IRPTC

PREVENTIVE TOXICOLOGY

Collection of Training Materials

Volume II, part I

ED NATIONS ENVIRONMENT PROGRAMME

UNITED NATIONS ENVIRONMENT
PROGRAMME (UNEP)

USSR STATE COMMITTEE FOR
SCIENCE AND TECHNOLOGY
(GKNT)

INTERNATIONAL REGISTER OF
POTENTIALLY TOXIC CHEMICALS
(IRPTC)

USSR COMMISSION FOR UNEP

PREVENTIVE TOXICOLOGY

Collection of Training Materials

Edited by N.P. Izmerov
Corresponding Member,
USSR Academy of Medical Sciences

Volume II
Part I

CENTRE OF INTERNATIONAL PROJECTS, GKNT

Moscow 1984

Collection of instructional materials on preventive toxicology is published by Centre of International Projects, USSR State Committee for Science and Technology under the USSR-UNEP/IRPTC Project "Control of Hazards Posed by Chemicals to Human Health and the Environment" implemented in cooperation with the Research Institute of Industrial Hygiene and Occupational Diseases, USSR Academy of Medical Sciences.

The collection is intended for toxicologists, hygienists and all those responsible for evaluation and control of harmful effects of chemicals to human health and the environment.

Materials of collection may be freely cited and reprinted with a reference to the source. Opinions expressed in instructional materials do not necessarily reflect the official policies of the United Nations Environment Programme. Since the published information was in exact conformity with the data available at the moment of publication, UNEP is not responsible for any errors or omissions or any consequences therefrom.

UNEP

CONTENTS

III. INDUSTRIAL PREVENTIVE TOXICOLOGY

IY. PREVENTIVE TOXICOLOGY OF AGRICULTURAL CHEMICALS

6300

Chapter III

INDUSTRIAL PREVENTIVE TOXICOLOGY

Edited by
Professor I.P. Ulanova

GOALS AND METHODS OF INDUSTRIAL TOXICOLOGY

I. V. Sanotsky

1. Definitions

Industrial toxicology belongs to a group of sciences which deals with justification, elaboration and implementation of preventive measures against the impact of harmful substances in the production environment. More fully this scientific field can be covered by term "occupational toxicology", since in such a case the description should be extended to include agricultural and transport toxicology. This group also includes the chemical hazards that occur under extreme conditions, such as in the work of astronauts or firemen.

The entire group of occupational chemical influences should be referred to the category of "hygienic toxicology" and placed there next to communal toxicology. A higher class should be termed preventive toxicology, and ecotoxicology should be included into it along with hygienic toxicology. Clinical toxicology with its multiple subclasses forms a parallel class.

Thus, industrial toxicology should be defined as the science of prevention, diagnosis and treatment of intoxications caused by industrial toxic chemicals and prevention of long-term effects of industrial poisons.

Industrial poisons are substances which man encounters in the course of his work as initial, intermediate, by- or end-products (in the form of gas, vapor, liquid, dust, smoke or mist) exerting harmful effects on working men in case of non-compliance with the labor safety and occupational hygiene requirements and, as a consequence, penetrating into the body in amounts inconsistent with its hereditary and acquired properties (in some cases this implies the quality of external exposure foreign to the body).

It becomes clear from the definition that all or almost all industrial chemicals are potential poisons.

The degree of virulence (toxicity) of a particular substance has long been measured in terms of its absolute quantity producing a definite biological effect.

The definition of toxicity as the ability of a chemical molecule to exert harmful action (the most common definition) is, in our view, inadequate, since, firstly, any substance can exert a harmful effect if present in a big enough quantity and, secondly, not every harmful effect, in our opinion, can be used to measure toxicity.

In his article "The Content and Goals of Toxicometry" N. S. Pravdin wrote, "despite large individual variations in the speed of the onset of death, the very moment of death can nevertheless be ascertained in a sufficiently objective manner. But even this scanty information is not available in regard to other parameters of intoxications".

Toxicity should be estimated on the basis of a lethal effect. Absolute toxicity is the value reciprocal of the absolute magnitude of the dose or concentration causing the death of animals.

On the basis of this principle we proposed sometime ago a continuous toxicity scale assuming for zero toxicity (0%) the value reciprocal of the toxicity of carbon dioxide whose median lethal concentration (CL^{120}_{50} m - the accepted notation for median lethal concentration in experiments on mice /m/ for 120-minute exposure /120/) is about 10^4 μm/l (10 μm$\cdot$m^{-3}); 100 per cent toxicity is conditionally taken to be the value reciprocal of toxicity of the most toxic group of organophosphorus chemicals whose LC_{50} is 0.0001 μm/l (10^{-7} μm$\cdot$m^{-3}).

In reality there are substances which are less toxic than carbon dioxide and more toxic than the organophosphorus compounds. Thus, the toxicity differential of the extreme compounds in the "continuous scale" (the real scale is discrete but every "step" in it is reduced to one per cent) will appear still greater. The position in the middle (50 per cent on the continuous absolute toxicity scale) of that system is occupied by hydrogen cyanide.

In practice, enlarged tabulation of toxicity offers a far more convenient alternative. We suggested grouping substances under three classes of toxicity: extremely toxic (50 per cent on the absolute toxicity scale), medium-toxic (25 to 49 per cent on the scale), and slightly toxic (0 to 24 per cent on the absolute toxicity scale).

The USSR National Standard GOST "SSBT.12.I.007-76. Harmful Substances: Classification and General Safety Requirements" sets out four classes of toxicity, just as in the classification of the Council for Mutual Economic Assistance (CMEA) (see Table 1). In the United States there are six toxicity classes.

As is evident from Table 1, in addition to toxicity, there is a wider class of concepts associated with the harmful effect of substances. Hazards of a substance is one of such concepts.

In industrial toxicology hazards mean a totality of properties determining the probability of intoxication (or, in broad, an unfavourable, noxious effect under actual production conditions or while using the chemical).

6300

Table 1

Parameter	Rates for classes of hazard			
	1st	2nd	3rd	4th
Maximum allowable concentration (MAC) of harmful substances in the air of a working zone, mg/m^3	Below 0.1	0.1-1.0	1.1-10.0	Above 10.0
Median lethal concentration, intragastric administration, mg/kg	Below 15	15-150	151-5,000	Above 5,000
Median lethal concentration, skin application, mg/kg	Below 100	100-500	501-2,500	Above 2,500
Median lethal concentration in air, mg/m^3	Below 500	500-5,000	5,001-50,000	Above 50,000
Index of potential inhalation toxicity (KVIO)	Above 300	300-30	29-3	Below 3
Zone of one-time effect $Z_{ac} = \frac{LC_{50}}{Lim_{ac}}$	Below 6.0	6.0-18.0	18.1-54.0	Above 54.0
Zone of chronic effect $Z_{ch} = \frac{Lim_{ac}}{Lim_{ch}}$	Above 10.0	10.0-5.0	4.9-2.5	Below 2.5

The hazard classification by the zone of specific (selective) effect was suggested by I. V. Sanotsky and V. N. Fomenko. This classification together with a proposed safety factor (its concept will be considered below) is presented in Table 2.

The "zone of irritant effect" concept was pioneered by G. G. Maximov in collaboration with I. V. Sanotsky and N. G. Ivanov in examining regularities of safe levels prognosticated by toxicometric values found in an acute experiment. Subsequently, this notion was extended to all types of systematic action by I. V. Sanotsky and his co-workers.

It was established that the more the systemic thresholds of single noxious dose exposure differ downward from those of strictly integral action (that is, at the level of composite indices reflecting simultaneously the state of several systems or of the entire organism), the more they approximate, in a single-exposure experiment, those harmful effect thresholds which will be found in a chronic and life-long experiment, the more hazardous is the substance concerned and the larger safety factor must be set up.

Table 2. Classification of hazards of industrial chemicals by zone of selective effect

Class of hazard	Extremely hazardous	Highly hazardous	Moderately hazardous	Slightly hazardous
Zone of specific (selective) effect $Z_{sp} = \frac{Lim_{ac\ integr.}}{Lim_{ac\ spec.}}$	>10	4–10	1–3	<1
Safety factor	>50	50	under 10	usual

Safety factor is the ratio of the value of the chronic effect threshold to the level of a sanitary standard, i.e. to the maximum allowable concentration. As a concept, the safety factor appeared as a consequence of our ignorance of species variations in sensitivity toward a given chemical (indeed, how anyone can compare human sensitivity and that of experimental animals).

The safety factor has been introduced to prevent harmful effects of substances on working men in case of an abrupt increase in their concentrations within the breathing area.

The magnitude of safety factor (I_s) is deduced as follows:

$$I_s = a \frac{KBLIO \; Z_{ch} \; Z_{sp} \; Z_{biol}}{Z_{ac} \; I_{cum}}$$

All of the abbreviations used therein have been explained in the text, except for I_{cum} (cumulation coefficient) and Z_{biol} (zone of biological effect). Both these parameters, in addition to the zone of chronic effect, reflect the cumulative properties of substance, that is, its ability to accumulate noxious action in the body as a result of both material accumulation and the build-up of functional changes.

The cumulation coefficient is usually determined in subacute experiment by comparing the results for doses causing lethal effect under a single exposure with the results attained for the total dose causing lethal effect in a subacute exposure.

$$I_{cum} = \frac{DL_{50}}{\sum_{0}^{n} DL_{50}} .$$

All of the parameters previously described belong to toxicometry, a system of principles and methods used to determine the toxicity and hazards of chemicals.

2. Goals of Industrial Toxicology

Back in the late 1920's, the initial years of an intensive industrialization campaign in the USSR, N. S. Pravdin, one of the forefathers of Soviet toxicology, formulated its principal goals as follows:

(1) hygienic expert examination of toxic substances;

(2) hygienic expert standardization of raw materials and products;

(3) hygienic expert rating (of harmful substances content in objects of the production and biological environments).

The sanitary standards limiting concentrations of hazardous substances in the air of a working zone and on the workmen's skin are most essential for industrial toxicology. Of some importance are also the so-called "exposure tests", or biological MACs which identify critical contents of noxious substances or their metabolites (and also the most vulnerable enzymes or other biochemical indicators, e.g. enzyme substrates, etc.) in biological materials: workmen's urine, blood and exhaled air. Sometimes such biological media are also taken into account as working mothers' milk, amniotic fluid, hair and nails (for some chronically acting metals which tend to accumulate in the body).

The tests, which make it possible to use toxicokinetic equations in assessing the total poison dose penetrating into the body via different routes, cannot, however, replace MACs and TSELs, since the exposure tests have been developed for comparatively few substances.

The hygienic standardization of raw materials and products (or toxicological limitation of admixtures), plays very important role because technical specifications of the manufacture of product admixtures are generally limited by technological criteria only. Some admixtures may have pronounced toxicity. For example, an admixture to virtually safe freons in the form of perfluoroisobutadiene which could appear as a by-product, is capable of causing acute intoxication. Or another example: petrol, as everybody knows, represents a complex mixture of hydrocarbons with certain additives. In some instances technical specifications require constraints on the composition of petrol of the same grade which are maintained in accordance with engineering requirements only. Meanwhile petrol of the same grade may include substantial amounts of especially hazardous aromatic hydrocarbons, sulfurous compounds and other chemicals. Limitation of their percentages according to medical indications is exactly what the hygienic standardization of raw materials and products seeks to achieve.

Toxicological (hygienic) expert examination refers also to the primary toxicological assessment of a product. As a rule, it comprises estimation of its toxicity (LD_{50}, and sometimes LC_{50} in case of saturating concentrations); local and sensitizing effect on the skin and mucous membranes, and on the eyes; and determination of the cumulation coefficient with subsequent calculation of TSEL.

Sometimes these need be supplemented with a list of tests to provide a more comprehensive characterization of a substance meant for sale, complete with recommendations as to the sensitivity levels of the control methods used. A toxicological safe data sheet, such as this, developed by the Soviet Toxicological IRPTC Center of the USSR Ministry of Public Health and the USSR/UNEP Project "Control of Hazards Posed by Chemicals to Human Health and the Environment", is offered for discussion by an international experts group.

3. Stages in Setting MACs

Studies in industrial toxicology should best be carried out in stages beginning with early phases of technological development. A schematic sequence of toxicological procedures in substantiating TSEL or MAC values, simultaneously with technological design stages is given in Table 3.

The table shows that generally the toxicological evaluation of a new chemical, prior to its introduction into the country-wide application, includes the following stages:

Table 3. Stages in technological development and toxicological assessment of industrial poisons (according to I. N. Sanotsky)

Stages of technological development	Stage of toxicological assessment
1. Process design	Preliminary evaluation: analysis of the literature, computation of toxicometric parameters
2. Laboratory design	Toxicological expert examination: acute and subacute animal experiments, toxicological tests of production engineering prototypes
3. Pilot-plant manufacture	Toxicological certification, substantiation of MAC: chronic experiments, investigation of long-term effects, examination of employees
4. Commercial manufacture design	Additional research: study of effective mechanisms, early and differential diagnosis, experimental therapy

Table 3 (cont'd)

Stages of technological development	Stage of toxicological assessment
5. Commercial manufacture	In-situ studies: examination of employees' working (and living) conditions, medical examinations, epidemiological studies, clinical verification of experimental data on prevention, diagnosis and treatment; adjustment of medical and technical requirements for production

This integrated approach pays off in terms of resource economy. It also provides for the implementation of one of the basic principle of hygienic standardization - toxicological research should precede the introduction of a chemical into the country-side application; in greater detail the general principles of sanitary standardization are dealt with in I. V. Sanotsky's lecture "The Concept of Preventive Toxicology and Underlying Principles of Health Standards in the USSR".

According to UNEP estimates, with timely use of the sanitary standards data, based on the toxicological research in the design, the costs of purification facilities would account from 6 to 12 per cent of the total project investment. If, however, such sanitary standards are developed only at the final stage of commissioning, the modernization costs would run up to 50 to 100 per cent of the investments, that is, the chemical plant will in effect have to be built anew.

3.1. Toxicological Analysis of the Literature

A review of data on the substances structurally similar to the chemical in question, should take into account the series of homologs and analogs. Within the series, inter- and extrapolation of toxicity and hazards are quite possible.

These operations, however, need by done with great caution since the first member in the series proves to have the greatest toxicity (despite general increment in toxicity in the homological series) and because the so-called "break" in toxicity due to higher melting and boiling points of the compound in the homologous series, accompanied with a simultaneous decrease in its solubility (usually observed starting from C_5-C_8 onwards).

Tentative Safe Exposure Levels (TSEL), or interim sanitary standards for the exercise of hygienic control during the early stages of process and production deisgn, may be suggested for use during the first stage of the toxicological evaluation - toxicological analysis of the literature. Thus the general correlation coefficient between the TSELs defined by physical and chemical properties and the MACs adopted in accordance with the complete program is, according to E.I. Lyublina and co-workers,0.5. The use of more comprehensive physical and chemical characteristics of the substance (electronic parameters, dipole, symmetry, etc.) for limited groups of chemicals, increases the correlation, which sometimes reaches +0.9. Yet this is not of frequent occurrence. G. G. Maximov with co-workers found that the use of multiple correlation techniques augments drastically the forecast reliability in relation to the levels of sanitary standards based on the physical and chemical properties.

At the present time, as an exception and if so permitted by the sanitary inspection, TSELs can be applied for designing the production facilities manufacturing substances of the 3rd and 4th classes of hazard. This may be done in case of limited exposure to the substances representing a well-studied group.

One such classification by the quality of effects of the chronic exposure to industrial chemicals in the lowest doses and concentrations as well as by the extent of irreversible changes has been proposed by G. G. Avilova. For instance, in line with the recommendations provided by toxicologists, chemists modified a number of production processes in synthetic dye manufacture with a view to avoid the formation of the potent carcinogen - naphthylamine (e.g. in I-acid production). In the pharmaceutical industry, the heating elements causing excessive heating of the reactors and causing harmful discharges into the air were eliminated. Another example: sulfuric acid was integrated into caffeine production at the stage of synthesizing cyanacetylmethyl urea to replace acetic acid with a view to decrease the temperature necessary for subsequent nitrosation; on the joint recommendations of toxicologists and hygienists some changes were introduced into production processes to avert the formation of especially hazardous semi-products. In the same manner, solvent replacements were suggested for some production processes. Thus, in the production of fatty acids methyl alcohol was replaced by butyl alcohol; in the petroparaffin manufacture benzene solvent is generally substituted by toluene; styrene-free glass-reinforced plastics and new grades of chloroprene-rubber latexes containing no residual chloroprene have been developed.

3.2. A toxicological experiment includes determination of the toxicometric parameters described above in addition to the investigations covered by the program of toxicological expert examinations of chemicals prior to their industrial application (the experts' requirements were referred to earlier); a chronic toxicological experiment, not infrequently of life-long duration, is carried out in order to establish the probability of long-term effects.

According to our data a study of accelerated ageing in small laboratory animals in response to the effect of chemical products has to take no more than nine months to one year upon completion of the chronic experiment which by itself may not be always sufficient to obtain the necessary information. Many authors insist on one year as the time sufficient for studying experimental carcinogenesis in mice, while others disagree, and favour a life-long experiment.

It is neither practicable nor necessary to examine here specific methods of studying particular integral, systemic, organic, cellular and biomolecular responses to noxious exposures. The important thing is to define the threshold of a harmful effect and to calculate the safety factor.

The basic principles of sanitary standardization are reviewed in I. V. Sanotsky's lecture "The Concept of Preventive Toxicology; Principles of Sanitary Standard Rating".

Following toxicological experiments, it is possible to recommend more credible TSELs than those calculated on the basis of physical and chemical properties. Simple calculations indicate that the MAC level in the USSR is, on average, 1,000 times lower than the LC_{50} level, though its variations are great. The highest reliability to date has been secured in predicting MACs of irritating agents by both the threshold of their irritant effect and Z_{sp}. The correlation coefficient of the MACs, found by express methods, and those established in compliance with the complete procedure, was above 0.9.

To assure comparability of research results obtained in different laboratories, it is necessary to have a comparable or, still better, standard set of experimental conditions shown in Table 4.

Table 4. Experimental conditions requiring standardization

Indices	Animal species	Exposure and other conditions	Notes
LC_{50}, LC_{16}, LC_{84}	Mouse (20±2 g) Rat (220±40 g)	2 hr 2-weeks 4 hr observation	Berens-Schlosser's method; Probit-analysis in Prozorovsky's modification; study of clinical picture and pathomorphology
LD_{50}, LD_{16}, LD_{84}	ditto	2-week observation	Index used if necessary
Irritating effect with skin or eye application	Mice (tail) Rabbits (back, ear) (1-2 kg)		Quantitatively estimated
Threshold of irritant effect	Any species Man (if safety guaranteed)	15 min 1 min	Use of most sensitive quantitative methods (to be distinguished from response threshold)
Threshold of acute effect Lim_{ir}	At least 2 species (rats mandatory, cats desirable)	4 hr, study 1/4 hr post-exposure	Use of at least 2 integral methods
Cumulation coefficient (I_{cum})	Mice	Intragastric	Leam's or Kagan-Stankevich's method
Threshold of chronic effect Lim_{ch}	At least 2 species (rats mandatory, cats desirable)	4 hr daily 5 days weekly, 4 months. At least 1 month post-exposure surveillance. Three concentrations at a time (Lim_{ac}) with 5-10-fold difference.No-effect, concentration must be established '	At least 2 integral and 2 pathogenetic methods (Histological tests mandatory). At first two-week and then monthly observations

Unified conditions of animals' keeping are required.

Proceeding from differences in animals' response ability, many authors believe that a three-fold error in establishing LD_{50} and LC_{50} is normal, since it is explained by unaccounted changes in the conditions under which the animals are kept.

The animal experiment would be worthless if it were not possible to extrapolate its results onto humans.

Some authors argue that such extrapolation is solely feasible in case of simultaneous use of many species (up to 10, including monkeys) and lines of laboratory animals. Others maintain that where there is a monotonous pattern of responses by four species of test rodents to the administration of the same poison (the coefficient of species differences should not exceed 3) there must be 70 per cent coincidence between the human and animal patterns of sensitivity.

In terms of the chronic and long-term effects, there has been a greater measure of coincidence observed in the response pattern of animal and humans, as well as a greater similarity between the response thresholds (Fomenko et al., reported it for 90 per cent of the cases).

Thus, the probability of error in extrapolating laboratory mammal data onto humans appears to be not so great. Still, it does exist. The answer lies in recruiting human volunteers for determining some toxicometric parameters (thresholds of the response, irritant and olfactory effects). To make this possible, the subjects must be guaranteed full safety in accordance with the Helsinki Convention and many international and national safeguards.

Another way to cut down experimental errors is to test the results of in-situ studies.

3.3. In-situ Studies

The correlation of employees' health and their working conditions is tested both during planned preventive medical check-ups and special epidemiological tests. The former's frequency varies usually from once in six-month to once in two years, depending on the toxicity of the substance in question and the specific production facilities.

Regrettably, epidemiological tests often take into account only the workers' occupation (a substance or, more frequently, a group of substances acting in combination with other factors), sex, and the duration of employment. In our opinion this is not enough; both the production facilities and the effective factors (concentration of hazardous substances in the breating zone, on the skin, temporal fluctuation of concentrations),must be thouroughly studied.

4. Conclusion

It is only a brief review of the principal goals of industrial (occupational) toxicology and the methodological procedures to attain these goals.

The accent was on the preventive side of the science as previously noted, the tasks of diagnosis, especially the early and differential one, as well as of treatment of intoxications and other forms of the diseases induced by the chemical agents present in the working environment (long-term effects, decline of total resistance to external impacts and other forms) also form part of industrial toxicology; they are dealt with in conjunction with the clinical symptoms of occupational diseases and other related disciplines.

The methods of industrial (occupational) toxicology are increasingly introduced into the related fields of knowledge, paving the way to toxicological (applied) genetics, applied teratology, "molecular toxicology", and a host of other sciences located at the cross-roads of the major avenues leading to the goals of prevention, identification and aversion of the immediate and long-term effects of chemical exposure.

The scientific potency of toxicology stems from the union of hygiene, clinical data and experiments that ties them together, said Prof. N. S. Pravdin. The practical potency of industrial toxicology lies in concerted work of chemistry, technology and medicine, aimed at developing safe production processes, at their timely adjustment on the basis of toxicological findings, and directed to drastic improvement of the working conditions.

BIOLOGICAL VALIDATION OF MAXIMUM SINGLE-EXPOSURE AND AVERAGE WORKING SHIFT ALLOWABLE CONCENTRATIONS OF CHEMICALS IN THE AIR OF THE WORKING ZONE

I.P. Ulanova

Sanitary-validated concentrations of harmful substances in the air of the working zone has always been a matter of primary concern for toxicology and industrial hygiene. In an industrial environment, even under continuous production conditions most favourable from the hygienic standpoint, toxic substances are known to exert intermittent action. This action is much more pronounced in batch production conditions because chemical concentrations reach higher levels due to loading and unloading operations, involving raw materials, semifinished and finished products, sampling, etc. However, the intermittent action of airborne concentrations of toxic substances has so far been underestimated both in shopfloor and laboratory tests. According to the norms adopted in the Soviet Union, the maximum allowable concentrations (MAC) of harmful substances must not be exceeded even for a short while. Only for some highly cumulative substances, such as cadmium oxide, metallic mercury, antimony, lead and its inorganic compounds, average working-shift concentrations have been approved. Undoubtedly, for most organic compounds exhibiting a chronic tendency for accumulation, concentration variations (within certain limits of course) are unimportant since their biological activity is determined primarily by the dose received by the body. In hygienic standardisation, such substances should preferably be treated in terms of time-weighed concentrations. However, lack of special research into intermittent action holds back taking final decision on hygienic standardization, since a hasty standard may be detrimental for workers' health or, on the other hand, a too stringent concentration may prove to be economically burdensome.

In most countries, the MAC of harmful substances for industrial environments are expressed in average working-shift concentrations, while in others (GDR, Rumania, United States, Czechoslovakia, Sweden, etc.) two values are used - the maximum and average working-shift concentrations. However, maximum single-exposure safe concentrations adopted in these countries are not well founded as evident at the WHO meeting where safe exposure levels were discussed for some metals and solvents. Some countries failed to provide scientific support for a safe range of maximum single-exposure and average time-weighted concentrations.

To resolve these problems, first of all, the damaging effect of poisons and adaptation to them, depending on the exposure conditions have to be studied experimentally on animals.

Experimental Data on Intermittent Effects of Chemicals

Relatively few studies have been carried out in which the body response to different exposures of chemicals was compared, with experimental conditions varying widely. The experiments in which continuous and intermittent exposures were involved can be singled out, the former implying uninterrupted effect of a toxic substance over a period of several hours and the latter, variations in its concentration within the same period. Thus, "continuous" should be used here with some reservation. Such an experimental approach is more consistent with the procedures commonly used in industrial toxicology. Another outstanding feature, is that these experiments, use the principle of equality of time-weighted concentrations throughout the day-long exposure. These two conditions determine the inequality in the effective concentrations in both types of exposure. As a rule, the intermittent exposure to chemicals was less favourable as compared to the continuous one (1,2,3,4,5,6,7).

In another group of experiments, the effective concentrations were maintained equal under intermittent and continous exposure, their duration being the same almost in all experiments, including breaks in intermittent exposure. Thus, the total amount of substances uptaken by the body during the entire experiment was higher under continuous exposure rather than in intermittent one. Such an experimental procedure is common, primarily, in day-to-day toxicology. The intermittent effect of chemicals in these experiments was often less dangerous than under continuous exposure to the same concentration.

Apparently, a lower overall concentration in the intermittent exposure is responsible for a less pronounced biological effect (8,9,10,11,12,13). However, in experiments with sulfur dioxide (14), formaldehyde (15), and methyl mercaptophos (16), the intermittent exposure was more effective in some respects than the continuous one.

It has been speculated that differences in the continuous and intermittent exposures to harmful substances depend on their physico-chemical properties of poisons and the associated processes of uptake by, accumulation in, and removal of these substances from the body (17,18). Of particular interest, in this connection, are experiments with ethanol and chloroform (17). Experiments in which equal amounts were inhaled indicate that, because of its high water solubility, ethanol is accumulated slowly in the blood, and the concentration fluctuations under the mask, produce no effect whatsoever on resorption of the poison as compared to continuous exposure. At the same time, in the case of chloroform which is poorly soluble and saturates the blood at a fast rate, the amounts inhaled at peak intensities exceeded those in continuous exposure.

Thus, a more pronounced effect of a fat-soluble substance, such as chloroform, under intermittent exposure can be ascribed to its higher content in the blood. Yet, when the concentrations were selected in such a manner that the blood content of the harmful substance was the same under both exposures, the intermittent exposure proved to be more dangerous in addition. According to (17), enhanced toxic effect in such instances depend on a decreased body adaptability, although no special research has been conducted to corrborate this assumption.

The literature give data on the adaptation to different exposure to methylene chloride (19) and xylene (20,21), their content in the blood being determined at equal air-borne average weighted concentrations. It has been shown that continuous exposure to methylene chloride at one tenth of the threshold acute effect and at its content in the blood being as high as 0.28 mg/ml virtually throughout the poisoning leads, in the final analysis, to true physiological adaptation. In this case, the poison content in the blood decreases toward the end of the experiment, whereas under intermittent exposure, when the poison content in the blood remained invariable, a pronounced pathological process was observed.

In experiments with xylene, with gradual adaptation of the body to this substance under both exposures, its content in the blood decreases in both modes, although the early developed adaptation to the intermittent exposure broke down after a short while. When the experimental procedure was slightly modified, namely, when effective rather than weighted average concentrations were made equal so that the estimated accumulation rate of xylene was lower under the intermittent exposure, the latter was still found to be less favourable than the continuous one(21). Adaptation breakdown was accompanied with a higher poison content in the blood, which, however, could be detected only after exposure to a high concentration.

As can be inferred from the foregoing, the poison content in the blood was determined while studying adaptation to substances exhibiting so-called "physical toxicity", that is to say they remain almost intact in the body. Substances displaying "chemical toxicity", are especially dangerous and have a capacity for specific action since they undergo transformations in the body (22). Such substances should be studied with due account for the possible development of adaptation as well as latent pathological process primarily in response to the specific effect. It should also be borne in mind that studies of chemicals under the intermittent effect in the context of establishing hygienic standards, provide reliable information only when the substances are taken in low concentrations. In this connection, we should like to single out a series of experiments involving the intermittent effect of chemicals, carried out at the Research Institute of Industrial Hygiene and Occupational Diseases, USSR Academy of Medical Sciences (23). The experiments followed a unified procedure in which continous exposure to poison lasted for four hours, concentration being relatively invariable. The intermittent exposure involved five 15-minute "peaks" and four 40-minute breaks during which clean air was let into the chamber. The 15-minute "peak" effect was selected on the assumption that in industrial environment a worker handling a short-time operation, say, in opening some equipment, may be exposed to increased concentrations of harmful substances for a short while.

One of the main preconditions for comparing both exposure patterns was equality of the total amount of the poison passing through the respiration zone of the animals in the groups being compared. To meet this conditions, almost equal average weighted air-borne concentrations of poisons in the test chambers were maintained for both exposures. This, in turn, provided for inequality of the effective concentrations under continuous and intermittent exposures. The concentration within a 15-minute peak exceeded the average weighted one by a factor of three, on the average.

The experiments were conducted at different levels and different exposure durations. Changes in the biological systems were studied at the level of a whole body as well as at systemic, organic, cellular, subcellular, and molecular levels. Particular attention was given to the state of the systems most susceptible to affection. Loading tests were used to reveal possible latent changes. Also determined was the content of the substance being tested or its metabolism products in the biomaterial.

The compounds selected for testing, such as carbon tetrachloride, benzene, tetrachloroethane, and dimethyl formamide, are widely used in industry and represent highly cumulative substances with a broad range of toxic effects. Apart from their effective concentrations, it is also important to take into account the time factor (for poisons cumulating in time) -that is, the time period during which compensated and noncompensated functional and structural changes are accumulated. The airborne concentration of substances in the test chambers was determined by gas chromatography.

Since the experimental studies of the changes in biological indices enabled the maximum and average weighted airborne concentrations of some substances in industrial environment to be established for the first time, let us now dwell upon their results.

Carbon Tetrachloride

When xenobiotic is present in a concentration corresponding to a level of 6 Lim_{ch} (12 MAC), its intermittent action is less favourable than the continuous one bath in the number of changed indices (17 under intermittent exposure and 9 under continuous exposure) and in the extent of these changes (Figure 1). That liver undergoes a more pronounced damage under intermittent exposure, is evidenced by the marked decrease in the rate of hippuric acid synthesis (on the fourth day), the appearance in the blood of mitochondrial enzymes (GDH and OCT), indicative of the severe lesions of hepatocytes, a perceptible increase in the activity of cytoplasmic enzymes (F-1-PA, SODH) in the blood as compared to continuous exposure, and a reduced content of cytochrome P-450 in the hepatic tissue, indicative of altered structural organisation of microsomes.

The observed inhibition of the reaction to loading with the substance (at the Lim_{ac} level), expecially as suggested by changes affecting the enzymes, which go beyond the physiological adaptation reaction, is indicative of a latent pathological process'.

The results of functional tests agree well with those of morphological analysis of the liver structure. The observed differences in the action of carbon tetrachloride under the two exposures cannot be explained by the dissimilar CCL_4 contents in the blood because it was approximately the same under both exposures, ranging from 0.62 to 0.78 mg (Figure 2) (23).

In an experiment with a chronic action threshold of CCl_4 equal to Lim_{ch} (2 MAC) (Figure 3), the changes were much less pronounced. In this case, the activity did not increase under intermittent exposure; on the contrary, the changes under continuous exposure were more manifest. Hence, the effect produced by intermittent and continuous action of the xenobiotic depends on its effective concentration.

Benzene

In the experiment with benzene, two levels of effects were involved. At 12 Lim_{ch} level (Figure 4), the test animals developed leukopenia with a more manifest effect on the blood system under intermittent exposure, which was indicated not so much by the number of changes as, primarily, by their extent and persistence.

At this level, the intermittent mode was definitely less favourable than the continuous one, The dynamics of the content of phenol - the main metabolite of benzene - in the urine of test animals was marked by a clearly defined increase beginning from the eighth day of exposure, was more pronounced in the intermittent mode (Figure 5).

When benzene was present in a concentration corresponding to 3 Lim_{ch} (Figure 7), the changes affecting the blood system resulted in moderate leukocytosis due to higher lymphocyte count. The exposure was not found to produce any effect on reaction nature. The phenol content in the urine of animals (Figure 8) was even higher under continuous exposure. Hence, the intermittent action of benzene at a low level does not enhance its harmful effect (25).

In a similar experiment with dimethyl formamide (Figure 9), no differences in the body response to continuous or intermittent exposure to xenobiotic at two levels equal to 2,6 and 7.8 Lim_{ch} were observed either (26).

Thus, as was shown in subacute experiments with four highly cumulative substances, the biological effect produced by varying concentrations as compared to the constant ones at the same average weighted concentration depended both on the physico-chemical and biological properties of the poisons as well as the level of their exposure. Three-fold concentrations of benzene and carbon tetrachloride are harmful only at a relatively high action level. For other substances (1,1,2,2-tetrachloroethane,dimethyl formamide), no difference was observed under continuous and intermittent exposures either at high (6 and 7.8 Lim_{ch}, respectively) or low (1.25 and 2.6 Lim_{ch}) levels. Hence, are greater variations in concentration allowable? To answer this question, special experiments were conducted with carbon tetrachloride at a low action level (Lim_{ch}).

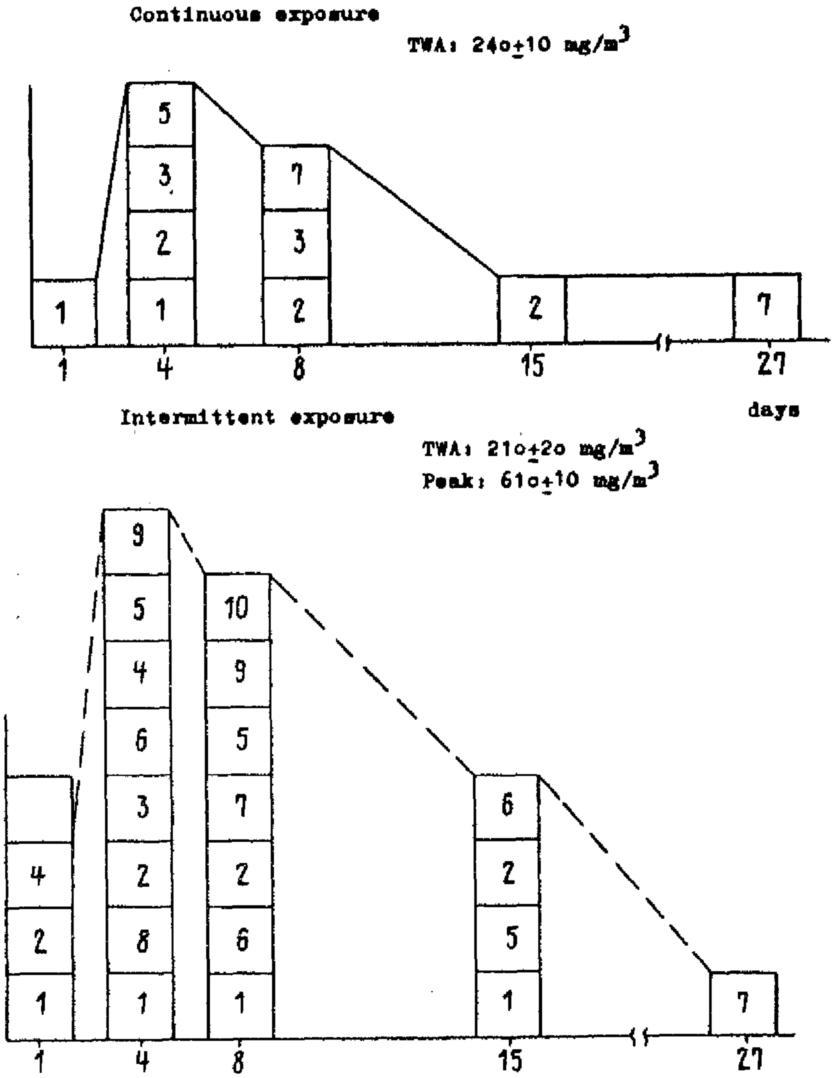


Fig.1 Changes in indicatirs at continuous and intermittent exposure to CCL_4 at the 6 Lim_{ch} level.
1-fructose monophosphate aldolase; 2-sorbitol dehydrogenase; 3-weight coefficient of the liver; 4-alanine amino transferable;
5- hippuric acid; 8-body weight; 9-urocaninase;
10- cytochrome - P-450.

6300

5-I

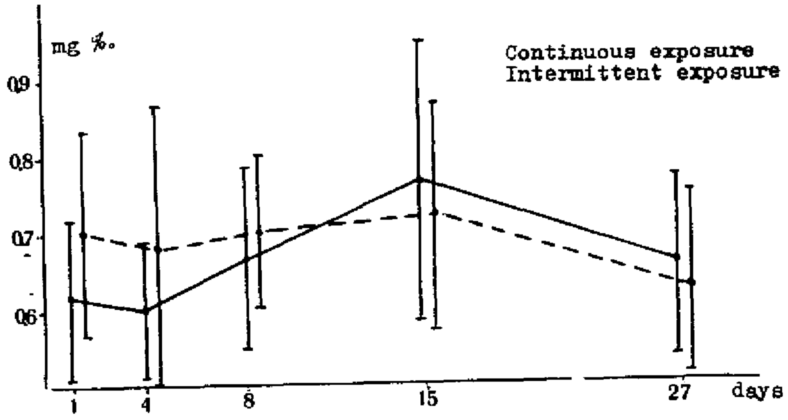


Figure 2: CCl_4 content in the blood of test animals ($6Lim_{ch}$)

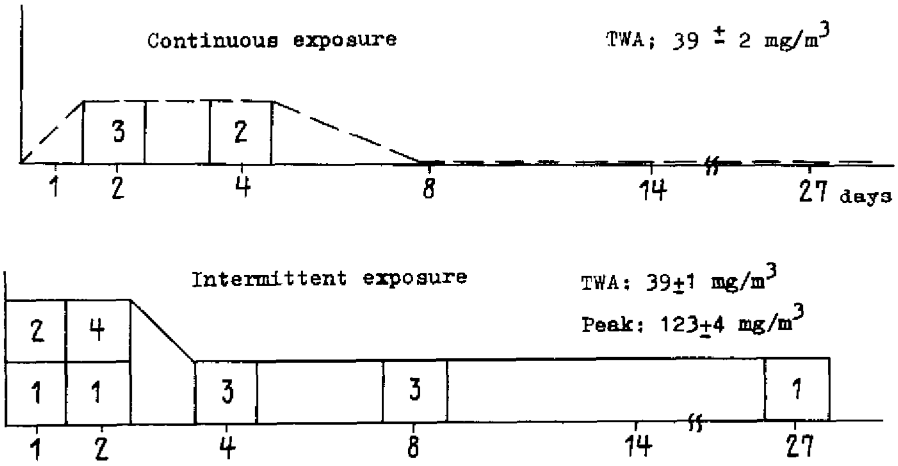
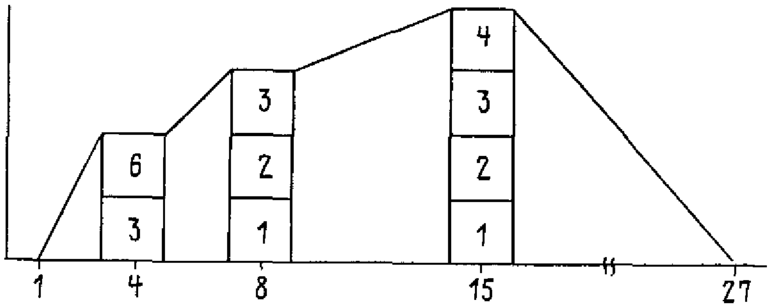


Fig.3 Changes in indicators at continuous and intermittent exposure to CCI_4 at the Lim_{ch} level.

1-fructose monophosphate aldolase; 2-bromosulfonphthalein test; 3-SIP

Continuous exposure

TWA: 48o±1.34 mg/m^3



Intermittent exposure

TWA: 49o±1.37 mg/m^3

Peak: 158o±44 mg/m^3

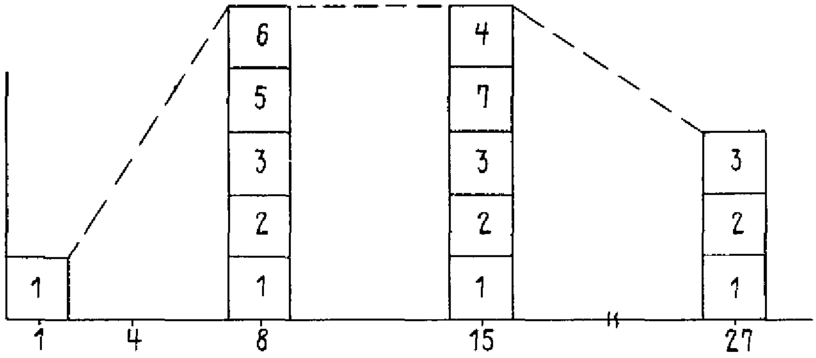


Fig.4 Changes in indicators at continuous and intermittent exposure to benzene at the 12 Lim$_{ch}$ level.
1-SIP; 2-leukocyte count; 3-lymphocyte count;
4-segmented neutrophils; 5-hemoglobin count;
6-reticulocyte count; 7-marrow cell count.

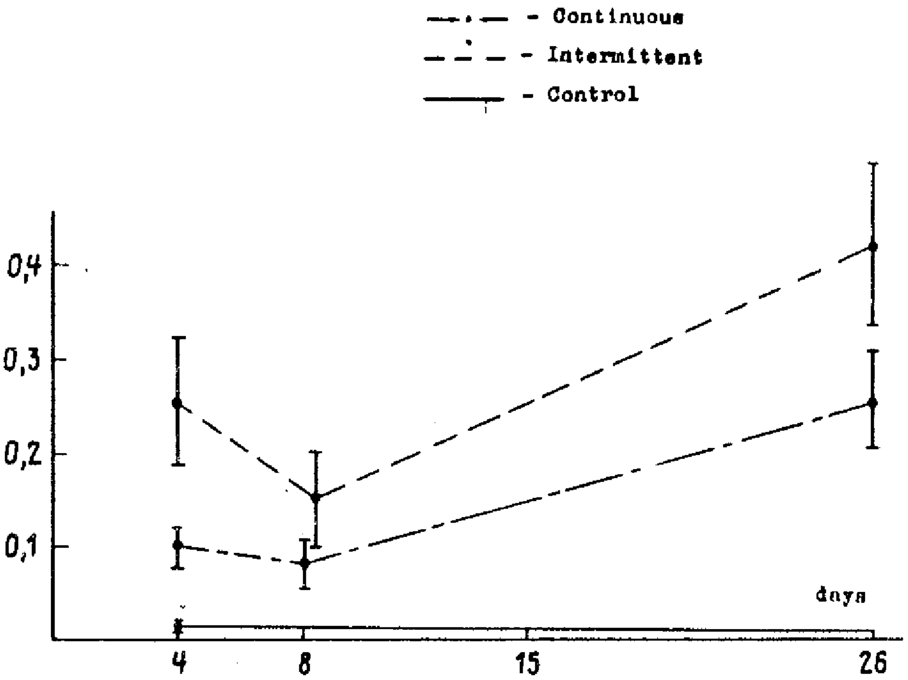


Fig.5 **Phenol** content in the urine of rats at continuous and intermittent exposure to benzene at the 12Lim$_{oh}$ level.

