1] The data obtained by A. I. Korbakova, S. N. Kremneva, N. K. Kulagina and I. P. Ulanova (1960).

cyclically and gradually proceeded. In the initial period, there was registered an increase of excitability (a decrease of the threshold of sensitivity to electrodermal irritations in rats, changes in the conditioned reflexes in rats with prevailing of the excitation process a decrease of excitability of the sciatic nerve in rabbits). Changes in the periferal blood occurred later than in the nervous system and were characterized by a primary decrease of the quantity of leukocytes followed by a decrease of the quantity of leukocytes with a shift to the left in the leukocytic formula. In some animals there appeared «young» forms of neutrophyls. The indicated changes in the blood, though appearing later, did not undergo the reverse development in the recovery period.

According to the scheme suggested by A. M. Rashevskaya et al. (1968), the first to be affected is the leukoblastic function (leukopenia, relative lymphocytosis in the peripheral blood), then occurs the damage of the megakaryocytic function (thrombocytopenia), and, finally, the damage of the erythroblastic function of the bone-marrow. Not all cases of intoxication, however, develop according to this scheme. For example, isolated damages of different formations in the bone-marrow may prevail.

The data on quantitative and qualitative characteristics of chronic intoxication with chlorobenzene, obtained in animal experiments, are given in Table 83.

Symptoms of lesions of the internal organs, especially of the liver, were revealed in workers subjected for a long time to exposure to chlorobenzene vapours at a concentration on the average one order higher than the MAC. Thus, Y. M. Stanislavsky (1940), N. D. Rosenbaum et al. (1947) found that 25% of the examined persons had enlarged liver, dystrophy of myocardium, a marked tendency to leukopenia with neutropenia and relative lymphocytosis.

To characterize the hazard of chronic poisoning with bromobenzene, T. A. Shamilov (1969) investigated two concentrations: 0.02 ± 0.0054 and 0.003 ± 0.00034 mg/l (Table 84).

Chronic effect of bromobenzene was characterized by polytropic changes in the nervous, hemopoetic systems and in the liver. Primary (one month and a half) changes were revealed in the morphological composition of the peripheral blood (leukocytosis). Changes in the functional condition of the nervous system appeared during the 3rd month of exposure. The STI values and the time of fixation of conditioned reflexes to weak and strong stimulants indicate the prevalence of the inhibitory processes in the central nervous system of animals. It should be emphasized that there simultaneously occurred disturbances in several processes, determining normal functioning of the nervous system, and, in particular, changes in the activity of monoamino-oxidase (T. V. Gnevkovskaya, T. A. Shamilov, 1968).

Table 83

Correlation between the degree of manifestation of the biological effect of chlorobenzene and the concentration under chronic exposure (for not less than 4 months)

Dose	Biological effect	Author
Inhalation 12.0—3.0 (rabbits), mg/l	Insignificant decrease of the hemoglobin quantity	E. N. Levina (1950)
4.6 (guinea pigs), mg/l	CL	Patty (1965)
4.6 (rats, mice), mg/l	Morphological changes in lungs, liver, kidneys	Patty (1965)
3.0—2.5 (rabbits), mg/l	CL	N. D. Rosenbaum et al. (1947)
1.0 (guinea pigs, rats, mice), mg/l	No changes	Patty (1965)
1.0—0.1 (rats), mg/l	Increase in the activity of cholinesterase of the blood, changes in muscular chronaxie	L. P. Tarkhova (1965)
Administration into the stomach 1.0—0.1 (rats), mg/kg	Dystrophic changes in liver, kidneys, mucous membrane of the stomach	P. F. Obukhov, (1955)
0.1—0.01 (rats), mg/kg	Changes in blood, inhibition of the activity of a number of enzymes, dystrophic changes in heart, liver, kidneys	S. P. Varshavskaya (1968)
0.001 mg/kg 0.02 mg/l	MAC (for water bodies)	

Leukocytosis and the tendency to an increase of the quantity of reticulocytes in the initial period of chronic exposure to bromobenzene are, apparently, a result of the stimulating effect of small concentrations on the hemopoetic system. A decrease of the leukocytes quantity in combination with a decrease of the quantity of thrombocytes and reticulocytes, observed later, may be considered as the inhibition of the hemopoetic processes (A. M. Rashevskaya, L. A. Zorina, 1968).

A decrease of the content of common protein in the blood serum indicates disturbances in the protein synthesis, which presents one of the symptoms of the liver dysfunction. Under exposure to bromobenzene, it can also be a result of a direct binding of SH-groups by bromobenzene.

The initial morphological changes, detected in the nervous system 3 months after chronic exposure, revealed themselves in the form of corrugation of cells of the cortex and of subcortial ganglions of the brain. At the same time, there was discovered a feebly manifested catarrhal-desquamative bronchitis. Hyperplasia of reticuloendothelial cells in the liver and around the lungs vessels, attesting to the existence of a protective-compensatory

Table 84

Indices characterizing the state of white rats and rabbits in the dynamics of chronic inhalation exposure to bromobenzene ($p<0.05$)

Indices		Examination period, months						Recovery period (1 month)	
		1.5		3		4.5			
		concentration, mg/l							
		0.02	0.003	0.02	0.003	0.02	0.003	0.02	0.003
Integral indices	Body weight (rabbits)	0	0	0	0	—	0	0	0
	Oxygen consumption (rats)	0	0	0	0	0	0	0	0
	Respiration rate (rats)	0	0	0	0	0	0	0	0
Nervous system	STI (rats)	0	0	+	0	+	0	0	0
	Fixation of conditioned reflex to a strong irritant (rats)	0	0	+	0	+	0	0	0
	Fixation of conditioned reflex to a weak irritant (rats)	0	0	0	0	+	0	0	0
Blood	Erythrocytes quantity (rabbits)	0	0	0	0	0	0	0	0
	Leukocytes quantity (rabbits)	+	0	0	0	—	0	0	0
	Neutrophils quantity (rabbits)	0	0	0	0	+	0	0	0
	Lymphocytes quantity (rabbits)	0	0	0	0	—	0	0	0
	Thrombocytes quantity (rabbits)	0	0	0	0	0	0	0	0
Liver	Activity of alkaline phosphatase (rats)	0	0	0	0	0	0	0	0
	Activity of alanine-aminotransferase (rats)	0	0	0	0	—	0	0	0
	Elimination of bromosulfalein	0	0	0	0	0	0	0	0
	Content of bilirubin and its fractions (direct, indirect) in blood serum (rabbits)	0	0	0	0	0	0	0	0
	Quantity of common protein in blood serum (rats)	0	0	0	0	—	0	0	0
	Content of SH-groups in blood serum (rats)	0	0	0	0	—	0	0	0
	Content of SH-groups in liver homogenate (rats)	0	0	0	0	—	0	0	0
Kidneys	Diuresis, relative density of urine (rats)	0	0	0	0	0	0	0	0
	Chlorides content in urine	0	0	0	0	0	0	0	0
	Morphological changes (rats)	0	0	(+)	0	+	0	0	0

Note: (+) initial morphological changes; + increase; —decrease; 0 no changes.

response, was found. Marked degenerative changes in the nervous system in the form of corrugation of separate groups of neurons in the cortex and in the hypothalamic region were discovered after 4 and a half months of exposure (the end of exposure). According to P. E. Snesarev (1950), the vital activity of the corrugated neurons is reduced to the minimum, which determines the exhibition of a number of physiological and clinical effects.

Indeed, in that period there were revealed a delay in the working out of the positive conditioned reflexes (to the light and the sound of bell) and an increase in the STI. At the same time, some decrease of the lymphoid elements content in the spleen was observed, which coincided with a decrease of the leukocytes quantity in the peripheral blood. There were also discovered a feebly manifested catarrhal bronchitis and slightly marked dystrophic changes in the liver and kidneys. Also revealed marked compensatory proliferative changes in the form of reproduction of reticuloendothelial elements in the liver and of histocytic elements in lungs and kidneys. A month after the end of exposure, the major changes in the animals appeared to undergo the reverse development. There should be, however, mentioned the degenerative changes of the germinal epithelium, occurring in separate seminiferous tubules with the appearence of gigantic cells. We do not have data from investigations of the testis in chronic experiments with benzene. In a replicated 8-day experiment at the Lim_{ac} level, disturbances in spermatogenesis were not revealed (E. M. Tchirkova, 1969). P. P. Dvizhkov and V. I. Fiodorova (1952) found out that subacute exposure to benzene at higher concentrations produces dystrophic changes in the testis and ovaries of the experimental animals.

T. A. Shamilov (1969) considered a bromobenzene concentration of 0.02 mg/l as the threshold concentration under chronic exposure. It should be emphasized that the thresholds of the integral and specific changes under chronic exposure appeared to be equal, which is typical. Therefore, we recommend to determine the selectivity of the poison's effect of this or that organ in acute and subacute experiments. Under chronic exposure to bromobenzene in a concentration of 0.02 mg/l, the periods of beginning of changes in the nervous system and peripheral blood composition of animals had no significant differences.

The indices of the hazard of chronic poisoning with benzene and bromobenzene are given in Table 85. For the other monohalides of benzene these indices have not been calculated because of the absence of the data on the Lim_{ac} and Lim_{ch}.

From the data in Table 85 follows that, though the values of the threshold concentrations of benzene and bromobenzene are equal, the potential hazard of chronic poisoning is one order higher under exposure to benzene, which has higher volatility.

Table 85

Main indices of the hazard of chronic poisoning with benzene and bromobenzene

Substance	Lim_{ch}	$Z_{ch}=\frac{Lim_{ac}}{Lim_{ch}}$	$\frac{Lim_{ch}}{C^{20}}$	$\frac{C^{20}}{Lim_{ch}}$
Benzene	0.02	55.0	0.000050	19 900
Bromobenzene	0.02	12.5	0.00076	1 305

The chronic effect zone of benzene is 4 times wider than that of bromobenzene, which also indicates higher hazard of chronic poisoning with benzene.

Some aspects of toxicodynamics and toxicokinetics. Selection of metabolic criteria of harmfulness

Similiarity of changes occurring under exposure to benzene and its chloro-, bromo- and iodo-derivatives is, apparently, to a great extent determined by qualitatively similar transformation of these substances in the organism. Irrespective of the way in which benzene enters the organism, from 16 to 42% of it is exhaled unchanged (Y. S. Teisinger et al., 1959; Parke, Williams, 1953); a very little amount is excreted with urine (0.1—0.2% of the resorbed dose).

Excretion of the main metabolites with urine, according to the same authors, is (in relation to the dose which entered the organism): from 9.7 to 42.4% for phenol; from 0 to 5.4% for pyrocatechol; from 0 to 3.3% for hydroquinone.

Benzene is deposited mainly in the adipose tissue (E. S. Mironos, 1969; I. D. Gadaskina, 1970), and in the bone-marrow (Duvoir et al., 1946).

A considerable amount of benzene in the organism is subjected to oxidation according to the scheme: benzene-phenol-pyrocatechnol and hydroquinone-oxihydroquinone. Oxidation of benzene occurs mostly in the bone-marrow and, to a much smaller extent, in liver. The largest part of phenol and polyphenol is eliminated in the form of conjugated compounds — combined with sulfuric and glucuronic acids (Walkey, Elkins, 1969; Dutkiewicz, 1965) — or may be bound by cystein and form mercapturic acid (Srbova et al., 1950).

Some authors explain the toxicity of benzene exactly by the effect of its metabolites on the organism (Fabre, 1954; Sato et

al., 1967; I. D. Gadaskina, J. I. Abramova, 1967); others relate it to the effect of the molecule of benzene itself, although not denying the role of its metabolites (N. V. Revnova, 1963).

Halides of benzene undergo in the organism similar metabolic transformations (exhalation in the unchanged form, oxidation, synthetic reactions with sulfur-containing aminoacids, in which the corresponding p-fluoro-, p-chloro-, p-bromo-, p-iodomercapturic acids are formed) (Young, Zbarsky, 1944; Stekol, 1947; Azonz et al., 1952). Under subcutaneous administration of monohalides in one dose (0.5 g/kg), 74% of fluorobenzene, 27% of chlorobenzene, 6% of bromobenzene and 3% of iodobenzene is exhaled unchanged. In this case, the quantity of the secreted mercapturic acid for the last three members of the series is almost the same (25%), while under exposure to fluorobenzene 1—2% of mercapturic acid is formed (Azour et al., 1952).

We assume that exactly the ability of fluorobenzene to be excreted from the organism unchanged explains the qualitative peculiarities of its biological effect and the lowest toxicity among the investigated monohalides of benzene.

Unfortunately, the presented data on the metabolism of monohalides of benzene have been obtained from model experiments, when the substances were being administered under the skin or into the stomach of animals. Therefore, using the example of bromobenzene, we have studied the elimination of its metabolites (p-bromophenylmercapturic acid)[1], as well as the excretion of glucuronic acid with urine under exposure to the compound by inhalation at different levels (CL_o, Lim_{ac}, Lim_{ch}). The results of these investigations are shown in Table 86 and in Fig. 32.

Table 86

Amount of bromophenylmercapturic acid (mg/24 hr) in a 24-hr portion of urine of white rats after a single 4-hr exposure to bromobenzene

Concentration of bromobenzene, mg/litre	Control	Test
16.5	4.1±0.9	16.3±3.0 $p<0.01$
0.25	5.2±1.0	16.7+2.0 $p<0.001$

Special attention should be paid to the increase of the quantity of metabolite in urine under the increase of the period of chronic exposure to bromobenzene in a concentration of 0.02 mg/l (Lim_{ch}) and to the maintenance of a constant quantity of meta-

[1] Determined by the titration (by iodine) method.

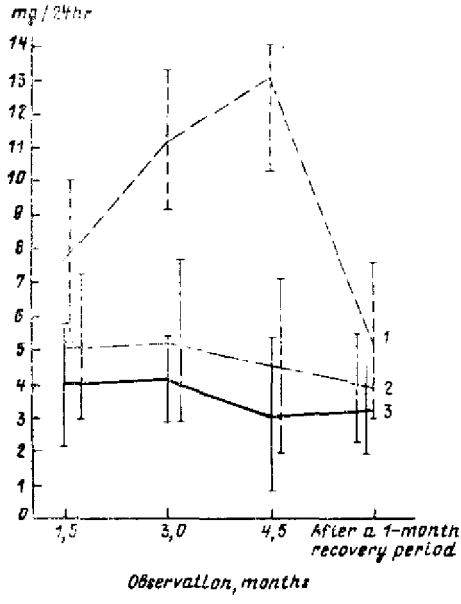

Fig. 32. Quantity of p-bromophenylmercapturic acid under chronic inhalation exposure to bromobenzene in different concentrations.

(1) 0.02 mg/kl; (2) 0.003 mg/l; (3) control.

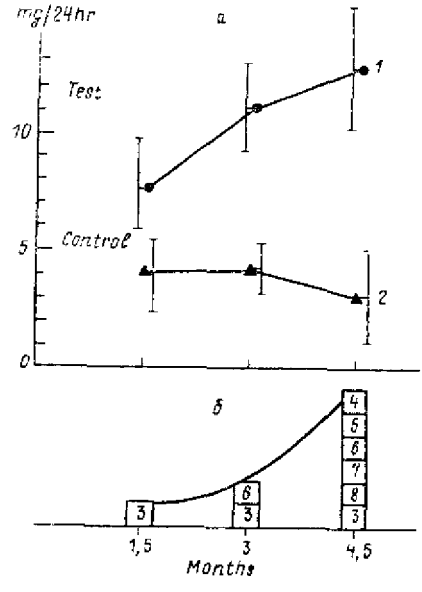

Fig. 33. Content of bromophenylmercapturic acid: (a) in the urine of rats; (b) indices changes under chronic exposure to bromobenzene.

(1) test; (2) control; (3) quantity of leukocytes; (4) SH-groups; (5) body weight, g; (6) STI; (7) quantity of reticulocytes; (8) quantity of thrombocytes.

bolite, slightly exceeding the control level under exposure to bromobenzene in a concentration of 0.003 mg/l (non-effective concentration, according to the indices under investigation). In our opinion, this difference may serve as the criterion of harmfulness (or harmlessness) of a poison in the corresponding concentration.

In order to prove the criterion of harmfulness of bromobenzene in a concentration of 0.02 mg/l, it seems important to compare the content of metabolite and the magnitude of changes (Fig. 33). When studing the content of p-bromophenylmercapturic acid in urine (the upper part of Fig. 33), one can see that after one month and a half of exposure the quantity of metabolite increased; by the 3rd month of exposure it increased by 46% in comparison with the level of one month and a half. In 4.5 months, the quantity of p-bromphenylmercapturic acid changed little in comparison with the previous period of investigation (by 16% only). Such an insignificant increase of the quantity of metabolite, under a sharp increase of the degree of intoxication of the organism, may, in our opinion, indicate overstress and exhaustion of this mechanism of detoxication.

When investigating the content of glucuronic acid in urine (in the same 24-hour test portion that was used for evaluating the p-bromophenylmercapturic acid content), there was found a significant increase of its concentration under the mentioned exposures; there was also revealed a correspondence between the degrees of the increase of the content of glucuronic and mercapturic acids. Taking into consideration the existence of a certain correspondence between the level of p-bromophenylmercapturic acid content (specific metabolite) in urine and the concentration of bromobenzene in the air, it is possible to recommend that special investigations be conducted for working out the exposure test according to the revealed metabolite.

Reactive capacity of monohalides derivatives of benzene

The metabolism of different compounds depends, to a large extent, on their physico-chemical properties and, first of all, on their reactive capacity.

Though no significant differences in the degree of toxicity of monohalides of benzene have been revealed, the toxicity appeared to increase gradually with the increase of the molecular weight. Under inhalation exposure (μM/L) fluorobenzene appeared 2 times and iodobenzene 7 times as toxic as benzene (see Table 76). This fact is, to a certain degree, explained by the instability of the carbon-halogen bonds in the molecules of these compounds.

A decrease of the energy of rupture of the carbon—halogen bonds in the molecules of monohalides of benzene corresponds to the increase of toxicity (according to the CL_{50} and DL_{50}) of the compounds (Fig. 34).

The investigation of the reactive capacity of separate monohalides of benzene by the method of electronic paramagnetic resonance (EPR) confirms the discovered regularity. As it is known, the EPR method is based on the resonance absorption of the energy of electromagnetic irradiation in constant field. The absorption of energy is proportional to the concentration of free radicals in the system.

The concentrations of the investigated substances are 10 times higher than the concentration of the stable radical (2,2:6,6-p-methyl-γ-pipiredol nitric oxide), therefore the rate of the radical destruction can be determined only by the reactive capacity of the substance. The method of EPR made it possible to obtain data demonstrating that the fastest destruction of the stable radical, as compared with C_6H_5Br and C_6H_5Cl, occurs in the C_6H_5I solution, i. e., the reactive capacity of C_6H_5I is higher than that of C_6H_5Br and of C_6H_5Cl in particular, which corresponds to the degree of toxicity or of the biological activity of the compounds (Fig. 35).

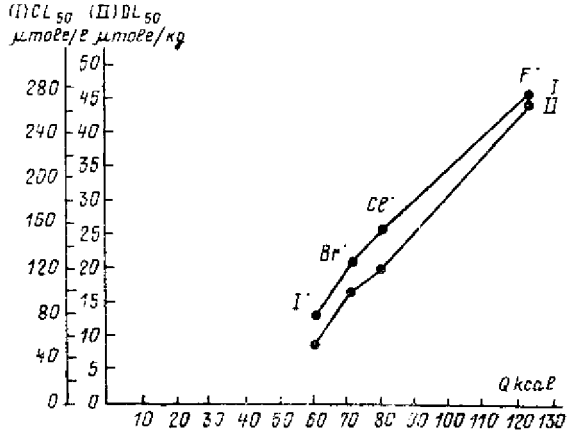

Fig. 34. Correlation between the energy of rupture of the carbon-halogen bond in molecules of monohalides of benzene and their toxicity according to the values of CL_{50} (I), DL_{50} (II).

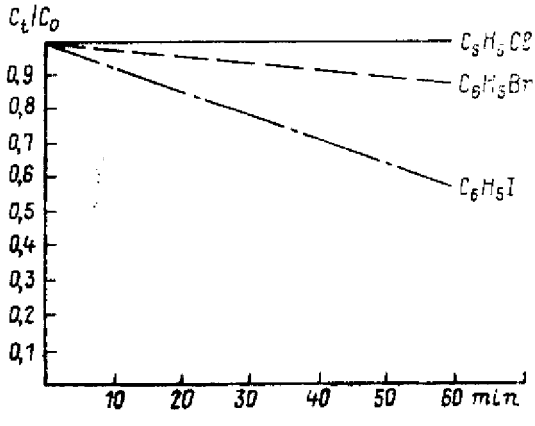

Fig. 35. Destruction of the RNO free radical in solutions of monohalides of benzene.

Conclusions. Values of the indices of the degree of toxicity and hazard of benzene and its monohalides are summarized in Table 87. The results obtained indicate that the introduction of the fluorine ion into the molecule of benzene produces almost no changes in toxicity, while the introduction of the ions of chlorine, bromine and iodine increases the toxicity and hazard of compounds, and in this case the difference is more marked at high levels of exposure and more feeble at the threshold levels. There was also demonstrated the correlation between the biological activity of monohalides of benzene and their reactive ability.

Table 87

Major parameters of toxicometry and hazard indices of benzene and its monohalides

Indices	Benzene	Fluorobenzene	Chlorobenzene	Bromobenzene	Iodobenzene
Lethal exposure					
DL_{50}, mg/kg	4 600	4 400	2300	2700	1800
Hazard class according to DL_{50}	IV	IV	IV	IV	IV
S	1.33	1.53	1.62	1.68	1.62
$\frac{1}{DL_{50} \cdot S}$	0.16	0.14	0.27	0.22	0.34
CL_{50}, mg/l	45.0	27.5	19.0	21.0	17.5
Hazard class according to CL_{50}	III	III	III	III	III
S	1.46	1.35	1.85	1.64	1.64
$\frac{1}{CL_{50} \cdot S}$	0.015	0.019	0.028	0.029	0.035
$CPPI_{CL}$	8.8	14.9	2.6	1.2	0.5
Hazard class according to $CPPI_{CL}$	III	III	IV	IV	IV
A single non-lethal exposure					
Lim_{ac}, mg/l	1.1	5.0	0.25	0.25	—
Hazard class according to Lim_{ac}	IV	IV	III	III	—
$CPPI_{CL}$	361.8	81.8	201.2	104.4	—
Hazard class according to $CPPI_{ac}$	III	IV	III	III	—
Z_{ac}	40.9	5.5	76.0	84.0	—
Hazard class according to Z_{ac}	III	I	IV	IV	—
Chronic exposure					
Lim_{ch}, mg/l	0.02	—	—	0.02	—
Hazard class according to Lim_{ch}	III	—	—	III	—
$CPPI_{ch}$	19 000	—	—	1305	—
Hazard class according to $CPPI_{ch}$	III	—	—	III	—
Z_{ch}	55.0	—	—	12.5	—
Hazard class according to Z_{ch}	I	—	—	I	—
I_{cum}	6.87	—	2.8	3.6	2.3
MAC, mg/m³	5	5	3	3	3

The potential hazard posed by benzene and its monohalides under a single lethal and a single non-lethal ($CPPI_{CL}$) poisoning corresponds to common hazard classes III and IV (according to the classification of the MACs Section). In case of chronic exposure, the potential hazard of poisoning with monohalides of benzene increases sharply. Contrary to expectations, the toxicity and the biological activity of the investigated compounds are equal according to the values of CL_{50}, Lim_{ac} and Lim_{ch}.

According to the magnitude of the acute effect zone, benzene and its monohalides are to be assigned to moderately or slightly hazardous compounds in terms of the development of acute poisoning. The hazard of chronic poisoning with benzene and bromobenzene is, judging from the experimental data, extremely high, which corresponds to the degree of hazard of chronic poisoning based on clinico-statistical data and hygienic observations. These phenomena are, undoubtedly, underlied by high cumulative ability of the investigated compounds.

The qualitative characteristics of the poisons were found to vary, to a certain extent, depending on the quantitative level of exposure. Thus, symptoms of the nervous system lesion prevailed in case of lethal poisoning. At the level of the acute effect threshold of benzene, first there was observed the reaction of blood in the form of leukocytosis, which is probably a result of a primary stimulating effect of benzene on the hemopoetic system. Under exposure to monohalides at this level, there were registered responses of both the blood and the nervous system. Chronic intoxication with benzene and bromobenzene was also accompanied by changes in the functional condition of the nervous system and in the composition of the peripheral blood.

The qualitative and quantitative indices of the hazard posed by the compounds under investigation have been taken into consideration in substantiating the values of their MACs for the air of workplaces. Thus, prior to 1968, the USSR legislation in force gave the MAC for benzene equal to 20 mg/m³. The results we have obtained, the data provided by B. A. Kurlyandsky (1970) and clinico-hygienic observations have made it necessary to revise the MAC for benzene.

A. M. Rashevskaya and L. A. Zorina (1968) cited cases of marked chronic intoxication with benzene with typical changes in blood, found in persons working under concentrations of benzene in the air of workplaces at a level of approximately 20 mg/m³, this level being periodically increased by up to times. V. A. Doskin (1969) has been observing the health conditions of workers from the first days of their contact with benzene. During the first year of work, in 1/3 of samples of the air the MAC (20 mg/m³) was not exeeded, but in 2/3 of all the samples of the air the MAC was found to be exeeded by 2—3 times. After 6—10 months of work under these conditions, 25 workers out of 300 had changes in

the blood typical for benzene intoxication. During the 2nd year of work under these occupational conditions, concentrations of benzene in 2/3 of all the samples appeared to be below 20 mg/m^3, and only in 2% of all the samples this level was exeeded by 4 times. However, even under such conditions, several cases of poisoning with benzene were revelead. At shoe-making factories, where benzene had been used before (after implementation of sanitary measures, the concentrations of benzene did not exeed the MAC and usually were at the level of 2—10 mg/m^3), single cases of negative effect of benzene on the organism were still encountered (O. N. Olimpieva, 1958; Z. A. Volkova, 1969). A. P. Volkova observed a decrease of the phagocytic activity of leukocytes and an increase of general morbidity among the workers engaged in synthetic leather production, exposed to benzene concentrations of 2—20 mg/m^3.

