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LONG-TERM EFFECTS OF CHEMICALS ON THE ORGANISM

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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 1979 Russian edition of *Otdalyonniye posledstviya vliyaniya himicheskikh soyedineniy na organizm* by I. V. Sanotsky and V. N. Fomenko.

PREFACE TO THE ENGLISH EDITION

This book is titled "Long-Term Effects of Chemicals on the Organism". In presenting them to readers as one hygienic problem the authors wished to project a system of view evolved in preventive toxicology on ways to assess chemically induced generative changes — embryotropic and gonadotropic effects and mutagenesis; somewhat less on accelerated ageing as highlighted by compromised vessels and myocardium; and in part also on chemical carcinogenesis. Admittedly, the views are debatable at many points.

Since this flow of information keeps expanding it was decided to supplement the monograph with a chapter reviewing current problems in the study of long-term consequencies from chemical environmental effects.

The volume is structured as an array of brief reviews based on the authors' "own" inputs — those obtained in the toxicology department of the Institute of Industrial Hygiene and Occupational Diseases, USSR Academy of Medical Sciences. They are just sufficient to address some dimensions of the problems concerned, as no exposition can claim completeness in a fast-changing information environment.

The authors hope for this volume to provide a foundation of fact for their own concepts. They hope it also to be a starting point of a creative debate that will help identify lines of convergence among the key concepts espoused by different scientific schools. Together, these will contribute to the cause of preventing the rise and origin of long-term effects in man constantly operating under chemical pressures — whether at work or at home.

We concur with the view at extension of total life longevity and the work capacity period in particular, as an essential social goal.

FOREWORD

In our industrial age, pollution of the human environment — occupational, communal, domestic and natural — by chemical compounds is an ever-increasing risk to public health.

Soviet scientists have done successful work on developing the basic concepts of sanitary regulation in the manufacture, application and utilization of noxious substances. Public health practitioners have put the programme into practice and, in collaboration with technologists, achieved impressive advances in protecting the environment against chemical contaminants. As a result, the incidence of acute and chronic poisoning has been markedly reduced and the rate of chemically-induced occupational mortality remains level, with a slight downward trend, despite explosive industrial growth.

Long-term, notably delayed, effects such as lesions of the vessels and heart; blastomogenesis, pareses and paralyses; sclerotic involvement of the body in organs, above all the lungs, kidneys, and liver; abnormalities of the bones; and injuries to the reproductive functions, involving gonadal alterations and chemical impairment of the foetus and progeny, and so on still, however, constitute a problem in the process of effective management.

The object of the present monograph is to discuss the goals of health-oriented regulation of the production, application and utilization of substances with the potential to exhibit their action both later in the life of the present generation and in subsequent generations that have not been directly exposed to the chemicals themselves.

The book inquires into changes in the reproductive function and the ageing processes of the cardio-vascular system resulting from exposure to chemical compounds; it also looks at some dimensions of chemical carcinogenesis.

It reviews certain points related to the theory of selectivity in the action of chemicals; to the criteria of harmfulness in establishing the thresholds of specific effects; and to research methods and findings on a few dozen toxic chemicals that are used as examples to demonstrate the fundamental general patterns of the dose/effect relationship. There is some discussion on whether it is possible, in principle, to interpret the magnitude of chemically-induced changes and introduce animal experiment data into sanitary practices.

The final chapters consider the prediction of long-term effects; strategies and tactical lines for future research; occupational health of women workers and protection of future generations against toxic insults.

The chapter on the results of studies on the ageing rate of the heart and vessels under the influence of chemical microdoses was written together with N. S. Grodetskaya.

Chapter 1

LONG-TERM EFFECTS OF EXPOSURE TO CHEMICAL COMPOUNDS IN MAN'S LIVING ENVIRONMENT: THE HYGIENIC PROBLEM

Introduction. The chemical, petrochemical, pharmaceutical, oremining and other industries; chemical technologies now gaining acceptance in all sectors of the national economy and the domestic sphere; synthetic livestock feeds and other products of microbial synthesis; and, finally, chemical food additives — all contribute to creating for contemporary man living conditions of a kind he has never known before. The hereditary qualities evolved in phylogenesis may, therefore, no longer correspond, to varying degrees, to the living conditions. The unity of organisms and the environment may be disrupted, and adverse effects of different severity will ensue. When the balance between organisms and their living environment is seriously upset, serious diseases of chemical etiology — acute, subacute, and chronic poisoning — will develop. When the equilibrium is upset only slightly, there follow non-specific, or pre-pathological states that, though themselves reversible, can nonetheless trigger a possibly irreversible general pathology. For example, a reduction of total immunity under the influence of microquantities of chemicals is known to be likely to promote an unfavourable course of an infection, but also result in impairment (and distortion) of regeneration processes, leading to tumour growth.

F. Fluri and F. Zanger focused long ago on the increased sensitivity to tubercular infection among factory workers chronically exposed to occupational hazards. It seems proper to transfer the relationships recognized to influenza, for example, despite the scarcity of research in the area. Sanotsky (1965) reported modified sensitivity to tetanotoxin in first-generation offspring that was a function of the doses of lead and other metals to which the female mice had been exposed during pregnancy (Fig. 1). Similar developments were observed following injections of thallium compounds in animals. The susceptibility to pneumococcus toxin varied directly with the thallium dose.

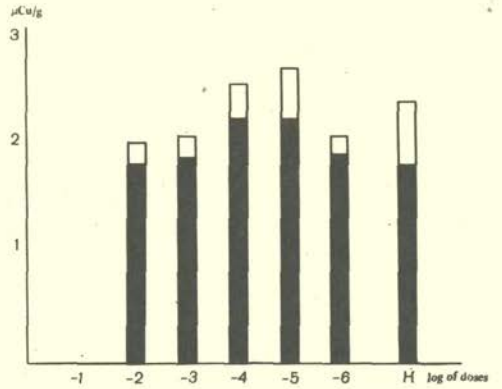

Fig. 1. Local response to tetanotoxin in offspring of albino female mice injected before gestation with different doses of heavy metals containing traces of radiothorium

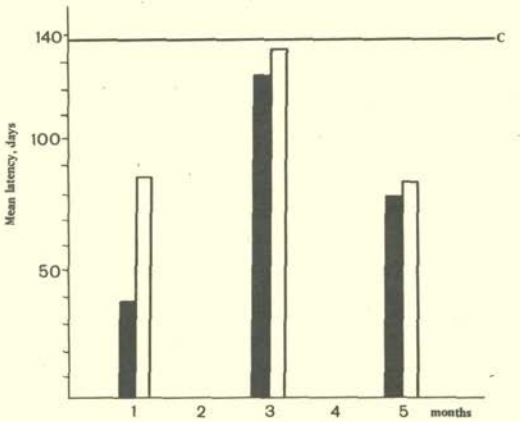

Fig. 2. Mean latency of tumours induced by benzo(a)pyrene against the stage of chronic intoxication at Lim_{ch} level (Kurliandsky et al., 1976).
C — control; blank columns — benzene; black columns — CCl_4

Fig 2. shows data from Kurliandsky et al. (1972) on a change of incubation periods in the development of blastomas induced by benzo(a) pyrene implantation, as a function of the state of the body in the various phases of mild chronic intoxication (at the level close to the threshold of chronic action, Lim_{ch}). The mechanism involved was controlled by a variety of processes, including ones that weakened (or strengthened) the immune properties. Considerable data have now been published on this.

Lastly, many chemical compounds that pollute the environment are capable of exerting a specific action on the organism (without major systemic toxic effects), which cannot be seen either during exposure or immediately after it, but only late in the life of the individuals exposed, not infrequently delayed for many years and even

decades since the period of chemical exposure. Still more serious for society is the manifestation of unfavourable, chemically-induced effects in subsequent generations. Sometimes these effects are evident, but at other times they represent a constituent of the so-called genetic load, which requires laborious and multidisciplinary investigations to identify its true causes. In dealing with the problem, some scholars (Abrahamson, 1976, and others) go to the extreme of stating the impossibility in principle detecting the underlying causes of the genetic load of latent mutations.

We cannot, of course, agree with such defeatism, though, man's generally postulated goal is not merely to cognize the world, but also to change it for the better. For the case in hand, this means weeding out the causes and long-term effects as such. With the current level of knowledge, however, the only realistic way to do it is to eliminate the causes and prevent the long-term effects of chemical exposure, as treatment remains an unresolved problem even though tremendous progress has been made in some areas (e. g., surgical treatment of congenital heart defects).

Thus, the long-term effects of exposure to chemical pollutants in man's living environment (and the plants and animals ecologically related to it) constitute, first and foremost, a hygienic problem, however grandiose this might seem.

State-of-the-art. A brief overview. Through the efforts of generations of hygienists and toxicologists, the scientific principles for studying chemical exposure have been developed; their purpose is to regulate the production and application of chemical compounds and the management of production waste. Some of these regulatory programme have focused specifically on justifying and enforcing the now numerous sanitary standards setting limits for the concentration of noxious substances in environmental media. The greatest success has been achieved in preventing a chemical impact on occupationally exposed workers.

The increase in the number and quality of sanitary standards in this sphere is illustrated in Table 1.

The untiring efforts made since the Revolution to provide healthier working conditions have resulted in a many-fold reduction in the incidence of both acute and chronic intoxication. The classical forms of chemically-induced occupational disease have virtually disappeared from the health records. Occupational mortality in the chemical industry has stabilized, in spite of the vast growth of production and significant expansion of the product range.

The 10th Five-Year Plan for the development of the USSR national economy provided for further expansion of the product range and an increase in output. Some related targets were as follows:

"In the chemical and petrochemical industry.

To increase production by 60 to 65 per cent. To increase the production of mineral fertilizers to 143 million tons (including 5 million tons of chemical feed additives), in 1980 with the average content of nutrients in mineral fertilizer of at least 40 per cent. To increase the rate

Table 1

Number of MACs for Harmful Substances in the Air of the Working Zone

Title of regulatory document	Year of approval	Number of substances covered
Decision of the People's Commissariat for Labour of the Russian Soviet Federative Socialist Republic of April 10 and August 30	1922	3
Decision of the USSR People's Commissariat for Labour, No. 232	1930	14
Branch Standard OST 90014—39	1939	40
National Standard GOST 1324—41	1941	79
National Standard GOST V—1324—43	1943	74
National Standard GOST 1324—47	1947	84
Normative Sanitary Rules 101-51	1951	90
Norm 101-54	1954, 1958	88
Sanitary Norms 245-63	1963	331
Sanitary Norms 245-71	1971	631
National Standard GOST SSBT. Air of the Working Zone. General Sanitary-Hygienic Requirements	1976	850

of building capacities for the production of phosphate fertilizers. To raise the production of concentrated and compound fertilizers ...

To considerably expand the production of plant protection chemicals and widen their range.

To make provision for increasing the production of synthetic resins and plastic by 90—110 per cent, and to improve the quality and increase the durability of plastics. To increase the output of new types of polymer materials, above all structural materials.

To secure in 1980 the production of household chemical fiber and thread with improved qualities. To boost the production of high-modulus fiber and texturised thread. To develop the production of high quality dye-stuffs, varnishes and paints.

To increase the production of synthetic rubber by 1.4—1.6 times, providing for a priority growth in the production of rubber which can completely replace natural rubber.

To ensure the maximum satisfaction of the requirements of the national economy in chemical products made in small quantities, including extra pure materials, chemical additives to polymer materials, auxiliary substances for textile production, conserving agents, catalysts and other products.

To expand the output and improve assortment and quality of household chemicals, as well as films and other types of materials for prepacking consumer goods, medical preparations and medical goods" [1].

The trend was carried forward in the Guidelines for the Economic and Social Development of the USSR for 1981—1985 and the Period Ending in 1990. It reads, "Our industry is steadily increasing the pro-

[1] Guidelines for the Development of the National Economy of the USSR for 1976—1980, Moscow, Politicheskaya Literatura Publishers, 1976, pp. 29—30.

duction and supplies to the collective and state farms, of chemical fertilizers and crop protection chemicals. The eleventh five-year period is to see a nearly 50 per cent rise in the production of chemical fertilizers. The chemical industry workers must see to it that more ballast-free and composite fertilizers are produced. At the same time it is necessary to improve the manner in which chemical fertilizers are used and distributed".

As a consequence, potential toxic emission into the environment is bound to increase.

The 24th, 25th and 26th Congresses of the Communist Party of the Soviet Union voiced special concern for environmental protection and the need to combine steps to speed up progress in science and technology with measures to prevent and avert hazardous polution of the air and water, and depletion of the land. More stringent demands are to be enforced on planning, economic management and design organisations as they design and construct new industrial projects or improve the environmental performance of existing plants.

Guidelines for the development of the USSR national economy, the 25th and 26th Party Congresses undertook to add new vigour to development and to transfer to production of the technologies and processes that ensure a drop in production waste and their maximum utilisation, along with closedcycle water management systems.

Another requirement is that a broader basis be provided for specialized lines of production responsible for the manufacture of the equipment, goods and materials needed for the initiation and operation of efficient waste-treatment facilities in industrial locations and urban areas.

Over and above national programme, the USSR is fulfilling commitments resulting from participation in inter-state organizations concerned with the protection of nature and the national use of its resources.

As a follow-up to the decisions of the 24th Congress of the CPSU, a number of legislative acts, such as the Fundamentals of the Legislation of the USSR and the Union Republics on Matters of Public Health; the Laws on Land Use, on Water Use, on the Protection of Nature and on the Use of Forests have been supplemented by articles that regulate sanitary protection from chemical pollution of occupational and social plant premises, the atmosphere of population aggregates, bodies of water used for commercial fisheries and hygienic, domestic and recreational purposes; soil, foods and other environmental media. In recent years, the Supreme Soviet of the USSR has considered measures for further improving the protection of nature and making rational use of natural resources. The USSR Council of Ministers has passed decision on protection of the Volga and Ural basins from contaminants. The CPSU Central Committee and the USSR Council of Ministers have adopted a wide-ranging resolution On Increased Protection of Nature and Improved Utilization of Natural Resources.

Finally, as the supreme measure of concern for the people's well-being, an article has been added to the new Constitution of the USSR granting Soviet citizens the right to health protection (Article 18,

Chapter 2, Section 1). In this manner, the Soviet Union has put together an exceptionally powerful economic and legal base for the initiation and maintenance of preventive programmes.

As such, the problem of research into the long-term effects of exposure to chemical compounds has not yet been sufficiently explored. It is known, though, that the average life expectancy provides an integral index of social well-being. In many ways, it is determined by infant mortality, fatalities from injuries and the causes of death in old age. It is generally recognized that in industrialized countries the principal cause of mortality in old age is cardiovascular disease (about 50 per cent) and malignant neoplasms (about 20 per cent). The incidence of these diseases has exhibited a slow but steady increase over the last decade. What, then, is the role played by the microquantities of inadequate chemical stimuli in the set of other causes? The answer is not sufficiently clear, but there are reasons for assuming a significant role for chemical micro-impurities.

To support the notion, workers from the toxicology department of the Institute of Industrial Hygiene and Occupational Diseases of the USSR Academy of Medical Sciences have established high specificity for the lesions of the vessels and heart caused by carbon disulfide exposure in comparatively low concentrations, insufficient to produce other effects. High selectivity has also been detected in the action of lead on the gonads. The latter is detectable at exposure levels so low that the porphyrine metabolism, for example, is barely affected (Table 2). The impact of esters of trichlorophenoxyacetic acid on the developing foetus is noted without systemic toxicological manifestations (Konstantinova, 1968, 1976).

A possible selective action of this kind may be directly related to signs of increasing infant morbidity and mortality, the most essential statistical indicator, which, in effect, constitutes the prime determinant of average life expectancy.

The chemical component in the causes of infant mortality is determined by mutagenic and embryotropic effects of toxics (the embryotropic impact, basically, may also involve elements of unfavourable mutations in the primordia of the body's organs).

It has long been known that mortality ensuing from congenital abnormalities greatly exceeds in developed countries fatalities from infantile infections. According to WHO estimates (1972), congenital developmental defects account for 1/5 of infant deaths, invalidism and mortality. A recent epidemiological study by Kantorovich and associates (1976) revealed a statistically significant increase in the incidence of congenital developmental abnormalities in the Russian Federative Republic of the USSR during the thirteen years from 1960 to 1973. The urban environment appears to be much more affected in this regard than the rural areas. A computer-based analysis of 80 thousand birth records indicates that the state of the mother's organism, as well as infections experienced during gestation (rubella in particular) are of major importance. In the geographical zones where the rate of congenital developmental defects is at its highest one can readily

Table 2

Morphological Indicators of Spermatogenesis and Urinary ΔALa * Content after Chronic Exposure to Lead Acetate

Indicators	Concentration, mg/m³, MAC level			
	I series		II series	
	0.03±0.0004	control	0.036±0.04	control
Index of spermatogenesis	3.67±0.04	3.68±0.01	3.69±0.01	3.7±0.04
Normal spermatogonia	24.86± ±0.75**	30.01±0.71	23.0± ±0.81**	27.5±0.78
Tubules with scaled-off spermatogenic epithelium	1.8±0.66	2.0±0.33	7.8±1.03***	3.17±0.3
Tubules with meiosis stage 12	4.0±0.45	4.0±0.85	3.16±0.28	3.2±0.56
ΔALa in urine in mg/kg creatinine	1.79±0.25	0.9±0.11	1.56±0.1	1.87±0.17

* ΔALa — delta amino-levulinic acid;
** $p<0.01$;
*** $p<0.02$.

Table 3

Mortality and Life Longevity among X-Ray Irradiated Progeny of Animals Exposed to Radiothorium in a Mixture with Metals

Group	Dose given to mothers, mC/kg	Irradiated progeny	Numbers dead at (days of exposure)													Mortality, %%
			six	seven	eight	nine	ten	eleven	twelve	thirteen	fourteen	fifteen	16 to 18	23 to 25	Survivals	Deaths	
1st	$1 \cdot 10^{-4}$	30	—	2	2	2	8	4	—	2	—	1	2	—	7	23	76.7*
2nd	$1 \cdot 10^{-5}$	30	2	6	2	8	—	2	—	2	2	—	—	3	3	27	90.0**
3rd	$1 \cdot 10^{-6}$	30	—	4	6	4	—	6	1	1	4	—	2	1	1	29	96.3
4th	Control	30	—	2	4	4	—	4	2	—	—	—	—	—	14	16	63.3

* $p<0.01$ in relation to the control group;
** $p<0.02$ — differences between the 1st and 2nd groups.

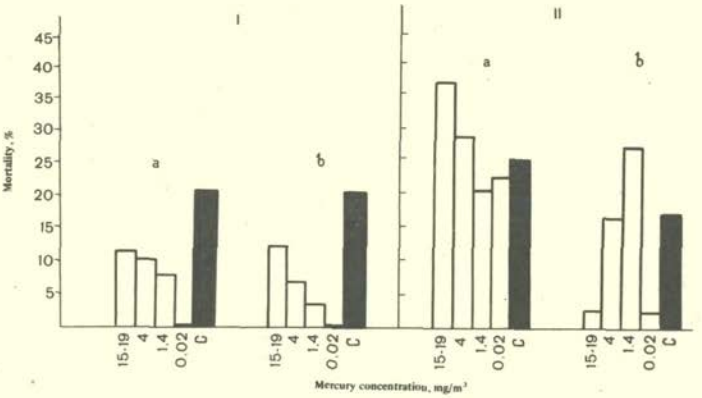

Fig. 3. Mortality among offspring of albino rats exposed to mercury vapours.
I — first-generation offspring; II — second-generation offspring; a — mercury vapours; b — mercury vapours + gamma rays (300 R); C — control

trace excess levels of environmental pollution by chemical compounds — a fact overlooked by the authors.

The manifestations of paradoxical responses in progeny (a prevalence of local responses to tetanotoxin with a diminishing dose range of harmful substances injected into the mother) discussed above (see Fig. 1) are obviously more than just isolated cases. The same experimental (Table 3) revealed in first-generation progeny increasing sensitivity to ionizing radiation with a decreasing toxic dose (the lowest doses in the selected range proved more effective).

In these instances there is a high probability of direct embryotropic effect.

In other instances, however, genetic action is obvious. For example, following mercury exposure as well as mercury and general gamma-irradiation of males (the females were intact), the first-generation progeny turned out to be more tolerant than the control group (other, concurrent findings were the faster development of conditioned reflexes and, conversely, a drastically weakened second generation) (Fig. 3).

One might get the impression that the discussion of long-term effects focuses exclusively on carcinogenesis, mutagenesis, and gonado-and embryotropism (including teratogenesis) of toxic chemicals (the problem of long-term cardiovascular pathology was mentioned only in passing). Indeed, this is the way many scholars construe the term.

In fact, however, long-term effects constitute a wide nosological group (Table 5). First, these are various degenerative processes leading to tissue atrophy. Such developments may be the outcome of chronic inflammatory processes, for example, in the mucous membranes of the respiratory and alimentary tracts, that develop in response to irritant chemicals. If compounded by connective tissue proliferation, they may lead to severe sclerosis of body organs (Table 4).

Table 4

Distribution of Pneumoconiosis (WHO Records, 1972, V. 26, No. 3, p. 125)

Country	No. of miners examined	Cases of pneumoconiosis	Morbidity, %%
Austria	2,096	—	2.9
Australia	—	—	2.9
The Netherlands	16,107	3,500	21.0
Sri Lanka	1,431	372	26.0
United Kingdom	—	—	11.5
United States	4,000	380	9.5
Zambia, 1967	43,500	1,305	3.0

Silicosis, for one, is undoubtedly among the most common long-term effects precipitating death. Toxic pneumosclerosis is just as subtle and insidious, because it covertly develops against a background of apparent habituation to the effect of irritating vapours and gases. Kidney sclerosis is a typical long-term manifestation of the effect of several heavy metals (uranium, lead, etc.). Of late, considerable attention has been focused in special literature throughout the world on vinylchloride as a strong carcinogen. Yet analysis of the literature indicates that the incidence of malignant tumours among exposed human continents is, in this case, very minor, whereas long-term vascular and osseous diseases are fairly common.

The frequency of cirrhosis of the liver induced by hepatotropic substances has not been assessed with sufficient accuracy, though the evidence now available suggests that liver cancer is often preceded by sclerosis (see, for example, Magee's publications on nitrosodimethylamine).

Degenerative developments in the nervous system in the longer term after chemical exposure are somehow left out of descriptions of long-term effects. Nevertheless Parkinson's disease polyneurites, pareses and paralyses, physchoses and other well-known long-term maladies in many countries cause pain and suffering to as many people as do the "fashionable" forms of long-term effects (Table 5).

Definition. The conceptual range of the long-term effect of chemical exposure should not, therefore, be restricted. In our opinion, the term "long-term effect" must be understood as the development of pathogenic processes and diseased states later in life in individuals previously exposed to chemical contaminants in their living environment and also in several generations of their progeny.

It would be sensible to apply the term "delayed effect" to stress the manifestation of diseases a long time after the cessation of chemical exposure.

The inclusion of the concept of long-term effects into a sanitary standard for the air of a working zone should thus be regarded as a major gain of Soviet medicine (National Standard GOST "SSBT.

Table 5

Effects of Chemical Environmental Contaminants on Mammals

Chemical	Systems affected in the long-term					
	nervous system	heart and vessels	lungs	liver	kidneys	bones
Nitroso dimethylamine				Cirrhosis, cancer		
Uranium					Sclerosis	
Carbon disulfide	Polyneuritis	Accelerated aging				
Beryllium			Cancer			
Silicon dioxide			Fibrosis			
Vinyl chloride		Peripheral vessels		Cancer		Osteodystrophy
Carbon monoxide	Psychoses					
Manganese	Parkinsonism					
Tricresyl phosphate	Pareses Paralysis					

12.1.005—76. Air of the Working Zone. General Sanitary-Hygienic Requirements)".

The concept of "maximum allowable concentration" (MAC) was defined in this publication as follows: "The maximum allowable concentration of a harmful substance in the air of the working zone is the concentration that, in the case of daily exposure in work conditions for eight hours daily (with the exception of non-working days) or during another period, but not more than 41 hours per week, through the entire working life, will not cause any disease or deviations from a normal state of health, detectable with currently available methods of investigation either during the work itself or in the longer term, in this and subsequent generations."

To summarize, the study of long-term effects in order to design a rationale for sanitary standards is today a compulsory and universal requirement. Yet not all toxicology departments are fully equipped to implement it.

Chemical impairment of the reproductive function (toxic effects on the gonads, foetus and progeny) are the best known of such impacts in quantitative terms (that is, action thresholds and compensability). Applied oncology has achieved undisputed successes because, in the world's first such venture, it has joined hands with toxicology to develop sanitary standards for carcinogens (the MAC of benzo(a)-pyrene in the air of the working zone is 15 $\mu g/100\ m^3$, and in the environmental air 0.1 $\mu g/100\ m^3$).

A much clearer view is beginning to emerge concerning the role of micropollutants in the gerontogenic impact (Ekshtat's term), and particularly in the accelerated ageing of the cardiovascular system. Indeed, it is these issues that can and must now be discussed in the monograph.

Everybody seems to agree that at least some facets of the problem under study are open to debate, this being a stark necessity, to renounce it would be to shun the effort to fathom the truth, that is to say, to refuse to provide a solid scientific basis for the prevention of chemically induced long-term diseases. It would also mean giving up regulatory control of production and waste-treatment processes as well as medicinal drug therapy and nutrition hygiene to the arbitrary rule of voluntarism. The result, now as on several previous occasions, will be either a direct damage to public health, sometimes quite impressive, in many countries or indirect health damage via unwarranted economic losses.

Discussion. It follows from the foregoing that the object of this monograph is to discuss the problems involved in regulating and standardizing the manufacture, application and utilization of diverse chemical compounds that have long-term, among them delayed, effects. Sanitary standards that impose restrictions, on the entry of harmful substances into occupational, communal, domestic and natural environments — and the biosphere as a whole — are known to be an essential component of sanitary rules and regulations.

Systematic presentation of the problems encountered in theoretical sexology, teratology, genetics and oncology cannot be the subject of a book intended for hygienists, preventive toxicologists, sanitary physicians and employees of the labour safety and health services.

Nevertheless, the authors hope that the major conceptual issues involved in evaluating the specific (selective) effects of substances on the reproductive function, the natural ageing rate of the cardiovascular system and selective oncogenic effects of chemical pollutants present in the biosphere will arouse interest among scientists in related disciplines.

The real problem is that theoretical geneticists, oncologists, teratologists and other scientists began to evolve within their respective agencies a service for dealing in practice with the problems involved in protecting the environment in general and occupational environment in particular from chemical pollutants. The decision should have received every measure of support, for the "capacities" of toxicologic institutions to handle applied genetics, teratology and oncology are clearly insufficient. Soon however, polar differences of opinion surfaced on the subject.

1. First, the specific pathogenic properties of chemical compounds began to be researched in isolation from their regular toxic properties. The researchers were thus led into abstract areas of high-dose exposures producing severe intoxication. As a consequence of the universal vascular disorders commonly associated with them, the

latter may give rise to considerable degenerative processes in various organs, e. g., the gonads involving abortions, foetal death, etc. On that basis the substances in question are entered into the registers of mutagens, teratogens or carcinogens as illustrated below.

Biologically active substances listed as carcinogens

1. Estradiol
2. Testosterone
3. Folliculin
4. Dioxycorticosterone acetate
5. Histamine
6. Glutamic acid
7. Gluconic acid
8. Glucose, lactose
9. Ethyl alcohol
10. Ammonia

Biologically active substances listed as mutagens

1. Alanine
2. Albumin
3. Arginine
4. Asparagine
5. Creatine
6. Creatinine
7. Cystein
8. Glutamic acid
9. Glutamine
10. Histamine
11. Adenylic acid
12. Catalase
13. Arylhydrocarbon-hydroxylase
14. Asparaginase
15. Liver enzymes
16. Deoxycholates
17. Ascorbic acid
18. Tocopherol
19. Adrenocorticotropine
20. Cortisone acetate
21. Diethylstilbestrol
22. Estrogens
23. Ethanol
24. Glucose

2. For the theoreticians, the primary interest lies with the qualitative characteristics, not infrequently of a quantal nature: "mutagen — yes or no?" On that basis, systems are designed for the primary „screening" of substances in order to identify potential mutagens. Earlier on, some toxicologists and hygienists also included this stage in their research efforts. More recently, however, most of them have become convinced that this is a waste of time and resources. For example, the teratogenic hazard of acetylsalicylic acid was revealed only at so high levels of exposure as to cast doubts on its real hazard as a drug. This also holds for other chemicals tested at the highest tolerable levels of exposure likely to be encountered at work, at home or elsewhere. For this reason, in choosing suitable exposure levels of poisons for determining their selective properties, one should either be based on their actually existing occupational, domestic and other levels of exposure or treat the issue academically. To this end, the entire spectrum of dose (concentration) — time — effect relationships should be defined according to their systemic manifestations and appropriate indices related to individual organs, with the registration of qualitative transitions in the body's responses to chemical stimuli. This is difficult because the mechanisms by which substances act are often dissimilar at different dose (or concentration) levels.

Committed theoreticians, being little-conversant with sanitary practice, often come up with recommendations that are unrealistic at best, and even detrimental to the cause of environmental chemical safety. Thus, many scientists still cling to the view that chemical bearing the qualitative label "mutagen" or "blastomogen" should n

be allowed to enter the environment at all. Sometimes has been said already of the embarassment this may cause them whenever essential food components or natural metabolites appear in the official lists of compounds banned for common use (mutagens or teratogens). Nevertheless, the proposed way of doing it seems the most radical and correct. The trouble, is that this entire situation cannot seem to be discussed. If the technological substitution of a teratogen, however weak, "potential" or non-specific, is feasible and if the substitution is warranted economically — it must be performed. Otherwise, comprehensive quantitative investigations based on medical and biological indications should be carried out and sanitary standards should be established (with due regard for the specific effects of the pollutants) to serve as medical-technological specifications for designers, on the one hand, and as a tool for sanitary-epidemiological inspection, on the other.

Experience has shown that technologies designed to ensure compliance with a sanitary standard, however unrealistic at first glance, are nonetheless feasible. For example, compliance with the newly established MAC for benzo(a)pyrene at 15 $\mu g/100\ m^3$ has been achieved in the air of electrolysis shops at an aluminium works by means of a fundamental change in the production process, involving the replacement of self-baking by prebaked electrodes. In underground mines where silicosis-hazardous dust concentrations were in the hundreds of miligrammes per cubic meter, dust-collection systems now ensure a safe exposure level of 1—2 mg/m^3, so new cases of the disease have been totally ruled out. At a number of production units new waste treatment facilities have brought down waste discharges to innocuous levels. Closed air- and water-supply cycles at plants virtually eliminated altogether the previous, counter-productive and ultimately impractical method of "detoxifying" effluents and discharges by dilution and dispersion.

These efforts currently constitute an engineering problem — that of multiplying manyfold the efficiency of these facilities, accompanied by an equally large reduction of the running costs and power intensity of treatment facilities.

3. The problem of extrapolating animal experiment data to human beings constitutes the centre of the debate. On this, too, there are two polar points of view. Some scientists argue that, because all living creatures have the same structure of genetic information, data developed from one animal species (and plants as well) can be transferred into hygienic practice. This explains why bacterial tests and those using in vitro tissue cultures (among them human tissues, most often lymphocyte cultures of the peripheral blood) are so common as to be included in the latest flowcharts of methods used in preliminary genetic selection [1] (the flowcharts of Bochkov /1972/, Dubinin /1977/, and others). Similar methods are being evolved in teratology.

[1] For assessment of the principle of "potential" hazard evaluation, see Chapters 2 and 5.

Table 6

Scheme for Studying Long-Term Effects in General Toxicological Evaluation, Consistent with the Technological Stages in the Development of Chemical Products

Stage of chemical and engineering design	Goal of toxicological investigations	Stage of toxicological evaluation	Content of general toxicological research	Study of Long-Term Effects	
				Content	Methods involved
I. Theoretical design of flowchart	Toxicological selection of suitable processes	A. Preliminary toxicological evaluation	Analysis of the literature on toxicity and the hazard of proposed feedstocks, reagents, catalysts, semiconductors, products and byproducts. Calculation of toxicometric parameters	Prediction of possible long-term effects by extrapolation and interpolation in series of structurally similar compounds, by correlation of different types of long-term effect	
II. Development of flowchart in the laboratory	TSEL	B. Expert toxicological evaluation	Acute and subacute animal experiments. Toxicological testing of prototypes. Setting acute action thresholds Lim_{ac}, cumulation coefficient and so on	For evaluation of acute action threshold — studies of gonadotropic, cytogenetic effects of key enzymatic systems linked with atherosclerosis; functional study of the cardiovascular system. Setting $Lim_{ac\ spec}$	Functional and morphological methods for evaluation of spermatogenesis (or ovogenesis); cytogenetic methods (analysis of animal somatic tissue cells; ECG, arterial pressure measurement)
III. Semi-commercial plant	Medical-technical specifications for the design of commercial production MAC	C. Toxicological certification D. Complete toxicological evaluation	Chronic animal experiments. Setting chronic action threshold, Lim_{ch}. Examination of workers	For setting chronic action thresholds — studies of gonadotropic, mutagenic, embryotropic and blastomogenic effects. Integrated research of cardiovascular systems. Setting $Lim_{ch\ spec}$	Functional and morphological methods for evaluation of spermatogenesis (ovogenesis), cytogenetic methods and those of dominant lethal mutations, embryotoxic and teratogenic effects, and transplacental tumour induction. Functional (ECG, AD, rheography), biochemical (lipoprotein metabolism and

IV. Design of commercial production		E. Additional toxicological investigations	Investigations into mechanisms of action of early and differential diagnosis, experimental therapy	Study of placental permeability toward ambryotropic effect. Additional investigations of mutagenic and blastomogenic effects and impacts on cardiovascular systems	connective aortal tissue), morphometric (aorta, myocardium musculoelastic vessels) Chemical and radiological methods to evaluate, placental permeability. Research methods for point mutations. Evaluation of blastomogenic effect in experiments of long duration (two years). Studies of cardiovascular systems
V. Commercial production (applications)	Adjustment of medical-technical specifications for production	F. Field studies	Study of working (living) conditions and of worker health status. Clinical testing of experim. preventive, diagnostic and therapeutic procedures	Epidemiological studies: by indications — study of gonadal function, cytogenetic blood analysis of workers, design of cards for recording natal cases, infant development, oncological and cardiovascular morbidity, longevity, and so on	Polling, gynecologic and sexologic studies, cytogenetic blood analysis of workers, ECG, arterial pressure measurement, study of lipoprotein metabolism parameters

The data derived by these methods lend themselves with great difficulty and perhaps even with a measure of "enforcement" to the kind of interpretation relevant to the needs of preventive medicine. The methods are, also, of course, out of the question when it comes to setting the thresholds for harmful effects of chemical environmental contaminants on man.

The other point of view that it is completely impossible to model human diseases (e. g. teratogenesis) on animals — is wrong (see Chapter 4).

The problem just outlined will be discussed in more detail in the appropriate chapters.

Interim summary results. The stage is thus set for examining the problems of long-term health effects of exposure to chemical environmental pollutants in the context of preventive medicine as hygienic problems.

Unlike many other medical and biological disciplines, preventive toxicology is essentially a quantitative science. The study of long-term effects by the methods of applied genetics, applied teratology and oncology, and applied pathophysiology or, in other words, by a set of morphometric, functional and biochemical methods fits nicely into the system of toxicometry with its required parameters [1] (CL_{50}, DL_{50}, Lim_{ac}, Lim_{sp}, Lim_{ch}, etc.) and their correlations (Z_{ac}, Z_{sp}, Z_{biol}, I_{cum}, K_s, etc.). The product of toxicometry consists of sanitary standards and other regulations setting safe levels of exposure for the production, application and utilization of chemicals in industry, agriculture, at home and in other spheres.

On several previous occasions we have validated the key principles for establishing sanitary regulations and standards for chemical compounds.

1. The principle of priority development and introduction of preventive programs before the test chemical comes into common use.

2. The principle of staged toxicological investigations, coinciding with the chemical and technological stages of a new product's development (Table 6).

3. The principle of the pre-eminence of medical and biological indications for the establishment of sanitary standards and regulations over any other approaches (technical feasibility or economic requirements).

4. The principle of thresholds intrinsic in all types of effects exerted by chemical compounds (including mutagenic and carcinogenic effects).

5. The principles of constancy of species abundance; the unity of the organism with its living environment; and the integrity of the organism as a biological system — underlying assessment of the criteria of harmfulness in the design of toxicometric schemes.

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[1] Methods for Toxicity and Hazard Evaluation of Chemicals. Ed. by I. V. Sanotsky. Moscow, Meditsina Publishers, 1970, 341 pp.

These and other major principles have been expounded by us, in different terms, in the literature, so the present monograph is intended to provide a supplementary treatment of them with special reference to the hygienic aspect in the overall problem of long-term effects.

As will become clear from the following presentation, investigations made by a group of researchers comprise the core of this book. Their beginnings go back to 1950, when one of the authors performed a detailed study on the effects of different radiothorium doses (in a mixture with heavy metals) upon reproduction in white mice. Follow-up investigations of the reproductive function in animals and humans were carried out on a growing scale until their current scope and intensity was attached. Thus, effective methodological approaches were designed, tested and updated; standardized methodological guidelines were developed for the system of institutions whose objective is to obtain comparable data from toxicological evaluation of the gonadotropic, embryotropic and mutagenic effects of new chemicals in order to define the thresholds of their harmfulness and develop hygienic standards and regulations. Several dozen compounds have now been examined, some general patterns identified and evidence secured that dictate, to a certain degree, the strategy and tactics for securing the occupational health of female workers and — in broader terms — affording health protection to future generations.

At the same time research data have been developed in increasing quantities regarding the ageing rate of the cardiovascular system and the way environmental pollution by trace quantities of chemical compounds becomes involved in the process. This data collection continues.

In cooperation with oncologists, a common tactical line of toxicologic research on carcinogens has been developed with a view to ascertaining the feasibility of appropriate preventive programmes.

Though the scientists have not been able to iron out all their differences, a major stride has been made toward the problem's solution, by the introduction into practice of new methods for predicting carcinogenicity and new sanitary standards and regulations.

The points just mentioned are covered more or less exhaustively in the corresponding chapters of the monograph. Constructive criticisms will be appreciated, particularly because the more general issues of concern to us here constitute an evolving set of problems.

Chapter 2

BASIC PRINCIPLES FOR EVALUATING CHEMICAL EFFECTS ON THE REPRODUCTIVE FUNCTION IN PREVENTIVE TOXICOLOGY

Impairment of the reproductive function by chemical compounds that gain access to the human body from the environment (gonadotropic, embryotropic and mutagenic effects) constitutes a particular toxic impact of an excess above normal background levels of chemicals in the environment. In other instances, this type of effect originates from deficits of chemical constituents in the environment, such as deficits of protein, vitamins, metals, etc.

We have already repeatedly discussed the basic principles of preventive toxicology underlying practical steps towards providing protection against exposure to chemical compounds (Sanotsky, 1967; Sanotsky, Ivanov, Fomenko, 1971; Sanotsky, Ulanova, 1975; Izmerov, Sanotsky, 1977; Sanotsky et al., 1977).

Some of the principles were briefly examined in Chapter 1. The set of closely related principles is now to be interpreted with reference to research into injuries to the reproductive function.

Priority of preventive measures. The principle of the priority of justification and implementation of preventive measures — prior to the ingress of the noxious factors that the measures are designed to control — requires, apparently, no clarification because it has been incorporated into public law (The Fundamentals of the Legislation of the USSR and the Union Republics on Public Health; the Law on Land Use; the Law on Water Use; as well as other legislative acts and national standards).

Nevertheless, conditions arise nowadays when absorption of new factors into man's living environment (occupational, communal, domestic and natural) is preceded neither by medical and biological assessment, nor by the design and implementation of preventive programme.

Failure to abide by the "priority principle" is likely to result in unfavourable changes in the status of human health and even in the development of pathological processes, to say nothing of indirect health damage from the destruction of some ecological chains and diversion of capital investment to cover damage caused by the chemical corrosion of equipment and plant, building materials, etc.

Authors outside the USSR estimate the cost of meeting medical-engineering demands for waste treatment facilities at 6 to 12 per cent of the project investment cost if provided for in industrial projects

with proper timing (at the pre-project stage), but as high as 50 per cent of the total cost in existing if they have to be re-equipped to comply with sanitary requirements.

As noted above, the staged pattern of research is a management tool for ensuring priority of preventive measures (Kosourov, 1963; Sanotsky, 1969; 1974; 1977; Zaugolnikov, et al., 1971; Krasovsky, 1976).

As applied to the study of the reproductive function and the blastomogenic and teratogenic effects of chemical compounds, the staged pattern of research is schematically represented in Table 6. An accompanying note is that several years ago, when the study of long-term effects was listed among investigations "according to indications", it was conducted exclusively under the stages called "Toxicological Certification" or "Complete Toxicological Evaluation".

Pre-eminence of medical indications over recent technological developments. The question of the priority of medical indications in the design of sanitary standards over their technological or economic feasibility is the single most "burning" point of the debate, past and present. No one denies, however, that sanitary standards must be progressive, that the medical and biological indications for their establishment must be the principal strategic line, and that their tactical enforcement over time and from project to project should be flexible, but only as long as newly-built projects accommodate the very latest requirements of preventive medicine.

What can the supporters of the "technological" or "economic" feasibility approach oppose to medical indications? Nothing, but tentative estimates and subjective assumptions.

As an example, when economists try to estimate the costs of implementing the SO_2 sanitary standards for the atmosphere of population aggregates they usually quote only the price of waste treatment facilities and the construction costs of coal desulfurization plants. They take no account of the fact that enforcement of the SO_2 standard cuts back losses from the adverse effects of sulfuric acid on the soil (the discharge from only one thermal power plant is tonnes of H_2SO_4), plants (devastation of pine woods), water bodies (fish death from water acidification), and construction materials (corrosion of bridges, roofs, plastics, and rubber).

While the above items can be estimated in terms, we deliberately leave aside at this point losses resulting from impairment of human health, which may not be always expressed in monetary terms. Even so, in the United States, for example, (Barret et al., 1973), the cost of health damage associated with the impact of air pollutants was estimated in 1968 to be 3,272 million dollars for sulfurous exposure and 2,788 million dollars for airborne particulates. Furthermore, by 1963 these morbidity and mortality losses had risen to 1.255 billion dollars from a 1958 figure of 400 million dollars (Ridker, Ronald, 1967). These estimates are merely tentative guides, but they are sufficient to demonstrate the good economic rationale of even the most stringent hygienic requirements.

It may be that the methods used to estimate economic losses caused

by environmental pollution are not efficient enough. Such a methodology is known be used in fish breeding. The task of designing a similar methodology for assessing losses from public morbidity and disability remains quite relevant if not altogether urgent.

The data base for an environmental control strategy must be rested on medical indications. This not only avoids an irreconcilable conflict with the pertinent economic requirements, but helps to secure, in using key approaches to future designs of new technologies, their all-round compatibility with sanitary and economic criteria.

Remarkably, the estimation of direct economic damage in the construction of waste treatment facilities has extremely limited applicability. Epstein (1976) revealed that, with the promulgation of a new standard for residual monomer concentrations in vinyl chloride plastics, the building costs of the "decontamination" system raised the question of shutting down the facility because of prohibitively high production costs. In practice, however, when this system for the follow-up processing of the plastics did become operational, it ultimately proved not merely feasible, but also economically advantageous.

A similar picture is observed in the manufacture of many other chemicals.

Choice of experimental model. Questions of applying data on animals to humans. Conclusive evidence of the impact of chemical compounds on the reproductive function is derived from the data of scheduled medical examinations and socio-hygienic surveys, which suggest that the function is damaged and life expectancy reduced among certain humans exposed to the chemical agent of concern. Completely credible information concerning the genetic hazard of industrial toxics could perhaps be obtained also from observations on the reproductive function and development of progeny among chemical workers and analysis of their rates of morbidity compared with those of their progeny [1].

In view of the essentially preventive character of Soviet medicine, however, these data should be considered belated. Besides, it is somewhat difficult to prove the role of occupational exposure to chemical compounds among the set of working and domestic exposures giving rise to reproductive function pathologies unless addition, experimental investigations are carried out and computer-based multifactor statistical analysis is used with suitable formalization of data inputs. At present, however, the analysis would come up against serious difficulties because of poor methodological support. In practice it is considered a virtual impossibility to locate and identify the appearance of such gross irregularities as dominant lethal mutations causing embryonal mortality, sterility and some decline in fertility with the immediate impact of a chemical. According to Potter (1962), undetected pregnancies account for a half of all conceptions, which is why the incidence of embryo deaths in utero is greater than the number of childbirths. In contrast, most spontaneous abortions are known to ori-

[1] Human Genetics and Public Health, WHO Records, No. 10, 1965.

ginate from chromosome aberrations, when they are present and detected in the embryo tissue cells, the causal factors of which were simply ignored in most studies (Stonova, Selezneva, 1968; Chebotarev, 1972, 1974). An important role must be assigned, therefore to an experiment for recognizing the leading pathogenetic factor, exploring the various facets in the injurious action of chemicals and helping define exposure levels harmless to the organism.

Experiments on test animals also constitutes the primary research tool at the stage when new compounds are synthesized in the laboratory. The data thus obtained could set the stage for the hygienic selection of a suitable technology on a scientific basis, but only if the selected experimental model is suitable. This is the most debatable issue now.

The great majority of data on the mutagenic activity of chemical compounds comes from experiments on classical genetic subjects such as the fruit fly Drosophila melanogaster, microorganisms and plants. The method of studying germ-coalesced mutations on the Drosophila in the genetical hazard assessment of chemical compounds also has a good measure of application in essentially hygienic works (Revazova, 1967; Rapoport, et al., 1968; and others). The subject has also been recommended for checking the genetic hazard of toxic chemicals (Chepinoga, 1967).

The reason that the models just discussed are now in common use has been suggested by a number of geneticists. Relying on the unity of the principal gene structures in all living creatures, they have argued that experimental findings in lower subjects make it possible to state the genetic hazard of chemical compounds for humans unconditionally (Rapoport, 1966; Auerbach, 1959; Schwartsman, Sondore, 1975). Liining (1966) suggested growing Drosophila larvae on a drug-containing medium as a means for evaluating drugs when first introduced. Such suggestions are probably based on the encouraging evidence derived from studying the effects of radioactive radiations, known to induce mutations in any organic species. They obviously, take no account of the fundamental species differences in toxicokinetics.

Yet the need to apply the principle of selective toxicity has long since been recognized for agriculture. The development of chemical products that help to get rid of the most diverse species of noxious organism without harming humans or animals is the key objective in suggesting new chemicals for common usage in farming. In particular, the selective mechanism of many agents among the most effective organophosphorus insecticides is based on two opposite mechanisms (Albert, 1971). Namely, in the insect organism such a chemical is converted into another, more toxic compound, while in that of a mammal it is changed into a less toxic agent. In the same monograph, Albert (1971) cites data on the conversion of phenols into beta-glucoronides in vertebrates and into phenyl-beta-glucosides, largely, in insects. In the same manner, in the insect organism cyclodiene compounds transform into corresponding epoxides, which are dangerous mutagens.

Still wider divergencies in metabolism exist between microorganisms. Bacteria are very easily injured by chemical compounds, as

their vital enzymes and nuclear substance are poorly protected. Advantage is taken of this in the development of chemical compounds exhibiting selective toxicity.

Manifestations of chromosomal variability differ in different species. This is due, in particular, to the disparate biochemical causes of mutagenesis indentified for them (Dubinin et al., 1965). As a result, even among lower organisms a chemical found to be mutagenic for one was not so for another (Table 7). This can be demonstrated even better in relation to the effect of chemical compounds, at the same exposure levels, on dissimilar species of organisms.

Table 7

Genetic Effect of Chemicals on Different Biological Subjects (from the literature and the authors' own data)

Chemical	Micro-organisms	Drosophila melanogaster	Mammals		
			human tissue in vitro	animal tissue in vitro	dominant lethal mutations
	*	**	*		
Ethylenimine	+	+	+	$+Lim_{ch}$	±
Propylene oxide	+	+		$-Lim_{ac}$	
				$-Lim_{ch}$	
Ethylene oxide	—	+		$+Lim_{ch}$	$-Lim_{ch}$
Epychlorohydrine	+	+	+	+	—
Urethane	+	+		+	—
Nitrosoethylurea	+	+	+	$+CL_{16}$	
Formaldehyde	+	+	---	$-Lim_{ch}$	
Butylated oxytoluene		+		$-Lim_{ch}$	
Zinc dimethylthiocarbamate [ziram]	+	—	+	$+Lim_{ac}$	
2,4-Dichlorophenoxyacetic acid	+	—	+	+	+
2,4,5-Trichlorophenoxyacetic acid	—	—	+	$+Lim_{ch}$	—
1-Naphthylmethylcarbamate	—	+	+	+	
Chloroprene	+	+	+	+	+
Dimethyl sulfoxide	—	—		—	—
Methylmethansulfonate	+	+		+	+
Benzene		—	+	+	+
Trimethyl phosphate	+	±	+	+	+
Hydroxylamine	+	±	+		—
Isoniazide	+	+	+		—
Chloridine		+	+	+	—
Hydrazide of maleic acid		+	+		—

Notes: * — doses with no cytotoxic potential;
** — at the LD_{50} level.

In highly organized organisms, their genetic structures appear, on the one hand, to be better protected from surrounding exposure insults because of tissue barriers and metabolic detoxification. On the other hand, possible mutations in them may be set off indirectly, by endocrinal

and neurohumoral disturbances, and not by any direct action on DNA (Koltsov, 1938; Klosovsky, 1963; Shakhova, 1965; Shtern, 1965; Swandon, 1959; Miller, 1965).

In recent years, most researchers have tended to favour the concept that, in order to "finalize" the conclusion concerning the genetic hazard for man posed by the agent under study, the effect needs to be ascertained through experiments on laboratory mammals species or on human somatic cells in vitro (Bochkov et al., 1972, 1975; Malashenko, 1975, et al; Kuznetsova, 1977).

Unfortunately, the alternative concept of chemical mutagen assessment cannot be recognized as suitable for the management of prevention problems. A mutagenic effect evaluated at high levels of exposure may fail to cause genetic lesions at realistic exposure levels (see Chapter 2).

A genetic model such as the in vitro study on the culture of human embryonic fibroblast tissues and peripheral blood leukocytes enables the pattern and frequency of changes in the normal set of human chromosomes to be observed during direct exposure to industrial toxics introduced into the culture medium (Varshaver, 1965; Waisfeld, 1965; Osetrova et al., 1968; Pogosyants et al., 1968; Revazova, 1973; Paul, 1963; Stepanis, Scherek, 1964; and others). A careful review of the genetic literature and a good knowledge of the laws of toxicodynamics in the integral organism are required, however, to show clearly that the in vitro study of chemical mutagenesis on human cells would seldom yield results consistent with those from a study in vivo. For example, the cytogenetic effect derived from human cells in an in vitro study simply failed to come through during in vivo exposures to diethylamides of lysergic acid (Gorey et al., 1970), Valium tranquilizers (Stenchever et al., 1969; Cohen et al., 1969) or chloropromazine (Shaw, 1970). The overdiagnosis arises because many of the protective systems inherent in the integral organism are missing from the tissue culture (Brusick, 1977).

There are also cases when the opposite happens, when more active substances form in the integral organism either during the metabolism of the effective agent or during its intervention into natural metabolic processes. Proof of this is provided by the cytogenetic activity of several compounds, readily identifiable in human cells when the compounds act on the integral organism in vivo but not detectable in vitro. The compounds referred-to include lithium chemicals (Friedrich, Nielsen, 1969), the cytostatics endoxan and S-cytoxan (Arrigni et al., 1962; Hampel et al., 1966), and several other agents.

Experiments in vivo should, therefore, be acknowledged as being more suitable, yet the validity of transferring data derived from animal experiments to humans still remains the key question in the area of research in preventive toxicology concerned with the mutagenic hazards of environmental factors (Sanotsky, 1965, 1972; Revazova, 1967; Bochkov, 1969, 1972; Fomenko, 1969, 1975; Zhurkov, 1975).