Carbon Tetrachloride. Different Conditions of Intermittent Action

It was demonstrated that up to five-fold concentration variations (intermittent exposure with five 10-minute peaks) as compared to continuous exposure to an equivalent average weighted concentration, CCl_4 did not intensify the toxic effect of xenobiotic: no sharp differences were observed between the exposures in the number of changes in the indices as well as qualitative aspects of the effect. When the CCl_4 concentration was varied up to ten-fold (intermittent exposure with five 5-minute peaks), the detrimental effect of the intermittent action is enhanced: the number of changed indices increased and the changes become more persistent (Figure 6). Thus, at the Lim_{ch} level and an average weighted concentration, three to five-fold variations in the CCl_4 concentration, are possible without adversely affecting the test animals.

Significantly, identical results were obtained in experiments with CCl_4 at 8 Lim_{ch} level (325 mg/m^3, conducted in Czechoslovakia (27). Equality of average weighted concentrations under continuous and intermittent exposure was a prerequisite here as well, but the experimental procedure was different. At the same average weighted concentration, the investigators created different numbers of peaks of varying duration, hence, the peak concentration was n-tuple the average weighted concentration: mode 1 - one 72-minute peak, one concentration was five-fold the other (peak cocentration - 1625 mg/m^3); mode 2 one 18-minute peak, one concentration was 20-fold the other (peak concentration - 6500 mg/m^3); mode 3 - six 2-minute peaks, one concentration was 20-fold the other (peak concentration -

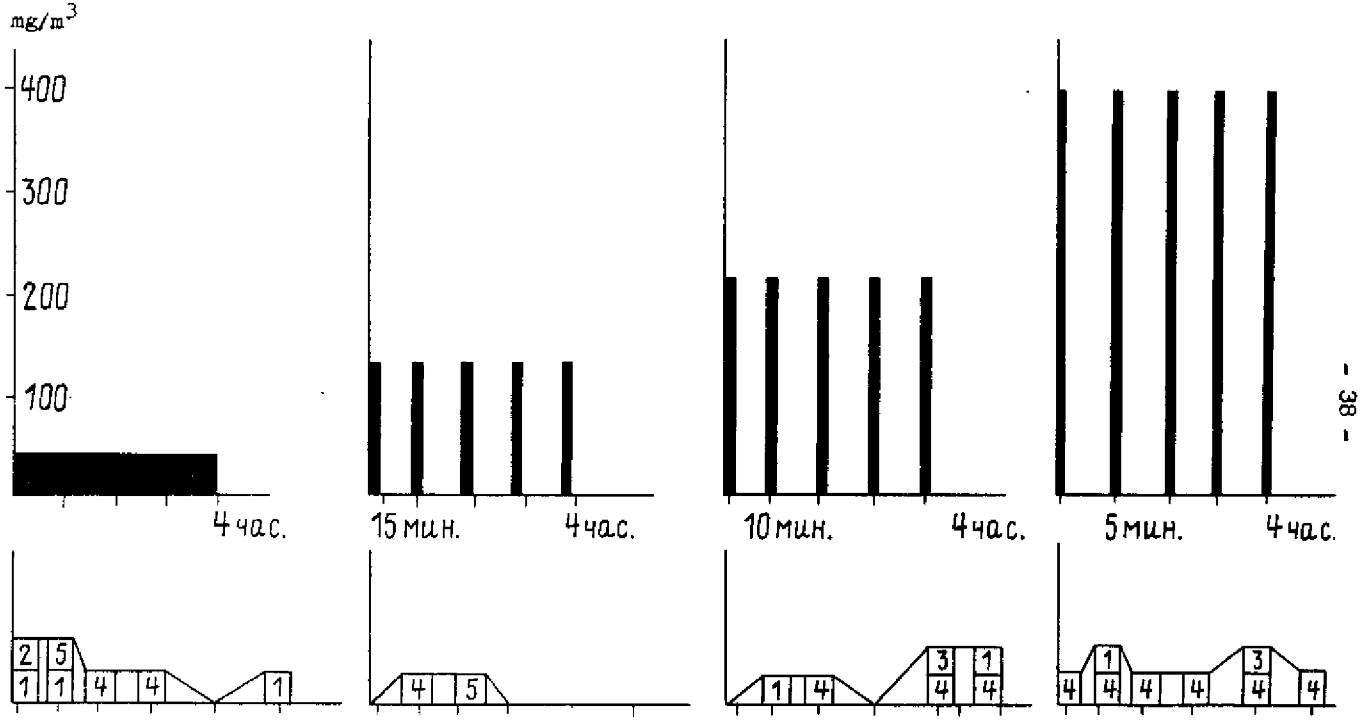


Fig.6 Changes in indicators at exposure to CCL_4. 1-FMPA; 2- SDH; 3-weight of the liver ; 4-SIP; 5-orientating reaction.

6500 mg/m^3). These experiments were aimed at elucidating the importance of not only the extent to which peak concentrations exceed the average weighted ones, but of the peak-to-peak interval as well. To assess the biological effect of CCl_4, a number of biological indices were used, adequate for determining changes in hepatic functions. The experimental results indicated that the peak concentration exceeding the average weighted one by a factor of five at the 8 Lim$_{ch}$ level, enhanced the toxic effect of CCl_4 under intermittent exposure as compared to continous one. Similar data were obtained when the peak concentration was 20 times higher that the average weighted one, regardless whether the exposure to the peak concentration was uninterrupted (18 minutes) or intermittent (six 3-minute exposures). These results corroborate the data obtained in the USSR according to which the biological effect produced by varying concentrations depends on the action level. It has been established in Czechoslovakia, for example, that at the 8 Lim$_{ch}$ the effect of CCl_4 concentrations varying by a factor of 5 is less favourable as compared to continuous exposure, while Soviet researchers have found that the same five-fold variations at a lower level (Lim$_{ch}$) do not enhance the toxic effect of CCl_4; that is, even when the peak concentration is five times higher that the average weighted one, the biological effect produced by varying concentrations also depends on the action level just as in the case of the three-fold concentration difference.

Validation of the Procedures Used for Establishing Double Sanitary Standards

As can be inferred from the above, it is expedient to introduce MAC values, both maximum and average working-shift ones more extensively to satisfy not only the health but also economic criteria (23). At present, general procedures must be developed for determining the permissible extent to which the maximum single-exposure concentration of chemicals may exceed the time-weighted one. Some recommendations to this effect can be found in the literature. D. Henschler (28) recommends, for example, to take into account the possible danger of a toxic substance, its action, half-life, and odour while substantiating the limit, frequency, and duration of the variations in concentration. These requirements are definitely essential but difficult to meet in practice. L.A. Timofievskaya, P. Schmidt, et al (29) suggest that the coefficient of tolerable limits be established within an interval from 1 for poorly cumulating to 3 for exceedingly cumulating substances, based on calculations of the chronic action zone.

A hygienic analysis of intermittent exposures brings to light some instances where the possibility of double standard is ruled out. A case in point is irritating poisons as well as substances with a narrow range of toxic effect and a sharply focused antienzymic action. It is known that even short-time presence of large amounts of such substances as nitrogen oxides, perfluoroisobutylene, hydrogen arsenide and fluoride in the air of workshops, can cause heavy poisoning within a few minutes. Therefore, for substances producing acute effect at short-time exposures, the only possible standard must be the maximum allowable single-exposure concentrations (30,31).

Conclusion

Experiments with harmful substances in low concentrations (1-6 Lim_{ch}) have shown that three- to five-fold variations under similar average weighted concentrations, do not cause any changes in the human body's responses both under intermittent and continuous exposures. This research pioneered the biological foundation for validating the average working-shift and maximum single-exposure concentrations for the air in the working zone. In this case, the term "maximum allowable single-exposure concentration of a harmful substances", as per the CMEA definition, implies the concentration of a substance in the working zone, time-weighted on the basis of short-time sampling (15 min.). An allowable harmful substance concentration time-weighted for a shift is a concentration determined by continuous or intermittent air sampling a time period not shorter than 75 per cent of working shift or a concentration time-weighted over the entire shift in production areas where workers are present all or part of the working time (32).

The qualitative and quantitative data accumulated so far concerning the effect of harmful substances allow the following recommendations to be made:

1. In the case of harmful substances exhibiting pronounced cumulative properties, the concentration time-weighted for a shift is determined along with the maximum allowable short-time concentration that may 3-5 times exceed the former.

2. In case of harmful substances characterized by specific effect (irritating, antienzymic, etc.), only the maximum allowable single-exposure concentration is determined.

Of course, the problem of intermittent effect in the context of hygienic regulation still remains to be resolved. Additional experimental and, what is more important, clinicohygienic studies are required.

6300

REFERENCES

1. E.V. Klenova, The Intermittent Effect of Industrial Toxins on the Body, Gigiena i sanitariya, 1949, No. 2, p. 23-31.

2. V.P. Paribok and A.A. Ivanova, Intermittent Effect of Poisons; in "Uchenye zapiski kemerovskogo gos. ped. in-ta", 1969, 10, p. 97-102.

3. V.P. Paribok and F.A. Ivanova, The Toxic Effect of Nitrogen Oxides During Continuous and Intermittent Exposure of Animals. Farmakologiya i toksikologiya, 1965, No. 4, p. 484-488.

4. B.I. Abidin, V.V. Kustov, V.I. Belkin, and L.T. Poddubnaya, Continuous and Intermittent Effect of Carbon Monoxide on the Animals. Kosmich. biol. i med., 1973, No. 4, p. 17-21.

5. I.D. Gadaskina, Ye.I. Lyublila, N.A. Minkina, and M.L. Rylova, Some Data Concerning the Effect of Carbon Monoxide on Animals at Continuous and Intermittent Exposures. Gig. truda i profzabolevaniya, 1961, 11, p. 13-16.

6. I.G. Samedov, Effect of Low Concentrations of Hydrocarbons on Some Physiological Functions of Animals at Different Exposures. Proceedings of the Scientific Conference on Industrial Hygiene, Toxicology and Pathology in Oil Refining and Petrochemical Industries, Baku, 1966, p. 20-22

7. P. Schmidt, I.P. Ulanova, G.G. Avilova, and S.M. Binnevis, Comparative Analysis of Adaptation to Continuous and Intermittent Exposure to 1,1,2,2-Tetrachloroethane. Gig. truda i profzabolevaniya, 1977, No. 2, p. 30-34.

8. Yu. Ye. Yakushevich, Experimental Results of Hygienic Evaluation of Continuous and Intermittent Effect of Benzene, Toluene, and Xylene. Gig. i sanitariya, 1973, No. p. 6-10.

9. M.A. Pinigin, A.S. Markarian, and V.S. Shurupova, Adaptation

Processes at Different Exposures to Chemicals (Continuous, Intermittent). Itogi nauki i tekhniki. Farmakologiya, khimioterapevt. sredstva. Toksikologiya, Moscow, 1973, vol. 5, p. 120-128.

10. Kh.S. Markarian, Comparative Toxicological Analysis of Aniline, and Dimethylaniline Inhaled Continuously and Intermittently. Abstract of a thesis, Moscow, 1972.

11. Yu.P. Bizin, Z.I. Pililyuk, and N.A. Usakova, Comparative Analysis of Continuous and Intermittent Effects of Carbon Tetrachloride on the Body. Proceedings of the Conference of Young Scientists (Institute of Biophysics), Leningrad, 1971, p. 26-27.

12. Yu.P. Bizin, G.M. Gorban, and Z.I. Pilipyuk, Comparative Study of Continuous and Intermittent Action of Carbon Tetrachloride on Animals. Gig. i san., 1977, No. 3, p. 27-29.

13. G.P. Tikhonova, G.M. Gorban, and Yu.P. Bizin, Comparative Experimental Study of Continuous and Intermittent Effects of Carbon Tetrachloride on the Microstructure of Animal Body. Kosmicheskaya biologiya i meditsina, 1970, vol. 4, No. 4, p. 20-25.

14. Z.P. Grigorevskaya, Comparative Toxicological Analysis of Continuous and Intermittent Effect of Sulfur Dioxide on Animal Organisms, Gig. i san., 1975, No. 3, p. 6-10.

15. I.K. Ostapovich, Concerning the Sensitizing Effect of Sulfur Dioxide and Formaldehyde at Different Inhalation Rates. Gig. i san., 1975, No. 2, p. 9-12.

16. G.P. Tikhonova, Continuous and Intermittent Effect of Methylmercaptophos an the Microstructure of Animal Organs. Farmak. i toksikol., 1971, No. 2, p. 235-237.

17. N.A. Tolokontsev, Comparative Study of Continuous and Intermittent Effects of Volatile Gases of Nonelectrolytes, Gig. i san., 1960, No. 3, p. 29-35.

18. E.I. Lyublina, N.V. Olyunin, Different Aftereffects of Narcotics Depending on the Frequency and Duration of Exposures. Transactions of the I.M. Sechenov Leningrad Society of Physiologists, Biochemists, and Pharmacologists, Leningrad, 1957, p. 18.

19. N.I. Maltseva, Comparative Analysis of Body Adaptation Reactions to Continuous and Intermittent Effects of Methylene Chloride with Determination of the Poison Content in the Biological Material.

20. E.I. Lyublina, Adaptation at Different Exposures to Industrial Toxins. Problema adaptatsii i gigiyene truda, Moscow, 1973, p. 14-19.

21. Ye.I. Lyublina, V.V. Dobrynina, and R.Ye. Livshits, An Experimental Study into the Intermittent Action of Noxious Vapors. Gig. truda i profzabolevaniya, 1977, No. 5, 27-31.

22. V.A. Filov, in "Quantitative Toxicology", Leningrad, 1973, p. 52-88

23. I.V. Sanotsky and I.P. Ulanova, New Approaches to Sanitary Regulation of Harmful Substances in the Air of the Working Zone. Gig. truda i profzabolevaniya, 1980, No. 8, p. 5-8.

24. N.P. Kazmina, A Study into Adaptation of the Liver to Continuous and Intermittent Effects of Carbon Tetrachloride. Gig. truda i profzabolevaniya, 1976, No. 3, p. 39-42.

25. E.A. Karpukhina, Comparative Analysis of Adaptation to Continuous and Intermittent Action of Benzene with Reference to Variations in Some Blood System Indices. 15th All-Union

Youth Conference, Erisman Institute, Moscow, 1978.

26. A.A. Germanova et al., Adaptation Reactions to Continuous and Intermittent Effects of Dimethylformamide. Toksicologiya novykh promyshlennykh khimicheskikh veshchestv, 1979, No. 15, p. 69-75.

27. David et al., Role of Time and Concentration on Carbon Tetrachloride Toxicity in Rats. Arch. Occupational and Environmental Health, 1981, 48, p. 49-60.

28. Henschles Begrenzug von Konzentrationsspitzen der Exposition genenüber gesundheitsschadlichen Arbeits. Arbeitsmed., Sozialmed., Präventivmed (Kurzzeitwerte), 1979, No. 9, 190-197.

29. L.A. Timofievskaya and P. Schmidt, The Past and Future of Unification of Industrial Toxicology Standards Between the GDR and USSR. Gig. truda i profzabolevaniya, 1981, No. 5, p. 21-24.

30. A.A. Kasparov et al., Modern Approaches to Regulation and Monitoring of Harmful Substances in the Working Zone Air, Proceedings of the Second All-Union Conference on Hygiene, Kiev, 1982, part 1, p. 55-58.

31. Methodological Recommendations for Experiments Aimed at Validating Sanitary Standards for the Air in the Working Zone. Problemy promyshlennoy toksikologii SEV, Moscow, 1982, p. 21.

32. I.P. Ulanova et al., Development of Methods for Establishing Threaholds of the Effect of Harmful Substances in the Air of the Working Zone, Gigiena, epidemiologiya, mikrobiologiya i immunologiya, 1980, 24, No. 3, p. 298-302 (Prague).

PRINCIPLES FOR VALIDATION OF THE MAXIMUM ALLOWABLE CONCENTRATIONS OF HARMFUL SUBSTANCES IN THE AIR OF THE WORKING ZONE

L.A. Timofievskaya

The development and substantiation of preventive measures to avert the effects of harmful chemicals in the workplace are the main problems of industrial toxicology.

The problems relating to standardization of harmful substances in the air of the working zone were treated on a systematic basis in the first third of the 20th century.

The first guideline for sulfurous anhydride, was set in the USSR in 1922. By 1956 an All-Union Commission under the USSR Ministry of Public Health was already working regularly to set MACs at that time.

The standardization in the Soviet Union was mainly based on the analysis of the literature data and health practice.

In the United States, similar efforts were undertaken in the 40's through the 50's *) after L.T.Fairhal suggested the term - Threshold Limit Value (TLV) for the use in hygienic standardization. Somewhat later, the term MAK-Werte appeared in Western Europe.

In view of urgent requirements of practical hygienic regulation, the need was recognized for the development of the theory for the rating procedures and standardization of the methods and approaches to the validation of MACs for noxious substances in

---

*) The Commission of Government Industrial Hygienists was instituted in 1938.

the air of the working zone. Concurrently, the key concepts of preventive toxicology were further updated.

One of the pioneering concepts of MACs defined them as "...such concentrations which, by a prolonged (during many months and years) effect upon human organism (and, moreover, in working conditions) induce no morbid changes and can be recognized as safe with the present level of our knowledge".

A large stride in hygienic standardization was made at the 1959 International Congress in Prague which formulated its concept and the basic goal. It was aimed at identifying such a concentration, whose prolonged inhalation over many years for 7-8 hours daily, will not induce marked physiological changes. The definition, slightly rectified, was later to become international.

While preparing a new hygienic standard consistent with the GDR "Air of Working Zone. General Sanitary Hygienic Requirements", the MAC definition generally accepted until 1975 was extended to incorporate an addition, proposed by Professor I.V. Sanotsky, pertaining to relatively long-term effects that occur in the present and subsequent generations.

Presently, the recognition of the need to protect progeny is included in the definition of the MAC concept for harmful substances, agreed upon by the CMEA member-countries.

The first TLV inventory published in 1948 features the concentrations that would induce no pathological effects, irrespective of the duration of the exposure.

In the 1950s the preamble to the TLV inventory said the objective of the TLV was only to improve hygienic conditions in the workplace rather than to set a safe value of the exposure.

From 1962, the preamble to the TLV defines the concept of the threshold limit value which, like the MAC concept kept being modified and updated over the years.

It should be noted that blistering criticism was levelled in the USA against the term "threshold limit values". For instance, back in 1956 H. Smyth doubted the validity of this term substituting it by "hygienic standard", which be assumed, will give a more accurate interpretation of the idea.

One of the early definitions of the TLV specifies it as a standard of the dose levels to which almost all the employees might be exposed regularly, day by day, without adverse effects. In 1965 the definition was supplemented with a reference to variations in individual sensitivity.

As of now, the definition of the TLV concept reads that "TLV specifies the concentration to which most workers can be repeatedly exposed daily without an adverse effect. Due to a broad variability in individual sensitivity to the effect of chemical concentrations at or below the TLV level, a small percentage of workers may feel discomfort and a still lower percentage develop more serious effects such as the aggravation of the previous condition or the initiation of occupational diseases".

It should be emphasized that in 1962 the inventory of the US standards indicated that TLV referred to time-weighted average concentrations. In 1965 the duration of the exposure was specified (7- or 8-hour workday). Besides, it made an exception for five substances with a carcinogenic potential and 29 "C" labelled (ceiling) substances for which the listed TLV values were the maximum ones and no exposure to them was allowed.

In 1976 the listing of TLV values was divided into three categories: average weighted concentrations for 7-8-hour workday and 40-hour working week (TWA), limit values labelled "C" and short-term MACs designated for 15-min exposure.

The MAK-Werte inventory in the Federal German Republic was published by the German Research Association in 1963. In 1970 the preamble to it addressed explicity the MAK-Werte concept and a host of relevant issues, for instance, skin, sensitization and carcinogenicity.

The only standard value given in the inventory identifies the time-weighted average. This and the subsequent FGR norm listings specify that MAK applies to pure substances and refer to the likelihood of individual sensitivity variations, depending on age, sex, constitution, dietary condition, and other factors. Special attention is paid to potential carcinogens.

The definition of MAK by the Senate Committee under Professor D. Henscher in 1980 says that "MAK-Werte is the maximum allowable concentration of a substance in the form of gas, vapor or suspended airborne particles in the ambient air which, with the present state of knowledge, even in a regular and usually prolonged, daily exposure during eight hours and with the observance of the average worktime of 40 hours per week (or 42 hours per week averaged for 4 successive weeks at plants operated on a four-shift basis), does not, in general impair health of the workers and their progeny, or places a burden upon them". The inventory provides the prerequisites for MAK validation, including the purpose of MAK, analytic control, approaches to rating mixtures and a number of specific effective factors such as odor, allergic manifestations, skin absorption, carcinogenic substances, etc.

Despite the significant dissimilarities between the definitions of MAK and TLV the respective US and FRG guidelines fit almost completely.

It should be underlined that three countries possess now the longest standard inventories; they are the FRG with > 450 chemicals, USA with > 550 chemicals and USSR with over 1000 chemicals. Such inventories exist also in other countries. The comparison of such inventories by the International Labor Organisation shows they are currently available in 19 countries. However, it should be noted that these countries rely in general on the TLV or MAC values and only in numbered cases do they depart from them.

By and large, the recommended MAC values are lower in the USSR than those in the United States. Judging by the TLV/MAC ratio, the substances differing two-fold or more, make 72 per cent of the total (the analysis was performed on the 220 chemicals, listed in the inventories of the USSR National Standards and those of the American Commission of Government Industrial Hygienists (ACGIH, 1980). A more detailed analysis has shown that a two- to five-fold difference is found in 48 chemicals or 22 per cent; a 5- to 10-fold difference in 42 chemicals or 18 per cent; and over ten-fold differences in 69 chemicals or 31 per cent. About 10 per cent of the US standards are more than 50 times higher than the corresponding USSR standards. The extreme values of the gaps between them apply to chloroprene

(TLV/MAC = 1800 times), caprolactam and selenium (MAC/TLV = 10 times). For 16 chemicals the USSR Standards are above the US counterparts (see Table 1).

Table 1

List of Chemicals with the MAC/TLV Ratio Over I

Chemical	Value in mg/m^3	
	MAC	TLV
1. Ammonia	20.0	18.0
2. -Anisidine	1.0	0.5
3. Bisphenol A (diphenylol-propane)	5.0	3.0
4. Vanadium pentoxide	0.1	0.05
5. Caprolactam	10.0	1.0
6. Cadmium oxide	0.1	0.05
7. Metallic cobalt and cobalt oxide	0.5	0.1
8. Perchloromethyl mercaptan	1.0	0.8
9. Selenium	2.0	0.2
10. Tantalum and its oxides	10.0	5.0
11. Phosgene	0.5	0.4
12. Zinc oxide	6.0	5.0
13. Metallic zirconium and its compounds	6.0	5.0
14. Vinylidene Chloride	50.0	40.0
15. Yttrium oxide	2.0	1.0
16. Trinitrotoluene	1.0	0.5

The analysis of the inventories in two countries brought to light essential differences between classes and categories of compounds. For instance, the differences between the MAC and TLV values appear to be minor for several irritant toxics and significant only for acetaldehyde and formaldehyde (36- and 6-fold respectively. See Table 2). Furthermore, the formaldehyde disparity was formerly wider still (72 times) - until its TLV was halved in the recent years. The above divergencies are apparently due to the Soviet acetaldehyde rating procedure which took into account not only its irritant properties but also a strong unpleasant odor, inducing reflex responses in low concentrations. The similar procedure in the USA had the only intent of avoiding excessive irritation and damage to the respiratory tracts in "habituated" employees.

The USSR formaldehyde MAC was reduced from 5 to 0.5 mg/m^3 in 1967. The rationale for the revision was extensive clinical hygienic and experimental evidence suggesting for formaldehyde a strong irritating potential when present in the air in the concentration of its formerly effective MAC value. The special literature in the USA also reports of the formaldehyde toxicity at 6 mg/m^3 or one fifth of that level. The TLV document package on formaldehyde shows that the 6 mg/m^3 concentration cannot ensure against irritation, which perhaps is the only reason why in 1972 its standard value was made twice as low, or 3 mg/m^3.

Irritants apart, the MAC-TLV disparities seem very minor in the case of aerosols, metals, metalloids and their compounds. For instance, in the list of chemicals, shown in Table 3, these disparities are more or less significant for lead, manganese

Table 2

MAC and TLV Values for Several Irritant Toxics

Chemical	Value in mg/m^3		
	MAC, USSR	TLV, USA	Disparity
1. Acrolein	0.2	0.25	-
2. Ammonia	20.0	18.0	-
3. Acetaldehyde	5.0	180.0	36
4. Diethylamine	30.0	75.0	2.5
5. Nitrogen dioxide	5.0	9.0	1.8
6. Chlorine dioxide	0.1	0.3	3.0
7. Iodine	1.0	1.0	-
8. Methylacrylate	20.0	35.0	1.75
9. Ozone	0.1	0.2	2.0
10. Sulfuric acid	1.0	1.0	-
11. Sulfurous anhydride	10.0	13.0	1.3
12. Toluene diisocyanate	0.05	0.14	2.8
13. Acetic acid	5.0	25.0	5.0
14. Formaldehyde	0.5	3.0	6.0
15. Hydrogen fluoride	0.5	2.0	4.0
16. Chlorine	1.0	3.0	3.0
17. Hydrogen chloride	5.0	7.0	1.4

and tellurium - 15, 16 and 10 times respectively (Table 3). It is essential to note that some 5 years or so ago, the US TLV of lead, chromium and mercury exceeded not only the Soviet MACs but also the standards accepted in other countries, e.g., the TLV value for mercury was reduced by half to match the International Recommendation resulting from a long-term program of dynamic epidemiological observations completed in the USSR. Of late, there have been reductions in the TLV values for chromium compounds and molybdenum.

Table 3

MAC and TLV Values for Several Metals, Metalloids and Their Compounds

Chemical	Value in mg/m^3		
	MAC, USSR	TLV, USA	Disparity
1. Beryllium and its compounds	0.001	0.002	2
2. Vanadium and its compounds, smoke of vanadium pentoxide	0.1	0.05	2
3. Cadmium oxide	0.1	0.05	2
4. Metallic cobalt and its oxide	0.5	0.1	5
5. Manganese	0.3	5.0	16
6. Molybdenum (soluble compounds)	4.0	5.0	-
7. Molybdenum (insoluble compounds)	6.0	10.0	2
8. Nickel (oxide, protoxide, sulfite)	0.5	1.0	2
9. Metallic mercury	0.01	0.05	5
10. Lead and its inorganic compounds	0.01	0.15	15
11. Metallic antimony dust	0.5	0.5	-
12. Tantalum and its oxides	10.0	5.0	2
13. Tellurium	0.01	0.1	10
14. Titanium oxides	10	15	1.5

Table 3 (continued)

Chemical	Value in mg/m^3		Disparity
	MAC, USSR	TLV, USA	
15. Uranium (soluble compounds)	0.015	0.2	-
16. Uranium (insoluble compounds)	0.075	0.2	3
17. Chromium anhydride, chromates, bichromates	0.01	0.05	5
18. Zinc oxide	6.0	5.0	-
19. Metallic zirconium and its insoluble compounds	6.0	5.0	-

The MAC and TLV values differ mostly in the series of chlorinated hydrocarbons (Table 4). Yet there is a recent tendency to bring some of them down, e.g. those of methylene chloride, chloroform, and dichloroethyl ester, etc. Significantly, the TLV values of some chlorinated hydrocarbons are still way above not just the respective Soviet MACs, but also those of other countries. One example is the average workshift concentration of dichloroethane of 50 mg/m^3 in Czechoslovakia and the GDR, 20 mg/m^3 in Sweden and 80 mg/m^3 in the FRG, Italy and Switzerland which are in contrast to that in the US of 200 mg/m^3.

Somewhat lesser divergencies occur in the solvents category except for the MACs of benzene and acetone that are respectively 16 and 12 times lower than their TLVs (Table 5). The benzene MAC value in the USSR was decreased from 20 to 5 mg/m^3, consistent with the data of experimental and sanitary hygienic observations maintained concurrently by three research organisations in the Soviet Union.

In the foreign literature, too, the need to lower the benzene TLV is widely recognized. In particular, R. Truhault (1968) recommended its reduction at least five times over. The largest disparity in this category is that of aniline (190 times) whose MAC was lowered from 3 to 0.1 mg/m^3 in 1971, as required by data from clinical hygienic and experimental observations.

Table 4

MAC and TLV Values for Some Chlorinated Hydrocarbons

Chemical	Value in mg/m^3		Disparity
	MAC, USSR	TLV, USA	
1. Benzyl chloride	0.5	5.0	10
2. Dichlorobenzene (ortho)	20.0	300.0	15
3. Dichloroethylene	50.0	790.0	16
4. Dichloroethane (1,2)	10.0	200.0	20
5. Methylene chloride	50.0	700.0	14
6. Methyl chloride	5.0	210.0	42
7. Tetrachloroethylene	10.0	670.0	67
8. Trichloroethylene	10.0	535.0	53
9. Chlorobenzene	50.0	350.0	7
10. Chloroprene	0.05	90.0	1800
11. Carbon tetrachloride	20.0	65.0	3.3
12. Ethyl chloride	50.0	2600.0	52

Table 5

MAC and TLV Values for Some Solvents

Chemical	Values in mg/m^3		Disparity
	MAC, USSR	TLV, US	
1. Amylacetate	100	530	5
2. Aniline	0.1	19	190
3. Acetone	200	2400	12
4. Benzene	5	30	16
5. Dimethylformamide	10	30	3
6. Xylidine	3	25	8
7. Xylene	50	435	9
8. Methylacetate	100	610	6
9. Unsaturated alcohols of the fatty series	2	5	2.5
10. Nitromethane	30	250	8
11. Nitroethane	30	310	10
12. Nitropropane	30	90	3
13. Nitrobenzene	3	5	1.6
14. Propylacetate	200	840	4.2
15. Carbon disulfide	1	60	60
16. Toluidine	3	22	7
17. Toluene	50	375	7.5
18. Cyclohexane	80	1050	13
19. Ethylacetate	200	1400	7
20. Ethyl, diethyl ester	300	1200	4

A similar comparative survey of the MAC and MAK-Werte, FRG, also identified a close fit for the irritant chemicals and an essential disparity for chlorinated hydrocarbons (up to 150 times). The differences between the hygienic regulations for chloroprene (720 times), styrene and alfumethylstyrene (84 and 96 times), aniline and nitroaniline (190 and 60 times), methyl and ethyl bromade (80 and 178 times) are enormous.

With respect to seven compounds, their USSR MACs are two to six times over the FRG MACs. On the other hand, the FRG MAC for quartz-containing dusts at 0.15 mg/m^3 is lower than in most other countries and nearly six times as low as in the USSR.

Among the metals, on lead, manganese and mercury the two listings differ most - by 10.6 and 10 times respectively. Of the solvents, mention must be made of acetone and toluene that depart from the corresponding MACs by a factor of 12 to 15.

The causes behind the discrepancies were analyzed by numerous specialists in the USA, UK, Italy and USSR.

As we see it, the standard listings differ because their underlying principles of hygienic rating are different.

In the USSR the fundamental principle of toxicological limitation on the amounts of noxious substances present in the environment is one of the thresholds intrinsic in their effects. The concept postulating the existence of a threshold of harmful effect for the majority of biological effects from chemical substances meets no objections. The threshold concept remains disputable, however, as regards the mutagens, blastomogens and radiation lesions. Dr. H.S.Stokinger, to cite one example, is biased, though with some reservations, towards the existence and necessary establishment of threshold levels for the effects of chemicals and Dr. H.Magnusson offers the definition of the latter's values, whereby a threshold can be defined as the value below which no effect will be produced, not even by a life-long exposure.

Researchers in several countries, not least in the United States, pay a special attention to different sensitivities of the methods used to validate MACs and TLVs, with a particular reference to conditioned reflexes as the method to study the nervous system. In fact, the frequent use of this highly sensitive method in the USSR is referred-to as one of the sources for the disparities that occur between the MACs and TLVs. For example, Professor H. Stokinger assumes that in the USSR they take as the limiting value any response of the body (whether physiologically meaningful or not) plus the safety factor of 10. Some of the studies published in the USA to validate individual TLVs employ less sensitive methods. Furthermore, in the Soviet studies designed for hygienic rating purposes the method of conditioned reflexes is held to be neither main nor mandatory. The rating programs in the USSR rely on a set of indices and take their hygienic significance into consideration.

Another fundamental principle of hygienic chemical rating in the USSR postulates pre-emption of medical indications. Permissible limits of exposure must be determined strictly on the basis of credible physiological and medical data. The limits must not be geared to technological feasibility but rather should be set up to stimulate technological and engineering innovations (material/process substitution, sanitary hygienic efforts, etc.). The latter are easier to achieve through preemptive efforts towards the validation of MACs and formulation of hygienic standards for environmental quality than through their design upon the inception of new production technologies.

In the USA and several other countries, techno-economic parameters outweigh.

Beyond the differences between the key principles of hygienic rating in the USSR and USA there are significant methodological divergencies and varied patterns of research management.

Presently, the hygienic rating of industrial chemicals in the USSR amounts to a three-staged procedure:

Stage I - determination of TSEL (Tentative Safe Exposure Levels) - is scheduled to be concurrent with the design of new compounds in the laboratory;

Stage 2 - the validation of MAC in animal experiments - is concurrent with semicommercial testing and production design; and

Stage 3 - correction of the experimental MACs by comparison of employee working conditions and health condition (epidemiological studies).

The US statutory document "Basic Principles and Methods of Data Acquisition for Validation of Threshold Limits in Air" consists of two sections, and namely: I - principles and methods of animal experiment and II - human data development. The latter section outlines the framework for research on human volunteers to assess irritant, narcotic and sensitizing action of chemicals. It defines the program of occupational and employee investigations needed primarily to classify the workers from a medical and anthropological viewpoint, as well as a technical program of research on the air medium at production facilities. Dr. H. Stokinger points out that US standards rely on sequential assessment of industrial practices in dealing with chemical hazards though the preamble to the methodological guide above favors the TLV establishment based on experimental animal data. The latter statement, though, is qualified by addressing the need for the TLVs thus established to be confirmed, as is commonly done in practice, by their verification in production environment.

Though a detailed examination of the document just mentioned a number of differences come to light in the approaches to the MAC validation from animal experiment inputs. For example, such parameters as Lim_{ac} whose assessment was strongly recommended by N.S. Pravdin, one of the founders of industrial toxicology in the USSR.

This is odd because not only does the threshold of an acute effect assist the validation of the dose and concentration levels of a toxic suitable for a chronic experiment but, more importantly, facilitates estimation of essential hazard criteria for the substance in question, such as Z_{ac}, Z_{sp} and Z_{ch}. Neither is the assessment of cumulative properties and estimation of I_{cum} recommended by the US methodological instructions, even though the methods of cumulation studies are valued by American toxicologists for the substantial advantages they can offer.

It is worth discussing briefly also the study of chronic effect from toxic chemicals. The US methodological guide sought to do it by conducting a two-year experiment on the grounds, as stated in the basic principles and methods of TLV design, that satisfactory data... should be secured from a lengthy chronic study because neither short-term research nor rapid methods are adequate for the purpose. Contrary to that statement of intent, no studies of this kind have been conducted even in regard of highly cumulative compounds.

H. Stokinger (1969) noted also that only 20 per cent of the chemicals on the US inventory were validated on the basis of a chronic experiment as a relevant data source. H. Smyth concludes from his analysis of 122 compounds that only 45 were recommended for the use after being tested in chronic experiments.

A closer scrutiny of the TLV validations for a number of chemicals suggests that short-term studies were the only supporting rationale for them. For example, the TLV validation for the super-mutagen ethylenimine had no data support apart from consideration of its acute irritating effect.

The findings of our experiments with ethylenimine exposure at the US TLV that had existed earlier included marked systemic toxic, gonadotropic and embryotropic effects, and overt mutagenic and carcinogenic effects. After these results were publicized the TLV of ethylenimine was lowered to 1 mg/m^3. However a follow-on research on the toxicity of ethylenimine revealed a strong effect of 0.7 and 0.4 mg/m^3 concentrations. Gonadotropic, embryotropic and mutagenic effects of ethylenimine became evident with even lower concentrations, ranging from 0.2 to 0.18 mg/m^3. The research inputs were taken as the basis for the validation of the USSR MAC for ethylenimine in the air of the working zone at 0.02 mg/m^3.

It is appropriate to note a significant share of the studies underway in the United States and other countries, e.g. Czechoslovakia, Finland, and Great Britain in order to clarify the fate of industrial toxics in the body. Similar experiments constitute an essential component in the study of acute chemical toxicity. The US methodological instructions allocate a large role to the assessment of a chemical's quantities exhaled in air and excreted in urine by comparison with its concentrations in the blood - for volatile solvents; and to the identification and distribution in the body - for the principal metabolites of low-volatile substances. These studies, in the opinion of the authors who compiled the instructions, provide a simple test to determine the exposure levels and thus promote more effective control of the occupational exposure to which workers are subjected. Studies such as these, deserve special attention since the questions related to kinetics and elimination of toxic chemicals are central to forecasting safe levels of exposure to chemical compounds.

It should be stated that another source of the basic differences between MAC and TLV arises from the disparate methods used in either country to do a chemical analysis, notably their selectivity and sensitivity.

The analysis into the causes for the TLV-MAC divergencies carried out by H. Magnusson led him to identify five basic essentials that account for these differences:

1. Different MAC determination procedures.
2. Different techniques for obtaining experimental and clinical data.
3. Special attention to the study of the nervous system.

4. Different mechanisms of official authorization (in the USSR, MACs are decreed by public law for everyone; in the USA, TLVs are endorsed by state committees without legal authority).
5. Sanitary inspection (in the USSR, it is performed by the full-time sanitary physician of Sanitary Epidemiological Stations, Ministry of Public Health, union inspectors and those of the Central Council of Soviet Trade-Unions; in the USA, by personnel of the HEW and Labour Departments, insurance companies and, seldom, by union inspectors).

It should be noted that the key rating provisions and the definition of the MAC concept have by now been standardized among the CMEA countries. Through their concerted effort and joint process of discussion, standard terms and definitions were adopted (MAC, TSEL, classification of toxicity and hazard, and design of experiment for MAC validation).

A pioneering list of guidelines suggested for use as international standards was the inventory made by the panel of expert toxicologists under Professor Truhaut (1963). Comprised of 28 chemical titles, it was endorsed by the 2d Symposium on MACs of harmful substances in the air of industrial workplaces.

At present, it seems possible to propose as an international inventory, a list, spanning the values of sanitary standards for 60 chemical compounds, whose MAC/TLV ratio is 2 or below.

In light of the proposed inventory, it is appropriate to draw attention to the recent trend for TLV reduction. As a rule the new US guidelines approach the Soviet MACs, thus offering an additional basis for the development of a single international listing.

We submit that, because of the Soviet Union's longer history of hygienic rating, its methods currently used are better-suited for the development and validation of the standards and establishment of more precise guidelines for them. The evidence to support the latter statement is an increasing amount of TLVs, subjected to a downward revision. Thus 59 TLV values were revised in the United States during the decade from 1962 through 1972. Furthermore, while over the first six years the TLV standards were decreased for 27 compounds on that list, their number reached 32 chemical titles in the next four years.

It is our feeling that we are advancing step-by-step towards the development of an international inventory - if not for all known chemicals then at least for many of those now in common use throughout the world. Possibly, these standards would allow a certain margin for variation. But in any case their development and adoption should be anticipated by relevant national practices in many countries.

SUMMARY

The present lecture sets forth basic principles and methods of validation for maximum allowable concentrations of harmful substances in the air of the working zone in a number of countries and, mainly, in the USSR, USA and GFR in the historical aspect.

Levels of standards for chemical compounds in the air of the working zone are compared in a number of countries and proposals are offered for elaboration of an international MAC list of harmful substances in the air of the working zone.

CHEMICAL ANALYSIS METHODS IN INDUSTRIAL TOXICOLOGY AND PRODUCTION ENVIRONMENT

S. Muraviova

Industrial enterprises of today use a wide variety of chemicals which in the form of gases, vapors, or dust may be hazardous for human health when they get into the working zone air. To prevent such risks, a special commission establishes, on the strength of the toxicity data for a large number of industrial chemicals, the maximum allowable concentrations (MAC) of harmful substances in the air of the working zone, and updates them from time to time to keep up with the latest research results.

Whether MAC is within the prescribed limits is determined by chemical analysis techniques used to measure the concentrations of harmful substances in the air and their conformity to the existing standards.

Chemical analysis of airborne harmful substances involves several successive stages: air sampling, extraction of the analyzed substance from the sample, and its quantitative determination.

1. Air Sampling

Air sampling is the key stage, because precise results and thorough analysis loose meaning in case of incorrect or inadequate sampling.

A proper choice of sampling procedure primarily depends upon the state of aggregation of an airborne substance (vapor, gas, aerosol) and its solubility in various solvents. Ammonia, divinyl, ozone and other substances, gaseous under normal conditions, are present in the air as gases. Liquids whose boiling point ranges from 230 to 250°C exist in a vaporous state. This very large group comprises organic solvents (aromatic hydrocarbons, chlorinated aliphatic hydrocarbons, lower acyclic alcohols, acids, etc.). Air also carriers the vapors of some solids with a comparatively high volatility (hexamethylene, diamine, iodine, camphor, naphthalene, phenol, etc.). Depending on production conditions (with and without heating) and application patterns, some high-boiling liquids and moderately volatile solids may be present simultaneously in the air as vapors and aerosols. This is the case, for example, when vapors evolving into the air at high temperatures cool and to a considerable extent condense to form a condensation aerosol (dibutyl phthalate, caprolactam, phthalic anhydride, polycyclic hydrocarbons, and other compounds).

Vapors and aerosols may also exist simultaneously, if the dispersed phase of an aerosol formed in spraying solutions or solids (e.g., in spray painting) is highly volatile.

Preliminary assessment of air pollution by vapors of volatile and low-volatile compounds requires knowledge of the volatility of these substances.

Volatility is the maximum concentration of vapors, expressed in units of mass per volume at a given temperature. Volatility (mg/l) can be calculated from formula:

$$L = \frac{16 \cdot P \cdot M}{273+t} \text{ mg/l},$$

where P is the pressure of saturated vapor at a given temperature, mm Hg;

M is the molecular weight of the substance;

t is temperature, °C.

If the volatility of a substance (e.g., sulfuric acid, dinonyl phthalate, etc.) at 20°C is much lower than the MAC value (10% and less), the presence of vapors in air can be neglected. Samples in this case are only taken to determine the aerosol. Should the MAC value be markedly exceeded (50 and more times), samples are only taken to determine the vapor content (e.g., ethylmercury chloride).

In production conditions, air temperature may fluctuate. The pressure of saturated vapor and, consequently, the volatility of a substance change with the ambient temperature. Since data on vapor pressure are not always available in literature, a formula has been suggested to calculate the approximate pressure of saturated vapors at various temperatures (S.D. Zaugolnikov et al., 1976). It expresses the correlative relationship between the boiling point of substances belonging to different classes of compounds, the saturated vapor pressure and the ambient temperature:

$$\lg P_t = 2.763 - 0.019 \cdot t_{b.p.} + 0.024 \cdot t^{o},$$

where P_t is the saturated vapor pressure (mm Hg) at t^{o};

$t_{b.p.}$ is the boiling point of the substance;

t^{o} is the ambient temperature.