On the basis of the cited data the MAC for benzene was lowered down to 5 mg/m^3.

Attention should be paid to the fact that, according to the norms in force in FRG in 1972, benzene was assigned to substances which present relably substantiated carcinogenic hazard for man.

As to the other monohalides of benzene, their MACs have not been established. We consider it possible to recommend the MAC for fluorobenzene (which is close to benzene, according to toxicometric parameters and the type of action) equal to 5 mg/m^3, and for chloro- and iodobenzene (by the analogy with bromobenzene) equal to 3 mg/m^3.

Thus, using the evaluation of toxicity and hazard of monohalides of benzene as an example, it has been shown that together with deviations of the indices from the general physiological norm, account being taken of the instability of these deviations, and evaluation of latent changes (the above-mentioned differences between adaptation and compensation), some metabolic indices (concentration of metabolite in biomedia, its dynamics, etc.) may serve as criteria of harmfulness. These criteria are additional indices serving for determining more precise thresholds of harmful effect of substances, on which methods for evaluating the degree of hazard presented by chemical compounds, used in hygiene and toxicology, are based.

Chapter 8. TOLUENE AND ITS CHLORINATED, FLUORINATED DERIVATIVES. DERIVATIVES OF FLUOROTOLUENE. EVALUATION OF TOXICITY AND HAZARD

Halogenated derivatives of toluene, nitro- and amino-derivatives of fluorotoluene are used in industry as polymerization catalysts in the production of rubber, plastic materials, as raw materials for organic synthesis (in production of dye-stuffs, fragrant substances, synthetic resins, tanning agents, pharmacologic preparations), etc.

These compounds may serve as an example of dependency of the biological properties on the replacement of the hydrogen atoms in the side chain by halogens; the same regularities have been revealed using the model of benzotrifluoride under its nitration and amination. Physico-chemical properties of the compounds of the series are given in Table 88. All chloro-derivatives of toluene are liquids. The larger the number of chlorine atoms in the molecule, the higher the relative density and the boiling point, the smaller the refractive index, the vapour pressure and volatility. Special attention should be paid to a sharp increase of the energy of rupture of the carbon-chlorine bond in the side chain of the molecule of benzene chloride, as compared

Table 88

Physico-chemical properties of toluene, its halides, nitro- and amino-derivatives of fluorotoluene

Substance	Molecular weight	Relative density	Boiling point, °C	Vapour pressure (20°C), mm of Hg	Volatility (20° C), mg/l	Refractive index	Bonds rupture, energy, kcal/mole
Toluene	92.14	1.8669	110.8	19.05	96.02	1.4969	83.0
Benzyl chloride	126.58	1.0994	179.4	1.0	6.9	1.5391	60.4
Benzal chloride	161.97	1.2557	205.8	0.3	2.7	1.5515	—
Benzotrichloride	195.5	1.3723	220.8	0.22	2.35	1.6012	72.0
Benzotrifluoride	146.11	1.1887	102.5	27.54	220.13	1.4114	114.0
m-Aminobenzotrifluoride	161.13	1.3047	187.5	0.93	8.20	1.4788	—
m-Nitrobenzotrifluoride	191.11	1.4318	201.5	0.54	5.65	1.4714	—

to the energy of rupture of the carbon-hydrogen bond in the molecule of toluene.

Introduction of the fluorine atoms into the side chain of the toluene molecule increases its relative density, the vapour pressure and volatility, but decreases the boiling point. Substitution of hydrogen in the ring of benzotrifluoride (BTF) with nitro- and amino-groups results in an increase of the relative density and boiling point of compounds, while the vapour pressure and volatility decrease. m-Aminobenzotrifluoride (m-ABTF) and m-nitrobenzotrifluoride (m-NBTF) appeared to be very close according to the cited parameters; the only exception is a slightly higher boiling point of m-NBTF, which determines its smaller volatility, and thereby its smaller hazard under real occupational conditions.

Characteristics of the hazard of lethal poisoning under exposure to halogenated derivatives of toluene

Values of the lethal doses and concentrations of toluene and its halogenated derivatives are presented in Table 89. The toxicity of chloro-derivatives of toluene varies considerably depending on two routes of administration: under administration into the stomach, they are slightly toxic (the DL_{50} is 1,300—1,500 mg/kg, T. V. Mikhailova, 1965); under administration by inhalation, they are highly or extremely toxic. Benzotrifluoride has been found slightly toxic under both routes of administration.

The toxicity and hazard of chloro-derivatives of toluene under acute lethal exposure have been assessed mainly in experiments with administration of the poison by inhalation.

We have registered an increase of toxicity of the substances from benzene chloride to benzotrichloride, i. e., under consecutive increase of the number of chlorine atoms in the side chain of the toluene molecule. In comparison with toluene, the toxicity of its chloro-derivatives has been found increased by over 2 orders. Introduction of the fluorine atoms into the molecule of toluene, on the contrary, decreased its toxicity. This dependency was especially distinct when the evaluation was made according to the continuous scale of toxicity, proposed by I. V. Sanotsky (1967). According to other classifications, all derivatives of toluene containing chlorine belong to either extremely or highly toxic substances (classes I—II). Toluene and benzotrifluoride belong to classes III—IV (Table 90).

Table 91 gives the hazard indices of lethal poisoning with halogenated derivatives of toluene.

Table 89

Lethal concentrations and doses of toluene and its halides under a single exposure

Substance	Effect	Dose, mg/kg (administration into the stomach)	Concentration, mg/l (inhalation)	Authors
Toluene	CL_{50}	—	30.0—35.0 (mice)	Spector, 1956
	CL_{50}	—	32.0±7.3 (mice)	I. P. Ulanova, 1971
	CL_{84}	—	50	
	CL_{16}		21.5	
	DL_{50}	7000 (rats)	—	Wolf et al., 1956
Benzyl chloride	CL_{50}	—	0.39	T. V. Mikhailova 1965
	DL_{50}	1500 (mice)	(0.58÷0.26) (mice)	
	CL_{84}	—	0.62	
	CL_{16}	—	0.23	
	CL_{100}	—	2.0 (cats)	F. Flury, F. Zernik, 1938
Benzal chloride	CL_{50}	—	0.21	T. V. Mikhailova, 1965
	DL_{50}	1400 (mice)	(0.45÷0.10) (mice)	
	CL_{84}	—	0.55	
	CL_{16}	—	0.08	
	CL_{100}		2.0 (dogs)	F. Flury, F. Zernik, 1938
Benzotrichloride	DL	11 000[1] (frogs)	—	Lehmann, 1928
	CL		1.0 (rats)	O. G. Arkhipova et al., 1968
	CL_{50}	—	0.06	T. V. Mikhailova, 1965
	DL_{50}	1300 (mice)	(0.09÷0.04) (mice)	
	CL_{84}	—	0.12	
	CL_{16}	—	0.03	
Benzotrifluoride	DL	6000[1] (frogs)		Lehmann, 1928
	CL_{50}	—	100 (mice)	B. D. Karpov, 1967
	CL_{50}	—	92.2	
	DL_{50}	10 000 (14 000÷ ÷7143) mice	(122.7÷ ÷69.4) (mice)	A. I. Halepo, 1969
	CL_{84}	—	149.2	
	CL_{16}	—	62.4	

[1] Administration into lymphatic duct.

Table 90

Evaluation of the toxicity degree of toluene and its chloro- and fluoro-derivatives (inhalation)

Authors of classifications	Toluene	Benzyl chloride	Benzal chloride	Benzotrichloride	Benzotrifluoride
S. D. Zaugolnikov et al.	IV—A	I	I	I	V
Hodge and Sterner	IV	II	II	I	V
MACs Section	III	I	I	I	IV
I. V. Sanotsky					
mg/l	21.5	50	53.5	61	15.0
μ mole/l	18	43.5	48.5	57	14.0

Table 91

Indices of the hazard of acute lethal poisoning with toluene and its halides in mice (inhalation)

Index	Toluene	Benzyl chloride	Benzal chloride	Benzotrichloride	Benzotrifluoride
$\frac{1}{CL_{50}}$	0.03 (0.0028)	2.56 (0.32)	4.76 (0.77)	16.6 (3.3)	0.01 (0.0015)
$\frac{CL_{84}}{CL_{16}}$	2.32	2.69	6.87	4.0	2.39
S	1.52	1.64	2.62	2.0	1.55
$\frac{1}{CL_{50} \cdot S}$	0.01 (0.0018)	1.56 (0.195)	1.82 (0.294)	8.3 (1.65)	0.0064 (0.0009)
CPPI	3.0	17.7	12.9	39.2	2.38

Note: The CL_{40} values expressed in mM/L are given in brackets.

Judging from the variability of lethal concentrations, the most hazardous compound is toluene, then in decreasing order follow benzotrifluoride, benzene chloride, and the least hazardous is benzal chloride. However, when the hazard is evaluated by the summation index $\frac{1}{CL_{50} \cdot S}$, its degree corresponds to toxicity. The most hazardous compound at this level of exposure is benzotrichloride, the least hazardous is benzotrifluoride. In view of the effective toxicity (according to the CPPI), the highest hazard is presented by benzotrichloride and benzyl chloride; the smallest hazard by benzotrifluoride.

Qualitative characteristics of the hazard. Under acute poisoning with toluene, its narcotic effect is especially marked (L. K. Hotsyanov, 1941; V. A. Pokrovsky, 1956), and morpholo-

gical composition of the blood undergoes changes (A. S. Faustov, 1967, et al.).

Chlorinated derivatives of toluene have irritant properties, which are particularly marked in benzyl chloride and benzal chloride. These poisons were used during the World War I as war tear gases (K. Los, 1963; A. I. Cherkes et al., 1964). All chloroderivatives of toluene affect nervous system and parenchymatous organs (N. V. Lazarev, 1938; Elkins, 1954; O. G. Arkhipova et al., 1963; V. V. Stankevich, V. I. Osetrov, 1963).

According to I. P. Ulanova et al. (1968), there was a distinct difference between the qualitative characteristics of the effect of chlorinated and fluorinated derivatives of toluene, whereas the clinical picture of acute poisoning with benzyl chloride, benzal chloride and benzotrichloride had much in common. Soon after inhalation exposure to chlorinated compounds of toluene mice, rats, rabbits exhibited symptoms of general excitation, of acute irritation of the upper respiratory tract. The state of excitation was replaced in animals by inhibition; sometimes rats and mice were falling on one side. Symptoms of motor automatism were found in mice, and spasmodic muscle twitchings were observed in rats. Mucosanguineous liquid oozed out from the nose, the cornea grew turbid. Death of animals poisoned with benzyl chloride and benzal chloride was usually observed during the exposure or on the 1st or 2nd day after the experiment. Cases when the animals died later were seldom registered. Under exposure to benzotrichloride, after 1—3 weeks the animals were becoming languid, were quickly loosing their body weight and finally dying.

Under acute poisoning with benzyl chloride and benzal chloride, there were registered more manifested symptoms of irritation of the respiratory tract of the animals, and under exposure to benzotrichloride the first affected were the nervous system, liver and kidneys.

Morphological investigations of the organs of animals which died during the exposure or on the 1st or 2nd day after the exposure to benzyl chloride, benzal chloride and benzotrichloride revealed perivascular edema in both the internal organs and the brain. Under exposure to benzyl chloride, perivascular edema was particularly marked in the pulmonary tissue, but phenomena typical for toxic edema of lungs (alveolar edema) were not registered. There were also revealed symptoms of irritation of the respiratory tract (from the nose mucous membrane to the mucous membrane of bronchi) in the form of fibrinoid layers, desquamation of the epithelium, edema and hemorrhages in the submucous membrane. The exposure to benzotrichloride produced sharp vascular changes and numerous hemorrhages in the brain. When the animals lived longer after the exposure to benzyl chloride, these phenomena were aggravated by pusnecrotic bronchitis, desqua-

mation of the mucous membrane with extensive leukocytic infiltrates in the submucous membrane, destruction of the latter and the opening of the muscular layer.

Under exposure to benzyl chloride and benzal chloride, a feebly manifested albuminous dystrophy of the liver cells and of the epithelium of the convoluted tubuls of kidneys were found in animals which died later. Under exposure to benzotrichloride, at the same periods the dystrophic changes in liver and kidneys were more marked (up to the fatty dystrophy of cells of the liver parenchyma and necrosis of the convoluted tubuls of kidneys).

The clinical picture of acute poisoning with benzotrifluoride was characterized by the absence of phenomena of marked irritation, which were typical for the exposure to the above-mentioned compounds. Immediately after the exposure, there appeared certain motor excitation, followed by languidness, disturbances in the coordination of movements and falling on one side. As a rule, the animals died on the first day after the exposure, and, in some rare cases, on the 2nd or 3rd day.

Microscopic investigation of the organs of animals which died at early periods after the exposure to fluorinated compounds of toluene (benzotrifluoride), or were killed at that period revealed very clear vascular disturbances in alli internal organs and in the brain (plethora, statis, perivascular edema and hemorrages). Dystrophic changes in separate neurones in the form of karyocytolysis with formation of cells-shadows, symptoms of neurophagia and vacuolization of protoplasm were observed in the cerebral cortex, in subcortical ganglion and in the thalamo-hypothalamic region (Fig. 36).

Despite the absence of manifest symptoms of irritation of the respiratory organs, the morphological investigation revealed plethora of the mucous membrane of trachea and pulmonary tissue with phenomena of perivascular edema, small local nidi of the edema of pulmonary tissue and traces of hemorrhages in the alveoli. With the increase of the life span those changes became less manifest, however, there simulteneously developed hyperplasia of lymphoid element around vessels, accompanied by intumescence of interalveolar septa. Albuminous and small-drops fatty dystrophy of separate groups of hepatocytes was observed in the liver. Albuminous dystrophy of the epithelium of some groups of convoluted tubules was found in kidneys. With the increase of the life span of the animals dystrophic processes in the liver slowered down; there appeared more than in the norm multinuclear hepatic cells, reproduction of reticuloendothelial elements proceeded up to the formation of histocytic nodes.

Thus, the substitution of hydrogen with chlorine in methyl group of toluene sharply increased the toxicity and hazard of compounds, attributing them irritant properties. Introduction of fluo-

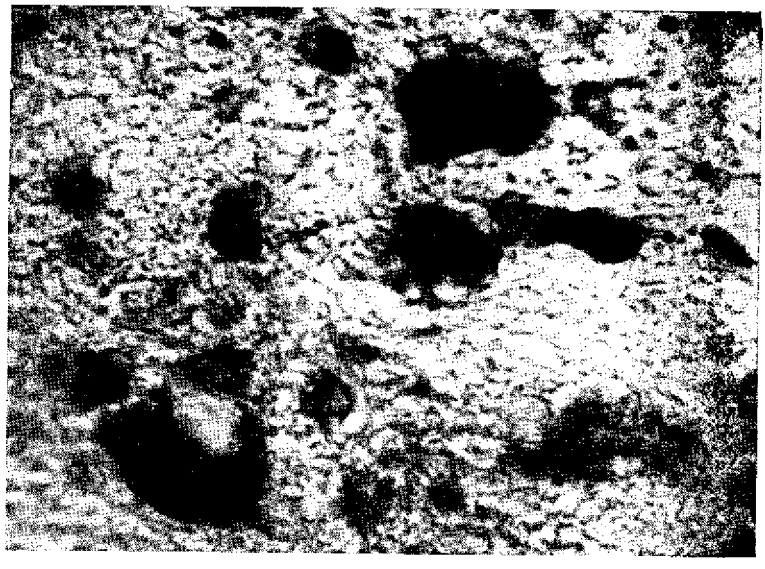

Fig. 36. Vacuolization of the nerve cells of the cerebral cortex.
Coloration by the method of Nissle, x900 (BTF, inhalation, the rat was killed on the 3rd day).

rine atom into the molecule of toluene slightly increases the toxicity of compounds, without changing the narcotic character of their effect.

Characteristics of the hazard of lethal poisoning under exposure to amino- and nitro-derivatives of fluorinated toluene

Values of the lethal doses and concentrations of amino- and nitro-derivatives of fluorinated toluene are given in Table 92. For comparison, the toxicity indices of BTF are also indicated. According to the values of median lethal doses, m-ABTF and m-NBTF, which have close toxicity, exceed the toxicity of BTF by dozens of times. Under inhalation exposure, the difference between the toxicity of BTF and of its amino- and nitro-derivatives is even more evident than under administration into the stomach (BTF is 100 times less toxic).

The continuous classification of I. V. Sanotsky (1967) reflects this dependency of the toxicity degree. According to other classifications, BTF is slightly toxic, but m-ABTF and m-NBTF are extremely or highly toxic. According to the classification of Hodge and Sterner, m-NBTF belongs to class III, i. e., to moderately toxic compounds (Table 93).

Table 92

Lethal doses (mg/kg) and concentrations (mg/l) of benzotrifluoride (BTF), aminobenzotrifluoride (m-ABTF) and nitrobenzotrifluoride (m-NBTF) for mice

Index	BTF	m-ABTF	m-NBTF
DL_{50}	10 000 (14 000÷7 143)	220 (323÷150)	520 (718÷377)
DL_{84}	16 750	405	870
DL_{16}	5 800	120	315
CL_{50}	92.24 (122.7÷69.4)	0.69 (0.81÷0.59)	0.88 (1.01÷0.77)
CL_{84}	149.20	0.88	1.10
CL_{16}	62.40	0.54	0.70

Table 93

Toxicity classes for BTF, m-ABTF and m-NBTF under inhalation exposure

Authors of classifications	BTF	m—ABTF	m—NBTF
S.D. Zaugolnikov et al.	IV	I	I
Hodge and Sterner	V	II	III
MACs Section	IV	II	II
I. V. Sanotsky			
mg/l	15.5	46.0	43.0
μ mole/l	15.0	42.0	41.0

Results of the calculation of the indices of the hazard of acute lethal poisoning are presented in Table 94. Thus, introduction of the amino-group into the molecule makes the hazard slightly higher in comparison with introduction of a nitro-group. In this case, the characteristics of the slope of the curve of lethal doses and concentrations for both compounds are virtually the same (administration of the poison into the stomach, judging from this index, is less hazardous than administration by inhalation).

Qualitative characteristics of the hazard from amino- and nitro-derivatives of fluorotoluene. It is known that introduction of amino (NH_2)- and nitro (NO_2)-groups into the molecule of toluene leads to the formation of substances having specific methemoglobin-forming and hemolysing effect.

M. S. Zakabunina (1954) has, however, shown that at low levels of exposure changes in the functional condition of the nervous system result from the direct effect of m-amino-toluene, and

Table 94

Some indices of the hazard of actute lethal poisoning of mice under administration of compounds into the stomach or by inhalation

Index	BTF	m-ABTF	m-NBTE
$\frac{I}{DL_{50}}$	0.0001 (0.0146)	0.00454 (0.73)	0.00192 (0.37)
$\frac{DL_{84}}{DL_{16}}$	2.88	3.33	2.76
S	1.69	1.83	1.66
$\frac{I}{DL_{50} \cdot S}$	0.059 (0.0086)	2.48 (0.398)	1.16 (0.22)
$\frac{I}{CL_{50}}$	0.0108 (0.00158)	1.449 (0.23)	1.136 (0.22)
$\frac{CL_{84}}{CL_{16}}$	2.39	1.63	1.57
S	1.55	1.27	1.25
$\frac{I}{CL_{50} \cdot S}$	0.0064 (0.009)	1.14 (0.180)	0.908 (0.176)
CPPI	2.38	11.88	6.42

Note: The DL_{50} and CL_{50} values expressed in mM/kg are given brackets.

are not determined by hypoxia. The urinary organs are being affected somewhat later, which is manifested in uremia, appearance of erythrocytes, hemoglobin and methemoglobin in urine. 0-Aminotoluene sometimes causes hemorrhagic inflammation of the urinary bladder. According to M. P. Slyusar (1963), nitrocompounds were found to damage the kidneys much rarely than amino-compounds, and they practically never damaged the bladder.

As has already been shown, BTF has a typical narcotic effect. In our experiments, the clinical picture of acute poisoning of rats with m-ABTF and m-NBTF was much similar, being determined by the formation of methemoglobin. Without a marked excitation period, the reflectory excitation was gradually decreasing (though the reflexes were preserved), adynamia and atony were developing, while narcosis usually was not observed. The animals died in the same way that under exposure to BTF, i. e., the breathing usually stopped during the first 24 hours, more seldom on the 2nd or 3rd day.

The microscopic examination revealed distinctly manifested disturbances in all internal organs and in the brain: large dilatation of capillaries, stasis, symptoms of perivascular edema, small perivascular and larger subarachnoid hemorrhages.

Along with the vascular disturbances, there were registered acute swelling of protoplasm of nerve cells in different regions of the brain, phenomena of karyocytolysis of separate nerve cells, hyaline thrombi in vessels. This may be related to hypoxia resulting from methemoglobinemia. With the prolongation of the life span of animals vascular changes in the brain disappeared, and by the 14th day practically could not be found.

The investigation of the nervous system by coloration, according to the method of P. E. Snesarev, revealed astrocytes with shortened processes, without processes (amoeboid dystrophy of astrocytes), or astrocytes in the state of decomposition into grains. Protoplasm and the nucleus of some nerve cells turned pink (central tinctorial acidophilia of the nerve cells). Those morphological manifestations presented, according to P. E. Snesaver (1949), a hypoxic test and indicated the brain hypoxia. Similar changes in the nervous system under exposure to aniline were observed by M. S. Tolgskaya (1955). Dystrophic changes in separate nerve cells of the cortex still could be observed 7 and even 14 days after the exposure to m-ABTF and m-NBTF. Reproduction of gliosic elements in the site of the destructed nerve cells and nidi of previous hemorrhages was also registered at that period.

Sharp plethora of the tracheal mucous membrane and of the pulmonary tissue with symptoms of perivascular dropsy and hemorrhages were found in the animals which died during the first 24 hours; symptoms of catarrhal-desquamative process were discovered in the trachea and in small bronchi. The same way as under exposure to BTF, after 2 weeks the indicated changes were becoming less manifest, but hyperplasia of lymphoid elements around vessels, however, developed together with intumescence of alveolar septa.

Microscopic investigation of the spleen revealed sharp plethora and vagueness of the picture (Fig. 37). Later, the mentioned changes gradually disappeared. However, by the 14th day after exposure to the poisons hyperplasia of reticuloendothelial and lymphoid elements was registered. Marked albuminous and small-drops fatty dystrophy of separate groups of hepatic cells was observed in liver, and alibuminous dystrophy of the epithelium of separate convoluted tubules was revealed in kidneys. By the end of observation (14 th day), the mentioned changes disappeared almost completely.

Hence, the toxicity and hazard of acute lethal poisoning under exposure to amino- and nitro-derivatives of benzotrifluoride are higher than under exposure to the initial product (BTF). The clinical picture of poisoning with m-ABTF and N-NBTF and morphological changes in the organs have some features typical for the effect of methemoglobin-formers and different from those ob-

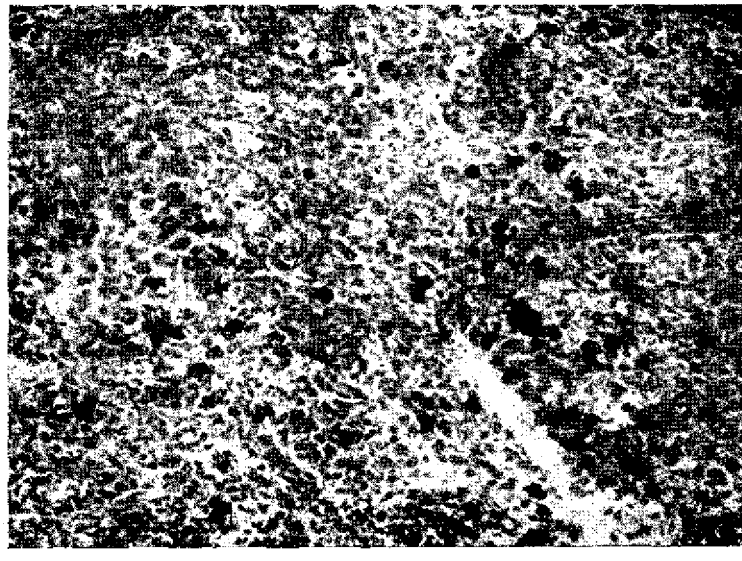

Fig. 37. Spleen. Deposition of the hemosiderin grains (m-NBTF, inhalation, the rat was killed on the 7th day).

Coloration with hematoxylin-eozine. X250.

served under exposure to BTF (cyanosis, dark-brown blood, karyocytolysis, hyaline thrombi in vessels and other symptoms of hypoxia of the brain). 2 hours after administration of the DL_{50} of m-ABTF and m-NBTF, 40.58 and 34.4% of methemoglobin, respectively, were found in the blood of the animals, together with changes in acidic erythrograms, attesting to an increased resistance of erythrocytes.

Characteristics of the hazard of acute non-lethal poisoning halogenated derivatives of fluorinated toluene

The data from the literature which may be used to characterize the hazard of non-lethal poisoning with halides of toluene are rather limited and hardly comparable (Table 95).

To make it possible to calculate the quantitative indices of the hazard of acute non-lethal poisoning, we present the threshold values, obtained by the Institute of Industrial Hygiene and Occupational Diseases of the USSR Academy of Medical Sciences using the unified scheme (Table 96).