A series of integrated studies — hygienic, clinical statistical, and empirical — revealed that gonadal disfunctions in workers handling

chemical agents are reproducible in spontaneously ovulating animals e. g., white mice and rats (Table 8).

Table 8

Results of Empirical and Clinical Hygienic Studies on the Long-Terms Effects of Several Industrial Toxics

Compounds \ Object of surveillance	Man	Laboratory animals
Chloroprene	+	+
4,4-dimethyldioxan-1,3	+	+
Tricresol	+	+
Phosphorus oxychloride	+	+
Tricresylphosphate	—	—
Dibutyl phthalate	+	+
Dioctyl phthalate	+	+
Phenol	+	+
Vinyl chloride	+	+
Ethylene oxide	—	+
Dimethyl phthalate	—	—
Chlorothene citrate	+	+
Chlorophen citrate	+	+
Butyl ester of 2,4,5-T [trichlorophenoxyacetic acid]	+	+
Zinc dimethylthiocarbamate	+	+
Carbon disulfide	+	+
Lead	+	+
Manganese	+	+
Caprolactam	+	+
Zinc ethylenebisdithiocarbamate	+	—

Of still greater interest in the context of interspecific extrapolations is the evidence of the genetic hazard of chemical compounds to humans and animals. Though extremely scanty, the evidence, as well as experience, show that the test animal model can be applied for this kind of research (see Chapter 5).

The question of whether animal experiments are essential for predicting the teratogenicity of chemical agents for man is debated in the literature. According to published data, most experimental investigations on the embryotropic action of chemical compounds are made on small laboratory rodents. Some authors prefer dogs and monkeys. Yet there is still a large proportion of studies performed on chick embryos. Their authors argue that the use of chick foetuses in applied teratology is practical on the grounds that their responses to the introduction of medicines are similar to those of the rodent embryo (Gebhardt, 1972). The argument is nonetheless disputable, as research findings from chick embryos are difficult to transfer to humans. For the moment, one certainty is that all substances teratogenic to man display a varying measure of teratogenicity in experiments on mice, rats and rabbits (see Chapter 4). Even so, the negative results from

the tests of chemicals on these animals are called into question by some authors (Manevich, 1966, Dyban, 1968), who point out that even thalidomide, a classical human teratogen, does not induce malformations in all animal species. One of the principal reasons for complexities in the interpretation of this experimental evidence is rooted in the information gap regarding the mechanisms of teratogenesis; the distinctive metabolic patterns of poisons in man and animals; and the differences in the interaction between two biological systems, the maternal and foetal organisms, in man and animals (The Report of a WHO Group of Experts, 1968).

Experiments nevertheless reveal a high rate of coincidence. A similar effect on foetal development in the occupational exposure of women workers and in animal experiments was shown in the literature for benzene, chloroprene, manganese, granosan and several other chemicals (see Chapter 4). Importantly, the toxic exposure levels inducing pathologies in human beings and animals have in many instances been the same.

As with the evaluation of general toxic action, experiments conducted on several test animal species ensure a more credible hazard assessment of the study chemical for man.

When deciding between highly-inbred (linear) and heterogenous (non-linear) groups of experimental animals, account must be taken of the fact that the large heterogeneity of the response in inbred animals is combined with its high selectivity (Malashenko, Surkova, 1973; Frohberg, Bauer, 1973). So, for poorly known compounds, the use of heterogenous groups or hybrid lines is more economical, because it cuts down the relative cost of the experiment. Indeed, the employment of inbred groups requires that the experiments be carried out in at least three strains of experimental animal. (L. Rossival, 1974). At present, a new line of tetrahybride mice, ♀ (CBA × $C_{57}Bl_6$) ♂ (C_{57} Bl_6 × DBA), showing remarkable viability and stability of indices, has been proposed for genetic research in the USSR.

Quantitative evaluation of disturbances. A study of the state of the reproductive function would typically employ morphological (histological) methods, though the results may be hard to interpret without reliance on associated biochemistry and the functional methods of research. Unfortunately, until recently changes in organs and tissues established by morphological methods were evaluated, with few exceptions, only qualitatively. Meanwhile every researcher is well aware that tissue sites with an impaired normal structure can be found in control animals. Without a quantitative characterization, even such seemingly "persuasive" evidence as photographs should be considered unsuited to the current level of knowledge, particularly for determining the harmful action thresholds of chemical compounds. It is also known that the individual features of organisms are fundamental for producing an effect of any magnitude, thus making it necessary to survey sufficiently representative groups and to evaluate the findings statistically.

For a morphological assessment, quantitative criteria are also needed because they permit minor structural changes to be identified that are invisible to the naked eye. Such criteria have been developed, in particular, for the testes (Nuzhdin et al., 1959), ovaries (Mandl, Zucherman, 1951, 1952; see Chapter 3), for assessment of embryotropic action (see Chapter 4), cytogenetic changes (see Chapter 5) and changes in the blood vessels (see Chapter 6).

Similarly, the tendency to segregate quantitative characteristics of changes from their qualitative features must also be criticised, because the relative number of chromosomal lesions, to take one example, makes little sense unless their quality (cohesion, fragments, bridges, etc.) is considered.

Relationship between test effects and the dose or concentration of a chemical agent. A review of numerous publications on lesions of the reproductive function caused by exposure to chemical agents and the latter's blastomogenic (or any other systemic) effects immediately reveals the clearly inadequate treatment of the relationship between the effect and quantity of the poison or its effective dose or concentration under appropriate exposure. Quite a few of the studies, clinical ones in particular, merely state that a certain substance produces an effect, sometimes supplementing this with data on the length of time it was employed. It is clear, though, that no conclusions about a poisons mode of action can be derived in such cases because it (inhibition or excitation of certain structures and functions) varies, not infrequently in opposite directions as the quantity of the chemical stimulus is changed. Thus, to define the dose — effect relationship is the first major problem in the study of a toxic effect on the reproductive function, blastomogenesis, and on the body's organs and systems in general.

The direct dependence of the magnitude of gonadal impairment on the test concentration of a chemical was experimentally ascertained from exposures to 1,2 dibrom-3-chloropropane (Torkelson et al., 1961), dimethyldioxan (Pashkova, 1969), lead (Yegorova et al., 1966; Golubovich et al., 1968; Chirkova, 1970), boric acid (Strongina, 1971), ethylenimine (Fomenko et al., 1976), organomercury and other pesticides (Vashakidze, 1966, 1970; Rybakova, 1964, Zhalbe, 1968), chloroprene (Davtyan, 1972), tretbutylperacetate (see Fig. 4), and aminopyrimidine (Fig. 5).

Over the last few years, the ebmryotropic effects of chemical compounds have been elucidated for many industrial chemicals of different categories. For most of them, a distinct effect — concentration relationship has been found, for example for the effects of chloroprene (Salnikova, 1971), dimethylformamide (Fig. 6), methylmercaptophos (Gofmekler et al., 1968, 1971), nicotine (Borinshtein et al., 1976) and other agents.

Genetic studies of different biological subjects mostly reveal the induction of mutations to be dependent on the concentration of the chemical agents, as exemplified by the effect of many depressants on mitosis. The many inert compounds acting in this manner include hexane and other aliphatic hydrocarbons, alcohols, eithers, chloro-

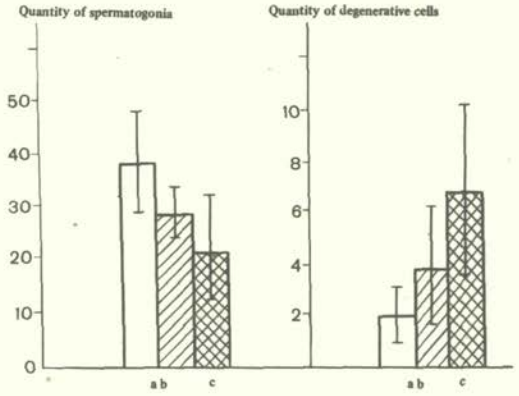


Fig. 4. Variation in the total of normal spermatogonia and degenerative cells in those chronically exposured to tretbutyl peracetate.
a — control; b — 1 mg/m³; c — 10 mg/m³

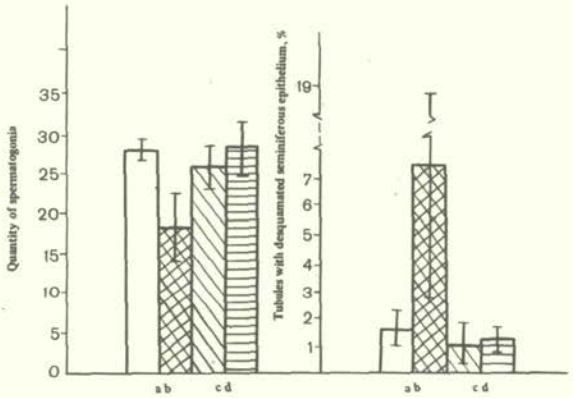


Fig. 5. Variation in some indices of spermatogenesis in rats 24 hours after a single four-hour exposure to aminopyrimidine in different concentrations.
a — control; b — 21.4 mg/m³; c — 61.9 mg/m³; d — 5 mg/m³

phorm, acetone, paraldehyde, acetamide, urethane, acetophenone, benzophenone, acetamylide, benzene, chlorobenzene, sulfonol (Östergen, 1951), as well as nitrogen and argon under pressure (Ferguson, Hawkins, Doxey, 1950). The effects disappear, however, as soon as the agent's concentration begins to decrease (Albert, 1971).

The incidence of mutation responses (to a single exposure) shows a linear relationship with the dose of triethylenmelamine (Generoso et al., 1973; Arnold et al., 1974), trifluoropromazine (Peterson, Legator, 1973), ethylenmethane sulfonate (Generoso et al., 1973), mitomycin C (Alder, 1973), giganton, methanesulfonate (Green et al., 1973), and lysergic acid's diethylamine (Sram et al., 1974) and tris-1-2-methylzirydinyl (Richardson, 1974; Waltenstein, 1974).

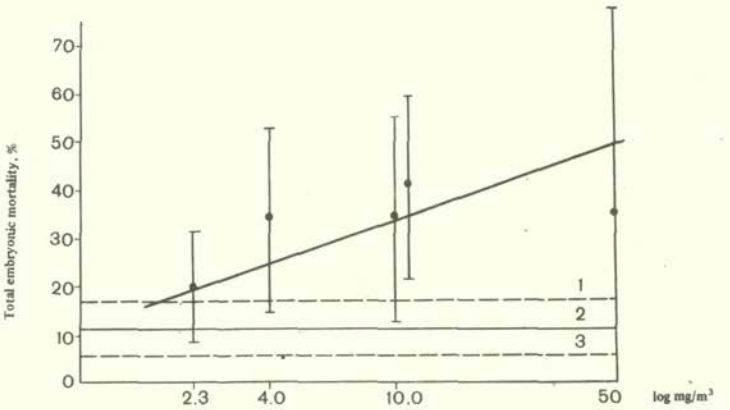


Fig. 6. Embryotropic effect/concentration relationship for dimethyl formamide.
1 — upper limit of confidence interval in control;
2 — general control;
3 — lower limit of confidence interval in control

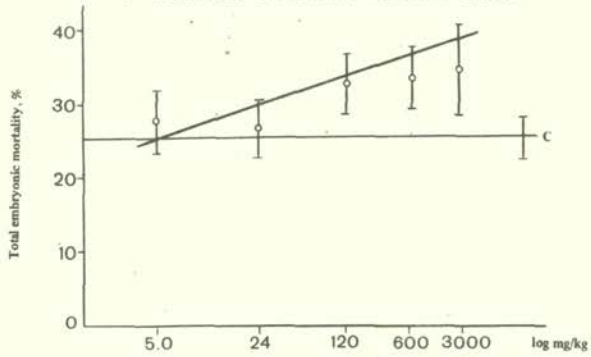


Fig. 7. Incidence of dominant lethal mutations in SHK mice against benzene dose.
C — control level

A direct correlation between the mutagenic effect and the concentration and dose was also shown for repeated and chronic exposure to morpholine (Fomenko et al., 1973), ethylene oxide (Strekalova, 1973), ethylenimine and chloroprene (Katosova, 1973, granosan, sevin and captan (Vashakidze, 1973), urethane (Suvalova, 1972) and benzene (Fig. 7).

On some occasions, the relationship proved more complex than expected. The organochlorine pesticide DDB produced a sinusoidally shaped dose — effect curve by the rate of overt mutations both in germ and somatic tissues ($r = +0.94$) (Matveeva et al., 1973).

In several other instances as well no direct dependence of the genetic effect on the dose (concentration) of a chemical agent can be found, for an altered dose (concentration tends to modify qualitatively the mode of action of toxics.

In view of this, it is all the more intriguing for preventive toxicology to explore the effect — dose (concentration) relationship for low levels of exposure (close to the action threshold). Workers in the toxicology

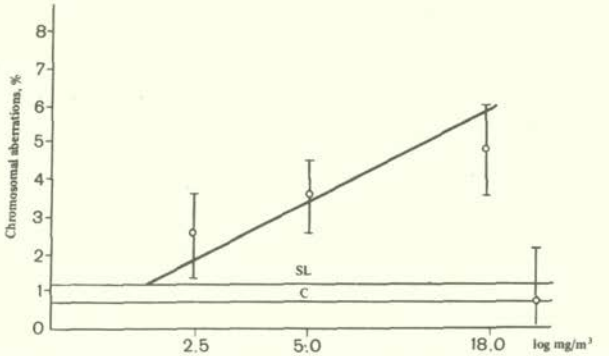


Fig. 8. Cytogenetic effect/concentration relationship for chloroprene (health survey of workers).

SL — spontaneous level, C — parallel control

department of the Institute of Industrial Hygiene and Occupational Diseases used chloroprene as an example (Katosova, 1973) to demonstrate empirically the logarithmic relationship between the cytogenetic effect and substance concentration that exists within the range of tested exposure levels. The observed distribution differed only slightly from the theoretical one with $\chi^2=10.79$, $n=5$, and $P=0.08$ ($\chi^2_{0.05}=12.6$) and followed a linear regression. That the effect is concentration-dependent was also shown from a survey of exposed workers (Fig. 8).

Intrinsic thresholds of effects. Preventive toxicology demands more than just quantitative evaluation of the dose — effect relationship: it needs an exhaustive quantitative evaluation that enables the full-scale utilization of experimental findings for finalizing the need for practical protective efforts. The important thing is to find the minimum effective (threshold) quantity of poisons, which means that no-effect doses and concentrations must be determined.

No one questions anymore the possibility of establishing the threshold of harmful effect for the majority of chemical compounds. It still somehow remains a contentious issue, however, in relation to mutagens and blastomogens, though the laws governing the interaction of a substance with the biosubstrate apply in this case as well.

The no-threshold concepts of chemical effects grew out of the abstract assumptions that there exists a monomolecular mechanism for the association of a mutagen with the nucleic acid basis, as well as for the extrapolations of the basic patterns of the effect from high exposure levels to low ones (Filippova, 1975), which are outside the scope of the study. Nevertheless, in most cases the intensity of the response by the organism declines as the dose of the stimulant does, especially if it is a chemical one, and the response falls to zero even before the dose does (Hatch, 1973).

What is wrong, then, with the no-threshold concept of mutagenic (carcinogenic) chemical effects? It is the authors' failure to recognize the non-uniform pattern of bodily responses to external effects and the

fact that the conversion of insignificant quantitative alterations into radical qualitative changes proceeds by leaps and bounds, as does in our case the conversion of physiology, adaptation and health into pathology, deadaptation and illness. In fact, this amounts to a denial of the fundamental differences between living and non-living matter and to deliberate abstraction from the constant renewal and regeneration of biological structures. Through such an approach, statistical distributions of individual sensitivities whose branches about at $-\infty$ and $+\infty$ are taken to be their basic pattern of distribution. To validate this assumption, the dependency of primary responses on the magnitude of exposure is extrapolated into an immeasurable region tending to zero.

The concept of thresholds inherent in the action of any noxious factors has been repeatedly examined by Sanotsky (1975, 1976, 1977). It has been shown that chemical mutagens are unable, in principle, to reach their "receptor" at low levels of exposure as long as the processes that eliminate the toxics from the (intact) organism and the latter's detoxifying metabolism remain in operation (many incidents of primary activation of chemical mutagens in the body are attended by processes leading to their subsequent clearance and metabolic detoxification).

Data from experiments with non-metabolizable and metabolizable substances indicate that a decrease in the dose administered to the body increases relatively the excretion of the substances and the detoxication (Sanotsky et al., 1976, 1977). Hypothetical substances are the only ones which, as they enter the body, would not be absolutely detoxified and excreted and, therefore, can reach the receptors in undiminished quantities. Such substances do not exist in reality.

It is well-known now that the simple bonding of a poison by the receptor is a long way from a biologically significant reaction with it. Thus, we have repeatedly utilized the data of Grigarzik and Passov (1958) suggesting that the binding (including firm binding) of lead ions by the erythrocyte (red-cell) membrane does not increase, the release of potassium and haemoglobin into the incubation medium. Mitova's data reveal that alcohol in vitro, when used in certain doses, changes the molecular structure of haemoglobin, but not its functional properties. Consequently, molecular restructuring, within certain limits of exposure intensity, can produce no functional changes in the biosubstrate. In the intact organism, the structural changes induced by chemical exposure are still less significant. Whether a biologically meaningful effect will arise depends on the substrate's "significance" for the cell's activity, on the availability of an alternative metabolic pathway and the rate of repair. It is known, for example, that in order to eliminate the mutant cells that crop up every minute, the body can activate its powerful immunity mechanisms, both humoural and cellular.

Levan and Biesel (1973), proponents of the genetic cancer theory, state bluntly that chemical carcinogens affect cells only if present in concentrations that exceed the threshold ones. The same view, but in

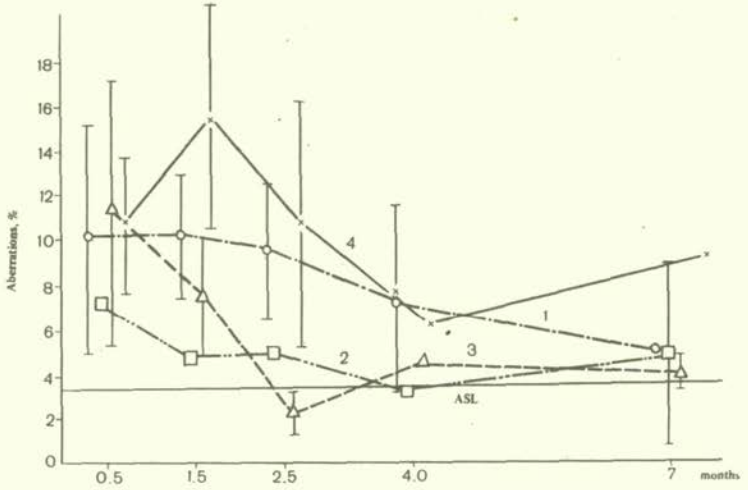


Fig. 9. Cytogenetic effect of butyl ether of 2,4-D(1), DDT(2), lindane (3), and prometryn (4) in the bone marrow of albino rats against time of exposure (Kulakov, Efimenko). ASL — average spontaneous level

relation to the manifestation of mutagenic effects, is aired by Mitrofanov (1969), Newill (1975) and other workers. Important data have been provided by Cohen (1969), who showed in an experiment on several biological models that the thresholds of mutagenic action really existed and that among other factors, they were partly dependent on the reparative activity of impaired genetic material. The processes of adaptation and generation such as the activation of detoxication, compensatory reproduction of molecules of the affected enzymes, switch-over to alternative pathways of normal metabolism, and activation of microsystems — have the object of adjustment to the new conditions of existence. An impairment develops solely when the rate of injurious processes (inactivation, degeneration, etc.) is greater than the rate of the adjustment and regeneration.

Our data suggest a threshold for the blastomogenic effect. Indeed, observation of a large experimental animal population until their natural death after ethylenimine inhalation revealed a clear-cut association between the yield of tumours and toxic concentration. The concentrations were defined at which the tumour formation rate approaches the control background level (Zaeva et al., 1968). For inhalation exposure to isopropyl aminodiphenylamine, an agent qualified by G. B. Pliss as mildly carcinogenic, a year-long experiment established the concentrations that are not carcinogenic for animals (Melnikova, 1969). Finally, Bruevich (1975) reports that even 3,4-benzpyrene, by dermal application, possessed a threshold level (as different petroproducts were applied to the skin), a fact later confirmed by Shugaev and Panchenko (1969). Needless to say, the threshold of an effect depends on the size of the statistical group, but groups of 100 individuals (methodolo-

gical instructions suggest a minimum group size of 50) produce a comparable effect with the general population.

Adaptation has been shown to take place at the subcellular, cellular, organic, organismic and population levels, with a continuing injurious exposure (radiation and chemical compounds). The documented occurrences include: reparation of chromosome rearrangements upon fractional irradiation (Dubinin, 1961; Yarmonenko, Palyga, 1964); the normal life of human populations against an increased (up to a certain limit) radiation background, as in highland or radioactive ore-mining areas; a decline in chromosome rearrangements in case of persistent chronic exposure to mutagens e. g. ethylenimine, prometryn, DDT, 2,4-D (Fomenko et al., 1973), ethylene chlorohydrine (Isakova et al., 1971); and rapid hereditary adaptation, e. g. of bacteria to antibiotics and of insects to insecticides. Since the law of natural selection does not hold for the human population, no exact factual evidence has been derived as yet with respect to man's real adjustment to living in a chemically polluted environment.

To the afore-mentioned evidence in favour of thresholds, it must be added that many of the known carcinogens are necessary for the body, either as food ingredients (selenium, arsenic, and some vitamins) or as internal medium components (sex hormones). The compounds take on a new quality only after they have passed a definite quantitative threshold.

It has already been pointed out that the method for setting safe exposure levels by mathematical extrapolation of data from high to low levels has won fairly wide support. It was used, in particular, to identify the MAC for benzo(a) pyrene (BP) for ambient air and other media. Thus, Yanysheva (1975) estimated the non-effective dose of 0.02 mg from a chronic experiment on rats with intratracheal administration of benzo(a)pyrene over a large dose range. The blastomogenic effect — chemical dose relationship thus identified was then described mathematically. By lowering the non-effective experimental dose 100-fold and assuming that the determined dose — effect relationship persists in the region of minimum dose levels, the author estimated the MAC by extrapolating the curve to the non-effective dose reduced 100-fold.

Though the attempt is certainly noteworthy, the author's principle has a number of disadvantages. First, this is an arbitrary model choice for extrapolation. Table 9 presents the results obtainable with the aid of some functions that approximate the experimental findings well in the region of average and high probabilities of the effect (the calculations were made by M. R. Zeltzer and G. A. Avilova). As seen in Table 9, the divergencies are rather significant. The difficulty in choosing a suitable model was underscored by Crenmer. He also showed that processing the dose-effect curves by three different methods gives excellent fits in the 8 to 92 per cent effect range. By contrast, extrapolation of the relationships into the minimum dose and effect region is fraught with gross errors (a more than 100-fold disparity in the results obtained by the different methods). The mere assumption of such extrapolations constitutes a serious deficiency of

the calculations. Seeking to correct the deficiencies noted above, the Mentel-Brian model utilized by Crenmer provides for the dose — effect curve to be constructed in the lowest dose region with a slope whereby a 10-fold increase of the dose alters the effect by one probit. Yet even this "rigorous" model, is much too imperfect.

We submit that, in dealing with the problem of thresholds in bodily responses to external impacts, the harmfulness-of-action theory is quite important. It states that, from the perspective of environmental protection, not all thresholds of the biosubstrate responses are essential, but only those that apply to harmful responses. This idea was formulated by N. S. Pravdin back in 1934. The theory was further evolved by his followers, and an increasing number of scientists have embraced his propositions in recent years. Weil (1972) indicates that for man, as indeed for any animal species, there exist dose levels incapable of exerting harmful action, though the effect they produce may be statistically meaningful.

Table 9

Results from the Extrapolation of Dose-Effect Relationship into the Region of Low Doses, Using Some Approximating Functions ($f_{\chi n}$)

Approximating function f (χ_n) (χ_n is effective dose, mg)	Neoplastic response on exposure to Yanysheva's non-effective dose χ=0.02 mg, %%	Neoplastic response on exposure to dose equiv. of benzo(a)pyrene MAC in air, %%	Dose producing neoplastic response level of 10^{-3}%, mg	MAC consistant with probability of neoplastic response of 10^{-3}%, μg/100 m³
$f_1(\chi_n)=1-e^{-1.55\chi_n}$	3.3	0.03	$7 \cdot 10^{-6}$	$3.5 \cdot 10^{-6}$
$f_2(\chi_n)=1-e^{-0.064\chi_n}$	0.13	0.0013	$1.5 \cdot 10^{-4}$	$0.75 \cdot 10^{-4}$
$f_3(\chi_n)=10\ln(\frac{\chi_n}{\chi}+1)$	6.9	0.1	$2 \cdot 10^{-6}$	$1 \cdot 10^{-6}$
$f_4(\chi_n)=32 \cdot \chi_n^{0.27}$	11	3.2	$3 \cdot 10^{-18}$	$1.5 \cdot 10^{-18}$

The approximating functions f_1, f_2 and f_3 were proposed by A. Ya. Yanysheva (1974).

While asserting a no-threshold character for primary responses to radiation exposure, Akoev and co-authors (1972), added the hypothesis of a threshold number for affected systems, that is, the hypothesis of a threshold for the harmful effect.

The threshold theory of the effect implies not the responses by biological substrates to the impact of external factors in general, but their meaningful biological (including medical) responses (see Chapter 8).

Under the accepted terminology in the Committee for the Establishment of Maximum Permissible Concentrations of Industrial Chemicals, the threshold of harmfulness should be taken to mean significant

deviations from the control as well as from the initial values of responses by the set of physiological systems maximum-sensitive to a particular effect which are found at the boundary between physiological variations, the physiological measure of protection, and the pathological process (Sanotsky et al., 1970). Reasoning from this basis, the threshold of mutagenic action for the study agents, for example, should be defined as the quantity of the latter (dose, concentration) whose action (given the methods used and a suitable statistical group of individuals) produces the minimum effect. In response to a toxic dose (concentration) exposure below that level, the frequency of mutations will not differ from those in the parallel or general control group (with the natural mutational background taken into account).

It is important that an assessment of the threshold of the harmful effect should rely on a set of methods — physiological, morphological and biochemical — along with the so-called loading tests (with the goal of identifying latent, temporarily compensated diseases). Practical examples to illustrate the applications of this approach are provided in the following chapters.

The threshold concept of effects plus appropriate harmfulness criteria constitute the corner stone of the theory of permissible exposure levels for environmental factors. It rests on the philosophical categories of quantity and quality inasmuch as, within certain limits, quantitative changes fail to cause fundamental qualitative changes in the given phenomenon. As long as the impact of a noxious environmental factor remains, within a certain period, below certain intensity, no qualitative changes occur in the body whose indices of vital functions (that is, health) are subject to homeostatic variations; and only an excess of a certain quantitative level results in different qualitative states — pre-diseased and then diseased.

Thus, while the threshold of primary, and inconsequential to the body, interactions between the tissues and exogenous and endogenous chemical compounds is sometimes difficult to establish, though it does exist in theory, the threshold of harmful effect signifying a change of imperceptible quantitative alteration into qualitative changes is more certain and obvious.

Supporters of the no-threshold theory of noxious effects have nothing to oppose to that, except airy speculations about the need to apply probability characteristics of stochastic (that is, random) processes. Why not extend the principle of randomness to the systemic toxic effect of poisons? Reasoning from the theory of random processes, individuals extremely sensitive to negligible toxic amounts as well as those insensitive to thousand-fold increases in lethal doses (e. g., of organophosphorus compounds) should appear very seldom, if at all. Indeed, no such cases have been documented so far, so experience (to date) is at odds with the hypothesis.

Another reason for taking a careful attitude towards the ideas of proponents of the no-threshold theory regarding harmful effect has to do with the arbitrary lowering of conventionally "safe" amounts (with a "probability of injury" equal to 10^{-5} or 10^{-6}). Though we

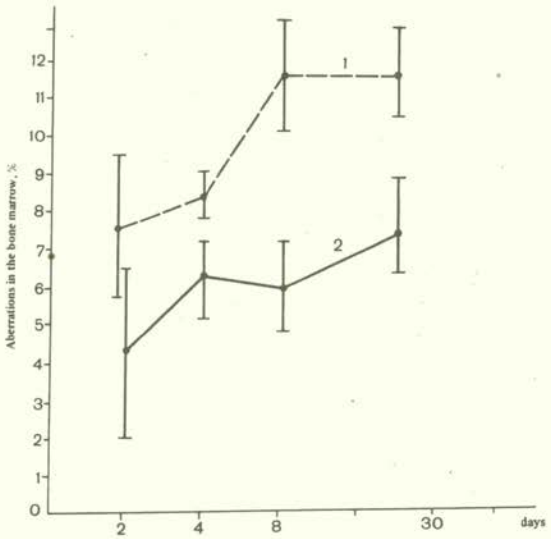


Fig. 10. Magnitude of cytogenetic effects of industrial toxic chemicals as a function of exposure.
1 — ethylene oxide (0.06 mg/l); 2 — ethylenimine (0.0006 mg/l)

uphold the principle of the priority of medical indications over technological or direct economic criteria, we do not ignore economics. The unnecessary and unwarranted understatement of the value of a medical standard has a pernicious if indirect, public health effect since it diverts resources from prevention and treatment to offset economic production losses.

Dependence of effect on the duration of exposure and surveillance. Several active compounds provide suitable examples for tracing the specific patterns of response in relation to the duration of exposure (they are ethylene oxide, morpholine, ethylenimine, and urethane).

Fig. 10 shows the relationship between the frequency of chromosome aberrations in the bone marrow cells of rats and the duration of exposure to ethylenimine and ethylene oxide in concentrations of 0.6 and 60 mg/m^3, respectively. As seen in the chart, the cytogenetic effect remained unchanged (for thirty days) after reaching a maximum increase at 2 to 8 days.

Similar data were obtained by Efimenko and Kulakov (1976) who gave per os to rats DDT, heptochloralindane, butyl ester, 2,4-D and 2,4,5-T, prometryn and zineb (carbomate) in doses of approximately equal effectiveness found within the limits of the chronic action zone (see Fig. 9). Cytogenetic analysis was performed at two weeks, and 1.5, 2.5 and 7 months after exposure. For all the test substances, the time — effect curve followed a unidirectional pattern in the sense that the number of chromosome rearrangements reached its maximum during the first half of the experiment schedule. Then, even though the

exposure was continued, the effect either became steady (prometryn) or returned to normal toward the end of the poisoning.

It is thus evident that, with time, protection mechanisms came into play (whether these was genuine adaptation or compensation for the disease process, it is impossible to say without further investigations).

A cytogenetic survey of employees in chloroprene and vinyl chloride production departments revealed no increases in the number of chromosome aberrations in peripheral blood lymphocytes, inspite of extended duration of employment ($r=0.01$ and 0.04 respectively). For example, the frequency of chromosome mutations in the duration — of-employment categories of under 5 and from 5 to 10 years was found to exceed almost equally the level of intrinsic control and the frequency of spontaneous chromosome abnormalities in the blood. Similar findings were reported by the General Hygiene Research Institute, Novosibirsk, for chronic ethylenchlorohydrine exposure. A marked effect (up to 11—12 per cent) was evident in the first half of the exposure period, but this returned to normal at the end of the experiment, when the chemical exposure concentration level equalled that of the chronic action threshold or was an order of magnitude greater (Ekshtat et al., 1971).

To conclude, the effect of mutagens coming from different chemical classes during continued exposure causes pathological manifestations to stabilize or even undergo complete regression. The data available reveal that the exposure levels at which the regression or stabilization occurs are specific for every toxic chemical.

In our experiments, a declining frequency of chromosome impairments over time was, in most cases, in no way connected with greater interference in the mitotic activity of somatic tissue. Despite the obvious effect of poisons, reparative processes overpowered destructive ones, giving rise to observed compensation. The absence of quantitative and qualitative changes in the peripheral blood at low-level exposures to the poisons mentioned above is presumably due to the remarkable plasticity of the hoemopoietic system.

Taking into account the mechanism by which chromosomes are restored upon fractional and chronic radiation exposure (Dubinin, 1966; Yarmonenko et al., 1968), it may be suggested that a cell population "adjusted to toxic effects" exists.

As shown demonstrably in a good many instances, the regaining of the normal characteristics of bone-marrow cells can presumably be viewed as an alternative compensation pathway for the disease process. It is important that the absence of chromosome aberrations in the terminal phase of the experiment (shown above) may not be evidence that no mutagenic hazard is presented by the study agent. The time taken to achieve a maximum cytogenetic effect is specific to each individual toxic agent or exposure level. The above factors must be considered design of hygienic standards in practice.

The crucial thing in the study of mutagenic effect in sex cells is to estimate the frequency of mutations in premeiotic cells because the

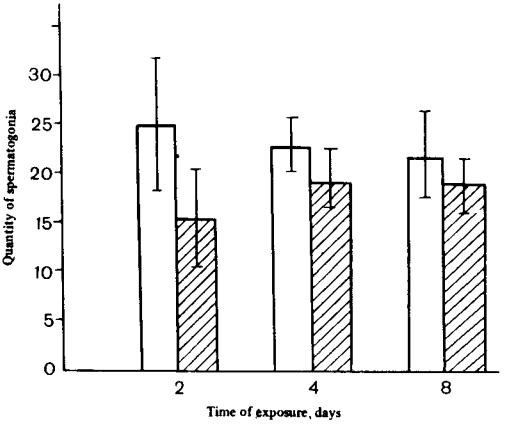


Fig. 11. Variations in the number of normal spermatogonia in seminiferous tubules on repeated ethylenimine exposure in concentrations of 0.6 mg/m^3.
Blank columns — control, hatched columns — experiment

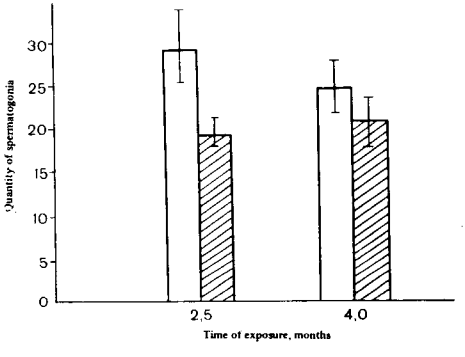


Fig. 12. Variation in the number of normal spermatogonia in seminiferous tubules on chronic ethylenimine exposure in concentrations of 0.6 mg/m^3. For symbols see Fig. 11

genetic alterations induced them persist throughout the reproductive period and may be passed on to subsequent generations.

The fundamental principles just discussed also apply to other occurrences apart from manifestations of mutagenic effect.

For example, there was a decreasing number of normal spermatogonia in rats after two days of ethylenimine inhalation exposure at a concentration on the level of the chronic action threshold. After a four-day exposure, their number was restored and was maintained at the same level after eight exposures (Fig. 11). This effect has also been established for chronic ethylenimine exposure (Fig. 12).

It is evident from the literature that many pharmacological drugs, when administered throughout the gestation period, displayed a lower teratogenicity than they did after a single administration of the total

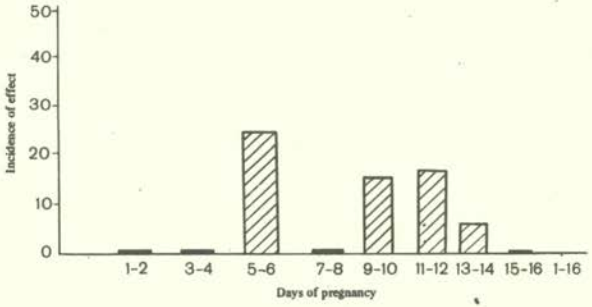


Fig. 13. Manifestation of teratogenic effect in albino rats exposed to a chloroprene concentration of 4.0 ± 0.7 mg/m^3 at different times during gestation. For symbols see Fig. 11

dose. This is due to the adaptation and adjustment processes during chronic toxic exposure (Dyban, 1968; WHO Report No. 364, 1968).

It seems more reasonable, however, to examine material-foetal adaptability by comparing the effects of equal doses (concentrations) of a chemical throughout pregnancy and at specific days during the gestation period.

Sal'nikova (1973), for example, showed that chloroprene induced no outward developmental defects when inhaled throughout pregnancy in doses of 4 ± 0.7 mg/m^3. By contrast, the same chloroprene concentration, if administered periodically (every two days) had the teratogenic effect of inducing hydrocephalus in the foetuses (Fig. 13). No such pathology was observed among control animals. Microanatomical analysis of affected foetuses revealed hydrocephalus on exposure to the poison at the 5—6, 7—8 and 11—12 the days during gestation. It was likewise found that inhalation of piperidine concentrations at the Lim_{ch} level throughout pregnancy and at particular periods (I. V. Silantieva's data) increased embryonic mortality to 42 per cent at nine days' gestation time. With daily piperidine exposures throughout pregnancy and single exposures at other dates, the embryo deaths did not depart significantly from those of the control group.

A similar, though less obvious, dependency of the effect upon the duration of exposure was reported for ethylenimine inhalation by pregnant animals.

Thus above evidence points to the likely development of adaptation to the impact of toxic agents during gestation. The adaptation is assumed to arise from the activation of the body's detoxification mechanisms during pregnancy.

The facts just described are significant not only from the point of view of the mechanisms responsible for the embryotropic effect of poisons, but also as an effective tool for sanitary standardization. They may also indicate that temporary relief of women-workers from production jobs for the duration of pregnancy, as many authors suggest, cannot ensure safety for their progeny. This is because pregnancy is not actually registered until eight to twelve weeks' gestation time,

by which time the foetus may have already been affected by adverse exposures. The only realistic way to afford prenatal protection to subsequent generations requires close consideration of possible embryotropic effects of chemicals during the setting and enforcement, of sanitary standards (see Chapter 4).

Preventive measures to protect the reproductive function in males are reviewed in Chapter 4. In the assessment of long-term effects overall, not merely their dependence on the duration of employment is important, but also the specific patterns of their development over time following a single exposure.

In general, the question of the exposure-dependent mutagenic effect of chemical compounds is a major one in the theory of chemical mutagenesis. With respect to alkylating substances with a delayed action, chromosome lesion would commonly affect the chromosomes emerging in the pre-synthesis stage, known to be most vulnerable to the effect of the alkylating agents. In the synthesis stage, the number of aberrations of the chromosomal as well as the chromatid type increases during cell division (Dubinin, Tarasov, 1969; Mitrofanov, 1969).

In addition to this distinctive mode of action, some alkylating substances are usually capable of inducing so-called prolonged mutagenesis — when chromosome rearrangements appear in subsequent generations of cells (Dubinin, 1968).

Quite a few authors hold the primary chemical mutagen to be capable of changing into a secondary mutagen which, in turn, induces mutations in subsequent generations of cell division (Sidorov et al., 1966).

Suvalova (1970) has discovered at the dose of 1 mg/g urethane can cause considerable disturbances in bone-marrow cells by as early as the third day of exposure. The numerical level of cells with chromosome aberrations declines only at 30 days after exposure.

An urethane dose of 0.1 mg/g was found to interfere actively with the workings of the chromosomal cell mechanism. In that event, however, the chromosome lesions heal faster and re-establish normalcy by the 14th day.

Since ethylenimine is classed with delayed-type mutagens, it was found necessary to discover, for its effect, the development pattern over time and stability after a single inhalation. To do so, rats were exposed to ethylenimine concentrations of 0.2 and 8 mg/m^3 and examined one, two, three, eight, fourteen and twenty-eight days following the single exposure (Fig. 14). An increasing number of chromosome rearrangements in the animals' bone marrow was registered on the third day after the inhalation of a 2 mg/m^3 ethylenimine concentration ($p<0.001$); on the eighth day, the disturbances multiplied in response to both larger and smaller dose exposures. At 14 days, the impaired cell division persisted only for the larger EI dose exposure. At 30 days no significant irregularities were to be seen anymore in the chromosomal apparatus of the dividing bone-marrow cells after either of these concentration exposures.

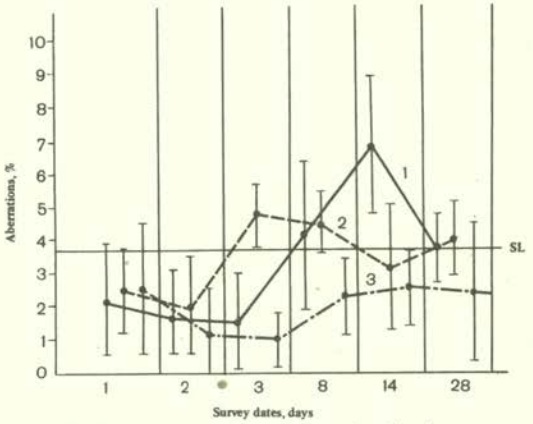


Fig. 14. Frequency of chromosome aberrations in the bone marrow of albino rats after a single ethylenimine exposure.

SL — upper limit of spontaneous level; 1 — 2.4 mg/m³; 2 — 0.8 mg/m³; 3 — control

From this evidence it can be inferred that most chemical compounds depend directly for their effect on the size of the dose (concentration), but indirectly on the duration of exposure. The cytogenetic effect produced by mutagens with a delayed action may not be in evidence until three to eight days after a single exposure. The latter must be considered in applying the index for determining $Lim_{ac\ spec}$ (see Chapter 2).

The concept of selectivity in the action of poisons on the reproductive function. The second leading principle in the toxicological research of long-term effects is that of identifying the specificity or selectivity of action.

A new parameter of toxicometry that crystallized in the process of investigating the long-term effects of exposure to chemical agents refers to the zone of specific action — Z_{sp} — suggesting the potency of some specific properties of the poison. This is calculated by comparing the threshold values for acute and chronic effects as defined by the integral characteristics and specific indices being studied (effect on the reproductive function, vessels, etc.):

$$Z_{sp} = \frac{Lim_{integr}}{Lim_{spec}}$$

Relative to the selective (specific) effect, it is thus possible to obtain data indicating pathological changes that are induced in the gonads, embryogenesis, cell division and other processes by a chemical agent in exposure doses and concentrations that have no effect as yet on the general condition of the body. Higher dose and concentration exposures can produce an observable pseudo-gonadotropic effect (see Chapter 3), associated primarily with vascular disorders and secondary hypoxia. The effect cannot be considered specific inasmuch as the simple mechanical compression of the testis vessels for a relatively brief time can set off degenerative developments in the seminiferous epithelium

similar to those that are likely to arise from severe intoxication. In the latter instance, the mechanisms affecting the testes and ovaries may well be different, but usually still non-specific and secondary. The same is true of embryotropic effect as detailed in Chapter 4.

For the gonadotropic effect of the kind discussed above, its specificity is not proved by us in any strict sense for the majority of cases, as the discrimination of an immediate lesion (more so physiological changes) of the gonads from impairment of the neurohumoral regulation mechanism presents a difficult practical problem. Yet the fundamental difference between the threshold of the selective gonadotropic effect and that of integral action offers a shortcut to a sufficiently valid declaration of specificity because, in that case, the regulation mechanism can be incorporated in the sexual system (in the generalized sense of the term).

The criterion of truth may be sought and found in the results of specific experiments. Poisons with specific effects on the gonads (or the immediate regulation mechanism of their function) are relatively few (Table 30, Chapter 4). Comparison of these values (Lim_{integr} and Lim_{spec}) has shown that, for example, tretbutyl peracetate, whose $Lim_{ac\ integr}$ is dozens of times higher than its $Lim_{ac\ spec}$ is a specific gonadotropic toxic agent. A similarly structured chemical tretbutyl perbenzoate, is not a specific gonadotropic toxic, by reason of having indistinguishable thresholds of individual and pathogenetic action. While the gonadal impact of lead is specific, the effect of mercury is not, according to our data.

Table 10

The Ratio of Single/Chronic Specific Gonadotropic Action Thresholds of Industrial Toxic Chemicals (in mg/m³ concentrations)

Chemical	Single action			Chronic action		
	$Lim_{ac\ sp}$	$Lim_{ac\ int}$	Z_{sp}	$Lim_{ch\ sp}$	$Lim_{ch\ int}$	Z_{sp}
Ethylenimine	0.8	10	12	0.16	0.4	2.5
Tretbutyl peracetate	20	150	7	1	1	1
1,3-chlorobrompropane	410	410	1	45.0	45.0	1
Aminopyridimine	22.4	61.9	3	11.4	11.4	1
Butyl ester * 3,4,5-T	0.1	50	500	0.1	0.01	0.1
Chloroprene	—	—	—	0.13	1.69	12

Note: * — doses (per os) in milligrams per one kg body weight.

The selectivity of toxic effect on the condition of the gonads has a significant manifestation at the level of the single-action threshold (Lim_{ac}), whereas, in chronic exposure, this specificity is usually lost, as shown in Table 10, using the example of Chirkova and Efimenko's experimental data.

The poisons listed in the Table were selectively gonadotropic after a single exposure (with the exception of 1.3-chlorobrompropane, for which the threshold levels according to the integral and organic parameters proved identical). Cases arise when the selectivity of the effect is extremely potent, as in butyl ester 3, 4, 5-T.

Chronic action exhibited altogether different patterns in that the gonadotropic effect and general signs of intoxication were seen largely at isoeffective levels; exposure to butyl ester 3, 4, 5-T induced no tangible gonadotropic action below the chronic action threshold according to the integral indices.

This was later shown not to be universally applicable. A study of the effect of chloroprene on the reproductive function in chronic exposure (5.5 months) was found to have clear-cut specificity (selectivity) for spermatogenesis. In the 5.5-month chronic exposure, the specific action zone amounted to 12 (R. M. Davtyan's data, see Table 10).

With respect to mutagenicity, in the chronic exposure to chemical compounds, in spite of its "staged pattern" (see Chapter 2), it is often evident at subthreshold levels according to the indices of systemic toxic effect, as was shown for chronic exposure to chloroprene (L. D. Katosova's experiments) when $Z_{spec} = 12$ and to ethylene oxide (A. E. Strekalova's experiments) when $Z_{spec} > 10$.

For this reason, the likely manifestation of selective action by agents in the chronic experiment as well should be considered in the design of experimental studies to evaluate the impact of chemical compounds on the reproductive function.

On the basis of the foregoing assessment of the research results concerning long-term effects on the reproductive function, the experiments are best started not with lethal exposure levels, but rather with ones below the threshold of acute effect in terms of integral indices. In the chronic experiment, one has sometimes to go lower ($Lim_{ch\ integr}$), indeed, on occasion, below the MAC established from the systemic toxic effect.

Some authors (Ryazanova, 1976; Kagan, 1969; Dubinin, 1976; Bochkov, 1976) disagree with this approach because they consider it necessary to determine first the so-called potential risk associated with the specific effect of a chemical compound at near-lethal levels and only then to go down to "realistic" levels — those low dose exposures obtaining in commercial applications of the chemical.

Starting the experiment with high doses and concentrations, apart from the "pseudoeffects" it produces which often may not be a sign even of a "potential" hazard, creates practical problems. The study of the chemical takes several times longer, much too long, of course, for today's needs.

Table 11 provides a classification of the potential risks associated with the effect of industrial toxics on the reproductive function. The integration of the risk classes into preventive recommendations makes them more relevant and valid (MAC, etc.). Though the classification presented in Table 11 is somewhat conventional, its further testing

Table 11

Classification of Potential Hazard from Effects of Industrial Toxics on the Reproductive Function

Classes of hazard	Z^{*}_{sp}	K^{**}_{s}
I. Extremely hazardous	>10	>50
II. Highly hazardous	4—10	10—50
III. Moderately hazardous	1—3	Up to 10
IV. Slightly hazardous	<1	Ordinary

Note: * Z_{sp} — the zone of specific (selective) effect;
** K_s — the recommended safety factor in calculating MAC from Lim_{ch}.

during hygienic standardization of chemicals will lead to proper appreciation of it.

Sanitary standardization of concentrations of chemical compounds with a specific effect on the reproductive function. One method used most effectively for preventing long-term effects is complete exclusion of any public exposure to a hazardous compound. Unfortunately, this is rarely, if ever, possible. Once it is accepted that a substance cannot be banished completely, the only solution is to set maximum allowable (safe) concentrations, e. g., for genetically active substances in the environment.

The question of the feasibility of hygienic standardization of industrial toxic mutagens is widely debated (Rapoport, 1966; Sanotsky, 1967, 1975; Fomenko, 1969; Filippova, Israel, 1976).

The ever increasing use of chemicals in our life confronts toxicologists with the need for hygienic standardization of chemicals with mutagenic, blastomogenic, gonadotropic, and embryotropic activity.

For chemical concentrations in the environment, their sanitary standardization relies basically on the threshold concept for all types of biological effect.

Several years ago "Methodological Instructions" were endorsed as guides in the sanitary standardization of blastomogens. Based on the data from animal experiments, the Instructions recommended the introduction of increased safety factors of $Lim_{ch\ integr}$ times 20 to 100. The potency of blastomogenic activity according to Shabad's 1971 classification was suggested as the criterion for selecting the magnitude of safety factors. The classification identifies all substances as strong, medium and weak. The strong group is of the chemicals that are carcinogenic to man and animals (Shimkin, 1968) together with substances found to be carcinogenic in experiments but not proved to be so for human beings. The medium-strong substances are carcinogenic in some instances and in the second half of the animal life-span ($K_s=20$). Finally, the weak agents are of dubious carcinogenicity because of conflicting evidence (K_s is usual).

More recently it became possible to set thresholds for blastomogenic action, so the safety factors lost much of their former import. They were replaced by interspecific extrapolation procedures, one example being that cited in Shabad, Sanotsky et al. (1976). The difficulties involved in the calculations as one moves from the effective to subthreshold levels are illustrated in Chapter 2.

In studies that deal with the gonadotropic and embryotropic effect of toxics, the threshold levels are usually clearly set out. Since, however, until recently, researchers lacked a single set of methods for determining the threshold of embryotropic effect, they found relatively high concentrations (doses) of substances somewhat difficult to adapt and utilize for sanitary standardizaton of industrial chemicals unless their systemic toxic effect on the maternal organism had been evaluated.

Chapter 3

GONADOTROPIC EFFECT OF CHEMICALS

State of the art. The mid-20th century is characterized by a significant increase in infertile marriages. With each year the problem is becoming more and more urgent, thus demanding thorough-going research and sensible management. Statistics reveal that, in the Soviet Union, 10 to 15 per cent of all marriages are infertile (Kunin, 1973). Yundoa (1972) estimated that 45 per cent of all divorces occur for medical reasons such as inability to have children, sexual dissatisfaction or infidelity for reasons of sexual disparity. Akunts (1971) holds infertility in 27.1 per cent of marriages responsible for their dissolution.

The previously deeply entrenched belief was that the women is to blame for marital infertility. It is now known, however, that the man is likely to be the "guilty" party in at least half of all infertile marriages. (Molnar, 1969; Kunin, 1973). Male sterility leading to infertile marriages accounts for 40 per cent of their total, according to Tiller (1953) and 34.6 per cent, according to the N. F. Zhordania Institute for Research on Human Reproductive Function of the Georgean SSR Public Health Ministry. A still higher proportion of this male pathology was perceived by Porudominsky (1964) — 50 per cent; Farris (1950) — 66 per cent; and Foel (1953) — 49 per cent. The cause is attributed to the extremely high sensitivity of the male sexual glands to all kinds of injurious factors, the reproductive structures of the testes being the most sensitive of their kind (Patanelli, Nelson, 1970; Jackson, 1970), which Leblond says (1970) respond, at a certain stage of spermatogenesis, to relatively minor environmental changes (Kushniruk, 1974).

The etiological causative factors of sterility are varied and many, so the immediate cause remains obscure in most cases. Until recently the problem area had been the exclusive domain of clinicians, that is, urologists, endocrinologists, gynaecologists, and psychiatrists. Sterility was often attributed to inflammation of the sexual organs, general endocrinal disorders and suchlike. Nevertheless, data accumulated in recent years by toxicologists, pharmacologists and hygienists authorize a much broader perspective on the problem of reproductive function disturbances. A new clinical-hygienic dimension of the problem has emerged, and the impaired reproductive function has been found to have a great deal to do with environmental impacts and occupational factors, notably the chemicals to which man is occupationally exposed at work.