Knowing the vapor pressure, one can calculate the volatility with ± (30-40)% error.

1.1. Air sampling into glass vessels and plastic bags

Only on rare occasions are air samples taken in glass vessels for laboratory analysis, e.g., for gas chromatography, when the sample size does not exceed 1-100 ml. But in most methods a sample of more than 2-5 l is usually required to determine the concentration of a harmful substance at a level of MAC and lower. A pipette with two cocks or a glass bottle is used to take air samples. Exchange or vacuum techniques are employed to fill those vessels with air.

Of late, polymer film bags (1-100 l) with a rubber hose and a screw clamp are increasingly used in air sampling. The advantage is that these bags are light and unbreakable. To prevent the sampled air from contacting with either rubber or corrosive metals, the bags are filled with the help of bellows coated with an inert film.

1.2. Air sampling into liquids, sorbents, and filter media

Sampling in liquids contained in a glass absorber is the most common technique of air sampling for chemical analysis. When air is sampled into liquids (inorganic and organic solvents), the analyzed substances either dissolve or enter into chemical reactions. The efficiency of absorption in a liquid largely depends upon the absorber structure design. Absorbers fitted with porous glass plates, Richter absorbers, having a 30 l/min or higher capacity, are the most efficient. The advantage of this sampling technique is that the harmful substance sought for can be simultaneously accumulated. Besides, the pretreatment of the sample is simplified, which, in case of stringent requirements and accuracy, is generally performed in the liquid phase, both for photometric and electrochemical analysis.

In recent years silica gel, activated charcoal and other solid adsorbents with a highly developed surface have been widely used to adsorb vapors and gases from air. Among solid sorbents, activated charcoal is the most commonly used.

At the rate of air passage up to 2 l/min the efficiency of adsorption from air by activated charcoal for most of organic vapors is equal to 99.99%. Silica gel is often used to sample chemical substances at low temperatures. Zeolites, graphitized carbon black, polymer sorbents, nonporous sorbents (potassium carbonate, copper sulfate, calcium chloride, etc.) and other substances are used in a number of cases to adsorb toxic vapors and gases. Their advantage is that desorption from these sorbents proceeds quite readily. Selective sorbents,such as synthetic molecular sievers, zeolites, are used to separate the components of a vapor-gas mixture, e.g., hydrocarbons, in the course of sampling. Adsorption of gases and vapors on film sorbents is a fast and efficient sampling technique. These sorbents consist of glass crumbs (ranging in size from 3 to 5 mm) treated with a film-forming solution and placed in glass tubes 17-20 cm long and 7 mm in diameter. The principle of sorbent tube operation is based on the ability of substances to interact with the film of a viscous sorbing solution when air passes through it.

Various fibrous filters are utilized to trap such highly dispersed aerosols as smokes, mists and dusts. AFA filters made of fine polyvinylchloride fibres are most extensively used. Analytical aerosol filters -AFA- are highly retentive and heat resistant (they can be used at air temperature ranging from -200 to ±60 - +150°C). The maximum capacity of filters with a working surface of 20 cm^2 amounts to 140 l/min. At a filtration rate of 40 cm/s, slippage through a standard oil mist is not more than 10%. Ultrafine fibre glass filters are also quite efficient in trapping aerosols. They are resistant to heat (up to 500°C), as well as to acids, alkalis, and other reagents; they also have a low hygroscopicity. At air stream velocity from 10 to 80 cm/s, the slippage of dioctyl phthalate aerosol through these filters is 0.01 - 0.08%.

AFAS-U (aerosol-sorption-charcoal) filters deserve special attention as they are capable of trapping simultaneously vapors and aerosols. They consists of fibrous filter material (FM) impregnated with OU-A or BAU fine-grained activated charcoal. At air stream velocity of up to 15 l/min, these filters can simultaneously adsorb a wide variety of organic vapors and aerosols.

Filters impregnated with a solid sorbent and chemical reagents are of great practical significance: AFAS-R filters to trap mercury vapors and AFAS-I filters to trap iodine vapors. Such adsorption is based on chemisorptive processes accompanied by the chemical interaction of the adsorbed vapor with the reagent applied on the sorbent. When used, these filters are placed into special cartridges - filter holders, made of duralumin, steel or plexiglass, the cartridges have a working surface of 10, 18 and 20 cm^2.

1.3. Air sampling equipment

Air sampling is carried out with the help of aspirators. For this purpose, aspirators of various models, vacuum cleaners, and other devices are used. They should be powerful enough to overcome the resistance created by absorbing solutions and filter media. They should provide for an adequate air aspiration rate for sampling. They should also be portable.

Hand-operated pumps are used to take air samples into plastic bags. When water supply lines are available, ordinary water-jet pumps can be used.

Flowmeters are used to measure the volume of air sampled by electrical measuring instruments.

2. Methods of Analysis

Photometric, chromatographic, electrochemical (polarographic) and other analytical techniques are most commonly used to determine microconcentrations of harmful substances in air.

2.1. Photometric method

The photometric method is based on selective spectral absorption of light when it passes through the analysed solution. Stained solutions absorb radiation in the visible spectral range from 400 to 760 nm, whereas unstained solutions - in the UV region from 200 to 400 nm. The nature of a substance and its concentration in solution determine the pattern and the degree of light absorption.

2.1.1. Photometric and spectrophotometric equipment

Soviet-made FEK-56, FEK-60, FEK-N-57 and other photo-electrocolorimeters are used for photometric analysis in the visible spectral region of 315-630 nm. SF-8, SF-9, SF-16, SF-26, and IKS-22 (USSR), as well as SF-PMO (FRG, Zeis), Uras (Hartman Braun) are used to measure optical densities and light transmission factors of liquids and solids over a wide wavelength range from 191 to 2,500 nm, including the ultraviolet and near-infrared regions.

2.2. Chromatographic methods

Of chromatographic methods, that of gas chromatography has at present found a wide application. As compared with other analytical methods, gas chromatography has several advantages: separation of compounds with similar chemical properties, detection of substances with poorly manifested qualitative reactions, for instance, N_2O, COS inert gases identified by their specific retention time, and, finally, a small volume of gas required for analysis.

In gas-chromatographic methods, air samples in the form of gaseous and evaporating components are injected into a stream of carrier gas and then passed through columns filled with solid adsorbing surfaces (adsorption chromatography) or nonvolatile liquids applied on solid surfaces (gas-liquid chromatography). Depending on different coefficients of distribution between the stationary (solid) and the mobile (gaseous) phases, individual components of the mixture move at different rates in the column, come out as separate fractions, and can be identified as individual substances in a mixture with the carrier gas. This method is distinguished by a very high selectivity, and, with appropriate detectors, also a high sensitivity.

2.2.1. Chromatographic equipment

Special instruments, chromatographs are used to analyse mixtures. The major components of a chromatograph are the column, where the mixture components are separated, and the detector. Flame ionization and electron-capture detectors, highly sensitive to halogen-containing compounds, are most commonly used. A helium detector has been used to determine gases.

Modern chromatographs are designed on a modular principle, incorporate a wide variety of detectors and computer facilities to process the chromatograms.

Soviet-made chromatographs LKhM-8MD, "Gazokhrom", and "Tsvet-100" are equipped with a variety of detectors, temperature programming devices and attachments.

Laboratorni Pristroid (Czechoslovakia) manufactures are Chrom chromatographs. Manufacturers that also produce chromatographs are: Varian and Perkin Elmer (USA), Pye Unicam (Great

Britain), Carbo Erba (Italy), Hitachi and Shimadzu (Japan).

In quantitative determinations of components by means of gas chromatography, three different methods are primarily employed: absolute calibration, internal standard, and area normalization.

2.3. Thin-layer chromatography

A modification of chromatography is the thin-layer chromatography whose underlying principle is the separation of substances, depending on their adsorption capacity. Separation is carried out in a thin layer of a sorbent coated on a special plate. Substances on the plate are distributed with the help of a solvent. A thin sorbent layer is the stationary phase, while the solvent is the mobile phase. A microsyringe or a micropipette is used to apply the sample on the start line of the plate. The plate is placed into a chamber containing the solvent which moves along the adsorbent layer under the action of capillary forces. The components of the analyzed sample migrate through the layer together with the solvent at different rates. When the solvent reaches the top end of the plate, separation is completed, the plate is removed, and the solvent is evaporated. The analyzed substances are developed on the chromatogram as zones or spots.

In thin-layer chromatography, silica gel, alumina, ion-exchange resins are used as adsorbents. Such solvents as alcohols, ethers, ketones, aromatic and halogen-containing compounds serve as the mobile phase. The basic characteristic of separation is R_f, the ratio of the distance between the start line and the spot center (a) to the distance between the start line and the leading edge of the solvent (s).

$$R_f = \frac{a}{s}$$

Its value depends on the adsorbent, the temperature and on other factors.

The stained spots can be quantitatively analyzed either directly on the plate or after their removal from the plate.

Thin-layer chromatography is simple and inexpensive.

2.4. Paper chromatography

Paper chromatography is not used as often as other analytical methods. It has much in common with thin-layer chromatography. The separation of substances is based on the same principle because chromatographic paper can be impregnated with solid adsorbents.

3. Polarographic Analysis

Polarographic analysis is used to detect metals and a variety of organic compounds. Polarography is an electrochemical technique in which the current is measured as a function of the potential during electrolysis. This technique is used to investigate substances capable of undergoing electrochemical oxidation or reduction, which results in a polarographic wave corresponding to the moment of ion outflux in the compound. If the solution contains different substances with a differing reduction or oxidation potentials, several waves are obtained on the polarogram. The height of the polarographic wave characterizes the concentration of a substance in the solution.

6300

The half-wave potential is essentially dependent on the background (extraneous electrolyte) used to raise the solution conductance. Therefore, reference tables list the values of potentials for quite specific conditions. Salts and alkalis of univalent metals, acids, ect. are used as background electrolytes.

Used as polarizing electrodes are: dropping mercury cathode, solid stationary and rotating electrodes made of different metals - platinum, gold, silver, etc.

The method of standards and additives, as well as calibration curves, are primarily used in quantitative determination of substances.

3.1. Polarographic equipment

The following Soviet-made polarographs should be mentioned: PPT1 (alternating current); multi-purpose PU-1, multi-purpose electrode UPE-6124. Among foreign models - OH-105 (Hungary), PA-2 (Czechoslovakia), and E-310 (FRG).

Air analysis today is also carried out with the help of such methods as atomic-absorption spectrophotometry, chromato-mass-spectrometry, neutron activation analysis, and the fluorescence method.

4. Gas Analyzers with Indicator Tubes.

Gas analyzers equipped with indicator tubes are employed to make a rapid analysis of air pollution by harmful substances. These analyzers are primarily used to monitor air quality in the working environment at MAC levels, as well as to determine lower concentrations.

The instrument consists of a hand-operated pump fitted with a steel spring which, on being pressed, expands the bellows, thereby sucking in 100 ml of air. The sampled air passes through the indicator tube designed for the detection of a particular pollutant. Air composition is identified by the change in indicator tube coloration.

The Soviet Union and other countries currently produce indicator tubes for the detection of more than 40 different pollutants (hydrogen sulfide, sulfur dioxide, carbon monoxide, aromatic hydrocarbons, nitric oxide, acrylonitrile, ammonia, acetone, vinyl chloride, dimethyl formamide, etc.).

5. Automatic Instruments for Air Pollution Analysis

It is expedient to use stationary automatic gas analyzers to control the air in the working environment for harmful substances of hazard classes 1 and 2, with MAC less than 0.1 and from 0.1 to 1.0 mg/m^3. An automatic analyzer is a programmed instrument for automatic collection of air samples, analysis of the content of a noxious substance, measurement and recording of analysis results.

Today in the USSR and abroad are manufacted stationary and portable gas analyzers. Among the Soviet-made gas analyzers are Gamma-1 and Sigma-1 for the determination of benzene, styrene, toluene, and chloroprene; IKRP-450 for mercury vapor determination; FL-5501M (multi-purpose) for airborne hydrogen sulfide, sulfur dioxide, ammonia, nitrogen oxide and ozone; FGTs for hydrogen sulfide, carbonyl chloride, hydrogen cyanide, and ammonia; Atmosfera for hydrogen sulfide, sulfur dioxide, chlorine, and ozone.

The Incometer (FRG) determines sulfur dioxide, nitrogen dioxide, fluorine-ion, and ozone, while the Techicon (USA, FRG) - hydrogen fluoride. Such instruments as Uras, Limas, Monocolor (FRG) are intended to determine chlorine, nitrogen dioxide, mercury vapors, etc.

REFERENCES

1. V. Leite. Determination of air pollutants in the working zone air. (Russian transl.) Leningrad, Khimiya, 1980.
2. S.I. Murav'eva et al. Sanitary and chemical control of air at industrial plants. Moscow, Meditsina, 1982.
3. E.N. Iovenko. Automatic gas analyzers and indicators for toxic and explosive compounds in air. Moscow, Khimiya, (in Russian), 1974.
4. A.K. Babko and A.G. Pilipenko. Photometric analysis. General Principles and Instrumentation. Moscow, Khimiya, 1968 (in Russian).
5. M.S. Bykhovskaya, S.M. Ginsburg, O.D. Khalizova. Methods of determination of harmful substances in air. Moscow, Meditsina, 1966 (in Russian).
6. N.Sh. Volberg. Methods of determination of harmful substances in air. Leningrad, 1968 (in Russian).
7. M.V. Lazarev et al. Harmful substances in industry. Moscow, 1976.(in Russian).
8. D.A. Vyakhirev and A.F. Shushunov. A manual on gas chromatography. Moscow, Vysshaya shkola, 1975 (in Russian).
9. A.A. Kasparov, E.N. Marchenko, N.I. Molodkina et al. Organisation of hygienic research to control air pollution of the working environment by chemical substances. Gigiena truda i profzabolevaniya, 1980, No. 8, 19-23 (in Russian).
10. A. Kaleimans. Gas chromatography. Moscow, IL, 1959, 32-35 (Russian transl.).
11. D.K. Kollerov. Metrological principles of gas-analytical measurements. Moscow, 1967 (in Russian).

12. I.M. Korenman. Photometric analysis. Determination methods for organic substances. Moscow, Khimiya, 1970 (in Russian).
13. T.A. Kryukova, S.I. Sinyakova, T.V. Arefyeva. Polarographic analysis. Moscow, 1959 (in Russian).
14. M.D. Manita, R.M. Salikhdzhanova, S.F. Yavorskaya. Modern methods of determining air pollution in populated areas. Moscow, Meditsina, 1980 (in Russian).
15. R.G. Pats and A.P. Vasilieva. Methods of AC polarography. Moscow, Metallurgia, 1967 (in Russian).
16. E.A. Peregud and E.V. Gernet. Chemical analysis of the air in the working environment. Leningrad, Khimiya, 1970 (in Russian).
17. E.A. Peregud, M.S. Bykhovskaya, E.V. Gernet. Rapid methods of determining airborne harmful substances. Moscow, Goskhimizdat, 1962 (in Russian).
18. T.V. Solovieva and V.A. Khrustaleva. Manual on methods of determining airborne harmful substances. Moscow, Meditsina, 1974 (in Russian).

FIBROGENOUS DUSTS. A CHEMILUMINESCENT METHOD OF STUDYING THEIR STRUCTURAL FEATURES AND MECHANISMS OF BIOLOGICAL ACTION

B. T. VELICHKOVSKY

Formulation of the Problem

Dust affects human organism in many different ways. Depending on its chemical composition, it can produce fibrogenous, toxic, irritating, allergic, carcinogenic, radioactive and photosensitizing effects.

Particular interest in pulmonary diseases caused by prolonged inhalation of fibrogenous dusts is determined by both the practical and the theoretical significance of this problem.

We are dealing here with the gravest and most widespread occupational pathology whose control is of paramount social importance. Dust-induced lung diseases are characterised by their irreversible course. Up to now there exist no specific methods of their treatment. Those who risk to be affected by fibrogenous dust are able-bodied persons engaged in industries of major importance.

All kinds of this dust are characterized by a special mechanism of their pathological effect on the organism.

At the basis of the action of dusts having a toxic, allergic, carcinogenic, irritating and photosensitizing action is the resorptive-chemical effect, i.e. the transfer of certain substances, specifically interacting with the biosubstrate, from the dust particles to the liquid tissue medium as a result of dissolution or extraction. Dusts containing radioactive elements or radon and thoron decay products adsorbed on the particle surface also exert a mediatory effect on the organism, caused by a certain kind of radiation.

As for fibrogenous dusts, for a disease to be initiated it is necessary that dust particles should be present directly in the respiratory organs, by themselves exerting a pathological effect on the tissues.

When studying fibrogenous dusts the investigator has to explain how silica, the most stable and chemically inert part of the Earth's crust, can produce such an unfavourable effect on the human organism; how finely dispresed solid particles, practically insoluble and nontoxic, can actually interact with living matter.

It is now generally accepted that the leading role in this process belongs to macrophages, whose destruction under the action of fibrogenous dust is the first link in the overall chain of subsequent pathological changes.

Studies performed in different countries have repeatedly confirmed the fact that no dust, even quartz dust, produces a direct fibrogenous effect without a successive replacement of the processes of phagocytosis, destruction and decay of coniophages. A direct contact of dust particles with the membrane of a phagocytizing cell is necessary for the realization of the harmful effect of silica, while the contents of dead macrophages activate fibroblasts, inducing the development of pneumosclerosis.

Therefore, in order to predict the possible fibrogenous properties of dust it is necessary to ascertain the structural and functional changes taking place in the cell contacting with a dust particle. This is usually done with the help of morphological (light- and electron microscopy) and biochemical methods (measuring the activity of cellular dehydrogenases in a test with nitrotetrazole blue, determining the yield of lysosomal enzymes, recording the hemolysis of erythrocites). These methods, however, are either not specific with respect to phagocytosis and can be related to other metabolic processes in the cell or do not allow a continuous detection of changes in macrophage activity in the course of phagocytizing. At the same time, it should be noted that it is the investigation of phagocytosis kinetics, including the earliest stages of the cell's contact with foreign particles, that facilitates the ascertainment of specific features characterizing the molecular mechanism of the interaction between phagocytes and fibrogenous dusts.

The most adequate for kinetic measurements is the chemiluminescence method.

Proceeding from the above considerations we had to verify whether the composition and the origin of dust samples affected the chemiluminescence of macrophages, whether the kinetics of chemiluminescent (CL) response depended on the dust properties, and, on the basis of this, to establish the possibility of using the CL method to predict the degree of fibrogenous effect for different dusts.

Materials and Methods

The tests were carried out on common male rats weighing 120-150 g. 20 ml 0.9% sodium chloride solution containing 5 u./ml heparin was administered intraperitoneally. The abdomen was massaged for 2-5 min and lanced, the contents were drawn off with a Pasteur pipette, filtered through a double gauze layer and centrifuged at 120 g for 10 min. The precipitate was washed twice and resuspended in 0.9% sodium chloride. The number of cells was counted, and the concentration brought up to

$1.5\text{-}2.0 \times 10^7$ cells per ml. All cell extraction operations were performed at 4°C. The viability of cells in the suspension was determined using the test with trypan blue; it was usually 92--96%.

CL of macrophages was measured on a chemiluminometer with a measuring cuvette 2 mm in volume, thermostatically controlled at 37°C. CL intensity was continuously recorded during 20 min after placing the cells in a measuring cuvette. Luminol in the concentration of 6.5×10^{-4} M was used as CL activator. The medium for CL measurements in all tests contained 0.9% sodium chloride, 0.005 M glucose, 0.0025 M magnesium chloride and 0.0065 M luminol. The final concentration of cells in the measuring cuvette was $1.5\text{-}2.0 \times 10^6$ cells per ml.

To estimate the extent of the inhibition of macrophage activity and viability by the dust in question, a standard dose of the same quartz dust sample, whose CL-response curve had previously been well studied, was added into the cuvette 5-10 min after the introduction of the analyzed dust.

Results

Background CL was observed to develop following the introduction of the peritoneal exudate cell suspension into the measuring cuvette, caused by the contact of cells with the cuvette walls. A further addition of dust particles into the system resulted in an increase in CL intensity. The kinetics of this response and its magnitude depend on the nature of the analyzed sample.

6300

Three types of CL response have been observed:

Type one - fast response with a high amplitude, short time to reach the maximum (1-1.5 min) and rapid decrease in CL intensity after the maximum has been reached. The total flash duration was, as a rule, 3-4 min. CL of this type accompanied the activation of macrophages by highly fibrogenous dusts: quartz, silica condenstae and chrysotile-asbestos.

Type two - slow response with slowly developing luminescence having a low amplitude. The time to reach the maximum was 6-8 min. After the maximum had been reached, CL intensity remained practically constant during 10-15 min. This type of CL response kinetics followed the contact of macrophages with low-fibrogenous dusts: coal, chalk, etc.

Type three - explosive response with an extremely short CL flash having a needle-like amplitude, after which the CL intensity curve fell below the level of spontaneous luminescence of microphages. Subsequent addition of a standard quartz dust sample (in contrast to the two previous types of CL response) failed to cause a new increase in CL intensity, which indicates the destruction of most of the cell elements in the measuring cuvette. Sometimes the CL curve went down at once even without a short flash. There was no response to subsequent introduction of standard dust in this case either. CL response kinetics of this kind was observed when microphages were in contact with one of the varieties of clinoptilolite zeolites, as well as some polymetallic dusts, e.g., the dusts of Yakutian auriferous gravels.

All the studied dust samples can thus be subdivided into groups, depending on the peculiarities of their luminescence.

It was considered necessary to ascertain whether CL kinetica and intensity depended on the consentration and the degree of dispersion of a dust sample.

The shape of CL response kinetic curve was shown to remain unchanged with a change in quarts dust concentration; the higher the dispersity (specific surface) of a sample, the lower the dust concentration necessary for CL amplitude to reach its maximum. For instance, for quartz whose specific surface is 2.75 m^2/g this concentration was equal to 6 mg/ml, and for SiO_2 condensate with a specific surface of 7.5 m^2/g the maximum was reached at a concentration of 1 mg/ml. CL intensity increased monotonously after the addition of quartz with a low specific surface (about 1 m^2) within the range of studied concentrations up to 8 mg/ml. A further increase in dust sample concentration in the measuring cuvette proved to be inexpedient because of considerable optical distortions due to the high turbidity of the solution.

The maximum value of CL response for crystalline quartz and amorphous silica condenstae was recorded with the same total surface of dust in the measuring cuvette: 0.015 m^2/ml or 10^{-8} m^2 per cell. However, with an equal total surface of the samples the amplitude of CL responce was always higher in quartz than in SiO_2 condensate.

Hence, the kinetics and the amplitude of CL responce of macrophages to dust depend not only on its chemical composition but also on its crystalline structure, as well as the size and the concentration of dust particles, in other words, on their total surface.

Interactions between macrophages and chrysotile-asbestos were marked by certain peculiarities. An increase in the concentration of this dust led to changes in CL response kinetics. At low chrysolite-asbestos concentrations, from 0.02 to 0.1 mg/ml, CL developed slowly, with the maximum reached only after 8-10 min. An increase in the quantity of added dust resulted in the growth of CL amplitude, and at concentrations from 0.25 to 0.5 mg/ml, CL reached its maximum 1 min after the introduction of the dust. Damage to macrophages caused by chrysotile-asbestos increased with the growth of its concentration. This was shown by experiments in which 1 mg/ml was introduced into the measuring cuvette several minutes after chrysotile-asbestos. The amplitude of CL response rapidly declined with the growth of chrysotile-asbestos concentration.

Dusts having the same composition and crystalline structure taken in a dose with the same total surface, but differing in their origin (quartzes from the Urals and from Liubertsy, near Moscow), produced CL responses of different intensities when activating macrophages. The value of CL responses of these dust samples correlated well with the degree of their fibrogenous effect (Table).

The concluding series of experiments was performed to study the mechanism of CL response initiation when microphages come into contact with fibrogenous dusts.

CL intensification by phagocytizing cells during the capture of different particles or soluble macromolecules, such as bacteria, latex, proteins, etc., results from producing active oxygen forms and their derivatives: superoxide radical anion ($^{\cdot}O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$) and singlet oxygen ($^1O_2$) (Fridovich, 1979; Sagone et al., 1980, and others). Reactions with the participation of these aggressive oxidizing agents are accompanied by light emission - chemiluminescence (M.N.Merzlyak and A.S.Sobolev, 1975).

To clarify the nature of CL response to quartz dust, certain enzymes were added to the cuvette: catalase which destroys hydrogen peroxide, or superoxide dismutase which interacts with

Table

Characteristics of properties of quartz dust samples, degrees of their fibrogenous effect and the values of CL responses of macrophages

Quartz dust sample	Content of SiO_2,%		Number of particles less than 5 μm in size, %	Adsorption of calcium cations, $\mu g/100\ A^2$	Weight coefficients of dry rat lungs, mg	Content in lungs of dust-affected rats		CL response intensity, rel.u.
	Total	Free				oxyproline, μg	lipids, mg	
Liubertsy	98.8	98.4	96.1	923	257 ± 10	5983 ± 459	981.4 ± 45.7	384
Urals	98.7	97.7	95.6	514	158 ± 12.1	4478 ± 441	47.4 ± 3.0	49

the superoxide radical anion. In the first case the luminescence intensity decreased by about one third, and in the second - by more than a half. CL response to fibrogenous dusts is, therefore of the same origin as in the case of other phagocytosis-activating agents, and is associated with the reactions of biological reduction of oxygen.

Only native, viable macrophages are capable of generating CL quanta in response to activation by quartz. Denaturation of cells by boiling, as well as their ageing for 24 hours at 4°C or 4-5 hours at 20°C, resulted in the disappearance of CL, both the spontaneous CL and that induced by dust particles. At the same time, the viability of cells, determined in the test with trypan blue, declined drastically.

Discussion

The data obtained have shown industrial dusts, differing in the degree of their fibrogenous effect, to be characterized by several types of CL development, differing in the amplitude, the time to reach the maximum luminescence value, the speed of CL intensity decline.

The first, fast, type of CL response manifests itself only following the introduction of highly fibrogenous dust samples, characterized by initiating the development of nodular forms of pneumoconiosis, as well as silicotuberculosis.

The second, slow, type can, most probably, be regarded as physiological, since it is retained for a long time at a constant level up to the completion of phagocytosis, with the macrophages retaining all this time their ability to respond to an additional irritant. Such a response is observed under the effect of low-fibrogenous dusts, whose prolonged action in high concentrations initiates the development of diffusive sclerotic forms of pneumoconiosis and of chronic dust bronchitis. CL response of a similar type was previously described for the absorption of bacteria and other phagocytosis-activating agents.

Of great interest is the third, explosive, type of CL response, caused by the destruction of macrophages upon the contact of dust particles with the outer cellular membrane. Such dusts are characterized by the development of a pathological process that can apparently be defined as fibrozing alveolitis.

The chrysotile-asbestos dust occupies an intermediate position. At low concentrations this dust produces a slow "physiological" type of CL responce, and at high concentrations - a fast responce caused by the impairmant of the regulation of the cellular membrane functions.

With the same total surface of different dusts, the amplitude of CL response will be the higher the more fibrogenous is the dust. Thus, the kinetics of CL response to two quartz dust samples of different origin proved to be dissimilar despite practically the same percentage of silicon dioxide and the respirable fraction particles in them.

It has been known for a long time that quartz dusts with the same percentage of free silicon dioxide and the same dispersity may differ in the degree of their fibrogenous effect. From the hemolytic and dehydrogenase tests, as well as the histomorphological and biochemical indicators obtained in a chronic experiment on test animals, it follows that the difference at the cellular level may be as great as three- - fourfold (T.B. Velichkovskaya, 1981). This, naturally, makes much more complicated the hygienic characterization and the substantiation of maximum allowable concentrations of a silicon-containing dust, since it is the percentage of free silicon dioxide which serves as the oficially accepted leading criterion based on which such assessments are made today. As seen from the table, the level of the fibrogenous effect of silica changes unidirectionally with the indicators reflecting the physico-chemical properties of the surface of dust particles, in particular, with the value of Ca^{2+} ions adsorption on the unit surface of dust particles and the nature and intensity of the CL responses of microphages. That is why the CL method can be used to determine the degree of the fibrogenous effect of industrial dusts.

The generation of active forms of oxygen into the environment constitutes an adaptation of phagocytizing cells, developed in the course of evolution. Free oxygen-containing radicals restrain the mobility of microorganisms, and in this way facilitate their absorption by phagocytes. Therefore, the formation of active forms of oxygen is far from always to be regarded as a pathological or a dangerous process.

The direct pathological consequences of the formation of active forms of oxygen by the phagocytizing cell manifest themselves only under the action of the dusts initiating the development of CL response of the explosive type. In this case, the intensive discharge of oxygen-containing radicals impairs the cytoplasmic membrane permeability so sharply that the result is the death of the cell itself. Such dusts have as yet been insufficiently studied. The specific nature of lesions caused by them has not so far attracted the necessary attention of therapeutists and occupational pathology experts.

When silica-containing and other highly fibrogenous dusts engendering the CL response of the fast type are inhaled, their effect on the macrophage is determined by the so-called cytotoxic effect. In the final analysis, its essence lies in a rapid destruction of phagolysosomes containing the particles absorbed by the cell. The destruction of phagolysosomes, in its turn, can have one of two causes. First of all, it would be logical to assume that, in this case too, the "culprits" are oxygen-containing radicals. They are released into the inner space of the phagolysosome by its membrane on coming in contact with dust particles, in the same way as they are generated into the environment upon contact of dust particles with the cytoplasmic membrane of the cell. This assumption is supported by the structural similarity of these membranes, since the phagosome is formed by cytoplasmic membrane immersion into the cell. The other cause of destruction can lie in an increase in the intraphagolysosmal pressure as a result of Donnan redistribution of ions, brought about by dust particles resembling polyelectrolytes in their surface properties. The second assumption is supported by a high sensitivity of phagocytizing cells to the changing osmotic pressure of the medium (B.T.Velichkovsky, 1980).

Dusts causing CL response of the slow type have a peculiar mechanism of pathological effect on the macrophage. There exists a widespread opinion that the differences between the high- and low-fibrogenous groups of dust are purely quantitative. It is assumed that coal and other low-fibrogenous substances can also produce a cytotoxic effect, although a less pronounced one. At the same time, recent findings tend to raise doubts about the validity of such ideas. Thus, the phagocytized quartz particles decrease oxygen consumption by macrophages, the cells loose their mobility and are rapidly destroyed, whereas coal dust, on the contrary, raises oxygen consumption by macrophages, coniophages remain mobile and retain their viability for an extended period of time.

The different nature of the action of high- and low-fibrogenous dust on the macrophage is also confirmed by the different CL response kinetics. During the phagocytosis of a low-fibrogenous dust,which causes CL response of the slow type, the macrophages that have absorbed a large amount of particles grow in size, and the number and size of lysosomes and mitochondria in them increases. These changes should be regarded as a typical "working" hypertrophy at the intracellular level. Prolonged development of such changes will also result in the death of the coniophage, caused by the same reasons and mechanisms as in the case of the decompensation of an excessively hypertrophic cardiac muscle.

The impact of dusts on macrophages, causing the three different types of CL response, is thus not the same in all the three cases. Each of these groups of dust has its own mechanism of destroying the cell that phagocytized them. At the same time, our findings lead to an important, in our point of view, conclusion: innocuous dusts do not exist. In certain conditions coniophages can be killed by any sparingly soluble dust. Silica, due to the specific properties of its surface,causes their death in such great numbers that subsequent reactions acquire a qualitative specificity; all lung diseases caused by dusts have one common and decisive feature of their development - the death and decomposition of macrophages.

Do the new data on the three different mechanisms of macrophage destruction by fibrogenous dusts change our notions on the physico-chemical properties of dust particles responsible for the development of dust-induced pathology?

There is an important distinguishing feature in the structure of fibrogenous substances. Their moleculee are formed by means of strong covalent chemical bonds. These are known to have a certain directionality in space. If these bonds connect dissimilar atoms, or the formed molecule is asymmetric, electron density shifts towards one of the atoms, and the probability of valent electrons staying in the field of this atom increases. The above shift causes the appearance of very small charges equal to the portion of the electron charge on the atoms joined by the covalent bond, as a result of which these atoms acquire polarity or a combined effective charge. The molecule containing a polar bond possesses a dipole moment. The more polar the covalent bond in the insoluble inorganic substance, the stronger the field of valent or molecular forces on the surface of its fracture.

Depending on how pronounced those properties are, all fibrogenous dusts can apparently be also subdivided into three classes. The first class should include natural polyelectrolytes of the zeolite type, on whose initial surface are located the functional groups capable of dissociating and entering into ion exchange reactions. When at physiological pH values their dissociation is sufficiently pronounced, the contact of a dust particle with the macrophage cytoplasmic membrane results in the development of a predominantly electrostatic type of interaction.If the surface of a particle acquires the oveall effective charge that is higher than a certain critical value, the arising CL response is of the explosive type, and a rapid contact destruction of the macrophage membrane takes place. This very situation can be assumed to be taking place during the phagocytosis of clinoptylolite.

The second class comprises substances of the silica type, on whose fracture surface appear chemical groups capable of both dissociating (free silanol groups) and forming hydrogen bonds (hydroxyl, siloxane, and other groups). The contact of such particles with the macrophage cytoplasmic membrane initiates the development of CL response of the fast type, and cell destruction is determined by the cytotoxic effect.

If a substance consists of the same kind of atoms or symmetric molecules, the covalent bonds are nonpolar, the predominant process taking place on the surface of fracture is that of physical adsorption, dust particles cause CL response of the slow type, and coniophage destruction is determined by the intracellular "working" hypertrophy of organelles and the decompensation of excessively hypertrophic cells.

REFERENCES

Fridovich I. The biology of oxygen radicals. Science, 1978, v.201, No.4359, p.875-880.

Merzlyak M.N., Sobolev A.S. In: "Developments in Science and Technology. Series: Biophysics. Moscow, 1974, v.5 (in Russian).

Sagone A.L., Pecker M.A., Wells R.M., Democko C. Biochim. Biophis.Acta, 1980, v.628, p.90-95.

Velichkovskaya T.B. Experimental Study of the Methods of Predicting the Fibrogenous Effect of Industrial Dusts and the Means of Pathogenetic Therapy and Prophylaxis of Pneumoconioses. Author's abstract of cand.thes. Moscow, 1981.(in Russian).

Velichkovsky B.T. Fibrogenous Dusts. Peculiarities of the Structure and the Mechanism of Biological Action. Gorky, 1980 (in Russian).

TOXICOLOGY OF SOLVENTS

A. M. Eitington

Solvents are chemical compounds or their mixtures capable of dissolving different substances, i.e. forming solutions that consist of more than two components.

Solvents may be classified on the basis of their purpose, physical properties, etc. The most common one is chemical classification which divides all solvents into organic and inorganic. This paper deals with the toxicology of a wide variety of organic solvents that are much more important for industrial and laboratory applications.

Organic solvents are rather widely used in the paint and varnish industry, in the manufacture of synthetic fibres, polymers, glues, smokeless powder, and celluloid, as well as in the rubber industry, perfumery, extraction of fats, dry cleaning, etc.

According to their chemical composition, solvents can be grouped into different classes of chemical compounds.

Some authors combine commercial solvents into three major groups or types: (1) alcohol; (2) ether; (3) benzene or chloroform.

Sometimes they are grouped into several classes on the basis of their distinctive characteristics.1.Alcohols, ethers and esters, ketones, aliphatic and alicyclic hydrocarbons are substances producing, for the most part, a narcotic (reversible) effect. 2.Trichloroethylene, methyl alcohol, carbon disulfide, that are, by their nature, different solvents, make up a group of chemicals causing persistent changes in the nervous system. 3. Benzene, toluene, xylene, chlorobenzene, and a number of other benzene derivatives, that are generally used in mixtures with esters, alcohols, and ketones, are poisons of the blood and the hemopoietic organs. 4. Substances causing degeneration of the parenchymatous organs; those are chlorinated hydrocarbons, as well as glycols and their derivatives.

Saturated Hydrocarbons

The solvents belonging to a group of saturated hydrocarbons are patent narcotics. The narcotic effect increases up to decane with the number of carbon atoms. For white mice, lethal concentrations of pentane, hexane, and heptane vapors amount to 380, 150, and 75 mg/l, respectively. In the case of chronic poisoning, the solvents of this series cause no severe organic changes but result in the instability of the CNS responses and the development of inflammatory and dystrophic changes in the parenchymatous organs. The maximum allowable concentrations (MACs) in the air of the working zone for all saturated aliphatic hydrocarbons C_1-C_{10} in terms of carbon amount to 300 mg/m^3.

Of the saturated alicyclic hydrocarbons it is necessary to mention cyclohexane, a common solvent for rubber, wax and bitumen. Its action is similar to that of aliphatic hydrocarbons; the narcotic effect, however, is more pronounced. It may cause pruritus and inflammation of the skin. The MAC for cyclohexane is 80 mg/m^3.

Aromatic Hydrocarbons

Benzene is the most toxic solvent belonging to the aromatic hydrocarbon series. High concentrations of benzene vapors have narcotic and spasmodic effects. Death is caused by the respiratory center paralysis. The central nervous system's functional disorders accompanied with dystonia effects, changes in the parenchymatous organs, primarily in the liver, are reported at the early stages of chronic intoxication by benzene. Changes in the blood are the major sign of chronic poisoning. Benzene's myelotoxic action is thought to be associated with the formation of oxidation products exerting a cytotoxic effect, with disturbed enzyme processes in the cells, and with a "peroxide" effect responsible for the so-called "radiomimetic" action of benzene. Phenols and polyphenols are the main products of benzene conversion. Benzene produces gonadotropic, embryotropic, and mutagenous effects. However, these disorders are believed to be secondary and caused by changes in other systems, which makes it impossible to speak of specificity of benzene's long-term effects.

In view of its high toxicity and, hence, high hazard level, benzene is replaced, whenever possible, in the USSR by less toxic solvents. Table 1 shows some parameters of the toxicity and hazard indices of some aromatic hydrocarbons and chlorobenzene.

Along with benzene, two other widely used solvents belonging to the aromatic hydrocarbon group, toluene and xylene, have a narcotic effect, when in high concentrations. Compared with benzene, the effects of toluene and xylene on hemopoiesis are much milder, yet more pronounced in the case of the CNS.

Table 1. Toxicity and hazard indices of some aromatic hydrocarbons

Substance / Index	Benzene	Toluene	Xylene	Isopropylbenzene (Cumol)	Chlorobenzene
LD_{60} (mg/kg)	4600	7000	-	-	2300
LC_{50} (mg/l)	45	32	50	25	19
Lim_{ac} (mg/l)	1.1	0.3-1.0	0.3-0.5	-	0.25
Z_{ac}	40.9	~49	~100	-	76
Lim_{ch} (mg/l)	0.02	0.05	0.05	-	-
Z_{ch}	55	~13	~8	-	-
MAC (mg/m^3)	5.0	50.0	50.0	50.0	50.0

Exposures to low concentrations of toluene and xylene result not only in the CNS disorders but also in changes in the redox processes, the functional state of the parenchymatous organs, and the adrenal glands.

All the solvents of the aromatic hydrocarbon series have an irritant effect on the skin and the mucous membrane and may gain entry trough the skin.

The MAC value for benzene is 5.0 mg/m^3, and 50.0 mg/m^3 for the rest of the above-mentioned solvents. A special mark is used to caution that benzene and chlorobenzene are dangerous when absorbed through the intact skin.

Mixtures of Volatile Petroleum Products

Benzines, mixtures of volatile petroleum products, are the most important solvents extensively used in industry. Benzine solvents are narrow low-boiling fractions of oil straight-run distillation.

As a narcotic, distilled benzine acts in the same fashion as methane hydrocarbons and cycloparaffins that make up the bulk of its mass. In the case of benzine exposure typical symptoms include the development of convulsions, excitation of the parasympathetic part of the nervous system, and respiratory disorders. Very high benzine concentrations may bring about instantaneous poisoning.

More moderate benzine concentrations (a one-hour exposure to a concentration equal to 9.5 to 11.5 mg/l) produce an irritative effect on the mucous membrane causing headache, vertigo, palpitation, psychic excitement, nausea, instability, and disturbing the motor system coordination which is followed by loss of consciousness.

Chronic poisoning by benzine vapors is characterized by functional neural disorders accompanied with myasthenia, flaccidity, fatigue, erethism, ataxia, and sleep disturbance. Women have been found to develop the menstrual cycle disorders. Experimental research of the benzine solvent at the Institute of Industrial Hygiene and Occupational Diseases, the USSR Academy of Medical Sciences, has not confirmed a possibility of specific effects of benzines upon gonads and fetus.

The MAC value for the benzine solvent (as carbon) is 300 mg/m^3.

Chlorohydrocarbons

6300 Chlorine derivatives of the methane, ethane and ethylene series are the most important solvents in this group of chemical compounds. In terms of their action, all these compounds are narcotics that exert a toxic effect on the CNS and internal organs. Some narcotics trigger more severe lesions of the parenchymatous organs and the liver, in particular (the so-called hepatotropic poisons), others mainly affect the nervous system.

Table 2 shows that the solvents belonging to the group of methane chlorine derivatives slightly differ from one another by the degree of acute toxicity and hazard. The clinical pictures of acute poisoning by these chemicals also have much in common and feature headache, vertigo, nausea, vomiting, mental confusion or loss of consciousness. A more severe irritant action on the mucous membrane is symptomatic of carbon tetrachloride; the signs of damage to the liver and the kidneys are more commonly reported concurrent with the CNS impairment effects.

Table 2. Toxicity and hazard indices for some chloromethanes

Substance / Index	Methylene chloride	Chloroform	Carbon tetrachloride
LD_{50} (mg/kg)	-	1000	9600
LC_{50} (mg/l)	63	21.2	34.5
Lim_{ac} (mg/l)	1.0	0.7	1.2
Z_{ac}	63	30	29
Lim_{ch}	0.25	-	0.05
Z_{ch}	4	-	60
MAC (mg/m^3)	50.0	-	20.0

Chronic poisoning by the solvents of this group exhibits prevailing signs of the affected nervous system, kidneys and liver. Carbon tetrachloride has a most pronounced effect on the kidneys and the liver, which is associated with the fact that among the mentioned compounds it is best capable of forming free radicals, which in turn results in a peroxide oxidizing destruction of the microsomal structures and the endoplasmic network of the cells.

All the mentioned compounds cause skin irritation, which sometimes results in dermatitides and eczemas.

The MAC value for carbon tetrachloride is 20 mg/m^3 and 50 mg/m^3 for methylene chloride.

Vapors of solvents - ethane chlorine derivatives - produce more powerful effects than the respective methane derivatives, though it mainly holds true for narcotic action. Dichloroethane and tetrachloroethane, and, to a lesser degree, trichloroethane are most nazardous with regard to hepatotoxicity.

Toxicity of ethane chlorine derivatives is believed to be caused mainly by their metabolities that are more toxic than basic products. Dichloroethane metabolizes to form chloroethanol and monochloroacetic acid; trichloroethane - to form small amounts of trichloroethanol and trichloroacetic acid. Tetrachloroethanes are partially converted to trichloroethilene to be changed to trichloroethanol and trichloroacetic acid.

The MAC value is 10 mg/m^3 for dichloroethane, 20 mg/m^3 for trichloroethane and 5.0 mg/m^3 for tetrachloroethane.