The data given in Table 96 indicate that, under acute exposure of the organism to halogenated derivatives of toluene, the first to

Table 95

Biological effect of toluene and its halides under a single exposure

Substance	Concentration, mg/l	Effect	Authors
Toluene	1.0—0.3 (rabbits)	Changes in the time of flexor reflex	E. I. Lyublina (1950) A. S. Faustov (1967)
Benzyl chloride	0.2 (cats)	Lim_{ir}	Y. Meyer (1927)
	0.085 (1-min exposure, man)	Lim_{ir}	Y. Meyer (1927)
	0.04 (10-sec exposure, man)	Lim_{ir}	Fieldner et al. (1931)
Benzotrifluoride	4.0 (rabbits)	Lim_{ac} Changes in the time of flexor reflex	B. D. Karpov (1967)

Table 96

Acute effect thresholds (mg/l) of halides of toluene according to integral and specific indices [the data by T. V. Mikhailova (1965) and A. I. Halepo (1969)]

Index	Animals	Benzyl chloride	Benzal chloride	Benzotrichloride	Benzotrifluoride
Rectal temperature[1]	Rats	0.05	0.1	0.1	3.9 (2.2)
Oxygen consumption[1]	Rats	—	—	—	3.9 (2.2)
STI[1]	Rats	0.1 (0.05)	0.1	0.1	2.2 (0.2)
Irritant effect	Cats	0.005 (0.001)	0.01 (0.005)	0.01	Not registered

[1] Indices going beyond the limits of natural fluctuations in the control of the given series of experiment. Non-effective concentrations are given in brackets.

appear were the specific effects: irritant (by chloro-derivatives of toluene), and the effect on the central nervous system (by benzotrifluoride). We should mention once again that at this level of exposure to toluene specific changes in the unconditioned reflexes of rabbits (E. I. Lyublina, 1950) are more emphasized than under exposure to trifluorotoluene.

The zone of specific irritant effect ($Z_{ir} = \frac{Lim_{ac}}{Lim_{ir(man)}}$) for benzyl chloride is 125, which, according to the classification of G. G. Maximov (1969), corresponds to the class of marked specificity. Benzotrifluoride has no specific irritant properties. Therefore, introduction of the atom of chlorine into the side chain of

the molecule of toluene causes an increase of the biological activity of the compounds at the Lim_{ac} level, while introduction of the fluorine atom, on the contrary, leads to its decrease.

Table 97 illustrates the hazard indices of acute non-lethal poisoning with toluene an its halides. The highest biological activity, according to the Lim_{lr} value, has benzyl chloride. The least

Table 97

Indices of the hazard of acute non-lethal poisoning with toluene and its halides

Index	Toluene	Benzotrifluoride	Benzyl chloride	Benzal chloride	Benzotrichloride
Lim_{ac}	0.3—1.0	2.2	0.1	0.1	0.1
Lim_{lr}	—	—	0.005	0.01	0.01
Z_{ac}	49	2	3.9	—	—
			20[1]		
			125.0[2]		
$\frac{Lim_{ac}}{C^{20}}$	0.0067	0.01	0.014	—	—
$\frac{C^{20}}{Lim_{ac}}$	147.7	100	69.0	—	—

[1] Z_{lr} (rats) $= \frac{Lim_{ac}\ (rats)}{Lim_{lr}\ (rats)}$

[2] Z_{lr} (man) $= \frac{Lim_{ac}\ (rats)}{Lim_{lr}\ (man)}$

active is benzotrifluoride. The potential hazard posed by these compounds at the considered level is the highest under exposure to toluene.

Characteristics of the hazard of acute non-lethal poisoning with nitro- and amino-derivatives of fluorinated toluene

Here we present the results of a single exposure to nitro-, amino-derivatives of benzotrifluoride and qualitative characteristics of their effect at this level.

When assessing the Lim_{ac} value for m-ABTF and m-NBTF, A. I. Halepo (1969) did not reveal differences in the STI values, rectal temperature, oxygen consumption, number of systoles, content of methemoglobin, acidic erythrogram (duration of hemolysis of erythrocytes, the peak-time corresponded to the maximum position of hemolysis), content of reticulocytes, the leukocytic formula under exposure to m-ABTF in a concentration of

0.008 mg/litre, and to m-NBTF in a concentration of 0,01 mg/litre. A concentration of m-NBTF equal to 0.03 mg/litre, and of m-ABTF equal to 0.02 mg/litre, which caused an increase of the STI value, should be considered as the threshold concentrations under a single exposure (Figs. 38 and 39). Under an

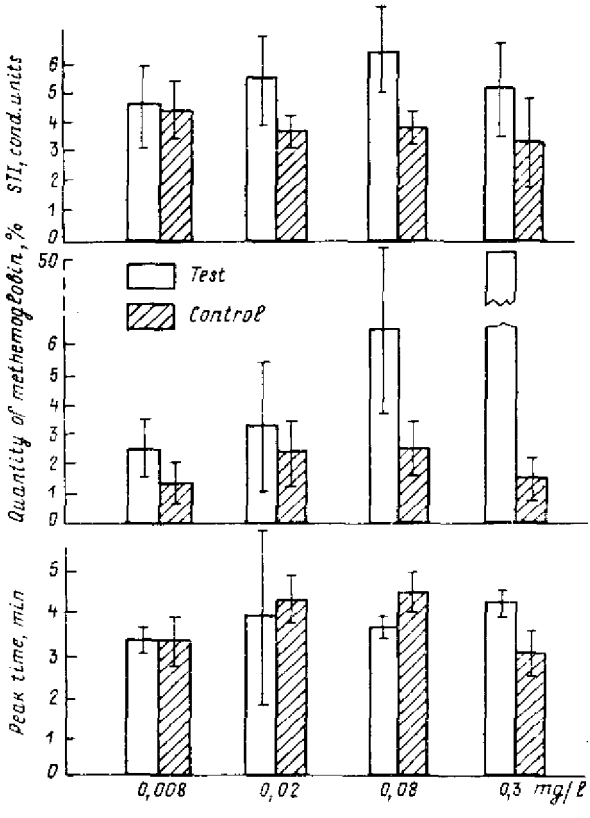

Fig. 38. Some indices of the state of rats during determination of the Lim_{ac} for m-ABTF.

increase of the concentration of the poison by 3—4 times, there were also registered changes in the peripheral blood: methemoglobin content increased, acidic erythrograms changed. However, there were no signs of changes in the formula of the blood composition at the investigated levels. No irritant effect of the compound was registered.

Consequently, it is not methemoglobinemia, but earlier disturbances in the function of the nervous system that determine the peculiarities of the effect of m-ABTF and m-NBTF at the Lim_{ac} level. Similar data for m-amino-toluene have been reported by M. S. Zakabunina (1954).

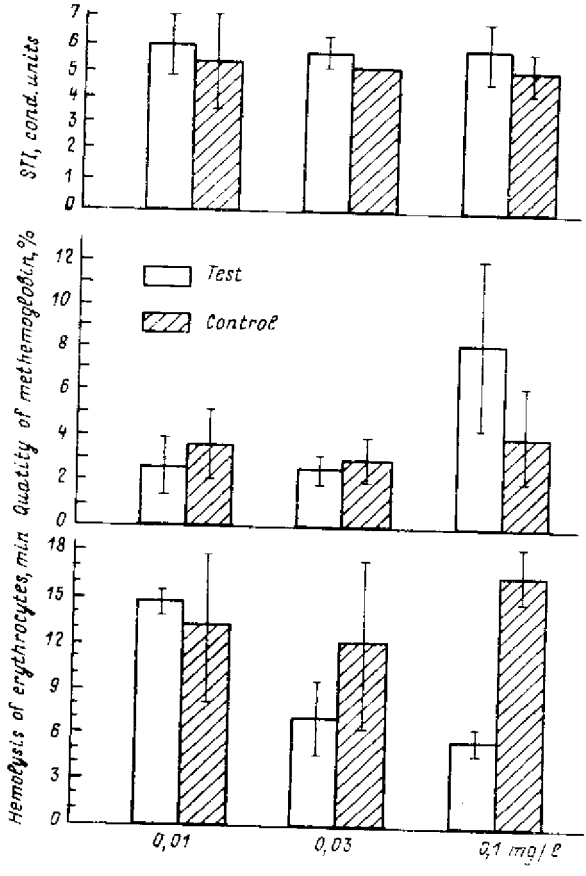

Fig. 39. Some indices of the state of rats during determination of the Lim_{ac} for m—NBTF.

Table 98

Some indices of the hazard of acute non-lethal poisoning with BTF and its nitro- and amino-derivatives

Index	BTF	m-ABTF	n-NBTF
Lim_{ac}	2.2	0.02	0.03
Z_{ac}	42	34	29
$\frac{Lim_{ac}}{C^{20}}$	0.01	0.002	0.005
$CPPI_{ac}=\frac{C^{20}}{Lim_{ac}}$	100	410	188

On the basis of the data given in Table 98 one may draw a conclusion that the biological activity of the considered amino- and nitro-derivatives of fluorotoluene at the Lim_{ac} level is approximately the same. The hazard of acute non-lethal poisoning, assessed by the Z_{ac}, is slightly higher under exposure to m-NBTF. At the same time, nitration or amination of benzotrifluoride increases sharply the biological activity of the compounds (by 100 times, while the character of the initial signs of the effect (changes in the function of the nervous system) remains the same.

The highest potential hazard ($CPPI_{ac}$) is presented by m-ABTF, then come m-NBTF and BTF.

Characteristics of the hazard of chronic poisoning with chloro-derivatives of toluene and fluorinated amino-toluene

Quantitative indices of the hazard of chronic poisoning with halides of toluene are shown in Table 99. The data from the literature about chronic effect of these substances are hardly comparable. Chronic intoxication with toluene has been investigated thoroughly, and is illustrated by experimental data as well as by the results of examination of working people.

Chronic experiments on animals revealed changes in the blood: a decrease of the quantity of erythrocytes and of the hemoglobin content, a decrease of the quantity of leukocytes, and sometimes, at the beginning of poisoning, lymphopenia (Engelhardt, Estler, 1935; et al.). Nevertheless, a number of authors deny the existence of any distinct regularity in the dynamics of changes occurring in the red and white blood (A. S. Faustov, 1967; Oettingen et al., 1942). Functional changes in the nervous system have been described by A. S. Faustov (1967), I. S. Gusev (1967) et al.; dystrophic changes in liver and kidneys are also considered possible (A. S. Faustov, 1967; Oettingen et al., 1942).

The examination of a large group of workers who had long been exposed to toluene vapours in concentrations, exceeding the established MAC by 2—12 times, revealed functional disturbances in the central nervous system (A. A. Dolmatov, 1965). Lesion of the peripheral nervous system was registered less often. Changes in the blood composition were similar to those revealed in the experiment. According to V. A. Pokrovsky (1956), under exposure to toluene, phenomena of emphasized anemia, and especially of leukopenia, are observed much more rarely than under benzene intoxication. However, some researchers (A. M. Rashevskaya, L. A. Zorina, 1968; Schwartz, Teleky, 1941; Wilson et al., 1948) came to the conclusion that under chronic intoxication with toluene a significant inhibition of the bone-marrow activity may oc-

Table 99

Relationship between the magnitude of biological effect of toluene and its halides, and the value of their concentration (chronic exposure for not less than 4 months)

Substance	Concentration, mg/l	Biological effect	Authors
Toluene	10—18 (rats)	Changes in the blood, dystrophic changes in liver, kidneys	Oettingen et al. (1942)
	2.6—0.5 (rats)	Changes in the function of the nervous system, in the morphological and biochemical composition of the blood, dystrophic changes in organs	A. S. Faustov (1967)
	0.05 (rats)	Lim_{ac} (by the same indices)	A. S. Faustov (1967)
	0.05 (rats)	MAC	
Benzotrichloride	0.1[1] (rats) 0.03[1]	Sharp irritant effect. Decrease of the body weight and leukocytes quantity, increase of blood pressure. Morphological changes in organs	O. G. Arkhipova (1963)
Benzotrifluoride	3.0 (mice, rats)	Ghanges in unconditioned and conditioned reflexes, albuminous and fatty dystrophy in liver and kidneys	B. D. Karpov (1967)
	0.2 (mice, rats)	No changes registered	B. D. Karpov (1967)

[1] A 20-day exposure.

cur if the type of the intoxication development is similar to that of the poisoning with benzene. Some authors point out the possibility of the liver lesion as a result of exposure to toluene in concentrations one order higher than the MAC. Inclusion of a chlorine atom into the methyl group of the molecule of toluene reduces the narcotic effect and sharply increases the irritant properties of the compounds, the same way as it occurs under acute exposure.

We do not have data from the literature on chronic exposure by inhalation to m-amino- and m-nitro-toluene. When, in a subacute experiment, these substances were administered to animals into the stomach and subcutaneously, morphological and biochemical changes were registered in the peripheral blood and in the functional condition of the nervous system, liver and kidneys

Table 100

Indices characterizing the state of white rats in the dynamics of chronic inhalation exposure to benzyl chloride [$p \leq 0.05$ (according to the data by T. V. Mikhailova, 1965)]

Index	Period of investigation, months									
	1		2		3		4		5	
	concentration, mg/l									
	0.01	0.002	0.01	0.002	0.01	0.002	0.01	0.002	0.01	0.002
Body weight	0	0	0	0	—	—	—	—	—	—
Threshold of neuromuscular excitability	—[1]	0	0	0	0	0	+	0	+[1]	0
Rectal temperature	0	0	+	+	0	+	+	+	+[1]	0
Hb content in blood	0	+	0	0					0	0
Leukocytes quantity in blood	—[1]	0	0	0	—				—[1]	—[1]
Erythrocytes quantity in blood	0	0	0	0					0	0
Content of hippuric acid in urine									—	0
Content of SH-groups in blood serum							0	0		
Content of albuminous fractions in blood serum	0	0			0	0			0	0
Diuresis	0	0			0	0			0	0
Concentration of protein in urine	0	0			0	0			—[1]	—[1]
Content of protein in urine in 24 hours	0	0			—[1]	—[1]			—[1]	—[1]

Note: Empty column: indices were note; determined; (+) increase; (—) decrease; (0) no changes.

[1] Changes went beyond the limits of natural fluctuations of median values in the control group of animals in this given series of experiment.

(M. Siza et al., 1959; P. I. Kosachevskaya, 1967). Formation of methemoglobin was not observed by the authors in the chronic experiment. Anemia, symptoms of vegetative dystonia (M. Siza et al., 1959), sometimes hepatitis with marked disturbances of the detoxication function of the liver (L. N. Kazinskaya, 1962; A. M. Rashevskaya, L. A. Zorina, 1968) have been revealed in working people, chronically exposed to nitro- and aminotoluene.

Characteristics of the hazard of chronic poisoning with chloro-derivatives of toluene have been given using the example of

benzyl chloride, which is the most important substance for industry and the least studied one in terms of biology at low levels of exposure. For this purpose, the effect of the poison was investigated in chronic experiment on white rats in two concentrations: 0.01 ± 0.0016 mg/litre (approximately one order lower than the Lim_{ac}) and $0.002 \pm 0{,}0003$ mg/litre (5 times lower than the Lim_{ir}). The results of the investigation are shown in Table 100.

Under chronic exposure to benzyl chloride in a concentration of 0.01 mg/litre, changes in the functional condition of the nervous system of rats occurred during the first month of exposure, then there increased their rectal temperature. Beginning from the 3rd month of exposure there was registered a decreas of the body weight of the animals, followed later by disturbances in the function of liver and kidneys. Moreover, the morphological changes in the internal organs of the animals were observed: rhinitis, tracheitis, bronchitis with hyperplasia of muciferous glands and round-cellular infiltrates in submucous membrane, as well as intumescence of the interalveolar septa in lungs. In some cases, these phenomena were accompanied by purulent bronchitis and catarrhal peribronchial pneumonia. Small dystrophic changes in the parenchymatous organs with significant quantity of casts were found in the lumen of convoluted tubules of kidneys. In a smaller concentration (0.002 mg/litre), benzyl chloride caused a decrease in the body weight of rats, an increase of rectal temperature and a reduction of the protein content in urine.

A concentration of benzyl chloride equal to 0.02 mg/litre has been conditionally accepted as the threshold concentration in a chronic experiment, though both of the investigated concentrations appeared to be effective.

The hazard of chronic poisoning presented by the series of amino- and nitro-derivatives of fluorotoluene has been studied on the example of fluorinated amino-toluene, which is more toxic and hazardous under single exposures (Table 101). The first concentration is 2 times lower than the Lim_{ac}, the second is lower than the first by one order. The results obtained indicate that m-ABTF in a smaller concentration caused periodically changes in the indices chosen to reveal the biological activity of the compounds. Under exposure to m-ABTF in a concentration of 0.01 mg/litre, animals exhibited periodically changes in the integral and pathogenetic indices.

An increase of the intensity of exposure to m-ABTF by approximately one order led to intensification of the specific changes in the blood: an increase of methemoglobin content in the blood during the 1st month, and an increase of the quantity of reticulocytes during the 4th month of exposure (Fig. 40).

Changes in the function of kidneys, though revealed only by the end of the exposure, remained even during the recovery pe-

Table 101

Indices characterizing the state of white rats in the dynamics of chronic exposure to m-ABTF by inhalation [$p \leq 0.05$ (according to the data by A. I. Halepo, 1969)]

Index	Period of investigation, months									
	1		2		3		4		5	
	concentration, mg/l									
	0.01	0.001	0.01	0.001	0.01	0.001	0.01	0.001	0.01	0.001
Body weight	0	+	0	0	0	0	0	0	0	0
Oxygen consumption	0	0	0	—[1]	0	0	0	0	0	0
Rectal temperature	0	0	0	0	0	0	0	0	0	0
STI	0	0	0	0	0	—	0	0	0	0
Respiration rate	0	0			0	0			0	0
Number of systoles	0	0	0	0	0	0	0	0	0	0
Methemoglobin content	+[1]	0	0	0	0	0	0	0	0	0
Acidic tolerance of erythrocytes	—	0	0	0	0	0	0	0	0	0
Hb content in blood	0	0	0	0	—	—	0	0	0	0
Quantity of:										
reticulocytes	—[1]	0	0	—	0	0	+	0	0	0
erythrocytes	0	0	0	0	0	0	0	0	0	0
leukocytes	0	0	0	0	+	+	0	0	0	0
granulocytes	0	0	0	0	0	+	0	0	0	0
agranulocytes	0	0	0	0	0	—	0	0	0	0

Note: (+) increase; (—) decrease; (0) no changes.
[1] Changes went beyond the limits of natural fluctuations of median values in the control group of the given series of experiment.

riod, which corresponded to the 3rd criterion of harmfulness, according to the definition of the MACs Section.

Microscopic examination of the organs of animals, carried out after a 1-month exposure, revealed dystrophic changes in the nervous tissue, appearing in the form of contraction of separate nerve cells in the cortex and subcortical nodes, vacuolization and karyocytolysis in subcortical nodes (Fig. 41). With the increase of the time of exposure, the mentioned changes in the nervous system did not intensify.

Feebly marked phenomena of catarrhal-desquamative bronchitis with intumescence of interalveolar septa and reproduction of lymphoid elements around vessels in the form of couplings occurred in the lungs. Dystrophic changes in liver (albuminous and fatty dystrophy) were observed only one month after the exposure. Hyperplasia of cells of the reticuloendothelial system

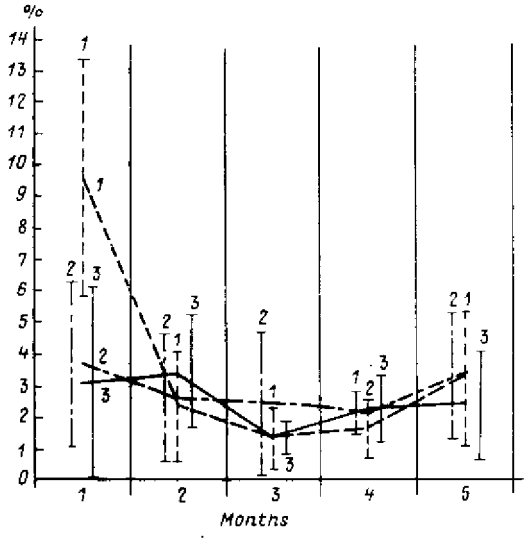

Fig. 40. Content of methemoglobin in the blood of rats in the dynamics of chronic poisoning with m-ABTF in different concentrations.
(1) 0.01 mg/l; (2) 0.001 mg/l; (3) control.

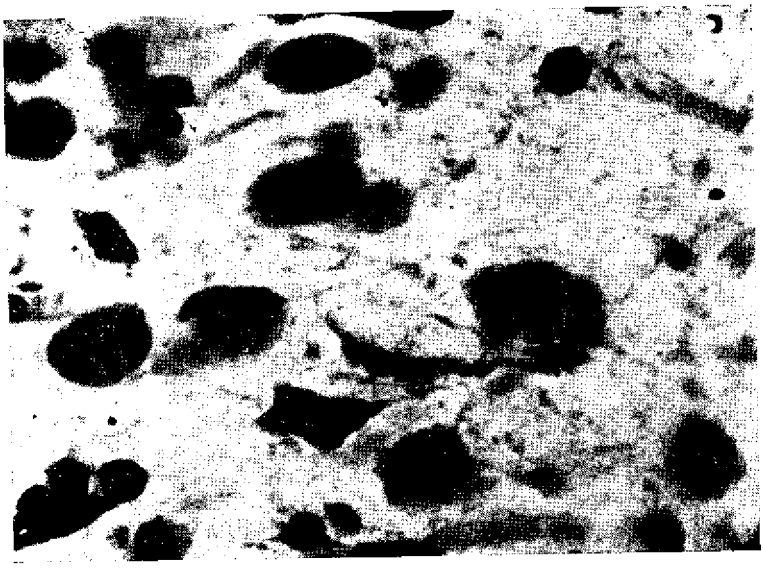

Fig. 41. Swelling and contraction of nerve cells in subcortical ganglions (chronic exposure, 0.001 mg/l of m-ABTF, x800).

with the formation of histiocytic nodes and appearance of multinucleate hepatic cells in quantities exceeding the norm were being registered throughout the chronic exposure. A feebly manifested albuminous dystrophy of the epithelium of separate convoluted tubules and slight interstitial process were revealed in kidneys. Those changes could be observed 5 months after the exposure, and remained during the recovery period (Fig. 42).

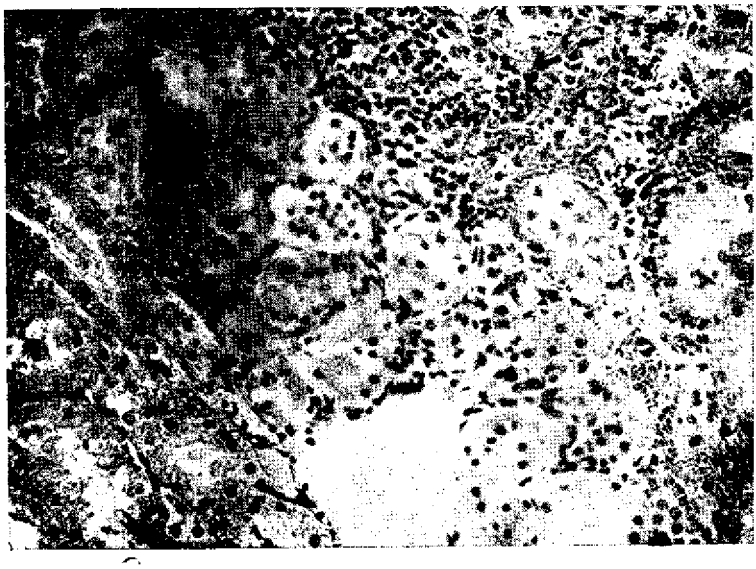

Fig. 42. Histiocytaric infiltrates in interstice of kidney (chronic exposure, 0.01 mg/l of m-ABTF, X250).

The presented data make it possible to consider a concentration of m-ABTF equal to 0.001 mg/litre to be close to the chronic effect threshold.

Thus, the substitution of the hydrogen atom by a halogen in the methyl group of the toluene molecule sharply increases the biological activity, changing the character of the substance's effect the same way as it occurs at the lethal and threshold levels under a single exposure.

As has already been stated, the chronic exposure to toluene at the Lim_{ac} level is characterized by changes in the functional condition of the nervous system and in the morphological composition of the peripheral blood. As to benzyl chloride, its effect is characterized also by morphological symptoms of chronic irritation of the respiratory tract and by changes in the function of liver and kidneys.

Inclusion of the amino-group into the molecule of benzotrifluoride increases its activity under chronic exposure by almost 3 orders (according to B. D. Karpov, the Lim_{ch} for BTF is 3 mg/l, and its non-effective concentration is 0.2 mg/l). In this case the effect of the substance on the nervous system, blood and the function of kidneys is being aggravated.

Indices of the real and potential hazard from the considered compounds have been calculated using their threshold values of acute and chronic effects (Table 102). For a comparison, this Table includes the hazard indices of several initial products.

Table 102

Some indices of hazard of chronic poisoning with derivatives of toluene and fluorotoluene

Substance	Lim_{ch}, mg/l	Z_{ch}	$\frac{Lim_{ch}}{C^{20}}$	$\frac{C^{20}}{Lim_{ch}}$
Toluene	0.05[1]	13	0.0005	1920
Benzyl chloride	0.001	100	0.0001	6900
BTF	3.0[2]	—	—	—
	0.2[3]	—	—	—
m-ABTF	0.001	20	0.0001	8200

[1] The data by A. S. Faustov(1967).
[2] Effective concentration, according to B. D. Karpov (1967).
[3] Non-effective concentration, according to B. D. Karpov (1967).

Thus, introduction of the chlorine atom into the side chain of the molecule of toluene, as well as introduction of the amino-group into the molecule of benzotrifluoride, increases the activity of the compounds and the hazard of chronic poisoning at the Lim_{ch} level. The potential hazard from the compounds at this level corresponds to their biological activity.

Changes in some indices of the hazard from chloro-derivatives of toluene, nitro- and amino-derivatives of fluorotoluene in view of peculiarities of their metabolism

A high chemical activity of the chlorine atom and a low mobility of fluorine in the side chain of the molecule are well known. Therefore, we have analysed the biological activity of the mentioned compounds (as in case of halides of benzene), taking account of the energy of rupture of chemical bonds, which are of great importance in thermodynamics and kinetics of chemical reactions.