The literature today contains considerable evidence concerning the influence of industrial chemical compounds on the gonadal function. Among the substances seen to cause these lesions via occupational exposure are benzene and its homologs, organochlorine compounds, and some metals, to name only a few (Sanotsky, 1968; Pashkova, 1969; Avkhimenko, Golubovich, 1969; Kushniruk, 1974; Dixon et al., 1975). Analysis, nonetheless, makes it clear that only a relatively minor fraction of the data published is applicable in the elaboration of preventive programme. This is because, in carrying out both clinical and experimental investigations, the researchers ignored possible manifestations of a toxic effect by the chemicals being studied. As a result the conclusions of most clinical studies neglect the general state of health, social and living conditions, and a thorough hygienic characterization of working conditions, among them concentrations of harmful substances present in the working environment. Most empirical research focused on the effects of industrial toxics in high doses and concentrations — as high as 200 to 500 MACs or more — which thus showed strong toxicity. Seen in this light, an altered function of the sexual glands could be a result of a severe lesion in other systems or organs. It is generally known, for example, that menstrual dysfunction can follow hemorrhagic diathesis (Kagan, 1950; Hamilton, 1927; and others) or a liver dysfunction which modifies the activity of estrogens (Teter, 1968). The ability to fertilize or, accordingly, to conceive — one of the integral indices by which the state of the sexual function is judged experimentally — may alter in the wake of a disturbance in the sexual instinct following an impaired state of the central nervous system (e. g., the narcotic effect of poisons, etc.). The issue will be treated in more detail below.

The low value of some works stems from their being poorly equipped methodologically. In many clinical survey programme, the conclusions were drawn exclusively from personal interviews and a general gynaecologic examination. In experimental research, too, no attempt was made at quantitative evaluation of the structural-functional elements of the testes and ovaries, nor was the choice of daily exposure and the total duration of the experiment anything but arbitrary.

A review of the research results dealing with the gonadotropic effect of dimethylacetamine and caprolactam (E. M. Chirkova's experiments) brings into a sharper focus the importance of quantitative evaluation of the sexual glands in determining the gonadotropic effect of chemicals. In response to a caprolactam concentration exposure of 124.6 mg/m^3, changes in the indices of gonadal function performance (Table 12) were manifested as a decreasing number of normal spermatogonia, a lower index of spermatogenesis and an increasing number of tubules in the 12th stage of meiosis. The changes were detected morphometrically and found to take place in the absence of any alterations in the functional condition of spermatozoa.

On the other hand, in studying the gonadotropic effect of bromine (E. M. Chirkova's experiments), the latter was discovered with the

Table 12

Morphological and Functional Indices (M±m) of the State of Seminiferous Epithelium in Rats [1] after 2.5-Month Caprolactam Exposure

Indices	Caprolactam concentration, mg/m³		
	control	124.6±14.02	10.62±1.63
Morphometric:			
Index of spermatogenesis	3.71±0.01	3.2±0.007*	3.7±0.02
Normal spermatogonia	30.2±1.3	18.3±0.98*	28.7±1.94
Tubules with desquamates epithelum, %%	2.7±0.98	2.5±0.7	2.8±0.52
Tubules with meiosis stage 12, %%	3.6±0.33	13.2±1.02*	3.82±0.48
Weight coefficients of testes, %%	0.84±0.04	0.73±0.06	0.79±0.05
Functional:			
Mobility of spermatozoa, min	290±13.1	270±10.24	282±8.59
Number of spermatozoa, mln.	60±7.8	56±9.45	58±8.64
Osmotic resistance, %% NaCL	2.4±0.2	2.7±0.55	2.5±0.12
Acid resistance, pH	3.8±0.11	3.9±0.18	3.95±0.22
Pathological forms, %%	23.2±2.98	25.6±1.43	27.3±3.1

[1] Statistical group — 8; * $p<0.05$.

Table 13

Functional and Morphological Indices of Spermatogenesis (M±m) in Rats Following Chronic Exposure to Bromine Vapours

Indices	Concentration, mg/m³			
	12.4±1.4	1.4±0.1	0.16±0.032	Control
Functional:				
Spermatozoan motility, min	210±13.3*	230±12.1*	302±18.9	290±12.8
Number of spermatozoa, mln	28.0±4.2**	44.0±8.0	63.0±6.0	67.9±9.0
Acid resistance, pH	2.85±0.14	2.75±0.23	3.0±0.18	3.0±0.19
Osmotic resistance, %% NaCL	2.2±0.02	2.2±0.05	2.35±0.02	2.2±0.02
Morphometric:				
Index of spermatogenesis, conv. units	3.2±0.1*	3.65±0.2	3.65±0.015	3.67±0.1
Normal spermatogonia, %%	17.2±0.92	24.3±0.84*	28.7±0.98	30.8±1.2
Tubules with desquamated epithelium, %%	1.2±0.13	0.95±0.26	0.75±0.24	1.23±0.26
Tubules with meiosis stage 12, %%	2.5±0.35	2.63±0.4	2.25±0.53	2.5±0.5

* $p<0.01$; ** $p<0.02$.

integrated use of functional and morphological indices (Table 13). The gonadotropic effect of bromine vapours by inhalation of their Lim_{ch} — level concentration of 1.4 mg/m^3 came into evidence as the total number of normal spermatogonia, a morphological index, tapered off and the time of spermatozoan motility, a functional state, was reduced.

The past few years have seen a remarkable qualitative improvement in the discipline studying the impact of occupational conditions on the gonadal function. It has been possible to show demonstrably the impairment of the menstrual and ovarian function among women workers in the manufacture of isoprene synthetic rubber, with dioxane as the primary chemical factor (Pashkova, 1968), and among the female employees occupationally exposed to styrene (Iziumova, 1972), caprolactam (Khadzieva, 1972), and manganese compounds (Mandzhgaladze, 1968).

Evidence has become available of the effects of industrial toxic agents upon the condition of the sexual function in male chemical workers subject to prolonged occupational exposure to manganese (Mandzhgaladze, 1969), boron (Strongina, 1971; Borisov, 1969), chloroprene (Davtyan, 1974); ethylated gasoline (Neshkov, 1971) and other chemicals.

Research data regarding the gonadotropic effect of chloroprene are cited in Tables 14 and 15 which summarize the findings of an employee survey by questionnaires, and spermatological and sexological investigations.

Despite the relatively small size of the examined employee groups, it seemed legitimate to compare the detected lesions as they appeared from complaints and according to objective criteria.

To begin with, in the under-five-year duration-of-employment category there were no employee complaints of impaired sexual functions and nor any significant changes found in the characteristics of the ejaculate. In the 5—9-year category, Ejoculatio praecox relativa, or relatively premature ejaculation, was identified in two out of three employees. The ejaculate showed a statistically significant decline compared with the control group in the number of spermatozoa per one ml, but it remained within the normal physiological limits. By contrast, the indices of the ejaculate volume and the amount of mobile spermatozoa dropped below normal limits (a decrease) and departed significantly from the respective control figures.

In the employee group with over 10 years' experience the pathology was seen to grow as suggested by sexological complaints of declining sexual performance (in three persons) and absolutely premature ejaculation, Ejaculatio praecox absoluta, tending strongly towards Ejaculatio ante portas (in three persons). None of the indicators of average characteristics of the ejaculate met the physiological standards and all differed significantly from the control group.

From a survey of workers exposed to antimonite ore dust (antimony-containing), Glushchenko (1977) disclosed changes of a functional character, among them a decreasing proportion of mobile

Table 14

Male Reproductive Function (Chloroprene) Exposure

Indices	Basic group	Control group
Number of examined employees	87	97
Pregnancies among workers' wives	381	419
Stillbirths	2	2
Spontaneous abortions	54*	19

* $p<0.05$.

Table 15

Results of an Ejaculate Study in Employees Occupationally Exposed to Chloroprene (I) and Antimonite Ore Dust (II) [1,2]

Duration-of-employment categories	Number of persons	Volume, ml	Quantities of spermatozoa				Fructose (in one h), mg/l
			total, mln	mobile, %%	normal, %%	morphologically modified, %%	
(I) up to 5 yrs	6	3.2±0.17	82.0±2.7	79.8±1.0	79.9±1.0	19.2±0.5	4,100±242
(I) 5 to 9 yrs	3	2.8±0.09*	69.3±4.9*	67.3±1.6***	78.6±3.2	21.3±3.4	4,000±740
(I) over 10 yrs	6	2.3±0.18**	52.9±3.9***	57.3±2.7***	67.0±3.1**	33.0±3.7**	1,930±38**
Exposed to (II)	14	3.3±0.6	61.5±13.8**	59.9±7.0**	74±7.4	—	—
Common control	9	3.2±0.5	102.8±2.2	77.4±0.7	80.9±1.0	20.1±1.2	4,230±384
Normal	—	3—5	60—100	75—80	No less than 80	15—20%	Over 250

[1] Accepted in the sexual pathology Lab of the Research Institute of Psychotherapy, Ministry of Public Health, Russian Soviet Federative Republic (Kunin, 1968; Molnar, 1969; Kagan, 1974).

[2] For the concentrations used, see Table 17.

* $p<0.05$; ** $p<0.01$ and 0.02; *** $p<0.001$.

spermatozoa in the ejaculate (see Table 15). Though the spermatozoa concentration differed from that of the control group, it did not go beyond the limits of physiological variations.

Even so, the drop in the average level to the lower normal limit may be seen as significant and meaningful because of the employees' comparatively short experience of work in that mine (under 6 years); moreover, the ejaculate obtained from 50 per cent of the examined workers showed a decrease of spermatozoa content to below the normal level to the point of total disappearance.

Comparison between the sexological and spermatological survey results in the employee group exposed to antimonite ore dust showed a reduction of the neurohumoral component in six persons, relatively premature ejaculation in three persons (Ejaculatio praecox relativa) and significant fall in the quantity of spermatozoa per one ml and their mobility.

The gonadotropic effect of chloroprene was confirmed in an experiment on laboratory albino rats. The findings of the experimental research on the gonadotropism of chloroprene (R. M. Davtyan's experiments) are presented in Table 16. That this experimental animal model can be applied in studying the effects of industrial toxics on the gonadal function was additionally shown by case-studies involving compounds of boron (Kasparov, Strongina, 1971); manganese (Mandzhgaladze, 1969); phenol (Kolosova, 1974), styrene (Iziumova, 1972) caprolactam (Khardzhieva, 1972) and a whole series of other chemicals — tricresol, phosphorus oxychloride, tricresylphosphate, etc.— listed in Table 17 (Pashkova's data).

Spermatogenetic irregularities due to the effect of chemical compounds are generally held to be linked with the metabolism of nucleic acids. The histochemical and biochemical RNA and DNA estimations in testes under a research programme concerned with the gonadotropic effects of ethylenimine, amidopyrimidine, tretbutyl peracetate, lead and other selective toxic chemicals revealed that a change in the morphological characteristics of spermatogenesis was accompanied by reduced RNA content in the cells of the seminiferous epithelium, largely in spermatogonia and spermatocytes (Egorova, 1962; Chirkova, 1969). With sharply defined degenerative changes in the seminiferous epithelium, as, for example, during subacute animal poisoning by ethylenimine in a Lim_{ac} — level concentration according to the integral indices (12 ± 1 mg/m^3 over a month), the researchers witnessed a large RNA reduction in all generations of the seminiferous epithelium, Biochemical tests of animals subjected to ethylenimine and lead exposure at levels inducing morphological and histochemical changes in the seminiferous epithelium shed light on alterations in the RNA content of the testis homogenate (Golubovich, 1975).

The problem of selectivity in the study of the gonadotropic activity of chemical compounds. In this context, the all-important question is how to differentiate between the primary selective gonadal lesion and damage to the regulation mechanism, in all its complexity.

Table 16

Functional (I) and Morphological Indices of Spermatogenesis (II) in Rats after Chronic Chloroprene Poisoning by Inhalation

Groups	Indices	Chloroprene, mg/m³			Control
		1.69	0.15	0.051	
I	Number of dead spermatozoa, %%	85.8±13.8*	67.7±12.4**	30.3±3.3	32.2±9.9
	Acid resistance, pH	5.9±0.46*	5.58±0.45**	3.25±0.53	3.8±0.53
	Osmotic resistance, %% NaCl	2.01±0.43	2.6±0.49	2.8±0.061	2.68±0.085
	Time of spermatozoan motility, min	91.2±44***	126±44.5*	296±15.7	333±15.4
	Number of normal spermatogonia	7.5±4.2*	9.7±3.1**	19.8±5.0	25.7±5.2
II	Index of spermatogenesis	1.68±0.62	2.18±0.7	3.18±0.44	3.02±0.46
	Number of tubules with desquamated epithelium, %%	9.7±5.6	2.6±0.8	2.28±1.5	2.28±1.2
	Number of tubules with meiosis stage 12, %%	2.5±1.4	2.6±0.54	3.28±1.0	2.57±0.9
	Testes weight coefficient, %%	0.37±0.065*	0.42±0.04*	0.52±0.05	0.5±0.038

* $p<0.01$ and 0.02; ** $p<0.05$; *** $p<0.001$.

Table 17

Clinical and Experimental Studies on the Gonadotropic Effect of Chemical Compounds

Chemical	Clinical		Experimental		MAC, mg/m³	
	exp. level, mg/m³	effect	exp. level, mg/m³	effect	general toxic effect	including long-term effect
Chloroprene	7—34	+	3.5—0.15	+	2	0.05
4,4-dimethyl-dioxan-1,3	15—50	+	30	+	10.0(1.4)	3.0
Tricresol	5—50	+	4	+	0.5	0.5
Phosphorus oxychloride	0.5—5	+	0.4	+	0.05	0.05
Tricresylphosphate	MAC level	—	1.0	—	0.5	
Dibutyl phthalate	0.5—5	+	6.1	+	0.5	0.5
Dioctyl phthalate	0.5—5	+	5.2	+		
Gasoline BR-1	100—850	—	300	—	300	300
Phenol	2—10	+	0.5	+	5	0.3

Symbols: + effect; — no effect.

To identify the gonadal response to a chemical stimulus as being primary or secondary is somewhat difficult because of the foreward and backward connection between the function of the sexual glands and other organs. The problem has a relatively simple solution at high exposure levels because, in that case, other organs are sure to be affected, but seems virtually unsolvable at lower levels of exposure.

About the only way left to the researcher is, therefore, to seek in-depth insights into the mechanism of toxic effect at low levels. Mandzhgaladze (1969) has managed to conclude from the transplantation of normal ovaries and introduction of sexual hormones into poisoned rat females that the predominant lesion of the gonads is causally associated with exposure to manganese compounds.

Yet the methods can hardly be suggested as a routine tool, as this would make the experiment overly complex. In our opinion, the key question in preventive toxicology is whether or not a chemical agent is selective (specific). The answer was previously said to be derivable from a comparison of survey results based on integral and specific indices (see Chapter 2).

Table 18

Specific (Gonadotropic) Action Zone (Z_{sp}) for Some Chemical Compounds by Inhalation

Compound	Lim_{int}, mg/m³	Lim_{sp}, mg/m³	Z_{sp}	Data from literature
Ethylenimine	10	0.8	12	Chirkova, 1970
Tretbutyl peracetate	350	20	7	Chirkova, 1970
1,3-chlorbrompropane	410	410	1	Chirkova, 1970
Aminopyrimidine	61.9	22.4	3	Chirkova, 1970
Chloroprene	1.69	0.15	11	Davtyan, 1974
Pyrrolidine	0.6	0.6	1	Chirkova, 1972
DMAA	24	24	1	Nakoryakova, 1974
Phenol	5	0.5	10	Kolesnikova, 1973
Benzine	300	300	1	Feller, 1973
Bromine	50	100	1	Chirkova, 1973
Lead		40	1	Chirkova, 1973
Butyl ester 2,4,5,-T*	50*	0.1*	500	Efimenko, 1974
Manganese	40	1	40	Mandzhgaladze, 1969
Boric acid	9.6	9.6	1	Strongina, 1971
Barium carbonate	1.15	1.15	1	Silaev, 1976
Boron**	1**	1**	1	Borisov, 1976
TMTD	0.45	0.45	1	Davydova, 1974
Trifluoperazine	0.66	0.66	1	Pavlenko, 1973
Lead acetate	0.01	0.01	1	Chirkova, 1978
Vinyl chloride	4.8	35	8	Glushchenko, 1978
Dehydroprednisolone acetate	0.4	0.4	1	Shashkina, 1976
Methyltestosterone	0.1	0.1	1	Shashkina, 1976

* per os, mg/kg; ** by ingestion in drinking water, mg/l.

Experimental evidence for selective gonadotropic action of some chemical compounds is presented in Table 18. It suggests clearly the maximum-specific lesion of the gonads for ethylenimine, chloroprene, tretbutyl peracetate, butyl ester 2,4,5-T, manganese, phenol and lead.

A BRIEF PROFILE OF THE STRUCTURE AND FUNCTIONS OF THE MALE SEXUAL GLANDS

If the principal methods recommended for the study of the specific gonadotropic action of chemical compounds are to be properly validated, a brief discussion of at least the essential points concerning the structure and function of the sexual glands is in order.

Male sexual glands. Sexual glands are known to be formed from several sources, that is, primary sex cells, the epithelium of the sexual torus and, finally, the mesenchyme. The primary sex cells form further spermatogonia and ovogonia. The epithelium of the sexual torus develops into the trophic syncytium (Sertoli cells in males and follicular cells in females), and the mesenchyme makes up the stroma, vessels and interstitial cells of the sexual glands.

Sex differentiation in embryos comes relatively fast following the laying-down of the primary gonads (Table 19). As such, the male sexual glands, as opposed to the female ones, take shape in the post-uterine development period.

Table 19

Comparison of the Time when the Sexual System Develops in Rats vs Humans (from Robson and Sullivan, 1968)

Structure	Days of gestation	
	man	rat
Primary gonad	42	—
Sex differentiation	49	13
Primary follicles in the ovary	100	17
Differentiation of metanephros	35	—

It is essential to note, in particular, that the system of sex develops in different biological species at different times during their embryogenesis. For example, the primary gonads originate in the human ovary during the first trimester of pregnancy (six weeks), but this occurs presumably during the latter half of gestation in the rat (see Table 19). A still greater difference between humans and rats exists in the development dates of primary follicles — in the first trimester and the final quarter respectively — but we shall return to this a little later.

The methodological value of the facts just discussed lies in the transfer of data obtained from animal experiments into public health practice.

In the post-uterine period chiefly under the influence of hypothalamus-hypophysic action, males finalize the development of the testes. In mammals, they represent a conglomerate of seminal tubules with their total ultimate attachment to the epididymis and ductus deferens, which also receive the excretory ducts of multitudinous accessory secretory glands. The latter's involvement in sperm formation is great, but a thorough examination of it is beyond the scope of this brief review.

A comprehensive summary of current views on spermatogenesis and ontogenesis has been presented by a WHO group of experts in report No. 333 (1968). The core of the spermatogenic epithelium is thought to be spermatogonia, which are always in the process of endless mitotic division, in which some of the dividing cells make up for the loss of spermatogonia, while the rest go through a series of irreversible transformations before they ultimately become spermatozoa.

The time taken to complete spermatogenesis, from the first division of spermatogonia till the spermatozoon's expulsion from the testes, is between one and two months for different species.

The time of spermatogenesis, as estimated in WHO Report No. 333 (1968), varies from 72 days in human beings to 48 days in rats and 35 days in mice.

Spermatozoa are believed to complete maturation in the epididymis, yet their mobility there is as low as in the seminiferous tubules of the testes. In man, the spermatozoa are pushed by the force of tubular peristalsis through the epididymis in the course of one to three weeks, depending on the frequency of ejaculation and a host of other factors. Even so, the life expectancy of spermatozoa, in the epididymis is sometimes greater (up to 70 days) (WHO Report No. 333, 1968).

The mature spermatozoon usually acquires motility only in the sperm (a spermatozoa suspension in the secreta of the accessory glands containing appreciable quantities of carbohydrates). The motility is maintained by oscillations of the tail, whose fibrillae contain an acemyosin-like substance. In the midtail, the respiratory processes are most intensive, usually working through the oxidation of exogenous products via the tricarboxylic acid cycle. A parallel process may involve energy accumulation by glycolysis of the endogenous products, though the process is about one-fifteenth of the total store of energy. In an unfavourable medium, the mobility draws energy by oxidation of endogenous substances, above all lipids. The degrees of motility and metabolic activity are comparable (Salusbury and Lodge, 1962).

With respect to the biochemistry of spermatogenesis, the major contributory factor is nucleic acid synthesis and, while the spermatogenic epithelium harbours the active synthesis and breakdown of both deoxyribonucleic, as well as ribonucleic acids, the mature spermatozoon contains little or no cytoplasm and thus virtually no RNA. This is assumed to account for the relatively low viability of mature male germ cells. The mature spermatozoon has only a quarter of the amount of nucleic acids that is found in first-order spermatozoon; it con-

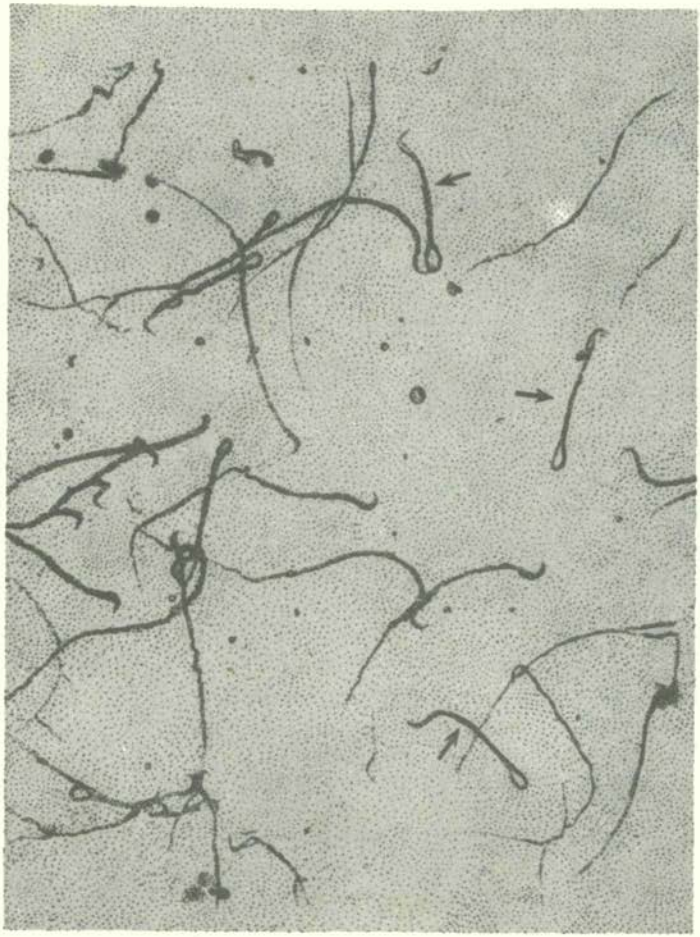


Fig. 15. Pathological forms of spermatozoa. × 200

tains also a half-complement of chromosomes, and undifferentiated ones at that (WHO, Report No. 333, 1968).

Whether spermatogenesis follows its normal course depends on a variety of factors, internal and external, such as the ambient temperature or an adequate vitamin supply. Should any of these conditions be disturbed, the spermatogenic epithelium develops degenerative processes, up to and including complete cessation of spermatogenesis and an emptying of the tubules or discontinued development at a certain stage of meiosis. When this happens, the spermatozoa not infrequently change their completion and the so-called degenerative forms of spermatozoa emerge (Fig. 15). The most common abnormality involves adhesion of the tail to the head. Researchers agree that morphologically anomalous spermatozoa are incapable of fertiliza-

tion but, if it should occur, the foetus is not viable. In other situations, as, for example, in case of sperm exposure to ionizing radiation, the spermatozoa are normal morphologically and functionally, but the fertilized ova usually succumb during early developmental stages. In some instances such seemingly "normal" spermatozoa are found to hold extraordinary quantities of DNA.

Selection of the methods for studying spermatogenesis

As with most other methods, the techniques used in toxicology need to be described in terms of relative sensitivity, specificity, reliability and, of course, labour-intensity. The research schedules and levels of exposure have been reviewed elsewhere (see Chapter 2).

Morphological methods. Morphological methods happen to have become the most widely used for studying spermatogenesis. Indeed, overt anomalies of spermatogenesis are identifiable even in macroscopic research, as the weight and size of the testis alter.

Yet it is indicators of initial alterations that might be set off by the lowest threshold levels of chemical exposure that are of primary interest to toxicologists. The pertinent methods were examined by these authors in the booklet "Experimental Research Methods for Establishing Thresholds for the Effects of Industrial Toxic Chemicals on the Reproductive Function" approved by the USSR Public Health Ministry, on June 10, 1977, No. 1744—77.

If the atrophy of the seminiferous epithelium becomes apparent on cursory inspection of the histological specimen through fixation in the Carnoy's fluid or 15% neutral formalin, embedding in paraffin, 6μm-thick sections, and staining with hematoxylin-eosin, any reference to the thresholds of effects is, of course, irrelevant (Figs 16, 17). This is not so in detecting changes unnoticeable to the naked eye, for which a series of indices have been suggested. Fogg and Cowing (1951) discerned four layers in the spermatogenic epithelium: 1. spermatogonia; 2. spermatocytes of the I and II order; 3. spermatids; and 4. spermatozoa.

It has been proposed that the number of layers of the spermatogenic epithelium in each tubule be counted in a hystological section of the testis and then the average condition of the tubules be measured by the formula:

$$I = \frac{\Sigma_a}{A}, \text{ where}$$

I is the index of spermatogenesis, a is the number of the SE layers found in each tubules, and A is the number of tubules counted (routinely, a total of 100 tubules is surveyed). It must be pointed out that, in practice, the Fogg and Cowing method is much too subjective and not very sensitive.

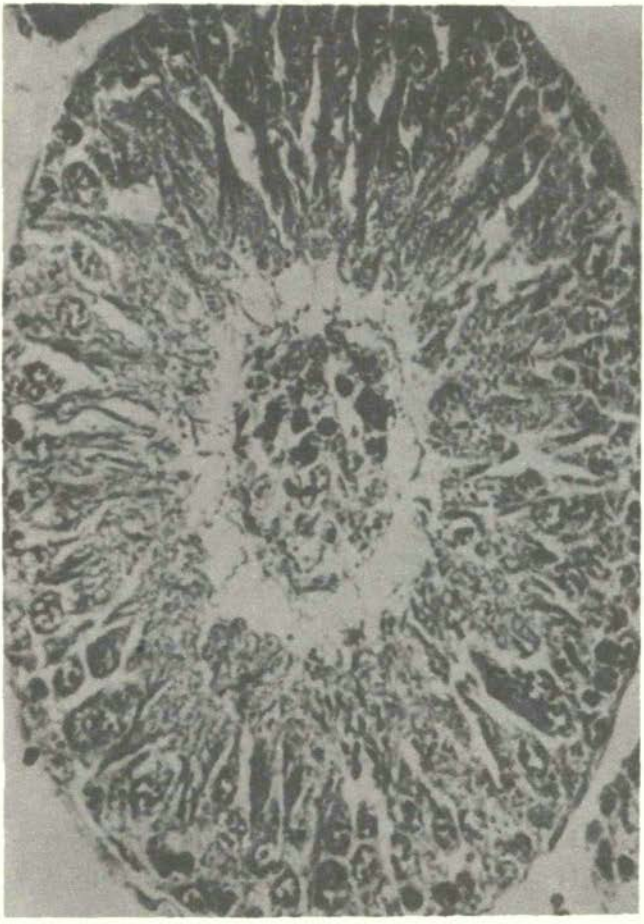


Fig. 16. Desquamation of spermatogenic epithelium in seminiferous tubule of the albino rat. ×200. Single exposure to tretbutyl peracetate in a concentration of 0.2 mg/l

A more objective morphological method is based on a count of normal spermatogonia (the cell layer next to the basal membrane), their normal numbers present in each rat tubule ranging commonly from 20 to 40. It is thought sufficient to examine 20 tubules (Arsenieva, Bakulina et al., 1967). A method for estimating the tubules with the 12th stage of meiosis (the metaphase of the second maturation division) is also quite objective, requiring 100 tubules to be reviewed.

The technique of counting the number of tubules with the desquamated spermatogenic epithelium (random travel of structural epithelial elements into the tubule lumen), by which 100 tubules are to be surveyed for one application, has proved a simple, objective and

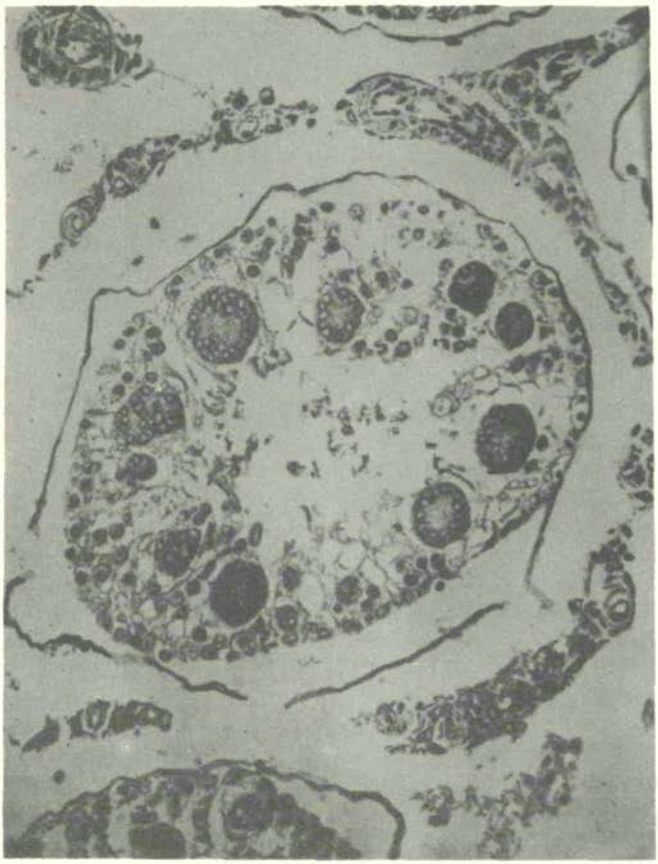


Fig. 17. Atrophy of seminiferous epithelium and giant cells in seminiferous tubule of the albino rat. ×200. Inhalation ethylenimine thirty-day exposure in a concentration of 0.01 mg/l

sensitive method. The time needed to perform complete quantitative evaluation of the seminiferous epithelium is 60 minutes per glass.

A comparison of the various morphological methods used for assessing spermatogenesis in terms of sensitivity to some occupational toxic exposures (Sanotsky, 1965; Sanotsky et al., 1967; Chirkova, 1970) has brought some useful insights. A reduction in the mean number of spermatogonia and numerical expansion of the tubules with desquamated spermatogenic epithelium were among the earliest responses to exposure, for example to tretbutyl peracetate, a specific gonadotropic agent from the category of organic peroxides, as also to lead acetate, caprolactam and aminopyrimidine.

Quite likely, though, different methods may be most sensitive to particular toxic exposures. Colchicine, for example, is known to stop cell division at the metaphase stage. An initial increase in the number of these metaphase-stage tubules in this and similar instances, may well provide the most sensitive index of modified spermatogenesis. Alkylating agents are considered extremely specific for particular types of the spermatogenic epithelium. Our data suggest, however, that ethylenimine, for example, is nonetheless, the first to modify the number of normal spermatogonia.

There must, therefore, be a possibility open for future researchers to approach the morphological assessment of spermatogenesis for applied purposes more economically in the sense of using a smaller set of methods than the previously recommended methodology for assessment in a chronic experiment of the alterations that happen to all morphological indices.

A relatively more simple procedure is to count degenerative forms among the mature spermatozoa. While, for big animals, the artificial vagina offers an easy way to procure sperm (Milovanov, 1962), obtaining it from small laboratory animals requires some effort. Though rhythmical electrical stimulation by rectal electrodes (Fox, Fox, 1967) does cause ejaculation, the quantity of the sperm thus produced is rather small. Mature spermatozoa are more commonly obtained from the tail portion of the epididymus by longitudinal dissection in a medium containing a dosed quantity of a physiologic salt solution (2 to 4 ml is the empirically proposed quantity for rats). The epididymus is moved vigorously for specified period of time (two minutes for rats /G. M. Egorova/) on a watch glass in order to get rid of some of the spermatozoa (the use of glass sticks is counter-productive if artefacts are to be avoided, so lengths of a washed rubber tube are used instead). The spermatozoa suspension is placed on slides, dried in air, fixed over heat and stained with any dye. The ratio of degenerative forms (Fig. 15) in relation to the spermatozoan counts (routinely per 1000) is estimated in the field of vision. Different ocular grids are applied and an MPR-1 microprojector may be useful.

It is a good practice to estimate, in parallel, the total number of spermatozoa in the sperm or suspension obtained from the appendage, using a leukocytic melangeur and a blood-count chamber or celloscope (though, according to our data, the resulting figures are somewhat lower in the latter case). It is possible to estimate, concurrently, the relative number of dead spermatozoa in the Goryaev chamber, because, unlike live ones, they stain and settle on the bottom.

Functional methods refer primarily to assessment of the state of spermatozoa in terms of the duration and speed of their movement, tolerance to pH changes, and osmotic resistance (Milovanov, 1962; Molnar, 1969).

The principal disadvantages of the methods for assessing spermatozoan movements include, from our point of view, on the one hand, their non-specificity, that is, failure to measure the ability to advance

towards the ovum. On the other hand, they can and usually are used to estimate the maximum travelling speed (in the capillaries) in the Goryaev chamber and elsewhere, or the maximum duration of movements (on the glass in a humid chamber).

Considerable information is provided by the photokinetic recording of spermatozoan mobility as applied by Chakyrev and Nachev (1963) for research on human sperm. The method permits simultaneous determination of the spermatozoan count per unit volume, the percentage of mobile forms, the type of motion maintained by the majority of spermatozoa, the rate of advance, and the maximum, average and individual speed of movement for the greater number of spermatozoa to an accuracy of 0.5 μm/s. The method is somewhat difficult to use in mass toxicologic surveys, however, because the study of animal spermatozoa would require a long period and the use of an individual microscope with a film camera.

One integral characteristic of the spermatozoan function undoubtedly derives from their ability to fertilize the ovum without feeding pathogenic information into it. A more detailed discussion of this is given below.

Biochemical methods. The first and theoretically most important single field, also of some applied significance, relates to the sex cell's energetics. Indeed, it is for good reason that a relatively small organ like the testis receives such a generous supply of blood (Annison et al., 1963). The intensity of the biochemical processes taking place in the spermatogenic epithelium is dictated by the need to keep an adequate flow of materials and energy to foster the normal growth and division of maturing cells and then the vigorous activity of the future spermatozoa. As noted earlier, spermatozoan mobility is comparable with their respiratory or glycolytic capability, since it occurs at the expense of ATP breakdown.

In some instances, the failure of the spermatozoan fertilizing ability has been traced positively to inhibition of the respiratory system.

A second critical area of research is the metabolism of nucleic acids, the substrate for the transmission of hereditary information.

Little information is provided by integral characteristics, such as the physico-chemical parameters of nucleic acids as determined at low levels of exposure. To accomplish a more thorough structural analysis of nucleic acids (determination of solitary molecular gaps or the order of the bases) requires a special laboratory. The most feasible determination, therefore, is that of the total balance comprising the total content of nucleic acids and the characteristics of their synthesis and disintegration.

In a study of testicular homogenates and their subcellular fractions, Golubovich (1975) found different modes of action of toxic chemicals (Fig. 18). When tested at low levels of exposure ethylenimine, for example, had no effect on the DNA content in the testicular homogenate but was responsible for substantial changes in the RNA metabolism. The drop in the DNA content in the homogenate appears

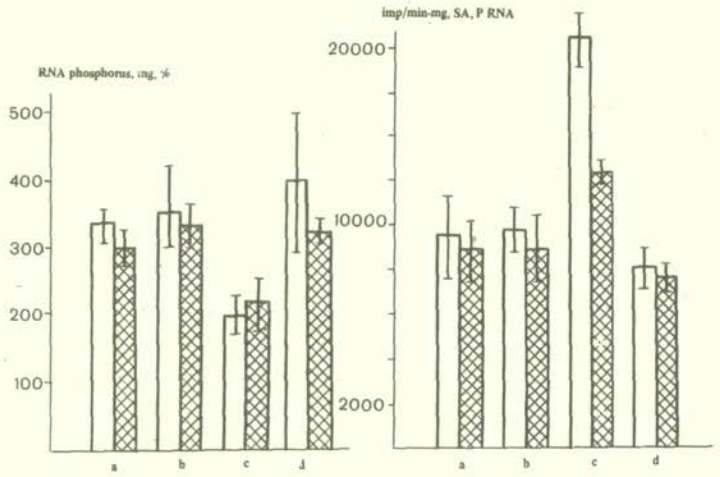


Fig. 18. RNA quantity and specific activity of RNA-cell fractions of the tests in the albino rat after a one-month ethylenimine exposure in a concentration of 12 mg/m^3.
SA — specific activity; a — homogenate; b — nucleus; c — mitochondria; d — supernatant. For symbols see Fig. 11

to have been caused by interference with its synthesis, since the RNA seeding with respect to phosphorus (studies using the redio phosphorus ^{32}P) declined more than the nucleic acid content. This decline of the DNA seeding was nowhere as obvious as in the mitochondria fraction isolated from the testicular homogenate. The injurious mitochondrial effect of ethylenimine can interfere with tissue energetics whose state depends directly on that of the mitochondria (see Fig. 18) (Golubovich and Orlyanskaya, 1974). Low-level lead exposure shifts the metabolic equilibrium towards RNA synthesis (the disintegration processes are inhibited and the RNA quantity in the testicular tissue is augmented). High-level exposures affect predominantly the process of synthesis (the nucleic acid content is reduced) (Golubovich and Orlyanskaya, 1974).

It must be realized, on the other hand, that homogenation of strongly differentiated tissues such as the testes tends to smooth over changes significantly, thus engendering a risk of misjudging the lack of visible changes in the homogenate as resulting from other developments, for example, an increasing nucleic acid content in some layers of the spermatogenic epithelium, with a corresponding decrease in other layers (Golubovich and Gnevkosskays, 1967; Sanotsky, 1967).

As the spermatozoon penetrates into the ovum, the activity of the hyaluronidase in the spermatozoan acrosome, as well as proteinolytic enzymes, are importantly involved in the process.

A BRIEF PROFILE OF THE STRUCTURE AND FUNCTIONS OF FEMALE SEX GLANDS

Mammal ovaries consist of the stroma, trophic tissue and the reproductive cells immersed in it. As distinct from male sex cells, a full complement of female sex cells (normally at the stage of the 1st-order ovocyte) is already available at birth and no regeneration of the ovocytes occurs during life.

The ovocytes of the first order discontinue development until the individual reaches sexual maturity. In the mature individual, the trophic (follicular) tissue begins to grow and the ovocyte increases in size. The first meiosis terminates only shortly before ovulation, however, (the emergence of a free ovum from the follicule into the abdominal cavity and its further progress into the uterus). It is generally assumed that most mammals ovulate at the metaphase stage of the second meiosis. By that time a follicular cavity, lined with stratified follicular epithelium, has been formed. Termination of the second meiosis with segregation of the second polar body usually occurs following the activation of the second-order ovocyte by the penetrating spermatozoon. The ovulating ovum is encased in a zone pellucidus thought to originate from follicular cells; on the outside it has a "corona" of sorts and an "ovigenic tubercle" substance containing, among others, hyaluronic acid.

It is known that not all follicles reach the stage of a vesicle, just as not all ovocytes ovulate. A greater proportion of them are killed by atrophy of the trophic (follicular) tissue surrounding them. This process, known as atresia, may gain considerable speed on exposure to toxic chemicals. Young ovocytes, with little or no surrounding tissue, show maximum sensitivity to external impacts. The follicle's development cycle depends for its duration on the age of the animal but, in any case, takes much longer time than does the sex cycle.

This duration is assumed to be 60 to 70 days in mice and several months in human females. It is generally recognized that, in mammals, the period during which the ovum remains capable of fully adequate fertilization is 10 to 20 hours after ovulation. The length of spermatozoan survival (in hours) in the female sexual tract is estimated, from summary data presented by the WHO Group of Experts in Report No. 333 (1968) as 6 in mice, 14 in rats, 22 in guinea pigs, 30 in rabbits, 48 in sheep, 72 in pigs, and 48 in humans.

As mentioned above, mammals are believed to have a second meiosis with the segregation of the polar body after the spermatozoa has already entered the ovum.

Data concerning metabolism in the ovum (non-fertilized and activated by fertilization) are insufficient because of the yet-unresolved difficulties involved in ova cultivation.

There is no doubt, however, that the embryo's development depends on factors bearing on the formation of the DNA hereditary information code, translation of the DNA into the RNA code, and the

processes involved in the application of the informational RNA code to synthesize characteristic proteins.

It follows from this that ovogenesis is not so well understood as spermatogenesis. Nevertheless, the hormonal regulation of the state of the female sexual tracts associated with the development stages of the ovum is somehow better understood than the normal regulation of the male sex system. Monitoring the hormonal state of a female individual is highly important for practical studies of the gonadotropic effects of chemicals.

The vaginal and uterine epithelium is known to undergo a series of cyclic changes, which set the stage for the timely reception of spermatozoa and then the fertilized ovum. The epithelium hypertrophies under the influence of the hormone of the developing follicle, which turns, after ovulation, either into a yellow body with a modified pattern of internal secretion when fertilized, or atrophies, if not fertilized, to make way for the hormonal activity of a new follicle with the proper replication of the cyclic epithelial changes.

SELECTION OF METHODS FOR STUDYING OVOGENESIS

As in the study of spermatogenesis, morphological methods have also been increasingly emphasized for research into the state of the ovocyte and its trophic tissue (follicle). Furthermore, like the investigation of the testes, common judgement argues against reliance on first-glance changes.

Morphometry. In order to determine the initial threshold changes of overriding interest to toxicologists, the relative proportion among the structural elements of the ovaries has to be quantitatively evaluated, as is routinely done at present.

Mandl (1951) and Zuckermann (1952) recommended making serial ovarian sections and counting over their entire surface area: a. primordial follicles and those having one layer of granulous cells, to be estimated in every tenth section (the result is correspondingly multiplied by ten); b. stratified follicles; c. follicles with a cavity, of which the follicles to be counted are those having, on the section, an ovum with a nucleus; d. atrophic bodies; e. yellow bodies. The elements "b" through "d" are estimated in every fifth section and the result is multiplied by five. The yellow bodies are defined from the median section or by visual inspection of the ovary, the idea being to count the total number of follicles in the ovary (Figs. 19, 20).

Because, over time, the ovarian connective tissue keeps growing and the tissue provides a certain measure of indication of ovarian ageing, many authors suggest that the area of the connective tissue be determined (planimetrically on the photograph or by projecting on the screen). A method still used by some workers in the absence of a planimeter involves cutting out from the photograph and weighing the silhouette of the connective tissue. This method is rather imper-

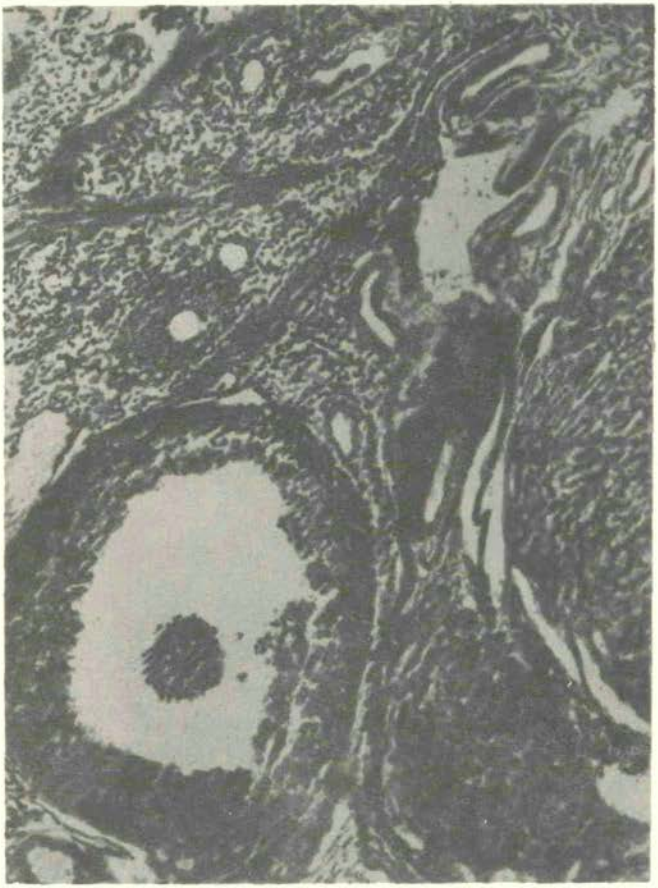


Fig. 19. General view of albino rat ovary. × 100

fect; a more productive technique, of course, features the application of photometric microscopes and microscopes with a television servo-system plus computer capability [Quantimet, Classimat T.A.S., etc.]. Not all laboratories have such instruments, available, however.

Numerous methods have been suggested for quantifying yellow bodies in small animals.

The relation of structural elements in the ovary gives an idea about the state of ovogenesis or its pathology [enhanced atresia, accelerated ageing of the organ, etc.].

Relevant examples employ average figures derived from investigation of low-level dimethyldioxane, dimethylacetamide and gasoline-solvent exposures for their effect on laboratory animals [Pashkova,s experiments, 1976]. The findings shown in Table 20 suggest a speed-up of the process of atresia.

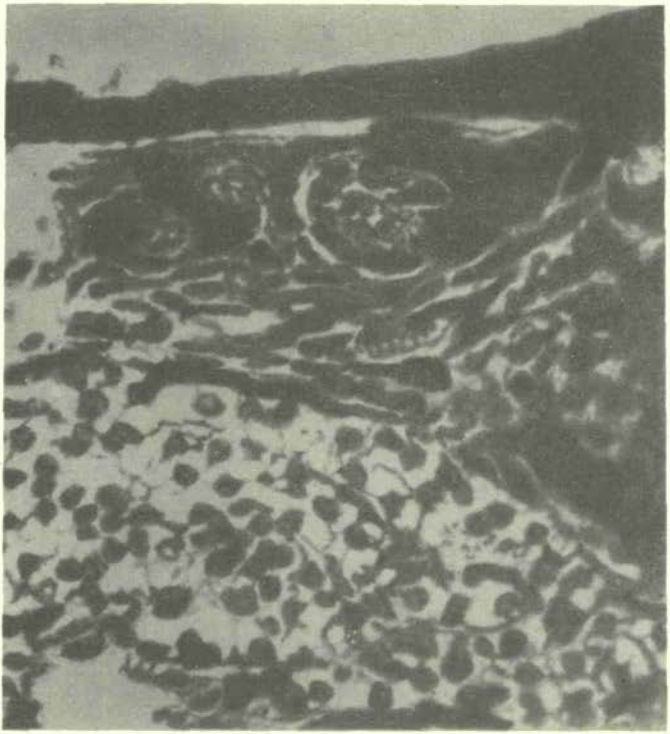


Fig. 20. Primordial follicles and ones with one layer of granulous cells (albino rat ovary). ×200

Functional methods. The function of the ovary is routinely judged by its hormonal activity, which contributes to the development of follicles. As previously mentioned, the most simple method is to examine the sexual cycle as determined in most cases from the state of the ovary. The method of vaginal smears which was proposed earlier by Stockard and Papanikolau identifies with ease phase, duration and pattern of the sexual cycle (the rhythm and duration of the phases). For practical gynaecological purposes, colpocytograms provide the means commonly employed in clinical hygienic investigations (Pashkova, 1969, 1976; Khairulina, 1973) because it permits an objective assessment of ovarian hormonal activity.

Integral methods for assessing the state of the spermatozoa and ova make use of the ability to fertilize and to conceive (for the ova, the assessment focuses on the female sexual system in its entirety, as the development of the ova and foetuses is known to depend not only on the state of ovogenesis as such, but also on the condition of the uterus and placenta). Male fertilizing capacity is indicated by the very fact of pregnancy in normal females and, more importantly, their fertility. It should be stressed that inability to fertilize may be less a result of spermatozoan deficiencies than of the defects in the central

Table 20

Composition of Structural-Functional Elements of the Rat Ovary Following Chronic Exposure to Dimethylacetamide (DMAA), 4,4-Dimethyldioxane 1,3 (DMD) and Gasoline

Chemicals	Concentration, mg/m³	Primordial follicles and those with one layer of granulous cells	Follicles with 2 or more layers of granulous cells	Graathian vesicles	Yellow bodies	Atresying follicles and atretic bodies	Total number of structural-functional elements
	Control	648.8	100.0	7.60	11.9	1448.7	2217.0
		83.3	11.3	0.40	0.39	79.1	77.7
		440.0	94.2	6.8	11.5	1327.5	1880.0
DMAA	1.4±0.15	72.5	11.5	0.53	1.94	90.1	91.5
							0.02
		305.0	84.2	5.8	8.5	1299.2	1702.7
	24±1.2	47.8*	6.2	0.35*	1.06**	68.1	76.5***
	Control	820	94.1	19.0	9.5	1120	2063.5
		56.4	10.2	1.4	1.2	87.1	85.8
		408.3	83.3	18.5	13.5	1837.3	2211.3
DMD	30±8	29.3**	14.7**	1.6**	0.66**	92.9**	22.9**
		380	140.8	16.6	14.3	1825	2299
	520±42	10.2**	12.9**	2.1**	1.1**	104.5**	64.3**
		668.0	72.5	9.1	8.66	1215.5	—
	Control	71.0	5.0	1.5	0.5	108.1	—
		766.0	68.5	6.1	9.8	1256.5	—
Benzine	37.0±4.1	115.5	16.8	1.3	1.6	99.5	—
	300.0±25.5	768.0	86.0	14.0	9.0	1333.0	—
		35.0	19.02	3	1.5	86.6	—

* $p<0.01$; ** $p<0.05$; *** $p<0.001$.

nervous system (impairment of the sexual instinct, etc.). Accordingly, the female's inability to conceive is greatly influenced not only by the gonads as such, but also and primarily by the state of the uterus and its mucous membrane.

On the other hand, the problem of tracking pre-gestational changes in the maternal organism is not yet completely resolved. The studies completed by Pavlova (1976) under out guidance have shown that the pathology caused by hydrogen chloride exposure in the maternal organism prior to pregnancy promoted an increase in foetal pathology on the "organ-to-organ" principle. To avoid artefacts in determining conception capacity and fertility (disturbance of maternal instinct and predation of progeny), some females should be sacrificed in late gestation. Along with fertility, it is necessary to estimate the pre- and post-implantation death of the ova; this is done by comparing the quantity of yellow bodies and the number of implantation loci.

Biochemical methods of ovogenetic research are seldom used nowadays on account of the prohibitively small mass of the ovary in laboratory animals. Endocrinological investigations into the hormonal activity of the various endocrine glands associated with the sexual

function is a more common option, particularly concerning the gonadotropic activity of the hypophysis.

Comparative male/female sensitivity to the effect of industrial toxics. The Communist Party and the Government of the Soviet Union focus primary attention on mother and child protection. In the USSR, women are granted paid ante- and post- natal leave; the right to extra unpaid leave of absence; relief from night-shift duties for the duration of pregnancy and nursing, and other benefits. The country operates a network of mother-and-child care institutions, women's consultation clinics, children's polyclinics, nurseries and kindergartens. A group of gynaecologists, pediatricians and embryologists were awarded a Lenin prize for designing methods for prenatal protection of the foetus.

Recruitment of women workers for jobs in the chemical industry posed researchers the important question as to whether it is possible to identify degrees of male and female sensitivity to toxics.

Extensive hygienic literature was published in the 1920s and 1930s on the effects of noxious environmental factors on the specific functions of the female organism such as gestation and lactation. On the basis of these, as well as other studies, in the early 1930s a list of occupations was drawn up contraindicated for female workers. For example, it was prohibited to engage women in lead- or mercury-handling jobs. At the time, this was the only protective measure that could be taken, for working conditions remained unfavourable in many occupations. One of the reasons for this was the failure to apply scientific expertise for effective management of hazards in the workplace, above all its potential for the hygienic standardization of harmful environmental factors.