Among the chlorine derivatives of ethylene, trichloroethylene and tetrachloroethylene are common solvents for resins and rubber. These solvents are known for their significant effect on the nervous system. Specific and persistent lesions of the nervous system are believed to be associated both with the action of the solvents themselves and with the impact of trichloroethanol and trichloroacetic acids, the products of their conversion.

Acute poisoning by trichloroethylene soon brings about a state of coma and prolonged unconsciousness. Myocardium is particularly responsive to trichloroethylene exposure. Cardiac disorders manifest themselves in extrasystole, tachycardia, and ventricular fibrillation. The impairements are followed by injury to the trigeminal nerve (its sensitive fibres),which manifests itself in complete insensibility of the facial skin and the front part of the tongue, extinction of gustatory and olfactory sensations, and a lack of reflexes from the nose mucous membrane and the cornea. Lesions of other cerebral (facial and optic) nerves are also reported.

Along with acute poisoning, chronic exposure to airborne trichloroethylene at a concentration ranging from 0.05 to 0.1 mg/l affects the nervous system, impairs the cardiovascular system, and may trigger cerebral disorders. A correlation between the degree of poisoning and the level of trichloroacetic acid in the urine has been acknowledged. In a clear case of poisoning, urine contains from 40 to 50 (or over) mg/l of trichloroacetic acid. The total amount of trichloroacetic acid and trichloroethanol equal to 100 mg/l should be considered critical for a possible development of initial signs of poisoning.

Upon exposure to tetrachloroethylene the above metabolites are formed in much lesser amounts, but the action of tetrachloroethylene itself on the parenchymatous organs, in particular, is more pronounced than that of trichloroethylene. For both solvents, the MAC stands at 10 mg/m^3.

It should be amphasized that many chlorinated hydrocarbons are regarded as compounds with potential carcinogenic activity. According to the IRPTC, the list of potential carcinogens includes carbon tetrachloride, chloroform, dichloroethane, trichloroethane, tetrachloroethane, trichloroethylene, tetrachloroethylene, i.e. those compounds that are commonly used as solvents. Though the inclusion of the said solvents in the list does not mean that they are carcinogens, this possible aspect of their biological action should be thoroughly and comprehensively studied.

- - - -

Alcohols

Alcohols find extensive applications as solvents of lacquers and polishers. Ethyl, propyl, isopropyl, and butyl alcohols are most commonly used. Although they have narcotic and irritant effects, alcohols, by and large, are less toxic than all the other solvents, except for methyl alcohol which is a potent nervous and vascular poison with a marked cumulative effect. The special toxicity of methyl alcohol is associated with the fact that it produces formaldehyde and formic acid. Whatever the route of methyl alcohol administration, it causes lesions of the optic nerve and the retina in the case of acute and pronounced chronic poisonings.

In chronic exposure, the poisoning progresses gradually and is characterized by irritation of the the mucous membranes, frequent respiratory disorders, neuritides, visual disturbance, narrowing of color vision limits, changes in electroretinogram, optic nerve atrophy, arteriostenosis and delatation of retina veins, as well as hyperemia of the vascular coat of the eye. Poisoning usually results from absorption of the toxic chemical through the skin concurrently with inhaling its vapors.

The MAC value for ethyl alcohol is 1000 mg/m^3; for propyl and butyl alcohols - 10 mg/m^3.

Among polymethylene (alicyclic) alcohols it is worth mentioning cyclohexanol, a common solvent possessing narcotic and irritant properties. The recommended value of the MAC for cyclohexanol is 10-15 mg/m^3.

Ethers

Ethers are used to dissolve alkaloids, fats, mineral oils, waxes, nitrocellulose, etc. By type of action, ethers are narcotics. They produce irritation of the mucous membrane of the eye and upper respiratory tracts. Some of them cause damage to the kidneys and the liver. Acute poisoning may result in bronchitis, pneumonia, damaged kidneys, neural disorders. Chronic poisoning causes nausea, vertigo, apathy, and disturbed sleep. Vegetative lability, increased tension reflexes, and cardiovascular lesions are reported. The MAC value for diethyl ether is 300 mg/m^3, while the tentative safe exposure level (TSEL) for diisopropyl ether equals 100 mg/m^3.

The ether of diethylene, a six-membered heterocyclic compound, is a common solvent of cellulose, resins, paints, and rubber. Diethylene ether or dioxane causes most severe injury to the liver and the kidneys bringing about irreversible changes. The MAC value for dioxane is 10 mg/m^3.

Dimethyldioxane as a derivative of dioxane is similar to dioxane in its toxic action. Given the specific gonadotropic effect of dimethyldioxane the MAC value for this chemical has been set at the level of 3.0 mg/m^3.

Esters

As a rule, lower aliphatic esters readily dissolve many compounds; they act by a whole molecule and display narcotic properties. The MAC value for methyl acetate is 100 mg/m^3; for ethyl acetate, butyl acetate, and propyl acetate - 200 mg/m^3. Methyl acetate is a component of wood-chemical or wood-alcohol solvents. The methyl acetate solvent contains no less than 50 per cent of methyl acetate.

Ketones

Ketones are another major component of the wood-chemical solvents. Ketones affect the central nervous system, and in the case of prolonged inhalation they will accumulate in the body. The slow rate of their excretion increases a possibility of chronic poisoning. The MAC value for acetone, methyl ethyl ketone, and methyl propyl ketone stands at 200 mg/m^3. The TSEL for methyl isobutyl ketone is 5.0 mg/m^3.

Cyclohexanone, an alicyclic ketone, is a more toxic solvent than any aliphatic ketones. Chronic poisoning has been found to retard the growth rate of test animals, to reduce O_2 consumption, to cause leukocytosis, eosinophilia, the CNS excessive excitability, and to change the weight of the internal organs, the adrenal glands, and the thyroid gland.

The blood displays increased activity of cholinesterase, and the reduced number of hydrosulfide groups. The MAC value for cyclohexanone is 10 mg/m^3.

Glycols and Glycol Ethers

Dihydric alcohols-glycols and their ethers - are known to be good solvents. It is unlikely that exposure to glycol vapors (except for the vapors of diethylene glycol) will result in acute poisoning for reasons of their low volatility. Ethylene glycol is highly toxic when it enters the organism orally affecting for the most part the CNS and kidneys since the chemical is a vascular and protoplasmic poison. Acute poisoning is accompanied either with preponderant damage to the kidneys or, in a less serious case, by transient effects associated with the nervous system lesions. The workers chronically exposed to ethylene glycol have been found to exhibit vegetative dystonia, reduced osmotic stability of leukocytes and phagocytic activity of neutrophils, as well as leukopenia and reticylocytosis. Changes in the blood may precede shifts in the state of the nervous system. The action of ethylene glycol is associated with both its own toxicity and that of its toxic metabolites. The chemical is believed to be able to metabolize yielding, in succession, glycolaldehyde, glycolic acid, aminoacetic acid and hippuric acid. Glycolic acid may, however, be converted to oxalic acid.

Diethylene glycol is much more toxic in the case of acute poisoning by inhalation. Exposed to diethylene glycol white mice die even at a concentration of 0.13 mg/l.

REFERENCES

1. Gadaskina, I.D. and Danishevsky, S.L. *The toxicology of solvents.* - In: "Manual on Labour Hygiene", vol. 2 (Moscow: Medgiz, 1963) (in Russian).
2. Shirokov, Yu.G. and Pashkova, G.A. *The toxicology of solvents.* Journal of the Mendeleyev All-Union Chemical Society 19, No. 2 : pp. 178-185 (1974) (in Russian).
3. Lazarev, N.V. (ed.). *Handbook on Harmful Substances in Industry* (Leningrad: Khimiya, 1976, vols 1 and 2, 1977, vol. 3) (in Russian).
4. Sanotsky, I.V. and Ulanova, I.P. *Criteria of Harmfulness in Hygiene and Toxicology in Assessing the Hazard of Chemical Compounds.* (Moscow: Meditsina, 1975): 328 (in Russian).

TOXICOLOGICAL EVALUATION OF MYCOTOXINS

L.V. Kravchenko

Mycotoxins are defined as toxic products of vital activities (secondary metabolites) of certain species of microscopic fungi which contaminate foods and livestock feeds and are a cause of alimentary mycotoxicoses in man and animals.

The economic damages caused by mycotoxins involve direct losses of food and feeds, sharp decline of their nutritive value, deaths, reduced weigh increment and reproduction rate of farm animals and their increased susceptibility to infectious diseases; and detoxification costs of contaminated products along with occasional, loss of markets for farm products.

Mycotoxins attract attention of different groups of researcher due to the fact that they are widespread and that most of them have carcinogenic, mutagenic and teratogenic properties. Some problems in mycotoxin studies and especially those concerned with protection of human health nowadays attract the focus of attention of such international organizations as the World Health Organization (WHO), United Nations Environment Programme (UNEP), International Agency for Research on Cancer (IARC) and International Union of Pure and Applied Chemistry (IUPAC).

Aflatoxins

Aflatoxins are best known among the mycotoxins. Their discovery came from a study of the etiology of an outbreak of non-infectious disease among poultry in England in 1960. Acute forms of the disease were characterized by development of hepatic necroses and before long hepatocarcinogenic properties in aflatoxins were ascertained.

The group of aflatoxins, at the present time, includes, in addition to major specimens, that is, aflatoxins B_1, B_2, G_1 and G_2, a number of their derivatives - aflatoxins M_1, M_2, B_{2a}, G_{2a}, P_1 and G_1 - similar in their chemical structure and biochemical action. By their chemical structure, aflatoxins are furocoumarins.

Of the four major specimens, aflatoxin B_1 is the most toxic and generally synthesized in maximum quantities. The ratio of individual aflatoxin toxicities can be shown with the example of LD_{50} for one-day ducklings: for aflatoxins B_1, B, G_1 and G_2 they are 0.36, 1.70, 0.78, and 2.83 mg/kg, respectively.

At first, aflatoxins were isolated from peanut and further research confirmed that it is the product which is most frequently and heavily (up to 8.6 mg/kg) contaminated by aflatoxins. Aflatoxins were then found in corn (up to 12.5 mg/kg) and - less often and in smaller amounts - in wheat, rice, barely, soya, beans, various nuts,cotton seed, dried and smoked fish, and in some vegetables and fruits. Aflatoxins can accumulate in milk, eggs, as also in tissues animals of received aflatoxin-contaminated feeds.

Acute Toxic Effect of Aflatoxins

It is no longer doubted at present that most mammals, including primates, birds, some species of fish, insects, microorganisms and higher plants are susceptible in varying degrees to the toxic effect of aflatoxins.

A feature of acute aflatoxin intoxication in a majority of animal species is speedy development of symptoms and high lethality rate.

The first symptoms to appear in all species include lack of appetite, loss of body weight or slow-down of its increment. In chicken the non-specific though characteristic symptoms of aflatoxicosis include ataxia, convulsions, opisthotonos (in grave states); in calves there are ataxia and malfunctions of the gastrointenstinal tract; and jaundice in swine and dogs. Multiple hemorrhages constitute yet another common symptom of acute aflatoxin poisoning.

LD_{50} values for some species are given in Table. 1.

Of the agricultural animals those maximum sensitive to aflatoxins include swine 3 to 12 weeks old, pregnant sows, and calves aged one to six months. Adult cattle are more resistant to aflatoxins and sheep have long been thought of as having absolute resistance to them. Of the poultry species, ducklings and turkey poults appear to be most sensitivie, pheasant nestlings somewhat less, and chickens and quails only relatively resistant.

The toxic effect of aflatoxins depends on animals' age, sex and diet. The common trend to all species is declining sensitivity with age and greater sensivity of adult males over adult females.

Table 1. LD_{50} VALUES OF AFLATOXIN B_1 FOR SOME ANIMAL SPECIES BY SINGLE INJECTION

Species	LD_{50} mg/kg body weight
Ducklings	0.34-0.56
Rabbits	0.3-0.5
Irridescent trout	0.5
Cats	0.55
Mink	0.5-0.6
Swine	0.62
Dogs	1.0
Guinea pigs	1.4-2.0
Sheep	2.0
Monkeys	2.2
Rats	
newborn	0.56
weaners	5.5
adult males	7.2
adult females	17.9
Chickens	6.5-16.5
Mice	9.0
Hamsters	10.2
Chicken embryos	0.025 μg/egg

Dietary patterns, as noted above, modify significantly the toxic impact of aflatoxins. This relates, first of all, to protein concentrations in the feeds. Decrease of proteins in the feeds has been proved to involve greater toxication of aflatoxins in piglets, chickens, rats and monkeys.

Carcinogenic Properties of Aflatoxins

Numerous studies of chronic intoxications by aflatoxins have shown that they belong to most potent hepatocarcinogens. Aflatoxins' carcinogenicity has been proved for rats (strains Fischer, Wistar and Porton, mice, ducklings, chickens, irridescent trout, salmon, mosquito fish, ferrets, dogs and monkeys).

In rats, inclusion of aflatoxin B_1 in the diet at 15 μg/kg resulted within 68 to 80 weeks in the development of hepatomas in 100 per cent of the animals which were also induced in 2 of 22 test animals by a concentration as low as 1.0 μg/kg. Dependence of the tumor incidence rate on aflatoxin B_1 contents in the ration is shown in Table 2.

Study of possible manifestations of the aflatoxin's carcinogenicity in offspring has shown that carcinomas developed in rat infants subjected to action of aflatoxin in prenatal (in uterus) or postnatal (via maternal milk) life.

Aflatoxin B_1 primarily induced hepatomas in rats, however carcinomas of the stomach, large intestine, kidneys and lungs were found in few rare cases.

Carcinogenic property is less pronounced in other aflatoxins - G_1, G_2 and B_2. Thus, the carcinogenic effect of aflatoxin B_2 on rats is hundred times weaker than of aflatoxin B_1.

In mice strong resistance to the carcinogenic effect of

Table 2. RELATIONSHIP BETWEEN CARCINOGENICITY OF AFLATOXIN B_1 IN RATS AND ITS CONCENTRATION IN RATION

Aflatoxin B_1 concentration (µg/kg ration)	Time of detection (weeks)	Incidence rate	
		hyperplastic nodes	hepatic carcinomas
0	-	1/18	0/18
1	104	7/22	2/22
5	93	5/22	1/22
15	96	13/21	4/21
50	82	15/25	20/25
100	54	12/28	28/28

the aflatoxin was discovered following its administration the animals (Table 3). By contrast, intraperitoneal administration of aflatoxin B_1 to infant mice at a dose of 1.25 µg/kg during the first seven days of life or 6 µg during three days developed hepatomas in 80 weeks after the administration.

Irridescent trout is remarkably sensitive. Addition of 0.1 g/kg of aflatoxin B_1 into its feed resulted in the development of hepatome within 20 months.

An hour's treatment of irridescent trout embryos with an aflatoxin B_1 at a concentration of 0.5 µg/ml produced hepatocarcinomas in 40 per cent of the affected fish.

Special interest goes to the evidence of a hepatocarcinogenic effect from aflatoxin exposure in primates - marmosets, African monkeys, macaques, and tupajidas (Table 3).

Other Manifestations of Biological Activity of Aflatoxins

Published reports on other toxicity indices of aflatoxins are scanty. Under experimental conditions teratogenic effect of aflatoxin B_1 was reported in hamsters, rats, chickens and Japanese grass snake (Ozyrias latipes).

Aflatoxin administered to hamsters on the eighth day of gestation induced malformations in 29.4 per cent of the fetuses. In 11.5 per cent of the mouse embryos exposed to aflatoxin on the eight day of intrauterine development, various kinds of defects were observed (cerebral hernias, anomalies in the gastro-intestinal tract). Administration of aflatoxin into the yolk-sac on the six day of incubation caused abnormalities to develop in 65 to 90 per cent of the embryos depending on the dose.

Aflatoxins B_1 and G_1 induce gene mutations in some bacteria, fungi and insects.

Aflatoxin Metabolism and Biological Mechanisms of Action

The half-life of aflatoxin B_1 in the body of different species varies from 12 to 15 hours. Aflatoxin metabolism in the liver is effected by the same microsomal enzyme systems as for other xenobiotics.

Aflatoxin B_1 is hydroxylated to form less toxic metabolites, aflatoxins M_1, B_{2a}, P_1 and Q_1, and aflatoxicol.

Table 3. CARCINOGENIC ACTION OF AFLATOXIN B_1

Species	Dose	Follow up time	Tumour incidence
Ducklings	30 µg/kg ration	14 months	8 out of 11-72%
Trout	8 µg/kg ratio	1 year	27 out of 65-40%
	4 µg/kg ratio	1 year	15%
Rhesus monkeys	100-800 mg, total	over 2 year	3 out of 42-7%
Marmosets	5.0 mg, total	2 years	2 out of 3
The Asian insectivor Tupajida	24-66 mg, total	3 years	9 out of 12-75%
Rats	100 µg/kg ratio	54-88 weeks	28 out of 28-100%
Mice	150 µg/kg ratio	80 weeks	0 out of 60
	1000 µg/kg ration	80 weeks	0 out of 30
Newborn mice	6.0 µg/g body weight (3 doses intraperitoneally)	80 weeks	16 out of 16-100%
Ferret	0.3-2.0 µg/kg	18-37 months	7 out of 9-78%

Meanwhile increasingly more evidence becomes available which has made it possible to suggest that biological activity of aflatoxins depends in large measure on the formation of epoxides, more active metabolites, during their metabolization. Moreover, it proved possible to deduce a certain relationship between toxic properties of the aflatoxins and the structure of their furfuran ring. B_1, G_1 and M_1, the most toxic of the aflatoxins, have a double bond in the terminal furan ring which may become epoxidized. In contrast, the double bond is absence in both B_2 and G_2 whose biological activity is, therefore, considerably lower. Results attained in studying biochemical potential of aflatoxins show that they are inhibitors for the synthesis of nucleic acids and protein. Although the entire molecular mechanisms of these disturbances have yet to be established. It is hypothesized that suppression of the nucleic acid synthesis is a consequence of binding aflatoxin B_1 (or its epoxide) with a DNA molecule or other chromatine component) disturbing its matrix properties. One cannot rule out a possibility of aflatoxins having a direct effect on the enzymes involved in the nucleic acid synthesis and on the structure of polysomes.

Aflatoxins Hazard for Human Health

A number of reports shows attest that acute and subacute intoxication man by aflatoxins is possible.

More frequently, acute aflatoxicosis is observed in children. All of the cases hitherto described occurred in the countries where the rates of food contamination by aflatoxins are quite high for a number of climatic and economic considerations. In Senegal and India these toxicoses were caused by peanut flour used as a protein source for treatment of kwashiorkor in children.

Reports of the epidemic outbreak of acute hepatitis in some Western Indian states in 1974 related it to the high level of corn contamination (2.5-15.6 mg/kg) by aflatoxin B_1. Interestingly, the disease rates in males were twice its incidence in females.

A study conducted in Thailand revealed that children at the age from one to thirteen years die annually by the hundreds from a disease recalling Reye's syndrome and featuring acute encephalopathy and adipose degeneration of internal organs. Post mortem examination of 23 children died of the disease revealed aflatoxins in the liver tissue (at 93 µg/kg), the contents of the stomach and intestine (at 127 µg/kg) as well as in the brain, urine, and bile.

Several reports that appeared in the past decade have attributed Reye's syndrome to aflatoxins. In 1972 aflatoxin B_1 was detected in the liver of patients in the United States, Czechoslovakia, and New Zealand. In 1977 Czechoslovakia published data on clinical examination of 27 infants and children aged from three days to eight years, who succumbed upon contracting Reye's syndrome. In the acute cases among them death came within one to 10 days as a result of coma, and in the subacute cases fibrosis and early stages of liver cirrhosis were identified. All the victims were identified as having in the liver aflatoxin B_1 at a concentration ranging from 20 to 2,760 µg/kg. The samples of powder milk abstracted for analysis were found to include aflatoxin B_1 (up to 5.4 mg/kg). The newborn cases of the disease were recognized as intrauterine aflatoxin poisonings.

In 1979 results published on examination of aflatoxin B_1 in the liver of children with Reye's syndrome showed that the toxin had been revealed at a concentration from 2.23 to 17.33 µg/kg in six out of eight patients. In two children aflatoxin was also found in the blood during the acute period of the disease.

Particularly noteworthy are results of studies concerning a possible correlation between the level of food contamination by aflatoxins and the incidence of primary liver cancer in man (Table 4).

Despite some difficulties with interpretation of currently available epidemiological data (lack of definitive records and identification of primary cancer in surveyed regions, role of other mycotoxins, vegetable alkaloids and viral infections in cancer etiology), the hazard of aflatoxins for human health is beyond question.

Ochratoxins

Ochratoxins and citrinine comprise the group of mycotoxins which predominantly affect the kidneys.

Ochratoxins were first separated from P.ochraceus in 1965. More recently, they were shown to have seven Aspergillus and six Penicillium species as their producers, while the toxins as such were discovered in wheat, barley, corn, oats, peanut, peas, coffee and other stuffs at concentrations up to 28 mg/kg. Residual quantities of ochratoxin A were detected in the liver, kidneys and fatty tissue of swine, in milk of cows receiving feeds contaminated by ochratoxin A.

By their chemical structure, ochratoxins are isocoumarins bound with L-phenylalanine.

Experiments on rats, mice, hamsters and chickens showed that ochratoxin A has teratogenic properties. Its teratogenicitic action on mice was much stronger than that of aflatoxin B_1. Following ochratoxin A injection, various abnormalities - cerebral hernias, anophthalmia and skeletal malformations - developed in 100 per cent of the fetuses on the 9 day of gestation.

No conclusive evidence has been secured to assert either mutagenicity or carcinogenicity of ochratoxins.

The current widescale interest in ochratoxins results from extensive proof of their involvement in the development of specific toxicoses among agricultural animals, namely nephropathies in swine and chicken. Epidemic nephropathies were reported from Denmark (up to 66 cases per 10,000 swine in some years) and the Nordic countries. In the kidneys of the affected swine ochratoxin A was generally revealed in concentrations ranging from 2 to 68 μg/kg.

Special attention should be paid to the hypothesis that

Table 4. INCIDENCE OF PRIMARY LIVER CANCER AGAINST CONSUMPTION OF AFLATOXIN IN FOODSTUFFS

Localization	Cancer indidence (per 10^5 population/year)	Consumption of aflatoxin (ng/kg body weight/day)
Kenya		
high above sea level	0.7	3.5
Thailand		
Songkhla	2.0	5.0
Swaziland		
high above sea level	2.2	5.1
medium-high above sea level	2.9	5.8
Swaziland		
medium-high above sea level	4.0	8.9
Kenya	4.2	10.0
Thailand		
Ratburi	6.0	45.0
Swaziland		
low above sea level	9.7	43.1
Mozambique		
Inhambane	13.0	222.4

NOTE. $\gamma = 0.9683$ ($P < 0.01$)

Table 5. DL$_{50}$ VALUES OF OCHRATOXIN A

Species	LD$_{50}$ (mg/kg body weight)	Route of administration
Chickens		
(white leghorn)	3.4	Per os
Turkey poults	5.9	Per os
Irridescent trout	4.7	Intraperitoneally
Guinea pigs		
males	8.1	Per os
females	9.1	Per os
Rats		
males	12.6	Intraperitoneally
	30.3	Per os
females	14.3	Intraperitoneally
	21.4	Per os
Female mice	22	Intrapetoneally
Quails	16.5	Per os

ochratoxin is etiologically important for causing in man a serious disease known as Balkan endemic nephropathy, which is rather common in some regions of Bulgaria, Romania and Yugoslavia. According to WHO estimates hundreds of endemic nephropathy cases have been reported from Bulgaria, around

3,000 in Bosnia, nearly 1,000 in Croatia, close to 4,000 in Serbia; 8,000 in Moravia and 3,000 in Romania. Common characteristics of the disease include prolonged chronic progression, histologically recognizable degenerative changes in renal tubules, interstitial fibrosis and hyalization of the glomeruli. One striking feature is the similarity of the clinical and pathomorphological pictures of the disease in man with the swine nephropathy caused by the ochratoxin. Studies carried out in endemic zones showed that a correlation could be established between the frequency of the ochratoxin detected in foodstuffs in endemic (12.8%) versus non-endemic (1.6%) regions, on the one hand, and the incidence of nephropathy there, on another.

The mechanism of ochratoxins action remains little-known at present. There is some evidence that RNA synthesis in the liver and kidneys is inhibited by intraperitoneal administration of ochratoxin A. Apart from this, synergism has been noted in the action of ochratoxin and citrinine on RNA synthesis. Ochratoxin A inhibits phosphorilase b activity, leading to an impaired glycogen metabolism in the liver.

Trichothecene Mycotoxins

Alimentary mycotoxicoses in man and agricultural animals can be explained by contamination of cereals by fungi of the genus Fusarium that appears every now and then at different regions in the form of "drunken bread" in the Far East and several West European countries, or as alimentary toxic aleukia in the USSR, or else as swine and cattle toxicoses caused by moldy corn in the United States.

Toxicologists know at present over 40 trichothecenes, indluding metabolites of different specimens of the genus Fusarium and several species from Myrothecium, Trichoderma and Cephalosporium. On the other hand, only four mycotoxins, namely T-2 toxin, diacetoxyscirpenol, nivalenol and desoxynivalenol (vomitoxin), are harmful as natural contaminants.

By their chemical structure, trichothecene are sesquiterpenes while their toxicity caused by epoxide present in them.

Acute poisoning by mycotoxins of this group is accompanied by lesions of the gastrointestinal tract and the cardiovascular and nervous systems. Typical signs of toxic action of the trichothecene mycotoxins involve the development of the hemorrhagic syndrome, rejection of food and vomition. Histological changes relate primarily to tissues with active cell division such as the lymphoid tissue, spleen and marrow, and resemble the changes caused by radiation, alkylating agents, puromycin and mitomycin.

Remarkably high resistance to the toxic effect of trichothecenes has been found in swine, cattle, sheep, horses, poultry and, among the laboratory animals, in cats, guinea pigs and rats. For mice, LD_{50} varies from 0.5 mg/kg, as in the case of verrucarin A, to 500 to 100 mg/kg, as for trichodermin (Table 6). For newborn mice, LD_{50} for fusarenone X amounts to 0.1 mg/kg only.

6300

The biochemical effect of the trichothecene mycotoxins is presumably a result of their inhibitory action on protein biosynthesis. All of the toxins in this group cause polysomal deaggregation, interferes with the initiation of translation (verrucarins, fusarenone X, nivalenol, T-2 and NT-2 toxins, and diacetoixyscirpenol) or with the extention and termination of translation (trichothecin, crotocin, trichodermin). It is noteworthy that the mycotoxins capable of inhibiting the initiation of translation possess far more obvious toxic properties than do the toxins which effect later stages of protein synthesis in the ribosome (Table 6). In fact, some trichothecenes can completely inhibit the activity of thiol-dependent enzymes.

Table 6. TOXIC PROPERTIES OF SOME TRICHOTHECENES AND THEIR IMPACT ON PROTEIN BIOSYNTHESIS

Mycotoxin	Type of translation inhibition	LD_{50} for mica (mg/kg)
Verrucarins	Initiation	0.5-0.75
Fusarenone X	-"-	3.3
Nivalenol	-"-	4.1
T-2 toxin	-"-	5.2
NT-2 toxin	-"-	9.0
Diacetoxyscirpenol	-"-	23.0
Trichothecin	Elongation and termination	250.0
Trichodermol	-"-	-
Trichodermin	-"-	500-1000
Crotocin	-"-	500
Crotocol	-"-	-

Zearalenone

Many of the fungi genus Fusarium, besides the trichothecene mycotoxins, produce also zearalenone, a micotoxin that presents a serious problem to animal husbandry. As a natural component, zearalenone is found in corn wheat, oats, barley and millet as well as in a variety of feeds where it occurs in substantial concentrations (0.1-3000 mg/kg). Its presence there has been documented in the United States, Canada, Australia, Japan and many European countries. In addition to zearalenone α = zearalenone has been revealed in corn and oats, the latter's activity being from three to four times greater than that of zearalenone.

Structurally, zearalenone is classed with resorcyclates. Both, zearalenone and its derivatives are strong estrogens. Swine have proved to be the most sensitive among domestic animals to zearalenone. Zearalenone contamination of feeds mainly corn and barley, causes grave vulvovaginites, abortions and sterility in swine. The hyperestro genic syndrome induced by zearalenone occurs also in cattle, chicken and turkey-poults. Among the laboratory animals, sensitivity to zearalenone has been reported for rats, mice, guinea pigs and monkeys.

Zearalenone administration to pregnant rats at a dose of 1-10 mg/kg caused skeletal anomalies in 12.8 to 36.8 per cent of the fetuses. No teratogenic effect of zearalenone has been revealed in mice after its administration at a dose of 20 mg/kg.

The mechanism of biological action of zearalenone relies basically on its ability to interact with specific binding sites of estrogens in the cells of the target organs.

Zearalenone has no immediate detrimental effect on man's health, but potential risks from the toxin still need to be appreciated because of the extremely wide distribution of its producers in nature and because it is provenly capable of accumulating in livestock tissues.

Conclusion

We have considered in brief current developments in studying mycotoxins which should without doubt be classed with extremely hazardous food contaminants occurring in nature. As could be judged from the previous discussion, the mycotoxin problem has ramifications in many areas. The surging interest among experts from diverse fields of knowledge in the problem of mycotoxins can be explained: first, by irrefutable proof of their real hazard for human health; second, by their exceedingly wide distribution; and third, by the rather impressive amount of the economic damage caused by them.

Assistance of specialists from other fields will certainly contribute to a more effective solution of the problem in future.

THE ROLE OF DIET IN THE METABOLISM OF FOREIGN SUBSTANCES

G.I. Bondarev, A.N. Martinchik

Production is man's foremost field of activity, involving real health risks from exposure to alien chemical substances. The rapid development of chemical industry and the wide use of chemical compounds in various other industries, the hazards associated with the chemical pollution of the working zone and the environment in general put forward the problem of searching for the means to prevent a possible harmful biological effects of xenobiotics.

Since the metabolic processes occurring in the body are based on the conversion of nutritive substances, and the dietary pattern of largely determines the metabolic background against which a foreign substance operates, it becomes possible for a diet to produce either a prophylactic or an adverse effect on the ultimate impact of xenobiotics (A.A. Pokrovskiy, 1979). Being a complex pharmacological agent, food is itself capable of induction (enhanced activity) or inhibition of enzymatic systems involved in the metabolism of alien substances. Any change in the activity of the enzymes responsible for the metabolism of xenobiotics under the influence of food may affect their biological action and either neutralize or enhance their toxicity.

Knowledge of the basic molecular nature of the biotransformation of foreign substances in the body and the possibility of its regulation by dietary factors lies at the basis of searching for food components or their combinations having therapeutic and prophylactic properties.

Biotransformation of xenobiotics.

Two phases or groups of reactions are identified in the reaction complex causing the conversion of xenobiotics in the living organism (Table 1). Phase I of the biotransformation includes the oxidation reactions mainly proceeding in the cell's endoplasmic reticulum. The most important of them is $NADPH_2$-dependent mixed-function oxidase containing cytochrome P-450. Phase II in the biotransformation of alien substances comprises various conjugation reactions of xenobiotics or their metabolites with endogenous substrates. Biosynthetic processes underlie all the Phase II conjugation reactions, while the essence of the Phase I reactions lies in the degradation of the xenobiotic molecule, with the exeption of the reduction reaction.

The general direction of xenobiotic biotransformation reactions is their conversion from fat-soluble into less fat-soluble (more water-soluble) metabolites, so making them more easily disposable from the body via the kidneys, investines and other routes. The oxidative and other transformations of xenobiotics not infrequently give rise to metabolites more reactive and toxic than the initial substance. This development was called "bioactivation" or "toxification" and in extreme cases, when it produces a fatally hazardous metabolite, "lethal synthesis".

Metabolites more toxic than the initial substances result from the metabolism of brombenzene, chlorbenzene, paracetamol (acetaminophene), iso- and iproniaside, etc. The carcinogenic effect of some polynuclear aromatic hydrocarbons is associated with the carcinogenic metabolites - arenoxides - formed in the course of biotransformation.

TABLE I.

Principal Classes of Conversion Reactions of Alien Substances in Living Organism

PHASE I REACTIONS:

A. Oxidation:

1. $NADPH_2$-dependent mixed-function oxidase, containing cytochrome P-450.
2. Dehydrogenase reactions:
   a. oxidation of acohols,
   b. oxidation of aldehydes.
3. Oxidases (monoaminooxidases).

B. Reduction:

1. Reduction of nitro-compounds.
2. Reduction of azo-compounds.
3. Carbonylreductases.

C. Hydrolysis:

1. De-estherification.
2. De-amidization.

PHASE II REACTIONS:

A. Conjugation:

1. Formation of glucuronides.
2. Formation of glucosides.
3. Acylation:
   a. acetylation,
   b. formation of aminoacyl derivatives.
4. Conjugation with glutathione and formation of mercapturic acids.
5. Sulfoconjugation.

6309

In each specific case one has to know exactly whether the process occurring is a genuine detoxification or the metabolites are more toxic than the original chemical, because that is exactly what will determine the choice of effective ways and means of prophylaxis - either the intensification or the slowing down of the biotransformation reactions.

The formation of toxic metabolites is most probable in the case of microsomal hydroxylation to be examined below.

Cytochrome P-450-containing mixed-function monooxygenase system.

The $NADPH_2$-dependent cytochrome P-450-containing the monooxygenase system of endoplasmic reticulim (EPR) is the most essential, and also the oldest, evolutional system of xenobiotics biotransformation. EPR is a membranous system of communicating cisterns and tubules permeating the whole cell cytoplasm. Every cell of the organism, with exception of erythrocytes, has endoplasmic reticulum. During homogenization, the endoplasmic membranes break into fragments which then become morphologically closed vesicules - microsomes - comprising the microsomal fraction of the homogenate. It is in liver cell microsomes that most enzymes are localized, which catalyze the conversion of xenobiotics and are usually examined in vitro.

The role of diet in the monooxygenase activity regulation.

The most common and widely accepted methods of ascertaining the role of food components in particular physiological or pathological processes are those modelling alimentary deficiency of the food factor or its prescription as a supplement to the standard or daily requirement. This is the aspect of the problem which is going to be treated here.

Food protein.

Insufficient intake of protein, the most essential nutrition component, with food decreases the concentration of cytochrome P-450 and P-450-dependent hydroxylase activity in the liver. The reduced activity of the monooxygenase system is an immediate causative factor increasing the toxicity of a large number of chemicals, among them pharmacological agents, to human beings and animals receiving a protein-deficient diet. Protein malnutrition often occurs in the developing countries. In the developed countries this state accompanies some pathological processes, e.g. chronic alcoholism, drug addiction, neurologic disoders, tumours, etc. It should be stressed that in these cases the dosaging of remedies should account for the decreased biotransformation function of endoplasmic reticulum with respect to medicines.

On the other hand, food containing excessive proteins, for example, 30 to 50 per cent of the daily calory content also adversely affects the ability of hydroxylase potential to be induced by phenobarbital. The induction potential is at a maximum when a protein content in the food equals to 10-20 per cent of the total calorific value of food. In a normal human diet the protein content lies within the same limits, on the average 12 per cent. However, besides, the quantity of protein, its quality is important, i.e. the balance of replaceable and essential aminoacids.

This dependence of the microsomal monooxygenase activity upon the protein content in food is clearly demonstrable in the case of xenobiotics bioactivation. A number of xenobiotics, industrial toxicants among them proved to be less toxic following a reduction of proteins in animal feed. A similar toxicity decline has been noted for carbon tetrachloride, carbon disulphide, heptachloran and octamethyl pyrophosphamide. If the products of xenobiotic metabolism are more toxic than the initial substance, protein insufficiency, because of the low activity of microsomal menooxygenases, will result in a smaller amount of toxic metabolites being formed. But if the initial substance is converted into less toxic metabolites, the process of detoxification will be more effective when the amount of proteins is adequate and weakened when it is inadequate. By way of example, for the capthane insecticide, its toxicity to rats that received no protein in their feed for twenty-eight days increases by a factor of 2,100! The toxicity of such widely used common drugs as aspirin (acetysalicylic acid) and phenacetin increases markedly with protein deficiency in the diet. Although the phenomenon of bioactivation is inherent not in the aforementioned four compounds but is characteristic of other xenoboitics as well (brombenzene, chlorobenzene, paracetamol, ipronisside, etc.) one cannot be absolutely sure, without experimental checking, that their toxicity will decrese with low-protein nutrition. Multifarious manifestations of dietary protein deficiency versus the biological impact of chemicals upon the organism do not permit an a priori answer to this question.

Lipids.

Nor does anyone question the importance of food fats for the normal functioning of the microsomal hydroxylases. As well as proteins, fats from the EPR membranes in many ways determine their properties and the catalytic potential of the membrane-bound enzymes.

Lipids account for 30 to 55 per cent in the dry mass of endoplasmic reticulum membranes. Phosphatidyl choline is a necessary component of cytochrome P-450 lipid environment. The absence of phosphatidyl choline results in cytochrom loosing its activity. The induction of the monooxygenase system by phenobarbital tends to increase the content of both the protein and the phospholipids, cholesterol and triglycerides in the microsomal membrane.

Endogenous lipids, like steroids or fatty acids, can interact with cytochrome P-450 to "occupy" the binding sites of foreign substances on cytochrome P-450, thus competing with them for the active sites. For the normal microsomal metabolism of xenobiotics essential polyunsaturated fatty acids are primarily required. A linoletic acid-deficient diet decreases considerably the activity of majour monooxygenases of the cell endoplasmic reticulum in rats. Conversely, with the addition of corn oil to rat feed, the microsomal monooxygenase activity increases, and the concentrations of linoleic and arachnoidonic acids in the microsomal membranes increase. From the hygienic of view, this is indicative of the importance of fats with a high content of polyunsaturated fatty acids, which are abundant in vegetable oils, in the human diet.

A diet containing oxidized fats and steroids induces monooxygenase reactions. Thus, corn oil oxidized by prolonged heating intensifies the transformation of 2-acetyaminofluorene into its carcinogenic metabolite, N-oxyacetylaminofluorene, in rats, thereby making the initial compound more carcinogenic. This example is yet another testimony to the pehnomenon of bioactivation, or enhanced carcinogeneity of the initial chemical in which food is by no means a minor factor.

With respect to the specific role of phosphatidyl choline in preserving the monooxygenase system of the xenobiotic metabolism functionally active, it has been found in our laboratory that the activity of certain monooxygenases and the concentration of cytochrome P-450 in rat liver are increased considerably by a combination of lipotropic factors. The complex included methionine, vitamin B_{12} and foleic acid, i.e. essential components for the metabolism and transport of methyl groups. When these essential nutrients were no longer present in the rats' diet, the activity of microsomal monooxygenases fell. The impact of the lipotropic complex can be mainly explained in terms of an increasing level of phosphatidyl choline and other phospholipids. Choline produces the same effect.

Carbohydrates

Carbohydrates (sugars) have a less demonstrative part to play in controlling the activity of microsomal enzymes. When oxidized via the pentosophosphate cycle, carbohydrates generate $NADPH_2$, the principal donor of reducing equivalents, necessary for the reaction of hydroxylation, as well as for the microsomal reduction of xenobiotics. The importance of carbohydrates as donors of glucuronic acid in the conjugation of xenobiotics and their metabolites will be examined below.

Vitamins and microelements.

Some vitamins and microelements are involved in the reactions of the microsomal metabolism of xenobiotics as co-factors within enzymatic systems. For example, riboflavin (vitamin B_2) in the form of flavinadenin nucleotide (FAD) enters into flavoprotein, an intermediate electron carrier from $NADPH_2$ to cytochrome P-450. Iron is a component of heme, itself a part of cytochrome P-450. Nicotinic acid (vitamin PP) forms $NADPH_2$. Experiments on animals have shown that, when there is no vitamin B_2 or iron in the rats' diet, the monooxygenase activity drops appreciably and, conversely, inclusion of vitamin B_2 and iron brings the enzyme activity back to normal. With insufficient magnesium, which is necessary for hydroxylation reactions, the enzymes activity also decreases. Although the direct involvment of ascorbic acid (vitamin C) in hydroxylation has not been positively established, this vitamin does, nontheless, alleviate the toxic effect of many xenobiotics: acetanilide, DDT, lindane, dieldrin, chlordane, hexachlorophene, benzene, phenol, and others. In scorbutic guinea pigs the microsomal enzymes activity and cytochrome P-450 content are noticeably reduced, while the prescription of vitamin C restores both these functions to normal.

Vitamin E plays a unique role in protecting living organisms against the effects of foreign substances. Biological action of some chemicals results from their ability to stimulate peroxidation of lipids in liver cells. The free radicals of fatty acids, thus formed, damage the biological membranes. By virtue of its being a most potent natural antioxidant, a substance neutralizing free-radical products, vitamin E protects cellular membranes from damage by lipid peroxidation products.

The foregoing makes it obvious that an inadequate intake of some essential nutritional factor with food disturbs to a certain extent the function of the microsomal monooxygenase system, decreasing the metabolic rate of foreign substances. Adequate and balanced ingestion of all the essential factors of nutrition is an indispesable condition for the normal functioning of microsomal oxidases with a mixed function.

The conugation mechanisms of the detoxication of foreign substances and diet.

Conugation is a biosynthetic process whereby foreign substances or their metabolities, formed during microsomal oxidation, produce complexes with readily accessible endogenous substrates, such as glucuronic acid, glutathione, acetyl, methyl, glycine, and sulphate. As a result of conjugation the molecule of a chemical substance becomes less lipid-soluble and more water-soluble and, therefore, more readily excretable from the body in faeces and urine. The conjugats of foreign substances are generally non-toxic for the organism and, from this point of view, conjugation represents a true detoxication process.

Conjugation with glucuronic acid is catalyzed by the endoplasmic reticulum enzyme, UDP-glucuronyl transferase.

Glycogen and glucose serve as a source of glucuronic acid (GA). The reaction of glucuron conjugation, in common with other types of conjugation, is based on biosynthetic mechanisms. Consequently, these reactions require heat for the synthesis or activation of the conjugating agent. On the whole, conjugation processes are more sensitive to energy exchange disturbances than the hydroxylation reaction. One of the major factors controlling the glucuron conjugation is the presence of a pool of glycogen in the liver, to provide GA formation. The substances forming conjugates with GA in the body tend to decrease the glycogen pool in the liver, which may impair the functioning of the other organs and systems. General fasting responsible for depletion of glycogen stored in the liver greatly interfered with the synthesis of glucuronides of foreign substances and endogenous substrates (bilirubin).

Let us now examine yet another type of the conjugation of foreign substances, namely, conjugation with glutathione (GSH). Conjugation with GSH constitutes the most important detoxication pathway for the compounds containing electrophilic groups. These are oxides, sulfoxides, halogen-containing aromatic and aliphatic hydrocarbons, alkylnitrites, diphenyl ethers, benzo(a) pyrene and other polycyclic aromatic hydrocarbons.

Glutathione represents a tripeptide, γ-glutamyl cysteinyl glycine. Conjugation with glutathione is catalyzed by glutathione-S-transferase (G-S-T), a sell-sap enzyme of the liver. So far, several forms of the enzyme have been discovered, differing in physico-chemical properties and substrate specificty.

Glutathione conjugates udergo complex biochemical conversions of the glutathione radical with subsequent acetylation and transformation into mercapturic acids excretable in urine and bile.