The energy of rupture of the carbon-fluorine bond in aromatic compounds is 85.6 kcal/mole, and 72 kcal/mole for the carbon-

chlorine bond. It is possible that exactly this fact determines distinctly marked irritant properties of chlorinated derivatives of toluene. At the same time, the peculiar features of toluene and its halides may depend on the routes of the poisons transformation in the organism.

According to publications, toluene is metabolized mostly by means of oxidation up to benzoic acid, which later binds glycerine (synthesis of hippuric acid), or glucuronic acid (synthesis of benzyl-glucuronic acid). J. Teisinger, S. Shkramovsky and J. Srbova (1959) in observing people found out that, when toluene is inhaled in concentrations of 0.217—2.009 mg/litre, only about 16% of the unchanged product is eliminated through the lungs. The rest of the toluene is practically completely transformed in the organism into benzoic acid, which is excreted with urine mostly in the form of hippuric acid in 24 hours, and only 10—20% is excreted in the form of glucuronides. No increase of phenol and cresols excretion with urine was registered in this case.

The content of benzoic and hippuric acids in urine of the people working in contact with toluene is used in various countries outside the USSR as «the exposure test», which serves to assess the dose of toluene absorbed per one working shift (Elkins, 1954; Piotrowski, 1967, et al.). Pazdezova, Srbova (1964) et al. consider the presence of metabolites of toluene in urine to be a diagnostic signs of poisoning.

Information on metabolism and elimination of chlorinated derivatives of toluene is even more limited. Stekol (1947) showed that an addition of benzyl chloride to the food of rats, kept on a casein diet, slows down the growth of the animals. Per os administration of L-cystein and DL-methionine prevented such effect. The authors came to the conclusion that mercapturic acids were formed as a result of combining of halogen-containing hydrocarbons with sulfur-containing amino-acids.

Formation of mercapturic acids is not the only way of the metabolism of chloro-derivatives of toluene in the organism. Bray, James and Thorpe (cited from Williams, 1959) found that 37% of benzyl chloride, administered to rabbits per os in lethal doses, is eliminated from the organism in the form of benzoic and hippuric acids. O. G. Arkhipova et al. (1963) observed a 2-fold increase, in comparison with control, of the content of hippuric acid in urine under subacute poisoning with benzotrichloride. There is an opinion (Stekol, 1947; Williams, 1967) that halobenzols may be excreted with urine combined with sulfur and in the form oxygenic compounds: glucuronides and ethereal sulfates.

We have found that under chronic exposure to benzyl chloride at a concentration one order higher than the Lim_{ac} the excretion of hippuric acid with urine increased. At the Lim_{ac} level, though

there was registered a tendency to an increase, the difference was not statistically significant. Under single exposures to benzyl chloride, benzal chloride and benzotrichloride at the Lim_{ac} level no increase of the amount of hippuric acid in the urine of rats was registered.

To answer the question on the possibility of combining of chloro-derivatives of toluene with sulfur-containing amino-acids, the content of free SH-groups in the blood serum (Fig. 43), and the content of albuminous fractions in the blood serum (Fig. 44) have been assessed under a single exposure to benzyl chloride at the Lim_{ac} level. On the basis of the reported data, one may assume that changes in the ratio of albuminous fractions of the blood serum under exposure to benzyl chloride is a result of a direct interaction between the poison and sulfur-containing amino-acids.

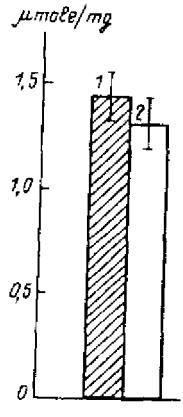

Fig. 43. Content of free SH-groups in blood serum under a single exposure to benzyl chloride at the Lim_{ac} level.
(1) test; (2) control.

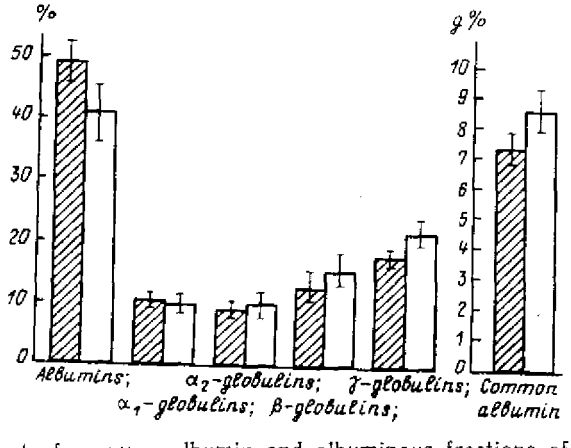

Fig. 44. Content of common albumin and albuminous fractions of blood serum under a single exposure to benzyl chloride at the Lim_{ac} level.
Hatched space indicates experiment.

Thus, under exposure to chloro-derivatives of toluene, the same as under exposure to toluene, there was registered the formation of hippuric acid, but to a considerably smaller extent (at a low level of exposure). Moreover, the final product of metabolism does not yet indicate that other stages of the process of transformation of the poison in the organism are similar. We have not found evidence of the existence of a direct binding of benzyl chloride by SH-groups of proteins and amino-acids at low levels of exposure in the chronic experiment. At the same time, under a single administration of the poison at the Lim_{ac} concentration, there were revealed changes in the albuminous spectrum of the blood serum, which were probably related to the liver lesion. This allows one to suggest the existence of a direct interaction between benzyl chloride or its metabolites and proteins.

m-Amino-benzotrifluoride and m-nitro-benzotrifluoride. The data from investigations demonstrate that the exposure to m-ABTF and m-NBTF at different quantitative levels brings about different types of changes. Under lethal concentrations, the substances cause the formation of methemoglobin. At the Lim_{ac} level, the first be to observed are the changes in the functional condition of the nervous system. Chronic intoxication with m-aminobenzotrifluoride is characterized by polytropic changes in the nervous system, peripheral blood, liver and kidneys.

The qualitative differences in the effect of m-ABTF and m-NBTF, typical of non-fluorinated amino- and nitro-derivatives of toluene, become evident when comparing toluene with trifluorotoluene (initial product). These properties are determined by the processes of the compounds metabolism in the organism. As has already been shown, toluene is transformed mostly by means oxidation of the side chain with the formation of benzoic, and then of hippuric acids or glucuronides. Nitro- and amino-toluene are transformed in the organism in a different way. In the process of oxidation, nitrotoluene is transformed either into nitrobenzyl alcohol, 25% of which is excreted with urine in the form of glucuronides, or into nitrobenzoic acid, which is excreted with urine in a free state (10% of the administered o-nitrotoluene). It is possible that p-nitrotoluene is oxidized faster than o-nitrotoluene, and that it is excreted with urine in the form of nitrobenzoic and nitrohippuric acids.

D. M. Rossiysky (1921) believed that all three isomers of nitrotoluene were transformed in the organism into nitrobenzoic acid. The most perfect, in his opinion, was the process of oxidation of para- and metaisomers, which are excreted with urine mostly in the form of corresponding nitrobenzoic and nitrohippuric acids. The only data on transformations of aminotoluene (toluidine) in the organism are that by I. D. Gadaskina (1965), who found that o-aminotoluene is oxidized into aminocresol in dogs, and that p-aminotoluene is, apparently, transformed into p-ami-

nobenzoic acid. As to amino- and nitrobenzotrifluoride, the possibility of formation of toxic products of transformation in the organism cannot be completely excluded. This concerns, in particular, derivatives of quinonimine, phenylhydroxylamine, which exhibit more manifest effect on the function of the central nervous system and which play the leading role in the formation of methemoglobin (A. M. Rashevskaya, L. A. Zorina, 1968).

Conclusions. Summarized data on the toxicity and hazard from the considered substances at different levels of exposure are given in Table 103. Thus, the potential hazard from halides of toluene under a single lethal ($CPPI_{CL}$) and a single non-lethal ($CPPI_{AC}$) poisoning is almost equal: mainly class III of hazard (moderately hazardous). The potential hazard of chronic poisoning with these compounds increases sharply. Toluene, m-ABTF and m-NBTF are potentially extremely hazardous in view of chronic poisoning.

Biological activity of the compounds of the considered series, according to the values of CL_{50}, Lim_{ac} and Lim_{ch}, remains relatively constant. Benzyl chloride and m-ABTF are exceptions. According to the CL_{50} and Lim_{ch} values, benzyl chloride belongs to extremely hazardous, and by the Lim_{ac}, to highly hazardous compounds. m-ABTF is highly active under single exposures, irrespective of the level, and extremely active in chronic experiments. The latter fact is, apparently, connected with the qualitative peculiarities of the effect of the poisons.

Though no increase of the methemoglobin content in the blood has been found, changes in the functional condition of the nervous system have been revealed very early.

It should be noted that the energy of rupture of the bond of the amino-group-carbon of the aromatic ring in the molecule of m-ABTF is very low, and is approximately equal to that of the chlorine-carbon bond rupture in the side chain of toluene (60.4 kcal/mole). At the same time, the bond of fluorine in the molecule of m-ABTF remains strong (80 kcal/mole), and it only slightly decreases in comparison with that of BTF (115 kcal/mole). Thus, in the series of the considered toluene derivatives, as the most probable transformations of the poison in the organism should be recognized the reactions of splitting off of chlorine and amine radical (Fig. 45).

The comparison of responses under a single exposure (Lim_{ac}) to different classes of the investigated halogenated hydrocarbons reveals, as a rule, similiarity of the changes in the functional condition of the nervous system. The changes, registered in the function of the nervous system, are determined by its high sensitivity.

By the magnitude of the acute effect zone, all compounds, except benzyl chloride, are moderately hazardous.

Thus, substitution of hydrogen in the methyl group of the toluene molecule by chlorine increased sharply the toxicity and

Table

Major parameters of toxicometry and hazard indices of halides of toluene

Index	Toluene	Hazard class	Benzyl chloride	Hazard class	Benzal chloride	Hazard class	Benzotrichloride	Hazard class	BTF	Hazard class	m-ABTF	Hazard class	m-NBTF	H
DL_{50}, mg/kg	7000	IV	1500	III	1400	III	1300	III	10 000	IV	220	III	520	
S									1.69		1.83		1.66	
$\frac{1}{DL_{50} \cdot S}$									0.059		2.48		1.16	
CL_{50}	32.0	III	0.39	I	0.21	I	0.06	I	92.2	IV	0.69	II	0.88	
S	1.52		1.64		2.62		2.0		1.55		1.27		1.25	
$\frac{1}{CL_{50} \cdot S}$	0.01		1.56		1.82		8.3		0.0064		1.14		0.91	
$CPPI_{CL}$	3.0	III	17.7	III	12.9	III	39.2	III	2.38	IV	11.9	III	6.4	
Lim_{ac}, mg/l	0.3—1.0		0.1	II	0.1		0.1		2.2	IV	0.02	II	0.03	
Lim_{ir}, mg/l	—		0.005		0.01		0.01		—		—		—	
Z_{ac}	49	III	3.9	I	—		—		42	III	34	III	29	
$Z_{ir} = \frac{Lim_{ac(rats)}}{Lim_{ir(man)}}$	—		125		—		—		—		—		—	
$CPPI_{ac}$	147.7	III	69	IV	—		—		100	III	410	III	188	
Lim_{ch}, mg/l	0.05	III	0.001	I	—		—		—		0.001	I	—	
$CPPI_{ch}$	1920	II	6900	I	—		—		—		8200	I	—	
Z_{ch}	13	I	100	I	—		—		—		20	I		
K_{cum}	—		—						6.53		10.65		10.4	
MAC, mg/m³	50.0		0.5		0.5		0.1		100		0.5		1.0	

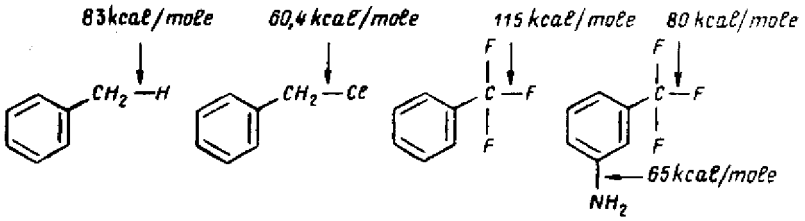

Fig. 45. Energy of bonds rupture in the molecule of toluene and its halides.

hazard of the compounds at all levels of exposure and attributed them irritant properties, which appeared to be the most manifested in monochloro-derivatives of toluene. Fluorination of the methyl group of toluene was found to decrease the toxicity of the compounds without changing the narcotic character of the effect. Biological activity of chloro- and fluoro-derivatives of toluene corresponded to the reactive capacity of the compounds (the rupture energy of the halogen-carbon bond in the methyl radical).

Introduction of an amino- or nitro-group into the molecule of BTF increased sharply the toxicity and hazard of the compound in comparison with the initial product at all levels of exposure. Changes in the quantitative characteristics of the hazard of the compounds were followed by the development of specific methemoglobin-forming properties, beginning from concentrations (doses) exceeding $3Lim_{ac}$, which was determined by high mobility of NO_2- and NH_2-groups in the molecule and by respective changes in the metabolism.

The maximum allowable concentrations for halides of toluene have been established taking account of their irritant properties. The Lim_{ir} for benzyl chloride is 0.0008 mg/l. These data have determined low MACs values for benzyl chloride and for other compounds of this series. For BTF, which has no irritant properties and which possesses considerably higher values of toxicometric parameters, the established MAC is 100 mg/m³. B. D. Karpov (1967) investigated the chronic effect of BTF and proved that cumulation did not occur under long-term inhaltion exposure to this product. Therefore, he recommended a MAC for benzotrifluoride equal to 100 mg/m³. In establishing the MAC for m-ABTF (equal to 0.5 mg/m³), the magnitude of the chronic effect zone (indicating high cumulative capacity of this product) has been used as a limiting index. The MAC from m-NBTF has been established by analogy with m-ABTF at the level of 1 mg/m³, since the hazard of acute poisoning from the latter appeared to be higher.

Chapter 9. HETEROCYCLIC COMPOUNDS. EVALUATION OF TOXICITY AND HAZARD

Characteristics of the hazard of lethal poisoning under exposure to imino-compounds

Imino-compounds are being widely used in the economy. The main physico-chemical properties of these compounds are given in Table 104.

Table 104

Main physico-chemical properties of imino-compounds

Substance	Molecular weight	Relative density at 20°C	Refractive index at 20°C	Boiling point under 760 mm of Hg (°C)	Volatility at 20°C, mg/l	pH	Ionizing potential, EV
Ethyleneimine	43.0	0.83		56.0	374	11.0	9.8
Pyrrolidine	71.0	0.85		88	194	14.0	8.9
Piperidine	85.15	0.8622	1.4530	106.3	93	14.0	8.7
Hexamethyleneimine	99.15	0.8770	1.4654	138.0	27	14.0	8.5

As can be seen from the Table, there is a decrease of volatility (and thereby, a reduction of the potential possibility of the poison's entry into the organism) from first member of homologous series to the highest homologues. These compounds are strong bases. These substances have good solubility in water and organic solvents.

Values of lethal concentrations of the considered compounds are presented in Table 105.

As follows from Table 105, there is decrease in the toxicity of the members of the series from ethyleneimine to hexamethyleneimine in a proportion of 1 : 4 : 16 : 27, which determines their assignment to different classes of toxicity.

Table 106 illustrates the hazard indices of lethal poisoning with imino-compounds under inhalation administration into the organism.

Table 106 shows that the most hazardous compounds are ethyleneimine and pyrrolidine, which are followed by hexamethylene-

Table 105

Lethal concentrations of heterocyclic compounds (for mice, 2-hr exposure, 2-week observation period)

Substance	Concentration, mg/l			Mean life span, days	Toxicity class (MACs Section)	Relative toxicity (I. V. Sanotsky)	Authors
	CL_{16}	CL_{50}	CL_{84}				
Ethyleneimine	0.24	0.4 (0.4 ÷ 0.46)	0.64	5.2	I	59%	G. N. Zayeva, L. A. Timofievskaya, 1968
Pyrrolidine		1.5	—	—	II	40%	G. N. Zayeva et al., 1974
Piperidine	2.3	6.5 (4.0 ÷ 10.7)	10.7	3.0	III	35%	L. A. Bazarova, N. I. Osipenko, 1967
Hexamethyleneimine	4.6	10.8 (6.5 ÷ 17.8)	17.4	2.3	III	25%	L. A. Bazarova, N. I. Osipenko, 1967

Table 106

Indices of the hazard of development of acute lethal poisoning with cyclic imino-compounds in mice under inhalation exposure

Index	Ethyleneimine	Pyrrolidine	Piperidine	Hexamethyleneimine
$\frac{1}{CL_{50}}$	2.5	0.6	0.15	0.09
$\frac{CL_{84}}{CL_{16}}$	2.7	2.7	4.7	3.8
S	1.6	1.6	3.0	1.9
$\frac{1}{CL_{50} \cdot S}$	1.5	0.4	0.05	0.048
CPPI	935	130	15	3.0

imine and piperidine. However, when assessing the hazard by the index $\frac{1}{GL_{50} \cdot S}$, the hazard degree in the series decreases from ethyleneimine to hexamethyleneimine. In view of the effective toxicity (according to CPPI), the most hazardous is also ethyleneimine, the least hazardous is hexamethyleneimine.

The type of clinical manifestation of acute intoxication has been the same for all 4 compounds, being characterized by irritation of mucous membranes of the upper respiratory tract and

eyes, by motor excitation, replaced by immobility before the death. Agony was accompanied by tonic convulsions. The median life span of experimental animals, strange as it is, decreased under exposure to these poisons at the level of CL_{50} from ethyleneimine to hexamethyleneimine, and was exponentially connected with the CL_{50} (Fig. 46).

When evaluating the life span of mice under conditions of saturating vapour concentrations of certain imino-compounds (at 20°), G. N. Zayeva et al. (1966) observed different kind of dependency; however, the life span in this case had linear dependency on the concentration (inversely proportional dependency) (Fig. 47). Morphological changes in the internal organs were characterized by marked vascular disturbances with symptoms of acute plethora.

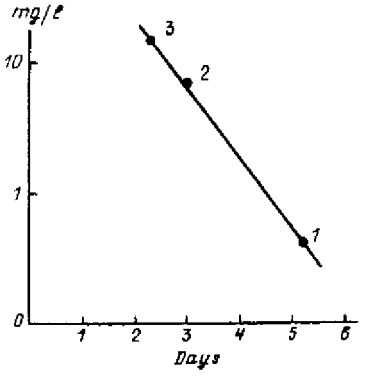

Fig. 46. Mean life span of mice under a 2-hour inhalation exposure to compounds at the CL_{50} level.
(1) ethyleneimine; (2) pyrrolidine; (3) hexamethyleneimine.

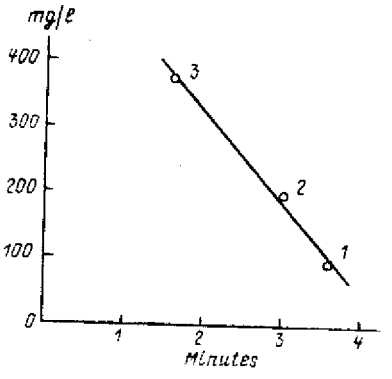

Fig. 47. Mean life span of mice in conditions of saturating concentrations of the compounds' vapours (under normal conditions).
(1) hexamethyleneimine; (2) pyrrolidine; (3) ethyleneimine.

Values of the lethal concentrations of imino-compounds have been determined under administration of the poisons into the stomach of rats (Table 107). High toxicity and alkalinity of the compounds have made it possible to determine parameters of toxicity for pure substances under this type of administration. In investigations there have been used 0.4% water solutions of ethyleneimine, 10% solutions of pyrrolidine and 8% solutions of piperidine and hexamethyleneimine.

Table 108 presents the hazard indices of lethal poisoning. Thus, the hazard indices of acute lethal poisoning under administration of the poisons into the stomach characterize ethyleneimine

Table 107

Lethal doses of imino-compounds under a single administration into the stomach of rats

Substance	Dose, mg/kg			Authors
	DL_{16}	DL_{50}	DL_{84}	
Ethyleneimine	—	17.3 (15.1÷19.5)	—	G. N. Zayeva et al., 1966
Pyrrolidine	—	250	—	G. N. Zayeva et al., 1968
Piperidine	346	371 (307÷449)	496	L. A. Bazarova, 1970
Hexamethyleneimine	326	360 (320÷417)	473	L. A. Bazarova, 1970

Table 108

Indices of the hazard of development of acute lethal poisoning with imino-compounds in rats under administration into the stomach

Substance	Index		
	$\frac{DL_{84}}{DL_{16}}$	S	$\frac{1}{DL_{50} \cdot S}$
Ethyleneimine	3.3	1.85	0.03
Pyrrolidine	2.0	1.4	0.0028
Piperidine	1.4	1.2	0.0022
Hexamethyleneimine	1.4	1.2	0.0023

as a compound presenting much higher hazard than other imino-compounds. Piperidine and hexamethyleneimine appeared to have equal degrees of hazard.

Evaluation of local irritant effect and cutaneous resorption of imino-compounds

Investigations of the irritant properties of the considered compounds have been undertaken in view of their highly marked alkaline properties.

The irritant effect thresholds for piperidine and hexamethyleneimine have been substantiated in tests on human volunteers. Their subjective sensations (A. A. Golubev, 1969) under a 1-min exposure were taken as the criterion of the irritant effect. The Lim_{ir} under exposure to piperidine has been found to be equal to 0.04 mg/litre and under exposure to hexamethyleneimine to 0.12 mg/litre. According to the classification of the hazard degree

Table 109

Indices of local irritant percutaneous effect of some imino-compounds

Substance	Minimum time for development of necrosis, min	Minimum concentrations of solutions causing necrosis, %	Lim_{ir}, moles	Response of conjunctive to 1 drop of 9% solution
Ethyleneimine	—	—	—	None
Pyrrolidine	0.5	0.5	—	+++
Piperidine	9	0.7	1/16	++
Hexamethyleneimine	12	3.0	1/8	+

From (+) to (+++) the degree of manifestation of the effect.

Table 110

The TL_{50} values under percutaneous application of imino-compounds to the tails of mice

Substance	TL_{50}, min	Authors
Ethyleneimine	0.12	G. N. Zayeva et al., 1966
Pyrrolidine	60	G. N. Zayeva et al., 1968
Piperidine	120 (80÷170)	L. A. Bazarova, 1970
Hexamethyleneimine	128 (90÷180)	L. A. Bazarova, 1970

by A. A. Golubev (1969), they belong to class II (highly toxic compounds). Values of the Lim_{ir} indicate that piperidine has a more marked irritant effect than hexamethyleneimine. The irritant effect of these compounds is not, however, specific, since the values of their general toxic effect thresholds are smaller than those of the irritant effect thresholds. In view of a high hazard presented by ethyleneimine, it was impossible to assess the mentioned characteristics for this poison.

As follows from Table 109, the indices used to characterize the irritant effect of imino-compounds are negative for ethyleneimine. It is related to the fact that ethyleneimine has extremely high cutaneous resorption, and local changes of the skin do not have time to develop because of the death of animals. The strongest irritant effect, according to the value of the minimum solution concentration, causing necrosis, and minimum time, causing initial symptoms of necrosis, has been revealed in pyrrolidine.

As one of the criteria for the quantitative evaluation of the toxicity and hazard of substances under percutaneous administration, we have used the index of TL_{50}, which in this given case of

contact with the poison indicates the death of 50% of the animals (Table 110).

As follows from Table 110, ethylenеimine can be absorbed very quickly through the intact skin. Then, the degree of manifestation of this property decreases from pyrrolidine to hexamethyleneimine.

Characteristics of the hazard of acute non-lethal poisoning with imino-compounds

One of the most important factors in the evaluation of the character of the effect and of the hazard degree of compounds is the determination of the acute effect threshold (threshold of a single harmful exposure), which characterizes the hazard of poisoning at low levels of exposure and makes it possible to estimate the specificity of a given effect. When determining the acute effect threshold, it is necessary to proceed from the physiological limits of adaptation of the organism, not from its damage (I. V. Sanotsky, 1967). The threshold concentrations for all 4 compounds have been determined on rats under a 4-hour inhalation of the poison's vapours.

According to G. N. Zayeva and L. A. Timofievskaya (1966), the acute effect threshold for ethyleneimine, assessed by changes in the indices of the conditioned reflexes, values of electrocutaneous irritation and changes in the leukocytic formula of the blood, is within the range of 0.03—0.06 mg/litre. According to G. N. Zayeva et al. (1974,) the acute effect threshold for pyrrolidine, determined by changes in the STI, is equal to 0.03 mg/l.

Determination of the threshold of

Concentration	STI, cond. units		Arterial pressure, mm of Hg		Steadiness of the skin capillaries (quantity of petechiae)	
	test	control	test	control	test	control
0.01	6.3±0.6	6.0±0.5	—	—	5.5±2.1	4.7±2.0
	p>0.05				p>0.5	
0.02	2.5±0.42	4.7±0.3	—	—	10.0±3.0	8.7±2.7
	p<0.05				p>0.5	
0.1	5.0±0.3	6.6±0.3	—	—	21.0±3.6	5.1±1.7
	p=0.05				p=0.02	
0.2	3.6±0.2	5.5±0.3	—	—	20.1±5.1	7.2±2.0
	p<0.05				p<0.05	
0.48	3.8±0.2	5.0±0.3	93	97	22.0±4.5	6.3±1.78
	p<0.02		p>0.5		p<0.01	
0.9	3.7±0.3	5.8±0.3	109±11.4	98±2.19	22.7±4.42	8.5±2.9
	p<0.05		p>0.1		p<0.05	

Results of investigations on determining the acute effect threshold for piperidine and hexamethyleneimine, obtained by L. A. Bazarova (1970), using some integral and specific indices, are given in Tables 111 and 112. As can be seen from Table 111, the minimum concentration of piperidine, causing changes in the STI, is 0.02 mg/litre. This concentration of piperidine may be considered as the threshold concentration under acute exposure. It should be emphasized that the STI value in the experimental group of animals exceeds the fluctuation limits of this index in intact animals. A larger concentration of piperidine — 0.1 mg/litre — causes not only a decrease of the STI value in the experimental animals, but also changes in the steadiness of capillaries of the skin and in the quantity of leukocytes.