Yet, even in those years, the comparative male/female sensitivities to the effect of industrial toxics became the subject of an ongoing debate. It was perceived by some that the considerable incidence of female gestation disorders and the high infant mortality might be due to the working conditions of the men married to these women. A wealth of data appeared on the occupational hazards of lead and mercury. In 1930, at the 8th National Convention of Obstetricians and Gynaecologists, E. M. Kudinovsky brought up the point of the substantial tolerance and biological resistance of the female sexual glands to the action of occupational toxics. Speaking in 1931, before the 6th International Congress on Occupational Diseases, Levi pointed out in his programme report that some specific functions (menstrual and genital) of the female organism made it more susceptible to the impact of industrial chemicals. As a comment on that statement, in 1938 N. V. Lazarev wrote that this concept of Levi's was "patently tendentious and unscientific". In following years factual experimental evidence and some clinical-hygienic data brought important insights into the issue.

Comparative sensitivity of general male and female responses to the effect of environmental factors. Some workers point to the greater sensitivity of females to chemicals like molybdenum or benzene (Hirokawa, 1955; Desoille et al., 1961) as well as nitro compounds

(Lazarev, 1938). After analyzing the findings of 283 acute experiments with a total of over 200 chemical substances, Krasovsky (1972) showed that, among the chemicals in the organophosphorus category, there were some to which female rats were three to five times more sensitive than males, and even five to eight times more sensitive, such as parathion, EPN and disistone. Kanevsky (1963) carried out a clinical survey of car-painting workers (118 males and 356 females) and found that females complained more often than males of headaches, irritability, and insomnia. In the morphological blood composition, they had twice as many detectable deviations as the males. Chevnetsov (1962) also argued for the greater vulnerability of the female organism on the basis of a survey of workers in cumol manufacture. Sosnovik (1934), Fridlyand (1950) and Kagan (1959) explored the picture of benzene intoxication, to find that it appears earlier in women. Even assuming greater female sensitivity, in general, to the effect of injurious environmental factors, this is far from being always the case.

Females are known, moreover, to show, by and large greater tolerance than males to ionizing radiation, starvation, cold, and narcotic drugs (Shettles, 1958). In the majority of animal species, the female has a longer life expectancy (Hennan, 1965). Females have also been shown to be more resistant to the toxic action of quite a number of chemicals (both by single and repeated administration to the body). Similar facts were reported from a study with low-dose exposures of mercury, pentobarbital, and ethanol (Heimburg, Schmidt, 1956; Aston, 1966), as well as such organophosphorus compounds as malathion, ronnem and methylparathion (Krasovsky et al., 1974).

The assertion that women are more sensitive than men sometimes feeds on poor knowledge of the issue. As an example, in their subjective health assessment of people who were exposed to a single 2 mg/kg acetophos dose, Krasovsky and co-workers (1972) noted increased sensitivity to the product among women workers, who made numerous complaints of poor health, general weakness, belching, the sense of a "lump" in the digestive tract and epigastral region, stomach aches, and inclination to vomit. The same symptoms, while also present in male workers, were not so severe. When estimated in objective research trials, the difference in blood cholinesterase activity between men and women, as also between animal males and females, did not exceed 15 to 20 per cent.

It has been repeatedly stressed in the literature that females are more sensitive than males to the effect of nitro compounds (Lazarev, 1938). More recently, however, Krasovsky (1975) has revealed that the intensity of methemoglobin formation in men and women, following exposure to nitro compounds, was actually the same, while the difference between the median lethal doses of the substances for males and females was no more than two-fold.

Table 21 provides data on the comparative sensitivity of males and females to some common toxics at the $DL_{50}(CL_{50})$ exposure level. As suggested by the Table, no major difference in sexspecific sensiti-

Table 21

Multiplicity of Sex-Specific Sensitivity Differences in Albino Rats to the Effect of Individual Groups of Toxic Substance *

Statistical parameters	Organophosphorus compounds	Organochlorines	Carbamates	Nitroamino-compounds
M±m	1.88±0.14	1.39±0.09	1.34±0.09	1.18±0.13
σ	1.14	0.51	0.30	0.26
n	68	36	12	15

Table 21 (contd.)

Statistical parameters	Inorganic compounds	Other	All chemicals
M±m	2.14±0.42	1.43±0.14	1.66±0.09
σ	0.95	0.71	0.99
n	5	28	149

* Cited from Krasovsky, 1973.

vity to toxics exists at the lethal level (though females are slightly less sensitive).

Similar data were obtained for lower exposure levels. It is worth noting that different authors come up with conflicting evidence on some individual toxics, in particular benzene (to which male individuals are said to be less sensitive but females absorb it more readily).

One is thus left to deduce that either sex-specific sensitivity to toxic chemicals is identical or that males show slightly higher sensitivity than females. To summarize the data on sex-related distinctions in the general responses to the impact of physical, chemical and psychic factors, it should be concluded that neither experimental data nor clinical-hygienic comparisons support the bias of emphasis in favour of "female labour" (except for some special cases). This is the basic tenet embraced today by the most members of the hygienic community, and the new hygienic design criteria for industrial plants do not envisage any distinctive sex-specific approach.

Comparative sensitivity of the reproductive function to the effect of environmental factors. Evidence from parallel studies of the ways in which industrial chemicals affect the reproductive function in males and females is limited. A comparison of experimental data on the gonadotropic action of toxics available in the literature indicates equal, indeed in some cases even greater sensitivity of the testes to the same exposure intensities. On the other hand, part of the available data does not lend itself to any such comparison, because the authors shunned the use of quantitative morphological methods in order to avoid subjectivism.

Clinical research data, too, are often unsuited for drawing definite

conclusions or offering relevant recommendations. An analysis of clinical inputs has been accomplished, among others, by R. A. Malysheva (1974) who is chairman of the Russian Soviet Federative Republic ad hoc subcommittee for the study of the effect of occupational factors on specific female functions. Admittedly, one of the key reasons for the slow progress and sometimes perfunctory research into this problem area stems from an insufficient competence and awareness among the obstetricians and paediatricians concerning the methods of hygienic and experimental investigations and their lack of contact so far with hygienists and occupational pathologists.

Even so, the more credible data, when closely examined, showed the following. For years, chloroprene exposure was stated to be a female health risk on the grounds of the adverse effects it has on the course of gestation and the development of progeny (Velkovich, 1940; Mirzabekyan, Akhverdyan, 1946; Mkhitaryan, 1962, and others). This was the motive for the ban on employment of women in chloroprene production. Yet the recent investigations completed in our laboratory have documented an impairment of the reproductive function in male workers engaged in chloroprene manufacture (R. M. Davtyan's research results), judging from the number of spontaneous abortions among the wives of workers in a chloroprene production department (see Table 14), the spermatograms of the worker group cited in Table 15, and the development of their children. Experimentally, the thresholds of the gonadotropic (in males) and embryotropic (Salnikova's study, 1973) effects of chloroprene were found to be identical.

A thorough investigation of the state of the sexual function in men (using the MSF/male sexual function/questionnaire proposed by Vasilchenko (1968)) and women (based on laborious compilation of case-histories and a gynaecologic examination) from the same production facility, together with experimental observations carried out simultaneously on males and females, have enabled Tarasenko, Kasparove and Strongina (1976) to prove conclusively the similar sensitivity of the female and male sexual glands to boric acid exposure.

In an equally conclusive manner, the identical sensitivity of the female and male reproductive function on exposure to manganese compounds was shown by Mandzhgaladze (1969) from a series of clinical-hygienic and experimental investigations.

The considerably greater proportion of studies (especially clinical) considering the impact of industrial chemicals on the reproductive function of the sexual glands in women rather than in men appears to be linked, first, to the shortage of competent expertise for studying sex function disturbances in males and, second, to the persisting concept among some experts that the protection of progeny has most to do with the protection of women. This concept is reiterated time and again at various conferences.

As for the manifestation of mutagenic effect, mass investigations on the spontaneous level of chromosome aberrations in the peripheral blood of numerous groups of the public, carried out at the Institute of Medical Genetics (Bochkov et al., 1973) have detected no sex-spe-

cific divergencies. Nor was any difference found in the sensitivity of male and female blood cells to mutagens. At the same time, a study of mutations in sex cells revealed greater sensitivity of spermatogonia compared with oocytes (Generoso, Russel, 1969; Russel, 1963, 1967; Deen, Blarr, 1976) resulting from distinctive patterns of spermatogenesis and ovogenesis. In males, stem cells (spermatogonia) mature gametes are known to produce throughout the reproductive period. In females, on the contrary, the formation of oocytes is complete before or shortly after birth, with no oogonia present during the mature period. This disparity of gametogenesis results in widely differing response to the mutagenic effect of various factors (Oakberg, 1968).

It has to be admitted that few hygienists seem to be aware of this, which explains why chemical mutagens are still regarded as being more hazardous to women. It is on these grounds that a strong point was made in the past for the withdrawal of women workers from immediate work exposure to these compounds (e.g., chloroprene).

Thus, the problem of protecting future generations does not fit into the limited framework of the female labour problem. In addition, the probable risks involved in the specific action on the reproductive function in both the male and the female organism should be considered.

With respect to the effect of noxious factors present in the working environment on gestation, a function unique to the female organism, it is absolutely essential, as our research makes clear, to discriminate in principle between two things. One of these is the mediation of embryonic developmental abnormalities via the maternal organism. The other is the specific embryotropic effect of noxious factors, which may have a threshold well below that of the maternal organism. The latter poses a real hazard and the extensive data obtained to date support the idea that lactating women's milk contains hazardous substances to which the women have been exposed at work or at home. Ionizing radiation, MWF, noise, vibration, physical and emotional stresses and many other factors combine to set off qualitative and quantitative changes in lactation. No comprehensive analysis on the dependency of the effect on the magnitude of extraneous impacts has yet been made, however. Nor are the thresholds of this effect to be found in the literature, with but extremely rare exceptions. This means it is particularly urgent to extend the scope of research in this area.

Thus, a brief review of comparative male/female sensitivity to the impact of industrial toxic agents sheds light on their essentially similar vulnerability. For the reproductive function, the principal way to afford prenatal protection to the foetus in those occupationally exposed to industrial toxic chemicals is to provide them safe working conditions, notably through establishing MAC and blocking out absorption through the skin and stomach. Some situations call for permanent or temporary (for the duration of pregnancy and lactation) withdrawal of women from occupational exposure to highly specific toxic

agents, ($Z_{sp} \geqslant 10$) (Sanotsky, Fomenko, 1974). The factors to consider have include the selectivity of the toxic's action on uterine tone, placental permeability, excretion of the chemical at effective concentrations in milk, selective sex-specific sensitivity and increased susceptibility to the chemical during gestation. On the other hand, it is essential to bear in mind that the adverse effects of a chemical may take hold very early in pregnancy (Fomenko et al, 1974), thus detracting from the effectiveness of these efforts. It is not the withdrawal of women from production, therefore, but the standardization of industrial toxics, with due consideration for their possible wide-ranging influence on the reproductive function of both the male and female organism, that must be seen as the ultimate remedy. In view of the foregoing, improvement and extension of methods suited to this kind of research is a fairly high priority.

Chapter 4

EMBRYOTROPIC EFFECT OF CHEMICALS

CHEMICALS WITH EMBRYOTROPIC POTENTIAL

Chemical exposure during gestation can be responsible for a variety of anomalies in foetal development, which most researchers separate conventionally into the following groups of effects: 1. teratogenic (histomorphological developmental abnormalities; biochemical, functional and other impairments in the functions of organs and systems, which become manifest in postnatal development); and 2. embryotoxic (intra-uterine death, reduction of the embryo's weight and size with normal differentiation of tissues). As discussed earlier, substances endowed with teratogenic and embryotoxic potential include a variety of chemicals finding industrial, agricultural, domestic and pharmaceutical applications. Consequently, it is a difficult task to a boundary between occupational and non-occupational (on- and off-the-job) exposures.

Drugs constitute the greater part of the chemicals so far explored for their embryotropic effects. Teratogenicity has been proved for a number of anticonvulsive drugs, antibiotics, hormones, and vitamins, both experimentally and clinically.

Of the chemical compounds widely employed in industry and agriculture, teratogenicity was confirmed for chloroprene (Nairit) latex (Velkovich, 1940; Mirzabekyan, Akhverdyan, 1964), the manufacture of industrial rubber items (Chervyakova, Barakovskih, 1974; Kolosova, 1974), phenol phormaldehyde resins (Korshunov, 1974) and some others. Published data of clinical and experimental investigations reveal the influence of granosan and DDT on embryogenesis and the development of offspring (Goloma, 1962; Komarov, Vasilkovskaya, 1968; Vashakidze, 1969, and others).

A health survey of women workers in an organosilicon varnishes and enamels plant disclosed a higher incidence of gestoses and abnormal labour (Petrova, Vishnevsky, 1972). Qualitative changes in the placenta were also discovered in the female employees of a synthetic rubber plant (Morozova, Shapin, 1973). These gestational anomalies were bound to affect the condition of the foetus.

There now exist registers, or extended lists of substances showing embryotropic effects (Manevich, 1965); Shepard, 1973; Sanotsky, Salnikova, 1979). To illustrate, some chemical teratogens are cited below.

Group	Chemicals
Chemical carcinogens:	7, 12-dimethylbenzo(a)anthracene, N-diethylnitrosomine, ethylnitrosourea, urethane, hexane
Hydrocarbons:	
Benzene and its derivatives:	meta- and paradiisopropylbenzene, chlorobenzene, benzene
Sulfur compounds:	hydrogen sulfide, carbon disulfide, sulfur dioxide
Chlorinated hydrocarbons:	chloroform, chloroprene, dichloriethane, methylene chloride
Amides:	diathylamide, methylformamide, dimethylacetamide
Amines:	diethylamine, hydrasine
Imines:	ethylenimine, piperidine
Ethers:	ethers of adipic acid, metacryl ether
Alcohols:	ethyl alcohol, phenylethyl alcohol
Acids:	di- and trichlorophenoxyacetic acids and their derivatives
Metals and their compounds:	mercury, tellurium, lithium, cadmium, sodium, copper, lead, zinc
Organophosphorus agents:	chlorophos, metaphos, carbophos, butyphos, parathion
Organochlorines:	DDT, dildrin, aldrin, dieldrin, endrin
Carbamates:	sevin, benlat, zineb, maneb, ramrod, dipterex, bromex, phthalan
Drugs:	
Hormonal drugs:	cortisone and its derivatives, estrogens, steroids
Antibiotics:	tetracycline, penicillin, levorin, fusidine, hystatin, streptomycin, monomycin
Sedatives, soporifics, tranquilizers:	chlorpromazine, trifluoperazine, imiprazine, chloracizine, aminazine, diazepane, pentobarbital, phenobarbital, trazodone
Antineoplastics:	cyclophosphamide, endoxane, metathrexate, pentoxyl

Because most studies provide no data on the thresholds of systemic toxic and specific effects, it has not been possible, however, to evaluate the selectivity (specificity) of embryotropic effect. This almost cancels out the practical value of these data for preventive toxicology.

In recent years, there has been an increasing number of papers not merely stating the embryotropism of a substance, but going on to analyze its mechanism (Broitman et al., 1966; Kotin, Repin, 1973; Bunrean et al., 1973; Collins et al., 1972; Forsthorfeld, 1973; Koshakji, Scheufler, 1973, and others). Indeed, the mechanism of embryotropic

effect amounts to a whole new subject and its coverage in the present book does not seem practicable. It does make sense, however, to briefly review permeability of the placental barrier to chemical compounds.

Placental permeability to industrial toxics. Environmental noxious exposures, as they transform in the maternal body, are likely to cause changes in the placenta. Until recently the role of the placenta in the initiation of the embryotropic effect had not received the attention it deserves.

The placental barrier is currently viewed as an integral unit in the complex chain of mother-placenta-foetus interrelations and only systemic study of the complex can be expected to yield good results in the effort to safeguard the health of future generations (Bodyazhina, 1963; Garmasheva, 1972; Otteni, 1973).

The placenta has been found to be a functionally complex organ sustaining the normal existence of the intrauterine foetus. The wide functional range of the placenta determines its specific duty during gestation.

In relation to the foetus, the placenta discharges five basic functions, namely: 1. respiratory (Bodyazhina, 1949); 2. trophic (Gorizontov, 1946; Kukina, 1967); 3. barrier (Brusilovsky, 1966; Shtern, 1927); 4. incretory (Berkovich, 1948; Pirogovsky, 1970); 5. excretory (Subbotin, 1957).

Placental permeability is close by related to its numerous functions.

Kubasov (1889) and Ott (1885) last century, and Arshavsky, Paducheva (1956) and Birger (1957) in more recent years discovered that a change of placental permeability occurs for a variety of reasons such as: 1. structure and type placenta; 2. the general condition of the maternal body; 3. period of pregnancy; and 4. structure of chemical compounds.

Korobova (1956), Brusilovsky (1970), Gorokhovsky (1972) and other authors identified an intimate relationship between placental permeability and its structural type. The placenta of the hemochorial type displays maximum permeability to chemicals.

That variations in placental permeability depend on the general condition of the maternal body, has been proved beyond doubt by a large number of workers in their respective studies (Vylegzhanin, 1937; Bodyazhina, Kurdiukova, 1964; Baksheev et al., 1968; Bekker, 1970; Bodyazhina, Kiriushchenkov, 1973; Vozovaya et al., 1974; Sholck, 1958). The barrier function of the placenta is impaired when the expectant mother suffers from an infectious disease, oxygen deficiency, radioactive radiation exposure, general malnutrition, and other factors.

Placental permeability was likewise proved to vary with the length of gestation. It was shown, furthermore, to differ with respect to various substances at different periods during gestation (Sholck, 1958).

In addition to the period of pregnancy, permeability variations of the placental barrier are brought about by exposure to numerous toxic compounds; e.g. nicotine makes the placenta permeable even to

Table 22

Permeability of Human and Animal Placenta to Some Chemicals

Chemical	Man		Test animals		Literature source
	embryotropic effect	placental permeability	embryotropic effect	placental permeability	
Mercury	+	+	+	+	Greener, 1925; Gang et al., 1972
Ethanol	+	+	+	+	Waltman et al., 1972
Lead	+	+	+	+	Carpenter et al., 1973 Elina, 1974
Herbicides of 2,4-D group	+	+	+	+	Konstantinova, 1973
Methylene chloride		+	+	+	Vozovaya et al., 1974
Carbon disulfide	—	+	+	+	Yaroslavsky, 1971 Salnikova et al., 1974
Tetracycline	+	+	+	+	Kiriushchenkov, 1973
Dimethylformamide			+	+	Sivochalova, 1975 Sheveleva, 1971
Orthonitrotoluene			+	+	Sivochalova, 1975 Kurliandskaya, 1948

substances otherwise incapable of passing through it under normal conditions.

Transplacental metabolism is known to occur not only by way of osmosis and diffusion, but also via the active transport of substances by the biochemical mechanisms known as "transporters" (Skow, 1960, and others). Lately, pinocytosis has been discovered to be importantly involved in placental permeability (Goncharuk, 1971; Strauss, 1971, and others).

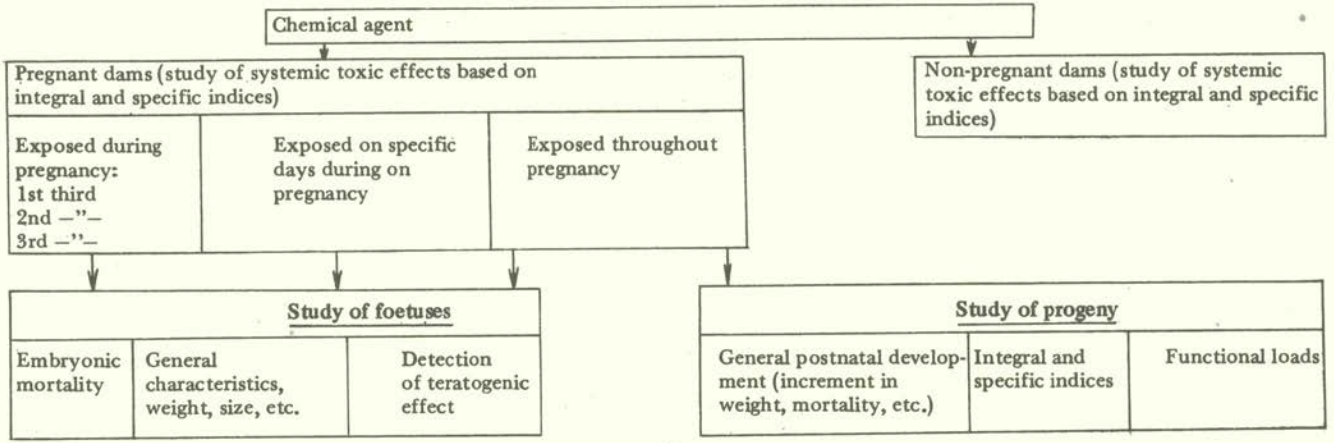
Although placental permeability for drugs is known sufficiently well, far fewer studies have dealt with placental permeability to industrial toxics. Meanwhile, the latter's exposure levels are commonly very high — so much so that this prevents comparison of clinical with experimental data. In a few cases, however, embryotropic effects can be traced to a poison's power of penetration through the placenta, as in dimethylformamide or tetracycline. Some relevant examples are given in Table 22.

By and large, analysis of the literature on the embryotropic effects of chemical compounds reveals a substantial research advantage of experimental over epidemiologic investigations. This is readily understandable, considering the structural complexity of occupational and domestic exposures, which makes the interpretation of clinical data so much more difficult.

The key role of experiments in the study of chemical compounds in order to validate prophylactic recommendations strongly suggests the need to examine the available evidence from the angle of experimental design. Experiments on the embryotropic potential of chemicals should be structured as shown in Diagram 1.

Diagram 1

Diagram of Experimental Research on Embryotropic Effect
(Inquiries into Thresholds and Specificity)



Methods for studying placental permeability. The methods used for investigating the placenta for its degree of permeability to chemical compounds may be direct or indirect.

The advantage of direct methods is self-evident. It derives from the possibility of estimating the amount of a chemical that passes through the placenta and its distribution in the embryo's tissues. The difficult point, however, is to develop adequate sensitivity for such a method.

The indirect methods for assessing the permeability of the biological membrane are less accurate. Yet a compromise between direct and indirect methods gives a most complete idea of the permeability of the placental barrier. In our work we apply both direct and indirect methods.

Thus, we have developed a chemical method to estimate DMFA present in the foetal tissues and the maternal placenta and liver (in collaboration with S. A. Osina, a junior research officer with the Laboratory of Industrial Hygienic Chemistry). Part of the development effort included definition of optimum conditions for complete DMFA extraction from these biological substrates. The DMFA determination was made in the fertilized ovum — at 12 days' gestation time; in foetuses and placentas — at 20 days; and in the liver and blood of pregnant rats at 12 and 20 days, following DMFA exposure at the Lim_{ch} and MAC levels.

DMFA inhalation at the MAC and Lim_{ch} levels can result in the deposition of the toxic in foetal tissues, as has been made clear by experiments (Sivochalova, 1975). At 20 days of pregnancy the index of placental permeability increases as a function of the toxic concentration in the air inside the exposure chamber (24 mg/m^3, Lim_{ch} level).

A microbiological method of diffusion into agar, sensitive to 0.05 μg per ml of analyzed volume, was useful for defining tetracycline (TC) in the maternal and foetal bio-substrates.

Sivochalova (1975) employed the method of so-called loading doses, i.e., on the day of sacrifice the controls and test rats were injected TC intraperitoneally (at a rate of 50 mg/kg) and killed one hour afterwards. The loading doses have been selected because the microbiological assay technique failed to capture the concentrations of the antibiotic in the foetal and placental tissues after the inhalation of TC at low levels (Lim_{ch} and MAC).

An index of placental permeability (IPP) — the ratio of the chemical's concentration in the foetal tissues (A) to the corresponding concentration in the maternal blood (B) in percentage terms: A/B 100% (Table 23) — was calculated in order to get an idea of transplacental transfer of the poison.

Besides direct methods for estimating the ability of a chemical to transfer across the placenta, our laboratory tested specific methods for studying placental permeability to several test substances, viz., 1. ^{131}J, 2. methionine ^{35}S, and 3. the dye — trypan blue.

1. For the purpose of radiometric investigations, radioactive iodine ^{131}J (without a carrier) is injected intraperitoneally in a dose of 5 μCi

Table 23

Tetracycline Concentrations in Placental and Foetal Tissues (n=10)

Bio-media, mg/g	Controls	Effect of tetracycline	
		MAC	MAC×10 Lim_{ch}
Foetus *	0.72±0.03	1.03±0.066**	1.51±0.047***
Placenta *	4.13±0.43	7.5±0.75	9.3±2.12****
Index of placental permeability	4.17%	6.92%	10.0%

* 20 days' gestation time; ** $p<0.001$; *** $p<0.01$; **** $p<0.05$.

per animal. Four hours after the injection, the animals are sacrificed and the tissues of the pregnant animals (blood, thyroid gland and liver), along with the placental tissues and the cervical portion of the foetuses, are subjected to radiometry.

Comparison of the resulting activities with the reference standard provides a yardstick for measuring marker concentrations in the tissues (isotope concentrations in the bio-substrates as a percentage of the dose of radioactive substance injected into the mother per one gram of raw tissue mass). Table 24 presents the results of a placental permeability study for nicotine exposure by subcutaneous administration from the first to the 19th day of pregnancy inclusively.

2. The procedure for applying methionine ^{35}S in placental permeability studies is essentially similar to that just described, apart from some modifications. Methionine ^{35}S is injected intraperitoneally in a dose of 10 μCi per animal, followed by their sacrifice one hour later. The use of methionine as an integral index of placental permeability makes the results much more difficult to interpret because the amino acid is vigorously involved in metabolism going on in the maternal body and placenta.

3. A freshly prepared 2-percent colloidal solution of trypan blue is injected into the tail vein (1 cm^3) and the pregnant animals are sacrificed 90 minutes later. Finely ground placental and foetal tissues are weighed and placed in weighing bottles holding an isotonic NaCl solution (2 cm^3 solution per one gram tissue per day). The assays are centrifuged at 3,000 rpm for eight minutes and subjected to photometry on a photoelectric colorimeter using a red light filter.

Importance of animal experiments. The question of how important animal experiments may be for predicting possible teratogenicity of chemicals in humans is still being discussed even though all substances teratogenic to man exhibit a varying degree of teratogenicity in experiments on mice, rats and rabbits. Many authors do not seem absolutely sure that negative results from testing some chemicals on animals indicate their zero teratogenic potential for man (Manevich, 1966). The difficulties and possibilities involved in animal data interpretation were discussed in Chapter 2.

Table 24

Content ^{131} J in Maternal and Foetal Bio-Substrates Exposed to Nicotine

Nicotine daily dose, mg/kg	Number of animals	Percentage of injected dose		
		Maternal blood	Maternal liver	Maternal thyroid gland
2.5	7	0.24±0.02	0.048±0.006	0.23±0.015*
0.5	7	0.22±0.03	0.045±0.005	0.31±0.09**
0.05	7	0.22±0.08	0.039±0.005	0.069±0.009
Controls	7	0.20±0.04	0.041±0.004	0.081±0.019

Table 24 (contd.)

Nicotine daily dose, mg/kg	Number of animals	Percentage of injected dose		
		placenta	foetus	foetal liver
2.5	7	0.179±0.028*	0.108±0.023	0.045±0.008
0.5	7	0.094±0.016	0.131±0.043	0.037±0.003
0.05	7	0.106±0.015	0.098±0.013	0.037±0.004
Controls	7	0.080±0.009	0.110±0.037	0.045±0.003

* $p<0.001$; ** $p<0.05$.

Most of these experiments make use of small laboratory animals (rodents) and only some workers prefer dogs and monkeys (Tanimura, Tukashi, 1972; Rao et al., 1973; Watanable Nobuo et al., 1973). Studies on chick embryos still claim a large share of the total.

Of late, sea-urchin eggs, the classic objects of embryology, have been suggested for use for preliminary evaluation of embryotropic effect from chemical environmental pollutants (the USSR-USA Workshop on Developmental Toxicity, Leningrad, 1977). Yet, as we see it, the findings may be of only limited use for solving some theoretical issues. This is because the research data gained from studies on chick embryos, sea urchins and other phylogenetically distant objects simply cannot be directly transfered to humans in view of the numerous fundamental metabolic differences between them. Wide divergences have now come to light in the species-specific sensitivity of animals to the same embryotropic agent (Smolnikova et al., 1973; Moriguehi, Mosahide et al., 1972, and others). Some pertinent information is given in Table 25. Thus, to conclude, no more than a few animal species seem suitable for experiments offering a more or less credible notion about the embryotoxic hazard of the same compound to man.

Studying specificity of embryotropic action. As mentioned earlier (see Chapter 2), the avaluation of a poison in terms of its specific (selective) action on a particular structure or function is usually based

Table 25

Animal Species Sensitivity to Effects of Chemicals Having Embryotropic Potential

Chemical	Mouse	Rat	Golden hamster	Guinea pig	Rabbit	Human	Cited from:
Chloroprene	Embryotoxic effect	Embryotoxic and teratogenic effects	—	—	—	Teratogenic effect	Akhverdyan, 1944; Sal'nikova, 1970; Sal'nikova, Fomenko, 1973
Benzene	—	Embryotoxic effect	—	—	Embryotoxic effect	Premature delivery, abortions, stillbirths	Tikhomirov, 1928; Butomo, 1935; Gofmekler, 1968, et al.
Nicotine	—	Same	—	Embryotoxic effect	Same	Abortions	Griner, 1925; Leshchinskaya, 1926; Gitelson, 1929, 1930; Mgalobeli, 1930; Sergeeva, 1939; King, Backer, 1966
Mercury and its salts	—	Same	Embryotoxic and teratogenic effects	—	—	Abortions, stillbirths, developmental anomalies	Griner, 1925; Grigorova et al., 1934; Snayder, 1971; Harris et al., 1972; Motter, 1974
Granosan	—	Same	—	—	—	Abortions, intoxications in newborn, retarded development	Goloma, 1962; Mukhtarova, 1963, 1966; Vashakidze, 1966
Butyl ester 2,4,5-T	Teratogenic effect	Same	—	—	—	Teratogenic effect	Konstantinova, 1974; Rassel, 1970; Courtney, 1970; Ngo Vihn Liong, 1973
Steroids	Same	Same	—	—	—	Premature delivery, abortions	Starkov et al., 1975; Rogoyski, 1972; Shah, Chaudhry, 1973; Green, Kochhar, 1973
Dipterex	Same	Teratogenic effect	Teratogenic effect	—	—	—	Staples, Golding, 1977

on comparison between the thresholds of systemic toxic (integral, non-specific) and the corresponding specific effect. The selectivity of toxic damage is measured by the magnitude of the specific action zone, Z_{sp}. Follow Z_{sp} of several chemical compounds (by embryotropic effect): dimethylformamide 10; dimethylacetamide 10; formaldehyde <1; ammonia <1; chloroprene 12; tetracycline 3; ethylenimine 1; urethane >1; 2,4-D$=1$; 2,4,5-T$=1$; vinyl chloride >1; dinyl <1; caprolactam — 1; phenol >1; gasoline 1; benzo(a)pyrene 10; hydrogen chloride <1; hydroperoxide of tertiary butyl 1; neozone D1; prednisolone 1; nicotine >1; and piperidine <1.

In evaluating embryotropic effect, it is wrong to estimate Z_{sp} by calculating the ratio of the threshold of a single exposure, $Lim_{ac\ integr}$, to that of the embryotropic effect. Indeed, such a correlation is only possible between the threshold values derived from experiments with equal exposure duration. In that case, it would be necessary to establish the threshold of embryotropic effect for a single administration and that would require, accordingly, that the effect be verified at different times during embryogenesis (see Chapter 4).

Relating Lim_{ch} to the threshold of the embryotropic effect as defined by repeated exposure to the agent under study is equally wrong, since the dose, actually given, i.e. the summary dose, does not conform in this case to the threshold value of the embryotropic effect.

Evaluation of a chemical for its systemic toxic effect on the maternal organism is a must in the study of its embryotropic effect. The only way to assess an effect properly in selectivity terms is by correlating within the same experiment, the threshold of the systemic toxic effect, as defined directly on pregnant females, with that of the embryotropic effect.

Routes of administration to the body. The route by which a toxic chemical is administered to the body in the experiment is naturally determined by the conditions of occupational exposure in the workplace. Since most substances enter in inhaled air, the inhalation route is the main one. Some low-volatile compounds with the potential of easy entry through unimpaired skin are, however, best studied by specified skin applications, as is done in the case of repellents. In some distinctly specified instances, model conditions can be chosen that involve routes of administration (intraperitoneal, subcutaneous, intravenous, etc.) that do not comply with occupational ones. Such conditions apply for several types of experiment investigating the mechanism of the action of a toxic, but should be avoided in the validation of a hygienic standard.

Pattern of exposure. Considering the actual conditions of toxic exposure at work, industrial toxicology is, in practice, concerned with embryonic sensitivity throughout and during the first trimester of pregnancy, when an expectant mother has not yet consulted a doctor or been transfered to another job. All major morphogenetic processes are completed during the early, pre-implantation period of embryonic development. It is, therefore, this period that determines the morphological and physiological distinctions of the developing organism.

The first trimester of pregnancy is also the time when the drug-induced manifestation of teratogenic effect presents a particular hazard in humans (Pumpianski, 1972). Neither is the use of drugs any more desirable in subsequent periods of pregnancy (from the fourth month on), for varying amounts of them are sure to pass transplacentally into the embryo's blood.

The sensitive and resistant periods of embryogenesis vary with different agents and depend on the degree of differentiation achieved during organogenesis and on the time of onset of specific biochemical reactions in them (Dyban, 1959, Agnew, 1972). Consequently, the recommendations on the teratogenicity testing of medicinal drugs emphasize the need to identify the period of embryogenesis that is most sensitive to the effect of the substance being tested.

Our experience with industrial toxic chemicals reveals marked changes in the pattern and severity of the embryotropic effect under different modes of administration of the test chemical. Thus, experiments were conducted (by L. S. Sal'nikova) to investigate the embryotropic effect of chloroprene exposure at the former MAC level throughout pregnancy and at different periods (by two-day sequences spanning one to 18 days' gestation time). No visible developmental anomalies were found after the continuous exposure, but the presence of a teratogenic effect was detected after exposure at specific periods: 5—6, 9—10, 11—12, and 13—14 days. The largest relative quantity — 23 per cent — of cerebral hernias in the embryos was observed with the minimum toxic exposure at 5—6 days of pregnancy. No such pathology was detected in the control (see Fig. 13 and Chapter 2.).

Pathological manifestations as a function of the dates of exposure to a 10% prednisolone ointment (applied to the skin in SHK mice) are shown in Fig. 21.

The above evidence is in line with the theoretical concepts regarding the development of a teratogenic effect. It indicates that, even though prolonged administration of drugs throughout pregnancy seems the logical answer, this may dramatically modify its metabolism in the maternal body and "camouflage" the teratogenic effect (Conclusions of the WHO Expert Committee, Technical Report No. 364, 1968).

It should be noted that the previously suggested and widely accepted critical periods of embryogenesis can hardly be fully applied because organs and tissues are constantly being laid down and differentiated daily and hourly, throughout pregnancy, and their impairment may have grave implications for the foetus. The main periods of organogeny in humans and ten animal species are listed in the works of Robson and Sullivan (1968) and Evans and Sack (1973).

We hold the view that it is most practical to test for the effect of a poison both throughout pregnancy as well as during its first third, provided, of course, the different times of organ development in humans and animals are taken into account (Table 26).

Selection of exposure levels. While drug teratology studies the effect of various compounds in high doses that are certain to affect the

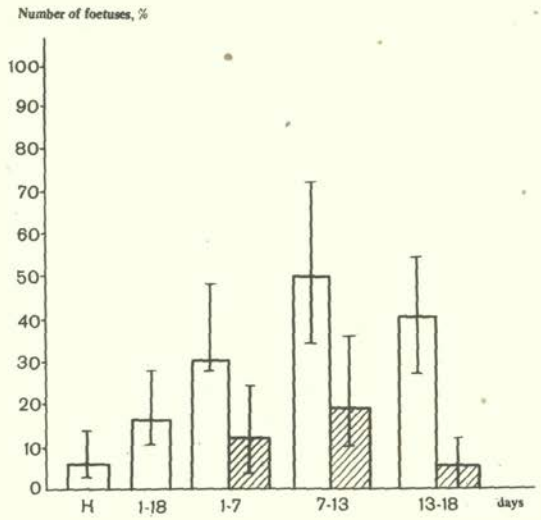


Fig. 21. Pathological embryogenesis against time of exposure to 0.5% prednisolone ointment (skin applications to pregnant albino rats).
Relative number of foetuses: blank columns — with hemorrhages; hatched columns — with general tumescence

Table 26

Times of Development of Some Organs and Systems in Humans and Experimental Animals (Robson and Sullivan, 1968; Evans and Sack, 1973)

Indices	Days of pregnancy			
	man	rat	mouse	golden hamster
Duration of pregnancy	267	21—22	18—20	15.5—16
Central nervous system				
Medullary plate and groove	18	9	6.5	7.5
Close of anterior neuropore	26	11	9	8.25
Closure of posterior neuropore	28	12	9.5	8.5
Development of cerebral appendage	33	13	—	—
Appearance of vascular plexus	35	15	—	—
Eye and ear				
Appearance of optic diverticulum	24	11	—	—
Formation of eye calix and lens	28	13	—	—
Development of optic nerves	49	17	—	—
Formation of eyelids	56	15	—	—
Accretion of eyelids	70	18	—	—
Separation of acoustic vesicles	28	13	—	—
Formation of labyrinth	49	15	—	—
Cardiovascular system				
Accretion of cardiac tube	23	11	—	—
Formation of intraventricular septum	49	17	—	—

Fig. 22. Scheme of macroscopic survey of foetuses according to Dyban (Section lines)

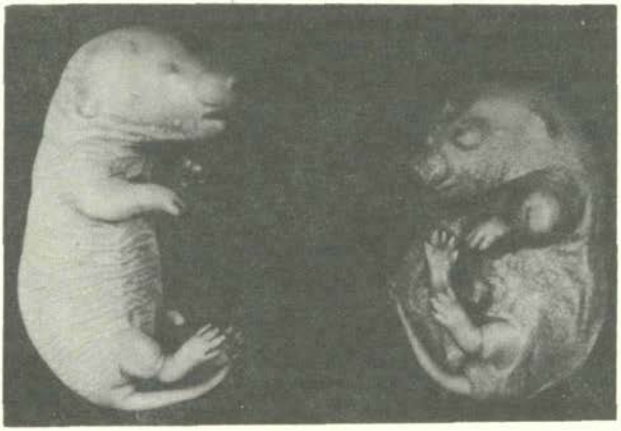


Fig. 23. Multiple malformations arising from benzo(a)pyrene exposure at 0.05 mg/kg; 1st to 15th day of gestation

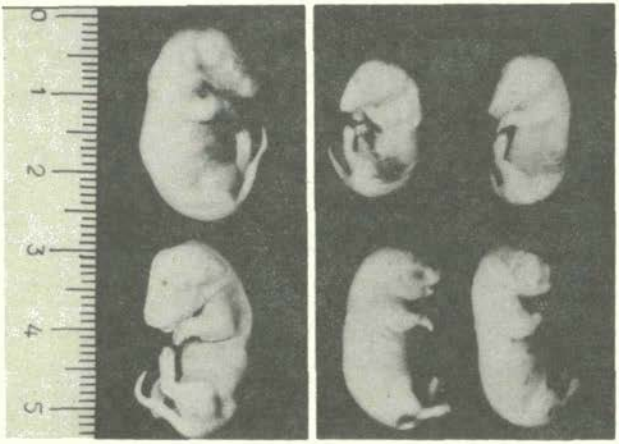


Fig. 24. Teratogenic effect of chloroprene

mother, the practical interest of industrial toxicology focuses on studies involving low levels of no consequence to the mother, but sufficient to reveal specific embryotropic effects. Some hygienic studies designed for evaluation, of embryotropic risks from pesticides do, however, make use of sublethal doses and concentrations.

Under the conditions just described, the maternal organism develops systemic poisoning and the changes of embryogenesis that arise cannot be relied upon for judging the specific effect of the test substance. We believe such an experiment is impractical even for a tentative evaluation, because the need for additional studies at lower levels of exposure unduly complicates, and extends the programme and increases its cost.

For the reasons given, the study of embryotropic effect should best begin, in our opinion, with exposure to concentrations of industrial toxics that are below the threshold of acute action for females and at the Lim_{ch} level and then, if necessary, go down to the MAC or lower still (see Diagram 1).

Study of embryonic material. Such methods as the determination of pre-, post-, and total embryonic mortality; of the weight and size of foetuses and the placenta; and macroscopic investigations (including A. P. Dyban's modification of the Wilson method, and the Dawson method) characterizing the teratogenic effect, are obviously the methods for evaluating the embryotropic effect of chemical compounds common to both toxicology and pharmacology. A scheme of foetal sections according to Wilson (1965) in Dyban's modification (1970) is given in Fig. 22.

Macroscopic techniques were used to detect the teratogenic effect of 3,4 benzo(a)pyrene (Fig. 23), chloroprene (Fig. 24) and some other chemicals.

Though the use of the methods is quite valid, it is not enough for complete identification of a possible teratogenic effect. For example, it does not give proper consideration to the state of the heart, although disturbed development of it in uterus is known to constitute the most common pathology. Greater information is provided by Staple's method of surveying foetuses macroscopically (Staples, 1975).

Study of progeny. It is widely known that developmental defects and functional inadequacies of various organs and systems often do not become obvious until after birth. This makes it essential to monitor the post-natal development of the offspring of animals exposed to industrial toxics throughout pregnancy or during some of its periods (Sanotsky, 1956; Dyban, 1968).

For example, analysis of embryonic material from the females exposed to an 0.6 mg/m^3 chloroprene concentration revealed no changes in total embryonic mortality that is the most sensitive index according to our data. On the other hand, observations of the offspring showed a significantly higher rate of post-natal deaths in this animal group. Similar data were obtained from estimating the body weight increment among newborn animals. The foetuses' weight was no different from that of the controls, though there was a decrease in weight

Table 27

A Study of Six-Month Old Progeny of VC-Exposed Rat Females

Indices	Concentration			
	MAC		1/10 MAC	
Sex	♂	♀	♂	♀
Hole reflex	0	0	0	0
Orientation response		0	0	0
Summated threshold index (STI)	—	0	0	0
Rectal temperature	0	0	0	0
Hippuric acid, mg/ml	0	+	+	+
Hexenal-induced sleep, min	0	+	0	0
Peripheral blood				
Haemoglobin	—	0	—	0
Erythrocytes, mln.	0	0	0	—
Leukocytes, thous.	0	—	—	—
Mass coefficients				
Heart	—	—	0	0
Lungs	0	0	0	+
Liver	—	0	—	0
Kidneys	—	0	—	+
Spleen	—	0	+	0

Symbols: + increase; – decrease; 0 — no effect.

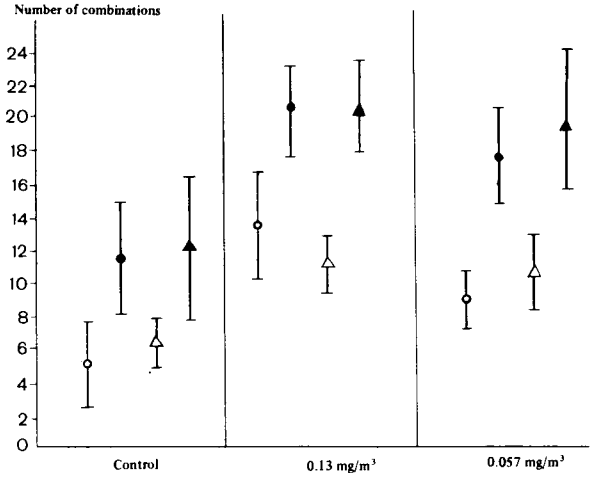


Fig. 25. Impaired elaboration of conditioned reflex to light and the sound of a bell in the offspring of chloroprene-exposed albino rats.
Circles — to the sound of a bell; triangle — to light; blank — initiation; black — reinforcement

increment in offspring at two-months of age (another factor to consider in such instances is the "lactation factor" — Sanotsky, 1950; Sanotsky, Avkhimenko, Fomenko, 1970).

Post-natal death was the most significant index in a survey of the progeny of rat females exposed to a 600 mg/m^3 concentration of dimethylformamide (an above-Lim_{ch} order), deaths accounting for 44.0±11.6 per cent against 14.1±4.7 per cent in the control.

Latent functional alterations in progeny can be discovered by a variety of functional loads. The Quick-Pytel test, when used to investigate impairment of liver function, demonstrated a drop in the hippuric acid concentration in the urine following a sodium benzoate load on the progeny of female rats exposed to the chloroprene at a concentration level equal to its former MAC of 3.6 mg/m^3. While there were no changes in the liver mass coefficients, this index did alter in the test females' offspring ($P < 0.001$] in response to an additional chloroprene load, compared with the control after a similar load).

The influence of vinyl chloride on embryogenesis was explored by Sal'nikova (1977) in a study involving its MAC-level concentration of 35 mg/m^3 or lower (4.6 mg/m^3). The VC impact was ascertained only during a survey of the progeny in the post-natal period (Table 27). Such an extreme burden as infection with cowpox virus induced a substantial decrease in the resistance of the offspring at six months of age, obtained from females exposed to vinyl chloride (35 and 4.6 mg/m^3) during pregnancy (1 through 20 days).

A survey of offspring in post-natal periods should encompass all the bodily functions. It is essential to examine the performance of the cardiovascular system, the nervous system (behavioural responses, state of conditioned activity, "memory", etc.) and sexual system (spermatogenesis and ovogenesis, fertility, etc.). Special emphasis, however, should be placed on the state of that system most selectively affected in the mother, i.e., dependence of the impact upon the mode of action of the toxic chemical (Pavlova, 1976). For example, a study of the progeny of females that had experienced chloroprene exposure during pregnancy revealed retarded development of reflexes to a strong and weak conditioned stimulus (a bell's sound and light) (Fig. 25). A VC-induced variation of motor activity is illustrated in Table 28.

Table 28

Induced Motor Activity in Progeny of VC-Exposed Rat Females

Concentration, mg/m^3	Length of time animals stayed on vertical string			
	n	3—4 days	n	9—11 days
Control	54	10.0±0.71	48	23.8±3.88
4.8±0.5	57	7.4±0.71*	32	20.6±3.20
35.3±3.5	23	12.0±1.4	16	98.3±8.49*

* $p < 0.05$.

Study of compensatory responses of the maternal organism and foetus

During pregnancy, the female organism undergoes a restructuring of her adaptive and compensatory-protective mechanisms so as in order to provide optimal nourishment for foetal development (Filimonov and Finikova, 1975).

A review of the literature (Beskov, 1972; Filimonov and Finikova, 1975; Persianinov, 1963; Lees et al., 1971; Winner et al., 1966, and others) indicates that, during pregnancy, the adaptive protective mechanism consists of three basic, interrelated systems: 1. the object of nourishment, the foetus, having a foetoplacental system of life support; 2. regional system of neurohumoural regulation, in while uterineplacental circulation and the contractive function of the uterus are essential effector elements; 3. a basic system of life support holding the two systems together, which comprises the maternal organism in its entirety. Homeostasis and dynamic balance of the maternal — foetal complex with environmental factors are effected by the system's physiological functions. Variation of general hemodynamics in the pregnant organism provides an essential mechanism for adaptation and compensation in the basic system. The contractive performance of the uterus is assigned a leading position in the study of the compensatory-protective mechanisms of the maternal organism in a diseased state (hemorrhage during labour or chemical exposure). Owing to the isometric contractions of the myometrium upon administration of various drugs, the uterine-placental circulation is likely to be upset by compromised venous outflow, blood filling, elevated intra-amniotic pressure, etc. Disturbances may also occur in the response of the maternal cardiovascular system.

Bearing this in mind, V. G. Filimonov (Filimonov and Kobozeva, 1967, 1969; Filimonov et al., 1972) proposed that the research methods of the compensatory-protective mechanisms be extended to investigations into several functions of the maternal body and foetus capable of inducing mobilization of the compensatory-protective responses. The functions are associated with a diseased state of the mother, application of exposure loads that enhance uterine contractions, and extreme states such as acute and subacute asphyxia of pregnant animals.

While experimenting with pregnant animals, their respiration rate and pattern, ECG, arterial pressure, electrohisterogram, intrauterine pressure, rheopletismogram of the uterine horn, oxygen dynamics of the myometrium and skeletal muscle are picked up by electrodes and recorded by instruments like a polygraph or an electroencephalograph. The functional state of the foetus in utero is determined from the foetal ECG, cardiotachogram, and length of "persistance" of the foetal heart biopotentials in extreme states, e.g., acute asphyxia. The specific pregnancy period must be carefully considered and all the functions just mentioned investigated in dynamics against that period. This will thus provide an insight into the mechanisms whereby toxic substances

influence the maternal organism and foetus, with particular reference to their direct or indirect effect on the foetus, and help develop effective prophylactic and therapeutic measures to counteract different forms of pathology during pregnancy and birth.

Investigations into the state of the compensatory-protective mechanisms, maternal as well as foetal, after drug exposure or in a pathologically occurring pregnancy, are central to a large number of studies. Filimonov and associated (1975) and Bials and co-workers (1972) looked at acute and subacute asphyxia in pregnant animals during experiments on rats and rabbits. They showed that the foetal cardiac electrical activity lasts longer with the increasing duration of the pregnancy and reaches its maximum late in gestation. This suggests that the adaptive-protective mechanisms of the body are brought into play to increase its resistance to the effect of pathogenic factors. The same authors found that the intensity of uterine contractions sustains the functioning of the so-called peripheral heart, since the foetus is tolerant to hypoxia. It is a long time before the reduction of gas exchanges developing in the maternal organism begins to tell on foetal cardiac functions.

Filimonov and Finikova (1975) used stimulation of the uterus in animal experiments (rats, rabbits, and sheep) by substances like oxytocin, prostaglandin and methylergometryn, to encourage intensified contractions of it in order to aggravate the pathological alterations in the foetus ensuing from acute blood loss. On the other hand, the cessation of the uterine contractive activity, as in acute asphyxia, combined with a change in the uterine-placental circulation, contributed to restoration of the functional normalcy of the foetus' cardiovascular system.

There are only a few studies on the impact of industrial toxic chemicals on the state of maternal and foetal compensatory mechanisms. One such is joint research by Sheveleva and Filimonov (1974) into some mechanisms involved in the embryotropic effect of dimethylformamide (DMFA) by inhalation exposure to a concentration of 400 ± 28 mg/m^3, the minimum effective dose in a chronic experiment. They established that the time of an intact cardiac function in the foetuses of test rats during middle and late pregnancy decreased when the pregnant rat developed acute asphyxia. Among the control animals' foetuses it extended with the inereasing time of pregnancy (Table 29). The functional state of the uterus in the pregnant animals appeared to be unaffected. The evidence discovered to date suggests a direct injurious action of DMFA on the foetus (Sheveleva and Osina, 1973).

Similar investigations by Sheveleva (1976) involved rat poisoning by the industrial toxic known as tertiary butyl hydroperoxide (TBHP) from 1 through 20 days' gestation time. They showed that the heart contraction-rate in 20-day foetuses of pregnant rats exposed to a TBHP concentration of 226 ± 28 mg/m^3 (Lim_{ir}) was higher than that of the control group 5 and 10 minutes after an oxytocin injection. With a toxic concentration of 2 ± 1.2 mg/m^3, a subthreshold dose according to the

indices of systemic toxic effect, similar changes were seen only 5 minutes after asphyxia. Toward the end of the experiment, the cardiac contraction rate did not diverge from the control and neither were any changes observed in the contractive behaviour of the uterus.