Of special interest, from the viewpoint of nutrition science, is the increased expediture (elimination with mercapturic acids) of the aminoacids cystein as a result of xenobiotics conjugation with glutathione. Insufficient cysteine or methonine intake with food constrains glutathione formation in adequate amounts, thus disturbing the detoxication of chemical agents via their conjugation with glutatione. It is well-known that glutathione insuffiency raises the liver toxicity of some industrial chemicals and drugs (brom-benzene, paracetamol, styrenes). Under the impact of such compounds GSH concentrations in liver decreases. Prescription of additional cysteine raises the glutathione level and protects the liver from damage.

The above data show that conjugation processes are accompanied by the consumption of some nutrients such as aminoacid cystein in the case of conjugation with glutathion and carbohydrates in the case of conjugation with GA. Antidotal properties of food and, on the other hand, antialimentary effects of a whole number of foreign substances manifest themselves in conjugation processes.

There are many ways for food and individual food components to affect the biotransformation of foreign substances, thereby raising the resistance of the organism to the harmful effects of xenobiotics.

The general principles of dietotherapy and dietoprophylaxis in the case of intoxication were formulated by A.A. Pokrovsky (1971). In brief, they are: 1/ utilizing the antidotal properties of individual food components; 2/ accelerating or decelerating the metabolism of toxicants, depending on the toxicity of initial substances or their metabolites; 3/ speeding up the elimination of toxic substances from the body; 4/ slowing down the absorption of toxic substances in the gastrointestinal tract; 5/ raising the general resistance of the organism; 6/ acting on the most vulnerable organs with specific nutrients; 7/ compensating for an increased expenditure of alimentary and biologically active substances during intoxication; 8/ using the desensibilizing properties of food components; 9/ using the antioxidizing properties of nutrients.

Dietotherapy and dietoprophylaxis at industrial enterprises in the USSR.

Clause 64 of the Fundamentals of Labor Legislation of the

USSR and the Union Republics states that therapeutic-prophylactic nutrition is to be provided free of charge to those working in especially harmful conditions, in accordance with the established norms. The issue of the such diets is regulated by Decree 4/II-I of January 7, 1977 passed by the State Committee of the USSR Council of Ministers for Labour and Social Problems and the Presidium of the Central Council of Soviet Trade Unions. It incorporates the List of industries, occupations and jobs which entitle the employees to receive free-of-charge therapeutic-prophylactic diets in view of especially hazardous working conditions; the diets, rates of distribution of vitamins, and rules for the provision of therapeutic-prophylactic diets.

Depending on the type of occupational hazard, the food is distributed as one of the six established diets. Diet I is intended for those occupationally exposed to X-rays and radioactive substances; diet 2 for those handling inorganic concentrated acids, alkali metals chlorine and its inorganic compounds, fluorine and its inorganic compounds, cyanides, phosgene, etc.; diet 3 for those working with lead and its inorganic components; diet 4 is to prevent intoxications with nitro- and aminocompounds of benzene and its homologs, chlorinated hydrocarbons, compounds of arsenic, tellurium, selenium, phosphorus, silicon, etc.; diet 5 for those exposed to mercury and its inorganic compounds, tetraethyl lead, brominated hydrocarbons, carbon disulphide, thiophos, compounds of manganese, beryllium, barium, etc.; diet 2a caters for those working with chemical allergenes, notably chromium and its compounds.

6300

The established amounts of food products in every diet have a definite therapeutic and prophilactic implication, that is why substitution of food products in the diet is only permitted in exceptional cases and strictly within the interchangeability norms approved by the USSR Ministry of Public Health (April 7, 1961). Current recomendations advise against incorporating into the diets fat kinds of meat, fish, corned beef, salted and smoked foods, as well as the substitution by them of any foods included in the diets.

All of the therapeutic and prophylactic diets are supplemented with vitamins: 150 mg of vitamin C in diets 1 and 3; 2 mg of vitamin A and 100 mg of vitamin C in diet 2 for those working with alkaline metals, chlorine and its inorganic derivates, cyanides and nitrogen oxides; 2 mg of vitamin A and 150 mg of vitamin C for those working with fluorides; 100 mg of vitamin C for those working with phosgene; 100 mg of vitamin C, 2 mg of vitamin A, 15 mg of vitamin PP and 25 mg of vitamin U with diet 2 a; 150 mg of vitamin C with diet 4, plus 4 mg of vitamin B_1 for those working with arsenic, phosphorus, mercury and tellurium compounds; 150 mg of vitamin C and 4 mg of vitamin B_1 with diet 5.

The therapeutic and prophylactic diets are given hot breaffasts or lunches before the work shift.

Advances in the biochemistry of foreign substances and nutrition science make it possible today, on the strength of the presently available precise data on the modes of conversion of foreign substances in the body, to speed up the detoxication and elimination of extraneous substances. Certain hopes are placed on the research into the antidotal properties of some nutrients.

It now becomes possible to use certain food components in controlling the processes of absorption and elimination of toxic substances from the body. This possibility has already been implemented in preventing lead poisoning. Lead compounds are removed from the body through the intestines. In this respect the rise of pectins, forming complex compounds with lead and preventing its absorption in the intestine deserves attention. Pectins are obtained from fruits, berries and vegetables. In 1970, the USSR Ministry of Public Health recommended giving 2 g of pectin in the form of pectin-enriched canned vegetable foods to workers exposed to the action of lead.

x x x

The fundamental molecular nature of the processes affording protection to the internal media of the organism, specific metabolic pathways of individual chemical substances and their groups, and the possible ways in which a diet can affect the metabolism of xenobiotics have been examined.

REFERENCES

1. Archakov A.I. Microsomal Oxidation, "Nauka", 1975 (in Russian).
2. Bondarev G.I., Vissarionova V.Ya. A diet as a factor of preventing occupational diseases. Vopr. pitaniya, 1980, No. 6, 6-9 (in Russian).
3. Bondarev G.I., Martinchik A.N. Diets against alien substances. Khimiya i zhizn, 1983, No. 3, 43-47 (in Russian).
4. Park Dennis W. Biochemistry of Alien Compounds. "Meditsina", 1973 (transl. from English).
5. Pokrovsky A.A. Metabolic Aspects of the Pharmacology and Toxicology of Food. "Meditsina", 1979 (in Russian).
6. Petrovsky A.A. (Ed.) Scientific and Technological Forecast on the Problem "The Principal Directions of Research to Obtain High-Quality Food Products Based on the Scientific Achievements in Agriculture, Biology, Chemistry, Biochemistry and Microbiology", Moscow, 1971, v. I (in Russian).
7. Aitio A. Conjugation Reactions in Drug Biotransformation. Amsterdam, 1978.
8. Campbell T.C., Hayes J.R., Merril A.H., Maso M., Goetchius M. The influence of dietary factors on drug metabolism in animals. Drug Met. Revs., 1979, 9, 2, 173-184.
9. Gillette J.R. Effects of induction of cytochrome P-450 enzymes on the concentration of foreign compounds and their metabolites and on toxicological effects of these compounds. Drug Met. Revs., 1979, 10, 1, 59-87.
10. Thurman R.G., Kauffman F.C. Factors regulating drug metabolism in intact hepatocytes. Pharmacol. Rev., 1979, 31, 4, 229-251.

Chapter 1V

PREVENTIVE TOXICOLOGY OF AGRICULTURAL CHEMICALS

Edited by professor Yu.S.Kagan

CHEMISTRY OF MODERN PESTICIDES AND TRENDS IN SEARCHING FOR NEW PESTICIDES

S. S. Kukalenko

One of the reserves for increasing the total outputs of agricultural products lies in eliminating crop losses to pests, diseases, and weeds. With this in view, a whole range of measures involving application of agrotechnical, quarantine, mechanical, biological, and chemical methods of plant protection is applied.

In terms of scope and efficiency, the chemical methods of crop protection hold a leading position in the total system of measures aimed at controlling pests, diseases, and weeds, and chemicals used for crop protection (pesticides) have come into prominence in agriculture and allied sciences. Such chemicals are produced and applied on an ever increasing scale, while investigations in this important field become more extended. Suffice it to say that nowadays, the world production of pesticides exceeds 2 million tons (active ingrediants) which is an increase of over 70 per cent as compared to 1970. It should be noted that recent years have seen a trend toward reducing pesticide production in some countries in favour of preparations whose per hectar application rates are low: between 10 and 100 grams. More than 1000 chemical compounds are now classified as pesticides, while the number of forms in which they are applied in some countries is in excess of 10,000.

Pesticides are being studied by scientists of various specialities including chemists (technologists, analysts, physical chemists and those specialising in chemical synthesis), biologists, entomologists, plant physiologists, phytopathologists, veterinarians, toxicologists, agronomists, physicians, specialists in agricultural machinery and others. Over 15,000 scientific reports and patents on pesticides are published annually; and all despite the constant growing stringency of standards with regard to pesticides in modern times new ones make their appearance each year. It is not accidental that pesticides are so much in the focus of attention - application of various chemical preparations in agriculture makes it possible to preserve no less than one-third of the potential yield and significantly reduce labour costs involved in crop cultivation.

International experience goes to show that if there is no systematic control of crop pests and diseases, it is possible, at best, to harvest no more than 37 per cent of the potential potato yield, 22 per cent of cabbage, 10 per cent of apple, 9 per cent of peach yield, etc. About two thousand years ago, there were more than 500 diverse types of animals known to ancient Greeks. Nowadays, the types of animals and microorganisms known to man exceed two million. As many as 70 thousand types of insects and mites attack all parts of crop plants during the vegetation period and in storage; out of this number, close to 10 thousand types inflict a substantial economic damage.

According to the data supplied by specialists, the number of various plant diseases which affect crop plants and fruits continually or periodically is about 30 thousand. Weedy plants are no less detrimental to agriculture: they deprive cultivated plants of moisture and nutrients available in soil, shade them and hinder their normal growth, infest the gathered grain with seeds poisonous for humans and animals, etc. In some cases, the yield may be lost completely.

On the basis of generalized data on the distribution of pests throughout the globe and the damage they inflict the scientists estimated in the early 1970s that, while the total annual world crop cost 140 billion dollars, pests, diseases, and weeds caused an estimated annual loss of 75 billion dollars, given the then level of pesticide consumption.

Insect pests and microorganisms destroying wood, paper, cotton, wool, and silk products, as well as gums, rubber, plastics, and leather inflict a considerable damage. In the tropics even optical glass can be subject to spoilage. Insect pests, mites, and other parasites are a serious hazard to livestock breeding, for many of them not only transmit infectious diseases but spoil the hide of the animal, disturb it, which results in reduced milk yields and weight gains. A serious threat to human health is constituted by insect pests, mites, and spiders recognized as infection vectors (encephalitis, typhus, relapsing fever, sleeping sickness, elephantiasis, and many other). Until recently, more than 100 million people the world over were infected by malaria, and, at present, in some areas of the globe, this disease kills millions of people yearly. What aggrevates the threat is a rapid reproduction of insects. To illustrate, within the season seven generations of the housefly can produce $3.5 \cdot 10^{12}$ individuals, whereas twenty generations of aphid can produce 35^{20} individual. A high mortality of these insects prevents indefinite increase in their populations in nature.

Plant protection chemicals (pesticides) employed for the control of pests and weeds are divided into the following major groups according to their application pattern.

Insecticides - to control insect pests. Individual groups of insecticides are designated more specifically (e.g., aphicides - preparations to control aphids).

Acaricides - to control mites, ovicides - to eradicate eggs of insect pests.

Nematocides - to control round worms (nematodes).

Limacides and molluscicides - to control various mollusca, including gastropoda.

Fungicides - to control plant diseases and various fungi.

Bactericides - to control bacteria and bacterial plant diseases.

Antiseptics - to protect non-metal materials against destruction by microorganisms.

Rodent poisons or rodenticides - to control rodents.

Herbicides - to control weeds.

Arboricides - to eradicate undesirable trees and shrubs.

Algaecides - to eradicate algae and other aquatic vegetation.

Pesticides also comprise chemicals intended to stimulate and retard plant growth (regulators), preparations to remove leaves (defoliants) and dry off the plants (desiccants) with a view to mechanizing labour-intensive harvesting of cotton, soy-beans, potatoes and many other crops; preparations for repelling (repellants), attracting (attractants), sterilizing (sterilizers) insects, and aphidants for retarding nutrition of insects.

Herbicides, insecticides and fungicides are employed most extensively in agriculture and other sectors of economy.

Pesticides are classified according to the method of penetration and type of action, as stomach poisons, contact poisons, and fumigants. This classification enables one to assess poison penetration and, therefore, poison application techniques.

Stomach insecticides bring about poisoning of insect pests through contact with integument.

Fumigants are chemical compounds which enter the bodies of insects and animals in the form of gas or steam through the airway. Such fumigants as acaricides poison pest insects and mites when passing through respiratory organs.

The above classification is somewhat conventional, for many pesticides possess stomach, contact and fumigant action. For instance, hexachloran, heptachlor, and other act more than one way.

Additionally, all pesticides fall into two major groups: contact and systemic poisons. Contact pesticides are those that kill or inhibit pests when applied directly to them. Systemic pesticides are those that are capable of entering plants, moving in their tissues and destroying harmful organisms (weeds, pathogens, pests).

According to the type of action, herbicides are classified as selective and nonselective chemicals.

By the chemical composition, pesticides are divided into the following three main groups:

- inorganic compounds (compounds of mercury, copper, boron, fluorine, barium, sulfur, as well as chlorates);

- pesticides obtained from plants, bacteria and fungi (pyrethrins, antibiotics, and phytoncides);
- organic compounds which are the most up-to-date pesticides (organic chlorine-, phosphorus compounds, derivatives of carbamic, thio-, and dithiocarbamic acids, diverse heterocyclic compounds, phthalamides, urea derivatives, phenol nitroderivatives, quinones, triazine derivatives, metalloorganic compounds, mineral oils, etc.).

Chemical compounds have been used for the control of pests and plant diseases from time immemorial. Use of sulfur to control mold was first mentioned by the outstanding Greek poet Homer in his epics "Iliad" and "Odyssey" written as early as a thousand years B.C. In 470 B.C., Democritus, famous Greek philosopher, suggested that plants should be sprayed with pure olive oil to prevent rots. In the 13th-16th centuries gardeners made sporadic attempts to obtain fruits of better aroma, taste, and colour, as well as to treat plants by inserting, under the cortex or into the stem pith, various species, making infusions of medicinal herbs and dyes, and sometimes arsenic to obtain poisonous fruits.

However, these experiments were accidental and empirical by their nature because at that time nothing was known about translocation or absorption of solutes in plants. From 1600 and up to the early 19th century British farmers resorted regularly to wetting wheat seeds in sea water for the smut control; common salt was used for that purpose somewhat later. In the 18th century with the development of Carl Linnaeus's species taxonomy, and with the discovery by Hulse in 1726 of the plant physiology theory, in particular, translocation of sap in plants the scientists became interested in pathogenic fungus classification. In that period attempts were made to treat some infected plants by injecting mineral salts and other substances. In 1705 Homberg was the first to recommend the employment of mercuric chloride as a wood antiseptic.

In 1885, in France, A. Millardet, managed to encourage, by means of an effective advertizing, a wide application of Bordeaux mixture for spraying grape vines to control mildew.

At the same period of time, solutions of sulfur and lime were used to combat down mildew in citrus.

By 1932 the development of such inorganic pesticides as copper-, sulfur-, and mercury- bearing compounds had reached its peak and signified the efflorence of the age of inorganic pesticides. However, by that time the following disadvantages related to the use of inorganic preparations had been formed: a narrow area of action on microorganisms, phytotoxicity to some plants, high toxicity to warm-blooded animals, especially with regard to mercury compounds. In this connection, as well as with regard to the advances made in the development of organic chemistry, intensive studies aimed at searching for new pesticides from among organic compounds.

A genuine revolution in chemical protection of crops was brought about, in the early 1940s, with the advent of contact insecticides belonging to the group of chlorinated hydrocarbons (DDT, HCCH, Aldrin, etc.) which are distinguished by a broad spectrum of action, high activity and low production cost. 1946 saw the beginning of manufacture of insectoacaricides belonging to the group of organic phosphorous compounds, and it was discovered that some of them possess systemic activity and show sufficient selectivity.

In 1942 there were reported findings of first field tests of dithiocarbamic acid (Ferbam) derivatives according to which those substantives can be used as effective fungicides.

Modern pesticides come under different classes of organic compounds.

The efficient utilisation of pesticides in controlling pests, plant diseases, and weeds depends, to a large measure, on the formulation of a chemical compound and the conditions under which it gets in contact with the foregoing targets. Various properties of compounds used as pesticides and the variety of their targets necessiate numerous formulations.

The selection of the formulation which may be the most efficient and economic under specific conditions is governed by the physico-chemical properties of the active principle of a preparation, its purpose and use pattern. The selected formulation determines, to a great extent, the technology used in producing the preparation.

Common formulations include wettable powders, emulsifiable concentrates, water suspension concentrates, pastes, oil solutions, tablets, granules, microcapsules, and dusts.

Wettable powders. When diluted with water, these powder-like preparations form sufficiently stable suspensions used for plant spraying.

Pastes. In terms of their properties, the wettable powders stand close to pastes which, apart from accessory agents, contain small amounts of organic solvent and water. To produce stable pastes, use is made of high-molecular water-soluble protective colloids (methyl-, and carboxymethyl cellulose). In paste production the components are emulsified in the colloidal mill at elevated temperatures.

Emulsifiable concentrates. These are pesticide-in-oil solutions in the form of droplets coated by surface-active substances ПАВ. When diluted in water, they form stable emulsions which do not laminate for a long time.

Use has been recently made of the so-called reversed emulsions wherein a pesticide dissolved in water is the dispersion phase, while oil is the dispersion medium. In this case, the pesticide aqueous solution droplets appear to be built into larger oil drops. Reversed emulsions are used for low-volume spraying; they ensure better adherence to plants and do not evaporate.

Extensive use has been made of pesticides dissolved in organic solvents for low-volume fine dispersion spraying to protect non-metallic materials against destructive attacks of microorganisms, and to disinfect premises.

Granular formulations. Applications of granular formulations are being used more and often as substitutes for powdered ones. Granular formulations can be prepared by impregnating prefabricated granules or such minerals as perlite and vermiculite, with liquid pesticides or their solutions, or by means of granulating powder-like preparations on appropriate carrier with subsequent dispersion.

Macrocapsulated formulations. These preparations consist of 5-100 capsules containing active ingredient whose shell is quickly destroyed under the impact of a particular substance, the sunlight, mechanical action or heating.

Dusts. These are fine mixtures of active ingredient and inert material - carrier - with a particle size of 3-30, except for some inorganic preparations containing no carrier, and consisting solely of active ingredient, e.g., ground sulfur.

The preparation's active ingredient may be either a solid or a liquid of various viscosity. Talc, kaolin, or some other inert material (chalk, gypsum, kieselguhr, tripolite, etc.) are used as carriers.

The basic lines of long-term research on pesticides are as follows:

(1) develop and introduce into agricultural herbicides with small consumption rates to protect basic crops against weeds, as well as growth regulators, defoliants, and desiccants;

(2) develop and introduce into agriculture insectoacaricides including pyrethroids with small consumption rates, which make it possible to prevent diverse insect and mite pests from developing resistance;

(3) develop and introduce into agriculture fungicides, including systemic chemicals with small consumption rates to control most hazardous diseases of major crops;

(4) develop and introduce into agriculture seed-treating materials with small consumption rates applied by pelleting, hydrophobizing and other methods to protect major crops, seeds, and shoots against pests inhabiting soil;

(5) develop and introduce into agriculture and forestry pheromones of insects, pests, including those of winter and cottonworm moths, clock beetles, leaf roller moths, garden webworms, and cabbage moths;

(6) carry out investigation with the aim of developing promising formulations (high-concentration wettable powders, granular and microgranular chemicals, generator-aid low-capacity sprayers and aerosols, capsulated and suspension chemicals);

(7) extend investigations in environmental protection for the manufacture of plant protection chemicals (PPC) (processing PPC production wastes ensuring maximum reuse of treated water, improving PPC production so as to significantly reduce volumes of wastes, developing efficient waste processing techniques employing such physical and physico-chemical methods as electrochemical, plasmochemical, ozonolysis, etc.).

Three factors are basically responsible for a current and future search for new pesticides:

- intensification of agricultural production requiring an ever increasing amount of pesticides;
- continuous need in new pesticides because of changes in pest populations and weed flora, disease forms, and cultivation of new crop varieties;
- necessity to search for pesticides which are less toxic to the environment and disintegrate fast

Higher production and application rates for herbicides and, correspondingly, their increased share in the pesticide consumption pattern are forecast for the period up to 1990.

Change in the relationship between individual groups of pesticides used in world agriculture
(per cent)

Pesticide group	1975	1980	1985 (forecast)	1990 (forecast)
Herbicides	41.8	46.0	50.6	55.6
Insecticides	35.4	32.6	30.5	28.2
Fungicides	18.8	17.9	15.8	13.6
Plant growth regulators	1.8	1.6	1.5	1.3
Fumigants	1.6	1.3	1.1	0.8
Others	0.6	0.6	0.5	0.5
Total	100.0	100.0	100.0	100.0

The herbicide group is characterized by a most rapid increase in consumption of triazines, carbamates and thiocarbamates, the derivatives of urea, aryloxyalkylcarboxylic acid, benzoic acid and other compounds.

The growth of production and application of organophosphorus compounds, dithiocarbamates, pyrethroids which are more selective and less toxic to man and animals, and reduced utilization of chlorohydrocarbons, arsenic compounds and other chemicals are predicted for the insecticide group.

Growth rates for the use of fungicides are predicted to be lower than those for herbicides and insecticides. Applications of dithiocarbamates, phthalimides, copper and heterocyclic compounds are expected to increase, while some countries are discontinuing the use of high-toxity mercury chemicals.

In the coming 5-10 years the following trends are expected to be prevalent in routine applications of pesticides:

- placing more stringent demands upon plant protection chemicals;
- rising in cost of research and development and prolonging the period of preliminary approbation for new types of pesticides;
- changing pesticide placement techniques so as to ensure their best utilization within the total complex of diverse protection methods (integrated plant protection methods);
- promoting pesticide-orientation of agriculture which is connected with industrial methods of crop cultivation though the recent years have seen extensive applications of integrated methods designed to control and implement agrotechnical and mechanical procedures of crop protection. The principle reason accounting for the preferential use of chemical method is that other methods cannot provide for a rapid and efficient pest control if crops are unexpectedly attacked on a wide scale.

The general requirements for new pesticides can be worded as follows:

1. Moderate persistence in environmental entities.
2. Lowest possible toxicity to man, animals, and other beneficial organisms.

- - - -

3. Relatively fast disintegration in soil, water, air and in the body of the warm-blooded anymals to form products which will be safe for humans, animals and cultivated plants.
4. Zero cumulation of the above chemicals in the body of humans, animals, birds and hydrobionts.
5. Highest efficiency in the control of noxious organisms with the lowest possible chemical consumption per unit of area under treatment.
6. Sufficiently high economic efficiency of agricultural applications.
7. No individual deleterious consequences for humans, animals and other living organisms under systematic continuous exposure to the above chemicals.
8. A possibility to alternate chemicals belonging to different classes of compounds so that noxious organisms could not form a habit to them, and the above chemicals could not cumulate in environmental entities.
9. Sufficient stability of chemicals during storage and their low aggressiveness towards apparatus designed for chemical utilization.
10. Availability of raw materials for the manufacture of chemicals and a possibility to arrange production on the required scale.

REFERENCES

1. Borisov V.M., Volfkovich S.I., Kukalenko S.S. et al. Chemization in agriculture. A handbook. Moscow, 1979.
2. Cramer H.H. Pflanzenschutz und Welternte. Leverkusen, Bayer, 1967, 523 S.
3. Wegler P. Chemie der Pflanzenschutz- und Schadlingsbekampfungsmittel. B. 1. Berlin-Haidelberg, N.Y.1970, 661 S.
4. Sevulescu A. Plant protection. Bucharest, "Meridiane", 1963.
5. Melnikov N.N. Chemistry and technology of pesticides, Moscow, 1974.
6. Proc. 8th British Insecticide Fungicide Conf. VIII, Brighton, 1975.
7. Zakharenko V.A. Economic efficiency of pesticides in plant growing. Moscow, VNIITEI-SELKHOZ, USSR, Ministry of Agriculture, 1977, pp. 1-52.
8. Kukalenko S.S., Volodkovich S.D. Systemic fungicides and seed-treating materials. NIITEKHIM, Moscow, 1981, Issue 1, pp. 1-68.
9. Kukalenko S.S. Gar K.A., Volodkovich S.D. Acaricide production and application methods. NIITEKHIM, Moscow, 1978, Issue 11, pp. 1-59.
10. Kukalenko S.S., Volodkovich S.D. et al. Chemical methods to control nematodes. Moscow, NIITEKHIM, 1977, Issue 5, pp. 3-29.
11. Kukalenko S.S., Volodkovich S.D. New systemic fungicides abroad. Khimiya v Selskom Khozyaistve, 1979, Moscow, Khimiya Publishing House, No.7, pp 30-36.
12. Kukalenko S.S., Gar K.A., Volodkovich S.D. Specific acaricides. Khimiya v Selskom Khozyaistve, 1980, Moscow, Khimiya Publishing House, No.5, pp. 29-33.

REFERENCES (continued)

13. Tikhonova R.A., Zakharenko V.A. et al. State of the art and trends in production and application of plant protection chemicals in capitalist countries. Khimicheskaya Promyshlennost za Rubezhom, 1979, Moscow, Issue 11, pp. 1-37.

GLOBAL SIGNIFICANCE OF PESTICIDES AND THEIR BIOLOGICAL IMPACT

Yu.S. Kagan

Discovery of pesticides is an outstanding achievement of modern science. Their application prevents harvest losses, caused by pests, diseases and weeds, worth billions of roubles annually. At the same time, pesticides, being biologically active substances, may affect the health of man. This is facilitated by the application of pesticides over vast areas, associated with the necessity of their universal use, by the persistence of many of them in the environment, their capability to accumulate in aqueous-nutrient chains and a possibility of their penetration into the human organism with vegetable and animal food, water and air.

Large-scale international trade in pesticides and foodstuffs treated with them creates conditions for extending the impact of pesticides onto the vast majority of the Earth's population.

Pesticides belong to the most widespread biosphere pollutants of global significance; that is why the problem of preventing their harmful effect on human health can no longer be solved by the efforts of one or even several countries. This calls for joint efforts on an international basis.

Firmly established at present are the facts of the presence of stable pesticides in various environmental objects, of their transport over great distances by air, river and sea water, of their migration from soil into plants and accumulation in vegetable and animal food /L.I. Medved and Yu.S. Kagan, 1966; L.I. Medved, 1973; E.I. Spynu, 1976; Yu.A. Kuchak, 1976/. The regularities underlying the behaviour of pesticides in the environment are primarily connected with their physical and chemical properties, namely: resistance to physical and chemical action, solubility in water and organic solvents, the rate and peculiarities of their metabolism in water, soil, vegetable and animal organisms.Large-scale application of stable chemicals in agriculture is obviously inadmissible. Examples of such substances are certain chlororganic hydrocarbons (DDT, dildrin, aldrin, etc.). Apart from high stability, these substances are characterized by their ability to concentrate in biological chains. Due to a high distribution coefficient their concentration in foodstuffs of animal origin is usually higher than in the plants eaten by animals. The presence of DDT in the human body has been proved on a global scale.

Of great importance in predicting the toxic action, distribution in the organism, penetration through cellular and intracellular membranes is the determination of oil/water partition coefficients. High partition coefficients are associated with the ability of substances to be easily absorbed through the skin, penetrate through the hematoencephalic barrier into the central nervous system and damage intracellular formations /Yu.I. Kundiyev, 1975/.

In recent years an attempt has been made at tracing the interrelation between electron density distribution in the molecules of certain phosphororganic pesticides and their anticholinesterase properties. The physiological activity of compounds is in this case greatly influenced by steric factors. This type of research is to be developed further (Yu.S. Kagan, 1981/.

Pesticides penetrating into the organism by different routes (through the mouth, respiratory organs, skin) may cause acute and chronic poisoning. Even before 1968 more than 34 thousand cases of acute pesticide poisoning had been described in the world literature /Pol'chenko, 1968/.

Cases of pesticide poisoning have been recorded on every continent. According to foreign literature, acute poisonings in Asia account for 44.3% (out of their total number), in America - 42.6%, in Europe - 10%, in Africa - 2.8%, in Australia and Oceania - 0.3%. It is of interest to note that Europe occupies the first place in pesticide consumption, while it is Asia and America who suffer most from pesticide poisoning.

Among the various chemical classes of pesticides causing acute poisoning the first place belongs to phosphororganic preparations (73.4%), then come chlororganic compounds (12.6%), arsenic-containing preparations (6.1%), zinc phosphide (0.9%), etc. Among the phosphororganic compounds, thiophos and other strong and highly toxic preparations have been responsible for most of the poisonings.

V.I. Pol'chenko (1973) found 86% of all the cases of acute poisoning to have been caused by strong toxic substances (LD_{50} below 50 mg/kg), 6% by highly toxic substances (LD_{50} 50-200 mg/kg), 2% by medium-toxicity preparations (LD_{50} 200-1000 mg/kg) and about 6% by the action of low-toxicity substances (LD_{50} over 1000 mg/kg). Analysis of these data confirms the conclusion that, as a rule, strong toxic substances must not be used in agricultural practice. In recent years it has become possible to reduce the hazard of some substances by imparting a new shape to them (pellets, micropellets), the use of which decreases the probability of affecting humans.

6300

21-2

Assessing the capability of pesticides to cause acute poisoning one should consider their toxicity not only when administered perorally , but also via other routes (skin, respiratory tracts).

The problem of percutaneous absorption of poison has numerous aspects: its dependence on the structure and the physicochemical properties of compounds, absorption mechanisms and pathways, pesticide permeability through different skin areas, the methods of studying skin-resorptive toxicity, etc. These questions are discussed at length in Yu.I. Kundiyev's monograph /1957/.

Among pesticides, extremely dangerous, as regards inhalation poisoning with vapours, are fumigants (prussic acid salts, methyl bromide, etc.). Most pesticides belong to the group of moderate- and low-hazard ones as their volatility is comparatively low, though there are exceptions to the rule (for example, high volatility and toxicity of dimethyl dichlorovinylphosphate - DDVP). However, when assessing the danger of inhalation poisoning, two circumstances should be taken into consideration. Even a relatively low volatility of numerous pesticides may lead to the formation of their vapours in the air in concentrations large enough to cause chronic poisoning.

The second thing is that many pesticides appear in the air in the form of the drop-liquid phase of aerosol or as dust. Depending on the aerosol particle size, pesticides can penetrate into various parts of respiratory tracts (bronchi, bronchioles, alveoles), be absorbed from there into the blood and produce a toxic effect.

An increasing number of recent publications have revealed that acute and chronic poisonings are not the only possible result of the unfavourable effect of chemical agents on human health.

Despite the continuous growth of the number of acute and chronic poisonings in many countries, attention of health experts is mainly focussed at present on the attempts to trace the relationship between the effect of chemical compounds and certain pathological conditions which disagree with the generally accepted concept of intoxication. Among these conditions of greatest importance are allergic diseases, hemopathies, disturbances of liver and sexual glands function, tumors, deformities, hereditary diseases.

The number of allergic diseases has sharply increased in the last decade. They often affect the most efficient part of the population. Their course is, as a rule, prolonged, recurring and frequently resulting in a steady loss of working capacity, which determines the great social impact of this type of biological effect of chemicals. There is no doubt that chemical compounds play an important role among the agents causing allergic effect on the organism.

In some cases this relationship can be traced directly (paraphenylendiamine, 2,4-dinitrochlorbenzene, isocyanates, numerous chlororganic and carbamide pesticides, medicinal substances). In other cases there exist either only assumptions or isolated observations of the allergic effect of compounds. Some chlororganic pesticides (DDT, etc.) have been classified by the World Health Organization among the substances capable of causing agranulocytosis and aplastic anemia. There has been described the appearance of hemolytic anemia after work with a mixture of heptachlore, dildrin and toxaphene, as well as due to the effect of cyneb fungicide. Porphyria was observed as a result of intoxication with hexachlorobenzene. A relationship was observed to exist between the growing number of hepatites and the effect of chlororganic pesticides, between the frequency of occurrence of bronchial asthma attacks and the use of phosphororganic compounds. It is quite probable that an important role in the progress of some pathological processes is played by the effect of pesticides on the immune reactivity and their participation in the mechanism of the organism's allergic restructuring.

Non-specific effects of some chemical substances are well known. A number of investigations have been directly devoted to pesticides. Pesticides with a differing mechanism of action (DDT, sevin, chlorophos) were found to affect immunity in doses producing no visible signs of intoxication. The phagocytic and adhesive properties of leukocytes change in this case, and their phosphotase activity declines. It has been established that certain herbicides (2,4 D, diuron and their combinations) suppress immunity. Experiments on white rats showed derivatives of carbamic, thio- and dithiocarbamic acids (carbine, eptam, maneb) to affect the immunological parameters and slightly enhance the susceptibility of rats to infection with a pathogenic culture of colon bacillus.

Allergic dermatites and eczemas would develop following a contact with DDT, hexachloran, carbine, cyneb, maneb, carbophos and thiophos. Besides retarded allergic reactions, have also been described reactions of an immediate type in the form of bronchial asthma, Quincke's edema.

Clororganic, phosphororganic, mercury-organic compounds, carbamates, cyanamide and fluorides are among those causing the appearance of chemical allergoses. Chemical agents may be semihaptenes and haptenes; in the organism they become antigens acquiring the ability to cause the formation of antibodies /I.E. Kovalev, O.Yu. Polevaya, 1981/.

Since the immune reaction is a major protective mechanism in the organism, it is quite natural that it can also occur in response to substances carrying genetically alien information.

Development of an allergic condition is one of the possible reactions to the intrusion of a chemical agent into immune processes. The reported cases, indicating that toxic substances may contribute to the progress of autoimmune processes, are constantly increasing in number. The autoimmune process may begin due to the formation of an autoantigene resulting from the damage of tissue proteins by the chemical compound. It has been proved that pathological liver antigenes and autoantibodies form in laboratory animals under the impact of some carcinogenic compounds, DDT, propasine. Antiliver antibodies have also been found in the organism of persons who were in contact with chlororganic and phosphororganic pesticides. It is necessary to study very seriously the role of autoimmune processes in the genesis of pathology developing under the impact of various pesticides.

Of particular interest in this respect are neurotoxic complications, often developing after poisoning with certain chlororganic and phosphororganic pesticides. An especially great number of polyneurites passing into severe pareses and paralyses have been described after chlorophos poisonings.

The issues under consideration are closely connected with the problem of remote consequences of the pesticide effect.

Remote effects of pesticides and other chemical compounds are usually defined as pathological processes developing in the organism long after the action of pesticides, as well as their influence on future generations. Among such effects are the impact on the cardiovascular system (accelerated progress of atherosclerosis, coronary insufficiency), certain nervous system failures (mental derangements, pareses, paralyses), changes in the lungs (development of pneumosclerosis), cirrhosis of the liver, etc. In recent years attention has been foccussed on blastomogenic, particularly carcinogenic, effects of different chemicals, pesticides among them, that are widely used now or proposed for agricultural practice, as well as on their mutagenic, teratogenic, embryotoxic and gonadotoxic effects. The problem lies in the difficulties of biological modelling of these processes, in the complexity of developing adequate models, and in the impossibility of predicting the above-mentioned pathological effects on the strength of short-term experiment data. As compared to the study of acute and chronic intoxications, data extrapolation from laboratory animals to man becomes more complicated. All this accounts for the slow accumulation of factual data on the possibility of remote effects of pesticides and difficulties in their interpretation.

Nonetheless, in recent years important data have been obtained for some widely used pesticides, which make it possible not only to assess their potential danger to human health, but also to develop theoretical aspects of this problem.

These data can be grouped as follows: 1) remote effects of pesticides on the physiological system of the organism and their influence on the progress of some pathological processes;

2) blastomogenic effect; the effect of pesticides on the progeny and future generations.

The effect of pesticides on the development of cardiovascular pathology is acquiring particular significance in view of the fact that the proportion of these diseases in the overall morbidity pattern has grown in recent years. Mortality due to cardiovascular diseases occupies the first place among all the lethal causes. Cardiovascular pathology has recently been observed to undergo rejuvenation, which is considered to be the resul of the impact of social, domestic and production factors among which chemical compounds play an important role /Shkhvatsabaya, Metelitsa, 1971/. As for pesticides in this respect, attention is primarily given to chlororganic compounds that can accumulate both in human and animal organisms. It has been found that DDT and lindan accelerate the development of experimental cholesterol atherosclerosis in rabbits. DDT has been noted to produce the greatest effect. Joint administration of DDT (0.001 LD_{50} daily during eight months) and cholesterol leads to a sharply expressed atheromatose alterations in the intima of aorta, particularly in its ascending part and in the arc, with the pathological process under the combined effect displaying a more universal character than in the case of the administration of cholesterol alone. DDT facilitates a wider development of the atherosclerotic process in the aorta, more distinctive alterations in the wall of coronary arteries. It was also found that DDT in small doses (0.001 LD_{50} - 6 months) raised the sensitivity of coronary cardiac vessels in rabbits to pituitrin, while daily administration of DDT or aldrin during 6 months in the dose of 0.01 LD_{50} resulted in a sharp intensification of pituitrinal coronary insufficiency, with some of the animals suffering from the development of infarction-like myocardial necroses /Yu.S. Kagan et al., 1974/.

Chlorophos intensifies the progress of liver pathology caused by the classical hepatoxic agent - carbon tetrachloride. Combined administration of chlorophos and carbon tetrachloride caused, already after 6 months, widespread cirrhotic changes and fatty dystrophia in rats, and in some of the animals the appearance of adenomas /G.A. Rodionov, L.Ya. Voronina, 1973/.

It is known that paracvat may cause development of pneumosclerosis.

Interesting data on the relationship between blood diseases and the effect of chlororganic compounds are presented by L.I. Komarova /1976/. In hemopoietic organs of those who died from leucoses and hypoplastic anemias the content of DDT and DDE was found to be higher than in hypodermic fatty tissue. Among 1083 examined patients with different forms of leucoses, hypoplastic anemia and diatheses,the frequency of contact periods with pesticides was 17.4%, for the patients with other diseases (non-hematological) - 6%.

In recent years some data have been published concerning the remote effect of pesticides on the nervous system. G.A. Vievskaya /1973/ discovered a number of mental derangements among people working with phosphororganic pesticides. Psychopathological symptomatology may include depression, schizophrenic syndrome, phobia, irritability. Phosphororganic pesticides are known to have a neurotoxic effect expressed in the development of pareses and paralyses. Neurotoxic properties are inherent in triorthocresolphosphate and, to a lesser degree, in chlorophos and dichlorovinylphosphate (DDVP). They affect axons in nervous cells, inducing the demyelination process in them. An important pathological link in this process is protein phosphorylation. Ataxy in animals receiving phosphororganic pesticides develops after 8-14 days, and paralyses - on the 13th-30th day. Neuropathologies can be caused not only by phosphororganic compounds but also by other pesticides.

Thus, a sufficient number of facts have at present been collected indicating that pesticides can either induce or facilitate the appearance of some unfavourable remote irreversible and slightly reversible effects (atherosclerosis, cirrhosis of the liver, pneumosclerosis, demyelation of nerve trunks) in highly important systems and organs, and these effects have to be predicted in advance and taken into account in the hygienic assessment of pesticides.

Among the most hazardous remote effects of pesticides is their blastomogenic action. Chemical carcinogenesis is now attracting attention of numerous experts. As for pesticides, experiments on animals have revealed differently expressed blastomogenic properties in a number of compounds. Weakly expressed blastomogenic properties have been found in chlororganic pesticides: DDT, aldrin, heptachlor, methoxychlor /O.P. Chepinoga, 1970/. V.S. Turusov /1975/ confirmed the carcinogenic effect of DDT and its metabolites, DDE and DDD, in experiments on C mice generations. The occurrence of hepatomas in males was observed to increase when DDT was administered with food in doses ranging from 250 to 2 mg/kg and in females - only from the dose of 250 mg/kg. DDE was found to be a stronger carcinogenic agent than DDT.

Morphological methods of investigations on mice and rats are indicative of blastomogenic hazard of some dithiocarbomates (TMTD, ciram, cineb, maneb).

Serious alarm was aroused by Giebel et al. /1973/ who reported on the presence of carcinogenic properties in chlorophos. In a later work Giebel et al. /1973/ observed in some experimental animals the development of myelinic leucosis, malignant and benign tumors under the action of chlorophos and phosphamide. Los et al. /1974/ attributed the carcinogenic effect of dimethyl esters of phosphoric acids to the presence of alkylating properties in them.

O.G. Petrovskaya /1975/ confirmed the data obtained by the German authors and discovered malignant and benign tumors of different localization and affected hematogenic organs in a considerable portion of low-cancerous mice of the C_{57} strain, black, and in Bestar strain rats receiving chlorophos in a 30 mg/kg dose through peroral, subcutaneous and epicutaneous application. Some authors, however, do not confirm the fact of chlorophos possessing carcinogenic properties. In the monograph on the assessment of the carcinogenic risk associated with pesticides is stressed the necessity of a further study of chlorophos carcinogeneity /JARC, 1983/.

Along with the carcinogenic hazard of pesticides, it is of primary importance to ascertain their influence on the future generations, which may be effected through mutagenic, embryotoxic, teratogenic and gonadotoxic effects.

Mutagenic effects of pesticides were examined in the book by A.I. Kurinny and M.A. Pilinskaya /1976/. They had collected literary data on the research into the mutagenic effect of 239 pesticides. Out of this number, mutagenic properties were identified on different objects for 119 compounds, i.e. 49.8%. If one takes into account the fact that the genetic activity of the majority of pesticides was tested on a single object only, more detailed investigations can be expected to reveal mutagenic properties in a greater number of preparations. At the same time, one must be careful in assessing the results obtained on models remote from man (plants, microorganisms, insects). It is quite natural that the greatest importance should be attached to data obtained on warm-blooded animals and humans. In many cases, cytogenic changes have been obtained only from large pesticide doses, far from those encountered in real conditions. Evaluation of the real danger from the genetic effects of pesticides requires a further study of all these problems.

Noteworthy are the data on embryotoxic and teratogenic properties of certain pesticides. Embryotoxic and teratogenic properties have been discovered in some phosphororganic preparations: thiophos, metaphos, DDVP, basudine and TEPP. However, only thiophos, basudine and TEPP caused fetus resorption in doses producing no intoxication in pregnant animals. V.A. Gofmekler and B.B. Khuriev /1970/ discovered the embryotic effect and altered fecundity of white rats under the inhalation effect of methylmercaptophos and chlorophos. A selective embryotoxic and teratogenic effect was identified by V.M. Voronina /1971/ in phthalophos whose structure contains the phthalamide group peculiar to a well-known teratogene - thalidomide.

Embryotoxic, gonadotoxic and teratogenic properties were found to be present in fungicides - the derivatives of dithiocarbamic acid. Gonadotoxic properties were discovered in sevin /A.I. Shtenberg, M.N. Rybakova, 1970/.

There have been reports on the gonadotoxic effect of a number of herbicides (atrasine, DNOC, etc.). In estimating these experimental data one should bear in mind that doses producing respective changes in laboratory animals are, as a rule, much higher than the quantities of pesticide that can penetrate into human organism with food. At the same time, some observations on humans confirm the possibility of a negative effect of pesticides on the fetus and the progress of pregnancy.Thus,Z.S. Saraimanova/1971/ observed frequent menstrual cycle disturbances, pregnancy interruption and lethal cases in women who had contacts with methylmercaptophos. E.A. Goloma /1962/ described a negative effect of ethylmercurchloride on intrauterine development of the fetus.