From the data in Table 112 follows that the minimum concentration of hexamethylenemine, corresponding to the criterion of harmfulness and causing changes in the STI, is 0.09 mg/l. This concentration is considered to be the threshold concentration under a single exposure. A higher concentration of hexamethyleneimine — 0.4 mg/l — causes changes similar to those observed under exposure to piperidine. Consequently, the nervous system appeared to the most sensitive to the effect of piperidine and hexamethyleneimine.

Indices for evaluating the hazard of acute non-lethal poisoning have been calculated by the lethal and threshold values of the compounds under a single exposure (Table 113). As can be seen from Table 113, the effectiveness of the compounds at the Lim_{ac} level is approximately the same it is slightly lower only in hexamethyleneimine. According to the degree of manifestation of the compensatory processes (Z_{ac}), the data appeared to be some-

Table 111

a single exposure to piperidine

Hb, g/litre		Erythrocytes, millions in 1µl		Leukocytes, millions in 1 µl	
test	control	test	control	test	control
142±2.7	146±1.4	8.2±0.36	8.17±0.36	10.7±0.45	11.0±1.04
p>0.1		p>0.5		p>0.5	
146±3.3	146±3.0	8.9±0.45	8.3±0.36	11.2±1.2	10.7±0.58
p>0.05		p>0.05		p>0.5	
143±4.8	139±5.0	7.9±0.17	7.9±0.37	10.2±0.83	17.0±2.56
p>0.05		p>0.5		p<0.05	
121±3.8	130±4.6	7.04±0.41	7.21±0.53	8.1±1.6	11.3±1.64
p>0.1		p>0.5		p>0.1	
138±3.0	135±4.0	7.05±0.37	7.3±0.3	7.4±0.14	8.5±0.28
p>0.05		p>0.05		p<0.01	
139±2.3	128±4.4	7.9±0.17	6.59±0.43	7.3±0.4	9.7±0.17
p<0.05		p<0.05		p<0.001	

Concentration, mg/l	STI, cond. units		Arterial pressure, mm of Hg		Steadiness of the skin capillaries (quantity of petechiae)	
	test	control	test	control	test	control
0.075	4.6±0.63	4.6±0.74	—	—	7.7±2.0	5.7±2.1
	p>0.5				p>0.5	
0.09	3.6±0.36	4.7±0.55	94.3±5.3	87.5±4.4	8.9±2.6	6.8±2.6
	p<0.01		p>0.05		p>0.5	
0.4	3.8±0.29	5.2±0.45	106.0±16.4	84.6±6.36	20.1±5.1	5.1±1.7
	p<0.05		p>0.05		p<0.05	
0.6	2.87±0.12	4.25±0.24	93±5.8	97±4.8	19.7±5.1	6.3±1.78
	p<0.001		p>0.5		p<0.05	

Table 113

Some indices of the hazard of acute non-lethal poisoning with imino-compounds

Index / Substance	Ethyleneimine	Pyrrolidine	Piperidine	Hexamethyleneimine
Lim_{ac}, mg/l	0.03—0.06	0.03	0.02	0.09
$Z_{ac} = \frac{CL_{50}}{Lim_{ac}}$	40	50	300	120
$\frac{Lim_{ac}}{C^{20}}$	$28 \cdot 10^{-6}$	$15 \cdot 10^{-5}$	$2 \cdot 10^{-4}$	$3 \cdot 10^{-3}$
$CPPI_{ac} = \frac{C^{20}}{Lim_{ac}}$	37 400	6 466	4 650	300

what contradictory, which can be attributed to the variability of the Lim_{ac}. However, the potential hazard of acute non-lethal poisoning (according to the $CPPI_{ac}$ and the thermodynamic activity) presented by ethyleneimine appeared to be 100 times higher than of hexamethyleneimine.

To assess the qualitative characteristics of acute intoxication with the considered compounds, investigations on some indices of the functional condition of separate organs and systems have been undertaken. The results obtained are given in Table 114.

The data in Table 114 indicate that under administration at isoeffective levels piperidine and hexamethyleneimine exhibit similar type of action. However, the degree of the changes manifestation is higher under exposure to piperidine.

Table 112

threshold of hexamethyleneimine

Hb, g/litre		Erythrocytes, million in 1 μl		Leukocytes, million in 1 μl	
test	control	test	control	test	control
130±5.6	138±1.4	7.687±0.42	8.4±0.08	11.5±0.47	10.2±0.37
p>0.25		p>0.5		p>0.05	
140±2.4	135±2.2	9.103±0.3	8.95±0.34	13.7±1.0	13.9±1.8
p>0.5		p>0.5		p>0.5	
155±4.2	142±3.0	10.8±0.66	7.6±0.34	8.0±0.87	12.6±2.37
p<0.05		p<0.001		p>0.25	
153±2.6	149±0.9	6.9±0.87	7.6±0.22	8.4±0.95	11.9±0.92
p<0.001		p>0.5		p>0.05	

Table 114

Some indices of intoxication in rats under a single administration of piperidine and hexamethyleneimine into the stomach in a dose equal to the DL_{16}

Index	Piperidine	Hexamethylene-imine	Control
STI, cond. units	2.8±0.4	3.5±0.24	6.8±0.3
	p<0.001	p<0.001	
Respirations per minute	237±10.9	159±13.6	307±1.54
	p<0.01	p<0.05	217±21.4
Arterial pressure, mm of Hg	152±4.42	129±7.29	85±3.3
	p<0.001	p<0.001	
Steadiness of the skin capillaries (quantity of petechiae)	12.8±2.7	10.7±2.07	4.2±1.9
	p<0.02	p<0.05	
Diuresis, ml	4.6±0.63	4.3±0.8	4.7±1.0
	p>0.05	p>0.05	
Protein, mg/ml	5.1±1.12	4.4±0.1	3.3±0.18
	p<0.05	p<0.05	
Hippuric acid, mg/24 hr	68±6.9	58.5±13.6	60.3±6.18
	p>0.05	p>0.05	
Hemoglobin, g/l	145±4.1	149±2.4	149±4.5
	p>0.05	p>0.05	
Erythrocytes, million in 1 μl	7.4±0.05	7.7±0.19	7.5±0.24
	p>0.05	p>0.05	
Leukocytes, thousand in 1 μl	15.8±2.64	14.5±0.74	16.1±1.6
	p>0.05	p>0.05	

Evaluation of the hazard of chronic poisoning with imino-compounds

Data on the results of chronic exposure to ethyleneimine in concentrations of 0.4±0.01 mg/m³ and 0.7±0.03 mg/m³ are shown in Table 115 (G. N. Zayeva et al., 1967).

Table 115

Indices characterizing the state of white rats in the dynamics of chronic inhalation exposure to ethyleneimine ($p<0.05$)

Index	Observation period, months								Recovery period
	1		2		3		4		
	Concentration, mg/m³								
	0.7	0.4	0.7	0.4	0.7	0.4	0.7	0.4	
Integral:									
body weight	0	0	0	0	0	0	0	0	0
STI	—	0	0	0	0	0	0	+	0
rectal temperature	0	0	0	0	0	0	0	0	0
Quantity in the blood of:									
hemoglobin	0	0	0	0	0	0	0	0	0
erythrocytes	0	0	0	0	0	0	0	0	0
reticulocytes	0	0	+	0	+	0	+	0	
leukocytes	0	0	0	0	0	0	—	0	
lymphocytes	0	0	0	0	0	0	—	—	
segmento-nuclear neutrophils	0	0	0	0	0	0	+	+	
Liver and kidneys									
A/G coefficient							—	0	
quantity of hippuric acid in urine, mg/24 hr							—	0	
quantity of protein in urine, mg/l							+	0	

Note: (+) increase; (—) decrease; (0) no changes.

As follows from Table 115, under a long-term exposure to ethyleneimine in a concentration of 0.7 mg/m³ there first appeared changes in the nervous system. One may assume that prolongation of the exposure would be accompanied by compensation of the mentioned disturbances. A stable increase of the reticulocytes quantity was being observed throughout the 2nd, 3rd and 4th months of exposure. By the end of the 4th month, the general quantity of leukocytes was found to decrease at the expence of lymphocytes, while the relative quantity of neutrophils increased. Changes in the functional condition of the liver were discovered during the 4th month of exposure. Exposure to a lower concentration of ethyleneimine (0.4 mg/m³) produced an intensification of inhibitory processes at the end of the 4th month. The character of changes in the leukocytic formula of the blood was analogous to that of the changes occurring under exposure to higher concentrations of the poison. This, evidently, indicates the existence of early lesions of the lymphoid tissue.

It is known that ethyleneimine and a number of its derivatives have cytostatic activity. Tissues of the hematopoetic organs and especially of the bone-marrow are the most susceptible to the effect of these poisons. Exposure to these compounds is accompanied by a decrease of the mitotic index in granular-erythropoetic systems as a result of disturbances in the mitosis initiation. Damage of the producing capacity of the bone-marrow causes changes in the peripheral blood picture.

According to the assumption of I. A. Rapoport (1966), a certain regularity may be observed in the mutagenesis, caused by organic substances. This regularity manifests itself in the fact that in every homologous series there is usually only one member with a high mutagenic activity. As a rule, it is the first member of the series. In view of this fact, it is advisable, when considering the biological activity of ethyleneimine, to pay special attention to its mutagenic, gonadotropic and embryotropic activity. In 1962, I. A. Rapoport carried out investigations on mutagenic activity of ethyleneimine using the method of study of the sex-dependent mutations of drosophila (at the level of the lethal doses). The author found ethyleneimine to produce an increase of the considered effect by 270 times in comparison with the control.

Investigation of mutagenic activity of ethyleneimine on mammals has been conducted using cytogenetic analysis of the bone-marrow, the method of dominant lethal mutations (L. D. Katosova, 1973), as well as observations of the development of the offspring of the first generation (G. N. Zayeva et al., 1967). It has been found out that under a single exposure (in concentrations of 2.4 and 0.8 mg/m^3) and under chronic exposure (in a concentration of 0.8 mg/m^3) ethyleneimine produced an increase of the number of chromosomal aberrations in cells of the bone-marrow of white rats (at the stage of anaphase-telophase). Induction of dominant lethal mutations was observed also after a 2.5-month exposure to the poison at a concentration of 0.8 mg/m^3. Thus, mutagenic activity manifests itself under single exposures to ethyleneimine in smaller concentrations and under chronic exposure earlier than general toxic effect ($Lim_{ac\ integr}$ is 10 mg/m^3; $Lim_{ch\ integr}$ is 0.4 mg/m^3). Examination of the 1st and 2nd generations from male rats which had been subjected to subacute and chronic inhalation exposure to ethyleneimine (G. N. Zayeva et al., 1967) revealed disturbances in the functional condition of the higher nervous system in females of the first generation, and a decrease in the vital capacity of the offspring of the 2nd generation.

I. V. Silantyeva (1973) investigated the embryotropic effect of ethyleneimine in accordance with the guidelines on methods for substantiating the thresholds of the effect of industrial poisons on the generative function. The author showed that a concentration of ethyleneimine equal to 0.01 mg/litre (Lim_{ac}) caused changes

in most indices throughout the pregnancy period. The effect of the product in a concentration one order lower (0.002 mg/l) was accompanied by changes in the quantity of yellow corpuscles, sites of implantation and in living embryos. And, finally, the effect of the poison in concentrations at the level of 1/2 of the Lim_{ac} did not bring about any changes in the indices. However, a single exposure in the mentioned concentration, undertaken on the 4th day of pregnancy, caused changes in the size of embryos. According to the author, a concentration of ethyleneimine equal to 1/2 of the Lim_{ch} (0.2 mg/m^3) may be considered as the threshold concentration by the embryotropic effect. Thus, values of the thresholds of embryotropic and general toxic effect are slightly different under exposure to ethyleneimine.

Investigation of the gonadotropic effect of ethyleneimine has been carried out by E. M. Tchirkova (1970). The threshold concentration ($Lim_{ac\ spec}$) of ethyleneimine under a single exposure, judging from the decrease of the quantity of normal spermatogonia (by 40%, as compared to the control), appeared to be the value of 0.8 mg/m^3. Those changes went beyond the limits of seasonal fluctuations. Hence, the gonadotropic effect is specific for ethyleneimine.

Information on the qualitative characteristics of chronic exposure to pyrrolidine has been obtained from an experiment on rats, exposed to the poison at a concentration of 2.6 ± 0.97 mg/m^3 (G. N. Zayeva et al., 1974). Already by the end of the 1st month, an increase in the excitability of the nervous system, which persisted throughout the experiment, has been found. A significant increase of the permeability of the skin capillaries, which persisted even at the end of the recovery period, has also been revealed (L. A. Bazarova, 1970): this corresponds to the 3rd criterion of harmfulness, according to the MACs Section classification. Under the mentioned conditions of the experiment, the product caused manifest changes in the testis (changes of the spermatogenetic index, etc.).

To evaluate the hazard of chronic poisoning with piperidine, L. A. Bazarova (1970) used two concentrations: 0.01 ± 0.001 and 0.02 ± 0.0003 mg/litre. The experiment was conducted on rats and rabbits (Table 116).

The experimental data show that chronic exposure to piperidine is characterized by changes in the functions of a number of systems and organs: nervous, vascular, hemopoetic, urinary. Exposure to piperidine at a concentration of 10 ± 1 mg/m^3 produced manifest changes in some indices. The beginning of exposure was accompanied by a decrease in the body weight. This index remained changed even at the end of the recovery period.

Early changes were registered also in the cardiovascular system. By the end of the 4th month of exposure, there occurred a stable increase of the permeability of capillaries, which remained

Table 116

Indices of the state of animals in the dynamics of chronic inhalation exposure to piperidine

Index	Examination period, months								Recovery period	
	0.5		1.5		2.5		4			
	concentration, mg/m³									
	10	2	10	2	10	2	10	2	10	2
Body weight										
rats	—	0	0	0	0	0	0	0	—	0
rabbits	0	0	0	0	0	0	0	0	0	0
STI	0	0	—	0	—	—	0	0		
Respiration rate (rats)	0	0	0	—	+	0	0	0		
Cardiovascular system										
ECG: systoles rate										
(rats)	0	0	0	0	0	0	0	0		
(rabbits)	0	0	0	0	0	0	0	0		
Arterial pressure										
(rats)	0	0	0	0	—	0	—	0	0	0
(rabbits)	—	—	0	0	0	0	0	0	0	
Steadiness of the skin capillaries (rats)	—	0	0	0	0	0	—	0	—	0
Permeability of the skin capillaries (rats)	+	0	0	0	0	—	—	—	—	—
Liver										
quantity of hippuric acid							—	0		
SH groups							0	0		
function of protein formation							0	0		
Kidneys										
diuresis								0		
relative density							+	0		
protein quantity							+	0		
Quantity in the blood of:										
hemoglobin	+	0	—	0	0	0	+	+	+	0
erythrocytes	0	0	—	0	0	0	0	0	+	0
reticulocytes	0	0	0	0	0	0	0	0	—	0
leukocytes	0	0	0	0	—	0	0	0	0	0
lymphocytes	0	0	0	0	—	0	0	0		
segmento-nuclear neutrophils	0	0	0	0	+	0	0	0		

Note: (+) increase; (—) decrease; (0) no changes.

irreversible even at the end of the recovery period. At the end of the exposure, there were changes in the ECG of rabbits: moderate fall of the ST segment and lowering of the T wave in respect to the isoelectric line. Pathomorphological investigations revealed focal productive myocarditis with initial phenomena of cicatrization. These changes appeared to be even more marked at the end of the recovery period, which indicates an intensification of the process in myocardium.

A long-term exposure to piperidine at the mentioned concentration caused lesion both of the tubular and glomerular regions of kidneys, which was confirmed by an increased quantity of protein in urine, by an increase of the relative density of urine and by a decrease of diuresis. Pathomorphological investigations of kidneys revealed sharply marked albuminous (up to hyaline-drops) dystrophy of the epithelium of convoluted tubules, and an en-largement of nuclei of endothelium with their polymorphism in some Malpighian bodies.

Throughout the period of chronic exposure to piperidine, there were being registered changes in the red blood, followed by an increase of the hemoglobin content at the beginning of exposure and at the end of the recovery period. The end of the recovery period was also characterized by an increase of the quantity of erythrocytes and by a decrease of the quantity of reticulocytes. Leukopenia in blood as a result of lymphopenia was found after 2.5 months of exposure. The testis changes were accompanied by a decrease of the number of N-spermatogonia, and by an increase of the quantity of tubules with peeled off embryonic epithelium, while the quantity of spermatogonia remained decreased even at the end of the recovery period.

A long-term exposure to piperidine at concentrations within the range of 2 ± 0.3 mg/m^3 was not accompanied by general toxic effect. We tend to consider the revealed changes in cardiovascular system as a manifestation of the specific effect of piperidine. We think that this concentration is close to the chronic effect threshold.

Indices of the hazard of chronic poisoning with ethyleneimine and piperidine are given in Table 117.

As follows from Table 117, ethyleneimine has a much higher real and potential hazard of chronic poisoning than piperidine. The difference, according to all indices, except that of the chronic effect zone, is one order.

Table 117

Major hazard indices of chronic poisoning with ethyleneimine and piperidine

Substance	Lim_{ch}, mg/l	$Z_{ch}=\frac{Lim_{ac}}{Lim_{ch}}$	$\frac{Lim_{ch}}{C^{20}}$	$\frac{C^{20}}{Lim_{ch}}$
Ethyleneimine	0.0004	25	0.000001	935 000
Piperidine	0.002	10	0.00002	46 500

Some aspects of the problem of metabolism of ethyleneimine and piperidine

Investigations carried out by G. N. Zayeva et al. (1974) showed that, under a single exposure to ethyleneimine at different levels and under different routes of administration into the organism, free ethyleneimine is found in blood and urine in a much smaller amount than the administered dose. It has been determined that there is a correlation between the level of the poison concentration in the air of exposure chambers and the quantity of free ethyleneimine in the blood of the experimental animals. Wright and Rowe (1967) showed that 50% of ethyleneimine, introduced intraperitoneally is excreted with urine within the first 24 hours, while a large quantity of it is eliminated in the form of metabolites, and only a small amount is eliminated unchanged.

Piperidine was detected in urine of man and animals by Euler in 1945. According to his data, man normally excretes daily with urine from 3 to 20 mg/litre of piperidine in the form of a salt, having properties of the pure product. Rabbits excrete daily with urine 0.42 ± 0.3 mg/mlitre of the poison. Euler thought that there was a correlation between the quantity of the eliminated piperidine and the intensity of the muscular work. For example, after physical training the quantity of piperidine in the urine of people increases. It has been determined that the amount of piperidine in urine varies considerably during 24 hours: more piperidine in the morning than during the day. Smokers have more piperidine in urine than nonsmokers. Thus, piperidine appears to be a natural metabolite, the quantity of which in the organism depends on a number of external factors. There are several hypotheses of the origin of piperidine in the organism. For example, Williams (1959) presumed that this compound was formed at the stage of intermediate exchange, probably from lisine and cadaverine by means of decarboxylation. The author showed that under per os administration of cadaverine to rabbits the quantity of piperidine in excretions increased, under administration of lisine this increase was insignificant. According to Nordenstrom (1951), piperidine is formed from cadaverine as a result of vital activity of the intestinal bacteria.

For preventive purposes, it is important to study the transformation of exogenous piperidine in the organism. Thus, under administration of the product to rabbits, piperidine is eliminated from the organism unchanged (Hildebrant, 1900). According to Novello, Harrow, Sherwin (1926), piperidine is not metabolized in the organism of warm-blooded animals. Absence of the differences in species sensitivity to the effect of piperidine (L. A. Bazarova, 1970) grounds the supposition that piperidine is not subjected to any transformations in the organism of man either.

According to V. V. Kustov and L. A. Tiunov (1969), in hygienic standardization of chemical pollutants in the environment which are natural metabolites, it is necessary to take into account their normal content in the biomedia. Thus, an increase of the carbon dioxide concentration in the inhaled air, corresponding to an increase of the concentration of carboxyhemoglobin in blood by 3%, is already significant. Similar data have been also cited for acetone and ammonia. Consequently, the usual limits of adaptability of «functional systems», responsible for natural metabolism of many products, are linked with the increase of concentrations of these products in the biomedia by not more than 2—3 times in comparison with norm.

L. A. Bazarova and N. I. Osipenko (1967) have found that the blood of intact rats contains on the average 0.09 ± 0.003 mg%, and the blood of rabbits, 0.14 ± 0.04 mg% of piperidine. The largest quantity of piperidine under a single exposure is revealed 30 min after its administration. An approximately equal quantity of piperidine in blood is found under exposure by inhalation and under administration into the stomach at the isoeffective level. A distinct correlation between the value of administered dose and the quantity of piperidine in blood has been discovered. The authors, however, consider it inexpedient to develop the exposure test on free piperidine, since the product represents a natural metabolite and its quantity depends, to a considerable degree, on a number of factors.

Morpholine, N-methyl- and N-ethylmorpholine. Evaluation of toxicity and hazard[1]

Morpholine and its derivatives are used in the production of synthetic rubber, in pharmaceutical and paint-and-varnish industry, in the production of plastic materials, bleaches, etc. These compounds are widely used in large-scale industrial production, therefore the number of persons working in contact with these poisons grows.

Morpholine, N-methyl- and N-ethylmorpholine are colorless liquids with sharp odour and high solubility in water and in a number of organic solvents. The main physico-chemical properties of these compounds are given in Table 118. Thus, the vapour pressure and volatility of N-ethylmorpholine are lower than those of other compounds, which is an index of a smaller potential hazard of acute poisoning. Good water solubility implies slow saturation

[1] The data by N. V. Migukina (1971).

Table 118

Some physico-chemical properties of morpholine and its derivatives

Properties	Morpholine	N-methylmorpholine	N-ethylmorpholine
Molecular weight	87	101	115
Relative density at 20° C	0.9998	0.9213	0.9872
Boiling point at 760 mm of Hg	130	115.6	138.3
Vapour pressure, mm of Hg at 20° C	11.1	16.6	6.1
Volatility, mg/l at 20° C	53	92	38
pH	11.9	10.2	8.1
Solubility in water	∞	Good	Good

of the organism (N. V. Lazarev, 1938; N. A. Tolokontsev, 1960). There also draws one's attention the consequent lowering of pH from 11.9 for morpholine to 8.1 for ethylmorpholine, which, undoubtedly, must influence the irritant properties of the compounds.

The data from the literature on the toxicity of morpholine and its N-alkyl-derivatives are rather few. There are some data on the DL_{50} for rats (1.6 g/kg) and for guinea pigs (0.9 g/kg). In a concentration of 12,00 ppm (about 43 mg/litre) the poison exerts a strong irritant effect on man (Shea, 1939). Smyth et al. (quoted from Patty, 1965) cite the data on the threshold value of the irritant effect of N-ethylmorpholine on man (220 mg/m^3). The CL_{50} value of morpholine for mice equal to 3 mg/litre, obtained by G. N. Zayeva and Tsirin (1966), is given, apparently, for aerosol of morpholine, which had been heated before being supplied into the chambers.

Characteristics of the hazard of lethal poisoning under exposure to morpholine and its N-alkyl-derivatives

Exposure to morpholine vapours under usual conditions, without heating the substance, produced only the minimum lethal concentration. This fact is apparently related to the capacity of the substance to react easily with CO_2 of the air, forming the salt (morpholine carbonate). Table 119 shows the results of acute experiments.

According to the given absolute indices of toxicity, the substances under investigation belong to class III, i. e., to moderately hazardous compounds.

Relative indices of the hazard of the investigated substances are presented in Table 120. From Table 120 follows that, according to all indices, the hazard degree of the substances in acute experiments is not high and is practically equal.

Table 119

Lethal concentrations of morpholine, N-methyl- and N-ethylmorpholine for mice under a single 2-hr exposure

Substance	CL_{16}, mg/l	CL_{50}, mg/l	CL_{84}, mg/l	CL_{50}, μ mole/l
Morpholine	10±1.5	12	15	136
N-Methylmorpholine	19.8	25.2	29.8	247
N-Ethylmorpholine	15	18	22	156

Note: The CL_{50} for N-ethylmorpholine was determined by the Van der Waerden (1940) method of one point. The CL_{50} for morpholine was assessed graphically.

Table 120

Indices of the hazard of acute lethal poisoning with morpholine, N-methyl- and N-ethylmorpholine under inhalation exposure

Substance	CPPI	Relative toxicity, %	$\frac{CL_{84}}{CL_{16}}$	S	$K=\frac{I}{CL_{50}\cdot S}$
Morpholine	4.4	25	1.5	1.23	0.06
N-Methylmorpholine	3.7	23	1.51	1.23	0.03
N-Ethylmorpholine	2.1	25	1.47	1.21	0.04

Values of the lethal doses of the compounds have been determined in experiments on rats and mice, and are shown in Table 121.

Table 121

Lethal doses of morpholine, N-methyl- and N-ethylmorpholine under administration into the stomach of animals

Substance	Doses, mg/kg		
	DL_{16}	DL_{50}	DL_{84}
Morpholine			
rats	950	1220±140	2370
mice	2000	2250±680	2810
N-Methylmorpholine			
rats	1300	1917±117	2340
mice	1580	1733±122.4	1900
N-Ethylmorpholine			
rats[1]	680	1200	1730
mice	1200	1460±75	1700

[1] DL_{50} in experiment in rats was determined by the method of one poi

Table 122

Indices of the hazard of lethal poisoning with morpholine, N-methyl- and N-ethylmorpholine under administration into the stomach of animals

Substance	$\frac{DL_{84}}{DL_{16}}$	S	$K=\frac{1}{DL_{50} \cdot S}$
Morpholine			
rats	2.49	1.6	0.0005
mice	1.4	1.18	0.0003
N-Methylmorpholine			
rats	1.80	1.34	0.0004
mice	1.2	1.09	0.0005
N-Ethylmorpholine			
rats	2.54	1.6	0.0005
mice	1.4	1.19	0.0005

The indices given in Table 122 fail to reveal any significant difference in the degree of the hazard of poisoning with the compounds under administration into the stomach, and characterize all 3 compounds as slightly hazardous (class IV by the mentioned classification).