Complementary research techniques. Studies published in recent years have suggested new methodologies in addition to the common methods of teratogenicity assessment (Kriiger, 1972). The use of the tissue culture is being explored as a possible way of elucidating the action of mechanism drugs in toxicological and teratological studies. Use of the post-implantation mammalian embryo culture is suggested as a means for evaluating metabolic responses to potential teratogens, even when the life and development of the embryonic culture is too short for observing malformations. Finally, there is a simple method for culturing during early organogenesis (Kochhar, 1975).

Method of culture. Pregnant DBA/2 mice were sacrificed at 12 days' gestation. The embryos were extracted from the uterus and freed from the envelopes in a sterile Ringer solution. Following estimation of somites, the embryos' fore and hind extremities were removed. The cultured extremities were from embryos with 34 to 42 pairs of somites, 75 per cent of the cultures coming from embryos with 36 to 40 somite pairs. The extremities were then thoroughly treated. The culture can then continue to grow until 40 to 45 somites are formed. The period covers the initial and early development of primordial extremities. The method requires special glassware and equipment to provide the effective nutrient and gaseous medium required for the growth of the culture. The procedure serves to monitor metabolic processes in the embryos within the first 24 hours of culturing.

Another noteworthy aspect in the employment of organ cultures is their preservation for a longer period (many weeks) sufficient for the development and differentiation of morphological and biochemical responses to the impact of teratogens. The point is that these functions are similar to those observed in vivo. Besides, this organ culture application is helpful in studying cell division, interaction and death, and also morphogenesis. The conclusion thus drawn is that it is possible to extrapolate data on a certain (notably one) organ culture to the whole organism. The method has much in common with the above-noted one of embryo culturing at the extremity laying-down stage.

Table 29

Contraction Rate of Foetal Hearts Following DMFA Exposure of the Mother

Group	Duration of exposure, days		
	8—14	15—20	1—20
Control	26±3.1	59.3±2.8	66.0±3.0
DMFA 400±28 mg/m^3	31±2.5	34±3.1 **	37.0±2.0 *

* $p<0.001$; ** $p<0.02$.

Kochhar (1975) suggests that model experiments (in vitro) are more sensitive to the effect of teratogens than in vivo experiments.

The methods developed in A. P. Dyban's laboratory for culturing fertilized ova, are thought to be more suitable and promising by a number of authors. It has been proposed that the serum of workers exposed to occupational chemical factors be used as the nutritive medium. The method needs to be tested for several industrial toxic chemicals and there is need for further research to specify the degree of reproducibility of the results and assess the possible impact of the "incompatibility factor" (human serum — rodent embryos).

An important new trend in experimental research deserves mention. Its purpose is to keep down the teratogenic activity of environmental factors with the aid of anti-teratogens or specially derived protective chemicals (Hood and Pike, 1972; Leller and Ivankovic, 1973). Yet the method is unlikely to find industrial and environmental hygiene applications.

SENSITIVITY OF PREGNANT AND NON-PREGNANT ORGANISMS TO CHEMICAL EXPOSURE

Pregnancy-related changes, functional and morphological, are known to modify bodily reactivity dramatically (Bodyazhina, 1963) and are thus likely to affect the response to chemical exposures. Yet the literature on the comparative sensitivity of the pregnant and non-pregnant organism is quite limited and controversial.

From experimental comparisons of pregnant and non-pregnant animal sensitivities to industrial chemical products, we ourselves have time and again been convinced that sensitivity of the body to a particular toxic chemical may rise and fall.

By way of example, we shall presently review data derived jointly with L. S. Sal'nikova, G. A. Sheveleva, M. V. Nakoryakova, G. E. Pavlova, and O. V. Sivochalova from studies of industrial chemicals; namely, chloroprene, dimethylformamide (DMFA), dimethylacetamide (DMAA), formaldehyde, cyclohexanone, hydrogen chloride, and tetracycline. The compounds are common in industries using female labour. All experiments were conducted on albino rat females weighing 200 ± 23 g. Inhalation exposure to the substances, the most common occupational mode, was researched in chambers with a dynamic supply of the chemical tested. The control animals were kept under similar conditions, with a dynamic supply of clean air. The magnitude of daily exposure, total duration, time and level of toxic impact, as well as the actual concentrations of the test toxics, are shown in Table 30.

For exposure to hydrogen chloride vapours, the sensitivities of pregnant nine days' gestation and non-pregnant animals were compared in terms of the "survival" index after a single exposure. For the other poisons, their effect was investigated by repeated exposure over 20 days, amounting actually to a total gestation period for the preg-

nant animals, and estimated in terms of systemic toxic effect. In these experiments, animals were examined on the 16th to 20th day of exposure. Integral indices characteristic of the chemicals tested were used and the statistical groups were composed of 8 to 32 animals.

Table 30

General Diagram of Experiments

Chemical	Concentration, mg/m³	Dose level	Length of exposure, hours	Duration of exposure, days
Hydrogen chloride	300—600	CL84	1	1
Formaldehyde	6.0±0.3	MAC×12	4	20
	0.4±0.02	MAC	4	
DMFA (amide of formic acid dimethylated under nitrogen)	10.7±1.03	MAC	4	20
	2.3±0.3	1/4 MAC	4	20
Cyclohexane	105.2±9.1	MAC×10	4	20
	11.5±2.9	MAC	4	
Chloroprene (Chlorobutadiene)	4±0.7	MAC×2	4	20
	0.6±0.08	1/3 MAC	4	
DMAA (amide of acetic acid)	300±25	$\sim Lim_{ac}$	4	20
	50±2.4	$Lim_{ch}\times 5$	4	
Tetracycline (polyfunctional hydronaphthacene compound)	1.0±0.06	Lim_{ch}	4	20
	0.18±0.01	MAC	4	

Hydrogen chloride. The research revealed the following:

According to the "survival" test the non-pregnant rats were more susceptible to the effect of hydrogen chloride vapour in lethal concentration (300 to 600 mg/m³) over a one-hour exposure. There were seven deaths out of the 32 non-pregnant females. In contrast, none of the pregnant rats died under similar conditions.

Formaldehyde. The non-pregnant animals proved more sensitive to formaldehyde exposure.

At a concentration of 6 mg/m³ or 12 MACs, formaldehyde changed the functional state of the kidneys in the non-pregnant rats by decreasing daily diuresis (4.8±0.5 ml/day against 6.9±0.6 ml/day in the control, $p < 0.05$) and chlorides (0.99±0.6 g/l from the control 1.33±0.13 g/l, $p < 0.05$) and increasing protein in the urine (1.9±0.2 g/l from 1.17±0.17 g/l in the control, $p < 0.05$) and the liver (reduced urinary excretion of hippuric acid following a sodium benzoate load of 106.2±18.8 mg/day, from 143.4±11.9 mg/day in the control, $p < 0.05$). Under the same conditions, the pregnant rats exhibited a change in only one index, a reduced level of blood haemoglobin (115±4 g/l against 133±5 g/l in the control, $p < 0.02$).

The subthreshold concentrations of the chemical proved equal according to the index of systemic toxic effect, reaching a level of 0.4 mg/m³ for both pregnant and non-pregnant rats.

Dimethylformamide. Among the non-pregnant rats, a DMFA concentration of 10.7 mg/m³ was responsible for disfunction of the

kidneys (an increase in the amount of chlorides in the urine to 1.25±0.14 g/l from 0.75±0.11 g/l in the control, $p<0.02$) and the liver (reduced urinary excretion of hippuric acid to 46.3± ±7.1 mg/day from 63.6±2.3 mg/day in the control, $p<0.05$). The pregnant rats had a reduced urinary excretion of hippuric acid (47.3±5.4 mg/day from 79.2±6.9 mg/day in the control, $p<0.01$).

Given a toxic concentration of 2.3 mg/m^3, the kidney disfunction persisted in the non-pregnant animals (chlorides in the urine equal to 1.24±0.18 g/l and to 0.75±0.11 g/l in the control, $p<0.05$) while the pregnant rats showed no detectable changes.

Cyclohexanone. After cyclohexanone exposure, the pregnants and non-pregnants exhibited an almost equal sensitivity to a concentration of 105.2 mg/m^3. The pregnant rats developed kidney disfunction (a reduced urinary chloride content of 0.69±0.2 g/l against 1.69±0.24 g/l in the control, $p<0.01$) and had a greater proportion of erythrocytes in the peripheral blood. Liver disfunction was registered also for the non-pregnant rats (a reduced protein content in the urine at 1.0±0.15 g/l against the control 2.1±0.3 g/l, $p<0.001$) on top of increased daily diuresis. To a concentration of 11.5 mg/m^3, the pregnant animals were somewhat more sensitive than the non-pregnant, revealing a decrease in the urinary chloride content to 0.97±0.1 g/l from the control 1.69±0.24 g/l, $p<0.02$) and of body weight increment. The non-pregnant ones proved no different from the controls.

Chloroprene. Chloroprene intoxication during pregnancy increased the sensitivity of the pregnant animals substantially compared with the non-pregnant ones.

Exposed to a 4 mg/m^3 chloroprene dose, the pregnant rats developed changes of several integral indices like body weight increment, oxygen consumption, spontaneous motor activity (SMA) and functional state of the liver (a rise in the content of hippuric acid in the urine to 103.11 mg/day, up from the control 68.0±3.6 mg/day, $p<0.01$). In contrast, SMA was the only index to change in the non-pregnant rats after a 20-day exposure. Additionally, the pregnant rats developed hypoproteinemia in response to a 0.6 mg/m^3 dose, whereas no alterations were observed in the non-pregnant females.

Dimethylacetamide. An examination of the effects of dimethylacetamide vapour in a concentration of 300 mg/m^3 in the pregnant animals showed increase levels of leukocytes in the blood and an impaired function of the kidneys (a reduced protein content in the urine of 3.8±0.6 g/l against 7.1±0.6 g/l in the control, $p<0.01$) and the liver (a lowered concentration of cholic acid in the bile, at 45.5±4.0 g/l against 698±50 g/l in the control, $p<0.001$), and enhanced spontaneous motor activity. In the non-pregnant rats, the content of protein in the urine was down to 2.6±0.4 g/l from the control 5.3±0.3, $p<0.02$. The DMAA concentration of 50 mg/m^3 caused SMA to rise and the urinary

protein concentration to drop to 2.9±0.4 g/l from 7.1±0.6 g/l in the control, $p<0.002$. The state of the non-pregnant females did not diverge from the control.

Tetracycline. Upon analysis of response to tetracycline exposure throughout pregnancy, the pregnant animals displayed a greater sensitivity than the non-pregnant ones. The effect of tetracycline at 1.0 mg/m³ on the pregnant animals increased their threshold of neuromuscular excitability (to 8.8±0.41 from the control 7.19±0.31, $p<0.001$); modified the functions of the kidneys (a greater proportion of urine, protein and chlorides) and the liver (decreased indices of the bromsulfaleic assay); and raised the mass coefficients of the liver and thyroid gland.

Tetracycline toxicity in pregnant rats was confirmed by pathomorphologic analysis of their internal organs, revealing heavy focal adiposity of hepatic cells, and the appearance of separate foci of necrosis at the Lim_{ch} concentration of the toxic.

Systemic toxic effect of the antibiotic dose of 0.18 mg/m³, or the MAC level, brought about a reduction in the excretory function of the liver (48.64±3.3 units of bromsulfalein, with $p<0.001$, against 70.9±2.02 units in the control) but caused no observable changes in any of the test indices among the non-pregnant rats.

Table 31

Comparative Sensitivity of Pregnant and Non-Pregnant Albino Rat Females to the Effect of Industrial Toxic Chemicals

Chemical	Concentration, mg/m³	Number of indices used	Number of modified indices	
			pregnant	non-pregnant
Hydrogen chloride	300—600	1	1	0
Formaldehyde	6.0±0.3	15	4	1
	0.4±0.02	15	0	0
Dimethyl formamide	10.7±1.03	10	2	1
	2.3±0.3	10	1	0
Cyclohexanone	105.2±9.1	13	1	2
	11.5±2.3	13	0	2
Chloroprene	4±0.7	13	1	4
	0.6±0.08	13	0	1
Dimethyl acetamide	300±25	12	1	4
	50±2.4	12	0	2
Tetracycline	1.0±0.06	25	—	8
	0.18±0.01		0	2

Thus, the research findings indicate that pregnancy, as a burden, can alter the body's tolerance to the impact of various toxic agents, towards both a greater or a lower resistance (see Table 25). Sensitivity variation in the animal's body exposed to a chemical agent often depends on the tested dose or concentration. It may be concluded from the foregoing that as concerns *a* chemical stu-

died in a suitable chronic experiment, it is far from always possible to rely on the unqualified use of these data as a key to the basic question in the validation of prophylactic measures: whether the embryotropic effect is, indeed, selective (Sanotsky and Fomenko, 1974). So, in studying chemical compounds for potential embryotropic action it is necessary to explore, in parallel, the responses of both pregnant and non-pregnant maternal bodies.

Conclusion. The methodological issues that bear on experimental research into the embryotropic effects of industrial toxic chemicals are now, on the whole, quite well developed. The use of Diagram 1 above has enabled the specificity (selectivity) of action to be assessed for several dozens of chemical compounds, thus establishing the threshold levels of exposure needed for the sanitary standardization of their concentrations in the air of a working zone.

Effective work towards the same objective is being done in the area of pesticide toxicology at the All-Union Research Institute for the Hygiene and Toxicology of Pesticides, Polymers and Plastics, USSR Ministry of Public Health; at the F. F. Erisman Institute of Hygiene, Moscow; A. I. Sysin Institute of General and Communal Hygiene, and other research centers.

Alongside this, it ought to be recognized that improvement of the ability to predict embryotropic hazards in experiments, particularly the transfer of animal data to humans, is still a very urgent task.

Chapter 5

MUTAGENIC EFFECT OF CHEMICALS

CLINICAL OBSERVATIONS

Mutations in sex cells. One consequence of chemical mutagenicity in the germ cells is the production of genetically abnormal gametes, resulting in the death of zygotes, embryos and foetuses and the birth of weak infants with developmental malformations (which may run in generations) and inheritable diseases (Bochkov et al., 1968, 1970; Bochkov, 1969, 1971, 1972; Kantorovich, 1976, Roberts et al., 1968 and others).

Observations of this kind are quite rare in clinical hygienic assessments of the occupational hazards posed by chemical processes and production units. Soviet toxicological literature of the last few years, with gynaecologists actively involved in dealing with the problems of female occupational health, has brought out some reports on the impact of factors in the working environment upon foetal development in the women workers exposed at child-bearing age to industrial chemicals. The data were discussed at conferences on the hygiene of female labour and incorporated in collections of scientific papers on the issue (e. g., the collections edited by Prof. R. A. Malysheva, 1974; or by Z. A. Volkova, 1976).

In evaluating published research results, it is difficult, however, to find out for sure whether a detected pathology results from exposure to a set of factors acting on the female organism before pregnancy or from the manifestation of the embryotropic effect (see Chapter 4). More reliable in this respect are data obtained from examination of male workers whose wives have no occupational contact with active chemical compounds. Such observations are very few. Thus, Kilian (1969) reported a rise in the number of still births and abortions among the wives of the workers occupationally exposed to ethylenimine. Similar data were derived by these authors (together with R. M. Davtyan) from a survey of male workers employed at a chloroprene plant. A questionnaire survey has established a higher rate of spontaneous abortions and still births in the workers' wives, compared with data for a control group of motor works employees. The data are summarized in Table 14.

Analysis of the questionnaires that describe the state of nervous, psychic and physical development, as well as the general health of the offspring, focused on frequent complaints from the experimental group workers about retarded early development, poor performance

at school, irritability, nervousness, and higher-than-normal susceptibility to diseases among their children.

Also noted during the survey was the birth of five mentally-retarded children having oligophrenia to five workers from the experimental group (clinically confirmed). Births of infants with signs of deviations at an early age or with psychic deficiency were found to have occurred at a spermatologically quite stable age of the workers themselves and a relatively young age of their wives, under 35 years old.

Despite the undeniably subjective nature of the questionnaire method of research, such data is definitely required, for guidance if nothing else, in order to evaluate the mutagenic safety of the chemicals extensively used in industry (in particular, during the clinical hygienic testing of MACs). It should be admitted, however, that the procedures for gathering such information have not yet been sufficiently elaborated.

Cytogenetic analysis of somatic human cells. It is generally believed that mutations in somatic cells lead to their death or functional alterations. The latter may well be the cause of birth defects that are either compatible or incompatible with life if the mutations have arisen during embryogenesis; or induce diseases in the individual in whose somatic cells the adverse mutations have emerged and become established (Bochkov et al., 1968, 1972; Prokofieva-Bel'govskaya, 1969; Bochkov, 1971, 1972; Zhurkov and Yakovenko, 1977).

Cytogenetic analysis of peripheral blood lymphocytes in humans who have been exposed to industrial toxics furnishes rapid and comparatively lucid information about the mutagenic effect of tested toxic chemicals on human beings.

The principle of blood culturing is based on the addition of phytohemagglutinine (PHA) to the leukocytesnutrient medium, causing their conversion into blastocyte-like cells with a high mitotic activity. Subsequent introduction of colchicine to the culture arrests mythoses at the metaphase stage. Then, with a hypotonic solution added to the medium, the cells swell and the chromosomes separate off from one another. At the metaphase stage, chromosomes appear as clear-cut structures, thus enabling easy detection of changes in the karyotype (the number of chromosomes in the nucleus) and of chromosome aberrations.

The classification of structural chromosome injuries proposed by Bochkov (1974) identifies the following types of aberration:

1. Aberrations of chromosome type: a. paired acentric fragments; b. point fragments (interstitial deletions); c. acentric rings; d. annular chromosomes (centric rings); e. chromosomes with more than one centromere (dicentric, tricentric, etc.); f. chromosomes abnormal for a given karyotype (in length, or in the arm ratio).

2. Aberrations of chromatid type: a. solitary fragments; b. exchanges of chromatid origin: asymmetric chromatid trans-

locations, symmetric exchanges between chromatids belonging to the group of quadriradicals; c. breaks at the centromere to form two telocentric chromosomes.

Cytogenetic analysis has to consider also achromatic injuries — chromatid and isochromatid gaps, or impaired integrity of one or both chromatids not accompanied by a shift in the fragments.

The nature of the gaps is conceived by researchers in a variety of ways. Some do not think of them as true aberrations (Revell, 1959), others (Evans, 1966) consider the gaps as potential injuries that have not actually emerged because they did not undergo reparation processes. If one assumes the existance of subchromatid exchanges to be real, the gaps can be regarded as fragmentation of subchromatids (Luchnik, 1973). In view of this, the gaps have to be analyzed separately.

Current literature offers quite convincing data, gained from cytogenetic analysis of peripheral-blood (less often of bone-marrow) lymphocytes, suggesting a mutagenic hazard for humans from occupational chemical factors.

Benzene. Chromosome injuries in peripheral-blood lymphocytes were noted in benzene-exposed workers (Tough and Court-Brown, 1965; Liniecki Tulian et al., 1971). Nevertheless, eight workers affected by benzene intoxication showed no detectable increase in structural chromosome lesions in their bone marrow cells, but did have cell aneuploidy (Sokolov et al., 1972).

Carbamates. Ziram and zineb inducted chromosome aberrations in the blood lymphocytes of production workers (Pilinskaya, 1971).

Arsenic. Cytogenetic disturbances in blood lymphocytes were identified in 13 workers with chronic arsenic poisoning (Peters et al., 1970).

Carbon disulfide. An increased rate of chromosome aberrations was observed in seven workers chronically exposed to carbon disulfide (Sokolov, et al., 1972).

Lead. The literature contains conflicting data on the influence of lead in the working environment on human somatic cells. A karyological survey of lead production workers reported a rise in the mitotic index and the number of mitoses with secondary chromosome aberrations (Schwanitz et al., 1970). An examination of bone-marrow cells in workers whose job is in some way associated with lead exposure showed an expansion of aneuploid cells due to hypoploidy [1], but failed to uncover chromosome injuries (Sokolov et al., 1972).

Ethylenimine. A cytogenetic blood analysis performed on 158 workers at an ethylenimine production plant (Kilian, 1969; cited from Sanders, 1969) revealed a greater frequency of chromosome disturbances, but they did not differ in a statistically significant manner from the chosen control group.

[1] Whether the effect is relevant to the assessment of mutagenic effect, is debatable.

Epichlorhydrin. A rise in chromosome aberrations in people occupationally exposed to epichlorhydrin has been documented by several researchers (Gromykhina et al., 1975; Kucerova, Zhurkov et al., 1977).

Evaluation of research results. Some studies cannot be accepted without reservation. For example, Gath and Thiess (1972) have not discovered any major influence on the pattern and frequency of aberrations in occupationally exposed workers, brought about by substances like benzene, ethylenimine and dimethylsulfate. Yet the comparatively high level of chromosome aberrations among the people in the control group (compared with data from other authors) casts doubts on their research results and does not permit an unqualified statement to be made on the safety of the chemicals for humans.

A critical condition for such studies is adequate knowledge of the key patterns of spontaneous mutagenesis. The Institute of Medical Genetics of the USSR Academy of Medical Sciencies has issued a methodological guide (1974) with a summary of the evidence obtained by the Institute's researchers from a cytogenetic analysis of 437 persons of different age and sex. Comparison of chromosome aberration frequencies among people of different sex and age has revealed no major differences between these groups, i. e., the rate of spontaneous chromosome aberrations will vary within the same range for males and females aged 0 to 70 years. For a complete evaluation of the chromosome aberration rate in the subject group, the number of individuals necessary for adequate research is decided by the size of the study group (number of employees at a plant, group of patients, etc.). On the basis of the computations that have been made, the recommended group size according to the numbers of individuals selected for this kind of research is as follows: 7 for a group of 10, 16 for a group of 50, 19 for a group of 100, and 23 for a group of 500 or more.

These authors collaborated with L. D. Katosova and G. I. Pavlenko on a cytogenetic survey (1976) of workers occupationally exposed to chloroprene, chloroprene latex, vinyl chloride, ethylene oxide, dimethyl acetamide and some repelants. These compounds, as also the articles made from them, are widely used in industry and in the home, meaning that large numbers of people are exposed to them in one way or another (Table 32).

The cytogenetic analysis was carried out in accordance with the requirements set forth in the methodological procedures recommended by the Institute of Medical Genetics. The cytogenetic effect was evaluated by comparison with both the data for the control group and the spontaneous level of the index being examined (Bochkov et al., 1974).

Chloroprene. While looking for a possible mutagenic effect of chloroprene, we examined 46 workers from three groups of production shops at two plants.

The first group was made up of 18 employees, 13 males and

5 females, from a shop producing chloroprene and making latex and rubber from it. The second group included 20 women workers handling chloroprene latex. The third group consisted of eight women packaging chloroprene latex products. The values of the airborne chloroprene concentrations in the working zone of the production shops are given in Table 17. Finally, the control group consisted of nine clinically healthy persons not occupationally exposed to any chemical hazards. The everage age of the workers examined in each group had no statistically significant difference from the controls.

The findings from the cytogenetic analysis of lymphocytes in the blood of the chloroprene workers pointed to an excess of aberrant cells in all three test groups over their level in the control group. It has not been possible to find out for any of the groups whether the cytogenetic effect was dependent on the workers' sex, age and duration of employment (see Chapter 2). When the effects on the three groups were compared in terms of magnitude, it turned out that the number of aberrant cells in the first group of those employed in chloroprene and chloroprene latex manufacture was statistically significant ($p<0.05$] and different from that in the third group.

Table 32

Cytogenetic Analysis of Peripheral-Blood Lymphocytes in People Exposed to Some Chemical Compounds

Chemicals	Groups	Concentrations, mg/m³	Number of workers surveyed	Number of metaphases	Number of aberrant cells, %%
Chloroprene [1]	First	45—2.7	18	1,666	4.77±0.57 *,**
	Second	7.0—3.0	20	1,748	3.49±0.51 *,**
	Third	1—4	8	648	2.5±0.49 *,**
	Control	—	9	572	0.65±0.56 *,**
Vinyl chloride [1]	Test	111—1.8	37	3,135	2.76±0.24 *,**
	Control	—	12	1,041	1.62±0.39
Dimethyl acetamide [1]	Test	5—10	20	1,673	4.9±0.5 *
	Control		16	1,395	3.7±0.36 **
Ethylene [1] oxide	Test	10.0—20.0	10	639	2.2±0.57 *,**
	Control		8	529	1.19±0.29 *,**
Dimethyl phthalate [2]	Test	Skin application for one month	17	1,646	1.9±0.42
	Control		10	926	1.56±0.28 *,**
—	Total control	—	45	4,463	1.25±0.38
—	Spontaneous level [3]	—	437	60,020	1.19±0.06

Notes: Statistical significance: * — compared to control;
** — compared to spontaneous level; $p<0.05$.
[1] Workers (for an explanation, see text);
[2] Volunteers (see text);
[3] The data of Bochkov et al., (1974).

As indicated by Table 32, the women workers in third group, packagers of chloroprene latex products, are exposed to lower chloroprene concentrations than those in the first group. The effect/concentration dependence thus identified may, however, be a random one, because the women workers in the second group experienced the same concentration exposures as those in the third, while the number of their aberrant cells did not diverge statistically significantly from that in the first group.

Analysis of the types of aberration found in chloroprene workers' blood revealed both chromosome and chromatide aberrations, but with a significant numerical prevalence of the latter. The aberrations of the chromosome type occurred chiefly in the form of paired fragments. Solitary fragments accounted for the most of the aberrations, — from 68 to 75 per cent in each group.

Vinyl chloride. We have examined 37 workers from two departments in the vinyl chloride polymerization process, to see if vinyl chloride has a cytogenetic effect. The plant's central laboratory records for 1973 and 1974 estimated that airborne vinyl chloride concentrations in the work environment of the "old shop" varied from 111 to 1.8 mg/m^3. The vinyl chloride MAC value of 30 mg/m^3 was exceeded three times or more in 20 per cent of the samples. Vinyl chloride concentrations in the working zone air of the other shop (where an innovative process has been operated since 1970) were found to have been at or below the MAC value during 1973—1974.

The surveyed workers' experience of handling vinyl chloride ranged from 1.5 to 28 years. The controls were 12 persons, four male and eight female residents of the same town, having jobs other than in chemical manufacture. The average age of the workers in the test group had no statistically significant differences from the controls.

A cytogenetic analysis of lymphocyte cultures demonstrated no appreciable differences ($p<0.05$) between the relative number of chromosome aberrations in the "old" shop workers (1.93 ± 0.25 with $n=23$) and those of the innovative process department (2.88 ± 0.57 with $n=14$).

Considering the workers' equal history of occupational exposure to the substance and the absence of any major variations between the effective vinyl chloride concentrations in both shops, the survey data have been combined. The results of analysis of the rate of chromosome injuries in the blood lymphocytes of the workers in the test and control groups are shown in Table 33.

In the experimental group of 37 employees only one worker, with five-years' work experience appeared to have no aberrant cells in the lymphocyte culture of the peripheral blood. Among the other workers examined, the frequency of aberrant metaphase plates varied from 1 to 5.33 per cent. In the experimental group cells with chromosome aberrations averaged 2.76 ± 0.24 per cent.

Within the 12-strong control group, no aberrant cells were found in three persons, 4 per cent in one, and 1 to 3 per cent in the remainder of the group, averaging 1.62 ± 0.39 per cent.

The chromosome injuries found in the experimental group were aberrations of the chromatid type (82.5 per cent), solitary acentric fragments predominating among them (76 per cent). Three chromatid exchanges were discovered, and all aberrations of the chromosome type were paired fragments. In relative numbers, the metaphases with gaps in the test group were no different from the controls, nor was there any association, between the chromosome aberration frequency and employees work experience and age.

To summarize, cytogenetic blood analysis of the chloroprene — and vinyl chloride — exposed workers attests to the possible mutagenicity of the chemicals under study.

Ethylene oxide. As suggested by a cytogenetic survey of employees from an ethylene-oxide production facility, out of ten examined employees only one person, with a two-year job experience, had no aberrant cells in his cultured blood cells. Among the rest, the frequency of metaphase plates with aberrations was above the spontaneous level, and as high as 12,5 per cent in a worker with a seven years service record. In the experimental group, cells with chromosome aberrations averaged 2.22 ± 0.5 (see Table 33). In the 8-strong control group, two showed no aberrant cells in their blood culture, four had one per cent and two, two per cent, giving an average of 1.19 ± 0.29 per cent.

Comparison of the cytogenetic survey findings regarding the workers exposed to ethylene oxide and the control group has revealed no significant disparities between their respective numbers of aberrant cells, but no final answer can be given until additional research is conducted among a larger worker group.

Dimethyl phthalate (DMP). The mutagenicity of DMP-based repellents was investigated in seventeen volunteers with no previous history of known extensive contacts with chemical compounds. The test repellent was applied to the skin for 26 days.

The average age of the subjects in the experimental and control groups was nearly equal, from 19 to 21 years. The findings concerning frequency and type of chromosome lesions in blood lymphocytes are presented in Table 32.

Of the seventeen workers examined in the experimental group, only one lacked aberrant cells in the lymphocyte culture of his peripheral blood; such cells represented 5—6 per cent in three subjects, 2—3 per cent in four, and stayed well within the spontaneous range of chromosome rearrangements in the remainder.

The major type of chromosome injuries were aberrations of the chromated type, 1.20 ± 0.21 per cent, or 60 per cent of the total, heavily dominated by solitary acentric fragments. Four chromatid exchanges were detected, and all aberrations of the chromosome type were paired fragments.

In the control group of 10, aberrant cells were missing in two and accounted for 4 per cent in one, for 2—2.6 per cent in four, and for one per cent in the rest.

Thus, the workers of the experimental and control groups did not diverge from each other as regards the number of cells with aberrations.

Dimethyl acetamide (DMAA). In order to study DMAA mutagenicity, 20 production employees with 2 to 6 years experience of work in the DMAA shop were surveyed doing the following jobs: chemical plant operators, heat treatment plant operators, and regeneration plant operators.

The control group was composed of 16 persons, eight males and eight females, all employeed in overhead departments such as information, personnel, accounting, and design, but working in the same production area. The subjects in the experimental group had no statistically significant variations in average age from the controls.

Data on the frequency and pattern of chromosome injuries in the blood lymphocytes of the subjects and controls are shown in Table 32. All 20 workers in the experimental group exceeded the spontaneous level in their numbers of aberrant cells, with two of them having 10 per cent, three 6—7 per cent, five 5 per cent and the remaining ten 2—4 per cent of chromosome aberrations. Throughout the experimental group, the average of cells with chromosome aberrations was 4.9 ± 0.49 per cent.

Among the sixteen controls, one had 1.43 per cent chromosome aberrations, two had 5 per cent, two 6 per cent and the rest varied from 2 to 4 per cent, or 3.7 ± 0.4 per cent on the average.

Chromatid-type aberrations outnumbered (77 per cent) other chromosome lesions in both groups, acentric fragments being dominant among them. Two chromatid exchanges were found in the test group whose relative number of metaphases with gaps did not diverge from that in the control group. No relationship was traced between the rate of chromosome aberrations and the workers' job experience or age.

Thus, the research findings have established among DMAA production employees a substantial excess of chromosome aberrations in the lymphocyte culture of the peripheral blood (almost four-fold) against the spontaneous level. Yet it is not possible to evaluate the mutagenic hazard of DMAA, because of the high level of chromosome aberrations in the control group, nearly three times their spontaneous level in the blood of healthy individuals. The effect is probably due to other factors or to DMAA acting jointly with other factors present in the surveyed production environment.

The DMAA example suggests the real difficulties in the assessment of a mutagenic risk from one particular job related factor. The magnitude of chromosome aberrations in human blood may well arise for any number of additional reasons unrelated to production (harmful habits, X-raying, past episodes of sometimes

latent infectious diseases, vaccinations, etc.). It would thus be wise, in our opinion, to combine clinical statistical surveys with properly arranged experimental investigations make it possible to discern the effect of a certain factor, establish the dose (concentration)/effect relationship and validate safe levels of the toxic chemical in the environment when it does not seem feasible to exclude it totally from production.

EXPERIMENTAL DATA

An essential point in the experimental evaluation of poisons for mutagenic hazard centres on the proper selection of exposure levels. In this respect, most mutagenicity studies have, indeed, made use of exposure levels of test chemicals high enough to produce common toxic effect. A study of mutagenicity should start with single exposure threshold levels according to integral indices, rather than with lethal or near-lethal levels. To establish the threshold of specific effect in a chronic experiment requires the doses and concentrations of substances to be used that occur at or below the threshold of chronic action by integral indices, sometimes even below the previously established MAC level.

Genetic models and methods. Another challenging issue in the assessment of chemical compounds for mutagenicity in human beings is the selection of a suitable genetic model.

The issue was treated in some detail in Chapters 1 and 2. As discussed earlier, the way to obtain most reliable data on the human mutagenicity of toxic chemicals is to study human cells in vivo and in experiments on the animal models with the closest affinity to man in their patterns of metabolism, protective systems, etc. (WHO, Technical Report No. 282, 1965; Sanotsky et al., 1968; 1970; Fomenko, 1969). A factor to consider is that different animal species and even strains show different sensitivities to mutagens (Malashenko, Surkova, 1973; Frohberg, Bauer, 1973). In the USSR, this has led to the suggestion that a new hybrid strain of mice be used — tetrahybrids, well suited for genetic research because of their remarkable viability and stable indices (high fertility and modest variability in weight and size). Even so, this animal species is not as yet used often enough in the study of chemical mutagenesis.

Wistar albino rats and SHK white mice are still the principal animal species used for the purpose. In the same way, cytogenetic analysis of somatic tissue cells and the method of dominant lethal mutations are still the most widespread tools for mutagenic research into laboratory animals.

Cytogenetic analysis of cells of animal somatic tissue. In animals, cytogenetic analysis is mostly performed on bone-marrow

cells, less often on the cells of a regenerating liver, embryonic tissue and leukocyte culture of the peripheral blood, and only in a few cases on corneal or intestinal epithelium. The bone marrow is capable of high mitotic activity that remains virtually stable throughout the day. The mitotic index amounts to 2.6—1.7 per cent in the bone marrow cells of albino rats (Akifiev, 1966; Fomenko, Katosova, 1968) and to 2.4 per cent (1.9—3.6) in those of white mice (Markaryan, 1966).

Since the bone marrow represents a heterogeneous population of cells, it provides a practical object for exploring the mutagenicity of factors rather than the starting mechanisms of injuries.

Anatelophase analysis of the animal bone marrow usually follows Jope's method (1942), taking into account the following types of chromosome injury (Fig, 26, 27, 28, 29): 1. fragments and rings (solitary and paired, or multiple); 2. bridges (chromatid and chromosome); 3. detached chromosomes or chromatids; 4. chromosome cohesion (considering the current view of this as chromatid aberrations that have not completely come through; their numbers should be computed separately).

The mitotic index is estimated by counting the relative number of prophases, metaphases, anaphases and telophases. The rate of spontaneous chromosome rearrangements in the rat bone marrow detected by anatelophase analysis, is variously estimated by different authors at 1—3 per cent; the cohesion rate at 1—5 per cent, and total injuries at 3.7 to 6.5 per cent (Movchan et al., 1967; Fomenko, Katosova, 1968).

We have analyzed variations in the spontaneous level of chromosome aberrations in the bone-marrow cells of albino rats (at the anatelophase stage) by seasons of the year, only to find no statistically significant distinctions (Table 33).

Table 33

Spontaneous Level of Chromosome Lesions in Bone-Marrow Cells of *Nondescript* Albino Rats

Index		Season				Annual average
		winter	spring	summer	fall	
Chromosome lesions, %%	M	3.44±1.4	3.67±1.3	3.38±1.33	4.7±1.35	3.7±1.4
Mitotic index, %%	M±σ	1.86±0.57	1.58±0.53	1.67±0.70	1.85±0.44	1.72±0.06
Number of animals		106	49	62	43	283

The metaphase analysis (Fig. 30) of animal bone-marrow chromosomes is carried out by Ford and Wollam's method (1963). The pattern of the chromosome rearrangements that the method helps to define has been described above in the section on cytogenetic

Fig. 26—29. Different types of chromosome rearrangement in bone marrow cells

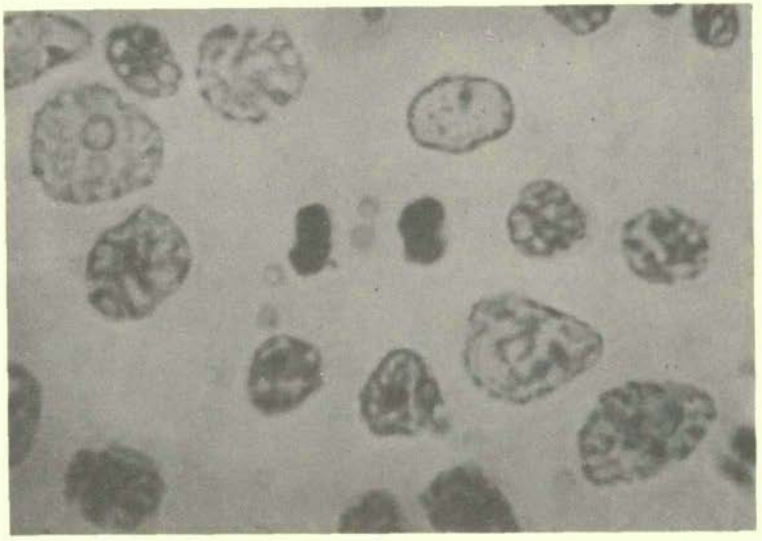


Fig. 26. Normal anatelophase

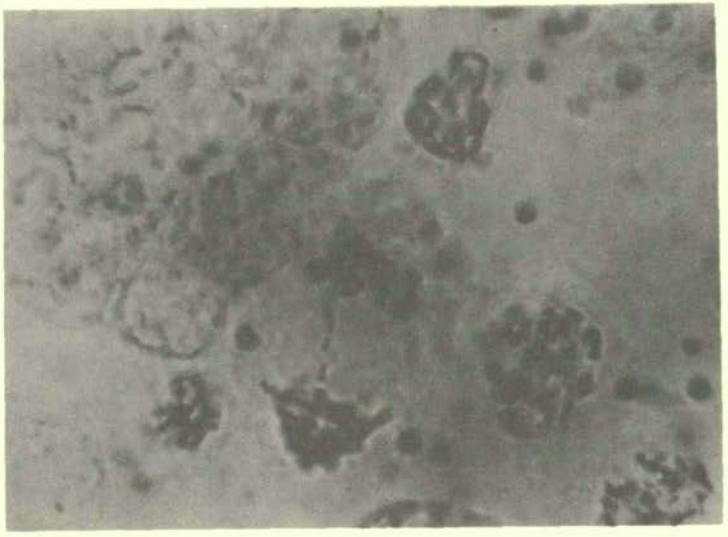


Fig. 27. Fragmentation of bridge

analysis of blood culture. Studies comparing the sensitivities of the anatelophase and metaphase methods are extremely scarce.

Katosova's study (1973) completed under our guidance sought to investigate the cytogenetic effect in mice, induced by chloroprene

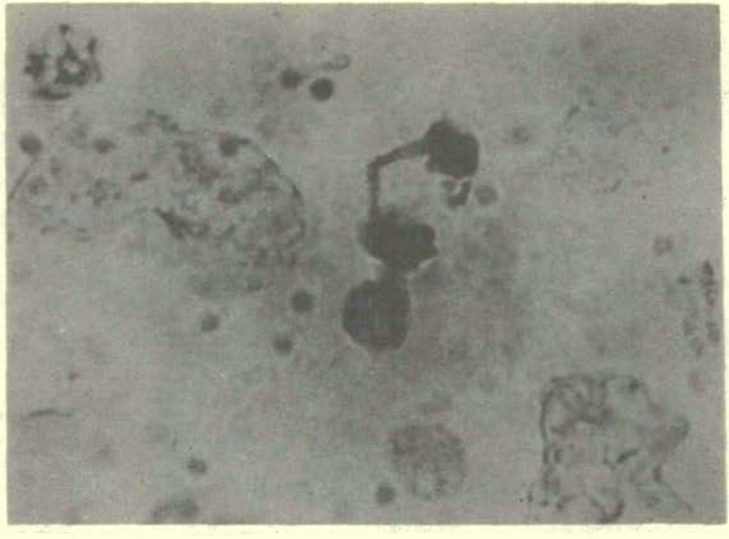


Fig 28. Chromosome bridge, a solitary fragment

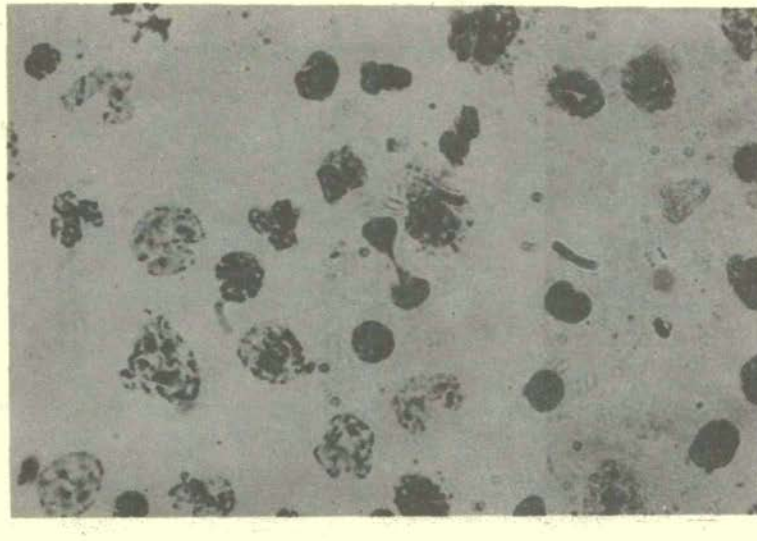


Fig. 29. Chromosome cohesion

inhalation, using both these methods. The percentages of bone-marrow cells with aberrations in mice poisoned by a MAC-level chloroprene concentration of 2 mg/m^3 were: 3.98±0.66 (1st series) and 3.2±0.22 (2nd series) in the case of anatelophase

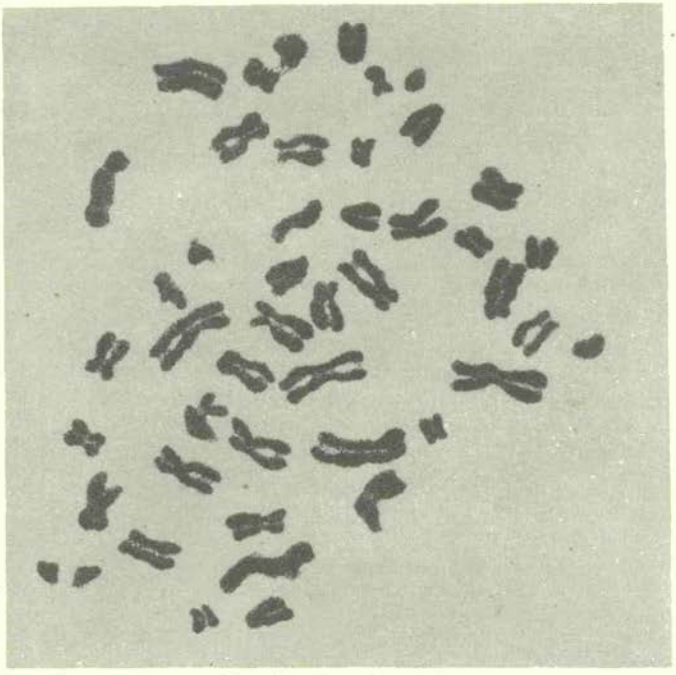


Fig. 30. Metaphase plate

analysis and 10 ± 0.68 and 10.9 ± 1.34, respectively, by metaphase analysis. Thus, at the metaphase stage, cell analysis has made it possible to detect three times the number of aberrations at the anaphase stage. On exposure to chloroprene concentrations of 0.32 ± 0.06 and 0.13 ± 0.01 mg/m^3, the cytogenetic effect was registered only by the metaphase method.

Greater sensitivity in the metaphase analysis of bone-marrow cells was mentioned by Markaryan (1966) and Strekalova (1971). Furthermore, E. E. Strekalova's study on the mutagenic effect of ethylene oxide demonstrated the ability of metaphase analysis to discover a cytogenetic response to exposure concentration levels only a tenth of those discoverable by the anatelophase method. With this in mind, the use of metaphase analysis is desirable for the mutagenic hazard assessment of chemicals based on cytogenetic analysis. Because, however, metaphase analysis of bone marrow cells requires a separate group of animals this may preclude its application in some instances. For this reason, it makes sense to use both methods in the hygienic standardization of mutagenic substances, but to do so with allowance for the different sensitivities of the specific cytogenetic techniques.

Induction of dominant lethal mutations. One method easily applicable for identifying mutations in germ cells during experiments with mammals is to estimate the frequency with which dominant lethal mutations appear in male germ cells on exposure to mutagenic factors exhibited in the first generation. The occurrence of dominant

Table 34

Comparison of Relative Magnitudes of Genetic Effect in Different Biological Specimens

Chemical	Test specimen	Exposure level	Effect (multiplicity of excess over control level)
Formaldehyde	Drosophila melanogaster	$\sim DL_{50}$	2—3
	Microorganisms *	$\sim DL_{50}$	8—10
	Human leukocyte culture in vitro	4—9 mg/l	0(cytostatic)
		0.4 mg/l	0
	Bone marrow in vivo	0.5 mg/m^3	0
Ethylenimine	Drosophila melanogaster *	$\sim DL_{50}$	10
	Human leukocyte culture in vitro	2×10^{-4}M	
		2×10^{-5}M	5—6
	Microorganisms (S. typhimurium TA 1950) in vivo	0.004 mg/kg	5.6
		0.04 mg/kg	10
		0.4 mg/kg	11
		4.0 mg/kg	15
	In vitro without activation	0.02 mg/l	6
		0.2 * mg/l	17
		2.0 mg/l	28
		20 mg/l	300
	In vitro with activation	100 mg/l	800
		0.02 mg/l	8
		0.2 mg/l	10
		2.0 mg/l	11.8
		20.0 mg/l	15
		100 mg/l	28
	Bone marrow in vivo	0.8 mg/m^3 (2 months)	2
		0.6 g/l (8 days)	1.7
	Dominant lethal mutations	0.8 g/l (2 months)	3.5
Vinyl chloride	Human leukocyte culture (in vivo)	30 g/l	1.7
	Microorganisms * (S. typhimurium TA-1530) in vitro	0.2—20 ppm (48 h)	12—28

* Data from the literature.

lethal mutations is assessed by the criterion of increased embryonic lethality in intact pregnant females mated with test males.

Quantitative evaluation of mutagenic effect constitutes part of the analysis of embryonic material; it involves counting the number of live embryos — A, dead embryos (resorption sites) — B, and corpora lutea — C. For each female, the following should be tested:

1. total embryonic lethality $\frac{C-A}{C}\times100\%$;

2. pre-implantation lethality $\frac{C-[A+B]}{C}\times100\%$;

3. post-implantation lethality $\frac{B}{A+B} \times 100\%$;

4. variation of total embryonic lethality — the index that can be applied for comparison of doses in terms of effectiveness or of results obtained at different dates, $1 - \frac{A/B \text{ exper}}{A/B \text{ control}}$

A review of the literature indicates that when being tested for mutagenicity, a substance, is likely to reduce the number of surviving zygotes both by inducting dominant lethals and by decreasing the number of fertilized ova following injury to the fertilizing capacity of spermatozoa [Malashenko, 1967; Pomerantseva, 1977; Adler, 1969]. The authors consider post-implantation lethality the most definitive genetic index for judging dominant lethal mutations.

The frequency of ovulations and embryo death before and after implantation may also be controlled by independent genetic systems, while the genes responsible for pre-implantation death exhibit a high degree of dominance (Bradford, Nott, Brinster et al., 1969, 1972). Since the data in the literature, too, are controversial, it is best to take both these indices into consideration.

Studies of this kind should preferably be structured so as to integrate the rate of dominant lethal mutations throughout the spermatogenetic cycle (for the length of the complete cycle of spermatogenesis in various animal species, see Chapter 2). On the basis of the duration of the spermatogenetic stages established for mice (Oakberg, 1955, 1956), it is conventionally assumed that, following the males' exposure to a mutagen, fertilization is effected by chemically injured cells for 1 to 7 days post-exposure, at the stage of spermatozoa; for 8 to 14 days, at the stage of late spermatids; for 15 to 21 days, at the stage of early spermatids, for 22 to 35 days, at the spermatocyte stage, and for 36 to 43 days, at the stage of the spermatocytes and spermatogonia, type B; in subsequent weeks it occurs at the stage of the spermatogonia, type A.

Registration of dominant lethal mutations throughout the spermatogenetic cycle indicated that the genetic sensitivity of sex cells at different stages of spermatogenesis depended on the impact of different chemical agents as much as on the dose (concentration) level of the chemical. For example, a study carried out in our laboratory (Domshlak et al., 1974) made it clear that administration of ethylenimine by inhalation and per os to C57BL/6 mice brought about a dose (concentration)-dependent alteration of genetic sensitivity at specific stages of spermatogenesis (Fig. 31). After inhalation exposure to a 0.06 mg/m^3 ethilenimine concentration, the most sensitive stages of spermatogenesis were the spermatogonia ($p<0.001$) and early spermatids ($p<0.05$); with the ethylenimine concentration increased to 7.3 mg/m^3, it was still the early spermatid stage ($p<0.01$) and the stage of spermatocytes ($p<0.01$). Ethylenimine in a concentration of 0.76 mg/m^3 proved ineffective and neither was the rate of dominant lethal mutations changed by

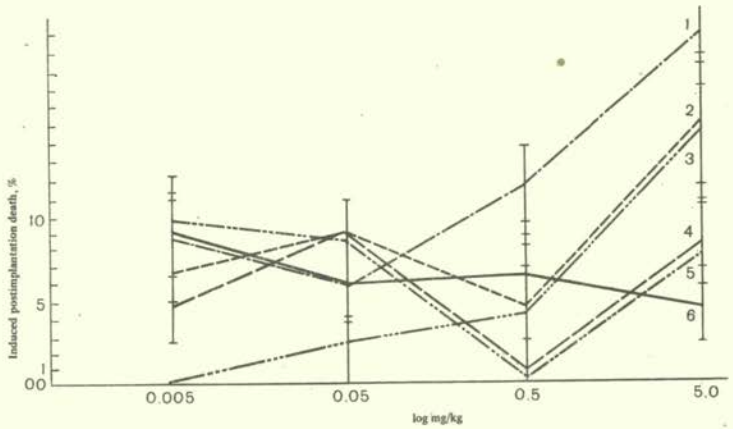


Fig. 31. Incidence of dominant lethal mutations on exposure to ethylenimine at different stages of spermatogenesis.

1 — early spermatids; 2 — late spermatids; 3 — spermatozoa; 4 — spermatocytes and B-spermatogonia; 5 — spermatocytes; 6 — A-spermatogonia

inhalation of the toxic in large concentrations, 75 and 137 mg/m^3, on exposure to the poison at the post-meiotic stages of spermatogenesis.

Following per os administration of ethylenimine to mice at a rate of 0.005 mg/kg, the highest genetic sensitivity was found at the stages of spermatocytes and spermatogonia ($p \leqslant 0.01$); with 0.05 mg/kg, at the stages of late spermatids, spermatocytes and spermatogonia ($p \leqslant 0.01$); and with a mutagenic dose exposure of 0.5 mg/kg, at the stages of early spermatids and spermatogonia ($p \leqslant 0.001$). The 5.0 mg/kg ethylenimine dose was shown to have produced a much higher frequency of dominant lethal mutations at the postmeiotic stages of spermatogenesis than did any of the other dose exposures of the mutagen. Variations in genetic sensitivity, analyzed by the rate of dominant lethal mutations, was also documented for endoxane (Briitinger, 1966), diethyl sulfate (Malashenko, 1971), and thioTEP (Malashenko, Surkova,1973). Large doses of the above mutagens tend to push up the frequency of dominant lethal mutations in spermatozoa, and low doses — in spermatogonia.