It should be noted that predicting remote effects of pesticides is at present one of the most important and difficult problems. The intricacy of this problem is associated with inadequate knowledge of the mechanisms of chemical carcinogenesis, teratogenesis and mutagenesis. This makes it difficult to reveal the relationships between pesticide properties resulting in the corresponding pathological effects and the chemical structure of pesticides. It is necessary to obtain new research data in this field, to clarify the dependence on the dose and application time of the substances, to compare the data obtained in experiments on animals with observations on humans, to carry out large-scale epidemiological studies.

Analysis of the occurrence of certain diseases in the zones of intensive pesticide application has shown the frequency rates of cardiovascular and nervous system disorders to be statistically much higher than in the control zone where the intensity of pesticide application was 3-4 times lower. Differences in the frequency of identified cardiovascular and nervous system pathology, as well as that of alimentary tract and respiratory organs, increase in the areas with a ninefold difference in pesticide application /Pol'chenko et al., 1976/.

These data confirm the possibility of a negative effect of pesticides on the health of the population and offer another proof for the necessity of the health standards of pesticide application to be universally observed.

A search for new pesticides must be carried out, with the presently available data on their possible negative effect upon human health being always taken into account.

REFERENCES

Giebel W, Loks K, Wieder G.P. Über die Kanzerogene hämatoksische und hepatoksische Wirkung pestizider organischen Phosphoverbindungen. "Arch.Geschwulstforsch." 1973, Bd 4, s. 311-328.

JARC Monographs in the Evaluation of the Carcinogenic Risk of Chemicals to Humans. 1983, v.30. Lyon, France.

Kagan Yu.S. General Toxicology of Pesticides. Kiev, "Zdorovye" Publ., 1981, 176 p. (in Russian).

Kundiyev Yu.I. Absorption of Pesticides through the Skin and the Prevention of Poisonings. Kiev, "Zdorovye" Publ., 1975, 199 p. (in Russian).

Kurinny A.I., Pilinskaya M.A. A Study of Pesticides as Mutagenes of Environment. Kiev, "Naukova dumka" Publ., 1976, 114 p. (in Russian).

Medved L.I., Kagan Yu.S. Hygienic significance of chemization in agriculture. In: "Introduction to Geohygiene". "Nauka" Publ. Moscow-Leningrad, 1966, p.219-250 (in Russian).

Pol'chenko V.I., Tkach L.I., Gumenny V.S. et al. Epidemiological aspects of studying morbidity in connection with pesticide application. In: "Hygiene of Application, Toxicology of Pesticides and Clinics of Poisonings. Moscow, 1976, iss.11, v.1, p. 125-128.

TOXICOLOGICAL ASSESSMENT AND HYGIENIC REGULATION OF PESTICIDES AND RELATED PROGRAMMES OF THE FAO/WHO AND OTHER ORGANIZATIONS IN THIS AREA

Prof. F.KALOYANOVA

I. TOXICOLOGICAL ASSESSMENT OF PESTICIDES

Classification by toxicity

To assess the harmful effect of pesticides and make decisions concerning their application, good scientific documentation is needed which can be used to establish criteria (see Table I).

Table I. Data required for toxicological evaluation of pesticides

Chemical and Physical Properties

- Chemical name in the IUPAC nomenclature
- Common and trade names
- Empirical and structural formula
- Physical state, colour, odour and formulation
- Molecular weight
- Melting point
- Boiling point
- Flash point
- Specific gravity
- Vapour pressure
- Solubility in water and in organic solvents
- Degree of purity, nature of impurities or additives and their percentage
- Thermal decomposition - hazardous products
- Stability under other conditions

Table 1 (cont.)

Toxicological Data (mammalian toxicology)

Acute toxicity

LD_{50} in rats (oral)

LD_{50} approximate in
other animals (oral)

LD_{50} approximate skin
application in rabbits

LD_{50} in rats (inhalatory)

Skin irritation in rats

Skin sensitation in
guinea pigs

Eye irritation in rabbits

Subacute toxicity

Oral application in 2 species of animals, one of which is not a rodent, with durability of 90 days; coefficient of cumulation with 3 doses and one control group, one of the doses is toxic.

Skin toxicity: 21 or 90 days, with 3 doses on 3 groups of rabbits or rats and same number of controls.

Inhalation toxicity with toxic concentration - one month.

Tests for demylinization are required for phosphoric organics and carbamates.

Chronic toxicity

Oral application in at least 2 species, one of which is not a rodent, with durability of 1-2 years; 3 doses and control group, one of the doses in nontoxic.

Inhalatory toxicity at least with 3 concentrations; the durability of the test with 2 of them is 4 months wit-determination of the threshold concentrations.

23-1

Metabolism

Data for 2 species of animals: penetration through organism, distribution, metabolic transformation.

Information of metabolism of pesticides and their products of transformation in the treated plant (metabolites or other products of transformation). If the products of transformation are different from the metabolites of the test animals, data for the chronic toxicological investigations of the metabolites of pesticides or other products of transformation may be required.

Reproduction

At least one species of animals - two species preferred.

At least 2 dose levels and a control group.

One of the doses must be toxic, the other without effect.

Usually 3 successive generations of rats - 2 generations are sufficient if the results are completed.

Two offspring of each generation are prefered. Both male and female should be treated for a period of 60 days from the period of growth; second and third generations are treated from the moment of cessation of suckling throughout the whole period of growth.

Data for teratogenicity; microscopic and skeletal investigation.

Data for mutagenicity and blastomogenic effects.

Human data

From industrial exposure, accidental poisoning and suicide as well as from controlled experiments on volunteers of special cases.

Other Toxicological Effects

Birds, fish, water and soil if available.

Persistence

Period of decomposition and mode of inactivation in the environment. Residues. Recommendations for safe handling, precautions taken during storage, transport and application, personal protective equipment, safe re-entry periods and waiting periods before harvest.

Maximum permissible levels; labelling information and treatment of intoxication.

Antidotes

Methods for chemical analysis in the environment and in biological media.

Registration and use of the compound in the parent and other countries.

To assist in the evaluation of these data, various classifications of pesticides are used in different parts of the world.

WHO recommended classification of pesticides by hazards was accordingly prepared (see Table 2) and adopted by the Twenty-eighth World Health Assembly which recommended the use of the classification by Member States, International Organizations, agencies, and regional bodies (WHO Chronicle, 29:397-401, 1975).

Guidelines on the use of the WHO recommended classification are issued regularly.

Table 2

Class		LD_{50} for the rat (mg/kg body weight)			
		Oral		Dermal	
		Solids[a]	Liquids[a]	Solids[a]	Liquids[a]
Ia	**Extremely hazardous**	5 or less	20 or less	10 or less	40 or less
Ib	**Highly hazardous**	5 - 50	20-200	10-100	40-400
II	**Moderately hazerdous**	50-500	200-2000	100-1000	400-4000
III	**Slightly hazardous**	over 500	over 2000	over 1000	over 400

[a] The terms "solids" and "liquids" refer to the physical state of the product of formulation being classified.

The classification used for the purpose of clearance in Bulgaria is given in Table 3. It represent adaptation and development of the classification used in USSR (Medved et al., 1968), by Kaloyanova (1977).

Table 3

Hygienic classification of pesticides

Factors	Extremely hazardous (Class I)	Very hazardous (Class II)	Hazardous (Class III)	Slightly hazardous (Class IV)
I	2	3	4	5
LD_{50} for rat (peroral)	To 50 mg/kg	50-100 mg/kg	100-1000 mg/kg	>1000 mg/kg
LD_{50} for rat (percutaneous)	To 100 mg/kg	100-500 mg/kg	500-2000 mg/kg	>2000 mg/kg
LD_{50} for rat (inhalatory, 4 hrs exposure)	To 200 mg/m^3; concentration of saturation is toxic level; provokes heavy acute poisonings	To 100 mg/m^3; concentration of saturation is above threshold level; provokes poisonings	1000-5000 mg/m^3; concentration of saturation causes slight effect and is about equal to threshold level	> 5000 mg/m^3; concentration of saturation provokes no effect
Coefficient of cumulation (k) $K = \frac{n\ LD_{50}}{LD_{50}}$	K = < I	K = I to 3	K = 3 to 5	K = > 5
Resistence period in environment; period of decomposition	Quite durable, above 1 year	Durable, 6-12 months	Moderately durable, 1-6 months	Slightly durable, under 1 month

Table 3 (cont.)

1	2	3	4	5
Blastomogenicity	Proved human cancerogens; strong cancerogens for test animals	Slightly cancerogenic for test animals. Effect in less than 20% of the animals with maximum non-toxic doses. Suspected cancerogenicity	No cancerogenic effect	No cancerogenic effect
Teratogenicity	Certain human abnormalities, reproductible in test animals; teratogenic activity with doses met in practice	Strong teratogenicity: 50-100% activity in test animals with doses not toxic for mother; effect with more than one type of test animal; polytropic effect	Provokes anomalies in less than 50% of offspring with doses not toxic for mother; teratogenic effect in one type of test animal; effects separate organs and systems; acting dose above 1/10 LD_{50}	No teratogenicity
Embryotoxicity	Not recorded in assessment	Selected embryotoxicity; manifests with doses not toxic for mother	Moderate embryotoxicity manifests with doses toxic for mother	No embryotoxic effect
Poisoning	Acute heavy poisonings possible in practical application	Probable acute poisonings	Acute poisonings only in exceptional conditions	Acute poisonings not probable

Table 3 (cont.)

I	2	3	4	5
Therapeutical possibility	No special treatment; poor therapeutical possibility	Certain antidotes; fair therapeutical possibility	Certain antidotes; good therapeutical possibility	Specific treatment; good therapeutical possibility
Irritation of skin	Very strongly irritant; chemical burning; acute toxic dermatitis from concentrated preparations; toxic dermatitis from working solution	Strongly irritant; provokes speedy development of symptoms; toxic dermatitis from concentrated preparation; cumulative effect of working solution	Irritant; cumulative dermatitis from concentrated preparation	Practically non-irritant
Irritation of eyes and upper respiratory organs	Working solutions with irritant effect	Working solutions with slightly irritant effect	Concentrations with irritant effect	Working solutions with no practical irritant effect
Allergy	Established allergic and photosensitizing effect in humans; positive evidence of sensitization of guinea pigs.	Established allergic effect in humans. Negative sensitizing test in guinea pigs	Presumed sensitizing effect on basis of chemical structure	No sensitizing effect

Notes: I. When decisions on preliminary sanitary control are being made, some pesticides may be put in an adjacent class of hazard. In addition to the limiting criteria, the remaining toxicological properties, type of formulation and economic importance are considered.

2. If the practical applications of pesticides indicates that conditions for creating vapours, liquids or solid aerosols of the preparation, near to those mentioned, are possible, the criterium for inhalation toxicity is taken as limiting.

Factors influencing the hazard

In agriculture and at sites which must be disinfected, different pesticides are often used during one season. Therefore, a combined effect of the various pesticides may be expected. In addition, the toxicological hazard of a given formulation is the combination of the inherent toxicity of the pesticide and all other ingredients.

Liquid formulations containing xylene and related materials are used for many insecticides. The use of volatile, toxic organic solvents increases significantly the occupational hazard.

Recently, more attention has been given to the effects of impurities in pesticide formulations. Marked differences in the toxicity of paration may occur due to its contamination with small quantities of the oxygen analogue paraoxon and the phosphorothiolate isomer, both of which are more toxic for mammals than in the parent insecticide.

Information about the composition of certain technical grade pesticides or their formulations is very often inadequate (toxaphene and chlorinated terpenes). Some pesticides are produced by several manufacturers, resulting in variations in the technical product, especially with respect to contaminants. The hazard from exposure to impurities in pesticide formulations comes during their production and use. The impurities may influence the metabolic pathways and degradation of the pesticide formulation in the environment or in the organism.

According to Rosival et al. (1980), the sources of impurities might be divided into main and secondary sources. In the former group, the basic material for pesticide production may contain impurities. The amines used in the production of alkylamine salts of phenoxyalkane carboxylic acids may contain N-nitrosoalkylamines. Chlorinated camphene may have impurities which affect the qualities of toxaphene. The manufacturing process includes reactions which inevitably result in pesticides that contain byproducts, such as organophosphorus thioates and dithioates, ethylenthyurea (ETU) formation in ethylenebisdithiocarbamate, dioxins in chlorphenoxyherbicides and pentachlorphenol.

Secondary sources include the unsuitable formulation of technical grade active ingredients, e.g. the izomerisation of organophosphorus thioates and dithioates and formation of hydrophilic degradation products. They also include unsuitable tank-mix preparations before practical application, such as ETU formation from ethylene-bisdithiocarbamate fungicides. Finally, a secondary source may be the unsuitable storage of formulations with products that result in physicochemical transformation.

The repeated, combined action of pesticides usually has an additive effect. Potentiation of toxicity after exposure to a combination of organophosphates may result from inhibition by one organophosphate of the carboxylesterases which either bind or hydrolyze a second organophosphate.

In a combined effect of pesticides, manifestations of antagonism which could lead to a reduction in the harmful effects are possible. Preliminary impregnation with chlor-organic compounds prevents, to a certain degree, the effect of phospho-organic compounds under experimental conditions.

6300 In addition, the combination of high temperature and humidity in hot climates and greenhouses enhances the degradation of pesticides in the working environment (organophosphates, synthetic pyrethroids and dipyridyliums). In such conditions, the increased absorption of the pesticides through inhalation and dermal contact may complicate evaluation of the hazard.

Bearing in mind method of application and the actual conditions under which the pesticide will be used, the pesticide should be adequately safe to humans and the environment, while providing adequate disease or pest control.

2. HYGIENIC REGULATION OF PESTICIDES

Regulatory control is one of the strategies used to limit the hazards of pesticides to human health, non-target species, and the environment. Legislation on the control of pesticides is complex. Human exposure may result at all stages of the life cycle of pesticides. Pesticide control legislation often consists of a number of different items, each concerned with a particular aspect of pesticide control. The legislation should cover all stages of the life cycle of pesticides: manufacture and formulation, transport, import and export, storage, labelling, sale, quality control, registration and use and disposal, environmental biological and monitoring. Legislation differs between countries and varies with climate, culture and economic conditions.

In most countries, enforcing legislative measures and updating and legislation are the responsibilities of the Ministry of Agriculture and the Ministry of Health and/or Labour. These ministries must ensure that legal requirements are adequately satisfied and that all sectors of the public are protected from any potential pesticide hazards.

Registration

All pesticides which are used should be authorized (registered) by appropriate authorities in the country of use. To avoid the negative effects of pesticide use, the toxic effects must be assessed and used as the basis for making decisions about registration. In most countries notification is obligatory. The notifier must submit the necessary data to the responsible department at the Ministry of Health. Usually, a consultative body that in- 6300 cludes toxicologists and other specialists gives recommendations for clearance. Decisions concerning the implementation of a given formulation and issuance of the clearance is the responsibility of the Ministry. Several categories of clearance are used: trials clearance, only for notifier's own personnel during trial studies; limited clearance, for limited amounts of the product; provisional commercial clearance for use for up to two years; and commercial clearance, no time limit.

The data necessary for pesticide registration are very similar in the different countries. The notifier is obliged to present toxicological data (see Table 1). Scientific data must give evidence that, the pesticide will not injure humans, crops, livestock, wildlife or damage the environment, if the label is followed. All pesticides must be classified for general or restricted use: pesticides with restricted use can only be applied by a well qualified applicator.

Increasingly the demands of national registration and health authorities include residue data on treated crops and commodities and in water, soil, air, wildlife and other ecotoxicological data.

The assessment of the human hazards arising from very small quantities of a pesticide in food and the environment is an important part of the overall risk-benefit evaluation now regarded as essential in most countries before a pesticide can be introduced.

Labelling requirements

Labelling is important for prevention of intoxications. Labels should be written in the language of the country-user. All labels should contain the following information: name (proprietary, generic, structural), registered use of product; active ingredients; mixtures; directions for use; pests to be controlled; crops of animals to be treated; dosage and method of application; warnings to protect the user and the environment; the statement "Keep out of Reach of Children"; name and address of manufacturer; and indications of toxicity according to the accepted practice in the country. The label should also state what protective clothing (chemically impervious aprons, boots and gloves, plus faceshields and sometimes respirators) and equipment are required during pesticide application, the re-entry period and first-aid instructions. Approval of labelling should be done together with registration of the pesticide.

Storage, Transport and Disposal Regulations

Secure storage of pesticides reduces the likelihood, illegal use by irresponsible persons. To protect consumers as well as cargo handlers, co-mingling of pesticides with other produce in common carriers is forbidden. Preparation of detailed cargo manifests is ordinarily required by law. This is extremely important in the event of vehicular accident, fire, or container leakage. Workers required to control the fire or load and unload the cargo must be apprised of the toxic potential of the chemicals and be provided with appropriate protective gear. There are international regulations for transport of dangerous goods including pesticides by air, seas, railway and roads.

Regulations covering disposal of unused pesticide and pesticide containers must combine effectiveness with practicality. Control should be aimed at discouraging the accumulation of used containers, and at safe disposal of residual pesticide.

There must be strong prohibition of improper disposal practices that lead to contamination of ground or surface waters. Burning pesticide bags must be done in areas where smoke will not affect local residents or nearby agricultural workers. Burial of containers must be allowed only in regions where residual chemicals will not lead to contamination of water sources. Ideally, suppliers should be required to pick up empty containers, recycling them whenever possible.

Regulations should specify that residual pesticide should not simply be dumped on the ground. The high concentration of pesticide at this point may present a serious hazard if rainwater forms a contaminated puddle from which livestock, pets, or wild animals drink. In the case of nearly all newly introduced pesticides, the best method of disposal of residual chemical is simply continued application to soil or crop at low concentration until the supply is exhausted. Nearly all such pesticides are effectively degraded in the natural environment, particularly when dispersed in low concentration. So long as amounts on crop or soil are not rendered excessive continued application presents the fewest hazards.

Environmental and Occupational Health Regulations

All countries are concerned with the safe use of pesticides at national and international scale.

More than 30 international organizations are concerned with pesticides. For the purpose of preventive medicine WHO, ILO and FAO play a very important role.

In the Headquarters of WHO in Geneva several departments are responsible for different kinds of activity in this area (see Fig. 2).

Vector Biology and Control Department

Meetings of experts or temporary advisors resulted in publishing on safe use of pesticides No 63/1979, 356/1967 and 513/1973. Bibliography information and data sheets for 56 pesticides have been distributed.

Environmental Health Department

It starts to prepare a comprehensive scientific monograph in the series "Environmental Health Criteria Document". Under No 9 is published the monograph on DDT and Derivates (1979). Some others (gramoxon, reglon, carbamates, 2.4. D etc.) are under preparation now. Short document series is also under preparation in order to reach the reader in the early stage of use of pesticides.

Occupational Health

On the field of occupational health WHO working group recommended same health based limits (see Table 6) in occupational exposure to pesticides.

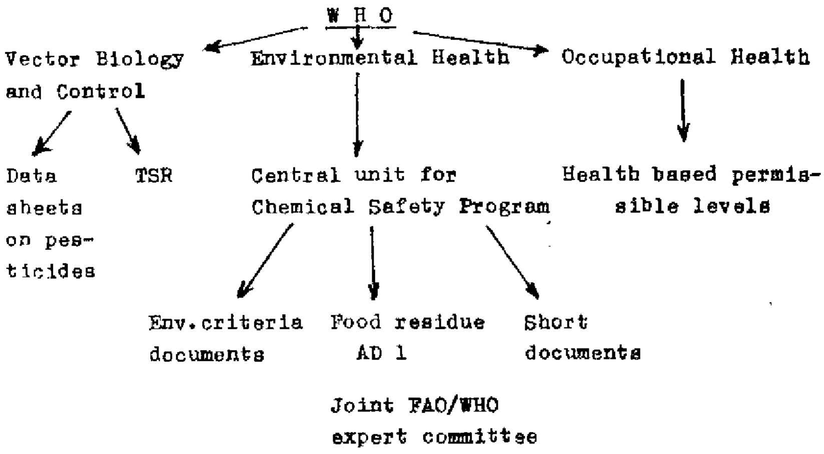
Table 6

Health based exposure limits (WHO)

Name of Pesticide	Environmental	Biological
malathion	no sufficient data	30% ChE inhibition
carbaryl	5 mg/m^3 (S)	30% ChE inhibition
lindan	0.3 mg/m^3 (S)	0.02 mg/l whole blood
DNOC	no sufficient data	20 mg/l whole blood

Still up to now no international activities are known for determination of water and soil permissible limits (PL). In USSR permissible limits for 10 pesticides for soil have been established (Kagan, 1981). In many countries standards for different categories of waters and for ambient air are adopted (Kaloyanova, 1977).

Fig. 2



Food safety. Very active international activity is done in this field jointly by FAO and WHO. Codex Alimentarius Commission plays principle role (see Fig. 1).

On the basis of scientific data acceptable daily intake (ADI) of many pesticides has been established (TRS WHO/FAO No 545 (1974), 612 (1977), 592 (1976) FAO Pesticide residues in food, 1977, 1978, 1979, 1980, 1981, Guide to Codex ML, 1978).

The Codex Guide to maximum residue limits lists recommended limits for more than 120 pesticides in a wide range of food commodities. These limits were proposed by the FAO/WHO Joint Meeting on Pesticide Residues in a series of reports and are based on an estimate of the maximum residue level expected in "good agricultural practice" after consideration of the acceptable daily intake (ADI) for the pesticide in question (Bates, 1982).

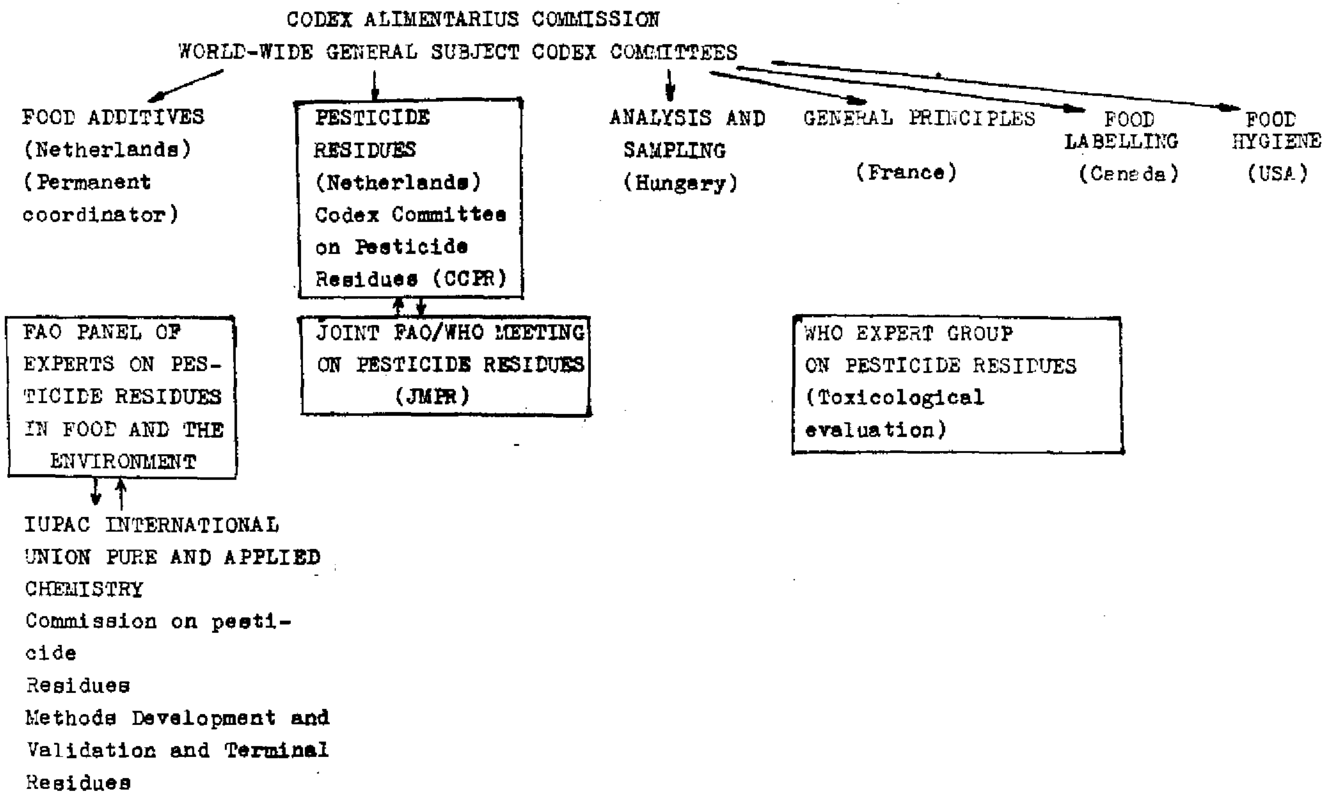
<u>Acceptable Daily Intake</u> (ADI) - This is the daily intake of a chemical, which during the entire lifetime, appears to be without appreciable risk on the basis of all the known facts at the time. It is expressed in milligrams of the chemical per kilogram of body weight (mg/kg). For a pesticide residue the ADI is intended to give a guide to the maximum amount that can be taken daily in food without appreciable risk to the consumer. Accordingly this figure is derived as far as possible from feeding studies in animals and/or man. ADI's are always subject to revision at any time in the light of new information.

<u>Temporary Acceptable Daily Intake</u> - This refers to an ADI which is recommended for a limited period during which additional biochemical, toxicological or other data can be obtained in order to establish an ADI. In cases where temporary ADI's are recommended by the JMPR, the recommendation will normally involve the application of a safety factor, the size of which will be dependent on the nature of the toxicity of the compound, but which will be larger than the normally used in estimating ADI's. Temporary ADI's are reviewed by the JMPR at subsequent meetings.

<u>Good Agricultural Practice in the Use of Pesticides</u> - For the purpose of Codex Alimentarius this is defined as the officially recommended or authorized usage of pesticides under practical conditions at any stage of production, storage, transport, distribution and processing of food and other agricultural commodities, bearing in mind the variations in requirements within and between regions, and which takes into account the minimum quantities

6300

Fig. 1


CODEX ALIMENTARIUS COMMISSION
WORLD-WIDE GENERAL SUBJECT CODEX COMMITTEES
FOOD ADDITIVES (Netherlands) (Permanent coordinator)
PESTICIDE RESIDUES (Netherlands) Codex Committee on Pesticide Residues (CCPR)
ANALYSIS AND SAMPLING (Hungary)
GENERAL PRINCIPLES (France)
FOOD LABELLING (Canada)
FOOD HYGIENE (USA)
FAO PANEL OF EXPERTS ON PESTICIDE RESIDUES IN FOOD AND THE ENVIRONMENT
JOINT FAO/WHO MEETING ON PESTICIDE RESIDUES (JMPR)
WHO EXPERT GROUP ON PESTICIDE RESIDUES (Toxicological evaluation)
IUPAC INTERNATIONAL UNION PURE AND APPLIED CHEMISTRY
Commission on pesticide
Residues
Methods Development and Validation and Terminal Residues

necessary to achieve adequate control, applied in a manner so as to leave a residue which is the smallest amount practicable and which is toxicologically acceptable.

Pesticide Residue - Under Codex Alimentarius, the term "pesticide residue" refers to any substance in food of man and animals resulting from the use of a pesticide. It also includes any specified derivatives, such as degradation and conversion products, metabolites and reaction products that are considered to be toxicological significant.

Codex Tolerance/Codex Maximum Residue Limit - For the purposes of the Codex Alimentarius these terms mean the maximum concentration of a pesticide residue that is recommended by the Codex Alimentarius to be largely permitted in or on a food commodity. The concentration is expressed in parts by weight of pesticide residue per million parts by weight of the food or commodity. In general, a *Codex tolerance* or *Codex Maximum Residue Limit* refers to the residue resulting from the use of a pesticide under circumstances designed to protect the food or food commodity against pest attack, according to good agricultural practice. When a residue results from circumstances not designed to protect the food or food commodity in question against pest attack the maximum concentration recommended is designated as a *practical residue limit*.

Methods of Analysis - The CCPR usually will accompany a tolerance recommendation with an analytical method of analysis that is considered as suitable to measure the residues covered by the particular Codex maximum limit. However, these are specifically not recommended as Codex "referee" methods to settle disputes involving pesticide residues in food moving in trade. CCPR does not consider that the absence of methods of analysis or sampling should retard the movement of tolerance recommendations through the ten-step Codex procedure.

In order to protect food and food from pesticides residue it is necessary to have in mind the recommendations summarized by Bates (1982):

- Pest control treatment should be made only when necessary. The requirements for pest control should first be established, followed by the application of the preferred method of control. In recent years the terms "integrated pest control" and "integrated pest management" have been introduced to embrace the appropriate use of pesticides in good agricultural practice.

- When pest/disease control is required in the early growing stage of the crop, a pesticide may be needed which has an adequate and acceptable degree of persistence. Thus, repeated applications of nonpersistent pesticides can be avoided. If plant quarantine and/or phytosanitary requirements make the application of pesticides close to harvest necessary, those which have a short persistence should be preferred.

- The agricultural use of persistent pesticides on crops for human consumption should be restricted as much as possible and be limited to the control of pests, weeds and diseases for which no suitable, alternative chemicals are currently available. In general, persistent pesticides should not be used on fodder crops and should be applied directly to animals for veterinary purpose.

- Formulations which combine maximum pesticide efficacy with minimum risk should be preferred. Supplementary adjuvants should be used only when their effect is known and where their use produces a significant improvement in performance. The quantity of pesticide applied should not be greater than minimum required to achieve the desired degree of control. The number of treatments should be determined by the desired degree of control and by the severity of pest/disease conditions.

- The method of application should be selected to ensure optimum pest/disease control with minimum contamination of the crop and the environment. Indirect treatment (such as soil application, seed dressing and treatment of alternate hosts) can, in some cases, be used to supplement or replace direct application to food crops. Application equipment should be maintained at all times and used according to manufacturer's instructions.

- Treatment should preferably be carried out when the pests/diseases are at the most vulnerable stage of development and when climatic conditions and cultural practices will ensure that the optimum effect will be attained from the treatment. In some instances, however, action may be necessary immediately after detection of the pest/disease species.

- The interval between last application and harvest should be as long as possible to permit the greatest reduction in pesticide residues. At the same time, the incidence of the pest, the degree of control required for a maximum utilization of the commodity and the vulnerability of the treated crop immediately before harvest should be considered. To this end, official preharvest intervals should be established and followed.

New Trends in Sanitary Legislation - Re-entry Intervals

An analysis of poisoning cases in Bulgaria has shown that many are persons not directly employed in plant protection but who work in areas sprayed with pesticides. For this reason, they had not been briefed or provided with protective means. Quite often, these poisonings occur in groups working in tobacco plantations up to 15 days after spraying or in persons who had picked flowers sprayed with organophosphate compounds.

We have worked out a method (Kaloyanova-Simeonova, 1970; Izmirova et al., 1973), for determining the minimum reentry intervals after application of pesticides or safe re-entry periods. Re-entry intervals are periods which must elapse after pesticide application before workers may go into fields or orchards to harvest the crop or perform any other tasks in order to avoid significant exposure to pesticides.

Re-entry intervals are difficult to implement because of the inherent complexity of modern pest control technology, i.e. multiple chemicals, formulations, and dosage rates, plus unpredictable climatic conditions affecting the degradation of residues. However, extensive research to identify safe re-entry intervals for a large number of pesticides applied to a great variety of crops is justified.

In Table 4 are presented re-entry periods established in Bulgaria.

If this period cannot be observed, persons working in sprayed areas must be warned of the risk and at least provided with protective gloves. The same problem arises in the United States, especially in California (Milby, 1974), where citrus, soft fruit trees and other crops that have heavy foliage are sprayed with toxic organophosphate compounds. Many episodes of poisoning, including fatalities, of workers have occurred from such residues, usually involving parathion. To deal with the problem, the State of California established worker re-entry safety standards for

Table 4

Minimum re-entry intervals for organophosphorus and carbamate pesticides in Bulgaria

Pesticides (days)	Hot House conditions (days)	Field conditions for raising tobacco
Dimetoat	3	7[a] - 5[b]
Intration	use prohibited	15[a] - 13[b]
Baifos	use prohibited	13[a] - 11[b]
Aktelik	1	-
Pirimor	5	5[a]
Unden	3	

[a] Application by tractor

[b] Aerial application

citrus fruit, peaches, grapes and apples. Federal standards have since been developed by EPA, but these are less stringent than are the California standards (Table 5).

The problem on safe re-entry intervals have been studied also by Spinu and all, 1980. Some mathematical equations are proposed for the calculation of the re-entry intervals.

If re-entry intervals are to be effective, regulations must require posting signs in treated fields or orchards at reasonable intervals along the periphery. The signs must warn against premature entry and state the data prior to which entry is illegal.

Table 5

Field re-entry intervals (FRI) in days or hours for selected pesticides - California and EPA regulations

Pesticides	FRI in California (Days)				EPA	(Hours)
	Citrus	Peaches Nectarines	Grapes	Apples	All other crops	All crops
Azinphosmethyl	30	14	21	14	-	24
Bidrin	2	2	2	2	2	48
Carbophenothion	14	14	14	2	2	48
Diazinon	5	5	5	-	-	-
Dioxathion	30	30	30	-	-	-
Endosulfan	2	2	2	2	2	-
EPN	14	14	14	14	14[*]	24
Ethion	30	14	14	2	2	24
Malathion	1	1	1	-	-	-
Methomyl	2	2	2	2	2	-
Mevinphos	4	4	4	2	2	-
Parathion-ethyl	30,45,60"	21	21	14	14[*]	48
Parathion--methyl	14[*]	21	14[**]	14	14[*]	48
Phorate	2	2	2	2	2	-
Phosalone	7	7	7	-	-	24

[*] If parathion and EPA used in combination or more than one pound per acre is applied. Otherwise the interval is 2 days.

[**] If encapsulated form is used the interval is 21 days.

" Depends on the amount per acre applied.

Source: Federal Register; May 16, 1975, State of California Dept. of Food and Agriculture, Feb.5, 1979

(The Table is adapted from M.Moses, 1980)

REFERENCES

Bates I. Safe practice in pesticide use: Pesticide residues in food and the environment. Interim document Toxicology of Pesticides, WHO/EURO, 1982, p.194-195.

DDT and its Derivates. WHO. Geneva. Environmental Health Criteria, 9, 1979.

Guide to Codex maximum limits for pesticide residues. Rome. Secretariat of the Joint FAO/WHO Food Standards Programme, 1978.

Izmirova N. et al. Determination of minimal period for safe work after treatment of the tobacco and cotton by organophosphorous compounds. In: Hygiene of application, toxicology of pesticides and clinic of intoxications. Kiev, 1973, 123-124.

Kagan I. General toxicology of pesticides. Kiev. Zdorovje, 1981.

Kaloyanova-Simeonova F. Prevention of intoxication by pesticides in Bulgaria. Whiter rural medicine. In: Proc. Fourth Intern.Congr.Rur.Med., Usuda, 1969 (Tokyo), 1970, p.35-37.

Kaloyanova-Simeonova F. /Pesticides - toxic action and prevention/. Sofia, Bulgarian Academy of Science, 1977.

Kay D. (ed.). The international regulation of pesticide residues in food. The Am.Soc.of Int.Law. 2223 Massachussetts Av. N.W. Washington D.C. 20008 - 15 I. 1975.

Medved L.I., Kagan I.S., Spinu E.I. Pesticides and problems of public health. Journal vsesoiuznogo chimicheskogo obstestva im. Mendeleeva, (1968), p.263-271.

Milby T., Chairman. Occupational exposure to pesticides. Report to the federal working group on pest management from the task group on occupational exposure to pesticides. - January 1974, Fed.

working group on pest management. Washington D.C. US Gov.print. office: 1975/0.551 - 026.

Moses M. Pesticides. In: Last J.M., ed. Public Health and Preventative medicine. IIth New York, Appleton-Century-Crofts, 1980, pp.731-750.

Pesticide residues in food: report of the FAO/WHO Joint Meeting on Pesticide Residues (FAO Plant Production and Protection Paper, 1977-81), Rome.

Recommended classification of pesticides by hazard. WHO Cronicle, 29, p.397-402, (1975).

Rosival L., Batora V. Consequences for field exposure of impurities in pesticide formulations. In: Tudoir W.F. and Heemstra-Lequin E.A.H., ed. Field worker exposure during pesticide application Amsterdam, Elsevier North-Holland, 1980, p.160.

Safe use of pesticides. Occup.Safety and Health Ser.Int. Labour Office, Geneva, 1977.

Spinu E.I., Bolotnij A.V., Soreva T.D., Ivanova L.N. Principles of establishment of safe re-entery intervals for people in treated areas by pesticides. Gigiena i Sanitaria, 1980, II, p.64-67.

WHO Chronicle (1975), No. 29:397-402.

WHO Technical Report Series No. 634 (1979); 677 (1982); 356 (1976); 513 (1973).

CONTROLLING THE ADVERSE EFFECTS OF PESTICIDES ON HUMAN HEALTH AND THE ENVIRONMENT

M.S. Sokolov,

the North-Caucasus Research Institute of Phytopathology
of the USSR Ministry of Agriculture, Krasnodar

Together with other controlled and uncontrolled pollutants, pesticides have turned into a constant ecological factor in many countries of the world. Therefore, one has to give them close attention although in the list of global pollutants pesticides come only at the end of the first dozen of the world's major pollutants (I).

Within the overall problem of protecting the biosphere from pollution, out of all landscape components the pride of place is given to the soil. This is really so because both nowadays and in the past the soil cover together with its microstructure performed the universal role of a biological adsorber, purifier and neutraliser of pollutants and a mineralizer of organic matter. At the same time hygienists repeatedly emphasize the role of soil as a source of polluting the contacting media, such as plants, the atmospheric air, surface and ground waters, with pesticide residues remaining in the soil (2,3,4).

Even the treatment of crops and plants (to say nothing of the soil itself) results in the major portion of applied chemicals (from 75 to 90%) finding its way into the soil surface. Besides, there are other pathways for pesticides entering the soil and polluting it: a drift of droplets, vapours and particles of pesticides during the aerial and surface treatments, irrigation waters, treated seeds, losses during the preparation and transportation of pesticide solutions, the surface and subterranean run-off, rainfall, products of the wind erosion, organic fertilizers, wastes from cattle-breeding farms, municipal garbage-dumping sites and plant exudates.

At present, it is difficult to find a land area (even in nature preserves) which would be completely free from pesticide residues. However, the so-called background concentrations of pesticides usually amounts to several fractions of a billion (mkg/kg) in terms of the soil mass. Under such low concentrations practically not a single pesticide may produce a negative impact on the soil and its biota. Therefore, in this lecture we shall discuss the impacts of only such background concentrations of pesticides which are significant and by several orders of magnitude higher than the above-mentioned background level. In other words, the effective concentration should be in the range of tenth fractions of mg/kg and over. This applies, first and foremost, to the soils which represent the main production asset either in agriculture or forestry.

In order to have a better idea of the negative impacts of pesticides on the soil, one has to agree on terms "the negative impact". In our opinion, such a notion should include at least changes in one or several major properties of soils:

- first, lower soil fertility;
- second, deterioration of the quality of crops, including the pollution of these crops with pesticide residues;
- third, the worsening quality of the biotic environments which maintain contact with the soil (surface and ground waters, the atmospheric air), i.e. the impossibility of their use either by humans or other organisms;
- fourth, the worsening of the soil's ability to self-purification[*] against both natural and xenobiotic compounds;
- fifth, the declining informative nature of soil as an eco-system, that is the impoverishment of its genetic stock.

It is therefore obvious how difficult it is to judge a priori whether a pesticide produces non-target negative impact on the soil or not. This question may be only answered after detailed studies based on the biological testing of a xenobiotic, on the assessment of its physico-chemical properties, and after assaying its fate and behaviour in such combinations as soil-water, soil-plant and soil-atmosphere.

Long ago researchers noticed that many aspects of pesticides' behaviour in the soil are subject to their interaction with the soil biota, primarily with microorganisms (bacteria, fungi, Actinomycetes, soil algae) and with diverse soil fauna. Scores of scientific works have been devoted to this problem, but nevertheless we still know very little about so-called non-target soil organisms and non-target (key) soil processes related to pesticides. An attempt to generalise the results of world-wide thirty-odd years research was under-

---

[*]
[**] (see next page).
The capacity to self-purification represents a complex of all natural processes aimed at restoration of the initial properties of a landscape as a whole or its separate components.

taken by the authors of a collective monograph "Pesticide Microbiology". The analysis of a large volume of published data, carried out by J.R.Anderson, demonstrates that out of twelve non-target soil parameters pesticides effected six, while herbicides effected only one, insecto-acaricides two and fungicides five parameters (5). Thus, fungicides adversely effected the process of nitrification. They also reduced the colonies of fungi and Actinomycetes, but increased the number of soil algea. Finally, "the breathing of the soil" and the activity of diverse enzymes was inhibited by fungicides, but stimulated by insecto-acaricides. Such data on pesticides were obtained, as a rule, by means of the ecotoxicological prediction under laboratory conditions. Some of the data used was based on the results of field research and monitoring.

Therefore, an objective assessment of the negative non-target effects of pesticides on soil is a complex matter. Nevertheless, persistent efforts have to be constantly undertaken for its step-by-step solution. The only realistic condition for this is to stop deteriorating the soil itself and the surrounding environments.

One of the methods allowing to speed up and facilitate experimental research in this field is to take up a precise inventory of pesticides and classify them by their effects on the biosphere as well as to substantiate their allowable rates for soil application. We believe that no complex experimental research for substantiating maximum allowable concentrations of pesticides for soil should be carried out for the entire range of pesticides at once. One can only recall that out of 171 pesticide compounds, recommended for use in this country, the application rates were established for only 26 preparations while the so-called tentative permissible concentrations (determined by calculation) were suggested for 14 compounds (6, 7). Therefore, the priority of rating must be given to the compounds which are most dangerous for the biosphere. Back in 1975, a list of 18 such compounds used in the moderately temperate climatic zone of the USSR was compiled. The list contained HCCH, Cyanoplav*),Granosan, Hexachlorobutadiene, Calcium Arsenate*), Nemagon*), Metaphos*), Sevin, Ziram*), Trisben, TMTD, polychloropinene*), DDT*), Zink Phosphide, Polychlorocamphene, DNOC, Carbathion and Octamethyl*). During the last 10 years eight of them were banned for use altogether and the application of the remaining ones was substantially restricted. The selection of priorities in rating specific compounds -- from the group of highly toxic and moderately toxic pesticides -- must be exclusively guided by the overall volume of thier application, i.e. by the annual amount in tonnes and the size of treated land areas. As far as the group of preparations which are relatively safe for the biosphere is concerned, it is advisable to use the method of calculations for their rating. For instance,

---

*) Banned for application now (6).

a method of linear regression equations which take into account the maximum allowable level of pesticides in crops, should be used (9).

Pollutants must first be classified by the danger they present for the biosphere and then rates of their application must be established, taking into account the minimum number of the most objective criteria (8). The procedure for assaying pesticides by eleven ecotoxicological criteria suggested below, includes five toxico-hygienic criteria, five eco-agrochemical and one ecotoxicological criterion proper.

The group of toxico-hygienic criteria for assaying pesticides includes:

I. Assessment by the maximum allowable concentrations in the residual amounts

- for crops (0-4)**),
- for drinking water (0-4).