Qualitative characteristics of the hazard. The clinical picture of the acute inhalation poisoning with morpholine, N-methyl- and N-ethylmorpholine is characterized by a marked irritant effect. Animals exhibit strong motor excitation, blepharospasm, lacrimation. By the end of the exposure, excitation is replaced by languidness and inhibition. In response to sound stimulus, spasms appear. Death occurs as a result of respiratory failure, while the heart continues to contract. The strongest irritant effect is that of morpholine. Death of the animals exposed to the substances occurred either during the exposure, or some hours after the exposure, but not more than 24 hours later.

When the lethal doses of the poisons were administered into the stomach of experimental animals, death occurred within two days. The animals looked scruffy and lost weight; sanguineous discharges oozed out from the nose and anus.

Acute plethora of all internal organs and brain was found during microscopic investigation of the organs of the animals which died after inhalation of the poisons. Microscopy of the organs of the rats which died within 24 hours after administration of the substances into the stomach revealed the same type of changes: plethora, hemostasis, diapedesis, and phenomena of oxygen deficiency of tissues resulting from these changes. In order to obtain a differentiated picture of the developing pathology in the animals to which the poisons were administered in the DL_{16} dose, the animals were killed on the 8th day, when acute symptoms in the survived animals subsided and changes in the «critical» or-

gans became prevailing. The most marked changes in the organs were observed in rats which were exposed to morpholine, the least marked ones were registered under administration of ethylmorpholine; the exposure to methylmorpholine held intermediate position.

Under exposure to morpholine, the following changes were revealed: dilatation of the liver capillaries; small-drops dystrophy in hepatic cells in the centre of lobes; grain dystrophy of proximal regions of convoluted tubules in kidneys; peeled off cells and hyaline casts in the lumens of the tubules. Pathological changes in other organs were not registered. Methylmorpholine caused analogous changes in liver; albuminous dystrophy of proximal regions of convoluted tubules was registered in kidneys. On the 8th day after the administration of ethylmorpholine, changes in the organs of experimental animals were not revealed[1].

Characteristics of the hazard of acute non-lethal poisoning with morpholine, N-methyl- and N-ethylmorpholine

The acute effect thresholds have been substantiated in experiments on rats under 4-hour exposures. Different functions of the animals have been investigated immediately after the exposure. The threshold effect was assesser by the following indices: STI,

Indices of the state of rats and a single

Concentration, mg/m³	STI, cond. units		Body temperature		Arterial pressure mm of Hg	
	test	control	test	control	test	control
1080	3.9±0.7	5.3±0.18	37.1±0.2	37.1±0.22	118±21.4	97±31.4
	$p<0.001$		—		$p<0.05$	
700	4±0.47	5.8±0.3	37.8±0.14	37.8±0.1	—	—
	$p<0.01$		—		—	—
280	3.0±0.2	6.3±0.3	36.8±0.2	37.0±0.21	118.3±17.2	132.5±17.4
	$p<0.001$		—		$p>0.05$	
120	4±0.92	5±0.77	37.2±0.24	37.0±0.3	101±10.4	98±8
	$p<0.05$		—		—	

[1] Pathomorphological investigations have been conducted by V. I. Govorchenko and V. I. Fiodorova.

respiration rate, arterial pressure, rectal temperature. The peripheral blood and functions of the liver and kidneys were analysed. Results were treated statistically. In view of a marked irritant effect of morpholine vapours, the threshold of the irritant effect was assessed on rats using the method of the vital coloration of pulmonary tissue by Y. I. Azhipa (1962), medified by A. L. Germanova (1970) (Table 123).

As can be seen from the data in Table 123, the morpholine vapours concentrations equal to 1,080 and 700 mg/m^3 caused changes in STI, respiration rate and produced leukopenia. Under exposure to the poison in concentrations of 280 mg/m^3 and 260 mg/m^3, there were changes in STI and respirations rate. A concentration of 120 mg/m^3 appeared non-effective. We presume that concentrations of 260—280 mg/m^3 are close to the acute effect threshold (Lim_{ac}), since the STI values of experimental animals went beyond the limits of natural fluctuations of STI of the intact animals.

Results of the substantiation of the acute effect threshold for morpholine by its irritant effect are given in Table 124. The results of the experiment made it possible to assess a concentration of 260 mg/m^3 as effective concentration, according to the irritant effect, and a concentration of 40 mg/m^3 as a concentration close to the threshold of the irritant effect for animals (Lim_{ir}^{kc}).

Since the experimental investigations of the acute effect thresholds revealed the presence of a marked irritant effect of morpholine (while the value of the Lim_{ir}^{kc} was smaller than the

Table 123

4-hr inhalation of morpholine

Respirations per min		Hb, g/l		Erythrocytes quantity, million in 1µl		Leukocytes quantity thousand in 1µl	
test	control	test	control	test	control	test	control
86±15	112±22	140±5.3	141±5.0	6.41±0.25	6.22±0.3	7.78±1.23	12.13±1.82
$p<0.05$		—		—		$p<0.05$	
78±3.5	97±5.5	138±2.8	140±3.0	—	—	10.85±0.5	18.6±0.72
$p<0.01$		—	—	—	—	$p<0.001$	
93±5	90±7.8	132±2.4	127±2.8	6.74±0.22	6.24±0.3	10.4±1.26	9.65±1.24
$p>0.05$		—		—		—	
86±6.2	88±5.7	154±5.2	154±6.6	7.40±0.31	7.52±0.28	10.4±1.13	11.6±1.27
—		—		—		—	

Lim_{ac}^{kc}, which attested to the specificity of the mentioned effect for morpholine in acute experiments), there appeared a necessity

Concentration, mg/m³	STI, conditional units		Respirations per min		Relative mass of lungs	
260	3.3±0.18	3.8±0.3	129±14.1 $p<0.05$	169±5.6	0.66±0.02	0.65±0.03
40	3.1±0.21	3.8±0.22	177±6.0	173±6.7	0.69±0.02	0.69±0.02
3	3.7±0.24	3.8±0.22	150±10.4	173±6.7	0.7±0.02	0.69±0.02

to substantiate the irritant effect threshold on human volunteers. During the exposure to the substances, which lasted 1 minute, subjective sensations of irritation of the upper respiratory tract and mucous membranes of the eyes were registered (Table 125).

Table 125

The threshold of smell and irritant effect for morpholine, N-methyl- and N-ethylmorpholine

Substance	Concentration, mg/m³		
	manifest irritation	irritant effect threshold	threshold of smell
Morpholine	33	16	3
N-Methylmorpholine	500	200	50
N-Ethylmorpholine	620	220	100

The investigations, conducted using the substances at the level of the acute effect threshold, revealed that the irritant effect was prevailing for morpholine. The magnitude of the irritant effect zone (equal to 7):

$$Z_{ir} = \frac{Lim_{ac}^{kc}}{Lim_{ir}^{kc}}$$

gives grounds to classify this effect as extremely marked (G. G. Maximov, 1969). The absolute morpholine concentration of 16 mg/m³, causing the sensation of irritation of the upper respiratory tract in 50% of people, makes it possible to assign this substance to class I (extremely hazardous compounds according to the classification of the hazard from irritants, A. A. Golubev, 1969). A decrease in the degree of manifestation of the irritant

Table 124

of morpholine by its irritant effect

Concentration, mg/m³	Coloration of pulmonary tissue during life-time[1]			
	accumulation		elimination	
260	0.79±0.0297	0.69±0.0158	0.028±0.0005	0.018±0.0015
	p=0.01		p=0.001	
40	0.667±0.022	0.638±0.0156	0.025±0.0008	0.016±0.01
			p<0.001	
3	0.66±0.0127	0.638±0.0157	0.016±0.0009	0.016±0.0012

[1] The data by A. L. Germanova.

effect of the substances makes it possible to assign the derivatives of morpholine to class III of the hazard according to this classification.

The conclusion that the 3 compounds under investigation have the same type of biological action has been made on the basis of observations of clinical manifestations of intoxication in animals in acute lethal experiments, especially of pathomorphological observations. Therefore, the STI has been chosen as the main index for determining the acute effect thresholds of N-substituted compounds of morpholine. Minimum concentrations of substances which caused a statistically reliable effect have been chosen as threshold concentrations. The value of 100 mg/m³ was found to be the threshold concentration for methylmorpholine, and 90 mg/m³ for ethylmorpholine.

Comparison of the effectiveness of action of the substances in absolute concentrations at the Lim_{ac} level, according to changes of the general index of STI in rats, shows that morpholine exhibits a less effective action on the nervous system than its derivatives. Relative values, however, and the acute effect zone, in particular:

$$Z_{ac} = \frac{CL_{50}}{Lim_{ac}}$$

permit one to consider morpholine as the most hazardous compound (class III). The Z_{ac} for this poison is equal to 43, and the Z_{ac} for N-methyl- and N-ethylmorpholine is equal to 252 and 200, respectively (class IV of the hazard by the classification of the MACs Section, 1970).

Thus, investigations of the substances in acute non-lethal experiments have revealed both the hazard degree of each compound and specific features of their effect.

Characteristics of the hazard of chronic poisoning with morpholine and its N-alkyl-derivatives

A concentration of morpholine of 70 mg/m³ caused in experimental animals an intoxication, characterized by changes in the nervous system, lesion of the lymphoid tissue and of the associated immunologic system, as well as by changes in the functional condition of kidneys. At early period of exposure to the substance (2 weeks), an increase of excitability of the nervous system in rats, a decrease of excitability of the nervous system in guinea pigs and leukopenia were registered.

Periods of compensation (2nd-3rd months of the exposure) and of decompensation of the pathological processes were revealed during the chronic experiment. The main changes in the nervous system (a decrease of excitability in rats and its increase in guinea pigs) and in blood were revealed at the end of the exposures. Together with leukopenia of the peripheral blood, occurring mainly as a result of lymphopenia, the following phenomena were registered simultaneously: a decrease of the quantity of globulins in the blood serum, inhibition of the phagocytic activity of neurophilous leukocytes of the blood and a decrease in the relative mass of the spleen, the histologic investigation of which revealed a decrease of the number of lymphoid elements of follicules. Spleen did not recover completely from those changes after the first month of the recovery period (3rd criterion of the hazard by the classification of the MACs Section).

A distinct cytogenetic effect of morpholine in a concentration of 70 mg/m³ was found when investigating the cellular composition of the bone-marrow of rats by the method of anatelophase analysis. The increment of the number of chromosomal rearrangements in the cells of the bone-marrow of rats reached $7.7\pm\pm0.2\%$ by the 4th month of the exposure, as compared to $2.5\pm\pm0.14\%$ in the control. Major categories of the rearrangements were represented by fragmentized arches and separately located fragments.

Under exposure of the animals to a morpholine concentration of 8 mg/m³, the character of the changes was the same as under exposure to the poison in a concentration of 70 mg/m³. These changes were, however, less manifested and did not exceed the limits of physiological fluctuations. Nevertheless, pathomorphological investigations of the internal organs of experimental animals revealed changes mainly in the lymphoid tissue: a decrease of the quantity of lymphoid elements in the spleen and in lymph nodes. After a month of the recovery period these changes were not registered any more.

Thus, the distinguishing features of chronic intoxication with

morpholine are the damage of the lymphoid tissue and the effect on the nervous system.

To characterize the hazard of chronic poisoning with morpholine, the magnitude of the chronic effect zone (equal to 35)

$$Z_{ch} = \frac{Lim_{ac}}{Lim_{ch}}$$

has been used (I. V. Sanotsky, 1962). This value characterizes the substance as extremely hazardous under chronic administration into the organism (class I, according to the classification of the MACs Section). The potential hazard, taking account of the volatility of the substance, is slightly lower (class II of the hazard).

Some aspects of the morpholine metabolism

According to Maller and Heidelberger (1957), morpholine is metabolically inert and stable. In investigating the metabolism of carcinostatic preparation OPSPA, the structure of which includes the morpholinic ring, the authors tagged it by means of radio-carbon (C^{14}). It has been found that morpholine was eliminated mostly (87%) with urine in the form of a simple salt.

In our own experiment, we have tried to compare the content of morpholine in the blood of experimental animals with its concentration in the exposure chambers. However, since there is no method which would allow one to assess the morpholine content separately from the content of other imino-compounds, we did not manage to find any correlation. The development of a chromatographic method for assessing morpholine in urine, which could be used as a basis for working out the exposure test, is urgent.

Main parameters of toxicometry of heterocyclic compounds — ethyleneimine, pyrrolidine, piperidine, hexamethyleneimine, morpholine, N-methyl- and N-ethylmorpholine — are given in Table 126.

As has already been shown, the most toxic compound under administration into the stomach is ethyleneimine. The value of its median lethal dose, determined for a 0.4% water solution, makes it possible to assign it to class II, i. e., to highly hazardous compounds in view of the development of acute lethal poisoning (classification of the MACs Section). Piperidine and hexamethyleneimine belong to the same class of the hazard. Morpholine and its derivatives belong to classes III and IV of the hazard of acute lethal poisoning. Values of the median lethal concentrations also increase from ethyleneimine to morpholine and its derivatives. The most distinct decrease of the hazard of actute lethal poisoning may be observed from ethyleneimine to

Main parameters of toxicometry and

Index	Ethyleneimine	Hazard class	Pyrrolidine	Hazard class	Piperidine	Hazard class
DL_{50}, mg/kg	17.3 (0.4%)		250 (10%)		371 (8%) 22.4 (100%)	II
S	1.85		1.4		1.2	
$\frac{1}{DL_{50} \cdot S}$	0.03		0.0028		0.0022	
CL_{50}, mg/l	0.4	I	1.5	II	6.5	III
S	1.6		1.6		3.0	
$\frac{1}{CL_{50} \cdot S}$	1.5		0.4		0.05	
$CPPI_{CL}$	935	I	130	II	15.0	III
Lim_{ac}, mg/l	0.01	II	0.03	III	0.02	III
$CPPI_{ac}$	37 400		6466		4650	
Z_{ac}	40	III	50	IV	300	IV
$\frac{Lim_{ac}}{C^{20}}$	0.000028	I	0.00015	II	0.0002	II
Lim_{ch}, mg/l	0.0004	I	—		0.002	
$CPPI_{ch}$	935 000	—		—	46 500	
Z_{ch}	25	I			10	II
$\frac{Lim_{ch}}{C^{20}}$	0.000001	I			0.00002	I
MAC, mg/m³	0.02		0.1		0.2	

the morpholine derivatives, following the values of the $CPPI_{CL}$. This index permits one to assign ethyleneimine to class I (extremely hazardous compounds); pyrrolidine, to class II; and piperidine and all the next compounds, to classes III and IV of the hazard. The indices of the acute non-lethal poisoning also characterize ethyleneimine as the most hazardous compound among those considered. All the other compounds, except morpholine and methylmorpholine, have approximately equal effectiveness.

The biological activity of ethyleneimine is especially emphasized under chronic exposure. Comparison of the Z_{ch} values, however, shows that morpholine presents a higher hazard. Piperidine is highly hazardous in view of development of chronic poisoning (class II). The type of the effect of the considered substances (ethyleneimine, piperidine and morpholine) in chronic experiment is different. The data obtained make it possible to substantiate the MACs for all these compounds. The MACs values for the most active ethyleneimine, and for the least active derivatives of morpholine, differ by two orders. The effectiveness of these com-

Table 126

hazard classes of heterocyclic compounds

Hexamethylenefmine	Hazard class	Morpholine	Hazard class	Methylmorpholine	Hazard class	Ethylmorpholine	Hazard class
360 (8%)		1200	III	1917	IV	1200	III
22.4 (100%)	II						
1.2		1.6		1.34		1.6	
0.0022		0.0005		0.0004		0.0005	
10.8	III	12.0	III	25.2	III	18.0	III
1.9		1.23		1.23		1.21	
0.048		0.06		0.03		0.04	
3.0	III	4.4	III	3.7	III	2.1	IV
0.09	III	0.28	IV	0.1	III	0.09	III
300		189		920		422	
120	IV	43	III	252	IV	200	IV
0.003	III	0.005	III	0.001	III	0.002	III
—		0.008	II	—		—	
—		662		—		—	
—		35	I	—		—	
—	—	0.001	II	—		—	
0.5		0.5		5.0		5.0	

pounds at the acute effect threshold differs only by one order. When substantiating the MAC for piperidine in the air of workplaces, the results of clinico-hygienic observations have been taken into account.

Chapter 10. PERSPECTIVES OF THE USE OF HARMFULNESS CRITERIA AND OF CLASSIFICATIONS OF THE HAZARD POSED BY COMPOUNDS IN HYGIENIC PRACTICE

As has been demonstrated, a large group of the harmfulness criteria established by the MACs Section is based on the concept of physiological norm. It is an important methodological principle of every investigation on the interrelation between the organism and the environment as well as of investigations in the field of biology and pathology, including medical genetics (G. I. Tsaregorodtsev et al., 1973), to take into account the biological norm of the organism's adaptive capacities. The development of the dialectics of interrelation between the economic and humane approaches to the transformation of nature under the conditions of socialism is among the most important tasks facing the science.

It should be remembered that there have been objections to the concept of phylogenetically motivated physiological norm. This issue has been considered in detail by G. Yugai (1973), who advocated the practical importance of this concept. We share this point of view, although the practical realization of this idea faces many difficulties, which have been mentioned above. Tables 1—6 of the Annex give physiological variations of some indices, calculated for mature white rats of the genetic inbred line of the Central Nursery of Laboratory Animals of the USSR AMS. The indices are given for two periods of observation. These data indicate that in some cases the physiological norm really appeared to be insufficiently stable, which may be related both to the sparsity of the material used in the investigation and to systematic errors in calculations.

Besides further efforts to improve conditions of the laboratory animals, when substantiating the criteria of harmfulness of given changes, we have used not only the data given in the Annex, but also the seasonal physiological variations of the indices for the intact animals acquired at the same time as the experimental animals. Nevertheless, the information given in Tables 1—6 of the Annex provides, in our opinion, an important reference-point for evaluating the harmfulness criteria according to the MACs Section classification.

The considered principles and practical methods for differentiating **simply effect** of substances from their **harmful effect** on

the organism for the purpose of determining the harmful effect thresholds, which underlie the methods for assessing the hazard from compounds, have made it possible to establish the hazard indices classifications, which can be used in systems of preventive and routine hygienic control. Using the analysis of major toxicometric parameters and their ratios, it is possible to propose classifications of the degree of the potential and real hazard presented by occupational, communal and domestic substances under a single and chronic exposures. The most developed are the classifications of the hazard from compounds for occupational conditions of exposure.

The application of the quantitative hazard criteria based on the data on the poisons' metabolism and determination of the correlation between various indices of the hazard and the MACs may lead to the substantiation of accelarated methods of hygienic standardization (limitation of toxic substances content in the media), and to the substantiation of other preventive measures. The determination of toxic metabolites in biosubstrates in comparison with the functional and structural changes enables one to differentiate, with a certain degree of reliability, between the stage of real adaptation and the compensation of a pathological process, and therefore to make proper substantiation of preventive measures. However, the concept of «critical organ», which is being persistently transferred to toxicology from radiobiology, cannot be at present recognized as «mature», especially as radiobiologists themselves are inclined to reconsider it. At the same time, the metabolic criteria seem to be promising in the field of complex hygienic standardization (N. F. Izmerov, I. V. Sanotsky, 1974).

Classification of poisons by the quality of the effect

The classification of poisons according to the quality of their effect has a high importance. First attempts to establish such classifications were made in military toxicology and were considered in the works of V. I. Glinchikov (1929), P. S. Sakharov (1937), F. I. Rachinsky (1940), A. I. Tcherkes, N. I. Lugansky, P. V. Rodionov (1964). These classifications were compiled on the basis of the chemical composition of the poisons. The shortcomings of the mentioned classifications consisted in the fact that substances with different chemical structure and the effect were often included in the same group. Moreover, different types of interaction between the poison and the organism and considerable changes of properties of substances under introduction of various radicals were not taken into consideration. As has been rightly noted by N. P. Kravkov, «a classification based on the chemical pro-

perties of substances only cannot be considered satisfactory enough, since the substances which are rather close to each other from the chemical point of view may exhibit different effect, while substances, having nothing in common in terms of chemistry, may produce the same effect.

The classification of G. V. Khlopin was of physiologico-toxicologic character. G. V. Khlopin (quoted from V. Glinchakov, 1929) divided substances into the following 5 classes: asphyxiators; eyes irritants or lacrimators; those causing fast poisoning with lethal outcome; those having slow toxic effect; aggravators and fumigators.

Very close to it is the classification of Vedder (quoted from V. Glinchakov, 1929): lungs irritants; vesicants; lacrimators; irritants; those affecting directly the nervous system; those disturbing the respiratory capacity of the blood. The same may be said about the classification of Lindeman (quoted from V. Glinchakov, 1929): substances producing general effect; substances affecting the skin; substances affecting reflexes; specific poisons affecting respiratory organs.

Kornubert (quoted from F. I. Rachinsky, 1940) based his classification on symptoms produced by substances, but, unfortunately, not always on the main symptoms from the viewpoint of pathogenesis (asphyxiators, lacrimators, toxic substances, vesicants, sternutators). Similar shortcoming has the classification of N. A. Soshestvensky (quoted from P. S. Sakharov, 1937), which distinguishes 3 types of poisons according to their action (asphyxiators, phlogogenic and reflectory). The classifications of Moignier and A. I. Tcherkes have been compiled taking account of the pathogenesis of intoxication.

The classification of Moigner (quoted from F. I. Rachinsky, 1940) looks as follows. (1) TS (toxic substances) exerting chemical effect on every living cell and killing it: TS having low solubility in lipoids of living tissues and high reactivity; their effect is fast, but superficial (like that of asphyxiators). TS having good solubility in lipoids, chemically stable, which affect slowly, but deeply (vesicant type). (2) Poisonous substances with general toxicity, affecting all living cells, but not forming chemical compounds with tissue cells (anesthetic type). (3) Irritant TS which affect nerve termini and separate cells, but neither kill nor exhibit soporific effect on them (lacrimators and sternutators type).

According to the quality of the effect, poisons have been classified also by Henderson and Haggard (quoted from F. Flury, F. Zernik, 1938). (1) Asphyxiant gases: I) common; II) biochemically effective. (2) Irritants. (3) Inhalation narcotics and congeneric compounds: narcotic gases not causing manifest consequences.

According to the classification of A. I. Tcherkes, A. F. Leschinsky (A. I. Tcherkes et al., 1964), substances are divided into the following classes: (1) Substances affecting the higher branch of the nervous system. (2) Substances affecting afferent (sensory) nerve termini: I) affecting receptors of the eye mucous membrane; II) receptors of the upper respiratory tract; III) receptors of the lower respiratory tract; IV) skin receptors. (3) Substances affecting the region of efferent nerves.

There were attempts (Oswald, 1924; Frankel, 1927) to reduce the variety of the effects of substances to 3 types: methane, benzene and ammonia. This division turned out to be artificial and did not find application. According to the character of the effect of poisons on the enzymatic systems, there have been many classifications (A. A. Pokrovsky, 1962; L. A. Tiunov, 1963; E. Y. Golubovich, 1970; M. Golddblatt and Y. Goldblatt, 1960, et al.).

L. A. Tiunov (1963) suggested the following classification. (1) Structural analogues of the substrate (antimetabolites). (2) Poisons representing the substrate of the effect of enzyme. (3) Poisons affecting the prosthetic group of enzymes: I) poisons disturbing the synthesis of the prosthetic group; II) poisons reacting with functional groups or with the metal of coenzyme; III) poisons which are structural analogues of enzymes. (4) Poisons affecting the protein part of the enzyme, the apoenzyme: I) poisons disturbing the synthesis of the protein part of the enzyme. Structural analogues of amino acids; II) poisons reacting with functional groups of the apoenzyme; III) protein denaturing poisons. (5) Poisons binding the activator necessary for functioning of the enzymatic system. (6) Poisons affecting one-component enzymes. (7) Poisons damaging intercellular structures. (8) Poisons causing inhibition of enzymatic systems as a result of lethal syntheses. (9) Poisons which are inhibitors of the free-radical reactions. (10) Poisons containing enzymes or acting like enzymes. (11) Poisons affecting the production of hormones, regulating enzymatic reactions.

Among recent classifications there should be mentioned the classification of E. Y. Golubovich (1970). (1) Structural analogues of the substrate which directly react with the enzyme. (2) Precursors of the structural substrate's analogues from which they are formed as a result of the «lethal synthesis». (3) Poisons affecting coenzymes and cofactors: I) structural analogues of coenzymes; II) compounds disturbing the synthesis of the prosthetic groups; III) compounds reacting with functional groups of the enzyme; IV) substances binding the activating metal. (4) Compounds affecting apoenzymes: I) protein denaturing agents; II) substances disturbing the synthesis of protein; III) compounds reacting with functional groups of the apoenzyme. (5) Compounds disconnecting the coordinated activity of certain enzymatic systems in space and time. (6) Poisons of the biological origin. (7) Poisons affecting the hormonal production and regulating fermentative reactions.