With the facilities available in a toxicological laboratory, it is very difficult to carry out studies of sensitivity evaluation at the various stages of spermatogenesis since they require too many test animals and too much time. A quicker answer can be given concerning the mutagenic hazard of toxic chemicals from an enquiry into the rate of dominant lethal mutations among animals exposed to the test mutagen throughout the complete cycle of spermatogenesis. Still, one gathers from the literature that, in most cases, low doses (concentrations) of a poison selectively affect stem spermatogo-

nia the frequency of mutations must, therefore, be analyzed 2—3 months after poisoning the animals, i. e., past the recovery period, if this kind of mutation in meiotic cells is to be identified.

Dominant lethal mutations constitute a particular manifestation of chemical mutagenicity. It would be hard to obtain clearcut mutations in mammals by exposing them to weak mutagens (Malashenko, Egorov, 1967). This is in no way because the mammalian gene structures are resistant to weak mutagens, but because, in addition to visible alterations of external traits, there are likely to be subtle biochemical inadequacies, psychic disorders, etc. (Rapoport, 1966). For this reason in order to obtain more reliable data, our laboratory carries out detailed research into the offspring. Indeed, by studying the conditioned activity of progeny we have been able to discover irregularities in the development and reinforcement of conditioned reflexes in relatively modest statistical test animal groups. For example, after intoxication of rat males by mercury in concentrations near the chronic effect threshold, reinforcement of the reflex to light and the sound of a bell was slowed down in the first as well second generations (Sanotsky et al., 1970). A study of the first-generation offspring of formamide-poisoned rat males yielded similar data. Chloroprene exposure by inhalation at a level of 0.06 mg/m^3, a subthreshold concentration in terms of toxic and specific effect on sexually mature males, interfered seriously with conditioned activity in the first-generation progeny.

Study of gene (point) mutations. The previously discussed methods for evaluating mutagenic activity in mammals fail to allow for possible manifestations of point (gene) mutations.

In recent years, tests have been proposed and tried out employing microorganisms as indicators of the presence of gene mutations. The studies were laid out in vitro (with and without preliminary metabolic activation of the test chemical) and in vivo, using the host-mediated assay (Fonshtein et al., 1973, 1977; Zakharov, 1973; Cerna, 1973; Malling, Frantz, 1974, and others). It is rather intriguing in this respect to try and identify mutagenic metabolites of chemical agents in the test animals' blood and urine (this method was developed at the Institute of General Genetics, USSR Academy of Sciences).

The most common in vitro method is based on the incubation of microorganisms with the test chemical, when the latter is introduced into a medium containing mammalian tissue homogenates (chiefly of the liver) with the co-factors necessary to trigger off the enzymes that perform metabolic conversion. The supporting rationale is that many chemicals tend to take on mutagenic properties exclusively as a result of metabolism. Workers from our laboratory (Domshlak et al., 1978) tested these methods to see if they were suitable for use in preventive toxicology. Studies with ethylenimine were designed to assess mutagenicity in systems in vivo by the host mediated assay and in vitro with and without metabolic activation (the mycrosomal liver fraction). Point mutations were re-

gistered with the use of sensitive strains of S. typhimurium. The findings (see Table 34) indicate that the rate of gene mutations in the system in vitro, without metabolic activation, under the effect of ethylenimine within the resulting dose range of 0.02 to 100 mg/l of the medium was greater than the level of spontaneous mutations by a factor of 6 to 800.

In the in vitro systems with metabolic activation and in vivo, the mutagenic effect was not as strong. In the former instance, the rate of gene mutations increased 8 to 28 times, in the latter (a dose range of 0.004 to 4.0 mg/kg) it became 5.6 to 15 times higher.

In the host mediated assay, the manifestation of the mutagenic effect of ethylenimine depended on the microbial strain used. In the same dose range, the rate of gene mutations grew 5.6-to 15-fold on the TA-1950 strain but was virtually absent from the TA-1532 strain. A review of the literature also reveals a clear-cut dependence of overt mutagenicity on the chemical type and microbial strain involved. This greatly reduces the usefulness of the proposed methods for preventive toxicology. Further research will be needed to estimate the feasibility and reliability of these methods.

Taking advantage of the possibility of evaluating the mutagenic hazard of chemical products and metabolites excreted in test animals' urine (see above), an attempt was made to compare the common animal tests and the proposed method in a study with zinc chloride (the study was conducted jointly with L. M. Kalinina and G. N. Polukhina from the Institute of General Genetics, USSR Academy of Sciences). The strains used to record point mutations were of TA-1537 S. typhimurium.

The findings have shown that following the short-term repeated administration of zinc chloride in doses of 120 (Lim_{ac}), 60 and 12 mg/kg into the stomachs of Wistar rats, the frequency of back mutations increased compared to the control over 2 to 7 days, dropped by the 11th day and increased again by the 15th. Concurrently, no increase was found in the rate of chromosome aberrations in bone marrow cells (the anatelophase method) nor in that of dominant lethal mutations in the spermatogonia of the same animals. Thus, the mutagenicity of zinc chloride was detected only in the test system judging by the output of point mutations.

The prospect of applying the method for determining the mutagenicity of the substances excreted in the urine in order rapidly to identify the genetic effect of industrial toxic substances on humans, poses a series of questions:

1. To what extent are the products excreted in the urine after the introduction of zinc chloride mutagenic to specially developed Salmonella strains?

2. Can they pose a mutagenic hazard for humans in the absence of structural chromosomal and dominant lethal mutations in rats?

3. What is the explanation for the weak mutagenic effect during

the first four days of the exposure and its drop by the 11th day following zinc-chloride administration in all doses?

4. How much is the documented effect associated with the effect of zinc chloride by the output of back mutations?

It seems promising, nonetheless, to carry on testing research methods on point mutations that use biosubstrates from workers exposed to the chemicals under study and to compare the method with the leukocyte culturing technique.

Summarizing the data at present available on the methods for evaluating gene mutations, it can be stated that microbiological tests for mutagenesis will gain wide acceptance in the assessment of mutagenic and carcinogenic properties by virtue of being simple, quick, informative, very sensitive and capable of identifying the end metabolite. For all that attention must be focused on the inherent limitations of the in vitro techniques, such as: 1. the lack of a natural entry route for the substance; 2. the failure to make sure that the metabolic processes in the in vitro system exactly duplicate those in vivo; 3. the failure to make it possible to investigate the role assigned to the chemical's distribution and elimination from the body; 4. the mutations that arise in the cells of indicator organisms cannot be identical to those found in mammals; 5. microbial tests cannot, by their nature, determine minimum effective (threshold) amounts of mutagens (carcinogens), which are basic to the limitation of their concentrations in the environment.

Specific locus mutations provide yet another method for evaluating the ability of test substances to cause gene mutations. Its key assumption is that animals untreated by a mutagen and which are homozygotous by a series of recessive genes are mated with those exposed to the study chemical and which are homozygotous according to the matching dominant alleles. When a normal allele in the sex cells of the treated animals mutates into a recessive one or a deletion appears in that locus, this is detectable by phenotypical changes in F_1 (offspring).

Because the method needs a large number of experimental animals (special strains of mice)[1] however, it is considered too costly for the extensive testing of industrial chemicals at hygiene-related research centres.

Sister-chromatid exchanges have been suggested by some authors as a suitable method for investigating the mutagenicity of chemical compounds (Perry, Evans, 1975; Wolff, Perry, 1975). The method is indeed effective for identifying a symmetric exchange of loci between sister chromatids with unaltered chromosome morphology.

A growing cell culture is treated with 5-bromdeoxyuridine for 28 h (the duration of two replication cycles). Then colchicin is added two hours before cell fixation. The cells are treated for

[1] 5- and 7-loci strains of mice were selected abroad (Russel, 1952—1969; Lyon, 1970) and one-locus strain, WR, by A. M. Malashenko in the USSR.

5—10 min with a 0.075 M KCl solution and fixed with a methanol/acetic acid mixture in a ratio of 3:1.

Following the treatment of the preparations with an acridine orange solution in a concentration 10^{-1}M, sister chromatids are distinguishable by staining with Giemsa dye.

In contrast to autoradiography, the method allows for greater resolution in the discrimination between sister chromatids.

A much greater frequency of sister exchanges than of chromosome aberrations was reported by Perry and Evans (1975) from a study examining the ability of X-rays and several chemical agents, notably mitomycin, quinacrine-yperite, N-methyl-N-nitro-N-nitrosoguanidine, biomycin, cyclophosphamide, etc. to induce sister chromatid exchanges. The authors suggest using the method to locate mutagens-carcinogens in the environment. Our data obtained from ethylenimine research confirmed the method's high sensitivity.

A method developed at the Institute of Medical Genetics of the USSR Academy of Medical Sciences tests chemical compounds on a leukocyte culture after pre-activation of a mutagen in the bodies of laboratory mammals (Chebotaryov, 1976).

The method consists essentially in taking serum from the animals a certain time (20—40 minutes) after their exposure to a mutagen and adding the serum to a nutrient medium where human lymphocytes are being cultured. Induction of aberrations in the culture is assessed by comparison with that observed in the parallel control.

The system has been proved effective with phosphamide as the test substance. It has also been proved that the level of aberrations depends both on the substance dose injected into the mouse and on the activity of the serum added to the lymphocyte culture (Chebotaryov, 1976). The proposed system cannot, however, as yet be considered universal and further work must be done on it.

To sum up the present discussion of the data on the accepted methods for studying the mutagenic properties of chemical agents, it is worth noting that the International Congress on Mutagenesis in Czechoslovakia (1975) adopted a programme for evaluating chemical mutagens for mutagenicity, as proposed by N. P. Bochkov, Director of the Institute of Medical Genetics, the USSR Academy of Medical Sciences. His mutagenicity assessment programme is composed of two subprogramme, screening and main.

I. The screening subprogramme includes: 1. a test on microorganisms with metabolic activation; 2. cytogenetic analysis of animal bone marrow cells.

II. The main subprogramme includes: 1. the screening subprogramme; 2. cytogenetic analysis of human peripheral blood.

Criticisms regarding the programme, in light of the objectives of hygiene and toxicology, were presented in Chapter 1. We recommend Diagram 2 to illustrate the importance of studying the mutagenic effects of chemical compounds for the purpose of hygienic standardization. The Diagram has been tested on a large number of the

chemicals previously examined by hygiene-related research establishments.

Some results of the experimental evaluation of chemicals

At the present time, at least half the studies concerned with mutagenesis use mammalian bone marrow cells. They have been used to study DDT, hexachloran, dieldrin, heptachlor (Markaryan, 1966), dipine (Akifiev, 1966), benzene (Matushita, 1966), thioTEP (Arsenieva et al., 1967), carbon tetrachloride, propylene oxide, di-n-propylamine, dinitryl perfluoglutaric acid (Fomenko, Katosova, 1968, 1969), ethylene oxide (Strekalova, 1970), 1,3-chlorobrompropane and chloroprene (Katosova, 1973). All of the investigations made predominant use of the anatelophase method.

Through analysis of the spontaneous level of chromosome aberrations in the bone marrow cells of albino rats (n=293) based on the method of percentiles (Sepetliev, 1965) the level of spontaneous pathology was estimated and a classification suggested according to the magnitude of the cytogenetic effect (Table 35).

Over 30 chemicals were thus assessed cytogenetically in the Department of Toxicology of the Research Institute of Industrial Hygiene and Occupational Diseases, the USSR Academy of Medical Science, and the effect concentration relationship was demonstrated for compounds including carbon tetrachloride, propylene oxide, di-i-propylamine, dinitryl perfluoglutaric acid, ethylene oxide, 1,3-chlorobrompropane, chloroprene, ethylenimine, and other substances (see Chapter 2).

Table 35

Classification of the Magnitude of the Cytogenetic Effect in Rat Bone Marrow Cells

Percentile *	P_{50}	P_{90}	P_{100}
Number of chromosome aberrations	2.7	5.0	8.0
	1.67÷3.60	3.6÷5.0	5.0÷8.0 and more
Magnitude of the effect	Normal	Poorly marked	Pronounced

* Processed by the percentiles method (Sepetliev).

Numerous chemicals have been proved capable of causing dominant lethal mutations in the sex cells of poisoned males, among them tripaflavine, hexamethylentetramine (Baldormann et al., 1967), triethylenmelamine (Cattanach, 1957), ethylenimine and diethyl sulfate (Malashenko, Egorov, 1967; Malashenko, 1971). This was done, however, using sublethal doses and intraperitoneal or intrascrotal injection of the mutagens.

As discussed earlier, data of value for industrial toxicology should be gathered by the inhalational entry route in concentrations

that actually exist in the work environment. Such studies are limited in number. Those accomplished under our guidance have demonstrated the induction of dominant lethal mutations in animals by chemical concentrations insufficient to cause changes in the indices of systemic toxic effect. This was the case with ethylene oxide, ethylenimine, chloroprene, cyclohexanone, tertiary butyl hydroperoxide, and other compounds. The above data have led the authors to conclude that the mutagenicity of the poisons tested was selective. It appears that, in preventive toxicology, analysis of the mutagenic effect during observation of the development of offspring is not practised as often as it should, even though it is common knowledge that the sustained ability to fertilize and conceive does not signify the wellbeing and adequacy of the reproductive function (Sanotsky, 1965, 1968, 1970).

Indeed, the transfer of untoward genetic information via spermatozoa (see Chapter 5) has been proved conclusively for exposures to a number of chemical agents, such as ethylenimine (Zaeva et al., 1967), mercury (Sanotsky et al., 1967), butyl ester 2,4-D (Konstantinova, 1968), manganese (Mandzhgaladze, 1969), granosan, dinitroorthocresol and sevin (Vashakidze, 1968, 1969), as well as several other compounds.

Male rats, when exposed to a single mercury dose of 0.02—0.04 mg/l, were seen to have a lower relative weight of the testes and less durable spermatozoid mobility (Egorova et al., 1962). It was also noted that the sperm of the males after exposure to a MAC level concentration is able to transmit information modifying the state of the progeny from healthy females (Sanotsky et al., 1967). Thus, in first-generation offspring, conditioned connections took a shorter time to develop than they did in the control, though the effect of the MAC-level mercury exposure on the fathers produced no visible changes. In the second generation, the stimulating impact of mercury induced unexpected inhibition.

A manganese compound induced changes in both second and third generation offspring of exposed males. These were a numerical expansion of dead and underdeveloped embryos, a lower viability of the offspring, a decrease in litter size, and also retarded sexual maturation and inhibition of the reproductive function (Mandzhgaladze, 1969).

Sensitivity of sexual and somatic tissue to the effect of mutagens. An important issue in chemical mutagenesis is the sensitivity of sexual and somatic tissue cells of mammals to the effect of chemical compounds. Radiation exposure causes the same types of mutation to appear in somatic cells as it does in sexual cells (Shapiro, 1966; Pomerantseva, 1977).

A comparison was made between the levels of manifestation of mutagenic effect in the cells of somatic (cytogenetic methods) and sexual tissues (the method of dominant lethal mutations) on exposure to low concentrations of chemical agents. Through cytogenetic analysis of bone marrow cells, the effect was discovered

at lower levels of exposure in the case of ethylenimine, chloroprene (Katosova, 1973), urethane (Suvalova, 1972) and 2,4,5-T (Efimenko, 1974). Hydrogen fluoride caused chromosome injuries in bone marrow cells after inhalation exposure to a concentration of 1.0 mg/m^3. Under the same conditions, the rate of dominant lethal mutations would not be augmented in the test animals (Voroshilin et al., 1975).

These occurrences may be due to the better-protected sexual system at the level of the integral organism because of the meiotic barrier that has developed in the course of evolution. Or it may be due to the varied capability of each of the tested methods to assist in evaluating qualitative alterations in the chromosomal machinery of cells. Beyond that, the examined cells in the somatic tissue were, naturally, a great deal more numerous than the sex cells, the state of which could be judged from the information available from analysis of embryonic material.

On the other hand, some of the data available today point to the essentially unidirectional effect of mutagens on both sex and somatic cells. For example, inhalation exposure to ethylene oxide (E. E. Strekalova's studies) in MAC-level doses produced a cytogenetic effect in bone marrow cells and a mutagenic effect in sex cells. A similar coincidence of the two effects at the same exposure levels was found in our study of the mutagenicity of tertiary butyl hydroperoxide (Katosova et al., 1977).

Other data suggest a sensitivity advantage for the method of dominant lethal mutations over cytogenetic methods. For example, R. Schram (1976) compared, in sensitivity terms, the dominant lethals test, cytogenetic analysis of chromosome aberrations in sex cells at the diakinesis-metaphase stage of the first meiosis, and the cytogenetic analysis of chromosome injuries in the bone marrow cells of mice, using a single intraperitoneal injection of a 1 mg/kg thioTEP dose. The comparison between the methods at their respective, most sensitive periods has shown the test for dominant lethal mutations to be the most sensitive technique (in fact, twice as sensitive as cytogenetic analysis of bone marrow cells).

We also observed higher sensitivity of the dominant lethals test towards the effect of some substances, notable benzene (at a level of 5 mg/kg intraperitoneally) and cyclohexanone (1.5 and 105 mg/m^3).

The data now available and the tissue-specific mode of action of chemical compounds based on their distinctive metabolism patterns make it impossible even to speak about extrapolation of somatic cell data to germ cells. Both methods — dominant lethal mutations (to judge mutations in germ cells) and cytogenetic analysis of somatic tissue cells — would thus have to be used for examining chemical factors for mutagenicity.

As for the cytogenetic analysis of meiotic preparations (of sexual tissue), most geneticists who have ever done research in this field would prefer to reject it as a necessary research tool because of its high labour-intensity and low information value. We are also of the same opinion, having some experience in using the method.

Conclusion. The problem of the mutagenic hazard evaluation of industrial toxics is in the stage of intensive work. In general, effective methodologic approaches to the experimental study of chemical mutagenesis to serve the purposes of preventive toxicology have already been designed. On the basis of the experience available in this sphere, we have proposed a diagram for investigating mutagenic risks from industrial toxic chemicals (see Diagram 2).

Chapter 6

LONG-TERM EFFECTS OF CHEMICALS ON THE CARDIOVASCULAR SYSTEM

Introduction. The long-term effects of chemicals on the cardiovascular system cover accelerated sclerotic changes in the heart and vessels of the myocardium, kidneys, brain and retina; faster progression of atherosclerosis and more severe course of its complications (infarctions, strokes, thromboses, etc.) and development of the hypertonic syndrome and vegetovascular distonias. Works by Soviet and foreign scientists bring into focus the risk associated with the rise of such complications in cases of acute and chronic intoxication by carbon disulfide, lead and other heavy metals, as well as carbon monoxide, fluorine and its salts. They also make it plain that the mechanism by which these and many other chemicals (hormonal active substances, antioxidants, microelements, vitamins, etc.) act on the cardiovascular system varies a great deal. It may apply variously to neurohumoral regulation disorders of the cardiovascular system, tissue hypoxia, inhibition or activation of the enzyme systems directly linked with the vascular wall, as also with the myocardium. Another focus of attention is the likely development of cardiovascular pathology in offspring, because of the effect that chemical compounds can have on the parental genetic machinery or immediately on the foetus (the genetic and embryological aspects in the impact of chemicals on the cardiovascular system). The theoretical and methodological facets of the problem are treated in other sections of this monograph.

Since there are monographs presenting data on the effects of particular chemicals on the cardiovascular system, the present section will review just some general points with respect to the problem of hygienic standardization applied to substances with long-term effects on the heart and vessels, plus methodological issues concerning predicting these effects experimentally.

General methodological approaches. Despite the large body of scientific information on the effect of chemical compounds on the cardiovascular system in late period of exposure and later in life after exposure (Orlova, 1953; Vasilieva et al., 1966, 1969; Vol'fovskay et al., 1969; Snegova, 1970; Roshchin, 1970; Konchalovskaya et al., 1972; Roshchin, Sanotsky, 1972; Monaenkova et al., 1975; Konchalovskaya, 1976; Vigliani, 1954; Hernberg et al., 1970, 1973; Gotta et al., 1972, etc.), it remains of little use for hygienic standardization and sanitary rating in particular. This is

mainly because all the above investigations involved comparatively high levels of exposure, so the state of the cardiovascular system was assessed against a background of severe intoxication, chronic or acute.

It is difficult to consider the relationship between the magnitude of an effect, the dose of a chemical and the latter's degree of selectivity, with a view to designing sanitary standards for chemical concentrations in the environment where there are several different factors — occupational, communal, and domestic — acting in concert. Psychic and emotional stresses, dietary imbalances, hypodynamia, smoking, alcohol, etc. are known to play a role in the development of cardiac pathology. While examining particular environmental influences, the likelihood of the same agent arriving concurrently from food, water, the indoor air of production spaces, ambient air, and via the skin cannot be ignored.

In this respect, an experiment can do a great deal more to ensure relatively stable conditions of exposure and rule out a number of associated factors, in order to clarify the causal relationship between chemical compounds and the development of cardiac and vascular pathologies.

Nevertheless, practical experimental prediction of the long-term effects of chemicals on the cardiovascular system comes up against certain difficulties. The main one arises from the lack, in the majority of test animals (monkeys and pigs excluded), of spontaneous atherosclerosis, hypertension and myocardial infarction, making it more difficult to transfer the test data to man.

There are numerous, generally known experimental means for simulating cardiovascular diseases. The principal experimental models of atherosclerosis are given in Table 36.

It is evident from Table 36 that none of the proposed experimental models is good enough for preventive toxicology, which studies the influence of low (threshold) chemical concentrations. In contrast, the induction of experimental atherosclerosis will require the administration to the body of substantial doses of biologically active compounds cholesterol, hormones (ACTH, cortisone), iodine acetate, etc. that trigger signs of intoxication. Given this background, the impact of minimum doses of industrial toxic chemicals may either go unnoticed or be considerably distorted.

Modelling other cardiovascular pathologies such as hypertension, myocarditis, coronary deficiency, etc. in a toxicological experiment is likewise beset with difficulties in the interpretation of the results obtained. Its use is therefore restricted to solving specific questions pertaining to the mechanism by which chemicals affect the cardiovascular system (Lukaneva, 1975).

In 1972 I. V. Sanotsky and associates have suggested an ingenious methodological approach to the study of long-term chemical effects on the cardiovascular system. The general idea is to act upon intact animals (without preliminary modelling of a cardiovascular

Table 36

Models of Experimental Atherosclerosis

Model	Literary data	Brief description
Cholesterol	Khalatov, Anichkov (1961) Myasnikov (1962)	Feeding rabbits with cholesterol in doses of 200 to 600 mg/kg/day for 4—6 months
Alimentary-cholesterol	Aptekar' (1964), Sidorov, Nikolaeva (1963)	Feeding animals (rats, rabbits) atherogenic diets with the addition of cholesterol for 3—4 months
Antithyroid	Leites, Gambashidze, (1963)	Injection of thyrostatics (to rats and rabbits) with the addition of common cholesterol doses for 3—4 months
Renal-cholesterol	Cited from Leites (1963)	Operative removal of the kidneys, induction of cellophane perinephritis and feeding (rats) with cholesterol for 2—3 months
Iodine acetate	Sidorenko, Dneprovskaya (1974)	Injection (to rats) per os of 7 mg/kg/day of iodine acetate for 5 months

pathology) with chemical compounds in minimum amounts and follow the rate of which natural ageing processes develop in the heart, vessels and entire organism.

At present, there is no generally accepted concept of ageing (Frol'kis, 1971; Streller, 1964; Comfort, 1967). Nevertheless, its main mechanisms and the set of symptoms peculiar to it have been well known for a long time. Structural and compositional alteration of the connective tissues, regular age-related changes in the pattern and intensity of the protein, lipid, carbohydrate, water and salt metabolism, and distinctive modes of regulation of vitally important processes in senility — are all nodular points one must be able to characterize in order to estimate the rate of advance of the ageing processes caused by chemical compounds. It is also expected that the test indices will be collated all along with the natural ageing processes in the controls.

Another fairly important point in validating the proposed methodology is whether chemically induced accelerated ageing of the body's systems and organs is possible in principle. This possibility is borne out, in particular, by Pashkova's evidence (1969) of premature ageing of the ovaries on dimethyldioxane exposure. Problems of gerontology are covered in studies on the effects of fluorine and its compounds (Gabovich, Tzipriyan, 1970). Much of the data cited above on the long-term effects of chemicals on the heart and vessels fit in nicely with the dynamics of accelerated ageing. The more characteristic signs of age alterations in humans include compromised elastic properties of arteries, sclerotic hardening, the emergence of vascular aneurisms in various organs

and neuroendocrinal disfunctions of cardiac and vascular regulation. Lastly, as one compares the initial processes leading to atherosclerosis and senile changes of the heart and vessels, a broad similarity is noticeable between the intimal metabolic criteria and the morphofunctional manifestations of age-related changes and atherosclerosis, Davydovsky (1969) described atherosclerosis as an immediate manifestation of age-related changes. Though presently many researchers do not concur with the concept in its entirety, they do acknowledge the existence of unquestionable and broad similarities between atherosclerosis and ageing (Vikhert, 1974).

VALIDATION OF EXPERIMENTAL DESIGN AND EXPOSURE LEVELS; SELECTION OF ANIMAL SPECIES

Natural ageing. The selected methodological approach demands that serious attention be focused on the natural ageing processes in experimental animals. A review of the literature on this subject reveals marked controversies and wide gaps in the data on normal age-related dynamics of many indices.

Hence there is obviously a need for systematic accumulation of such data on both biochemical and functional parameters of the state of the cardiovascular system and morphometric criteria. It is essential to stress the importance of acquiring data from sufficiently large (of at least 50 to 100) and homogeneous groups of intact animals, since their varying conditions of existence can influence the state of the cardiovascular system greatly. A special note is in order for the active role that the concentrations of such microelements as copper, iron and zinc in livestock forage and drinking water play in the dynamics of age-related alterations in the heart and vessels. Their entry into the body of test animals varies over a wide range (Klauder, Pettering, 1977) and is likely to enhance the effect of chemical compounds (Cerklewski et al., 1977).

As seen from Table 37, natural variations in control data ($M \pm 2\delta$) must be considered in order to gain an idea of the wide variations in the indices employed. Not infrequently, the spread of variations in one age group is so wide that it covers the other two groups completely. If so, the hygienic significance of identified alterations is hard to avaluate, thus necessitating multiple replications of laborious experiments. Analysis has shown that most information is obtainable from the ratio between the connective tissue and myocites in the myocardium, the thickness of myocardial muscular fascicle, heart coefficients by mass, the cholesterol level in the blood, the quantity of oxyproline in the aortal wall, and the thickness of the elastic membranes in the vessels of a musculo-elastic type.

The different rate of ageing deserves note. Indeed, most indices changed in the period between 2—6 and 12—16 months, followed by a relative stabilization.

Thus, a life-span of 12 to 16 months should be considered sufficient for evaluating the ageing rate of the heart and blood vessels.

Table 37

Some Morphological and Biochemical Indices ($M \pm 2\sigma$) of Intact Albino Rats (Males) and Three Age Groups (own data)

Indices	Age, months		
	2—6	12—18	24—36
Biochemical			
Phospholipids of serum, m mole/l	120±34.9 n=117	197.4±6.7 n=75	135.7±6.8 n=46
β-Lipoproteids of serum, g/l	889±43 n=62	627±52 n=115	732±52 n=51
Cholesterol of serum, m mole/l	0.88±0.02 n=63	1.07±0.03 n=118	1.46±0.09 n=53
Oxyproline (aorta), mg/g of dry tissue	31.1±0.84 n=43	32.3±0.65 n=60	35.7±0.96 n=35
Hexosamines (aorta), mg/g of dry tissue	9.5±0.14 n=48	10.4±0.21 n=63	9.9±0.26 n=35
Hexuronic acids (aorta), mg/g	7.6±0.09 n=40	9.2±0.1 n=50	9.6±0.16 n=36
Morphological			
Thickness of left ventricular wall, mm	3.25±0.06 n=56	3.50±0.06 n=92	3.4±0.07 n=35
Thickness of right ventricular wall, mm	1.54±0.04 n=56	1.31±0.04 n=92	1.53±0.05 n=35
Thickness of adventitia (aorta), μm	62.7±7.3 n=35	105.5±4.2 n=44	100±12.1 n=10
Thickness of intima (aorta), μm	8.9±0.29 n=35	9.04±0.34 n=44	9.3±0.14 n=10
Number of elastic membranes (aorta)	7.9±0.26 n=35	8.75±0.23 n=44	7.4±0.8 n=10
Thickness of elastic membranes (aorta), μm	5.4±0.1 n=35	3.8±0.06 n=40	4.3±0.2 n=10
Thickness of elastic membranes (vessels of musculoelastic type, caliber ~50 μm)	1.53±0.04 n=26	1.6±0.03 n=28	1.78±0.08 n=28
Tension * of (the same) arteries (*conv. units*)	0.62±0.02 n=28	0.7±0.02 n=28	0.61±0.03 n=28
Thickness of myocardium muscular fascicle, μm	12.6±0.3 n=40	14.2±0.4 n=30	
Connective tissue	0.22±0.01	0.28±0.1	
Myofibrils (myocardium)	n=40	n=30	

* Notes: hypertension — 1; normotonia — 0,5; reduced tension — 0, n — number of animals.

Time of research. The influence of chemical compounds on the cardiovascular system was investigated in a chronic experiment (four-months long, by inhalation, or one year-long, per os) and in the longer term (9 to 12 months) upon completion of the chronic experiment. The time of exposure is specified in the standards accepted in industrial and communal hygiene. The duration of the recovery period is such that it allows changes in the test animals to be compared with natural age-related alterations in the control animals

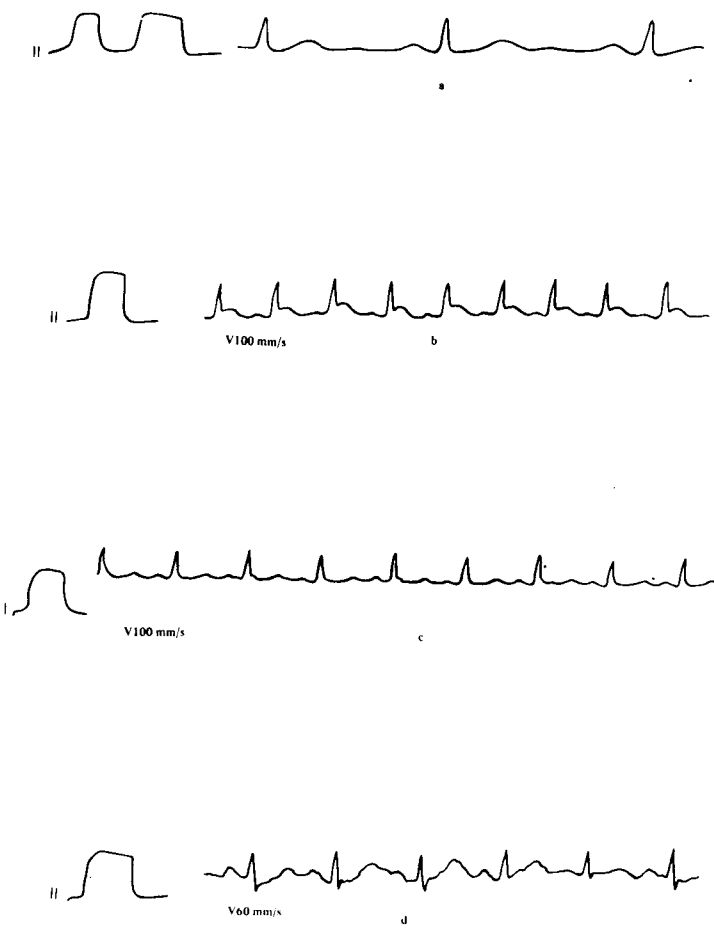


Fig. 32. Human ECG(a), rat's (b), guinea pig's (c) and rabbit's (d)

of both middle (12—16 months old) and senior age (24—36 months old).

Species sensitivity. A rather essential point is the selection of a suitable species of animal for the experiment. Possible differences in species sensitivity must be carefully considered. Extensive discussion of species-related ageing patterns of the heart and blood vessels is impracticable in this monograph, two or three species of laboratory animals should be used in parallel to give cogency to the data obtained.

For an ECG evaluation, rabbits or guinea pigs should be examined along with rats, the most widespread and commonly used animals in toxicological research. The former have an ECG which is more like the human ECG than that of rats (in whom, as is known, the S—T interval is missing). Shown in Fig. 32, for the sake of comparison, are the

ECG of the rat, guinea pig, rabbit and man. The rat is nonetheless the generally accepted gerontological model at present, due to the major similarity of its myocardial and vascular metabolism with man's. The use of mini-pigs and monkeys is rather desirable for this kind of investigation.

The statistical groups designed for research on long-term effects should be at least 30-strong, considering the wide natural variability of the normal indices used. It should be noted with respect to some indices with a wide variability that expansion of the statistical groups to 100 head (phospholipids, coagulative blood system) might be necessary.

METHODS OF RESEARCH

Functional methods. The most integral functional evaluation of the state of the cardiovascular system can be achieved with a polycardiographic method which allows, furthermore, an integrated assessment to be made of the central as well as the peripheral parts of the system. The methodological difficulties of handling small laboratory animals have not yet, however, been ultimately resolved. Accordingly, the accent is currently made on the evaluation of specific methods for examining the heart and the vascular bed.

Electrocardiography enjoys wide popularity as a clinical method for the early diagnosis of functional and organic changes in the heart. There are several constraints on the method, like the need for filtration of muscular biopotentials, fast rhythms, etc., when applied in experiments on small laboratory animals (Mikishova, 1969). Once they are cleared away, estimating the rate and rhythm of heart contractions and, moreover, identifying variations of myocardial excitability, conduction and contractility become distinctly possible. The use of adequate functional (physiological and pharmacological) tests not only provides a more intimate knowledge of the hygienic significance of recognized changes, but also promotes a better understanding of the mechanism by which poisons act on the myocardium.

Table 38 quotes the results of applying the pharmacological loads normally employed for toxicological experiments.

For pulse integration, a variety of schemes are used including that illustrated in Fig. 33 (M. M. Moshkov).

Arterial pressure constitutes the most important single integral test characterizing the tension and rigidity of the vascular wall. The use of functional loads seems absolutely essential for estimating this index as well.

Rheo- and plethismography are common clinical methods which should find practical application in the toxicological experiment, where they can assist with the description of both central hemodynamics (systolic volume and cardiac output) and the peripheral part (elastoplastic properties of peripheral arteries of the cardiovascular system) (Verich, 1975).

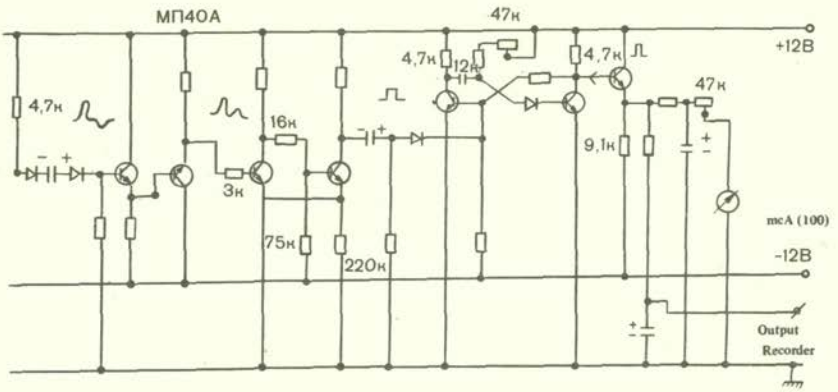


Fig. 33. Diagram of integrated pulse recording in small laboratory animals (M. Moshkov, 1976).

Table 38

Functional Pharmacological Loads on the Cardiovascular System

Medicines or drugs (name)	Dose and administration route	Observation schedule	Normal effect	Mechanism
Adrenaline	4 μg/kg of 10% solution intraperitoneally	After ten minutes	Increase and enhancement of heart contractions	alfa- and beta-adrenoreceptors
Pituitrine	0.15 units intravenously	After thirty seconds	Increase of AD, capillary construction	Smooth musculature
Caffeine	30 mg/kg of 10% solution per os	After ten minutes	Increase and enhancement of heart contractions	Nervous system
Atropine	50 mg/kg of 20% solution under skin	After thirty minutes	Increase of heart contractions	m-cholinoreactive systems
Proserine (Neostigmine)	1 mg/kg of 0.05 solution under skin	After 30 and 60 minutes	Conductance disturbances, arythmia	Anticholinesterase effect

The state of the capillary bed requires as many objective methods as possible for its assessment. The methods include capillarography and tests for the permeability of the capillary bed to colloidal and other stains and for the stability of the capillary wall against mechanical and other impacts.

Biochemical methods. Because it has been proven possible to compare biochemical ageing criteria for humans and animals in order to construct a system for forecasting long-term chemical effects, this discipline deserves special attention. If the analysis is further buttressed by indices of lipid metabolism and data on the state of the connective tissues (according to the levels of oxyproline, hexosamines and hexuronic acids), as well as on the coagulative and anticoagulative blood systems, it might be possible to uncover more mea-

ningful man/animal correlations for ageing processes. By virtue of having extremely narrow ranges of natural variations, the indices of the state of the connective tissue proved useful in determining the hygienic importance of the changes so identified. The research results regarding the indices of haemostasis showed their age-related dynamics to be somewhat ambiguous and largely dependent on the initial condition of the test animals. The results obtained from comparative evaluation of the biochemical and electromechanical methods for assessing coagulative blood properties in three animal groups, by age, are shown in Table 39 (E. Ya. Golubovich's experiments).

Table 39

Indices of Coagulative and Anticoagulative Blood Systems in Three Rat Groups, by Age

Indices	Age, months		
	6—8	18	24—26
Biochemical	n=60	n=45	n=45
Total coagulability, s	64.3±1.5	73.8±2.7	75.0±2.5
Recalcification time, s	50.6±2.0	110.7±8.8	142±7.4
Blood tolerance to heparin, s	313.2±29.9	224.7±26	304.8±16.4
Thrombin time, s	9.9±1.3	45.2±3.0	112±18.5
Concentration of fibrinogen, mg/l	41.8±2.2	86.6±6.8	180.2±19.4
Fibrinolytic activity, %%	3934±186	4332±412	5598±380
Electrophysiological (coagulogram)	47.5±3.6	17.1±4.1	10.6±3.2
T_1c	n=75	—	n=30
T_2c	55.3±1.99	—	66.0±6.5
Tc	182.9±3.7	—	234.0±11.0
T_3c	125.8±3.58	—	167.2±8.7
A_5c	465±13	—	920.7±90.6
F_5 conv. units	4.4±0.3	—	2.1±0.15
	0.86±0.05	—	0.4±0.03

Note: The correlation coefficient for biochemical and electrophysiological indices of the coagulative blood system is r±0.57; n is the number of animals.

A note is in order that the change of coagulative blood properties may occur not only because of a compromized state of the vascular wall (the thrombogenic theory of atherosclerosis and the contribution of increased coagulability to the development of coronary heart disease), but also because of impaired liver function.

The evaluation of haemostasis is also important in dealing with the long-term implications of chemical exposures for the cardiovascular system, because the functional lability of the coagulative and anticoagulative blood systems depends, heavily on the state of the neuroregulatory mechanisms (the adrenalin-heparin, histamin-heparin, and other complexes). The latter seem to be fundamentally implicated in the development of cardiovascular pathology.

Studying regulation of cardiac activity and vascular tension is of primary importance in elucidating the mechanisms by which chemical compounds affect the cardiovascular system.

Morphological methods. Bearing in mind the minimum (threshold) magnitude of the chemical exposures under study, absolute preference should be given to quantitative morphological criteria. A broad range of the morphometric indices involved in structural characterization of the heart and blood vessels (both large and small organ arteries and veins) are helpful in estimating the significance of the functional and biochemical alterations detected in the same animals (Grodetskaya et al., 1976).

Thus, the integrated management of investigations is a vital requirement for evaluating the long-term effects of chemical compounds on the cardiovascular system. Another requirement is for maximum correlation of experimental data with those resulting from a clinical-hygienic examination of workers occupationally exposed to minimum concentrations of chemicals for prolonged periods (the description of the surrounding environment must be as accurate and distinct as possible).

Methodological recommendations are now available for evaluating the state of the heart and vessels in a toxicological experiment with the express purpose of setting hygienic standards for the concentrations of chemicals in the air of the working zone (Trakhtenberg, Sanotsky et al., 1977). They refer to the methodological aspects in the study of long-term effects on the cardiovascular system. The routine use in hygienic standardization of the principles and methodological approaches outlined in these instructions will further the objectives of unifying and subsequently generalizing experimental data.

Hygienic standardization of compounds with long-term effects on the heart and blood vessels. The methodological approach discussed above has been effectively applied for estimating experimentally the rate of ageing processes in relation to a number of chemical compounds, in order to establish sanitary standards for their concentrations in man's environment.

Carbon disulfide. The selective action of carbon disulfide on ageing processes was demonstrated in a chronic experiment with CS_2 concentrations of 10 and 1 mg/m^3. At these concentrations, carbon disulfide produced no systemic toxic effect (Martynova et al., 1976). But immediately after the chronic experiment (4 hours/day for 4 months — the 1st group) and in the longer term (nine months — the 2nd group), indices relating to the ageing rate changed so much that some of them exceeded the limits of the physiological norm (Table 40).

Comparison of the experimental data with clinical hygienic studies (Saitanov et al., 1977) suggested a certain correlation between the ECG changes in employees long-exposed at work to the above-mentioned concentrations of carbon disulfide and the structural alteration of the myocardium in the experimental animals. Some of the patients surveyed developed myocardial dystrophy as evidenced by

Table 40

Changes in the Heart and Blood Vessels Following Chronic Exposure to Carbon Disulfide

Group	Indices	Concentrations, mg/m³			
		100	10	1	Parallel control
1st	**Biochemistry of aorta:**				
	Oxyproline, mg/g	34.5±1.2 *	33.4±1.06	33.1±1.06	30.7±1.03
	Hexosamines, mg/g	13.4±0.45	13.0±0.41	13.2±0.35	13.6±0.44
	Hexuronic acids, mg/g	10.1±0.12	10.4±0.19	9.8±0.2 **	10.4±0.13
2nd	**Morphometry of myocardium, μm**				
	Connective tissue/ myocites	0.31±0.01	0.36±0.01 *	0.28±0.01	0.28±0.02
	Average thickness of myocites bundles	2.78±0.09	3.01±0.21	2.90±0.1	2.93±0.05
	Morphometry of aorta, μm				
	Intima	2.6±0.1	2.7±0.1 *	2.8±0.2	2.4±0.1
	Media	22.2±0.6	21.8±0.7	19.8±1.5	22.2±1.4
	Adventitia	8.6±1.1	9.7±0.9	11.6±0.9	10.1±0.8

* $p<0.05$, ** $p<0.02$

clinical signs (a slightly extended left heart margin, dullness of heart beat, and frequent and feeble pulsation in roentgenoscopy), as well as altered repolarization of the ventricular ECG complex (abatement or two-phase pattern of the T wave). The genesis of the changes perceived is rather complex. Interference with neuroendocrinal regulation, excessive concentrations of catecholamines, a disturbed fat and protein metabolism (Dumkin, Ryzhkova, 1969) and electrolytic imbalance (Milkov et al., 1974) are all too likely to play a role in altering the myocardium in the case of chronic exposure to carbon disulfide.

Data from orthostatic and physical tests indicate undisputed changes in the regulation mechanisms of cardiac activity. Results of pharmacological tests with potassium chloride and inderal confirm the perceived involvement of the altered catecholamine concentrations and electrolytic metabolism in the pathogenesis of recognized myocardial dystrophia. If, furthermore, the clinical data and results of the functional tests are considered the non-coronary genesis of the disturbance found in the myocardial repolarization stage becomes patently obvious (Saitanov, Grodetskay et al., 1977).

We have discovered no hypertrophic and hyperplastic processes in the myocardium of experimental animals in conjunction with diffuse

sclerosing, which corroborates the above suggestion. Unfortunately, the lack of spontaneous atherosclerosis in rats precludes a closer correlation of the recognized changes with human atherosclerosis.

In carbon-disulfide exposure, the experimental animals (the experiment was carried out three times) exhibited no major changes of cardiac function and biochemical indices. In contrast, during human atherosclerosis the indices change very frequently, so all or certain of these parameters, such as arterial pressure, and the levels of cholesterol, ß-lipoproteids and phospholipids in the serum, are taken as the basis for deciding whether or not the test chemicals pose "atherogenic" risks to the public.

It would seem more logical to focus on the study of pathomorphological material giving quantitative evaluation of the magnitude of atherosclerotic changes (Hernberg et al., 1973).

MAC-level carbon-disulfide concentrations in the air of the working zone (1 mg/m^3), while incapable of producing a systemic toxic effect, do modify several parameters responsible for accelerated age-related dynamics of changes in the circulatory system. This is hypothesized from the recognized alteration of the biochemical composition of the aortal connective tissue and the consequent change of one of its morphometric characteristic, namely, morphological signs of rearranging in the connective tissue of the musculoelastic vessels. In this respect, a warning sign is a change of the cardiovascular system that Sal'nikova and Chirkova (1974) located in the first-generation offspring of females exposed to the given carbon-disulfide concentration.

The question of the mechanism by which carbon disulfide acts on the cardiovascular system is an important problem in its own right. Before the problem of the long-term effects of carbon disulfide on the heart and blood vessels is finally resolved, thorough clinical hygienic research will be needed to acquire data on the health of persons exposed to carbon disulfide in minimum concentrations, those who retired after prolonged exposure to it, and their progeny.

The hazard inherent in carbon disulfide was confirmed in experiments with thiuram exposure, the poison for which carbon disulfide is the principal metabolite in the body (Chernov et al., 1969). We have found structural changes in the myocardium (Table 41) following chronic thiuram exposure of rats.

As is evident from the table, chronic thiuram exposure (TMTD — tetramethylthiuram disulfide) in both tested doses was unable to produce a systemic toxic effect, but did cause hypertrophy of muscular fascicles in the myocardium, probably because of the animals' predisposition towards hypertension (Zhilenko et al., 1976).

In a study with ethylphenylthiuram (according to N. V. Khoroshilova's data, EP-thiuram is a compound less toxic than TMTD, as its metabolism follows a somewhat dissimilar path, producing less carbon disulfide; Table 42) no changes of the myocardium were detected in the long-term after chronic exposure, though there was a concurrent change of peripheral blood as well as some biochemical indices (doses of 100 and 50 mg/kg for 9 months). Both these studies provide indirect

Table 41

Changes of the Myocardium Following Chronic Thiuram Exposure

Thiuram dose, mg/kg	Indices		
	connective tissue/myofibrils	nucleus/cytoplasm	thickness of myofibril fascicles, μm
0.05	0.23±0.01	0.055±0.005	2.75±0.04 *
0.005	0.23±0.01	0.045±0.003	2.75±0.04 *
Control	0.25±0.02	0.051±0.03	2.41±0.02

* $p<0.001$.

Table 42

Dynamics of CS_2 Expulsion in Exhaled Air in Rats (μg/2h) on Exposure to Threshold Doses (Lim_{ac}) of TMTD and EP-Thiuram

Compounds	Dose, mg/kg	Time of observation after exposure, days		
		1st	2nd	3rd
TMTD	100	69.5±32.0	266.0±43.5 *	77.9±24.4 *
EP-thiuram	500	3.9±2.2	8.8±4.6	1.9±0.9

* $p<0.001$.

evidence linking TMTD-induced myocardial changes to carbon disulfide.

Klimov's data (1976) indicate changes in a number of indices of fat metabolism and aortal rearrangements among test animals' toxic responses to the effect of the ethers of O-phthalic acid, dihydroisophoron and some other chemicals in near-MAC concentrations. But, unlike carbon disulfide, these toxics set off these changes at the same levels that trigger systemic toxic responses.

Exposure to **sodium fluoride** (Pankratova, 1974) modifies the lipid metabolism and composition of the connective tissue of the aortal wall and markedly changes the functional indices of the cardiovascular system (Table 43). Signs of ageing of the cardiovascular system were seen to develop at the same levels of exposure to fluoride and its compounds that caused specific changes of glycolysis and mineral metabolism (Fig. 34).

Myasnikov (1965) singled out the phase of vasomotor instability in the development of atherosclerosis. Very early in the development of pathological process more attention should be paid to regulation disfunctions than to the signs of impaired lipid metabolism. Quite likely, changes in the functional state of the circulatory system determine both premature ageing and atherosclerosis.

On chronic exposure to fluorine, a diminished heart rate of a phasic character was registered first. When neostigmine tests were applied

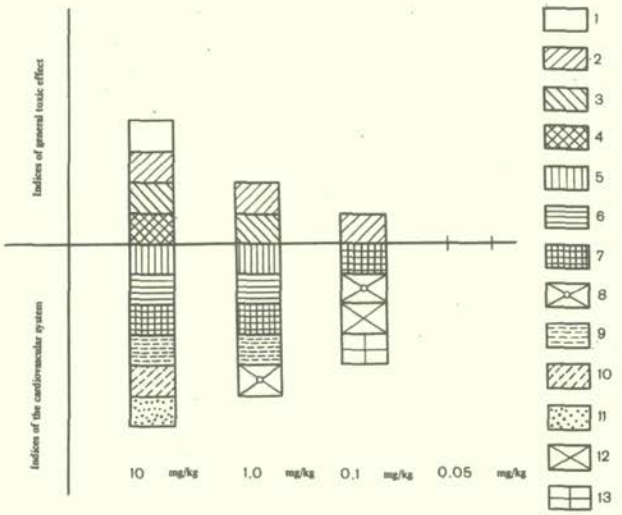


Fig. 34. Ratio of integral/specific (cardiovascular system) indices altered by chronic exposure to sodium fluoride.

1 — body weight; 2 — mass coefficient of internal organs; 3 — Summed Threshold Index (STI); 4 — respiration rate; 5 — arterial pressure; 6 — heart rate; 7 — capillary resistance; 8 — hexuronic acids; 9 — heart mass coefficient; 10 — cardiometry; 11 — Mass Coefficient of Peripheral Heart (MCPH); 12 — *hexosamines/oxyproline*; 13 — *β-lipoproteids*

in the phase of apparent wellbeing, which could have been mistaken for adaptation, they revealed latent and temporarily compensated changes of myocardial conduction. Arterial pressure in the test animals fell repeatedly during the year and, on some occasions, the pressure drops were outside the limits of physiological variations.

Changes of capillary resistance also occurred in phases in the sense that the periods of lowered resistance were succeeded by normal ones. Significantly, the phases of modified regulation of the myocardium, vessels and capillary resistance coincided, suggesting a generalized process responsive to common regulation.

That impaired lipid metabolism is involved in the emergence of vascular changes in old age (as also in human atherosclerosis) is well-known. It is commonly judged from the blood concentrations of cholesterol, phospholipids and ß-lipoproteids. While no changes took place in the phospholipid and cholesterol levels under the effect of sodium fluoride, the concentration of ß-lipoproteids was nearly halved by a fluorine dose exposure of 0.1 mg/kg (in one-year-old animals). Similar changes were noted in the intact animals of the senior age group (two to three years old).

Also noted were compositional and structural changes of collagen and elastic fibres. The effect of fluorine in two doses (10 and 0.05 mg/kg, paradoxical in effect) induced a reduction of collagen

Table 43

Results of Chronic Sodium Fluoride Exposure

Chemicals	Sodium fluoride dose over a year				
	control	0.05 mg/kg	0.1 mg/kg	1.0 mg/kg	10.0 mg/kg
Oxyproline (aorta), mg/g	31.0±1.21	33.3±1.02	34.2±1.41	33.1±1.41	30.6±0.9
Hexosamines (aorta), mg/g	9.1±0.3	9.3±0.43	8.5±0.29	8.7±0.28	8.9±0.33
Oxyproline/hexosamines	0.29±0.01	0.28±0.03	0.25±0.01 *	0.26±0.01	0.29±0.01
Hexuronic acids, mg/g (of aorta)	7.5±0.22	8.1±0.19	6.7±0.30 *	6.8±0.25 *	6.9±0.29
GAG (aorta) histochemistry, conv. units	4.23±0.25	4.4±0.51	3.73±0.33	4.82±0.26	5.16±0.32 *
Cholesterol (serum), mmole/l	1.21±0.11	1.70±0.37	1.47±0.27	1.49±0.37	1.21±0.13
Beta-Lipoproteids (serum), mg/l	495±86	478±71	263±26 *	563±62	474±52
Phospholipids (serum), mmole/l	267.4±10.1	288.1±41.3	267.4±26.0	262.3±26.0	255.8±26.0

* $p<0.05$.