2. Effects on the organoleptic properties

- for crops (0-I),
- for drinking water (0-3).

3. Volatility (vapour pressure) - (0-3).

4. The per-oral toxicity (DL_{50}) for warm-blooded animals (1-4).

5. Ability to accumulate in the organism of warm-blooded animals (0-3).

The group of ecological and agrochemical criteria of pesticides includes the following:

**) In brackets we list arbitrary scores of compounds' toxicity (8).

6. Persistence in soils (2-8).

7. Effects on soil fermentation processes and the biota (0-2).

8. Migration along the soil profile (0-3).

9. Translocation into cultivated plants and a phytotoxic effect through the soil (0-3).

10. Response to solar radiation (0-I).

11. The coefficient of selectivity belongs to the ecotoxicological criteria of pesticides (0-3).

Those compounds which score over 20 points by all eleven criteria are referred to as highly toxic; those scoring between 20 and 13 points to moderately toxic, and those which score less than 13 points are considered as low toxic.

A toxic metabolite (or metabolites) of a pesticide whose share of the toxic residues amounts to no less than five percent (in biological media, soil or water) should be assessed similar to the initial compound. The initial compound should be given such a score of points which corresponds to the maximum value.

Since the first two groups of criteria were discussed in detail earlier (8), let us explain the meaning of an ecotoxicological criteria proper, first suggested by Metkampf for insecticides and which he called the coefficient of selectivity (10). In this particular case it is suggested to calculate this coefficient as the ratio of the average lethal dose DL_{50} (per-oral exposure, in mg/kg) to the total dose of the preparation applied to the soil during the vegetation period (kg/hectare). Let us explain this in more detail by taking one and the same target (flax) and two different preparations (2M-4X and Lynuron). Herbecide 2M-4X is moderately toxic for warm-blooded animals, its DL_{50} for mice being 566 mg/kg, the average recommended dose being I.I kg/ha and its coefficient of selectivity being 514. Lynuron is only slightly toxic for warm-blooded animals, thus its DL_{50} for mice being 2300 mg/kg, the average recommended dose being 0.4 kg/ha and the coefficient of selectivity being 5750. Therefore, by using the coefficient of selectivity as a guiding principle, one can say that the use of Lynuron is preferable to 2M-4X. Similarly, when two slightly toxic herbicides are applied to sugar beets, then in case of Dalopan its coefficient of selectivity is 423, while that of sodium trichloracetate is 150. Consequently, it is preferable to use Dalapon than sodium trichloracetate. Of course, it is reasonable to compare only the mutually-replacable preparations which are close by their toxic effect.

In the soil, as is known, residues of vegetative and living organisms are utilized by the saprophyte heterotrophic microflora. As researchers were able to prove, the decomposition of certain xenobiotics in general correlates with the availability of the heterotrophic microflora and with the cellulosolytic activity of the soil (11, 12, 13). Under similar conditions of moisture, the disintegration of organic xenobiotics greatly depend on the temperature of the soil. Under the field conditions, in order to obtain a generalized characteristic of activity of heterotrophic microorganisms and to assess the self-purification ability of various soils from pesticide residues, it is convenient to use the debris-to-litter coefficient (14, 15). In conditions of an undisturbed landscape this coefficient expresses the ratio of the organic matter reserves accumulated in the litter to the annual vegetative shedding. It characterises the rate and seasonal intensity of the biological turn-over of carbon and of the activity of the saprophyte microflora which play an important part in the decomposing of organic pesticides. One research gives the average values of this coefficient compared with the actual quantity of energy coming in with the natural vegetative shedding in the different landscape and geochemical zones of the country (16).

For some restricted areas characterized by a specific type of soil, the degree of pesticide accumulation in the soil may be better described by the residue-load coefficient (17). This is a dimentionless value expressing the ratio between the amount of a pesticide which has survived in the soil by the beginning of the next post-application year to the total amount of the preparation applied throughout the year (17). The experimental data required for its calculation may be easily obtained in conditions of a microfield test whose methods have been improved in the research institute of the USSR Academy of Sciences (18). Calculations carried out in accordance with the formula (17) demonstrate that with the value of the residual-load coefficient being in the range of 0.3-0.9,it will take three to seven years for the preparation concentration to double in the soil.

Finally, it is possible to co-measure the self-purification ability of the soil within a specific region with a load (or dose) of highly toxic pesticides which find their way into the landscape each year. On small-scale maps such data can be easily combined with the use of pesticides and conditions of their detoxication. Methods of drawing up such maps were described and several types of such small-scale maps were prepared by the research institute of the USSR Academy of Sciences jointly with research workers from Moscow University (16,17). At last, by taking the value of the seasonal and annual run-offs from the water-catchment area and knowing the average value of the debris-to-litter coefficient for the area, one can calculate the average annual and maximum possible discharges of pesticide residues into the water basin and their expected concentrations in the river estuary.

According to the calculations which took stock only of those pesticides which are used in agriculture, such USSR rivers as the Kura, Prut, Southern Bug, Amudarya and Dniester were effected the most (17). On the contrary, such rivers as the Volga, Ural, Rioni and Terek do not practically contain residues of highly toxic pesticides. Such rivers as the Don, Kuban and Syrdarya occupy an intermediate position in terms of their pollution with pesticide residues. In the USSR the basins of the Sea of Azov - the Black Sea, the Aral Sea - the Caspian Sea and the Baltic Sea are characterised by the maximum (calculated) load of residues of highly toxic pesticides.

The country's other water-basin areas may be called zones with minimum potential pesticide residues.

To sum up, the ecological and toxicological assessment of pesticides and a forecast of their effects includes the following basic stages:

- first, the classification of the entire range of pesticides used in specific countries from the point of view of ecology, agrochemistry, toxicology and hygiene;

- second, a series of tests (in laboratories and under the field conditions) involving primarily the compounds which are highly toxic for the biosphere and the preparation of their rates for soil application.

Any wide-scale use of pesticides must be proceeded by these two stages.

Finally, objective predictions for assessing the effects of the application of pesticides within a landscape, region or a river basin must be prepared for all those agricultural areas where ecologically highly toxic pesticides are widely used. Such predictions must by all means take into account the self-purification ability of individual landscapes and river basins.

As potential pollutants of the biosphere, pesticides hold a place at the end of the world-top-ten pollutants list. Such rating, of course, is conventional since some countries such as Japan and Italy, being the world's top pesticide manufacturers, apply some 16-18 kg of pesticides per hectare of arable land, whereas the figures for the U.S.A. and the U.S.S.R., themselves the world's major pesticide producers, are in the range of 2-2.5 kg/ha. Social and political considerations also add up to different and sometimes diametrically opposite attitudes towards the environmental pollution, including the pollution with pesticide residues.

There is no reason either to underestimate or overestimate a possible potential damage done by intensive applications of pesticides. Highly emotional statements on this issue may be found in the press both now and in the past. However, since pesticides and other agricultural chemicals are used for treating crops which are used as foodstuffs or fodder, pesticide residue concentrations must be strictly controlled in food-stuffs, soil, water, plants and other objects of the environment. The U.S.S.R. has the strictest requirements in this respect.

The characteristics of possible types of residual effects of these agricultural chemicals and an analysis of the data on pesticide monitoring available in the world, allow us to assume that at present we observe the residual pesticide effects on the landscape, regional and basin scale (20). The most pressing task of ecotoxicologists is to eliminate the residual pesticide effect on the global scale. To achieve this goal, intensive experiments, pesticide monitoring and the use of conceptual and mathematical modelling are needed. This information would allow to predict the fate and possible negative impacts produced by pesticides and other agrochemicals on the soil, other landscape elements and the biosphere as a whole.

References

1. Almeida, M.A.O., A Myth of the Equilibrium in Nature, UNESCO Courier, 1973, I, 25-28, in Russian.
2. The Fate, Transformation and Array of Pesticides and Their Metabolites in the Soil, Transactions of the First All-Union Conference, Pushchino-on-Oka, 1973, 13-16, 22-25, 27-29.
3. Goncharuk, E.I., Nashtein, S.Ya., Tsipriyan, V.I. Methods of Rating Toxic Substances in the Soil, Gigiena i sanitariya, 1974, 12, 19-23.
4. Perelygin, V.M., Theoretical Foundations of Hygienic Rating of Toxic Substances in the Soil, Gigiena i sanitariya, 1975, I, 29-33.
5. Anderson, J.R., Pesticide effects on non-target soil microorganisms. in: Pesticide Microbiology. Ed. by I.R.Hill, S.J.L. Whright, Academic Press, London, 1978, 313-533.
6. A List of Chemical and Biological Chemicals to Control Pests, Plant Diseases and Weeds, Including a List of Growth Regulators of Plants, Permitted for Agricultural Application in 1982-1985, Goschimkomissiya, Moscow, 1982, Part I and 2, pp. 126, 179.
7. Krechkovsky, E.A., Hygiene of Application of Agricultural Chemicals, Reference Book, Kiev, Zdorovye Publ., 1978, 237.
8. Sokolov, M.S., Strekozov, B.P., The Succession and Certain Principles of Rating Pesticides in the Soil, Khimiya

v selskom khozaistve, 1976, 5, 63-66.

9. Medved, L.I., Spynu, E.I., Sova, R.E., Calculated Hygienic Rating of Pesticides in the Soil, *Gigiena i sanitariya*, 1979, II, 22-24.
10. Metkampf, R.L., The Development of Target-Oriented and Biologically Decomposable Pesticides. in: The Strategy to Control Pests, Plant Diseases and Weeds in the Future, Moscow, Kolos Publ., 1977, 139-159.
11. Ananyeva, N.D., Galiulin, R.V., The Effects of 3,4-DCA on the Content of Saprophyte Bacteria in the Grey Forest Soil. *Khimiya v selskom khozaistve*, 1980, 2, 56-57.
12. Ananyeva, N.D., Sokolov, M.S., Tolstova L.A., The Accessibility of Propanide Bound with Clayish Materials and Pure Culture of *Pseudominas aurentiaca* I. in: Wirkungsmechanismen von Herbiciden und syntetischen Wachstumregulatoren. Herausgegeben von H.R.Schatte, 1979, S.235-243.
13. Galiulin, R.V., et al. The Decomposition of Lynuron, Monolynuron and Their Derivatives in Different Soils Depending on Temperature Conditions, *Agrokhimia*, 1979, 6, 106-116.
14. Rodin, L.E., Bazilevich, N.R., The Dynamics of Organic Matter in the Biological Turnover of Matter in the Basic Vegetative Species, Moscow-Leningrad, Nauka Publ., 1965, 253.
15. Glazovskaya, M.A., Technobiogeoms as the Basic Physico-Geographical Objects of the Landscape-Geographical Forecast, Geography Series, Moscow University Press, 1972, 6, 23-35.

16. Sokolov, M.S., Glazovskaya, M.A., Methods of Compiling Small-Scale Maps of the Use and Detoxication Conditions of Pesticides, in: Methods of Ecological and Toxicological Modelling and Forecasting, Pushchino, 1979, 20-29.

17. Sokolov M.S., Sokolova, E.B., et al., The Ecological and Agrochemical Assessment and Forecast of Pesticide Effects, Biology Series, USSR Acad. of Sciences Press, 1980, 5, 359-369.

18. Galiulin, R.V., Studies of the Fate of Xenobiotics Under Standard Soil Conditions, in: Problems and Methods of Ecological and Toxicological Modelling and Forecasting, VINITI, 1978, No. 532-78, 123-131.

19. Hamaker J.W., Mathematical Prediction of Cumulative Levels of Pesticides in Soil. in: Organic pesticides in the environment, 1966, 122-131.

20. Kovda V.A., et al., Pesticide Effects and Prediction of Polluting Land Areas with Their Residues, Biological Series, USSR Acad. of Sciences Press, 1977, No. 1, 120-124.

OCCUPATIONAL HEALTH ASPECTS OF PESTICIDE APPLICATION IN AGRICULTURE

Yu. I. Kundiev

In practice, an intoxication hazard depends not only on the toxicity of the agent that causes it, nor merely on its ability to enter the body by a particular route, but also on the sanitary and hygienic set of working conditions specific to its application. The extent of chemical contamination on the exposed parts of the workmen's body and clothes, as also the airborne amount of a pesticide in the working environment is decided, largely, by the treatment process applied, by design characteristics and the working condition of the equipment, plant and machinery used, and by compliance with safety rules and regulations.

In different countries safe working conditions are ensured through their national health services which may have some peculiarities. However, there exist some universal principles of preventive measures.

General Principles of Preventive Measures

The Soviet Union's system of effective hygienic, sanitary engineering and other measures is targeted on preventing the adverse effects of pesticides on workers handling them in agricultural field uses. Its key elements include hygiene-based selection of new pesticides, standardization of their application procedures, substitution of strong toxic agents by their less hazardous counterparts, improved operation of machinery and plant to meet current sanitary requirements, proper job management, and other considerations.

An important measure refers to the use of personal protective equipment and observance of personal hygiene.

Medved (1974) formulated the key features of pesticides and their application procedures essential for the understanding and development of preventive efforts.

The first feature relates to the impossibility of preventing pesticide circulation in the biosphere. Following crop spraying or dusting, incorporation into soil and other application techniques, the pesticides will persist in the environment until their complete disintegration.

The second feature has to do with the strong biological activity of pesticidal agents. As distinct from other chemicals, the pesticides are designated to kill unwanted live organisms by deliberately affecting the processes that support their vital activities. But because the various different classes of live organisms share in common the principal systems of their vital activities, pesticides are bound to affect adversely not merely the farm pests, but also man and domestic livestock.

Finally, the third feature addresses purposeful efforts to produce relatively high pesticide concentrations designed to wipe out pests in the areas near the objects being treated. Although the concentrations may pose risks to those occupationally exposed, still they cannot be lowered for failure to achieve the desired effect on crop production.

Hygiene-based Selection of Pesticides

The selection is based on the hygienic evaluation of pesticides available in their classification according to the basic criteria of harmfulness (health hazard).

If by any one of the criteria a chemical belongs to Class I of the hygienic classification, this means it is very hazardous for human beings and warm-blooded animals and not to be registered for use in farming practices. The Class 2 pesticides are permitted for limited use and those of Calsses 3 and 4 - for wide application, given strict compliance with the hygienic (health-based) regulations and standards. The decision to have a pesticide identified with a particular class is based on the limiting criterion.

The hygienic selection rules out the use in agricultural production of potent toxic chemicals - those with DL_{50} by intragastric exposure below 50 mg/kg; chemicals with strong skin-resorptive toxicity; highly volatile substances with a saturating concentration equal to or higher than their toxic concentration; supercumulative toxicants with a cumulation coefficient below unity; and very persistent compounds with over two years' period of decomposition into non-toxic components. It is not permitted to apply substances capable of deleterious long-term effects in their actually employed doses and concentrations (tumor growth, allergic effects, impairment of the reproductive function).

Substitution of Hazardous Pesticides

Toxic substances which are likely to be damaging to health or the environment have to be gradually withdrawn. The damage, though, has to be documented beyond doubt from clinical hygienic comparisons and the findings of epidemiological, ecological, etc. research efforts.

Two examples of implementing this measure were expulsion of DDT from agricultural uses in the USSR and substitution of mercaptophos for less toxic organophosphoric agents.

Hygienic Regulation of Pesticide Application

This, first of all involves the scientifically validated standards and rules whose strict observance can effectively prevent pesticide poisonings among the persons occupationally exposed to pesticides by handling them in the field, as well as pesticide contamination of foods, the air of population aggregates, water bodies, and soils. The methodological approaches to pesticide hazard evaluation are reviewed below.

Standards and Regulations

Under Soviet sanitary (health) legislation, a hygienic standard for the concentration of a harmful chemical in an environmental medium shall guarantee no diseases or deviations of the health status in the short and long term and no harmful effects on subsequent generations.

The hygienic standardization procedure for the pesticides consists of three basic stages, namely:

1. Validation of calculated hygienic standards using the physical and chemical constants of the subject chemicals and the findings of a short-term experiment.
2. A complete program of investigations to establish chronic action thresholds and examine long-term effects.
3. Hygienic study of pesticide concentrations in environmental objects and drawing clinical hygienic parallels with the major goal of correcting the hygienic standards.

The criteria currently used as official hygienic standards are tentative safe exposure levels (TSEL), approved for a term of two years. The work to establish TSELs for pesticides occurs usually at the same time with their State Acceptance Tests in agriculture. If the Tests yield positive results the TSELs of common pesticides are further replaced by MACs. Since for a chemical in limited use conducting the complete hygienic experiment is not practical its TSELs can be merely reapproved for a second term of service. Another procedure to establish a pesticide MAC, besides the development of a calculated hygienic standard, involves a shortened standard-setting program. This will be done whenever a new pesticide comes from their well-explored class or when standards for this pesticide have been established in some environment and need to be established for another environment.

The experiments carried out under the complete program include discovery of the threshold doses and concentrations in acute and chronic trials. The harmful action threshold in the chronic experiment, as defined by the limiting criterion of harmfulness, is taken as key to determining the hygienic standards. Its reduction many times over (a safety factor) to account for the substance's degree of hazard, differences in species-specific sensitivity, etc. permits prediction of maximum allowable safe (harmless) levels for human beings.

The hygienic standardization of pesticides must be an integrated procedure conducted simultaneously for different environmental objects. The core of this integrated procedure for hygienic standardization is identifying the maximum permissible dose for humans. It is calculated from the baseline subthreshold dose found in the chronic experiment by selection of appropriate safety factors (Kagan, 1981).

A measure that is singularly effective in setting hygienic standards for pesticides involves preliminary sanitary supervision over their early period of utilization in farming practices. The chemicals permitted for use are exclusively limited to those which appear in the List of Chemical and Biological Controls of Pests, Diseases and Weeds Recommended for Agricultural Use. The list is prepared by the State Commission for Chemical Controls of Plant Pests, Diseases and Weeds under the USSR Ministry of Agriculture. It is annually updated and coordinated with the USSR Ministry of Public Health. Not to be listed there are potent toxic chemicals (PTC) or the pesticides capable of inducing severe pathology, deleterious long-term effects, etc.

The List specifies the use rates of pesticides, the agricultural crops to be treated, treatment replications, and the date of the last treatment before harvesting. Pesticide inclusion into the List requires having the full set of toxicological experiment data and all the hygienic standards and specifications relative to the pesticide's concentrations in all environmental media.

As suggested by the extensive experience of Soviet hygienists and toxicologists, it is good and practical to exercise a differentiated approach to pesticide standardization.

At present, the scientific coordinating agency in charge of the research work on pesticide hygiene and toxicology includes in its organizational structure a special commission with the duty of hygienic standardization for pesticides in conditions of their field application. Recommendations have been developed showing ways to standardize the requirements for validating MACs of pesticides and listing the data needed to establish hygienic standards.

The differentiated (case-by-case) approach to imposing standards on specific field applications of pesticides in agriculture has been practised in the USSR since 1980.

The MACs for harmful substances present in the air of the work area, approved for use in agriculture are as follows:

afugan - 0.05 mg/m^3, hazard class I (appr. by Deputy Chief Sanitary Physician of the USSR, April 7, 1980, No. 21, 68. 80);
anilata - 10 mg/m^3, hazard class 3 -"-
euparene - 1 mg/m^3, hazard class 2 -"-
chloramp - 10 mg/m^3, hazard class 3 -"-
actellik - 2 mg/m^3, hazard class 3 (appr. by Chief Sanitary Physician of the USSR, February 25, 1961, No. 2502-81)
bronocot - 1 mg/m^3, hazard class 2 -"-
diquat - 0.05 mg/m^3, hazard class 1 -"-
phthalan - 0.5 mg/m^3, hazard class 1 -"-
amiben - 50 mg/m^3, hazard class 4 (appr. Deputy Chief Physician of the USSR, April 22, 1982);
heterophos - 0.05 mg/m^3, hazard class 1 -"-

In sum, ten MACs have been approved for pesticides in the air of the working zone as applied in agriculture.

Hygienic standardization for pesticide concentrations in soil is an important method to prevent noxious pesticide effect. The USSR has been the world's first country to pioneer the hygienic standardization of exogenous chemicals in soil, pesticides included (E. T. Goncharuk, 1977). Commencing from 1977, MACs and methods for determination in soil have been designed and endorsed for the following pesticides: dilor (0.5 mg/kg), heptachlor (0.05 mg/kg), zineb (1.8 mg/kg), metaphos (0.1 mg/kg), DDT (1.0 mg/kg). In all 25 pesticide MACs have been approved by now and in 1981 a standard listing the indices to judge the sanitary status of soil came into effect; its title GOST is 17.4.2.01-81. Pesticide concentration in soil is one among the indices in the standard.

As concerns prevention of occupational poisonings and obtaining safe quality foods, both are importantly linked with the so-called expectancy period (the time elapsed from the last pre-harvesting treatment of food crops until the harvesting date) and pesticide application rates. As new scientific data and field experiences become available, they are used to perfect the Sanitary Rules for Pesticide Storage, Transport and Application in Agriculture, along with a host of instructions, methodological guides, etc.

Updating methods and procedures for pesticide application and designing new machines and devices consistent with hygienic specifications have the dual purpose of minimizing air pollution in the working zone, exposed body parts and clothes of the workmen and environmental objects, as well as increasing efficiency and thereby decreasing the needed personnel who become occupationally exposed to toxic chemicals in their assigned jobs. Plants are treated nowadays using the airborne and ground devices capable of high-efficiency operation. Pesticide application by hand is prohibited and mechanization is extended from principal also to auxiliary operations. Expert evaluation is practised with regard to the machines and devices used for chemical plant protection.

6300

All of the plant-spraying and dusting equipment must be in good repair and carefully checked in advance, prior to field application. The entire process of filling the sprayers on spreader tank trucks and other hermetically sealed vessels with mechanically operated mixers needs to be fully mechanized procedure relying on pumps, ejectors, etc.

Plant protection methods and procedures are constantly updated and new chemicals are developed in a variety of forms (pellets, aerosols, microcapsules, etc.). Work is always in progress to design new agents, new processes and methods, and new applications, thus favourably influencing the sanitary-hygienic working conditions and occupational health.

One example is a change from the ordinary method of full-volume spraying to low-(LS) or ultralow-volume spraying (ULS) that changed fundamentally one of the most widely used pest control techniques. The key feature of the latter methods is a considerably reduced pesticide application rate (in litres per hectare) in conjunction with an increased concentration of the active ingredient in the solution. The pesticide agents thus used contain additives to improve on their dispersion, adhesion and several other physico-chemical properties that distinguish the agents from those used formerly. The application equipment, too, differs widely from the one previously used. In monitoring working conditions has to beat in mind that, because of the finely dispersed particles thus created, their time of persistence in the air increases a great deal (to 5.5 hours with 5 /uk) and so does the probability of their penetration into the body via the alveoli.

The method to control plant pests, weeds and diseases by pesticide incorporation into soil is gaining ever broader acceptance, because it cuts the pesticide expenditure and reduces the risk of air pollution in the working zone. The use of pelletized forms of pesticides considerably improves the working environment, and the scope of spraying application is being gradually narrowed.

The maximum safe sprayer designs are tractor-mounted air-blast and boom sprayers as well as devices operated under the lateral-blast principle. The sprayers are mounted on or operated in unit with tractors equipped with cabins. Hose sprayers seem impractical for hygienic reasons.

Pesticide applications in agriculture are unique in that the applicators are exposed, as a rule, to a set of different pesticides acting jointly. This is because, first, pesticides are normally used in a wide selection to suit different purposes (seed desinfection, crop spraying, soil fumigation, etc.); second, because the existing system requires alternating the agents in use so as to prevent the selection of insect forms resistant to particular pesticides; and, third, due to the application of composite chemicals with a view to instantaneous destruction of insects, fungi, etc. Agricultural workers such as croppers, horticulturists or livestock breeders are generally exposed intermittently, at varied intervals (days, weeks or months). Yet some of the employee groups like toxic chemicals storage personnel, agricultural aircraft crews or granaries fumigators experience, as a rule, simultaneous exposure to a set of pesticides.

Combined pesticide effects should be increasingly emphasized at present because of the current trend for expanding the use of composite pesticides. Soviet researchers gained evidence for their enhanced toxic effect with the intermittent mode of action or with alternating high- and low-level exposures.

Highly mechanized pesticide and mineral fertilizer applications, involvement of tractors and airplanes, working outdoors in all kinds of weather and all seasons of year exposes the workers, besides chemical impacts, to a number of physical factors like intensive noise, vibration, high and low temperatures, etc. The concomitant action of pesticides and physical factors aggravates the untoward effects of pesticides.

Observation many-year data indicate that, apart from pesticide handling (storage, transport, use), the subsequent operations of tending the treated crops present an added risk from residual amounts of pesticides and their metabolites. Such instances were reported from fields treated with polichloropinene, HCCH, and 2,4-D both, during the next few hours or days after application and several weeks later. This depends importantly on the properties of the pesticide in question, namely, persistence, volatility and metabolism. The conversion rate of pesticides is dictated by the type of soil, temperature and humidity. Other factors to consider are the replication of treatments, and the pesticide doses applied. For two treatments they are set up to be lower than for one treatment, the per hectare rates being the same. Higher air temperature contributes to faster pesticide break-down, with some of their metabolic products capable of causing intoxications. For example, PCP metabolizes to form chlorocyan, phosgene, hydrogen chloride and other highly toxic chemicals.

Widescale use of mineral fertilizers affects the environment and the exposed workers. Some mineral fertilizers containing ammonia or fluorine, if improperly stored, release as they decompose noxious vapors hazardous for persons exposed to them.

The research data gained in the recent years have provided the basic input for the Sanitary Rules Regarding Storage, Transport and Application of Pesticides (No. 1123-73 of September 20, 1973) and Mineral Fertilizer (No. 1049-73 of April 13, 1973), approved by the USSR Ministry of Public Health.

Hygienic Concerns in the Use of Toxic Chemicals in Animal Husbandry, Hot-houses, Conservatories and Seed Verification Laboratories

In animal husbandry pecticides are used as insect killers against blood-suckers, subcutaneous botflies, ectoparasites and also as means to control dermatoses and other diseases. They can be administered internally for therapeutic purposes or employed as local or general external applications by spraying, placing the animals into special baths, etc.

While treating rams with dicresyl, a derivative of methylcarbamic acid, by immersion into baths or spraying manually, its concentration within the workers' respiration areas is many times the MAC. Also, skin contamination of the personnel occurs. For these reasons, the animals should be treated by the mechanical method, outdoors or effectively in ventilated indoor rooms, at specially equipped sites (asphaltor cement - covered), with their subsequent thorough cleaning and detoxication.

In hot-houses and conservatories toxic chemicals are applied to treat the soil and the surfaces of growing plants by spraying, dusting and gassing, and to control soil pests and diseases. To improve the working conditions, pesticide formulation and application will have to be mechanized, rational work and rest procedures to be validated, carbon oxide concentrations to be lowered and the microclimate to be perfected by providing rational ventilation and controlling air humidity.

In seed verification laboratories the employees are likely to experience exposures to such chemicals as granosan, TMTD, heptachlor, and some other via the respiratory organs or through the skin. Again, rational airing and the use of personal protective equipment is necessary.

Hygienic requirements for toxic chemicals warehouses. Such warehouse should be located outside of population aggregates, at least one kilometer distant from residential buildings, livestock sheds, water supply sources, and other facilities. On special occasions, if so permitted by the health service, the warehouses may be operated in case of a sanitary protective zone less than one kilometre wide. Indoors, the warehouse should consist of at least two sections, a larger section for storing chemicals and a smaller section for lockers with the workmen's ordinary and working clothes, a shower, a wash stand, personal protective equipment and personal hygienic kits, a first-aid kit, and vacuum cleaners; a space must be set aside for keeping and storing records. The warehouse must be dry, well-illuminated and effectively ventilated, with walls covered by dust- and gas-proof lining easily washable. The floors should be smooth, cement or concrete. The personnel may be present only during actual operations (delivery and issue of chemicals, completing papers, etc.). The workday should not exceed six hours, or four hours where granosan is stored and handled.

Proper work management requires appointment of specialized teams to transport and apply pesticides and their regular instruction, skill upgrading, safety training, personal hygiene education, etc.

Preliminary and routine medical examinations are the most essential therapeutic preventive measure. They should cover both the full-time employees assigned to work with pesticides and those hired for seasonal jobs. The medical examinations of the workers are carried out at the local therapeutic-prophylactic establishments and guided by the official list of contra-undications against pesticide exposure.

In closing, it should be noted that until very recently the guiding criterion in designing preventive programs to provide a healthier working environment and in validating MACs has been, largely, pesticide toxicity. This is being revised to pay greater attention to other properties of a chemical agents, such as cumulation potential and the ability to cause long-term effects (malignant neoplasms and mutagenic, teratogenic and embryotoxic action). A great deal is expected from the findings of the epidemiological investigations now increasingly underway in the USSR and other countries.

COMPREHENSIVE HYGIENIC RATING OF PESTICIDES IN THE ENVIRONMENT

E.I.Spynu

Hygienic rating of pesticides was carried out for the first time in the world in the USSR in the 1930s (L.I.Medved). In the half-century since then the principles for elaborating these rates are being continuously improved. The basic principles for standardizing the methods of toxicological and hygienic regulation were outlined in the 1950s.

Much research effort was addressed to these problems in the 1960s. Realized at the same time was the idea of comprehensive hygienic rating of pesticides in all environmental objects.

The suggested principle of comprehensive rating of pesticides (E.I.Spynu, E.A.Antonovich and K.K.Vrochinsky, 1967) consists in that the composite content of a preparation in different media (food-stuff, water, air) should not exceed the maximal permissible daily dose for man (D_m, MPD). The D_m is an integral reflection of the biological activity of a substance with regard to its toxicity, its potentials to cause so-called long-term side effects (carcinogenic, mutagenic, embryotoxic, etc.), and of its comparative sensitivity in the human model.

Unlike the above described principle, the former practice of isolated rating of chemical substances in individual media envisaged substantiatine the rate of a substance in a given medium solely on the ground of its permissible dose, regardless of the fact that in reality the human body is subject to simultaneous entry of various chemicals from all the media. Such an approach is still practised by the FAO-WHO, since the established MPD values for pesticides are used when calculating rates for food-stuffs only.

We believe that the health of the population may be adequately protected only by obtaining information on the behaviour of chemical substances in different media and making a comprehensive assessment of their influence on the human body. What is needed is not only a quantitative and qualitative characterization of a given substance, but of the products of its transformation as well.

According to a number of authors (E.A.Antonovich, 1976; Dugan, 1973, and others), up to 7-10 various substances may be present at the same time in food-stuffs, water and the air. It has been demonstrated that upon the interaction of pesticides with environmental objects the following may take place:

- the active principle of a pesticide preparation may be retained in various media for a long time;
- it may undergo conversions with the formation of qualitatively new destructive products and metabolites differing in chemical structure, biological activity and stability. Thus, depending on the character of the process, these or other chemical substances and the products of their transformation may enter the human body together with food, water and air.

Mathematical modelling of the nature of the integral effect of various pesticides demonstrates that reactions of different type may be involved. Along with a cumulative build-up of the effect, its potentiation and antagonism were observed. Of no small importance is the route of entry of a substance into the body. The nature of interaction varies with different substances, which is apparently due to the specific features of their toxicodynamics. I.V. Sanotsky (1968) believes that most frequently occurring at the level of threshold concentrations is the summation or independent action of an effect. Yu.S. Kagan (1981) underlines that the summation of toxic effects has been established both for pesticides of similar chemical structure, and for those which are very different in structure but have a similar mechanism of toxic action. A.I.Korbakova (1972) maintains that for assessing the hazard of the combined effect of chemical substances one may use the principle of cumulative toxicity. Our analysis of the combined action of a number of pesticides prompts the opinion that summation is the predominent type of reactions in the multiple entry of small pesticide doses.

This makes it possible to represent the principle of comprehensive rating of pesticides as the formula:

$$D_m = \sum_{i=1}^{3} D_i$$

where D_m is the maximal permissible harmless dose;

D_1 is the pesticide dose in the food ration;

D_2 is the pesticide dose inhaled with atmospheric air;

D_3 is the pesticide dose ingested with water.

In accordance with the outlined principle of pesticide rating the following order of investigations is suggested.

The investigatory program includes the following stages:

1. The establishment of D_m in animal experiments (D_m/an.).
2. The establishment of D_m for man on the basis of materials on the comparative sensitivity of humans and animals to a chemical and determination of the corresponding correction r to D_m/an.

3. Establishing the actual contamination of environmental objects (K_1, K_2, ..., K_1) with a given pesticide, or making a relevant judgement according to analogous preparations with similar physico-chemical properties and chemical structure.

4. Calculating the rate for each medium (D_1), proceeding from the D_m/hu. and the K_1 value.

5. Comparing the D_m/hu. with the combined quantity of pesticides which may be taken up by the human body upon exposure to the air, water and food-stuffs.

Here three variants are possible:

(a) the combined pesticide intake by the human body is equal to, or less than D_m/hu.

(b) the combined pesticide intake by the human organism upon exposure to different media is greater than D_m/hu.

6. The recommendation of MAC and PRQ upon the condition indicated in point 5(a) is made, proceeding from the actual contamination levels of individual media.

Also taken into account here are data on the organoleptic properties of the water, food-stuffs and the air containing the test substance, and the effect of the detected quantities of substances is determined regarding indices characterizing the nutritive value of the food-stuffs, the sanitary condition of the reservoir, etc. Ratings are established by the critical indicator.

7. Given the condition indicated in point 5(b), the recommended MAC and PRQ values for the pesticides are below the detected level of actual contamination of different media. For reducing the pesticide content in the environmental objects down to the recommended values to the toxicological criterion, appro-

priate regulations regarding pesticide application should be introduced (limiting the objects to be treated, reducing expenditure rates, increasing the "expectation time", etc.).

Let us dwell briefly on the main stages of the suggested investigation program.

When establishing D_m for animals the most sensitive specific and non-specific tests should be used, allowing to detect the poison influence at the earliest stages of intoxication. The utilization of the ample experience accumulated in this field by Soviet and foreign researchers allows to resolve this task. We feel that, when selecting the tests, special attention should be given to the utilization of tests involving functional loads and additional introduction of the poison.

Much less elaborated are approaches to the establishment of harmless doses of chemicals for human beings. Very helpful in tackling this question may be a comparison of the responses of humans and animals to the effect of the pesticide in concentrations determined in the working zone during pesticide trials. The vital prerequisite of such animal experiments is the accurate reproduction of the pesticide concentrations and exposures recorded in production conditions. A comparison of the character of human and animal responses to the effect of the pesticide under the same conditions will provide some quantitatively expressed idea about their comparative sensitivity to a given poison. Toxicologists, however, have but few sensitive tests which can be used for investigations in humans. This is due to the fact that the considerable number of tests used in experiments with animals cannot be applied to the study of working people. The search for adequate tests should be stepped up and special attention, from our point of view, should be given to the study of the metabolism of poisons, to determining the content of the chemical and its metabolites in the blood and feces, depending on the degree and conditions of exposure to the chemical concerned. This will make it possible to establish the dependence between the quantitative content of substances in biological media and the severity of various functional disorders of the organism. Studies along this line at different stages of intoxication will permit to determine the content of the chemical in different body media of diagnostic importance. The value of this type of investigations is unquestionable.

Tangible help for determining the comparative sensitivity of humans and animals may come from an analysis of material about cases of human intoxication with the chemical concerned, or by compounds of the relevant chemical group as well as about the morbidity of persons having been exposed to these substances. The emphasis should be placed on a correlation between the actual conditions of exposure and the severity of the pathological disorders.

It is of great importance to investigate in man the irritating effect of small quantities of pesticides and their possible allergizing action.

The following materials are used when determining the D_m for humans - the D_m/hu. value; data on the severity of long-term side effects, findings on the comparative sensitivity of humans and animals, information about the persistence of the pesticide and its capacity to increase its concentration in the end links of food chains, and also information on the possibility and degree of pesticide excretion with breast milk.

The rating of pesticides in different media is carried out with account of the following basic factors: (a) the rates for food-stuffs are established for each kind of food, proceeding from their proportion in the diet so that the overall pesticide content in the whole food ration should not exceed the permissible daily dose; taken into account here (b) is the possibility of the transformation and metabolism of the pesticides in the process of technological and culinary treatment; (c) the MAC value in water is established (with account of the possible migration of pesticides throughout the reservoir at such a level that their content in fish and waterfowl should not exceed the PRQ (K.K.Vrochinsky, 1967); (d) when rating pesticides, data is taken into account about the organoleptic properties of water, food-stuffs and air, and also indicators characterizing the sanitary conditions of the water reservoirs, etc., the rates being established by the critical indicator.

Investigating the actual pesticide contamination of food-stuffs, water and the air should be one of the main tasks in the matter of pesticide rating. This is of special importance since, in accordance with the demands of hygienists, a number of low-toxic pesticides are at present being synthesized and introduced into agriculture. If, in the course of rate setting, one should be guided by the results of toxicological investigations alone, the PRQ and MAC values could be in some cases by one or even several orders of magnitude greater than the actual combined content of these substances in plants, water and air. In our opinion, such a view of the problem has no justification whatsoever. As a matter of fact, it is impossible to gain a thorough understanding of the diverse manifestations of the adverse effects of a new substance in the relatively short time available.

It is absolutely clear, that the tests used for rating and characterizing the effect of a new substance cannot rule out some other aspects of the compound's deleterious effect. This is strikingly demonstrated today in connection with the detection of many so-called side effects of pesticides (mutagenic, embryotoxic, teratogenic, carcinogenic, co-carcinogenic, allergic, etc.). Hence, the hygienists' concern with establishing the lowest possible rates of chemical substances in different media.

This prompts the suggestion that recommended rates should not be higher than the actual pesticide content in various media revealed when observing the conditions of their application. The selection of the rate should be made by the critical criterion (hygienic, toxicologic e.a.).

In the past 15 years rates have been set for 60 pesticides in different environmental objects, elaborated on the principle of comprehensive hygienic rating of pesticides. The work has been carried out by institutes concerned with the problem "Scientific Principles of the Hygiene and Toxicology of Pesticides, Polymers and Plastics".

On the basis of the aforementioned we believe that the suggested comprehensive approach to the rating of pesticides in environmental objects offers the following advantages over the current practice of separate rate-setting for pesticides in individual media.

1. The elaboration of the ratings takes into account the actual entry of a preparation into the body upon human exposure to different media.

2. The rates are established in accordance with the actual pesticide contamination of the environment.

3. The time of elaborating the maximal permissible doses and concentrations of chemicals in water, air and food-stuffs is considerably reduced.

Therefore, the suggested principle allows to establish hygienic rates in different environmental objects with greater certainty and lower expenditure of means and assets. The idea of comprehensive hygienic rating realizes the thesis set forth by N.S. Pravdin and N.V. Lazarev about the integrated triad: experiment - hygienic studies - clinical observations.

Along with the traditional rating of chemicals in foodstuffs, the air and water, operating in this country since 1978 are rating for pesticides in the soil. Since the world at large lacks such hygienic regulation, let us shed more light on the matter.

It has been established that in the process of agricultural cultivation about half the chemicals used get onto the top layer of the soil. Also flowing onto the soil are preparations washed by rain from the surface of plants, while some pesticides are brought directly into the soil when they are introduced with treated (disinfected) seeds and by other routes. As a result the soil, in comparison to other objects of the biosphere, is a place of maximal accumulation of stable substances. The soil, in addition to being a depository, is also an active link in the complex chains of pesticide migration in the environment (soil-water-food and other chains).

Available at present are numerous data (hundreds of thousands of analyses), both in the Soviet and in the foreign literature, concerning the actual soil contamination with pesticides. The quantities of pesticides in the soil vary from hundredth parts to units of mg/kg. The time of their retention in the soil may be counted in months and years.

An analysis of the quantitative aspects of pesticide migration in the soil-water, soil-air, and soil-plant chains indicated that one of the most important factors determining the possibility and degree of pesticide penetration into contact media is their content in the soil. However, it is quite impossible to evaluate the pesticide content in the soil of our planet from the hygienic standpoint for want of established hygienic standards. While these are lacking the implementation of appropriate measures to prevent the accumulation of stable pesticides in the soil and, indeed, in the biosphere as a whole is impossible. The need to establish admissible pesticide contents for the soil is obvious.

We have suggested a principle for the hygienic rating of permissible pesticide contents in the soil; it consists in determining the safe pesticide quantities in it, at which the levels of preparations in the media in contact with the soil will not present a human health hazard, nor will they negatively affect the soil general sanitary indices (E.I. Spynu et al., 1970). The basic criteria for evaluating the hazard of pesticide contents in the soil are the degree of contamination of plants, water and the air, and also changes in the general sanitary condition of the soil.

Taken for the standard rating is such a content of the pesticide in the soil when its content in the media exposed to it does not exceed the permissible residual quantities established for it, nor does it impair the general sanitary conditions.

For establishing the maximal permissible contents of preparations in the soil, a study is made of the quantitative correlations between the pesticides' concentration in the soil and their penetration into the vegetation, the air and water in contact with the soil concerned. This takes into consideration the influence of the following factors on the degree and nature of migration of preparations through the soil-water, soil-air, and soil-plant chains:

1) conditions of application (rate of expenditure, frequency of treatment, form of preparation);

2) the physico-chemical properties of the pesticides (solubility in water, in fats, volatility, stability at different pH values, etc.);

3) special features of the media (type of soil, humidity, relief of terrain, species and varietal features of plants, depth of ground waters, etc.);

4) climatic conditions (temperature of the soil and air, the rainfall, etc.).

The studies were conducted with the use of the preparations under production conditions, and also in experiment (lysimeters, vegetation vessels, soil columns, etc.).

Experimental rating in the soil consists of the establishment of four indicators, reflecting transition and interactions within the soil-plant, soil-air, soil-water and soil-microorganisms systems. The rating is set by the critical indicator.

The program for pesticide rating in the soil includes the following stages:

1. Carrying out studies in natural and experimental conditions.

Experimentally established are:

(a) the stability of the pesticide in the soil depending on multifactorial influences;

(b) quantitative aspects of its migration within the soil-air, soil-water and soil-plants ecosystems;

(c) the degree of influence on the sanitary conditions of the soil.

Studies in natural conditions are:

(a) the actual content of the pesticide and products of its transformation in the soil with account of the preparation's migration into different media and the factors determining these prodesses;

(b) the influence of the preparation on the biological activity of the soil.

2. An analysis of the results of the natural and experimental studies for selecting the critical indicator and establishing the MAC value.

3. The development of regulations ensuring the MPC of the study pesticide in the soil.

The availability of pesticide ratings for soil makes it possible to assess from hygienic positions their actual content and elaborate measures to prevent the contamination of individual elements of the environment by establishing appropriate regulations and recommendations (expenditure rates and frequency of application of preparations, plant species grown on soils containing an increased quantity of pesticides, calendar dates for carrying out operations and the time-limits of conducting agrotechnical measures connected with the loosening of the soil).

Here is a list of the official ratings for the content of pesticides in the soil.