There is a number of classifications based on the degree of manifestation of a given specific effect of poisons. Thus, N. I. Shumskaya, O. G. Alexeeva and N. K. Kulagina (1967) developed a classification of substances having the sensitizing effect; L. M. Shabad (1966) established a classification of blastomogenes; A. A. Golubev (1969) systematized compounds according to the irritant effect. A. A. Golubev based his classification on the value of the threshold concentration, causing irritant effect (Lim_{ir}) in man (Table 127). N. G. Ivanov (1974) supplemented this classification with the Lim_{ir} values for different spe-

Table 127

Classification of substances having irritant properties (according to the Lim_{ir} value)

Hazard class	Indices	Changes in subjective sensations of man	Changes in the respiration rate of rabbits	Changes in the respiratory system of rats	Hypersalivation in cats
I	Extremely irritant	Less than 20	Less than 500	Less than 50	Less than 900
II	Highly irritant	20—200	500—5000	50—500	900—9000
III	Moderately irritant	201—2000	5001—50 000	501—5000	9001—90 000
IV	Slightly irritant	More than 2000	More than 50 000	More than 5000	More than 90 000

cies of laboratory animals, using separate indices which had been calculated on the basis of comparison of the data from the literature.

It is well known that the general character of the poison's effect on the organism depends on the exposure level, i. e., the qualiative manifestation of the poison's effect at high concentrations often differ from the result of a long-term exposure at small concentrations. Thus, benzene in high concentrations exhibits narcotic and spasmodic effect, while under chronic exposure it affects the blood and hemopoetic organs. Aminobenzotrifluoride in large concentrations produces formation of methemoglobin and symptoms of hypoxia, while under chronic exposure it causes changes in the functional condition of the nervous system which are not related to hypoxia. It should be also taken into account that many poisons have several properties.

A new classification based on the type of the poisons' effect at low levels of exposure has been suggested as an example:

(I) extremely hazardous;
(II) moderately hazardous
(III) moderately hazardous;
(IV) sligthly hazardous.

I. Substances producing selective effect at remote periods:
1) blastomogenic;
2) mutagenic;
3) atherosclerotic;
4) causing sclerosis of organs (pneumosclerosis, nephrosclerosis, etc.);
5) gonadotropic;
6) embryotropic.

II. Neurotoxic poisons:
1) spasmodic and neuroparalysing poisons;
2) narcotics, causing damage of the parenchymatous organs;
3) substances with a pure narcotic effect.

III. Substances affecting the blood:
1) substances causing inhibition of the bone-marrow;
2) substances causing changes in hemoglobin;
3) hemolytics.

IV. Irritants and caustics:
1) substances irritating eye mucosa and the upper respiratory tract;
2) substances irritating the skin.

This classification, of course, needs some improvement. In particular, it does not cover numerous types of the effect and aspects of pathogenesis of intoxications. Inclusion of a substance into a given group indicates only the predominant effect of this substance under real conditions of production and application. Nevertheless, this classification allows one, to a certain extent,

to characterize the quality of the poison's effect in order to assess quantitatively the hazard posed by its effect to the organism.

The degree of irreversibility of changes could have been adopted as the basis of a classification of the hazard from substances according to the type of the effect. Each class of compounds has different degrees of the irreversibility of the effect, while, e. g., irreversible sterilization does not present a direct threat to life, the same as the death or damage of an embryo is not included in the classification of the causes of death, since, from the legal point of view, man «begins» only after the fetus is born. The mentioned types of the effect, however, present a high social danger, therefore, we assign them to the most hazardous types of the effects of poisons. It means that irritants, which we have assigned to the least hazardous substances according to the type of the effect, though they can produce irreversible changes, do not, however, present, from the hygienic point of view, such a high hazard as the class of substances mentioned above.

The same substance may simultaneously have several types of the effect, which complicates its assignment to this or that class of the hazard according to the quality of the effect. Therefore, the application of the hazard classification, based on the types of the effect of substances, is possible only if the limiting rial design and to ventilation equipment.

Medico-technical requirements and industrial production

The classification of the hazard from chemical substances, which we have developed together with S. D. Zaugolnikov[1], Y. S. Kagan et al., serves as a reference-point in the work of the MACs Section of the All-Union Problem Commission on Scientific Grounds of Labour Hygiene and Occupational Pathology. This classification has now found its reflection in the hygienic standards adopted by the USSR Gosstroy (the USSR Council's of Ministers State Committee on Construction). In the MACs list (CH 245-71) there has been included a special clause «Degree of the hazard posed by compounds», which specify the requirements to the industrial design and to ventilation equipment.

> 3.7. In designing the production of harmful substances of classes I and II of the hazard in closed premises, there should be, as a rule, provided the installation of production equipment in isolated cabins, premises or

[1] In accordance with the hazard classes, S. D. Zaugolnikov et al. (1970) have developed also requirements to marking, transportation, individual means of protection, etc.

zones with the control of this equipment from control panels or operator's zones.

5.13. It is allowed to provide the entry of the air from adjacent premises if harmful or unpleasantly smelling substances are not discharged in them, or if harmful substances belong to class IV of the hazard and their content in the incoming air dies not exceed 30% of the maximum allowable concentrations in the air of workplaces.

In this case, there should be provided the balance between the organized drawing in and drawing out of the air in order to meet the present standards on the purity of the air, regarding ventilation.

Note. The arrangement of exhaust ventilation with mechanical induction which is not compensated by the organized drawing in of the air is not permitted in buildings and constructions with stove heating.

5.16. For recycling, it is allowed to use the air of the premises where there are no discharges of harmful substances, or if the discharged substances belong to the class IV of the hazard and the concentration of these substances in the air supplied into the premises does not exceed 30% of the maximum allowable concentrations. In this case, requirements of clause 5.17 of the present norms should be taken into consideration.

It is allowed to provide the work of inlet systems for recycling during nonworking hours in cases when the possibility of residual discharges of harmful substances of classes I and II of the hazard is excluded (CH 245-71).

Certain medico-technical requirements have been developed for the use of substances in agriculture (for example, for pesticides). Special regulations on methods of work (All-Union Institute of Hygiene and Toxicology of Pesticides and Plastics, Kiev) provide for the possibility of the use in agriculture of only those substances the DL_{50} of which exceeds 50 mg/kg. The time of decomposition of substances regulates the time of treatment and awaiting.

Thus, F. Kaloyanova and A. Bainova (1973) proposed a new type of hygienic standards for agriculture: the minimum period of safe work (following the example of quarantine periods existing in food toxicology). The safe period of work is determined by the initial concentration of pesticides on plants. The safe period for the beginning of work after the plants treatment with organophosphorous pesticides was determined by means of a complex investigation of washes from leaves, from the workers' hands and

by exposure tests on the cholinesterase inhibition. Nomograms for practical use have been suggested. In the number of the items necessary for determining the MACs there has been included the requirement of the absence of changes in the alimentary properties of plants, etc.

Much less developed are medico-technical requirements for communal and everyday life conditions of exposure (except the MACs and ARQ). Although these values substantiate the necessary height of discharges, the magnitude of sanitary-protective zones and put some requirements to building materials, polymers used for medical and alimentary purposes, etc., one should, however, agree that medical regulations on production and use of chemical products, despite some principal achievements, still require further theoretical development.

Thus, A. S. Arkhipov et al. (1974) have noted the insufficiency of medico-technical requirements to the waste disposal in chemical industry, resulting in a situation when in a number of cases the protection of the factory grounds and residential areas against pollution is not completely ensured by basic calculations. We should try not to consider every industrial production as something original and unique, since such an approach leads to the substantiation of local preventive measures only and does not provide for formulation of generalized requirements.

Enlarged characteristics of substances (classifications based on particular practically applicable indices) must be used as the basis for putting appropriate requiments to equipment (for example, to sampling units, flanges, seals fittings etc.). The same is valid for the individual means of protection.

A number of gaseous and vaporous substances (oxygen, carbonic acid, nitrogen, hydrogen sulfide, carbon monoxide, carbon disulfide, dichlorethane, aniline, benzene, benzol, etc.) can enter the organism through intact skin and cause the development of intoxication. It is quite evident that the means used at present to protect the organism from the entry of the mentioned substances by protecting the respiratory organs only are insufficient. It is necessary to use also different means of skin protection (special clothes, in particular).

Complex evaluation of the hazard presented by substances

Great attention has been recently paid to investigations directed at a complex evaluation of the impact of chemical pollution of the environment on living organisms for the purpose of establishing complex hygienic standards.

It has been shown that in conditions of the field works in agriculture workers get only a tiny specific dose of pesticides from

the air, which presumably is the most contaminated working zone (zone of respiration). The specific dose of the poison intaken by the same workers from communal objects (the atmosphere of residential areas, water of reservoirs used for communal and domestic purposes) does not exceed 5%, while the specific dose of pesticides, intaken by the same people from food, is 92—95% (L. I. Medved et al., 1971). Thus, only a complex evaluation of all objects of the environment may protect agricultural workers from the effect of pesticides.

The same is true for many other substances. Thus, for example, carbon oxide — one of the most widely used occupational poisons — is, at the same time, a widely used communal and domestic poison. Symptoms of the effect of this poison on the organism (carboxyhemoglobinemia) have been revealed in 4/5 of persons living in apartments with natural gas supply (V. Z. Martynyuk, D. G. Devyatko, 1956). Regions of extracting and processing of oil may, to a full extent, be assigned to artificial geo-chemical provinces, since similar changes in regulation of the cardiovascular system have been found either in people working in the industry or in nonworkers (Z. Z. Zagidullin, 1966).

Certainly, the sources of discharges of many substances may be mainly (or exclusively) occupational or domestic. However, the intensification of chemization of everyday life and development of new branches of chemical industry are accompanied by an increase of the «metabolism» between different objects of human environment. Thus, up to recent times, natural food and fungous toxins were considered as mainly domestic poisons. Now, however, because of the development of the bacteriological industry, similar substances should be assigned to occupational poisons. Solvents, incluiding those aromatic or chlorinated, have long been mainly occupational poisons. Now, in spite of certain limitations, these compounds have become everyday life poisons.

Cases of a complex effect of substances present in different environmental objects attract once again the attention to the inadquacy of the hygienic evaluation of the living conditions of man from a narrow standpoint of a particular Problem Commission («Scientific Foundations of Labour Hygiene and Occupational Pathology», «Scientific Foundations of Hygiene and Toxicology of Pesticides, Polymers and Plastic Materials», «Nutrition of a Healthy and Sick Person», «Hygiene of Residential Areas», etc.) of a given department.

E. I. Spynu (1967, 1968) demonstrated that the quantities of pesticides, entering the organism, although being at the MAC level for each medium, exceed the safe dose (D_m), if summated. The author suggests to calculate this dose by the formula:

$$D_m = \sum_{i=1}^{4} D,$$

where D_1 is the dose of pesticide in alimentary ration; D_2 is the dose of pesticide intaken with inhaled air; D_3 is the dose of pesticide intaken with drinking water; D_4 is the dose of pesticide intaken with the inhaled atmospheric air (the author does not take into account D_5, i. e., the dose of pesticide absorbed through the skin under everyday life conditions, although the cutaneous resorption may be rather significant).

The necessity to determine the specific value of the MAC for each environmental object makes it difficult to use the method of calculation offered by E. I. Spynu. The author suggests the simplex method for designing the investigation.

A much simplier approach has been proposed by A. I. Korbakova et al. (1971). They believe that the formula of the summation of the effect is applicable for the evaluation and limitation of a complex effect of substances on man from different media:

$$\frac{C_{occup.}}{MAC_{occup.}} + \frac{C_{atm.}}{MAC_{atm.}} + \frac{C_{water}}{MAC_{water}} + \frac{D_{food}}{ARQ_{food}} \leqslant 1.$$

Unfortunately, hygienic standards are being established for environmental objects not always at the same time, which makes the realization of this proposal difficult.

Moreover, it is known that hygienic standards are being established on the basis of different principles. Resorptive effect, for example, is used only in establishing the MACs for the air of workplaces, the average daily MACs for the atmosphere of residential areas (the maximum single MAC is determined by the reflex effect), sometimes for water used for public and domestic purposes (by «toxicological» limiting index), for food (with the safety factor of up to 100). A significant difficulty consists also in the fact that the toxicity of the same substance may appear rather different under different routes of its entry into the organism (Table 128), which is related to changes in its metabolism, changes of interreception zones and to other reasons.

Although the ration of the real quantity of a poison in a given environmental object to its hygienic standard will not be changed in this case, the hazard characteristics under different methods of the substance administration often appear different. Thus, for irritants, the local concentration is more important than the absolute dose entering the organism. As an example, we will cite the data by A. M. Klyachkina (1973) on the effect of bromine. Under inhalation, it is a specific irritant poison, the effect of which produces changes in the respiratory system, having decisive importance at all levels of exposure. Enteric administration produces changes characteristic for the effect of the

Table 128

Toxicity degree of substances under administration into the stomach and by inhalation (I. V. Sanotsky, K. K. Sidorov, 1975)

Substance	Method of administration	
	into the stomach	inhalation
	DL_{50}, mg/kg	CL_{50}, mg/m³
M-Aminobenzotrifluoride	220 (III, moderately toxic)	440 (I, extremely toxic)
Benzyl chloride	1500 (III, moderately toxic)	390 (I, extremely toxic)
Benzal chloride	1400 (III, moderately toxic)	210 (I, extremely toxic)
Benzotrichloride	1300 (III, moderately toxic)	60 (I, extremely toxic)
Bromoacetopropylacetate	600 (III, moderately toxic)	149 (I, extremely toxic)
2-Vinylpiredine	420 (III, moderately toxic)	460 (I, extremely toxic)
Perfluoroadipinic dinitrile	1955 (IV, slightly toxic)	62 (I, extremely toxic)
Perfluoroglutaric dinitrile	997 (III, moderately toxic)	58 (I, extremely toxic)
Mesidine	372 (III, moderately toxic)	290 (I, extremely toxic)
Fluorochlorocarbon liquid	7600 (IV, slightly toxic)	930 (II, highly toxic)
2-Chloroethanesulfochloride	240 (III, moderately toxic)	250 (I, extremely toxic)

Note: Roman numerals indicate the class of toxicity according to the classification of the MACs Section.

bromine ion (changes in the nervous system, thyroid gland, etc.). Moreover, under inhalation, the effect has usually an intermittent character, while when the toxic substance is intaken with mater the effect is more continuous (monotonous).

The most feasible seems at present the establishment of two hygienic standards of the complex effect: that for the evaluation and control of the exposure by inhalation, and that for the control of the enteric exposure. If we consider the dose which has entered the organism, than a high importance may have the exposure tests (the MACs in biomedia, fluctuations of the enzymatic indices, etc.), which represents integral tests of resorption of the substance by the organism from the environment, with the account being taken of the cutaneous resoption (Tables 129—131, according to E. Piotrovsky, 1970). The exposure tests, however, cannot substitute for hygienic standards on the content of substances in the environment, and should be used together with them.

The classification of the hazard degree of substances under complex exposure has not been worked out yet due to the mentioned reasons. Its development will become possible only after all necessary data are collected.

Table 129

Substances for which comparative exposure tests are available

	Toxic substance	Substance under investigation	Medium
1	Aldrin, dildrin	H. E. C. D.	Blood
2	Antimony	Sb	Urine
3	Arsenic	As	Urine
4	Benzidine	Benzidine	Urine
5	Ethylene chloride	Ethylene chloride	Urine
6	Chromates	Cr	Urine
7	Hydrogen cyanide	Rodanides	Urine
8	DDT	DDA	Urine
9	Dichlorobenzene	Dichlorophenol	Urine
10	Dinitro-o-cresol	2-Amino-4-nitro-o-cresol	Urine
11	Fluorides	F^-	Urine
12	Formaldehyde	Formaldehyde	Urine
13	Malathion	p-Nitro-m-cresol	Urine
14	a-Methylstyrene	a-Phenyllactic acid	Urine
15	Parathion	n-Nitrophenol	Urine
16	Trinitrotoluene	2,6-Dinitro-4-amino-toluene	Urine
17	Uranium	U	Urine
18	Vanadium	V	Urine

Table 130

Substances which do not have interpretation in terms of biology

Toxic substance	Substance under investigation	Medium
Cadmium	Cd	Urine Blood
Manganese	Mn	Urine Blood
Nickel	Ni	Urine
Selenium	Se	Urine
Tellurium	Te	Urine
Thallium	Tl	Urine

Table 131

Substances for which quantitative exposure tests have been developed

Toxic substance	Substance under investigation	Medium
Aniline	p-Aminophenol	Urine
Benzene	Phenol	Urine
Carbon disulfide	Products of iodizide reaction	Urine
Ethylbenzene	Almond acid	Urine
Methanol	Methanol	Urine
Nitrobenzene	n-Nitrophenol	Urine
Lead	Lead	Urine
Styrene	Almond acid	Urine
Carbon monoxide	CO—hemoglobin	Blood
Toluene	Benzoic acid	Urine
Trichloroethylene	Trichloroacetic acid	Urine

Evaluation of the hazard from substances under mediate effect

All the data presented above concern mostly the direct effect substances on man. The mediate effect via ecological chains has not been yet sufficiently studied.

Approaches to the general evaluation of the allowable load on the external medium have been considered by Y. A. Izrael as well as by Y. A. Anokhin and I. I. Glazunov. They have suggested the following analytical expression of the general effect of pollutants:

$$A = \int\limits_{\vec{R}}\int\limits_{t} \sum_{m}\sum_{l}\sum_{l}\sum_{s} M(m) N_m(\vec{R}, t)(1 + \nu_m)\varepsilon^{j}_{mll} dt d\vec{R},$$

where A is general damage; $\vec{R}$ is space, t is time;

$N_m(\vec{R}, t)$ is distribution of organisms of the species;

$M(m)$ is the mass of organisms depending on their ecological significance; the magnitude of dependency on the role of the organisms' mass in the stability system;

ε_{mll}^{j} is the measure of biological effect;

m is the level for a given organism;

j is the character of the medium (air, soil, substerrenean waters, etc.);

li is the characteristic of heterogenicity (pollution, irradiation, heat, etc.);

$(1+\nu_m)$ is the characteristic of correlation between external factors, $\nu=0$ under independent effect, $\nu=1$ in case of additive effect, $1\leqslant\nu<1$ in case of antagonism, $\nu>1$ in case of synergism.

The analysis of this abstract scheme shows that the method of dispersion and dilution of pollutants does not ure the protection of the biosphere.

A short review of systems of mathematical modelling of the influence of the environmental factors on the dynamics of the population has been presented by M. Y. Antonovsky. The author has reported his own data on the dependency of the effects on the genetic structure of a population. These models may play a significant role in predicting the effects of pollutants and in the prevention of the pollution of the biosphere and of its different objects.

When analysing a given situation, most authors distinguish mainly two links of the chain: the pollutant concentration in the medium and its concentration in the organism (of plants, animals, and sometimes in man's tissues). We don't think it possible to dwell on the unsolved tasks of this global problem. In short, one can conclude that ecologists do not, as a rule, follow the dependency of migration, accumulation or discrimination of substances in alimentary chains on doses (concentrations). The same error is being committed in investigations on the combined effect of substances: the regularities observed at lethal levels of exposure are being unconditionally transferred to low levels of exposure. Certainly, the migration of substances in alimentary chains, their transformations depend on their effective masses. Relatively low levels of pollution for a particular species of living organisms probably stimulate accumulation and metabolism, while high levels inhibit them. In a certain range, the concentration of poison in the environment (each previous alimentary link may be considered as an object of the environment in respect to the next alimentary link) may appear connected by a direct dependency with processes of accumulation and transformation of poisons. It is quite possible that the development of a new branch of science — the ecological toxicology — will direct the investigations of migration, transformation and effects of substances on ecosystems at the study of the dose-effect relationships for each specific case and for a chain of cases as a whole.

In substantiating safe levels of exposure in branch toxicologies, for example, in hydrobiology and ichthyology, there are being applied simplified methods, which were used in industrial toxicology in 30-ies and 40-ies. Thus, Johnson (1974), describing the techniques accepted in the USA for evaluating safe levels of ex-

posure of fish to water pollutants, pointed out that a standard safety factor ($K = \frac{Lim_{ch}}{CL_{50(96hr)}} = 0.02$) was being used in many cases.

In practice, however, this value increases up to 0.05, and even up to 0.1 of the $CL_{50(96hr)}$ (it should be noted that in 30-ies and 40-ies in industrial toxicology there was accepted a standard safety factor equal to 1/10 of the Lim_{ac} value). Equalizing the theoretical and methodical levels of different branches of preventive toxicology is a necessary prerequisite for theoretical generalizations, which may serve as the basis for developing preventive measures.

In view of the afore-mentioned facts, a particular attention at the present stage of the struggle for sanitation of the biosphere should be paid to methods for disposal of industrial wastes. The known methods of wastes disposal by burning, buring, dissolving are hazardous is spite of their seeming simplicity, as it has been more than once demonstrated: they can contaminate the environment and create artificial geo-chemical provinces (T. U. Tashbekov, Y. U. Hasanov, 1970; A. Z. Zahidov, N. T. Tadzibayeva, S. N. Hayatayev, 1972). The primary task is to use the hazard classifications of substances in order to provide regulations on introduction of closed technological cycles of water- and air-supply with removal of pollutants and their transformation into secondary raw materials for industry. The determining unfavourable properties of substances in this case are their stability in the environment and the capacity to transform into more hazardous compounds. We would like to propose some simple rules (I. V. Sanotsky, 1974a).

1. If a toxic substance cannot be completely eliminated from the industrial, communal and domestic environment, and from the biosphere in general, its quantity in objects of the environment must be subjected to toxicological limitation on the basis of the substantiation of the harmful effect thresholds.

2. If the rate of decomposition and elimination of a substance in the environment does not exceed the rate of its entry, the balance should be restored taking account of the thresholds of harmful effect of the substance on living organisms.

3. If a substance is being concentrated in the environment, including alimentary chains, its discharge must be prohibited, in particular, by means of closed cycles of air- and water-supply.

Economic expediency of these requiments (in spite of their subordination to medical requiments) must be substantiated on the basis of long-term scientific-technical and technico-economic forecasts. The latter is feasibly only in conditions of planned economy and under productional relations which are typical for socialist society. However, the published results of investigations on economic mediation of the unfavourable effect of environmen-

tal pollution on man belong mainly to scientists from capitalist countries. This fact also determines the selection of indices to be used, for example, to evaluate economic significance of sickness and mortality, and methods of calculation.

If the damage from the effect of atmospheric pollution on sickness and mortality rate of the population, in case of diseases of the respiratory organs, was estimated in 1958 in the USA in 400 million dollars (Ridker, Ronaldg, 1967), then in 1963 these losses increased up to 1.255 billion dollars. If this sum is added by losses from cancer and cardiovascular diseases, caused by the pollution of the environment, then this sum would increase up to 2.08 billion dollars (Lave, Seskin, 1970).

Thus, the cost of detriment to health, caused by the effect of atmospheric pollution in 1968 in the USA, was 3,272 under exposure to SO_x, and 2,788 billion dollars under exposure to suspended particles. Total damage[1], caused by the atmospheric pollution only, was evaluated in 16 billion dollars in 1968 (Barret et al., 1973).

Certainly, the cited figures should be considered approximate, but, nevertheless, they are rather instructive. We do not share the opinion, spread in the USSR, that the «damage» (indirect) caused by morbidity cannot be estimated in terms of money. It goes without saying that life, moral and aesthetic losses cannot be estimated in money. But, nevertheless, economic criteria for the evaluation of the effectiveness of protective measures (e. g., increase of labour productivity, reduction of wasted working time, etc.) may and must exist. Methods developed by researches of capitalist countries cannot be applied in «intact form» to socialist system. Therefore, it is absolutely necessary to consolidate common efforts of scientists of socialist countries directed at the establishment of the criteria for economic assessment of the damage.

In view of the afore-mentioned, preventive medicine, and especially preventive toxicology, must be assigned to manufacturing branches, since the results of their investigations not only directly provide for realization of the most important elements of scientific and technical progress, but, by preserving human health and associated ecological systems, may find a direct positive economic evaluation.

Concluding the description of the state of the problem of the criteria of harmfulness of the effects of chemical compounds, serving for evaluation of their toxicity and hazard, it should be emphasized that the main attention, naturally, has been paid here to occupational exposure, since toxicology of occupational substances is at present the most developed branch of preventive to-

[1] Property, materials, health and flora.

xicology. We have tried to remind the reader one well-known, but often forgotten fact: people engaged in manufacturing process are at the same time inhabitants of residential areas, while residents of populated areas spend 1/3 of their life under conditions of occupational exposure to poisons.

Only a complex evaluation of the human environment and of the direct effect on human organism of chemical compounds from different objects of the biosphere, as well as of the effects, exerted by poisons mediately through other types of economic damage, may lead to a socially motivated success in the way of protection of the environment and rational use of its resourses.

Chapter 11. SUBSTANTIATION OF BIOLOGICAL FEASIBILITY OF DETERMINING MAXIMUM SINGLE AND MEDIAN PER WORK SHIFT ALLOWABLE CONCENTRATIONS OF CHEMICAL COMPOUNDS

The material presented in this monograph on the criteria of harmfulness in hygiene and toxicology has made it recently possible to improve the accepted methodological approaches to hygienic standardization of harmful substances in the air of workplaces. This refers to the substantiation of toxicological feasibility of determining maximum single and median per work shift allowable concentrations of harmful substances.

The necessity of the biological substantiation of these two hygienic standards has arisen out of practical needs and is related to the problem of intermittent exposures.

The atmosphere of the working environment is known to be always in the state of flux so that harmful discharges reach peak concentrations at some moments through the manufacturing process and then decline or even totally disappear. The dynamism of the atmosphere within the workers' respiration zone results from the staged pattern of the manufacturing process, operation of ventilation units, temperature changes, workers' displacements, and the use of the individual means of protection of the respiratory organs. Even with a continuous manufacturing process (hygienically the most favourable one), there may be still large variations in the concentrations of harmful substances luring the work shift.