Table 44

Cardiometry in Rats Chronically Exposed to Sodium Fluoride

Dose, mg/kg	Left ventricle, mm	Right ventricle, mm	Height of heart, mm	Width of heart, mm	Left/right ventricle	Relative heart weight
Control	3.5±0.7	1.0±0.7	14.5±0.24	20.0±0.57	3.7±0.4	0.248±0.015
1.0	3.3±0.18	0.97±0.09	14.7±0.34	19.0±0.46	3.4±0.8	0.278±0.008 *
10	3.7±0.25	1.24±0.09 *	15.0±0.35	20.0±0.57	3.5±0.2	0.314±0.008 *

* $p<0.05$.

contractility (identified on the model of collagen filaments from rat tails). The 0.1 mg fluorine dose modified the hexosamines/oxyproline ratio of the aortal wall in one-year-old animals (Table 43). The hexosamines-oxyproline relationship, the value which Sobel (1962) believes can be the test for determining "biochemical age", increased in the adult control albino rats 1 to 1.5 years of age and decreased in the "old" animals aged 2 to 3 years. The initial rise in the index fell to the period when formation of the animal organism was nearing completion and was largely due to the increase in the hexosamine level. The subsequent reduction of the index in "old" animals is attributable to the expansion of non-collagen proteins in the aortal wall, unaccompanied by a change of the oxaproline level. The fluorine ion has thus caused several indices of protein and fat metabolism to change in a way typical of accelerated ageing. Such a trend in the processes is also observed in the case of atherosclerosis.

With age, the intima and the internal third of the middle tunica of the artery become accumulation sites of an intermediate homogenous and metochromatically stained substance of a mucoid nature. This is causally linked with a buildup of acid mucopolysaccharides (AMPS), mainly at the expense of the chondroitin sulfate B and heparitin sulfat complexes labelled by the quantitative histochemical method on A. V. Tutnova's MUF-5 cloth spectrophotometer. The augmented AMPS content in the aortal media of the test animals given the fluorine dose of 10 mg/kg over one year points to changes common to ageing vessels. This is in contrast with the reduced AMPS biochemically detected in the aortal wall. Such a disparity between the results of biochemical and histochemical investigations of the contents of acid mucopolysaccharides is frequently to be found in the literature. One possible explanation is that the histochemical method (staining with toluidine blue at pH=4.0) identifies the entire AMPS complex, whereas the biochemical method (the level of hexuronic acids) shows the total AMPS content minus keratan sulphate. In addition, the AMPS content may vary at different levels of the aorta, as some AMPS (chondroitin sulfate B, heparitin sulfate) increase in quantity and others (hyaluronic acid) decrease with progressive ageing. The identified alteration of the AMPS amount in the aortal wall way be largely a result of interference with carbohydrate metabolism.

Animals exposed to fluorine in water for a long time exhibited no morphological changes either in the wall of the elastic type vessels (aorta) or those of the musculoelastic type (e. g. mesenteric arteries). This suggests that there might be a lengthy period of compensation for the functional and biochemical irregularities noted above. A macroscopic assessment of the morphological substrate of biochemical changes in the myocardium (inhibition of glycolysis) by the cardiometry method has demonstrated the unequal probability of compensation at different exposure levels to fluorine ion. The changes induced by the 10 mg/kg fluorine dose referred not only to the relative weight of the heart, but also to a modified thickness of the ventricular walls (Table 44), thus giving proof of crude morphologic rearrangements.

Fluorine ion in certain doses (0.1 to 10 mg/kg) thus produces, by prolonged exposure, certain changes in the cardiovascular system which can be regarded with a high degree of confidence as a manifestation of accelerated ageing.

By the inhalation entry route into the body and by complex exposure (inhalation plus per os injection), sodium fluoride changes functional and biochemical indices in a similar manner. The effect is thus potentiated to be way above its level associated with the toxic's separate entry via the respiratory tracts and gastrointestinal tract.

The data gained from the experiment with sodium fluoride may serve to substantiate a revision of the MAC for fluorine and its inorganic salts in the air of the working zone, on the one hand, and support the validity of the hygienic standards for fluorine ion concentration water bodies, on the other.

The evidence resulting from **papaverine** exposure suggests, when analyzed, a similar trend for the toxic's action, irrespective of the route of entry into the body. At all levels of exposure and in the long-term of nine months after inhalation, changes in the indices showing the systemic condition of the body were noted along with a specific, hypotensive effect of papaverine. The latter was responsible for a modified tension of the musculoelastic vessels, an immediate result of the chemical's vasobilative and hypotensive effect (Fig. 35).

A more in-depth assessment of the state of the body also integrating the characteristics of the biochemical composition of the connective tissue of the aorta immediately after the chronic experiment with a papaverine per os administration (over one year — the 1st group and in the longer term, after nine months — the 2nd group) has revealed changes in the concentration of hexuronic acids that sum up the content of mucopolysaccharides in the aortal tissue (Table 45).

Table 45

Biochemical Indices in Chronic Exposure to Papaverine (a study of test animals' aortas)

Indices	Group	Dose, mg/kg		
		10	1	control
Oxyproline, mg/g	1st	35.8±1.6	32.4±1.6	36.0±1.5
	2nd	37.4±4.3	33.8±1.1	33.7±2.1
Hexosamines, mg/g	1st	11.0±0.3	10.8±0.4	11.3±0.4
	2nd	12.0±0.3	12.0±0.6	1.3±0.32
Hexuronic acids, mg/g	1st	10.8±0.12 *	10.2±0.08	10.1±0.09
	2nd	10.1±0.24	10.2±0.21 **	9.7±0.007

* $p<0.01$; ** $p<0.05$.

The data thus obtained were used for hygienic standardization of the chemicals analyzed and for a further updating of the existing

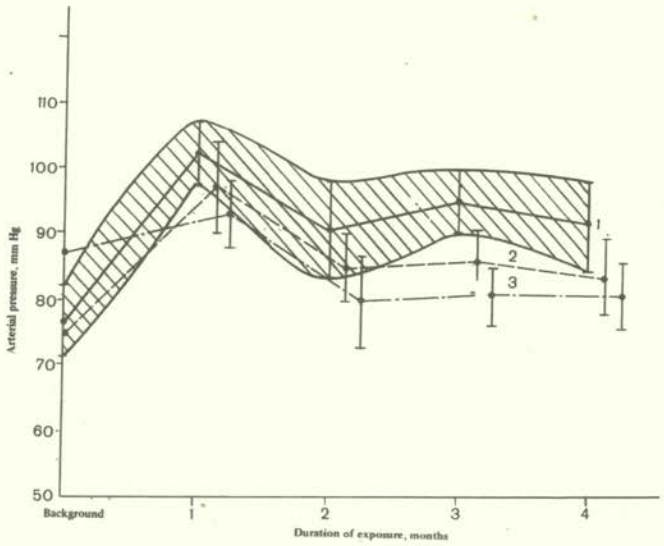


Fig. 35. Variation of arterial pressure in the rat after chronic papaverine inhalation exposure.

1 — control (hatched strip denotes confidence intervals); 2 — 0.1 mg/m^3; 3 — 1.0 mg/m^3

MAC for the air of the working zone (1 mg/m^3 for carbon disulfide and 0.1 mg/m^3 for papaverine).

The experimental evidence accumulated over the years on the impact of **lead** in minimum concentrations is conflicting, primarily because of the different lead compounds that were used to model lead intoxication. The variability of the toxicities of different lead compounds and their degrees of cumulation in the body at lethal levels, is widely recognized. This may also be true of exposures to minimum amounts of different lead compounds. Although the "acetate" model of lead intoxications is employed more frequently than other, if not altogether justifiably, this is done without due regard for the possible combined effect of acetic acid and lead ions.

The findings of a subacute experiment with exposure to metal lead and lead acetate in concentrations at the level of the existing MAC in the air of the working zone, designed to test the "acetate" model of lead intoxication, are presented in Fig. 36 (Kudarov, 1976). One week after the exposure began, the left ventricular wall was found to be thickened, probably because of the heavier load on that compartment of the myocardium. Besides, the same group had an increased quantity of reticulocytes in the peripheral blood and a declining content of total seral protein.

A fortnight after the onset of the exposure, neither test group diverged practically from the control in any of the chosen indices. After one month (Fig. 37) of lead acetate exposure the 2nd-group

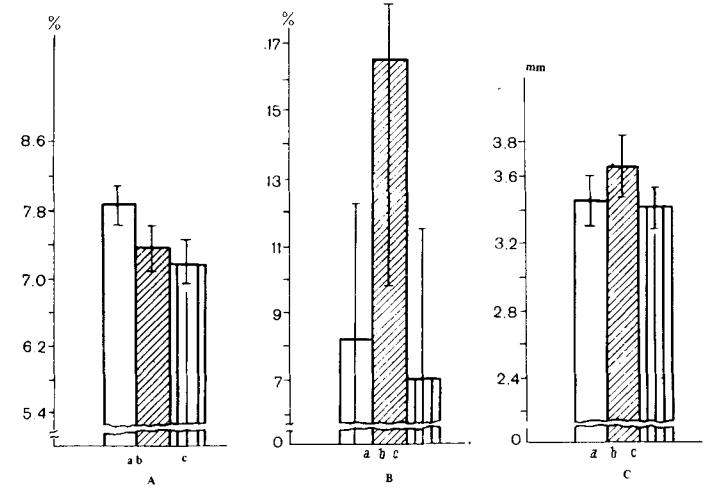


Fig. 36. Results of one-week subacute exposure to lead acetate and metal lead.
A — total protein of blood serum. B — quantity of reticulocytes. C — thickness of left ventricle; a — control; b — Pb-metal (0.01 mg/m³); c — Pb-acetate (0.017 mg/m³)

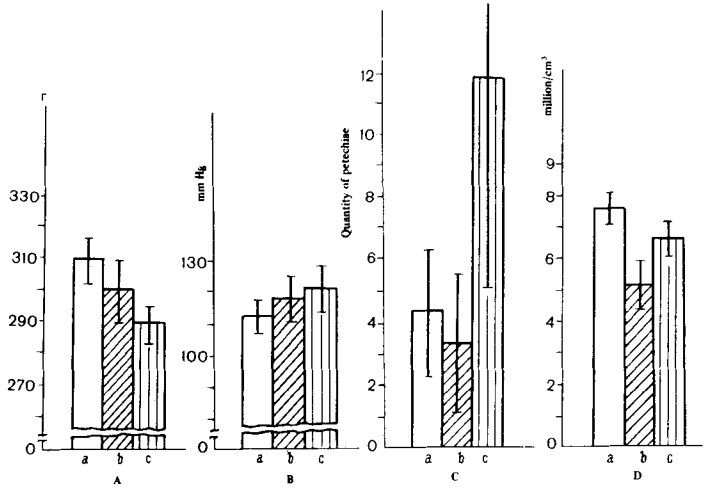


Fig. 37. Results of one-month subacute exposure to lead acetate and metal lead
A — body weight; B — systolic arterial pressure; C — capillary resistance; D — quantity of erythrocytes; a — control; b — Pb-acetate (0.017 mg/m³); c — Pb-metal (0.0108±0.008 mg/m³)

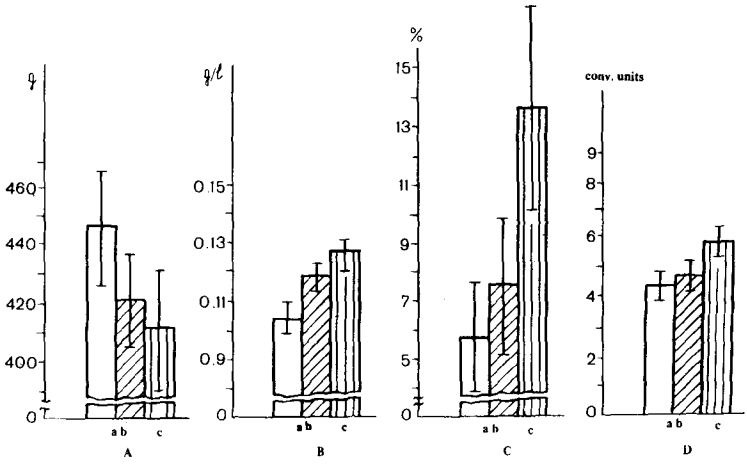


Fig. 38. Results of one-month chronic exposure to lead acetate and acetic acid.
A — body weight; B — haemoglobin concentration; C — quantity of reticulocytes; D — STI; a — control; b — acetic acid (3 mg/m^3); c — Pb-acetate (0.036±0.004 mg/m^3)

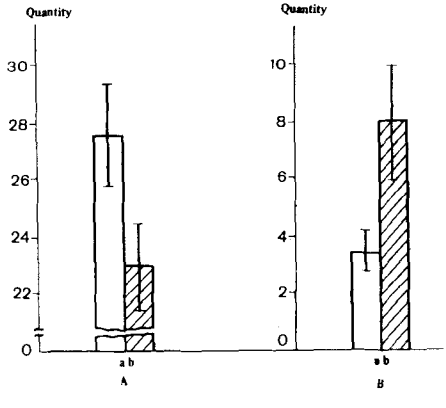


Fig. 39. Gonadotropic effect of lead acetate.
A — normal spermatogonia; B — tubules with desquamated epithelium; a — control; b — Pb-acetate (0.036±0.004 mg/m^3)

animals developed more numerous changes than did the control or the 1st group. They occurred not just in the indices specific to lead, such as the quantity of peripheral blood erythrocytes and the resistance of skin capillaries, but also in the integral indices relative to the condition of the body, that is, body weight and systolic arterial pressure. Also, there was stronger anaemization resulting from the effect of metal lead.

Thus, some qualitative dissimilarities were brought to light in the experiment with parallel exposure to metal lead and lead acetate.

With a view to specifying the role of acetic acid ion, a chronic experiment was carried out, in which exposure to the MAC-level concentration of lead acetate went in parallel to that of acetic acid. The latter's concentration was estimated so as to have the 0.01 mg/m^3 concentration of lead (from lead acetate) in the air of the inhalation chamber matched by an acetic acid concentration of about 3 mg/m^3, this being the MAC of acetic acid in the air of the working zone (Fig. 38 through 41).

Dynamic observation of the animals during this trial series in the chronic experiment has revealed the tested concentration of acetic acid to be virtually innocuous (see Fig. 38). A one-time decrease in animal body weight in this group (the 2nd month of the exposure) and an expanded quantity of haemoglobin are within the range of natural variations of the indices in the control animals and cannot be considered important and meaningful for the body. Among the animals exposed to lead acetate, numerous changes characteristic of lead intoxication were recorded both during dynamic observation throughout the four-month experiment and after its termination. This series of trials has once again confirmed the gonadotropic effect of lead (see Fig. 39) and its absence in animals exposed to acetic acid.

A morphometric analysis of the musculo-elastic vessels of animals exposed to lead acetate revealed some thickening of the vessels's middle tunica (10.5 ± 0.2 μm against 10.0 ± 0.1 μm in the control, $p < 0.05$ and, as a consequence, a reduced ratio between the thickness of the middle tunica and intima (0.52 ± 0.01 against 0.55 ± 0.01 in the control, $p < 0.05$). The diminished content of ß-lipoproteids in the same group (409 ± 44 mg/l against 581 ± 47 mg/l in the control) agrees well with the previously recognized dynamics of accelerated ageing processes (Sanotsky et al., 1976). The myocardium of the animals exposed to lead acetate, when morphometrically assessed, exhibited a decreased average thickness of the muscular fascicles (Fig. 42). In the animal exposure to acetic acid, these changes were not significant by comparison with those in the control.

The lack of alterations in the composition of the connective tissue of the aorta in both test animal groups is consistent with their absence established by morphometric analysis of the aorta.

It seems obvious that the effect, other than that of metal lead, noted in the subacute experiments arose not from acetic acid ion, but rather from its combination with lead ion. It should be stressed that the changes due to the effect of lead acetate and metal lead

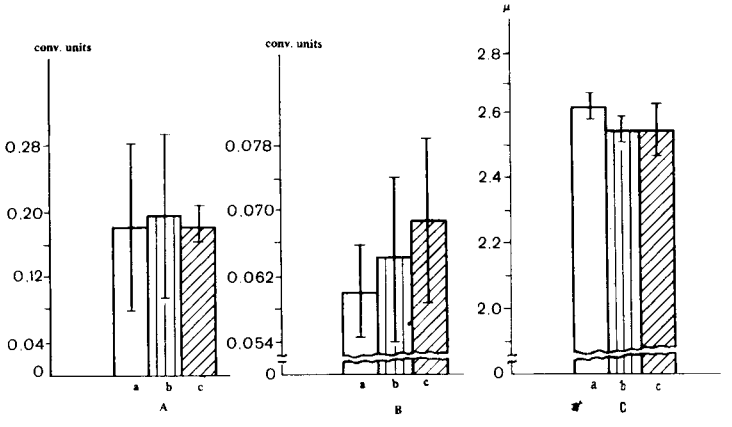


Fig. 40. Results of chronic action of lead acetate nine months after poisoning.
A – connective tissue/myocites ratio; B — nucleus/cytoplasm ratio; C — thickness of myocardial muscle fascicles;
a — control; b — Pb-acetate (0.036 ± 0.004 mg/m^3); c — acetic acid (3.0 mg/m^3)

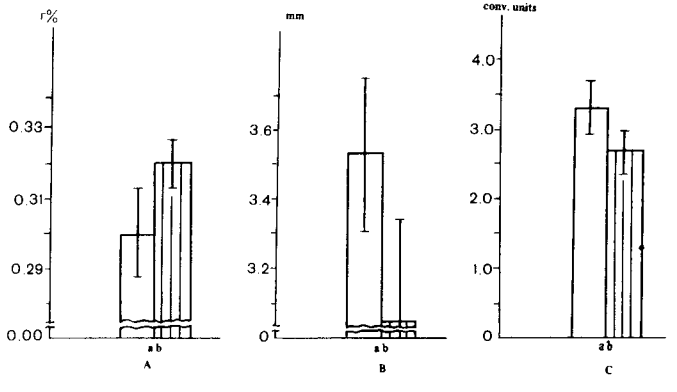


Fig. 41. Results of myocardial morphometry after chronic exposure to lead acetate.
A — mass coefficient of heart; B — thickness of left ventricle; C — left/right ventricle thickness ratio;
a — control; b — Pb-acetate (0.036 ± 0.004 mg/m^3)

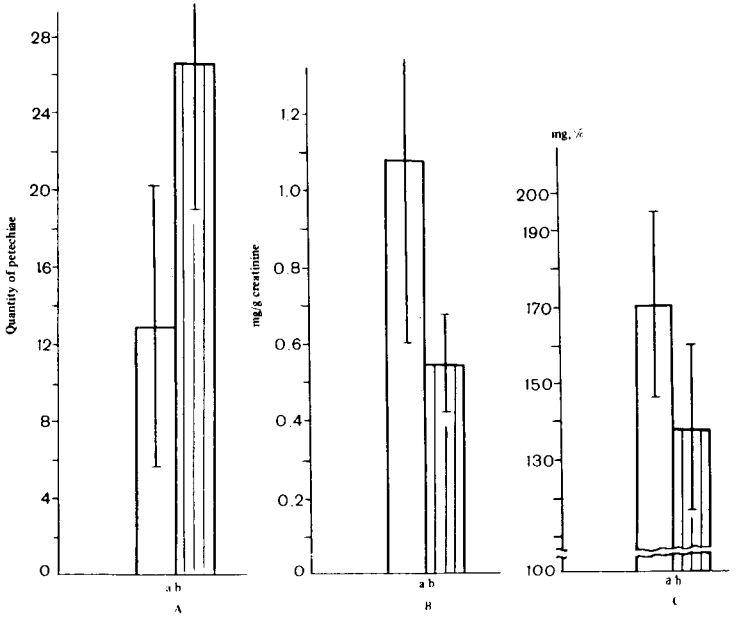


Fig. 42. Results of chronic exposure to lead acetate in MAC-level concentrations in the air of the working zone.

A — capillary resistance; B — ALC in urine; C — glycogen in myocardium; a — control; b — Pb-acetate (0.036 ± 0.004 mg/m^3)

show the same trend. A minor degree of potentiation of the effect in the case of the lead acetate exposure will be taken into consideration by us in further evaluation of the short- and long-term effects of the poison.

During additional trials in the chronic experiment, a more detailed analysis of the test animals' ECG involving functional pituitrin and neostigmine loads has revealed solitary and, in our view, largely random deviations of some parameters between the test animals and the controls (Table 46). However, a morphological analysis of the heart

Table 46

Heart Rate in the Rat Following Chronic Inhalation Exposure to Lead Acetate Compounded by Neostigmine Load

Animal groups	Background	Time after load, min	
		20	30
Control	380±27	323.3±17	333.3±21
Lead acetate (0.01 mg/m^3)	400±21	370±11 *	371.3±16

* $p<0.05$.

(see Fig. 37) disclosed serious changes not merely in terms of its relative with (probably due to the lagging increment of body weight), but also in the thickness of the left ventricular wall. This animal group was found to be predisposed to hypertension.

Histometry of the left ventricular myocardium in the test animals yielded results no different from the control. In the animals chronically exposed to lead acetate, their elastic membranes were morphometrically found to have been thinned, with no other parameter altered from their control levels ($2.62 \pm 0.13 \mu m$ down from $3.05 \pm 0.12 \mu m$ in the control). The data may be regarded as suggesting changes in the elastic properties of these formations (a declining ability to contract), which is but a phase of elastic degeneration.

At nine months through the recovery period, there was a noted (see Fig. 42) reduction of the Δ-ALA urinary content, a sign of compensatory rearrangements in porphyrin metabolism, along with lowered resistance of the capillary wall and a decrease of glycogen in the myocardium. The latter two signs are somewhat alarming in view of the previously established (immediately upon termination of the four-month experiment) trend displayed by the action of lead on the heart and blood vessels. The lower quantity of the glycolysis substrate in the myocardium might suggest a strain on the processes of energy supply for cardiac activity.

Conclusion. Our data prove that a short-term biological experiment enables one to see whether the action of chemical agents is indeed targeted on the heart and blood vessels, provided the integrated approach is taken to evaluating the state of the cardiovascular system. It is worthy of note, however, that additional efforts are needed to ascertain the hygienic importance of the recognized changes.

In our judgement, a particular hazard looms from the chemical compounds with a potential for selective action on the heart and blood vessels. The degree of selectivity can be estimated from the general rules (the ratio of the integral/specific action thresholds). Of the toxic chemicals examined by us, carbon disulfide and TMTD are the only ones with a certain potential for selective action on the processes responsible for the ageing of the cardiovascular system. For lead, sodium fluoride and papaverine, the effect is seen at the same levels as the systemic toxic effect. Interim data on the influence of other industrial toxic chemicals (vinyl chloride, vitamin PP, antimony-containing ore, etc.) also point to the risk of accelerated ageing of the heart and blood vessels on exposure to these compounds' doses and concentrations that give risk to a systemic toxic effect.

The experimental results from exposures to lead acetate, sodium fluoride, papaverine and carbon disulfide suggest some predictive value for the methodological approach used to estimate the development rate of ageing processes in the heart and blood vessels.

There is an obvious need for large-scale epidemiological and clinical-statistical surveys of employee groups handling chemical compounds at work. There are gaps to fill in our present knowledge of possible chemical effects on the origin, development and outcome of

cardiovascular pathologies. The difficulties associated with selecting suitable test groups from among workers with adequate work experience and the general public, exposed at work or at home to a large variety of chemical agents, including agricultural, domestic and communal pollutants, should not discourage investigators. Special attention to the state of the heart and vessels is needed where extensive worker and public contingents are exposed to the analyzed chemical.

Results will be most suitable and reliable if obtained through synthesis of broad clinical-epidemiological (with due regard for findings from analysis of autopsies) and integrated experimental studies. The evidence thus gained will serve the purpose of reliable health protection and prevention of cardiovascular diseases.

Chapter 7

PREDICTION OF THE LONG-TERM EFFECTS OF INDUSTRIAL TOXIC CHEMICALS

Because the research into long-term effects from exposure to industrial toxic chemicals requires considerable time and effort, it is essential to be able to predict such effects. The emergence of malignant neoplasms, congenital development defects and other injuries of the reproductive function, as well as premature ageing are perceived to originate from specific structural characteristics of the effective agent.

PREDICTION BASED ON CHEMICAL STRUCTURE

The literature describes numerous attempts to trace the relationship, if any, exists between biological activity of chemical carcinogens and their physical and chemical properties, such as electron configuration of molecules, the ability to form free radicals, etc. (A. Rietan, V. Rietan, 1955; Higer, 1961; Emmanuel, 1975, and others). Comparison of these data shows, nonetheless, only a certain degree of correlation between the biological effect and physicochemical properties. Similar and not very dependable correlations were found for mutagens and teratogens. More encouraging results from prediction, for example, of the potency of mutagenic effect were shown demonstrably with respect to one chemical series by Rapoport (1966) and others.

On the other hand, a clear view of such relationships can help identify particular groups of substances as being active mutagens, blastomogens or embryotropic agents. For example, these are groups of chemicals known to show genetic activity (Nikiforov, 1965; Kovalenko, 1967; Dubinin, 1970, etc.). These classifications, while rather conventional and sometimes conflicting with the generally accepted nomenclature in chemistry, prove useful in practice. Given below is just such a composite classification of chemical mutagens compiled from literary data inputs.

Name of class	Examples of class members
1. Alkylating agents:	yperites, ethers of sulfuric and alkansulfonic acids, epoxides, ethylenimines, diazo compounds, lactones, etc.
2. Nitro compounds:	hitrous acid, nitrosamines, nitrosamides, nitrosoguanines, etc.

3. Hydroxylamines:	hydroxyl amine, etc.
4. Peroxides:	tretbutylperacetate, etc.
5. Aldehydes:	formaldehyde, etc.
6. Antimetabolites: (among them structural analogs of nucleic acids):	azaguanine, azacerine, urethane, etc.
7. Salts of some variable valency metals:	manganese, aluminium, etc.
8. Acridine dyes:	proflavin, acridine orange.

Principal classes of chemical having blastomogenic effect were established as consisting of polycyclic aromatic corbohydrates (PAC), alkylating agents, azocompounds, aromatic amines, oxycompounds, some metals and metalloids, nitrosamines, microbial carcinogens (aflatoxins, etc.).

There are also groups of substances known to be embryotropic agents.

Major classes of chemicals with an embryotropic effect

1. Unsaturated hydrocarbons
2. Aromatic hydrocarbons
3. Amides of acids
4. Polychlorinated compounds
5. Oxycompounds
6. Antibiotics
7. Vitamins
8. Cytostatics, etc.

Comparison between the recognized and previously examined classes of blastomogenic, mutagenic and embryotropic agents indicates that the same agent may be capable of producing all such effects.

With respect to the impact of chemicals on the gonads, there is not yet any generally accepted approach to forecasting this effect through analysis of the structure of a chemical and the involvement of specific functional groups. Only for a relatively few chemical compounds like antimetabolites, alkylating agents, etc. have certain links been suggested between their chemical structure and the effect on the gonads (Avkhimenko, Golubovich, 1969; Chirkova, 1970).

Thus, the proposed method for predicting these forms of long-term effect is not reliable. Chemicals categorized as potentially hazardous on the basis of their chemical structure are much too numerous, while among structurally similar compounds sometimes only one may be capable of affecting inheritable structures. For example, two chemicals as closely related as ethylene and propylene oxides, when tested for mutagenicity in our experiments, proved to be rather remote from each other (mutagenicity for mammals was found in ethylene oxide only). An equally wide disparity was detected by us in the ethylenimine series, notably for piperidine and cyclosiloxanes. On the other hand, this method, in conjunction with other techniques, offers a way of defining, in general terms, the compounds to be further investigated for possible risks associated with long-term effects.

It must be noted that, in principle, the answer concerning the particular properties (biological included) of substances should be sought precisely in the structure of its molecule, but the clue to the mystery is yet to be found.

PREDICTION BASED ON IN VITRO TESTS

A large number of rapid tests have been proposed to predict certain long-term effects. Central to most of them is the search for precursors of mutagenic (and carcinogenic) effects through experiments in vitro.

The following tests are those most often used for "screening" carcinogens.

Ames' test. This is conducted on Salmonella typhimurium (strains TA-1535, 1538, 98 and 100) in a medium where the microsomal liver fraction (S=9) of test animals (rats) is added to make metabolic activation of the test chemical possible if necessary.

The test chemicals is added in varying proportions to the incubation medium, and back mutations are defined by the type of proof-reading shift or the substitution of bases.

Cell transformation test. This involves estimating the growth of somatic cell cultures on semisolid agar, which is achieved by using fibroblasts from newborn Syrian hamsters (BHK 21/C 13), diploid fibroblasts of the human lungs (WI=38) or human liver cells. A microsomal fraction (S=9) of liver cells is added to the incubation medium to allow for possible metabolic activation of a carcinogen. Blood serum is not added. Then the likelihood of colonies forming in a liquid medium after six-to eight days incubation with five different concentrations of the test carcinogen is estimated and Cl_{50} is computed. The surviving cells are transferred to semisolid agar. The growth rate and number of transformed colonies are calculated at Cl_{50} and, if the colonies show a 2.5-fold increase over the control, the test result is said to be positive and the test substance a potential carcinogen.

Williams' and Rabin's test. The endoplasmatic reticulum isolated from the rat liver is freed from ribosomes and kept under observation using an isotope technique. If, during in vitro incubation with a suspect carcinogen, the endoplasmatic reticulum shows a degranulation of 10 per cent or more in comparison with the negative control, the response is considered positive.

Purchase and associates (1976) showed an approximately equal rate of positive responses from the evaluation of 58 recognized carcinogens and 62 non-carcinogens according to the Ames, cell transformation, and Rabin tests (91 and 93, 91 and 97, and 71 and 71 per cent, respectively).

Nevertheless, the authors single out the Ames and cell transformation tests as being more suitable for preliminary screening.

In our view, the in vivo tests that the authors used merely for comparison are more reliable. For example, the implantation test or the test for atrophy of sweat glands can perhaps be considered quite promising.

Implantation test. This involves assessing the structure of a fibrose capsule and surrounding tissues three months after surgical subcutaneous introduction into the mouse of a small circle made of the Millipore filtre and coated with a suspension of the test chemical in gelatin. The state of the capsule is evaluated in terms of a conventional scale from 1 (low) to 5, as specific elements appear. A statistically significant excess of the medium score is believed to be a positive response.

Test on murine skin sweat glands. The test chemical is applied to murine skin and a numerical reduction in the sweat glands compared to hair follicules is held to be a positive sign of carcinogenicity.

Tetrazole reduction test. The test chemical is applied to murine skin and post-exposure skin samples are placed in a tetrazole water solution. The reduction rate of the colourless tetrazole into stained formazane is estimated by colourimetry. A statistically significant increase in the tetrazole reduction rate is taken to suggest a carcinogenic hazard in the test chemical. Purchase and associates report that the implantation test gives 95 per cent positive reactions with non-carcinogens against merely 37 per cent positive responses to the effects of carcinogens. The tetrazole reduction test offers, respectively, 73 and 40 per cent positive responses. The test for sweat gland atrophy is positive in two-thirds of the trials, relative to both non-carcinogens and carcinogens.

One of the latest systems for forecasting carcinogenicity was proposed by Montesano and Tomatis (1977). It consists of genetic tests in vitro as well as in vivo; tests on microorganisms or mammalian tissue cultures (with or without metabolic activation); the host mediated method; studies using Drosophilla; cytogenetic analysis of mammals in vivo; dominant lethal mutations; investigation of neoplastic cell transformations in culture; and research into DNA reparations.

We consider mammalian studies comparing the thresholds of the various effects involving an impact on the cell's developmental machinery to be more promising.

CORRELATION OF SPECIFIC LONG-TERM EFFECTS

This method has been tested by us for several years as part of the research programme to establish correlations between the gonadotropic, mutagenic, blastomogenic and embryotropic effects of chemicals. One important consideration is the varying amount of work needed to investigate each of the above effects.

Studies by Soviet and foreign investigators have showed the correlation of mutagenic with blastomogenic (Rapoport, 1969; Platonova, Pogosyants, 1969; Bryan et al., 1969) and of blastomogenic with teratogenic effects (Elis et al., 1964; Elis, 1972).

On some occasions, though, the effects did not coincide. In part, this is because mutagenicity was investigated primarily on Drosophilla and microorganisms, while blastomogenicity on mice and other mammal species. Yet the use of the same test subject and preferrably the same tissue is an absolute necessity for such studies (Platonova, 1969).

We have analyzed a number of chemical compounds, and the data obtained are summarized in Table 47. It follows from the Table that, as animals were exposed to the same compounds (chloroprene, ethylenimine, urethane, 1,3-chlorobrompropane, tryphthazine, etc.), a positive correlation appeared between the specific effects.

It must be emphasized that the experimental data suggesting mutagenic and gonadotropic action for chloroprene fit the clinical findings from a questionnaire and spermatological survey (see Chapter 5). From an immediate survey of exposed workers the three effects decisive for the impact of the poison upon the generative function were found to be closely interrelated. In addition, there is reason to suspect chloroprene of having blastomogenicity.

A similar concurrence of all the specific effects under study has been discovered in the action of organomercury compounds from the carbamate category on rats (Vashakidze, 1970) and also for a number of other chemicals.

Published experimental and clinical data show concurrent embryotropic, mutagenic and gonadotropic effects produced by metals, such as manganese (Mandzhgaladze, 1965, 1969), cadmium (Kar, Das, 1962, etc.), mercury (Sanotsky et al., 1967; Panova, 1974; Goncharuk, 1977) and lead (Egorova et al., 1966; Golubovich et al., 1968; Panova, 1974; Krasovsky et al., 1977).

Several other compounds (ethylene oxide, cyclohexanone, formaldehyde, aminopyridine, carbon disulfide, etc.), when tested on animals, exhibited manifestations of some effects and the absence of the other ones.

No mutagenic/gonadotropic correlations in the sense of gonadotropic effect unaccompanied by a mutagenic effect were detected for amidopyridine exposure.

Carbon disulfide exposure of pregnant rats throughout the gestation period in concentrations of 2.2 ± 0.68 and 13.3 ± 2.8 mg/m^3, the level of the former MAC, displayed systemic and embryotropic toxicity. The same toxic, at the same exposure levels, however, had neither a predominant influence on the gonads nor a mutagenic (cytogenetic) [1] action. Finally, the third category of poisons

[1] Sokolov and associates (1972) reported an increase in chromosome aberrations in the lymphocyte culture of the peripheral blood from a survey of carbondisulfide intoxicated workers.

Table 47

Correlation of Effects [1]

Substances	Concentrations	Levels	Effects			
			M	G	E	B
Dimethyl acetamide	20.0 mg/m^3		+	+	+	
	1.0 "	MAC		−	−	
Morpholine	70.0 "	10 Lim_{ch}	+	−		
	8.0 "	Lim_{ch}	+	−		
Tryphthazine	0.66 "	10 Lim_{ch}	+	+		
	0.06 "	Lim_{ch}	−	−		
Benzene	583 "	1/2 Lim_{ac}	+	−		
	23 "	Lim_{ch}	+	−		
Cyclohexane	105.2 "	10 MAC	+	−	+	
	11.5 "	MAC	+	−	−	
Urethane	1.0 "	10 Lim_{ac}	+	+	+	+
	0.1 "	Lim_{ac}	+			−
Binary mixture	200.0 "	Lim_{ch}	+	+		−
(methyl and ethyl	32.0 "	1/10 Lim_{ch}	−	−		−
carbamates)	1.0 "	1/100 Lim_{ch}	+			+
	0.01 "		−			−
Methyl carbamate	1.0 "	10 Lim_{ac}	+			−
	0.1 "	Lim_{ac}	−			
	0.01 "	1/10 Lim_{ac}	−			−
Dinyl	1130 "	1/5 DL_{50}	−	−		
	570 "	1/10 DL_{50}	−	−	+	
	280 "	1/20 DL_{50}	−	−	+	
Ethylenimine	2.4 "	1/4 Lim_{ch}	+	+		
	0.8 "	Lim_{ch}	+	+		
	12.0 "	Lim_{ac}	+	+	+	
Chloroprene	0.16 "	1/10 Lim_{ch}	+	+		
	1.69 "	Lim_{ch}	+	+	+	
	0.13 "	1/10 Lim_{ch}	+	+	+	
	0.05 "	MAC	−	−	−	
Aminopyrimidine	11.4 "	Lim_{ch}	−	−		
	1.4 "	1/10 Lim_{ch}	−	−		
1,3-chlorobrompro-	45.0 "	Lim_{ch}	+	+		
pane	5.4 "	1/10 Lim_{ch}	−	−		
Ethylene oxide	112.0 "	100 MAC	+	−		
	30—60 "	20 MAC	+	−		
	1—3 "	MAC	+	−		
Carbon tetrachlo-	40.0 "	Lim_{ch}	+	−		
ride						
DMFA	10.0 "	MAC	−	−	+	
	2.5 "	1/4 MAC	+	+	+	
Formaldehyde	5.0 "	MAC	−		+	
	0.5 "	10 MAC	−		+	
3,4-BP	1.0 "		+		+	
	0.01 "		−		−	
HPTB	107.0 "	~/0	+	−	+	
	17.0 "	Lim_{ch}	+	−	+	
Lead	0.5 mg/kg		−	+		
	0.05 "		+	+		
Manganese	26.0 "	1/2 Lim_{ac}	+	+	+	
	6 "	1/10 Lim_{ac}	+	+	+	
Neozone D	20 "	~Lim_{ch}	−		+	±
	1 "	1/10 Lim_{ch}	−		+	±

Table 47 (contd.)

Substances	Concentrations	Levels	Effects			
			M	G	E	B
Butyl ether	0.4 mg/kg		+	+	+	
2,4,5-T	0.001 "	Lim_{ch}	+	—		

Notes: M — mutagenic, G — gonadotropic, E — embryotropic, B — blastomogenic.

[1] The data used were those derived by the laboratory of General Toxicology, Research Inst. of Ind. Hygiene and Occup. Dis., USSR Academy of Medical Sciences; Institute of Agr. Hyg., Saratov; and Inst. of Ind. Hyg. and Occup. Disease, Georgia, USSR.

are those that produced none of the effects being studied in test animals. This should probably be seen as a negative correlation of the effects (according to selected parameters).

While judging the effectiveness of predictions based on a concurrence of specific long-term effects, it should be borne in mind that they are probably associated with both the direct and indirect action of toxic chemicals on cell division processes, and with the effective concentration (dose) level.

The physiological characteristics of the functions examined, the dynamic pattern of variation in some indices, the accuracy of the methods used, and several other factors can hamper the evaluation of the degree of correlation between the effects.

Drawing on their experience these authors have developed the following concepts on that matter. Overall, the results from studies on the gonadotropic effect of several chemical compounds from different classes proved of little use for predicting other effects, as no stable correlation was established between the gonadotropic and these other effects.

A large prognostic potential relative to the blastomogenic and, to a lesser extent, the embryotropic effect has been identified in the cytogenetic analysis of the somatic tissue of test animals.

A review of the literature indicates, therefore, that direct correlations of genetic, embryotropic, blastomogenic and, to some extent, gonadotropic actions do exist. Yet the concurrence of their action thresholds, the most essential characteristic for preventive purposes, is not very common. Apart from that, one important fact is the much earlier manifestation of the cytogenetic effect on exposure to blastomogens, as well as lower action thresholds than that of blastomogenicity (e. g. in the case of ethyl carbamate, ethylenimine and other toxics). This fact is rather significant, as it can give added reliability to a hygienic standard.

A note is in order that the gonadotropic effect is normally exhibited during the first half of the chronic experiment (within the duration of one spermatogenetic cycle) and thus lends itself to relatively rapid evaluation.

TRANSPLACENTAL INDUCTION OF TUMOURS AS A PROGNOSTIC METHOD

Recent years have seen much concern displayed over the possible manifestations of chemical blastomogenicity in offspring through transplacental exposure. This was triggered off by tragic episodes in which tumours of the genital organs appeared during adolescence in the daughters of women who had undergone hormonal treatment during pregnancy.

Studies of transplacental blastomogenicity have been completed with regard to polycyclic aromatic hydrocarbons (Shabad, 1927; Andrianov, 1971; Tomatis, 1965; Bulay, Wattenberg, 1971); the aminoazo compounds; orthoaminoazo toluene and dimethylaminoazo benzene (Fuks, Fridman, 1953; Gel'stein, 1961; Titova, 1973); the fluorenes: 2-acetylaminofluorene (Shabad, Kolesnichenko, Sorokina, 1975); the carbamates: urethane (Gripiute, 1955, 1957; Kolesnichenko, 1968, 1973; Zaeva et al., 1968; Kolesnichenko, Mikonova, 1971; Larsen 1947; Klein, 1954; Vesselinowitch, 1973); pineb (Kvitnitzkaya, Kolesnichenko, 1970) and other chemicals such as 6-methylthiouracil (Napalkov, 1971; Napalkov, Alexandrov, 1968), aflatoxins (Crice et al., 1973) and DDT (Shabad et al., 1975).

Examination of the literature and the experience of their own research enabled L. M. Shabad and his associates (1975) to deduce several general principles.

Under transplacental blastomogenesis, tumours appear when carcinogenic substances are administered during the last-trimester of the gestation period, i. e., at the foetal period or organogenesis.

The embryonic organism has been noted for its considerable sensitivity to the effect of carcinogenic agents. This results in a shorter latency of tumour development and their multiplicity and malignancy. In some instances, the effect via transplacental exposure was stronger than in the adult individuals given the same dose of the chemical. The spectrum of tumours resulting from transplacental exposure is wider and, not infrequently, includes kinds of tumour that never arise from exposure in the post-natal period.

Some compounds are blastomogenic exclusively after transplacental exposure. In our joint studies with G. I. Zaeva and L. S. Sal'nikova urethane exposure in mice (at a rate of 0.1 mg/kg, the threshold level by blastomogenic effect, administered subcutaneously) caused tumours lung (adenomas) as early as the third month in the life of their offspring. A research on the blastomogenicity of neozone D (phenyl-alfa-napthylamine) at the Lim_{ch}^{*} level of 20 mg/kg, according to L. S. Sal'nikova's data, showed the substance to be incapable of having carcinogenic action by transplacental exposure. Benign tumours thus identified, however, surpassed the control background significantly.

To conclude, transplacental tumourigenicity must be considered

as a possible specific long-term effect in evaluating the embryotropic effects of environmental factors. The method shows promise for use in determining the threshold of blastomogenic action for soluble chemical agents.

EXPERIMENTAL PREDICTION OF THE LONG-TERM EFFECTS OF TOXIC CHEMICALS ON THE CARDIOVASCULAR SYSTEM

The problem defined by the title is as yet in the research stages. Preliminary data suggest that an effective key to it may be comparison of pathogenetic indices relative to the impact of each specific poison in short and long (chronic) term experiments. Sodium fluoride and papaverine were used to demonstrate the possibility of predicting, even in short-term experiments, the degree of hazard resulting from the long-term effects that may arise. To this end, a set of biochemical, functional and morphological indices must be closely considered, as well as the responses of the body to pathogenetic loads.

Relative to prediction of the atherogenic effect from genetic alterations, it is worth considering the now widespread hypothesis concerning somatic mutations as a cause of ageing (Comfort, 1967). Although the mechanisms involved are not understood well enough, it would seem to be of interest to verify the correlation of cytogenetic effects (using the bone marrow as the most common kind of tissue in the research into mutagenic effects) and the accelerated ageing processes of vessels under the influence of chemical agents. Chapter 6 referred to the scarcity of data on the influence of chemical compounds upon the vascular ageing processes. Most compounds known to be involved there were also proved, however, to be mutagenic in clinical or experimental observations (see Chapter 5), such as e. g. carbon disulfide [1], lead, tetramethyl-thiuram-disulfide, etc.

For all that, any conclusions on the correlation between the effects would be premature and further data need to be accumulated to support the foregoing hypothesis. Admittedly, there does not yet exist an adequately grounded system for predicting the atherogenic effect of chemicals from their mode of action, but substances that affect the adipose and carbohydrate metabolism, along with antioxidants and thyrotropic agents, are suspect in this respect. Similarly, the study of substances that can influence vascular tension, arterial pressure and the myocardium requires a guarded approach. In fact, additional experiments need to be made in this entire field.

Conclusion. Real conditions are now available for predicting long-term effects from exposure to chemicals used in industry. On

[1] The data are controversial.

the other hand, the forecast system is not yet adequate and needs further elaboration. Furthermore, some lines of investigation are not promising.

Predictions based on the distribution of a poison in the body, notably in the gonads, foetal tissues, etc. are often taken as the basis for drawing definitive conclusions. A method such as this was recommended for the prediction of gonadotropic risk from metals (Shtabsky, 1976; Krasovsky et al., 1976). The ability to accumulate in and thereby damage the gonads has been ascertained for manganese (Mandzhgaladze, 1969), boron (Borisov, 1976), lead (Krasovsky et al., 1977) and other metals. The same has been proved for the effects of mercury (Carpenter et al., 1973), lead (Jang et al., 1972), tetracycline, dimethylformamide, etc. In contrast, numerous substances without the potential for selective accumulation in the appropriate tissues produce specific effects. For these substances, the degree of tropism may or may not coincide with their pathologic impact. For example, zinc is known for the role it plays as a microelement in the process of cell respiration and intermediary metabolism and for its ability to accumulate in the gonads and foetal tissues. Lead, tellurium and other elements can build up in the osseous tissue without damaging it.

Other chemical compounds, however, are perfectly capable of harming tissue without accumulating in it. For example, beryllium, copper, and selenium, while not accumulating in the sexual tissues, injure the gonads. Thus, while making no further criticisms prediction of the gonadotropic and embryotropic effects of poisons on the basis of indices of their incorporation into the appropriate tissues, has several limitations, but it can be used if proper consideration is given to all the above comments.

Chapter 8

SOME ASPECTS TO THE PROBLEM OF THE SO-CALLED NORMAL INDICES OF VITAL FUNCTIONS IN EXPERIMENTAL ANIMALS IN THE LIGHT OF CURRENT CONCEPTS ON THE CRITERIA OF HARMFULNESS FOR CHEMICAL COMPOUNDS WITH LONG-TERM EFFECTS

As noted earlier, one primary concern in testing chemical compounds is the criteria of harmfulness in evaluating recognized changes (Sanotsky, 1972; Sanotsky et al., 1974; Sanotsky, Ulanova, 1975). The prime idea of preventive toxicology — the concept of thresholds with respect to all types of effect, including blastomogenic and mutagenic — largely depends for its realization on the availability of well-defined qualitative and quantitative characteristics describing the state of health as opposed to the diseased state. The threshold of a harmful effect implies changes that not only differ significantly from their "intrinsic" parallel control, but pass beyond the limits of physiological variations in a given species and a given season of the year.

PRINCIPLES FOR ASSESSING NATURAL VARIATIONS IN INDICES OF VITAL FUNCTIONS

Currently, the principal approaches to the assessment of natural variations amount, in effect, to their evaluation from data of a substantial series of observations on control groups, involving deviations within the range of 1.5 to 2σ. Consideration of physiological variations within the range of 2σ, accounting for 95 per cent of the whole general population, seems to be most appropriate from the statistical point of view, because 95 per cent is the probability value most commonly used for biological investigations.

Recommendations for the evaluation of the hygienic import of experimentally detected changes, aided by sigmal deviations of seasonal and yearly variations were integrated into an official document (Decisions of the Commission for the Establishment of Maximum Allowable Concentrations of Chemical Compounds in the Air of the Working Zone, Leningrad, 1969).

A critical review of the proposed approach for describing "normal" physiological variations has led to the conclusion that in some ca-

ses, this approach undoubtedly promotes accuracy in establishing the threshold of the harmful effect. It is most applicable and acceptable for indices that remain stable and clear-cut during the period of observation on the animals — such as the composition of peripheral blood or the diameter of the cell nuclei in the follicular epithelium of the thyroid gland as an indicator of its functional performance. Table 48 below shows that, among the functional indices of the thyroid, the cell nucleus diameter in the follicular epithelium turned out to vary little in the control, regardless of seasons of the year. The values we have found in the control groups agreed with our data cited above. On the other hand, the above index changed ahead of all other morphometric indices (on the same tissue), forcing us to recognize its high information content and hygienic importance (the criterion of harmfulness) when the test group indices extend beyond the 2σ limit.

Comparison of the most sensitive index of spermatogenesis — i. e., the quantity of spermatogonia in the seminiferous tubules in test animals — with natural variations of the index in the controls (a 320-strong statistical group) led to the identification of statistically meaningful changes. The great experience of work in that area has repeatedly convinced us that natural variations of the control (even minus seasonal variations) are sometimes very large. In fact, some control groups can significantly differ ($p<0.05$) from one another statistically. For this reason, no statistical significance relative to the control, which is identified in a separate experiment, can be seen as suggesting the manifestation of harmful effect from a chemical, since the same value characterized the control in another experiment.

Table 48

Natural Variations in Functional Activity Indices for the Thyroid Gland (in Rats)

Indices	Average value	Number of cases	Number of groups	Standard deviation, σ
Integration of ^{131}J (after 24 h)	29.77%	151	20	10.39
Height of follicular epithelium	2.78 conv. units of eyepiece micrometer	114	24	0.46
Diameter of nuclei of follicular epithelium	23.43 conv. units of eyepiece micrometer	55	10	2.01
„Conversion" index for iodine bonded with plasma proteins	70%	60	10	11.6

In that case, the calculation of "physiological variations" (and consideration of seasonal fluctuations) is absolutely necessary.

As work in that direction was carried out, the natural variations for a number of indices proved to be so wide as to prohi-

bit their use. In a specific observation, the numerical values of particular indices may well be within the "physiological" limits (those derived from a large number of observations), irrespective of the essentially injurious action of a chemical. In other words, the physiological variables of some indices employed in toxicology prove to be non-stationary on certain occasions, which makes it difficult to validate a general population for them. It turned out that the principal indices of the kind discussed here include, first of all, the respiration rate, SMA (spontaneous motor activity) and a few others. It is worthy of note that responses to be registered with the aid of these indices are controlled by a set of factors, the important ones being the type of higher nervous activity and individual characteristics. In practice, this fact is ignored when the results are evaluated, thus generating the very heterogeneity that hinders the assessment of the responses.

The approach seems to be equally difficult to use in evaluating foetal death in utero since, among test animals, the embryo mortality rate varies from 14.0 to 34 per cent.

Thus, for the determination of "normal" indices, in addition to quantitative estimates, account has to be taken of the qualitative character of particular indices. This concept is in line with Professor Yugai's idea (1973) about the restricted applicability of the purely numerical (average statistical) approach in defining the norm.

Conscious of the disparate variability inherent in the different indices of vital functions in laboratory mammals, Sheftel and Sova (1976) suggested that the key indices be classified and assumed a different degree of certainty based on whether the indices concerned are "plastic" ($p<0.05$) or "rigid" ($p<0.1$). Although we disagree with the proposed evaluation procedure, the actual idea of classifying indices according to their degree of variability certainly deserves attention, as it does determine the necessary size of statistical groups. In evaluating the age-dependent state of the cardiovascular system substantial variations ($>\pm 1-2\sigma$) were noted only for a minor number of indices, rather integral in character (weight of animals, absolute and relative weight of the heart, thickness of heart chamberwalls, external dimensions of the heart, and so on) (see Fig. 34). Most of the indices used in gauging the intensity of metabolic processes, along with a number of functional characteristics of the vascular bed, are subject to broad variations in every age group, depending on the general condition of the animals. The hygienic significance of changes in these indices due to chemical exposures should presumably be assessed in similar terms as the cumulative data of the parallel control until complete standardization in the quality of laboratory animals is assured.

As for estimates of physiological variations based on 2σ, it should be noted that the 2σ approach is only feasible if the examined distribution is normal. Indices enjoying wide currency in our field, such as the number of chromosome aberrations, total

embryonic mortality and some others, do not follow a normal distribution. Fig. 45 shows a quantitative distribution of chromosome aberrations in bone marrow cells (using anatelophase analysis). The graph actually shows a Poisson distribution.

The limits of physiological variations were defined by the method of non-parametric analysis or that of percentiles, often applied in developing standards (anthropometric, somatometric, etc.) given that the number of observations is equal to 100—200 or more. The 50 percentile, known as the median, is one for which variations from 1.67 to 3.6 are assumed to be normal (see Chapter 5 and Table 37).

FACTORS BEARING ON THE MAGNITUDE OF NORMAL INDICES

Among the aggregate of factors influencing natural variations in the control, systematic and random errors occupy a certain place, but the methods so far used for estimating them are grossly inadequate, even though systematic errors reach 10 per cent in colorimetry and as much as 15 per cent in sampling.

Another cause, at least in some instances, may be that our accepted "norm" is, in effect, a latent pathology. Shown in Figs. 43 and 44 are variations of some biochemical indices and quantities of chromosome aberrations in the bone marrow of control animals — non-inbred albino rats from different consignments — over several successive years. The graphs suggest significant ($p<0.05$) divergencies in the values of the indices among the animals from different consignments in the control. As seen in Fig. 45 (from the example of chromosome aberrations), the indices of some control groups surpass the spontaneous level for the test animal species, i. e., the animals are actually sick. Large variations in the animals' general reactivity (judging from the inflammatory response to protein and turpentine injections) were reported by Shcherbakov (1976). Finally, major differences were captured in the responses of animals received from a nursery, as compared with identical animals after vitaminization or of animals born in the vivarium of a research establishment.

The foregoing demonstrates the need to develop standards for the quality of laboratory animals and properly adjust the regulations concerning the maintenance and feeding of such animals (Sanotsky, 1970). The question of reliability as regards animal research methods (particularly in evaluating the impact of low-intensity factors) requires special treatment.

The ways in which collective or individual maintenance of animals or the types of hierarchy established within animal groups can affect the manifestation of a toxic response in animals have been discussed repeatedly. This is all the more important for studies concerned with the state of the reproductive function in test animals (Sanotsky et al., 1971). For example, many experimentors

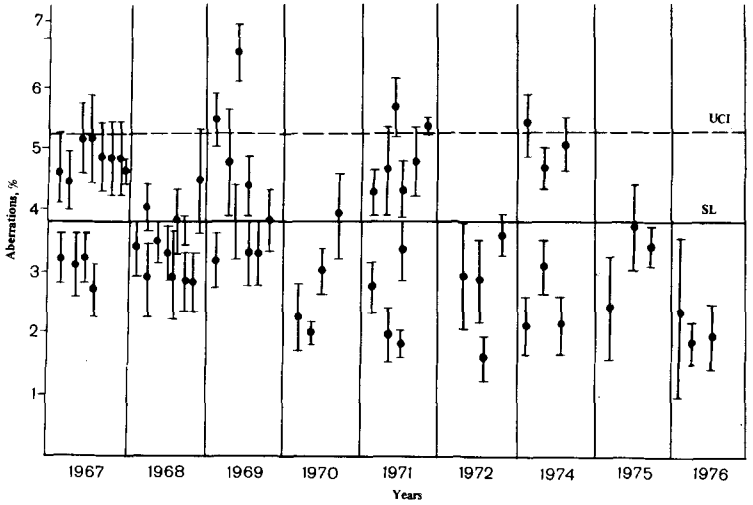


Fig. 43. Chromosome aberrations in the bone marrow of intact albino rats received from nurseries of the USSR Academy of Medical Sciences over several years. UCI — upper confidence interval of spontaneous level; SL — spontaneous level

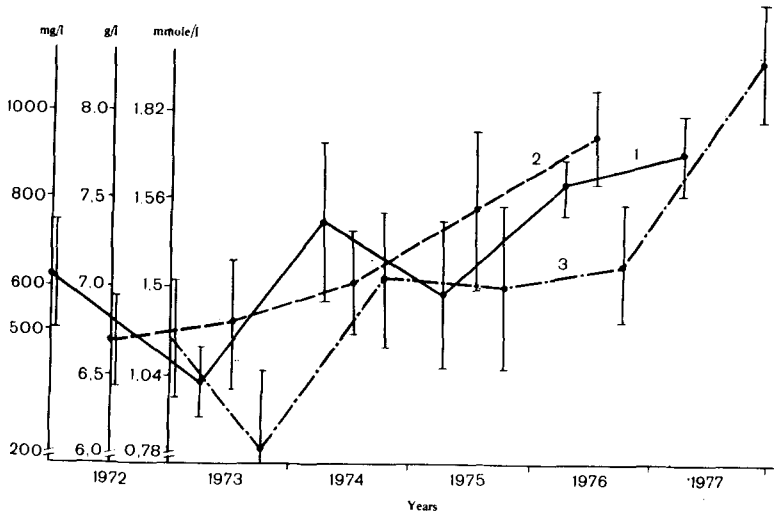


Fig. 44. Natural variations (M+m) in some biochemical indices of three-month-old albino rats received from nurseries of the USSR Academy of Medical Sciences. 1 — β-lipoproteids; 2 — total protein; 3 — cholesterol

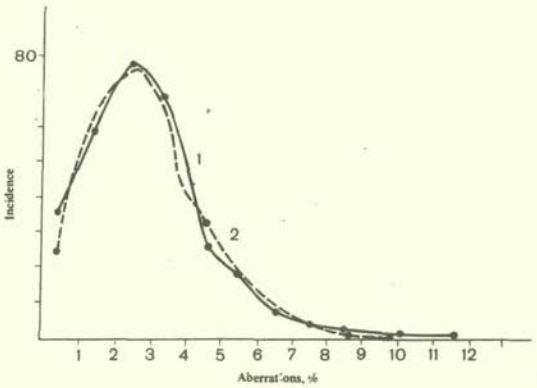


Fig. 45. Distribution pattern of chromosome aberration rate in the bone marrow of control animals.
1 — empirical, 2 — theoretical

allow the animals to be moved about daily from one cage to another.

Although the dietary composition and the feeding rates are set down in mandatory orders, the system of supervising their observance remains unsatisfactory.

It is known, however, that failures to comply with the feeding standards can by themselves be detrimental. As an example, a change of embryotropic effect following a reduction of dietary proteins was reported by Stenberg and Gorchinsky (1976).

The unfavourable aftermaths of A, E and other vitamin shortages have often been described and this applies equally to the role of microelements — selenium, manganese, zinc, etc. These issues have been tackled in recent WHO publications (1975). Petering and associates (1976) showed how diminished copper concentrations in the diet (<8 ppm) induced degenerative and sclerotic changes of the myocardium, aorta and other vessels, similar to the accelerated ageing of the organs.

Conclusion. Thus, the problem of evaluating the hygienic significance of experimentally recognized changes has a direct bearing on the concern for the quality of laboratory animals and conditions under which they are kept.

Unless the quality of laboratory animals is standardized, the regulations for their maintenance adjusted and the feeds kept uniform and supervised on a regular basis, experiments dealing with long-term effects will be very difficult.

There is need to study in depth the credibility of the results obtained with the use of SPF — animals. Linear animals continue to be used widely in toxicologic research in general and studies of the reproductive function, in particular. For example, the rates of in utero death in intact tetrahybrid mice (with a variability index of 3) are more stable than in non-inbreds (variability indices of

17 and 55 in SHK mice and non-inbred albino rats, respectively). On blastomogenesis scientific opinions vary.

The numerical size of statistical groups is known to be of major importance for the reliability of the results obtained. Faced with the difficulty of increasing the size of the experimental group, the control group may well be increased. The latter is allowed to include the double number of animals in the test group.

It is a good practice to divide experimental and control animals into two subgroups and survey them separately, so as to estimate the amount of information noise.

The findings of one-time and subacute experiments, unless reproduced many times, can be taken largely as being random. Experience shows that precise reproduction of the results cannot be achieved even at the level of lethal doses and concentrations. For low levels of exposure, the total number of incidental factors appears to play a more significant role than does a short-term chemical exposure of minimum intensity. Only through multiple reproduction of the experimental conditions can a good estimate be made of the degree of correlation between recognized changes and the chemical exposures applied. In order to resolve the issue of the criteria of harmfulness for recognized changes by correlation with the physiological norm, certain conditions need, therefore to be provided, these being the key to the effectiveness of the programme.

Chapter 9

SUMMARY RESULTS AND FUTURE PROSPECTS

It is clear from the previous discussion that considerable efforts are being made to advance the field of preventive and hygienic toxicology that studies the long-term effects of chemical exposure on man and related animals and plants.

The concept of long-term effects, previously taken to mean, in principle, interference with the reproductive processes and blastomogenesis, has now been extended to cover cardiovascular pathology and ageing processes, both their general pattern and specific forms. Unlike the earlier practice of studying long-term effects for the hygienic (including, toxicological) evaluation of a chemical factor only when indicated, this has now been made mandatory. Formerly, the methodological basis for studying long-term effects would be sought in the research and development going on in related scientific disciplines. The investigations of chemical compounds aimed at preventing their long-term effects are now no longer conducted by blindly following the recommendations of classical geneticists, oncologists, teratologists and other members of the theoretical disciplines.

These recommendations, and the methods contained in them, when tested for applied uses, have been found to be irrelevant in many ways to the validation of sanitary regulations for the manufacture and use of chemicals, or for the protection of the public against chemical exposure. The modified character of the contemporary "chemical" pathology (the ill-defined and nonspecific manifestation of clinical forms and the vast incubation period of delayed diseases) was considered for our Methodological Guides for research into the effects of chemical compounds upon the reproductive function (mutagenic, gonadotropic and embryotropic action) with a view to establishing the thresholds of harmful action needed for sanitary standardization (USSR Ministry of Public Health, No. 318, 1977).

In cooperation with Kiev research institutes, methodological guides were developed for experimental research into the cardiovascular system. These incorporated forecasting methods of long-term pathology. The Anticancer Committee of the USSR Ministry of Public Health has approved the Methodological Guides for the study of the blastomogenicity of chemical agents (No. 819—869).

These and other methodological documents present a necessary minimum package for encouraging various research centres to de-

velop comparable data, so as to make possible not only the establishment of sanitary regulations, but also broad theoretical generalizations. Most these methodological guides state emphatically that their purpose is by no means to curb the initiative of individual researchers or research groups in their search for suitable new methods and systems of methods.

Relying on these methodological aids, testing procedures and modifications of research techniques, in the recent years hygienic research establishments have studied scores of chemical compounds in an effort to define safe levels of exposure for them in terms of long-term and delayed pathological manifestations. As an illustration, Table 49 cites data developed by the toxicology staff of the Institute of Industrial Hygiene and Occupational Diseases, USSR Academy of Medical Science. Table 50 identifies specific chemicals by name.

As was stressed earlier, not all of the compounds were found capable of selective action on the target organs and systems. Consequently, the levels specified in the sanitary standards derived from data on systemic toxic properties, have been properly adjusted, but not for all the compounds studied. Nevertheless, the very fact of the sanitary standards being borne out is of great scientific and practical importance.

Table 49

Quantities of Industrial Chemicals Examined for Long-Term Effects in the Toxicology Lab of the Research Institute of Industrial Hygiene and Occupational Diseases

Considered effect	Number of tested chemicals	Those examined for	
		validation of MAC	revision of MAC
Accelerated ageing of vessels	10	2	1
Embryotropic	28	7	4
Gonadotropic	50	22	5
Mutagenic	44	16	3
Blastomogenic	10	3	1

The approach we have just discussed is an example of the practical utilization of theoretical findings that are fundamental to the guiding set of principles for sanitary regulation at work and at home. The principal guideline, now as before, focuses on thresholds of harmful effect most closely associated with the rate of regeneration, reparation and adaptation both within the life-span of one generation and in subsequent generations. The oft- repeated proposition is that an injury occurs solely when the development rate of the pre-pathological process exceeds that of recovery and adaptation.

Restorative systems of structures and functions have now been discovered at all levels of biological organization, not least at

Table 50

Industrial Toxic Chemicals Examined for Long-Term Effects

Chemical	Types of action examined			
	mutagenic	gonadotropic	embryotropic	blastomogenic
Ethylenimine	+	+	+	+
Mercury	+	+	+	
Carbon tetrachloride	+	+	+	
Phosphorus oxychloride	+	+	+	
Formaldehyde	+	+	+	
Gasoline BR-1 "Galosha"	—	+		
Morpholine	+	—		
Tryphthazine	+	+		
Dimethylperfluoroadipinic acid	+			
Benzene	+—	—		+
Propylene oxide	+	—		
Cyclohexanone	+	—	+	
Urethane	+		+	+
Binary mixture (ethyl and methyl carbamates)	+	+		
Methyl carbamate	+			—
Arsenical anhydrite	+	—		—
Dinyl	—	—	+	
Ethylene oxide	+	—		+
Chloroprene	+	+	+	
Aminopyrimidine	—	+		
1,3-chlorobrompropane	+	+		
PL-86 rubber	—	—		
Tertiary butyl hydroperoxide		+		
Phenol	—	+		
Ammonia			+	
Neoprene latex	+	+	+	
Dimethyl acetamide	—	+	+	
Ditretbutyl peroxide	+	—		
Phosphorus oxychloride	+	+		
Dimethyl formamide	—		+	
Lead			+	
Carbon disulfide	—	—	+	
Pyrrolidine	—	+		
Piperidine		+	+	
Dimethyl dioxane		+	+	
Di-n-propylamine	+			
Epoxyfurfuryl ether		+		
N-butyl-2-dibutylthio urea		—	—	
2-chlorethane sulfochloride		—		
Tretbutylperbenzoate		—		
AO-40 (a phenol derivative)			+	
Ionox (")			+	
Extract-4 (petroleum oil)			+	
Stabiloil-18 (")				—
Furadonine		+	+	
Furacillin		+		
Furazoline		+		
Butyl ether 2,4,5-T	+	+	+	
Butyl ether 2,4D	+		+	
Furaginum		+		

Table 50 (contd.)

Chemical	Types of action examined			
	mutagenic	gonadotropic	embryotropic	blastomogenic
Vinyl chloride	+	+	+	+
Caprolactam		+	+	
3,4-benzo(a)pyrene	+	+	+	+
Tetramethylthiuram disulfide	—	—		
Bromine		+		
Iodine		—		
Prednisolone			+	
Nicotine	+	+	+	
Tetracycline			+	
Captan	—	+		
Methyl-γ-trifluopropyl cyclotrisiloxane	+	+		
PFMS 13 (a mixture of cis- and transisomers of dimethylphenyl cyclosiloxanes)	+			
Dimethyl phthalate	—	—	—	
Diethyl toluamide	—	—	—	
R-162 (N-benzoyl piperidine)	—	—		
DI-2-ethylhexyl phthalate	—			
Neozone D	—			
Zinc chloride	+—	—		

Note: + presence of effect; — absence of effect; in all other cases the effect was not examined.

that of nucleic acids. The systems become more active in response to outside impacts, as does, for example the activity of "lidase", which leaps up by 80 per cent under ionizing radiations (Zasukhina et al.). The same authors go on to state, however, that the restorative system of nucleic acids does not work for some specific exposures. As in most other cases, nothing is said about the quantitative and qualitative characteristics of the phenomenon. What quantities of a particular stimulus will result in impairment of the restorative and adaptive systems? This is a central question.

With each passing year, the substances subject to hygienic evaluation increase in number. Because the research on the probability of and conditions giving rise to long-term effects from the action of chemicals on groups of workers and the general population is compulsory nowadays, the problem of staged progress (synchronous timing of chemical and technological development and sequential continuity in the study of long-term effects) is gaining a position among the leading problems of hygiene and toxicology. Qualitative evaluation of the "potential" (but not real!) hazard does not solve the problem, because of the many basic errors inherent in it. Evaluation of the potential hazard of long-term effects cannot and should not be undertaken at high exposure levels (partially lethal or maximum tolerable). Rather, it must be arranged at threshold and subthreshold (according to integral parameters) levels, as this enables the measurements to be quanti-

tative and thereby to be of use in predicting the thresholds of specific effect by prolonged entry into the body, and therefore the TSELs also (Tentative Safe Exposure Level).

At later stages of the research (but before design, production and application begin) the study of unfavourable effects (in terms of the time of exposure or the set of indices involved) should be extended in depth and in breadth. The idea is to allow the experimental data, in conjunction with that gained for limited, pre-exposed employee groups (laboratory attendants, plant operators, service and maintenance personnel at semi-commercial plants and on pilot lines), to be used as basic inputs for raising TSELs to the rank of MACs.

The next stage in the research into long-term effects, as mentioned above (see Chapter 2), consists of clinical studies (outpatient or hospital) combined with a thorough assessment of man's living environment (occupational, communal, domestic, and natural). Under the existing regulations (Methodological Guides for Establishing MACs for Harmful Substances in the Air of the Working Zone, 1969) such studies must be scheduled within three years of the substance's registration for use in the national economy. By that time, only early signs of corresponding changes (not lesions) will have appeared in most instances. Later consistent with the phases of the chronic process, the changes may disappear from view as they sink to a lower, latent level of biological organization. To locate and interpret these latent changes hygienically from the viewpoint of the criteria of harmfulness is yet another goal for the future.

Early and progressing pathological changes in the reproductive system, as also the galloping development rate of the cardiovascular pathology can be seen if the exposure levels are relatively high. With low exposure levels (minimum effective), the latency of a disease is prolonged to the limit of life and well beyond that.

For the reason suggested, the introduction of an efficient system for surveilling populations until the end of their life-span, that is, monitoring the state of health of disabled chemical workers, pensioners and communities located in geochemical provinces, both natural and anthropogenic, constitutes one condition vital for rapid progress. Investigations into the causes of death and post-mortem studies are equally essential for achieving ultimate success.

The difficulties involved in interpreting the results of clinical hygienic correlations and epidemiological researches are well-known. They are also known to multiply in studies on the long-term effects of chemical etiology. The multitude of factors responsible for such phenomena unavoidably invites the use of multifactor analysis.

Soviet researchers (Ivakhnenko et al., 1971, and others) proposed a method for collective assessment of arguments and tested its suitability for assigning a new pesticide to a certain class on the basis of the time the chemical remains on crops. This was

done using the values of its 22 characteristics, such as physicochemical properties, mode of application, chemical parameters of the target crops, and climatic parameters. Comparison of the resulting data, calculated and factual, showed that the prediction accuracy so achieved is quite appreciable, in the order of 80 per cent.

It should be noted, however, that repeated verifications are needed here to ascertain the accuracy of the conclusions, when largely subjective data, like those coming in by way of questionnaires, are incorporated into any system of multifactor analysis. Gathering accurate information on all points of interest to the investigator and properly checking the data necessitates laborious organization and considerable effort and resources.

At the particular stage we describe, in order to establish a relatively stable situation for the effect of extraneous factors and rule out multiple side-effects, it would be practical for the researcher to resort once again to the experiment in an attempt to pick out the leading factors, determine the relationship between the effect and the chemical dose (concentration) in the environment and the duration of exposure. As this is done, all problems involved in interspecific extrapolation still hold.

The problem of long-term effects from chemical exposure is, of course, not just a hygienic issue. Its solution is virtually impossible without bringing the interdisciplinary and interdepartmental interests involved in a highly efficient complex of resources and relations managed in a synchronous and coordinated manner. Through strenuous efforts, the extended creative human life-span, a paramount social goal, must, and undeniably will, be achieved.

Chapter 10 (additional)

CURRENT PROBLEMS IN RESEARCH INTO THE LONG-TERM EFFECTS OF ENVIRONMENTAL CHEMICAL IMPACTS

Numerous recent publications disclose wide-ranging interest among geneticists, embryologists, oncologists, and genotologists in the practical management of public health objectives relating to the prevention of long-term adverse chemical effects. The pace of the date inflow is particularly rapid for the mutagenic, teratogenic, and blastomogenic hazard assessment of different chemicals. Yet a closer look at the data shows them to be extremely difficult to compare and still more difficult to apply in handling hygienic problems. Not only were they derived by using different methods, exposure levels, observation time-frames and biological targets (as noted already in Chapter 2) but more importantly, the systems used for theoretical analysis of the test data were diametrically opposed and the underlying scientific concepts bore no comparison.

It appears that, while hygienic toxicologists dutifully read the literature in related fields, workers in these fields look up toxicological literature all too infrequently. As a result, such routine phenomena as individual sensitivity, or species-specific variations in responses to a particular chemical, or inversion of effect, and so forth are sometimes revelations to them.

One aim of current studies is to provide a sound experimental, clinical and theoretical rationale for a methodology for studying long-term chemical effects with the express purpose of designing a system of prophylactic measures.

If viewed from the applied perspective, that is, as a problem for prophylactic medicine, this should best be undertaken on the basis of the fundamental concepts of preventive toxicology that have proved valid in experience. The validity of formulating recommendations on limiting environmental concentrations of chemicals with indicators of the systemic toxic effect as the basic input has been ascertained from hygienic standardization of substances with a potential for specific effect.

The sets of methods we have developed and tested for identifying the thresholds of deleterious chemical effects on the reproductive function (gonads, embryogenesis, and mutagenesis), on the ageing of the cardiovascular system and other similar conditions, found approval and acceptance when methodological recommendations on that subject for the CMEA member — states were endor-

sed; they are published in a series of methodological papers entitled Problems of Industrial Toxicology (CMEA, 1982).

It is gratifying to note the widespread concern that those engaged in theoretical science have shown in the last few years for the implementation of their research results. This was evident from the purely toxicological and pharmacological notes to many reports at the Annual Meetings of the European Environmental Mutagen Society (1982, 1983, 1984) from the now discernible, well-defined tendency to use lower level doses and concentrations of mutagens in experiments; from the attention being focussed on qualitative and quantitative, distinctive manifestations of chemical effects and metabolic patterns depending on the effective exposure level; and from a recent focus on the difficulty of extrapolating data from lower to higher biological specimens, especially to man.

On the other hand, some very recent publications (the WHO-published Hygienic Criteria for the State of the Environment or Principles and Methods for Toxicity Assessment of Chemicals, Part I/WHO, Geneva, 1978/; Methodologic Guides of OESR, 1981; G. Gobinet, 1982; S. Smeels, 1982) suggest numerous points in the study of long-term effects that still need to be coordinated on the international level.

The most important of these include prediction of particular long-term effects; the transfer of the data developed in experiments on animals, plants, microorganisms, insects, etc., into sanitary practice; and the feasibility of establishing safe exposure levels. In some way or another, these points are reflected in corresponding chapters of this book. Awareness of new data and the need to analyze those previously obtained and published by us require, however, complementary treatment of the most topical issues.

I. PREDICTION OF BLASTOMOGENIC EFFECT ON THE BASIS OF THE MUTAGENIC HAZARD OF CHEMICALS

Researchers nowadays seem to be increasingly inclined to recognize a close link between mutagenic and carcinogenic effects. The modern mutation theory of cancer proves a substantive role for gene, genome and chromosome structural mutations in carcinogenesis. The effect of carcinogens was shown to be able to produce a favourable environment for the appearance of a spontaneously mutating cell capable of uncontrollable reproduction (in case this combines with malfunctions of the body's defence systems) (Vainio, Sorsa, Hemminki, 1981; Vogel, Zijlstra, 1981; Mc. Cann, Horn, Kaldor et al., 1982).

Granted the interrelationship between the effects, many authors suggest that various rapid genetic techniques be applied for evaluating the potential blastomogenic hazard of chemical mutagens (Table 51). It is widely believed that there is a high correlation

between the carcinogenic and mutagenic effects detected with the aid of the Aimes system (80—85%). True carcinogens, however, are fewer in number than potential carcinogens, which are recognized on low-organized specimens with no regard for the level of toxic exposure. We have shown earlier that there is as yet no way to define safe chemical exposure levels for humans using microbiological specimens, this being the ultimate task of research in preventive toxicology. Mammals seem a more reasonable alternative for estimating the quantitative patterns in the rise of mutations and neoplasms, even though this involves considerable observation time, money and hard work. For the reasons suggested, long-term research programmes in experimental carcinogenesis to serve the purposes of hygienic standardization are extremely rare, and limited, in fact, to recognized carcinogens only. Of the total list of over 1,000 chemicals standardized in the USSR according to indicators of systemic toxic effect, no more than 10—15 standards include blastomogenic effects. Given the multi-factor etiology of cancer, the number of investigations taking clearly a straight line to setting sanitary standards for carcinogens is not yet sufficient. Nor, of course, can they be taken as the basis of preventive sanitary supervision for reasons of ascertaining things post factum.

Thus, the problem of predicting a likely blastomogenic effect of industrial chemicals from quantitative correlations and discovering the real risk they present in the work environment remains particularly relevant today.

For several years now our group has been testing various genetic techniques to see whether they can actually predict the blastomogenic activity of chemical compounds.

The mutagenic activity of toxic chemicals was estimated in vitro — by a variety of microbial tests with and without metabolic activation; in vivo — by host mediated assays, cultivation of microorganisms in the urine of poisoned animals, spot tests, cytogenetic analyses of animal bone-marrow cells and human peripheral-blood lymphocytes, micronucleus tests, and sister chromatid exchanges.

Estimates of blastomogenic activity were derived by the traditional method involving long-time observation of the test animals, as well as by the method of transplacental induction. The findings were published in several papers (Sanotsky et al., 1978; Zaeva et al., 1979; Domshlak et al., 1981).

A comparative evaluation of several genetic methods employed ethylenimine as the test chemical (Table 52). Preliminary experiments looking into the latter's metabolism and toxicokinetics enabled tested exposure levels (doses and concentrations) to be compared on different biological specimens during in vitro and in vivo experiments. Ethylenimine was determined to have caused mutations in all test systems and the lymphocyte culture of peripheral donor blood to have been more susceptible to the toxic effect of the poison than S. typhimurium TA 1950. An important fact for practical applications has been the proven high informative value and sen-

sitivity of the cytogenetic methods that are available for use not only in animal experiments, but in epidemiological studies as well.

Parallel mutagenicity-carcinogenicity studies of several chemicals differing in chemical structure and type of biological impact in experiments on laboratory animals showed the following: the cytogenetic effect in the bone-marrow cells of laboratory animals was manifested earlier (Table 53) and at lower levels of exposure while no blastomogenic effect (using adequate statistical animal samples) could be recognized (Table 54). An examination of workers by the cytogenetic method (cultivation of peripheral blood lymphocytes from employees exposed to mutagens at work) revealed an excess of chromosome aberrations on exposure to a number of chemical carcinogens (Table 55). Of the substances tested, vinyl chloride and dimethyl sulfide are human carcinogens. The compounds were seen to increase chromosome aberrations in the lymphocytes of the surveyed employees' blood, as did formaldehyde, a potential carcinogen according to current views (Treichmann, Šram, 1976; Sanner et al., 1982).

With respect to the blastomogenic activity of chloroprene, which showed cytogenetic activity at relatively low exposure levels of 1—4 mg/m^3, the literature furnishes conflicting evidence.

The blastomogenic activity of high-level lead doses was documented in animal experiments and its cytogenetic effect was revealed in exposed workers who were affected by lead concentrations above its MAC. **No significant excess of cells with chromosome aberrations was found at the MAC level.**

Styrene produced mutagenic (specifically, cytogenetic) responses in both experimental animals and in a survey of occupationally exposed workers (Vainio et al., 1976; Norppa et al., 1979. etc.); its blastomogenic activity, though not detected (R. Sram et al., 1982), is still presumed (Letterberg, 1981).

Table 51

Short-Term Tests Determining Mutagens and Carcinogens *

Test type	Number of tests
Point mutations in bacteria	7
Inhibited bacteria growth	2
Phage tests	3
Insect mutagenesis	4
Mammalian mutagenesis	16
Cytogenetic mammalian tests	13
Other eucaryote tests	21
Transformation of mammalian cells	17
Transformation of virus-infected cells	4
DNA binding	2
DNA break and other injuries	9
Inhibited DNA replication	2
DNA reparation	7

* — adapted from M. Sorsa (1982).

Table 52

Comparison of Research Results on the Mutagenicity of Ethylenimine by Different Methods on Isoeffective Levels

Methods		Levels	$<Lim_{ch}$	$\sim Lim_{ch}$	1/5 Lim_{ac}	$\sim LC_{50}$	$>LC_{50}$
in vitro	microbial tests	1. Without metabolic activation		+	+	+	+
		2. With metabolic activation (Microsomal liver fraction)		+	+	+	Death of cells
	cell culture	3. Cytogenetic analysis of human peripheral blood lymphocytes	—	+	+	Cytotoxic effect	
		4. Sister chromatid exchanges	+	+	Cytostatic effect	Cytotoxic effect	
in vivo		1. Host-mediated assay		+	+	+	
		2. Dominant lethal mutations	±	+	+		
		3. Spot test		+	+		
		4. Cytogenetic analysis of animal bone-marrow cells	+	+	+		

+ — effect observed; — — no effect observed; ± — effect observed at one spermatogenesis stage

Table 53

Dates at which Cytogenetic and Blastomogenic Effects Appeared in Experiments with Rodents Exposed to Different Carcinogens

Chemicals	Doses (concentrations)	Dates when effects appeared	
		mutagenic	blastomogenic
Ethyl carbamate	0.1 mg/g	3 days	12 months
	1.0 mg/g	3 days	3 months
Ethylenimine	12.0 mg/m^3	2 weeks	1—1.5 years
Vinyl chloride	30 mg/m^3	2 weeks	1.5—2 years *
Dimethyl sulfate	20 mg/m^3	1 week	>2 years
	2 mg/m^3	1 week	>2 years

* Adapted from B. A. Kurliandsky et al. (1980).

Table 54

Comparison of Chemical Exposure Levels Causing Recognizable Mutagenic (in vivo) and Blastomogenic Effects in Experiments

Chemical	Concentration, mg/m³	Exposure levels*	Effects	
			mutagenic	blastomogenic
Ethyl carbamate	1.0	10 Lim_{ac}	+	+
	0.1	~Lim_{ac}	+	−
Methyl carbamate	1.0	10 Lim_{ac}	+	−
	0.1	~Lim_{ac}	−	−
Ethylenimine	12.0	~Lim_{ac}	+	+
	0.8	~Lim_{ch}	+	−
CCl_4	>300.0		+*	+*
	40.0	~Lim_{ch}	+	
Neozone D	20.0	~Lim_{ch}	−	−
Vinyl chloride	30.0	Lim_{ch}	+	+*
	4.8		+	
Dimethyl sulfate	20.0		+	+
	2.0	~Lim_{ch}	+	+
	0.2		+	−
Formaldehyde	**			+*
	23.7		+	
	3.8	~Lim_{ch}	+[1]	
	0.5		−	

*— adapted from the literature. **— concentration described as "subtoxic".
[1] — excess number of gaps ($p<0.02$).

No cytogenetic effect among the workers surveyed was associated with dimethyl phthalate, ethylene oxide or dimethyl acetamide; nor was there any evidence of this found in the literature available to the authors.

Thus, to summarize, both experimental studies and the survey of exposed workers showed not merely all-out correlation of the cytogenetic and blastomogenic effects of a number of chemicals from different classes, but also the ability of the cytogenetic effect manifest itself earlier on and at lower levels of exposure.

As in previous comparisons, here, too, a rather high carcino- and mutagenicity correlation was found, according to the indicator used. It is nonetheless not absolute, of course, as cases are known when no carcinogenicity whatever was established for strong mutagens such as nitrous acid, hydroxylamine, analogs of nitrous bases, and so on. The substances' failure to be tumorigenic should probably be attributed to organ-specific DNA reparation, the inhibitory effect that various chemicals present in the internal and external media have on cell transformation processes, and other impacts (Nomura, 1981; Stichet et al., 1974; Robett, 1982; etc.). On the other hand, while epigenetic mechanisms of carcinogenesis also exist (J. Weisburger, E. Weisburger, 1981; Yamasaki, 1982; Roberfroid, 1982), they seem of no serious consequence in real life.

Table 55

Cytogenetic Effects Among Workers Exposed to Industrial Chemicals

Chemical	Concentration, mg/m³	Groups	No. of workers surveyed	No. of abberrant cells	p
Chloroprene	45—2.7 7.0—3.0 1.0—4.0	I II III control	18 20 8 9	4.77±0.57 3.49±0.51 2.5±0.49 0.65±0.56	<0.001 <0.001 <0.05
Vinyl chloride *	111—1.8	I control	37 12	2.76±0.24 1.62±0.39	<0.05
Ethylene oxide	100—200	I control	10 8	2.2±0.57 1.19±0.29	>0.05
Dimethyl acetamide	5—10	I control	20 16	4.49±0.5 3.55±0.4	>0.05
Lead	0.03—0.51 0.04—0.025	I II control	18 12 45	3.5±0.51 2.31±0.5 1.25±0.38	<0.05 >0.05
Dimethyl sulfate *	~5.0	I control	14 13	2.59±0.24 1.57±0.21	<0.01
Formaldehyde *	0.5—3.0	I control	40 13	2.97±0.65 1.4±0.3	<0.05
Dimethyl phthalate	30—50 **	I control	17 10	1.9±0.42 1.56±0.28	>0.05

*— blastomogens;
**— dose by skin application (mg/kg).

In addition, account must be taken of the difficulties involved in identifying weak blastomogens in traditional animal experiments, and still more so in epidemiological investigations.

The data in Table 52 suggest that sister chromatid exchanges were the most sensitive indicator for ethylenimine. In the literature they are also reported to be highly susceptible to and, presumably, predictive of blastomogenic effects (Sorsa, 1979; Wolff, 1981; Waksvik et al., 1981). Our research in conjunction with the Institute of Medical Genetics, USSR Academy of Medical Sciences, on the mutagenicity of formaldehyde, indicated that the number of sister chromatid exchanges correlated negatively with induced unscheduled synthesis of DNA. This led Titenko and Chebotarev et al. (1983) to formulate the hypothesis that a study of these indicators and structural chromosome lesions in groups surveyed performed concurrently with a study of the same indicators, plus the effect of a recognized mutagen in vitro, makes it possible to identify "direct" and "indirect" mutagens. Using the system, it may be possible, in testing chemicals for mutagenic hazard, not only to predict blastomogenic risks, but also to define the test chemical as either a promoter or an initiator. The data obtained for formaldehyde led to its categorization as an "indirect" mutagen because of its ability to damage reparation systems, and to the suggestion of its "promoter" role in blastomogenesis.

2. THE POSSIBILITY OF EXTRAPOLATING ANIMAL TEST DATA TO HUMANS

The possibility is closely linked with species-specific metabolic patterns of chemicals in the body. As in the toxicity study of chemicals, it is necessary here, too, to select the experimental model using species sensitivity data and to pick for experiments animal species close to humans. Experience indicates that the commonly used laboratory animals provide a reliable model.

Some scientists still question the credibility of animal experiments for determining the potential hazard from teratogens and establishing permissible levels of chemical concentrations in the environment.

In practice, however, such a situation can be thought of only in casuistic cases. Doubts may be raised for another reason: whether there was all-out compliance with the conditions necessary for identifying the degree of species sensitivity by the indicators of systemic toxic effect, or perhaps the set of indicators was inadequate and the observation time-frame not long enough. Thus, in some animal experiments the experiments fail to consider postnatal development of progeny (Table 56), whereas such a study can reveal an adverse effect for lower levels of toxicant exposure than those defined from developmental studies in utero.

In light of the foregoing, a broader scope of research in expe-

Table 56

Constraints on Studies of the Embryotropic Effect of Industrial Chemicals

Chemicals	Levels of exposure	Embryonal death	Microanatomy of foetus	Examination of progeny	
				load-free	loaded
Chloroprene	Lim_{ch}	+	+	+	+
	1/10 Lim_{ch}	—	—	—	+
Carbon disulfide	10 MACs	—	+	+	+
	MAC	—	—	+	+
Neozone D	Lim_{ch}	—	+	—	+
DMAA	10 MACs	—	—	—	+
Vinyl chloride	Lim_{ch}	—	+	+	+
	1/10 Lim_{ch}	—	+	+	+

Table 57

Man-Animal Comparison of Mutagenic and Gonadotropic Effects

Chemical	Level	Mutagenic effect		Gonadotropic effect	
		man	animal	man	animal
Chloroprene	> MAC	+	+	+	+
	~MAC**	+	+	+	+
Vinyl chloride	MAC **	+	+	+	+
Dimethyl acetamide	10 MAC	—	—	+	+
Ethylene oxide	$Lim_{ch\ integr}$	—	+		—
Dimethyl phthalate	MAL	—	—		—
Lead	MAC	+	+	+	+
	>MAC	—	—	+	+
Antimonite	MAC	—	—	+	+
Manganese	$Lim_{ch\ integr}$		+	+	+
Epichlorohydrin *	>MAC	+	+		
Benzene	>MAC	+ *	+		
Formaldehyde	<Lim_{ch}	+	+		
Dimethyl sulfate	Lim_{ch}	+	+		
Chromium	~MAC	+	+	+	+
Styrene	>MAC	+ *	+		
Ethylenimine	>MAC	+ *	+		
Benzene	>MAC	+ *	+		
TMTD *	>MAC	+	+		
Cyclphosphine *	>Lim_{ac}	+	+		

* — data from the literature;
** — reduced after new findings.

rimental teratogenesis is now required. Table 57 compares, for a selection of different chemicals, the mutagenic and gonadotropic effects ascertained from a survey of exposed workers and an experiment on small laboratory animals (rats and mice).

From the research results summarized in Table 57 a definitive opinion can be formed as to the suitability and reliability of the

experimental model (laboratory mammals) for evaluating the effects mentioned above. Processing the data by the goodness of fit criterion — χ^2 has shown the latter's value for the examined series to be 0.21. For a reliable difference at the level of $p=0.05—0.01$, the tabulated value of χ^2 corresponds to 3.85—6.63, i. e., there is no difference between human responses and animal experiments. The examples in Table 57 therefore demonstrated the validity of immediately relating the results of animal experiments to humans, provided the most sensitive animal species was properly chosen and the observation techniques were suitable.

3. RECOMMENDED METHODS FOR MUTAGENIC HAZARD ASSESSMENT

Closely allied with the problem of the relevance of animal data for man is the other problem of the reliability of the methods used to evaluate long-term effects. Nowhere is the problem so urgent as in identifying mutagenic injuries at all levels of genetic structures (chromosomic and genomic in the germ and somatic tissue). Experience to date in experimental work and in testing new, previously unused, methods for preventive toxicology provides ample evidence for additional recommendations and refinement of some existing outlooks on the subject.

Determination of gene mutations likely to be triggered off by chemical impacts has been a weak point in experimental research into mutagenesis. Lately, great hopes have been placed on the spot test, a technique for defining mosaics by distinctive colour of the hair. The method was tested by us while studying a number of chemicals, including ethylenimine and dimethyl sulfate (Table 58). The test animal line we employed was described by Domshlak et al. (1981). The method, though, was modified for pregnant females as the exposure target to make it really feasible with respect to the number of animals used ($F_1=180—200$). It is currently presumed, however, that the effect identified by this modified technique can suggest not only true mutations, but recombinations, as well. Though the method for assessing gene mutations needs to be further improved; it is of use now, from our point of view.

The same question of credibility also applies to the studies carried out on occupational groups. Our research jointly with the Institute of Medical Genetics on formaldehyde went a long way towards estimating the informative value of different methods, relating an identified pathology to the work environment, and comparing the results produced by different methods.

A survey of workers from different departments at a furniture factory with varied formaldehyde concentrations of 0.5 to 3.0 mg/m^3 involved the use of the following techniques: analysis of chromosome aberrations and sister chromatid exchanges; exami-

Table 58

Frequency of Hair-Colour Mosaics Observed in First-Generation (F_1) Female Mice in Response to Ethylenimine (EI, per os) and Dimethyl Sulfate (DMS, by inhalation)

Compound	Concentration (dose)	Levels	No. of mice, F_1	No. of mosaics	Frequency of mosaics, %%
EI	0.4 mg/kg	$\sim Lim_{ac}$	197	51	25.88±3.12
	0.08 "		198	77	38.88±3.46 $p_1<0.001$; $p_2<0.01$
	0.008 "		198	85	42.22±3.51 $p_1<0.001$; $p_2<0.01$
	control		958	211	22.02±1.34
DMS	20 mg/m³		140	53	37.85±4.09 $p<0.01$
	2.0 "	$\sim Lim_{ch}$	144	43	29.86±3.8
	0.2 "		157	50	29.94±3.5
	control		787	191	24.27±1.53

p_1=compared to the respective level in the control.
p_2=compared to a level of 0.4 mg/kg.

Table 59

Methods Recommended for Mutagenic Hazard Evaluation of Industrial Chemicals (a Minimal Set)

Tissue	Animals	Humans
	Methods	Methods
Germ	DLM	Records of family history (abortions, stillbirths)
Somatic	Cytogenetic analysis (bone marrow) Estimation of mosaics by hair colour (spot test)	Cytogenetic analysis (lymphocytes) Unscheduled DNA synthesis SCE

nation of unscheduled DNA synthesis, both spontaneous and induced by a recognized mutagen — thiophosphomide; special procedures for estimating the functional state of immuno-competent lymphocytes and their degree of sensitization to formaldehyde, the principal occupational allergen. It will be seen from Table 55 that formaldehyde-exposed workers had an excess of chromosome aberrations ($r=0.31\pm0.12$; $F_{1.49}=5.32$; $p<0.05$). No significant correlation was reported between the occupational harmfulness and the level of TPA-induced aberrations; between the occupational harmfulness, spontaneous SCE rate and unscheduled DNA synthesis; or between the induced SCE level and occupational harmfulness. A significant negative correlation was established between occupational harmfulness and TPA-induced unscheduled DNA synthesis in vitro (r=

$= -0.28 \pm 0.12$; $F_{157} = 4.68$; $p < 0.05$). Certainly, as has been noted already, these findings provide primarily useful insights into the mode of action of formaldehyde, and, moreover, their use can be extended to make some general recommendations.

Evaluation of cytogenetic survey results convinced us time and again that, for individual cases (persons), the rate of chromosome aberrations is no indicator of the level of exposure. It is, nonetheless, a good and reliable indicator of the magnitude of exposure for the entire group. This was documented, for example, for chloroprene (3 groups of surveyed employees /Chapter 2/), lead, and other toxicants. This view is consistent with the perception on the subject available in the literature (Sorsa, 1980; Bochkov, 1982).

Analysis of chromosome aberrations found in the lymphocyte culture of specific employee groups exposed to industrial poisons is definitely an important method, but hardly sufficient at the present stage for evaluating chemical mutagens. What is needed, in fact, is some additional way of estimating the influence of chemical agents on the state of the reparation systems. Though, as many studies have shown, sister chromatid exchanges do reflect reparation processes, it is not yet clear how far this indicator can be trusted. A fairly straightforward method for determining the magnitude of reparation, which may indicate the intensity of mutagenic effect, is to assess the level of unscheduled DNA synthesis in the lymphocytes of the test group at the G_0 stage.

Importantly, in the evaluation of formaldehyde for mutagenic effect on furniture factory employees (in the leukocyte culture), this indicator was reported to have changed in a more significant way ($p < 0.001$) than did the chromosome aberrations ($p = 0.05$). This makes it possible to recommend for wider practical use in cytogenetic employee surveys.

Thus, because gene mutations pose greater risks to the population than chromosome failures one would ordinarily explore along these lines and because the state of the reparation systems may probably be a measure of the hygienic significance of recognized alterations, it is absolutely necessary to test the methods under a broader protocol.

Drawing on the experience of mutagenicity assessment of chemicals, the following minimal sets of tests can be recommended for evaluating mutagenic effect in both animal experiments and worker surveys (Table 59).

An immediate study of genetic changes in humans implies recording the various effects from mutagenic action. This involves, first of all, measurement of the rate of spontaneous abortions, childbirths with congenital developmental defects, stillbirths, and infant mortality as compared with the controls. As part of the procedure for recording these parameters, genetic effects are to be estimated in the germ cells. These mutations may be either of a gene or of a chromosomal and genomic nature. To identify them,

questions are asked in an interview during a medical examination or in a questionnaire (to be completed by a medical officer).

The questionnaire survey should be arranged among male workers. The questionnaire should inquire about the rate of spontaneous abortions and developmental birth defects. A good example was a questionnaire that proved quite informative even with a modest number (150) of workers surveyed as part of the chloroprene study. In females, discovery of a mutagenic effect induced by industrial chemicals on the germ tissue is known to be a practical impossibility, primarily because of the distinctive features of their ovogenesis. Apparently, major factors in this must be the effect, direct or indirect, they have on the function of the gonads, and the damaging of the foetus (embryotropic effect). Workers from the Institute of Medical Genetics estimate that, to ascertain a two-fold increase in the mutation process, 85 pregnancies need to be followed, whereas, to measure a 10% increase in the mutagenic process, 6,000 pregnancies need to be analyzed.

The key question in a cytogenetic worker survey is how to decide the number of persons to be examined (the sample size). Bearing in mind the previously established fact that the rate of chromosome aberrations obeys the Poisson distribution, the necessary sample quantities (the minimal quantity of surveyed subjects) were established, with at least 100 metaphase plates analyzed per culture to a probability of 95% (see Chapter 5).

The Institute of Medical Genetics has now reported a further refinement of the minimal sample size for cytogenetic analysis, sufficient for surveying occupational groups (Table 60).

In view of the data now available on the numerical dependence of chromosome aberrations in the lymphocyte culture on the season and the likely influence of other environmental factors, the survey arrangements must always include provision for a parallel, suitable control. Another useful move is to compare the resultant data with a recognized spontaneous level (1.19 ± 0.06, according to the Institute of Medical Genetics) so as to detect the possible contribution of those other environmental factors responsible for an excess of chromosome aberrations in both groups of subject.

An important question in interpreting the data of a cytogenetic worker survey is whether the increase in chromosome aberrations in the lymphocyte culture is somehow related to possible disturbance of the body's immune system. The hypothesis was formulated by Kerkis and Skorova (1974), who demonstrated the relationship between these effects on a group of patients.

The formaldehyde experiments, we staged together with Dueva on a group of 60 people in order to test the hypothesis, revealed no such relationship, casting doubts on previously established perceptions with regard to chemical compounds.

In an animal experiment, we established a more than 80% concurrence of mutagenic effect in somatic and sexual tissue cells. Moreover, data now emerging on the application of the specific

locus test to register point (gene) mutations also indicate a similarity of the results with data on the rate of induced chromosome aberrations in bone-marrow cells (for the case of dimethyl sulfate and ethylenimine) and dominant lethal mutations (for ethylenimine).

Table 60

The Necessary Scope of Studies for Conducting Cytogenetic Surveys of Occupational Groups (adapted from N. P. Bochkov)

Rate of increase in chromosome aberrations — percentage growth from control	Necessary for analysis		
	Number of metaphases	Number of individuals	
		with p=0.05	with p=0.01
by 25%	38,300	63	117
by 50%	10,600	17	32
by 100%	3,100	5	9

CONCLUSION

As of today, therefore, the most relevant issues in the study of long-term of chemical effects are: prediction, relevance and validity of animal test data extrapolation to man; and sound formulation of preventive programmes.

A brief review of new findings and a further analysis of the data cited above show the realistic possibility of managing these issues in practice. On the other hand, it must be noted that there are also other ways to approach the solution of the problems discussed here. One that we feel deserves considerable attention is to try to see whether long-term effects may be predictable from some physico-chemical properties, biological reactivity and several other parameters, with particular reference to the alkylating activity of chemical compounds as a prognostic clue to their mutagenic and blastomogenic effects (Hemminki, 1980).

The prime guideline for this kind of prediction should be the use of low-organized biological specimens such as microbes, insects and the like. The experience of toxicologists and pharmacologists points, however, to severe constraints on the results if they are to be applied to man. For the overwhelming majority of such cases, the only way to predict a chemicals' toxicity for humans is to test them out in experiments on laboratory mammals. Qualitative differences in intoxication patterns between animals and humans are few and far between; of much greater concern, however, is the ability to discriminate between them by quantitative parameters, with the difference likely to point either way. In our work, chemicals from different classes were taken to demonstrate the similarity of the results obtained from humans and small laboratory animals (rats and mice) according not only to qualitative, but also quantitative criteria.

Alternatively, species-specific divergencies in mutagenesis are obvious because such differences do exist in the metabolism. The distinctive metabolism rates and patterns may also be responsible for dissimilar indicators in animals of different lines (Tiunov, 1981;

Benesova, 1982). Data extrapolation is the most accurate when suitable data are available on the processes of the metabolism, distribution in the body, biotransformation, sorption and absorption of the test chemical in humans and experimental animals. In practice, data of this kind are of limited availability, which is why some of the relevant recommendations insist on using as many as 11 animal species (as suggested by different sources), among them mini-pigs, monkeys, rats and dogs, depending on the similarity of some metabolic aspects between these animals and humans.

Sanotsky and Ulanova (1976) argue that, in most cases (up to 70%), no significant difference exists in the species-specific sensivity of various biological species to chemical agents (for their purpose, the significant difference is threefold or more).

The methodological problems of data extrapolation with respect to specific effects are much easier to tackle if the study of long-term effects is perceived, overall, as one of the stages in the toxicometry of chemical substances designed for their hygienic limitation in the environment. The indicators of systemic toxicity that were recorded in several animal species, as required in the Methodological Recommendations on Experimental Research for MAC Validation (CMEA, 1982), may be sufficient to establish the relative significance of species variations. With a variation in species sensitivity of three times or more, the probability of total agreement between the results obtained from laboratory animals and human responses is substantially reduced. In that event, all that needs to be done is to increase the accepted safety factor and expand the set of experimental models.

The procedure requires considerable extensions when specific recommendations need to be developed for selection of the safety factor as one moves from test animal data to prediction of safe exposure levels for man. Besides estimation of the key indicator — the degree of selectivity of action (see Chapter 2) — a means for monitoring species sensitivity to the poison must be integrated with a qualitative characteristic of toxicity, which is a function of quantitative exposure levels.

Extrapolation of effects from higher to lower levels is possible but difficult. Not only does it involve mathematical difficulties, as is emphasized in the literature, but also, and more important for toxicologists, the mode of action of the poison, or pathogenesis, seems to vary with exposure levels. Caffeine is an obvious example. Some time ago geneticists tended to treat it as a mutagen (see Chapter 2) and a "ban" on the consumption of tea and coffee ensued. This is no longer the case, now that the literature strongly suggests that caffeine has a remarkable ability to suppress the formation of neoplasms and inhibit the transformation of cell cultures (Nomura, 1981). The same is true of other substances (e. g. vitamin A, ascorbic acid, etc.). In other words, questions of data extrapolation should be exclusively handled within the dose limits, keeping the mode of action of a chemical agent qualitatively similar; of practical

value, therefore, are low, real-life doses and concentrations of the test chemical.

Warnings concerning the danger of long-term effects evolving from occupational contact with chemicals may be much more effective if data are available directly for human subjects. Such studies on human volunteers must be managed in accordance with the Helsinki Declaration (revised in Tokyo in 1979) and Article 7 of the International Pact on Civil and Political Rights endorsed by the UN General Assembly (WHO, 1976); there must also be a complete data profile on the chemical's toxicity for animals.

On the one hand, this provision may give rise to a negative reaction because it complicates the methodology for identifying long-term effects. On the other, one shares Paget's (1970) concern that unavailability of such data may, in fact, lead to uncontrolled experimentation on human groups, even though the safety of the doses and concentrations being tested has been ascertained only from animal experiments. For this reason, humanistic considerations would require greater scope for studies on volunteers testing the safety of chemical concentrations suggested for general use in practice (Principles and Methods for Evaluating the Toxicity of Chemicals, WHO, Geneva, part I, 1978).

For substances with long-term implications of the kind examined above, it is possible to recommend a series of predictive tests, such as studies of chromosome aberrations, sister chromatid exchanges and unscheduled DNA synthesis in the culture of peripheral blood lymphocytes before and after exposure to the chemical under study, and of sex hormones. Judging from experimental test data, such a manifestation can be effectively registered within three months. Half a year appears to be sufficient for observations on volunteers.

The scope of the present discussion for each relevant aspect does not cover fully the area of research focused on the long-term effects of chemicals that are gaining such broad acceptance in the economy and in everyday life. The continuous inflow of new data and ongoing broad discussion of methodological issues are cause for optimism. The problem is being tackled successfully and will no doubt be eventually resolved.

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