Periodical manufacturing processes are characterized by even greater intermittence, due to sharply increasing concentrations when the plant is opened to charge the feedstock, transfer intermediate products, discharge finished products, and take process samples. Nevertheless, in production as well as in experiments the factor of intermittence of the airborne toxic concentrations fails to be properly considered. According to the definition accepted in the USSR, the majority of the MACs values of harmful substances present, in effect, their maximum concentrations which shall not be exceeded even for a brief period of time. Only for some highly cumulative substances, such as cadmium oxide, metal mercury, antimony, lead and its nonorganic compounds, have the median per work shift concentrations been also accepted. Doubt-

less, concentration variations (surely, up to a certain extent) are insignificant also more many organic compounds having a chronic-concentration type of action, since the biological effect of such substances is determined by the dose of substance which entered the body. For these substances, their median time-suspended concentrations present a more valid guide to hygienic standardization. However, the lack of specially arranged investigations on intermittent exposures in terms of hygienic regulations makes it difficult to adopt final decisions, since an underestimated standard may result in detriment to the workers' health whereas an injustified overestimation of the standards involves much higher economic expenditures.

In most countries the MACs of harmful substances for the working environment are, in fact, median per shift values and some countries (GDR, Romania, USA, Czechoslovakia and others) make use of two values: maximum and median per shift concentrations. At the same time, the substantiation of specific methods for determining the maximum single concentration levels is insufficient (the fact ascertained during the discussions on international recommendations at the WHO meeting on the substantiation of safe exposure levels using health indices for a number of metals, solvents, etc.).

The key issue in this respect is the absence of a scientifically substantiated possible range of the maximum single and median time-suspended concentration.

To solve the stated issues, it was found necessary to compare in animal experiment the responses of damage and adaptation to poisons, depending on the level and regimen of exposure.

Scientific workers from the Toxicology Department of the Institute of Industrial Hygiene and Occupational Diseases, USSR Academy of Medical Sciences, have conducted experiments with a number of substances in common industrial use: fat-soluble (carbon tetrachloride, benzene, methylene, chloride, tetrachloroethane), and water-soluble (dimethylformamide and ethanol). Earlier, N. A. Tolokontsev showed that under high levels of exposure the intermittent action is hazardous only in case of exposure to fat-solubles: the fact attributed by the author to a high saturation rate of the organism. We have carried out experiments using two levels of exposure: relatively high (6—12 Lim_{ch}) and low (the level of the chronic effect threshold, Lim_{ch}).

In order to compare the results of investigations, practically equal median suspended concentrations of the substances under study were produced in the air of exposure chambers either under monotonous or intermittent regimens of exposure (the concentrations were determined by chromatographic methods). On the basis of the preliminary data, as the monotonous regimen was conditionally taken a 4-hr exposure to poisons at a relatively constant concentration. The intermittent regimen comprised five

15-min «peaks» and four 40-min intervals during which fresh air was supplied into the chamber. The «peak» concentration was 3 to 10 times that of the median suspended concentration.

The duration of the experiment was 1—1.5 months. In order to detect latent changes, a load with the same substance and, in the case of the benzene exposure, a blood-letting load were applied at different periods of the experiment.

Table 132 summarizes the results of the comparison of the effects registered under monotonous and intermittent regimens of exposure to the substances under consideration under different intensity of exposure. The data were obtained with the participation of G. G. Avilova, A. I. Halepo, N. M. Maltseva, N. P. Kuzmina, A. L. Germanova, E. A. Karpukhina, S. I. Muravieva, G. S. Pavlovskaya, and L. P. Anvaer.

Table 132

Comparison of monotonous and intermittent regimens of exposure to some chemical compounds

Substance	Median suspended concentration under monotonous and intermittent regimens	Peak/median concentration ratio under intermittent regimen	Results of investigation
Carbon tetrachloride	Lim_{ch}	3.1	No difference
	Lim_{ch}	5.0	The same
	Lim_{ch}	10.0	Interm. more hazard than monoton.
	$6Lim_{ch}$	3.2	The same
1,1,2,2-tetrachloroethane	Lim_{ch}	3.25	No difference
	$6Lim_{ch}$	3.2	The same
Dimethylformamide	$2.6Lim_{ch}$	3.0	No difference
	$7.8Lim_{ch}$	3.24	The same
	$3.1Lim_{ch}$	2.82	The same
Benzene	$12.0Lim_{ch}$	3.22	Interm. more hazard than monoton.

The animal experiment has demonstrated that at the minimum effective levels (the more so, at the MAC level) the maximum single concentration may be several times higher than the monotonous concentration if the median exposure concentration is maintained throughout the experiment and no deterioration of the state of the test animals occurs.

There should be mentioned that in the experiment with benzene

the produced quantity of phenol[1] corresponds to the degree of manifestation of intoxication. At the 12 Lim_{ch} level, the phenol content in the urine of the test animals is much higher under intermittent regimen (Fig. 48).

At the 3 Lim_{ch} level, under monotonous regimen, the phenol content in the animals' urine on the 4th and 8th day was somewhat higher than under intermittent regimen, however, by the 26th day of the experiment it decreased down to the control level (Fig. 49).

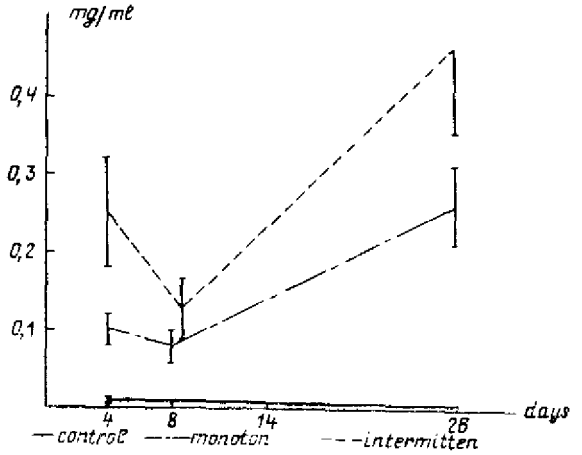

Fig. 48. Phenol quantities in urine of rats under monotonous and intermittent exposure to benzene (at the 12 Lim_{ch} level)

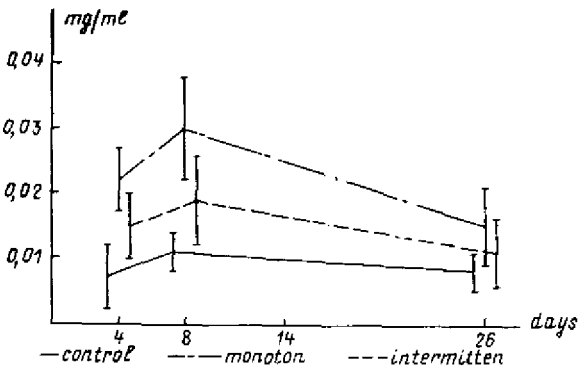

Fig. 49. Phenol content in urine of rats under monotonous and intermittent exposure to phenol (at the 3 Lim_{ch} level)

[1] The method for the phenol content assessment was developed by E. A. Karpukhina and G. S. Pavlovskaya; it is based on the reaction of hydrolysis of phenol derivatives with subsequent elimination of phenol by air aeration and its gas-chromatographic assesment.

The investigation of the processes of adaptation of the organism under different regimens and levels of exposure, which we carried out together with Y. G. Antomonov and A. B. Kotova (the Institute of Cybernetics, Ucrainian Academy of Sciences), has shown the possibility of constructing a mathematical model of the adaptation.

It was confirmed that at high levels of exposure the adaptation to the intermittent regimen is weaker than to the monotonous regimen and at low levels of exposure the monotonous regimen can be substituted by the intermittent regimen without any damage to the adaptive ability of the organism.

Thus, it appears necessary to make a wider use of the median per shift and maximum single concentrations, which meet not only medical, but also economic requirements (I. V. Sanotsky, I. P. Ulanova, 1980).

It is surely necessary to develop general methods for determining the permissible range for the case when the maximum concentration exceeds the median time-suspended concentration. Henschler (1979) recommends to take into account the degree of hazard, type of action, half-elimination, and smell of substances when substantiating the limits, frequency and duration of the variations.

These requirements are hard to fulfil in practice and, to some extent, indefinite. L. A. Timofievskaya, P. Shmidt et al. (1981) suggest to establish the coefficient of permissible limits in the range from 1 to 3 on the basis of the calculated chronic effect zone (Table 133).

Table 133

Values of the $\frac{MAC_{max}}{MAC_{median}}$ ratio for different hazard classes (Z_{ch})

Hazard class	Degree of cumulation	$Z=\frac{Lim_{ac}}{Lim_{ch}}$	$\frac{MAC_{max}}{MAC_{median}}$
I	Extremely cumulative	10	3.0
II	Highly cumulative	10—5	2.0
III	Moderately cumulative	5—2.5	1.5
IV	Slightly cumulative	2.5	1.0

In order to assess the importance of maximum and median concentrations, it would be advisable to perform an analysis of clinico-hygienic observations. For the working environment, however, the factor of intermittence remains still insufficiently studied. Let us cite two recent examples.

Arkhipova et al. (1977) have found that under exposure to significant «peak» concentrations of toluene (2 orders of magni-

tude higher than the MAC level) the workers exhibited only hematological changes of adaptive character. This study is important for the comparison of the toxicokinetic indices, obtained from experiments, with clinical indices. Along with the maximum concentrations, the median time-suspended concentrations were determined using a highly sensitive gas-chromatographic method. Clinico-hygienic observations of the process operators engaged in butyl rubber production and working in contact with methyl chloride (N. I. Simonova, 1982) have shown that under 1.3 to 3-fold variations of the methyl chloride concentrations (even in conjunction with other unfavourable factors) the workers did not exhibit pathological changes (the period of observation was 6 years). In case of larger variations, the intermittent exposure to methyl chloride had adverse effect on the workers.

The «peak» concentrations are of particular importance in the case of exposure to irritants, i. e., the substances having a limited zone of toxic effect and sharply directed antienzymatic action. As is generally known, even short-term significant discharges into the air of workplaces of such compounds as oxides of nitrogen, perfluorobutane and hydrogen arsenite lead within several minutes to severe intoxication. Therefore, the maximum single concentrations must be recognized as the only possible standard for the substances capable to cause acute intoxication under short-term exposures.

Certainly, the problem of intermittent exposures cannot be considered to be fully resolved today as regards hygienic standardization. There will be needed further experimental and clinical investigations. For hygienic practice, it is necessary to design individual control instruments and develop appropriate methodological guidelines on harmonization of the methods for monitoring the maximum single and median time-suspended allowable concentrations of harmful substances in the air of respiration zone.

Annex

Average body weight of

Season	Males, for 3 years					Males for 1 year				
	n	M	1σ	1.5σ	2σ	n	M	1σ	1.5σ	2σ
2 months										
Winter	—	—	—	—	—	—	—	—	—	—
Spring	6	145.0	12.3	18.4	24.6					
Summer	6	178.0	3.9	5.8	7.8					
Autumn	7	155.0	9.1	13.6	18.2					
Annual	19	159.5	9.1	13.6	18.2					
3 months										
Winter	19	254	13.5	20.2	27					
Spring	22	266	30.2	45.3	60.4					
Summer	16	245	13.2	19.8	26.4					
Autumn	6	225	15.0	22.5	30.0	15	241	17.8	26.7	35.6
Annual	63	253.2	21	31.5	42.0					
4 months										
Winter	16	274	15.2	22.8	30.4	143	292	29.4	44.1	58.8
Spring	15	812	42.9	64.4	85.8					
Summer	16	291	17.5	26.2	35.0					
Autumn	—	—	—	—	—					
Annual	47	291.7	15.5	23.2	31.0					
5 months										
Winter	—	—	—	—	—					
Spring	67	316	33.5	50.2	67.0	27	372	26.7	40.0	53.4
Summer	—	—	—	—	—	27				
Autumn	15	337	13.0	19.5	26.0					
Annual	82	320	30.0	45.0	60.0					
6 months										
Winter	—	—	—	—	—	9	444	31.0	46.0	62.0
Spring	93	361	37.0	55.0	74.0	16	387	31.2	46.8	62.4
Summer	30	352	36.8	55.2	73.6					
Autumn	11	337	17.4	26.1	34.8					
Annual	134	357	35.9	53.8	71.8					
7 months										
Winter	—	—	—	—	—					
Spring	82	394	48.4	96.8		25	457	46.2	69.3	92.4
Summer	45	407	37.1	74.2						
Autumn	7	388	18.5	37.0						
Annual	134	398	43.9	87.8						

Table 1

mongrel white rats, g

Season	Females, for 3 years					Females, for 1 year				
	n	M	1σ	1.5σ	2σ	n	M	1σ	1.5σ	2σ
2 months										
Winter	—	—	—	—	—	—	—	—	—	—
Spring	47	117	13.4	20.0	26.8					
Summer	—	—	—	—	—					
Autumn	—	—	—	—	—					
Annual	47	117	13.4	20.0	26.8					
3 months										
Winter	64	218	17.9	26.8	35.8	90	227	17.7	26.5	34.5
Spring	47	204	9.2	13.8	18.4					
Summer	10	193	6.3	9.4	12.6					
Autumn	48	183	9.7	14.5	19.4	15	197	17.8	26.7	35.6
Annual	169	203	13.3	19.9	26.6					
4 months										
Winter	8	210	3.4	5.1	6.8					
Spring	170	234	22.4	33.6	44.8					
Summer	30	235	17.9	26.8	35.8					
Autumn	77	223	11.2	16.8	22.4	15	256	20.9	31.5	41.8
Annual	285	231	15.4	23.0	30.8					
5 months										
Winter	57	259	20.2	30.3	40.4	15	275	25.1	37.7	50.0
Spring	110	262	23.8	35.7	47.6					
Summer	64	258	22.9	34.4	45.8					
Autumn	28	256	15.1	22.6	30.2					
Annual	259	260	22.3	33.4	44.6					
6 months										
Winter	63	276	21.8	32.7	43.6	12	301	17.9	26.9	35.8
Spring	69	274	27.1	40.6	54.2	90	279	26.1	39.1	55.2
Summer	92	274	22.7	34.0	45.4					
Autumn	30	284	20	35	40					
Annual	254	275	23.5	36.2	47.0					
7 months										
Winter	71	285	25.4	38.1	50.8	11	327	5.9	8.9	11.8
Spring	8	286	37.7	56.6	75.4	30	285	32.8	49.2	65.6
Summer	148	286	26.6	39.9	53.2					
Autumn	20	275	24.8	37.2	49.6					
Annual	247	285	26.5	39.7	53.0					

Season	Males, for 3 years					Males, for 1 year				
	n	M	1σ	1.5σ	2σ	n	M	1σ	1.5σ	2σ
8 months										
Winter	—	—	—	—	—					
Spring	—	—	—	—	—	36	462	49	73.5	98
Summer	60	432	34.8	52.2	69.6					
Autumn	—	—	—	—						
Annual	60	432	34.8	52.2	69.6					
9 months										
Winter	—	—	—	—	—					
Spring	—	—	—	—	—	10	471	56.8	85	113
Summer	46	470	36.5	54.7	73.0	16	523	32	48	64
Autumn	20	454	59.0	88.5		6	325	46.3	69.5	92.6
Annual	66	465	31.3	46.9						
10 months										
Winter	—	—	—	—	—					
Spring	—	—	—	—	—					
Summer	59	499	60.3	90.4	120.6	16	543	59.2	88.8	118
Autumn	—	—	—	—	—					
Annual	59	499	60.3	90.4	120.6					
11 months										
Winter	—	—	—	—	—					
Spring	—	—	—	—	—					
Summer	—	—	—	—	—					
Autumn	6	129	12.2	18.3	24.4					
Annual	6	129	12.2	18.3	24.4					

Continued

Season	Females, for 3 years					Females, for 1 year				
	n	M	1σ	1.5σ	2σ	n	M	1σ	1.5σ	2σ
8 months										
Winter	17	295	16.3	24.4	32.6					
Spring	35	306	30	45	60					
Summer	63	892	23.9	35.8	47.8	30	296	32.8	49.0	65.6
Autumn	32	314	35.9	38.8	51.8					
Annual	147	301	25.3	37.9	50.6					
9 months										
Winter	23	313	31.9	47.8	63.8					
Spring	—	—	—	—	—					
Summer	38	523	33.0	48.5	66.0	29	304	32.3	48.4	64.6
Autumn	—	—	—	—	—					
Annual	61	319	32.6	48.9	65.2					
10 months										
Winter	—	—	—	—	—					
Spring	—	—	—	—	—					
Summer	—	—	—	—	—					
Autumn	—	—	—	—	—	30	295	27.3	41.0	54.6
Annual	—	—	—	—	—					
11 months										
Winter	—	—	—	—	—					
Spring	14	312	28.8	43.2	57.6					
Summer	—	—	—	—	—					
Autumn	—	—	—	—	—	6	325	46.5	69.8	99
Annual	14	312	28.8	43.2	57.6					

Some indices of functional condition of liver and

Season	Hippuric acid (mg/24 hr)					Elimination of bromosulfalein, % of administered dose					Diu	
	n	M	1σ	1.5σ	2σ	n	M	1σ	1.5σ	2σ	n	M
												For 3
Winter	160	95.7	25.1	37.6	50.2	54	82.5	10.6	15.9	21.2	94	4.44
Spring	184	101.2	20.1	30.1	40.2	12	80.5	16.8	25.2	33.6	90	5.21
Summer	115	84.7	18.3	27.4	36.6	45	75.0	10.9	16.3	21.8	74	4.83
Autumn	175	60.7	21.0	31.6	42.1	50	81.7	10.1	15.2	20.2	66	4.70
Annual	634	88.0	21.0	31.6	42.2	161	79.9	16.4	24.6	32.8	324	4.79
												For 1
Winter	94	64.9	68.8	103.2	137.6						80	7.4
Spring	54	80.5	65.3	97.9	130.6						52	8.6
Summer	64	92.3	76.9	115.4	153.0	No data					56	7.8
Autumn	85	84.6	66.1	99.2	132.2						60	7.5
Annual	297	73.5	69.3	103.0	138.6						248	7.8

Composition of peripheral

Season	Hemoglobin, g/l					Erythrocytes,	
	n	M	±1σ	±1.5σ	±2σ	n	M
Winter	104	136.9	93	139	18.6	42	6.61
Spring	23	143	75	117	150	84	7.2
Summer	24	136	115	172	230	50	5.02
Autumn	58	134.3	69	103	138	16	6.38
Annual	209	135	108.8	132	176	200	6.15

Table 2

kidneys of rats for 2 periods of observation

rests, ml			Protein in urine, mg/ml					Chlorides in urine, mg/ml				
1σ	1.5σ	2σ	n	M	1σ	1.5σ	2σ	n	M	1σ	1.5σ	2σ
years												
1.25	1.87	2.50	95	6.0	1.44	2.16	2.88	75	2.2	0.5	0.75	1.0
1.11	1.66	2.22	83	6.96	2.74	4.11	5.48	77	1.77	0.4	0.6	0.8
1.07	1.60	2.14	79	6.29	2.15	3.22	4.30	84	1.95	0.58	0.84	1.16
1.26	1.89	2.52	86	6.68	1.86	2.76	3.72	56	1.84	0.5	0.75	1.01
1.16	1.75	2.34	85	6.48	2.05	3.06	4.09	73	1.94	0.47	0.74	0.8
year												
1.9	2.85	3.8	80	2.8	1.0	1.5	2.0	80	1.9	0.2	0.3	0.4
0.8	1.2	1.6	36	4.0	0.1	0.15	0.2	30	1.5	1.0	1.5	2.0
0.6	0.9	1.2	49	2.3	0.7	1.0	1.4	56	1.8	0.4	0.6	0.8
0.2	0.3	0.4	54	2.3	0.8	1.2	1.6	54	1.8	1.0	1.5	2.0
0.9	1.3	1.8	219	2.8	0.7	1.4	1.4	220	1.7	0.6	0.9	1.2

Table 3

blood of rats (for 3 years)

million/μl			Leukocytes, thousand/μl				
±1σ	±1.5σ	±2σ	n	M	±1σ	±1.5σ	±2σ
0.67	1.05	1.37	42	13.02	2.44	3.66	4.88
0.95	1.42	1.9	84	12.27	2.5	3.75	5.0
0.97	1.45	1.94	64	13.03	2.94	4.41	5.88
1.68	2.52	3.36	16	10.96	2.2	3.3	4.4
0.96	1.44	1.92	206	12.56	2.62	3.93	5.24

Ta

Relative weight of internal organs of rats, in %of the body weight

Season	Heart					Spleen					Season	Lungs			
	n	M	1σ	1.5σ	2σ	n	M	1σ	1.5σ	2σ		n	M	1σ	1.5σ
							For 3 years								
Winter	8	0.3	0.03	0.05	0.06	8	0.3	0.06	0.09	0.12	Winter	8	0.7	0.11	0.17
Spring	55	0.3	0.04	0.06	0.08	40	0.3	0.06	0.09	0.12	Spring	45	0.6	0.8	1.2
Summer	127	0.3	0.03	0.05	0.06	127	0.3	0.02	0.03	0.04	Summer	109	0.4	0.15	0.23
Autumn	22	0.35	0.05	0.08	0.1	76	0.38	0.17	0.23	0.34	Autumn	16	0.55	0.23	0.35
Annual	212	0.31	0.037	0.06	0.08	51	0.32	0.08	0.11	0.15	Annual	178	0.56	0.32	0.49
							For 1 year								
Winter	31	0.34	0.02	0.03	0.04	31	0.27	0.04	0.06	0.08	Winter	31	0.62	0.04	0.06
Spring	40	0.26	0.04	0.06	0.08	40	0.26	0.08	0.12	0.16	Spring	40	0.48	0.02	0.03
Summer	20	0.28	0.01	0.015	0.02	20	0.29	0.07	0.105	0.14	Summer	20	0.5	0.02	0.03
Autumn	98	0.32	0.04	0.06	0.08	98	0.31	0.1	0.15	0.2	Autumn	98	0.56	0.01	0.015
Annual	189	0.3	0.02	0.03	0.04	189	0.28	0.072	0.1	0.14	Annual	189	0.54	0.02	0.32

For 3 years

Winter	—	—	—	—	—	82	17.7	2.73	4.10	5.46	Winter	122	3.59	0.71	1.07	1
Spring	8	0.5	0.11	0.17	0.22	81	14.2	2.34	3.51	4.68	Spring	207	3.93	0.5	0.75	1
Summer	20	0.49	0.09	0.14	0.18	106	16.0	2.26	3.39	4.52	Summer	220	3.36	0.73	1.09	1
Autumn	—	—	—	—	—	81	14.0	2.01	3.02	4.02	Autumn	180	3.65	0.42	0.61	0
Annual	14	0.495	0.1	0.155	0.2	350	15.5	4.46	6.69	8.92	Annual	729	3.63	0.59	0.88	1

For 1 year

Winter						50	15.0	2.6	3.9	5.2	Winter	24	2.8	0.8	1.2	1
Spring											Spring	24	4.3	0.7	1.05	1
Summer											Summer	28	3.2	0.32	0.51	0
Autumn											Autumn	24	3.4	0.3	0.45	0
Annual											Annual	100	3.5	0.6	0.9	1

Ta

Some physiological indices of white rats

Season	Respirations per min					Systoles per min					Season	Arterial pressure, mm of H			
	n	M	1σ	1.5σ	2σ	n	M	1σ	1.5σ	2σ		n	M	1σ	1.5σ
							For 3 years								
Winter	279	165	21.8	32.7	43.6	223	474	47.6	71.4	95.2	Winter	61	102	9.37	14.06
Spring	248	166	20.3	30.4	40.6	160	480	40.0	60.0	80.0	Spring	143	112	8.95	13.43
Summer	193	165	25.2	37.8	50.4	175	467	50.4	75.6	100.8	Summer	116	118	8.73	13.095
Autumn	320	170	19.0	28.5	38.0	234	500	43.3	64.9	86.6	Autumn	82	102	14.17	21.26
Annual	1040	167	21.6	32.4	43.2	802	480	45.3	67.9	90.6	Annual	402	109	10.31	15.96
							For 1 year								
Winter	128	157	16.5	24.7	33.0	65	502	35.0	52.5	70.0	Winter	—	—	—	—
Spring	112	172	20.8	31.2	41.6	100	465	48.0	72.0	96.0	Spring	30	102	11	16.5
Summer	106	160	18.0	27.0	36.0	48	490	52.5	78.7	101.0	Summer	60	94	12	18
Autumn	168	168	22.5	33.7	45.0	72	520	41.0	61.5	82.0	Autumn	36	86	8.8	13.2
Annual	524	164	19.4	29.1	38.8	285	494	44.1	66.1	88.2	Annual	126	94	10.3	15.9

Table 6

Oxygen consumption, litre/kg×hr

Season	For 3 years					For 1 year				
	n	M	1σ	1.5σ	2σ	n	M	1σ	1.5σ	2σ
Winter	57	1.57	0.29	0.43	0.58	40	1.60	0.36	0.54	0.72
Spring	42	1.65	0.56	0.84	1.12	40	1.91	0.38	0.57	0.76
Summer	65	1.57	0.38	0.57	0.76					
Autumn	32	1.55	0.96	1.44	1.92	72	1.94	0.18	0.27	0.36
Annual	196	1.58	0.55	0.82	1.09	152	1.77	0.28	0.42	0.56

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58	26	naturally
62	27	toxicity
69	Table 18(3)	0.3
76	24	Iodobenzene
100	20	researchers
103	Table 39(7)	quantity
105	19	follows
108	25	mitotic
118	22	G. N. Kra-
120	43	significant
125	Table 48	$K_2 = \frac{DL_{50\ min}}{DL_{man}}$
	12	slight
	15	sensitivity
129	5	straight
160	14	peculiarities
161	Table 70	Methyl chloride Carbon
162	23	of
166	Table 71	volatility
171	Table 78	under
	last	agranulocytes
172	Table 79	under
177	33	disfunction
	38	subcortical
180	12	Similarity
	28—29	pyrocatechin
188	Table 88	Volatility
193	6	tubules
	10	tubules
199	last	fore
203	8	times)
206	6	0.0003
	12	decrease
216	27	inhalation
222	Table 111	Concentration
240	21	neutrophilous
247	24	Goldblatt
252	31	benzine
253	30	including

Continued

Page	Line	Should read
	34	inade-
255	Table 128(7)	2-Vinylpiridine
	4	matter
287	1	radiation
288	52	poisoning
	56	concentrations

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