Maximal Allowable Concentrations of Chemicals in Soil (MAC)

Nos.	Pesticide	MAC, mg/kg of soil	Limiting index
1.	Atrazine	0.5	Translocational (soil-to-plant transfer)
2.*	Atrazine	0.01	Phytotoxic
3.	Basudin	0.2	Translocational
4.	Banvel D	0.25	"
5.	Betanal	0.25	"
6.	HCBD (Hexachlorbutadiene)	0.5	"
7.	HCCH (Hexachloran)	0.1	"
8.	-HCCH (Lindane)	0.1	"
9.	Dalapon	0.5	"
10.	DDT and its metabolites (sum total)	0.1	"
11.	Dilor	0.5	"
12.	Gardona	1.4	"
13.	Heptachlor	0.05	"
14.	Malathion	2.0	"
15.	Kelthane	1.0	"
16.	Linuron	1.0	"
17.	Methyl parathion	0.1	"
18.	Polychlorocamphene	0.5	"
19.	Polychloropinene	0.5	"
20.	Prometrin	0.5	"
21.	Propanide	1.5	"
22.	Rogor	0.3	"
23.	Sevin	0.05	"
24.	Simazine	0.2	"
25.*	Simazine	0.01	Phytotoxic
26.	Fenuron	1.8	Aqueous
27.	Fozalon	0.5	Translocational
28.	Phthalophos	0.1	"
29.	Chloramp	0.05	"
30.	Dipterex	0.5	"
31.	Zineb	1.8	"

Footnote:

*1 - an MAC value of 0.01 mg/kg is recommended for soils intended for the cultivation of crops sensitive to Simazine and Atrazine: cereals (oats, wheat, rye, barley), vegetables (cucumbers, cabbages), technical crops (sugar beet, sunflower), fodder crops (vetch-oats mixture, alfalfa, winter rape).

2 - the presence of Simazine and Atrazine residues in soils intended for tobacco cultivation is impermissible.

Vigorous work is going on towards the further substantiation of rates for chemical substances in the soil (E.J.Goncharuk, 1981; V.M. Perelygin, N.I. Tonkopy, 1978; S.J.).

Calculating the Actual Pesticide Intake by Man

The above described approach to determining the actual dose of pesticide (D_a) finding its way into the human organism (with food, water and atmospheric air) is reflected by the following formula:

$$D_a = \sum_{j=1}^{3} D_j \qquad (1)$$

where D_j is the dose entering the human body with the j-th object (food-stuffs, water, atmospheric air).

The possible intake of a pesticide with food is calculated, proceeding from the residual quantities of the preparation in the concrete food-stuff with account of its proportion in the daily food ration of a definite population group:

$$D_1 = \sum_{j=1}^{n} C_j Q_j (1 - q_j) \qquad (2)$$

where D_1 is the daily pesticide dose that may be ingested with food-stuffs (mg);

C_j is the concentration of the preparation in the j-th product (mg/kg);

Q_j is the quantity of the j-th product in the daily food ration (kg), determined either by human physiological needs, or by a special study of the question;

q_j is the coefficient of pesticide destruction in the process of culinary treatment of the j-th product.

The amount of pesticide introduced into the organism with drinking water may be described by the equation:

$$D_2 = C_w N(1 - \zeta) \qquad (3)$$

where C_w is the concentration of the preparation in the water of the local water source (mg/l);

N is the daily rated water consumption by man (l);

ζ is the coefficient of pesticide destruction in the water mains purification plants in the process of water treatment.*

The next formula (4) is used for determining the pesticide dose which may enter the human organism with atmospheric air:

$$D_3 = C_v V k_v \qquad (4)$$

where C_v is the pesticide concentration in atmospheric air (mg/m^3);

V is a man's daily air exchange (m^3);

k_v is the pesticide absorption coefficient in the respiratory paths.**

---

* The destruction coefficients (q and ζ) are taken into account for pesticides for which appropriate information is available. When unavailable, the coefficient is taken for 0.

** When this information is unavailable the coefficient is taken for 1.

Thus we arrive at a general formula for calculating the real (actual) pesticide dose entering the human body:

$$D_a = \frac{C_j Q_j (1 - q_j) + C_w N(1 = \zeta) + C_v V k_v}{M} \qquad (5)$$

where M is the average weight of a person (in the given group of the population) in kg.

Approaches to the Assessment of the Actual Pesticide Intake in Humans

The combined pesticide dose entering the human body may be calculated on the basis of the mean or maximal levels of contamination of environmental objects. It is advisable to use maximal values only for calculating highly dangerous pesticides (with likely long-term consequences, etc.).

The existing levels of pesticide contamination of the biosphere should be assessed by comparing the actual dose (D_a) of pesticide residuals intake from different media against the maximal permissible daily dose of the preparation for man (D_m), which is an integral reflection of the substance's biological activity with account of its toxicity, its potential for causing so-called long-term side effects, and also the comparative sensitivity in the human and animal models:

$$APL_j = D_a \cdot D_{ij}^{-1} \qquad (6)$$

where APL_j is the actual pesticide load;

$D_a D_{ij}$ are, respectively, the actual and the maximal admissible intake of the j-th pesticide.

At present, Soviet and foreign investigators (annual reports by the FAO/WHO Expert Committee) have substantiated the D_m values for more than 60 pesticides.

In cases when $D_a < D_1$ it can be maintained that the system of pesticide application accepted under the conditions of the given region does not present any substantial risk to the population group concerned. If, however, $D_a > D_1$, the degree of the danger related to such an excess must be determined. Bearing in mind that during the transition from the subthreshold dose in the chronic experiment to the D magnitude, a safety margin of 30 to 200 is used; the following classification is suggested.

The contamination of environment objects is recognized to be:

1) potentially dangerous when $APL = 1$-3, i.e. D_a exceeds D_m from 1 to 3 times;

2) dangerous when $3 < APL \leq 10$;

3) highly dangerous when $APL > 10$.

Guided by the feedback principle, at $APL > 1$ more rigid regulation is necessary, and possibly a revision of the conditions of pesticide application. Let us examine the latter case in greater detail.

When calculating the levels of actual pesticide intake by man, it is established which food-stuff or environmental object is the main source of this intake. Next analyzed are the cause-and-effect relationship between the conditions of pesticide application and the degree of contamination. The results of this analysis serve as the basis for correcting hygienic ratings for the content of the preparation in environmental objects (the air, water, soil) and food-stuffs, and also the hygienic regulations ("expectation time", etc.) and conditions of pesticide application.

One should bear in mind that residual quantities of 7 to 10 pesticides and their metabolites may be detected simultaneously in the food ration, in the water and air. It is advisable, therefore, to evaluate the combined action of the pesticides on the human organism with regard to the partial contribution of each preparation to the overall actual pesticide load (APL_{Σ}).

The majority of investigators today point out that, given the combined effect of chemical substances at the threshold level, predominantly observed is cumulation, or "independent" action. A review of data on the combined effect of pesticides (Yu.S. Kagan, 1981) in the main confirmed this regularity. Since the potentiation of toxicity in combined action has been established only for some pesticides (γ-HCCH and TMTD, Sevin and Butiphos), we suggest the utilization of the summation principle for the hygienic assessment of the combined action of pesticides. Taking account of this principle, we use the following formula for calculating the APL_{Σ}:

$$APL_{\Sigma} = \sum D_{aj} \cdot D_{ij}^{-1} \qquad (7)$$

where APL_{Σ} is the summary actual pesticide load;

D_{aj}, D_{ij} are, correspondingly the actual intake and the maximal permissible dose of the j-th pesticide.

For evaluating the danger of the APL_{Σ} the same classification should be adopted, we believe, as for the APL_i, that is, in cases of determining the hazard of overall contamination with one pesticide.

6300

An Example of Calculating the Actual Pesticide Intake by Man

Let us assume that when studying the actual pesticide contamination of environmental objects, phosphamide was detected simultaneously in food-stuffs, in the water of the reservoir serving as the source for drinking and domestic needs, and in the atmospheric air (see Table). In order to calculate the pesticide intake injested with food-stuffs, we determine the quantity of food-stuffs containing phosphamide in the daily food ration. Since beet-root is subjected to cooking, we take into account the coefficient of the preparation's destruction, which is 1.0.

Initial Data for Calculating D_a. Degree of Phosphamide Contamination of Food-Stuffs and Environmental Objects

Nos.	Food-stuffs and environmental objects	Concentration of phosphamide
1.	Cabbage	0.55 mg/kg
2.	Beet-root	0.06 mg/kg
3.	Cucumbers	0.18 mg/kg
4.	Apples	0.30 mg/kg
5.	Cherries	0.31 mg/kg
6.	Water in reservoir	0.012 mg/l
7.	Atmospheric air	0.009 mg/m^3

When calculating the intakes of the preparation by the organism with water and atmospheric air, we take the daily water consumption to be 3 l, the daily air exchange as 12 m^3, the phosphamide destruction coefficient in water treatment as equal to 0.65. We take into account that at the concentration detected in the atmospheric air (0.009 mg/m^3) the pesticide will be entirely absorbed in the respiratory paths, i.e., $k_v = 1.0$.

According to the FAO/WHO recommendation, we assume the weight of the human body to be 60 kg.

Substituting into formula (5) the values of phosphamide contamination of the food-stuffs, water and atmospheric air, the daily consumption of food-stuffs, water and air exchange, as well as the appropriate coefficients, we calculate the actual pesticide intake of the human organism:

$$
\begin{aligned}
D_a = {} & \frac{0.55\ mg/kg \cdot 0.2\ kg + 0.06\ mg/kg \cdot 0.05\ (1.0 - 1.0) +}{60\ kg} \\
& \frac{+\ 0.18\ mg/kg \cdot 0.1\ kg + 0.3\ mg/kg \cdot 0.15\ kg + 0.31\ mg/kg \cdot}{60\ kg} \\
& \frac{\cdot\ 0.03\ kg + 0.012\ mg/l \cdot 3\ l(1 - 0.65) + 0.009\ mg/m^3 \cdot}{60\ kg} \\
& \frac{\cdot\ 12\ m^3 \cdot 1}{60\ kg} = 0.005\ mg/kg.
\end{aligned}
$$

A comparison of D_a with the maximal admissible daily dose of phosphamide for man recommended by the FAO/WHO at the 0.02 mg/kg level, demonstrates that in the example presented, the current practice of pesticide application poses no significant risk to the health of the population. The actual pesticide load in the example examined will be:

$$APL_{phos} = 0.005 \cdot 0.002^{-1} = 0.25$$

An Example for Calculating the Total Actual Pesticide Load

Let us assume that a certain group of the population is being exposed to the combined action of three pesticides - phosphamide, dipterex and hexachloran. As has been established above, the APL for phosphamide is 0.25. Let us further assume that the actual dipterex intake for the same group of the population is 0.003 mg/kg, and that of hexachloran is 0.001 mg/kg. The respective APL will then be:

$$APL_{Dip} = 0.003 \cdot 0.005^{-1} = 0.6$$

$$APL_{HCCH} = 0.001 \cdot 0.002^{-1} = 0.5$$

Hence the total actual pesticide load (phosphamide, Dipterex and hexachloran) will be:

$$APL_{\Sigma} = 0.25 + 0.6 + 0.5 = 1.35$$

Since the total actual pesticide load exceeded the accepted margin (1), it may be maintained that this group of the population is exposed to the effect of preparations at a higher than safe level, and thus the degree of pesticide contamination of environmental objects is assessed as potentially dangerous. The case calls for an analysis of the causes responsible for the increased pesticide intake by the human body, first of all, of Dipterex, whose proportion in the total actual pesticide load is the highest. On the basis of such an analysis preventive measures are developed.

Thus, comprehensive rating of pesticides in environmental objects, on the one hand, and the all-round evaluation of the chemical contamination of the biosphere, on the other, will make it possible to reveal the critical factors, to establish the ways in which they affect man, and to control on this basis, the quality of the environment for purposes of protecting the public health.

REFERENCES

Balk F., Koeman J.H. (1984). Future hazards from pesticide use with special reference to West Africa and Southeast Asia. For the Group on Environmental Pollutants of the IUCN Commission on Ecology and the World Wildlife Fund. Commission on Ecology Papers Number 6, Gland, Switzerland.

FAO Plant Production and Protection Paper 28, Second Expert Consultation on Environmental Criteria for Registration of Pesticides, FAO, Rome, 1981.

Koeman J.H. (1982). Ecotoxicological evaluation: The ecoside of the problem. Ecotoxicol.Environ.Safety, 6, 358-362.

CUTANEOUS ABSORPTION OF PESTICIDES AND PREVENTION OF POISONING

Yu. I. Kundiyev

According to statistical data reported by different countries, the inhalation of harmful substances present in the air of the working zone accounts for 80-90 per cent of all occupational poisonings in industry. At the same time inhalation makes up only 27% per cent of all occupational poisonings by pesticides in agriculture. The rest is either the result of cutaneous absorption (33%) or the co-called combined cases (about 40%).

A variety of factors are responsible for the cutaneous absorption of a substance, with the substance properties playing an important role, too.

The substances soluble in fats and lipoids are known to be readily absorbed through the skin. With a few exceptions, electrolytes either do not penetrate through the mammalian skin at all or their absorbtion is very weak.

E. Overton (1924) believed cutaneous absorption to be controlled by the fat solubility of substances. The above statement was based on the assumption that cell membranes consisted of lipoids. Overton's theory established a quantitative dependence of absorption through any cell membrane on the degree of substance solubility in lipoids.

Later on that theory was criticized, and numerous exceptions to the rule of Overton were found (M. Jacobs, 1924, et al.) Still subsequent investigations revealed that lipoid-soluble substances readily penetrate through the skin, as well as through other cell membranes, whereas the substances insoluble in lipoids fail to be absorbed. The quantitative dependence between the solubility degree and absorption cannot be considered constant. Certain substances, distinguished by slight solubility in lipoids, are absorbed more readily than well soluble ones. It has been proposed that a high solubility of substances in fats, combined with a certain degree of water solubility, is the optimum condition for cutaneous absorption.

Today, this point of view is generally accepted. There are numerous examples indicative of the fact that not only fat but also water solubility are important for absorbing any substance through the skin.

Thus, it is not the degree of fat solubility but the partition coefficient in the oil-water system that does play an important role in the cutaneous absorption of substances.

Apart from partition coefficient, other factors of physi. chemical properties of substances are of prime importance, in particular, the size of a molecule, its steric configuration, the presence or absence of symmetry. A. Giese (1959) provides data pointing to the greater significance of the partition coefficient. However, all other conditions being equal, differences in the molecular size, even if they are small, are of certain importance for absorption.

For toxicologists and pharmacologists, absorption of chemical substances through the skin essentially implies that from the site of their application on or contact with the skin surface these substances, either through the stratum corneum and deeper layers or through the hair follicles and the excretory ducts of the sebaceous and sweat glands, find their way into the dermis, then into the blood flow, and affect the body. Proceeding from this assumption it is expedient to discuss henceforth the methodological questions pertaining to the cutaneous absorption of chemicals.

All methods to be employed for this analysis could be classified into three groups.

The first group methods are based on detection of cutaneously absorbed substances or their breakdown products in the fluids and tissues of the body; the second group methods - on identification of the body general or local reactions triggered by the cutaneously absorbed substance; the third group methods - on measurement of the substance elimination from the application site.

The methods of the first group can be used not only to ascertain the fact of cutaneous absorption, but also to determine its absorption rate.

Albino mice, rats, guinea-pigs, rabbits and cats are most commonly used as experimental animals. One should take into account, however, that structurally and functionally the skin of these animals differs appreciably from the human skin. According to Stejskal (1928) and other authors, owing to the great thickness of the stratum corneum and the entire epidermis, the great length and tortuosity of the gland excretory ducts, the human skin is less permeable as compared to the skin of a large variety of laboratory animals. In permeability, the human skin is approximated only by that of suckling-pigs (R. Tregear, 1961).

Numerous researchers emphasize that to ascertain whether the substance is cutaneously absorbed in effective amounts or not it is more advantageous to use smaller test animals since they show the highest body surface-to-weight ratio (the unit of the body weight accounts for a larger area of the integument).

Which substances then may virtually pose a hazard of poisoning as a result of their absorption through the intact skin? First of all, these are the substances possessing a certain degree of toxicity. In this case, considering a comparatively modest rate of cutaneous absorption against, for instance, that of pulmonary absorption, these substances should be capable of causing poisoning when absorbed in minute amounts, i.e. their toxicity should be extremely high. The rate of absorption of these substances through the skin is often compared with the absorption rate from the alimentary tract. Of great importance is the fact that the ability of these substances to dissolve in fats and lipoids is combined with water solubility. A certain role is played by the substance consistence or form in which it is encountered in the working environment. All other conditions being equal, viscous, gluey liquids pose a serious hazard, for they easily stick to the skin and are securely held by it. A variety of physical and chemical processes are responsible for the mechanism of the substance fixation on the skin. Probably adhesion forces, electrostatic attraction and adsorption on the surface skin structures play an important role. One should also bear in mind a possibility of chemical interaction of substances on the skin surface (chemisorption, formation of complex compounds, etc.).

6300

Of course, it is important to consider other properties, too. If a substance penetrates through the skin, its action will manifest itself after accumulation of an effective toxic dose, which is known to be associated with the processes of biotransformations and excretion. A quantitative aspect of these processes is well described by A. Golubev, E. Lyublina, N. Tolokontsev, and V. Filov (1973).

It the 1930s N. Lazarev and co-authors, S. Rothman and other researchers studied various groups of chemicals as regards the risks of poisoning in case of absorption through the intact skin. It was noted, that among these groups of chemical substances, hydrocarbons, chlorohydrocarbons, ethers, alcohols, esters, metallo-organic and sulfurous organic compounds, aromatic amino- and nitro compounds were capable of being cutaneously absorbed. However, only some of the above-mentioned groups pose a practical danger of poisoning through the skin. Thus, because of low toxicity, hydrocarbons, ethers and esters, and alcohols constitute no hazard. Owing to high volatility, chlorohydrocarbons, sulfurous organic compounds are much more hazardous when absorbed through the lungs. Only aromatic amino- and nitrocompounds, as well as metallo-organic compounds were classed at that time with the hazardous substances causing poisoning through the skin. And the interesting thing is that with respect to metallo-organic substances N. Lazarev correctly predicted that the chemicals belonging to this particular group would be able to induce chronic occupational poisonings when absorbed cutaneously. As it will be demonstrated below, this prediction has been fully confirmed.

A dramatic flourish of chemistry in the last decades has given rise to a large number of new substances, including those belonging to the groups earlier studied by N. Lazarev. To illustrate, N. Lazarev pointed out that because of low toxicity and volatility esters are not hazardous for humans from the viewpoint of cutaneous poisoning. Today there have emerged new low-volatile esters of phosphoric and fluoric acids which are extremely hazardous owing to their high toxicity combined with their ability to penetrate through the skin, as well as such highly toxic chlorohydrocarbons as the organochlorine compounds of diene synthesis.

In this connection the necessity arises for discussing some groups of chemical substances again as regards the risks of cutaneous poisoning.

The physical properties of nitro and amino compounds of the aromatic series possess a large variety of physical properties, with low volatility being common to them. They are either high-boiling liquids or crystal substances, this fact being of prime importance for comprehending the possibilities of poisoning by these substances. Their low volatility is often combined with high toxicity, and, consequently, cutaneous poisoning is more probable than poisoning through vapor inhalation.

The representatives of this class of substances, dinitrophenol compounds, find use in agriculture as herbicides.

According to the data of E. Burkatskaya (1974), dinitrophenols show varied toxicity, when applied to the skin. Dinitroorthocresol is least toxic with this pathway: the dose of 500 mg/kg has been found to kill no rats, while the dose of 1135 mg/kg has been lethal to 50% of test animals (rabbits). The LD_{50} value of dinitrophenol for rats has been found to equal 540 mg/kg. According to the evidence of the author, the most toxic are dinitropropylphenol and dinitrofluorine-butylphenol. When applied to the skin in doses of 300-500 mg/kg, these substances kill most experimental animals.

Comparison of the degree of toxicity of DNP, DNOC and DNBP applied to the skin with partition coefficients in the oil-water system has demonstrated an important fact: among these substances, DNBP shows the highest toxicity, when cutaneously absorbed; its partition coefficient has an average value (partition coefficients equal 3.3, 460, and 120, respectively).

Of great interest are the data on contamination of the skin in agricultural workers handling dinitrophenol pesticides. Batchelor (1956) found that in one hour an average of 63.2 mg of DNOC settle on the open parts of the skin of the workers spraying fruit trees with the chemical, whereas only 0.4 mg is inhaled during same period. Much the same results were obtained by H. Wolfe and associates (1961) when they observed workers exposed to DNBP: in one hour 88.7 mg of the chemical settled on the skin, whereas only 0.18 mg were inhaled.

The relevant literature provides reports on poisonings of people handling dinitrophenol herbicides.

It should be emphasized that in the case of percutaneous absorption many nitro and amino compounds of the aromatic series produce not only systemic resorptive but markedly local effects. Thus, in humans 2,4-dinitrophenol, 2,4-dinitrochlorobenzene, 2,4,6-trinitrotoluene, nitroanilines and other substances induce burning, dermatitis, and sometimes chronic eczema .Other chemicals such as nitrobenzene, dinitrocresols, aniline, and xylidine are absorbed asymptomatically, and the fact that they have penetrated the skin may be overlooked.

Starting from the 1940s, i.e. from the beginning of a wide practical use of organophosphorus compounds (OPC), the literature has provided data on the ability of these chemicals to penetrate the intact skin in the amounts poisoning and killing animals. In 1943 H. Hodge and J. Sterner found that triorthocresylphosphate easily penetrates the intact skin of the vertebrates. In 1949, A. Lehman studied the toxicity of the thiophos insecticide when applied to animal skin. Since then, Soviet and foreign literature has provided a great body of data on the toxicity of OPC when absorbed via this route. These data have been generalized by T. Fredriksson (1958), F. Malkinson (1960), and Yu. Kagan (1963).

The majority of the authors engaged in the study of the OPI toxicity in the case of cutaneous absorption emphasize that the chemicals produce no irritant effect. Consequently, their cutaneous absorption occurs asymptomatically and may remain unnoticed until resorptive action takes places.

An extensive application of OPI entailed a multitude of insecticide poisoning cases. H. Abrams, D. Hamblin, J. Marchand (1950), P. Bidstrup and co-authors (1950), H. Desoille and co-authors (1951), L. Teleky (1955) and other researchers reported tens and hundreds of OPI, largely of thiophos, poisonings which became very common abroad.

S. Okinaka (1955) reported 1,777 cases of poisoning out of which 306 were fatal. In 1957 K. Ueda made public the evidence on 7,047 poisoning incidents occurred in Japan, one-third of them being fatal.

Yu. Kagan (1963) drew up an inventory of OPI poisonings which had occurred in various countries of the world, incroporating as many as 12,000 cases. This inventory attests that poisonings were reported in all countries either producing organophosphorus insecticides or utilizing them to control farm crop pests.

V. Polchenko (1972) carried out case studies of 18,394 insecticide poisoning cases reported abroad of which 12,517 cases were the result of the OPC effects.

Of great interest are reports on poisonings which occurred in California where pesticides are most commonly utilized. In 1960 G. Kleiman, J. West, and M. Augustine reported that OPI, and most frequently thiophos, mercaptophos and TEPP, were responsible for one-third of all occupational diseases. In 1962 this state reported 827 poisoning cases caused by insecticides and other agricultural chemicals. Thiophos, phosdrine and carbophos poisonings are most frequently reported. Absorption through the skin is considered responsible for 60% of all poisoning incidents. Most of the poisoning cases have been registered among citrus pickers (Reports, 1964).

Foreign literature on OPI occupational poisonings evidences that most of the cases arise from application of such chemicals as thiphos, mercaptophos, phosdrine, and trithion which are highly toxic and readily absorbed through the skin. Many poisonings have resulted from violation of safety rules and careless handling of these highly toxic chemicals.

Improved pesticide management in agriculture, adequate instruction of population and other measures yield positive results. Thus, in 1953-1954 in Japan the annual number of nonfatal thiophos poisonings equalled 1,800 whereas in 1959-1960 this figure did not exceed 550 (K. Ueda, 1962). However, many countries, including developed ones, still report a large number of poisoning cases. Today Japan annually registers an average of 300 thiophos poisonings. K. Ueda (1966) emphasizes that inadequate system of preventive measures hampers further reduction of the poisoning level in that country. The resorption of thiophos through the skin is primarily responsible for poisoning cases.

Over the last 20-30 years a variety of chlorinated alicyclic and aromatic hydrocarbons which are comparatively low-volatile crystal solids or liquids have found wide practical applications largely as pesticides.

Several poisoning cases have been reported among industrial and agricultural workers as a result of cutaneous absorption of hexachlorane γ-isomer (Report to the Council of Pharmacy and Chemistry, 1951).

Attempts have been made abroad to use hexachlorane γ-isomer as an antiscablous agent. Such applications of the chemical in effective insecticidal doses have been found to be a hazard of systemic toxic effects owing to a comparatively large degree of resorption through the skin (R. Horton, and co-authors, 1948).

Polychlorocyclodienes, otherwise known as the diene synthesis chemicals, are the most hazardous of this group of substances, due to their physico-chemical properties. Most of the diene synthesis insecticides are low-volatile compounds, readily soluble in fats, benzene, xylene, cyclohexane, but only slightly soluble in ethyl alcohol and almost insoluble in water. They are also distinguished by high stability in soil and other environmental media. Of these chemicals, alodane, chlordane, heptachlor, aldrin, dieldrin, and endrin should be primarily referred to.

When absorbed through the skin, the lethal doses of aldrin, dieldrin, endrin and other chemicals are of the same order of magnitude as in the case of oral administration (the cutaneous-oral coefficient approximates unity).

Despite the fact that these chemicals were used in agricultural practice for a short time, foreign literature mentioned hundreds of human poisonings with dieldrin, chlordane, endrin and other substances. Many of these cases resulted from contamination with pesticides of the skin or clothes of workers.

The application of such diene pesticides as aldrin, dieldrin, endrin, and isodrin has been prohibited in Soviet farming because of their high toxicity, especially in the case of cutaneous absorption, and pronounced cumulative properties.

Among compounds of other classes utilized as pesticides, organic compounds of mercury pose severe hazard to human health. It was found that these compounds are capable of entering the body through the intact skin in the amounts which would poison or kill test animals. However, pertinent literature provides no evidence as to the toxicity parameters of mercury organic compounds, when absorbed cutaneously. Much more attention is given to toxicity determination when chemicals are inhaled as vapors or dust. This is quite natural, for mercury compounds display a comparatively high volatility.

Extensive use has recently been made of liquid mercury-containing seed-treating materials. Other conditions being equal, liquid chemicals are known to be more hazardous than dusts from the viewpoint of percutaneous poisoning.

The derivatives of carbamic, thio- and dithiocarbamic acids have recently found expanding applications in agriculture as insecticides (aryl esters of N-methyl-carbamic acid), herbicides (alkyl esters of N-aryl-carbamic acids), and fungicides. The insecticidal properties of esters of N-alkyl-carbamic acids are responsible for their anticholinesterase action.

In the case of cutaneous absorption, the substances of this group show low toxicity. Absorbed through a variety of routes, including the skin, most substances belonging to this group exhibit mild cumulative properties.

Among the derivatives of dithiocarbamic acid, the salts of N-methyl- and N,N-dimethyldithiocarbamic acids and N-ethylene-bis-dithiocarbamic acid are of greatest practical significance. Tetramethylthiuram disulfide (TMTD), zineb, ziram, and carbathion are most widely used. And the interesting thing is that some chemicals are employed not only as pesticides but, as in the case of ziram and TMTD, as vulcanization accelerators.

Physico-chemical properties of the majority of this group of substances (powders or crystals which are practically insoluble in water and hydrophobic organic solvents) are responsible for their poor absorption through the skin. When applied to the skin, the LD_{50} values of cuprosan, cuprocine, zyneb, polycarbacine, maneb, polymarsyn, TMTD, edition exceeds 2-3 g/kg. Only certain substances showing comparatively high solubility in water and organic solvents are toxic when applied to the skin. Carbathion (sodium N-methyldithiocarbamate (dihydrate)) is one of these chemicals.

Chemicals containing alkaloids, nicotine and anabasine, exert skin-resorptive effects. They are classified as pyridine derivatives. The water solutions of sulfates, anabasine sulfate and nicotine sulfam, are produced for agricultural purposes. The salts of anabasine and nicotine were found to be absorbed through the skin in far smaller amounts than free bases.

Of practical importance are two more pyridine derivatives: 1,1-dimethyl-4,4-dipyridyl chloride (paraqat, gramokson) and 1,1-ethylene-2,2-dipyridyl (diqat, reglon). According to V. Makovsky (1971), paraqat shows high toxicity, when administered orally or applied to the skin. The LD_{50} varies with different types of animals amounting to 30-50 mg/kg and 250-300 mg/kg. Diqat was found to be less toxic: the LD_{50} equals 100-150 mg/kg, and 500-600 mg/kg (cutaneous absorption). These chemicals possess no cumulative properties. Owing to its high toxicity and persistence in the environment the use of paraqat is banned in the Soviet Union.

The results of numerous observations and experimental data point to the fact that a variety of environmental factors influence the process of cutaneous absorption, which is primarily attributable to functional and not infrequently to structural changes in the skin barrier.

Analysis of occupational poisonings, including those caused by poisons absorbed through the skin, shows that in a hot season they drastically increase in number.

An improved absorption of chemicals through the skin at higher ambient temperature is generally associated with reduced viscosity of the skin fat and its easier mixing with a substance.

Increased blood circulation promotes an increased penetration into the blood of substances which under normal conditions are absorbed through the skin, but have no considerable effect upon the absorption of substances which do not generally penetrate the skin.

Chemical substances are frequently used as solutions, emulsions, ointments, etc. They exert effects in combination with solvents, emulsifiers, excipients, and other accessory chemicals.

Such solvents as methyl and ethyl alcohols, hexane, acetone augment the cutaneous absorption of other chemicals. This effect stems from the washing of part of lipids present in the surface layers. These solvents produce no effect on the structure of the barrier layer.

The results of numerous investigations suggest that accessory substances aid cutaneous absorption of the main product by way of substance dissolution, surface tension reduction, decrease in volatility, surface lipids dissolution, destruction of stratum corneum structure, and local hyperemia development. Those solvents, emulsifiers and excipients which themselves readily penetrate the skin generally function more efficiently as "transport agents" for the basic substance.

Toxicological investigations on test animals generally aim at revealing the ability of substances to penetrate through the intact skin. To this end, new chemicals are largely utilized. Identification of lethal and toxic doses, as well as cumulative properties of a substance absorbed in this particular way greatly contributes to the value of these investigations.

Lethal effect is the principal objective criterion of a substance toxicity, while a half lethal dose (the LD_{50}) is the most statistically significant value. At the same time, as N. Pravdin

(1933) emphasized, toxicity is a relative value to be cognized through comparison. This is responsible for numerous attempts to classify substances according to degree of toxicity and hazard. Some classifications include toxicity when substances are absorbed through the intact skin.

The USSR Academy of Medical Sciences, divides substances in terms of toxicity and hazard into 4 classes (I. Ulanova and M. Pinigin, 1974). This classification incorporates the cutaneous LD_{50} as one of the criteria:

Class	LD_{50}, mg/kg
I (extremely toxic substances)	below 100
II (highly toxic)	100-500
III (moderately toxic)	500-2,500
IV (slightly toxic)	over 2,500

A pesticide classification suggested by L. Medved (1969) provides a deeper insight into a comparative degree of risk to be poisoned through the skin. Apart from the LD_{50} this classification accounts for a cutaneous-oral coefficient:

$$C = \frac{LD_{50}\ \text{cutaneous}}{LD_{50}\ \text{oral}}$$

This ratio implicity characterizes the degree of substance cutaneous absorption.

Depending on skin-resorptive toxicity with due regard to the cutaneous-oral coefficient (C), this classification of pesticides envisages three groups of substances:

Group	LD_{50}, mg/kg
I (pronounced toxicity)	less than 300, $C < 1$
II (marked toxicity)	300-1,000, $C = 1 - 3$
III (mild toxicity)	over 1,000 mg/kg, $C > 3$

It has been already mentioned that to assess a possibility of chronic poisoning, it is necessary to study the toxic properties of a substance, when repeatedly applied to the skin.

The practical hazard of poisonings stems not only from the toxicity of a substance and its ability to penetrate through a particular route, but also from specific sanitary- and hygienic conditions of its manufacture and employment. Production processes, design features as well as the working order of the machinery and instruments used, and the safety regulation observance are primarily responsible for the contamination of exposed parts of the workers' body and clothes with a chemical substance along with its entry into the air of the working zone.

To develop efficient measures for prevention of poisoning it is of vital importance to generalize the data of experimental and toxicological investigations and the findings of a hygienic study of labour conditions.

QUANTITATIVE CRITERIA OF THE HAZARD AND HYGIENIC CLASSIFICATION OF PESTICIDES

Yu.S. Kagan

The ever increasing use of pesticides, preparations characterized by a high biological activity, in all countries necessitates ascertaining their potential hazard and actual danger to human health. A health hazard is understood as the likelihood of acute and chronic poisonings, as well as of pathological processes that do not fit into the notion of a poisoning. These processes may occur immediately after the action of chemicals or much later. The problem is, therefore, to possess sufficiently clearly defined criteria making possible not only a qualitative but also a quantitative characterization of pesticide hazard, because the ultimate goal of hygienic toxicology is to establish harmless concentrations of chemicals.

Pesticides exert diverse effects on living organisms which are manifested as acute or chronic poisonings, allergic, carcinogenic, teratogenic and other effects. Pesticide hazard is characterized by the probability of the occurrence of such effects. Better explored is the question concerning the quantitative criteria of the toxicity of chemicals. Three types of relationships are relevant for the quantitative assessment of chemical compounds: the "dose-effect", "time-effect" and "dose-time" dependences.

The dose-effect relationship has been studied most thoroughly. It is usually followed "from top to bottom", i.e. from the upper toxicity limit, beginning from lethal doses and concentrations, whose effect cannot be registered. The "dose-effect" dependence being of probabalistic nature, associated with the species and individual sensibility to the substance in question, it is important not only to determine the nature of this relationship at different intensities of the effect, but also to have quantitative probabalistic indicators allowing evaluation of the result obtained for not only a particular limited sampling of test animals but also for a more general set. Probabalistic determination of toxicity parameters for several experimental animal species also facilitates the extrapolation of experimental data onto humans.

It is only natural that regularities observed at the level of lethal and toxic doses are easier to study and record. That is why experimentators are quite justified when they first of all want to establish the upper limit of the toxicity of a substance. The aim of this stage of investigations is to determine the values of DL_{50} (a dose killing 50% of experimental animals) and CL_{50} (the corresponding concentration).

The values characterizing the upper toxicity limit are usually established for the pathways of the pesticide entering the organism in the conditions of its production and application: peroral entry, application on the skin and inhalatory uptake.

Since most pesticides are low-volatility compounds, when estimating their inhalation poisoning hazard one should bear in mind the possibility of their entering the respiratory tracts in the form of hydroaerosoles or dust. This is possible when dust-inducing pesticides are produced and packed in workshops and when they are applied in agriculture by dusting and spraying. The danger of poisoning in this case is determined by the size of aerosole particles, the depth of their penetration into the respiratory tracts, the rate of absorption from the respiratory into the gastroenteric tract.

Along with the establishment of the DL_{50} and CL_{50} values, of paramount importance in toxicology is the finding of the lower toxicity limit - the threshold of toxic effect. Here it is worthwhile to consider at some length the notion of a threshold and the problem of establishing threshold concentrations in toxicology.

As for the threshold of biological effect, this notion, according to Horsefall's apt remark, "has the resilience of rubber articles: you can't push it away" (cit. after N.A. Tolokontsev, 1973). The magnitude of a threshold dose will actually depend on which indicator has been chosen to establish it and on the sensitivity of the methods used. The higher the sensitivity of the methods the lower the threshold. In theory, even negligible amounts of biologically active substances will react with biosubstrates and, therefore, produce an effect. Non-effective doses do not exist. The problem is how to assess the arising changes, whether or not they are harmful or are within the range of fluctuations observed under the conditions of normal vital activity. It is thus not so much the establishment of a threshold of any effect, but that of harmful effect, i.e. the task is to determine the doses and concentrations of substances that cause initial changes, bordering on norm and pathology. It was in this context that the founders of industrial pathology in the U.S.S.R., N.S. Pravdin, N.V. Lazarev and A.I. Cherkes, treated the problem of threshold doses and concentrations.

In determining the biological significance of a shift it is important to study biochemical, physiological and morphological parallels. If biochemical shifts are accompanied by prolonged functional changes, involving irreversible structural transformations, such shifts must be regarded as harmful. Analysis of the behaviour of the changes with time is of major importance for a proper assessment of hygienic implications. Persistent and, especially, progressing shifts should undoubtedly be regarded as harmful. The method of functional loads, revealing the stress on the adaptive mechanisms, can prove to be useful in the assessment of shifts. When application of the investigated dose of a substance causes a stable stress on adaptive processes, a shift of this kind should be regarded as unfavourable, and this must be taken into account when one establishes the threshold of harmful effect. The DL_{50} load value of a poison can also be used to assess the state of adaptive processes. If a poison, applied in a DL_{50} dose, kills all the test animals, this indicates a weakening of the organism's adaptive functions; if, conversely, all the animals survive - their resistance to the poison is increasing (naturally, one must consider here the likely differences between the experiment and the control, taking into account the size of small sampling).

Integral shifts assessed at the level of an organism are more significant from the standpoint of the criterion of harmfullness, yet in establishing the harmful effect thresholds it is also important to take into consideration the changes occurring in particular organs and physiological systems, as well as the shifts at a cellular and molecular levels. Much depends on the biological role of the system and the nature of the observed shift. Each particular case calls for a thorough analysis of concrete shifts, with due account for the nature of pathology caused by a more expressed effect. If the tendency in the changes brought about by threshold effects coincides with the basic nature of the patological process, resulting from the application of large doses of a given substance, such shifts are certainly to be taken into account. Any alternative or graduated indicator of the effect produced by a chemical can be analyzed in this way.

Having established the upper and the lower toxicity limits we get an idea of the zone of acute toxic effect. Essentially, this notion is similar to that of the range of therapeutic effect exerted by drugs (the ratio between their toxic and therapeutic doses); gradually the term was made more specific so that now most toxicologists define "the zone of acute effect" as the ratio of lethal doses or concentrations to the threshold of toxic effect (Yu.S. Kagan, 1965; N.A. Tolokontsev, 1973; I.V. Sanotsky and I.P. Ulanova, 1975).

The fundamental criterion of the hazard of substances, serving as the starting point for their hygienic rating is the threshold of chronic effect. The problem of establishing the threshold of harmful effect in a chronic test is extremely complex and critical because ultimately it is the accuracy with which this threshold has been defined that determines the correctness of the hygienic standards set up. The issue has been discussed in detail in a number of general textbooks and specific manuals on toxicology (I.V. Sanotsky, 1970; O.N. Yelizarova, 1972; I.V. Sanotsky and I.P. Ulanova, 1975; N.A. Tolokontsev and V.A. Filov, 1976 and others).

The condition of laboratory animals is judged from a complex of biochemical and physiological tests that reflect integral and specific responses of the organism to the action of poison. One of the tested doses is assumed to be a threshold dose, and then, after establishing the safety factor, this concentration or dose is reduced to the level prescribed by the hygienic standards. One can fully agree with M.A. Pinigin and G.N. Krasovsky (1979) that the threshold of harmful effect is a region (range) of minimal effective doses. With such an approach the established threshold doses and concentrations will, naturally, be only approximate, since these quantities depend on numerous factors: sensitivity and adequacy of the tests employed, the test animals species, the distance (interval) between the tested doses, the chronic test duration, etc.

The worked out quantitative criteria of pesticide hazard served as the basis for the elaboration of their hygienic classification. When assessing the hazard posed by pesticides one should bear in mind the two categories of people who can come in contact with the chemicals: persons exposed to pesticides in the course of their production and application and practically the entire population, consuming the foodstuffs containing pesticide residues, drinking the water and breathing the air where pesticides may be contained in the form of vapours or aerosols. In the former case, pesticides can come under the classification of chemicals by their hazard rate, which forms part of labour safety standards: "Harmful Substances. Classification and General Safety Requirements" (GOST 12.1.007-76). In the latter case, a different approach is needed as it is not work with pesticides during fixed working hours which is in question, but the risk of pesticide ingress by different pathways into the organism of people belonging to different age groups, having a different state of health, etc. For the population of greatest importance is the persistence of the active agent, as well as the cumulative properties, ability to accumulate in the tissues of the organism. For those who come in direct contact with pesticides of greater importance is their skin-resorptive toxicity.

Apart from the hazard of acute and chronic intoxication, also important is the capacity of pesticides to cause unfavourable long-term effects (carcinogenic, mutagenic and teratogenic). It is likewise necessary to estimate the probability of various kinds of pathology: allergic effect, embryotoxic, gonadotoxic and other negative properties.

The grouping of pesticides based on their toxicity and hazard rate, their capacity to cause various pathological processes, including long-term consequences, is a classification essential for proper decision-making in the field of hygiene and, therefore, has been called "hygienic" (L.I. Medved, Yu.S. Kagan, E.I. Spynu, 1968). The hygienic classification of pesticides takes into account different criteria of pesticide hazard, with the principle of a limiting criterion being used to refer the pesticide to a particular hazard group when the overall hazard of a pesticide is evaluated. This is the property primarily determining the hazard of a pesticide. For example, if a substance has a very high toxicity and belongs to the group of strong poisonous substances, without featuring any other negative properties, it should be referred to the 1st class of hazard. If a substance has a low toxicity, but displays a pronounced carcinogenic or mutagenic effect, it will still be referred to the 1st class of hazard.

The hygienic classification of pesticides is based on the following principal criteria: persistence, volatility, enteric toxicity, skin-resorptive toxicity, cumulative properties, blastomogenic properties, teratogenic properties, embryotoxicity, allergenic properties. The hygienic classification of pesticides is given below.

HYGIENIC CLASSIFICATION OF PESTICIDES

(based on the principal criteria of harmfulness)

I. Enteric toxicity:

Strong poisonous substances	DL_{50}	< 50 mg/kg
Highly toxic	"	20-200 mg/kg
Toxic	"	200-1000 mg/kg
Slightly toxic	"	> 1000 mg/kg

II. Skin-resorptive toxicity

Strongly pronounced	DL_{50}	< 500 mg/kg, skin-oral coefficient 3
Pronounced	"	500-2000 mg/kg skin-oral coefficient 3 - 10
Slightly pronounced	"	> 2000 mg/kg skin-oral coefficient > 10
Not pronounced		no signs of intoxication with maximum possible doses of pesticide applied on the skin

III. Hazard of agents based on degree of volatility: (in a chronic test)

Strongly pronounced	saturation concentration higher than or equal to lethal
Pronounced	saturation concentration higher than toxic
Slightly pronounced	saturation concentration exerts threshold effect
Not pronounced	saturation concentration exerts no threshold effect

IV. Cumulation:

Hyper-cumulation	Cumulation coefficient (C_{cum})	< 1
Pronounced	"	within 1 - 3
Moderate	"	" 3 - 5
Slightly pronounced	"	> 5

V. Persistence:

Highly persisitent	Half-life period	1-2 years
Persistent	"	from 6 months to 1 year
Moderately persistent	"	1-6 months
Slightly persistent	"	up to 1 month

VI. Blastomogenic properties (after L.M. Shabad, 1966):

Obviously carcinogenic	known to cause cancer in humans, strong carcinogenes in tests on animals
Carcinogenic	carcinogenic properties proved in tests on animals, not proved on humans
Slightly carcinogenic	weak carcinogenes in tests on animals

Suspected to possess blastomogenic properties.

VII. Teratogenic properties:

Obvious teratogenes	teratisms observed in humans, experimentally reproduced on animals
Potential teratogenes	teratogenic properties established experimentally on animals in actually encountered doses
Suspected teratogenes	availability of experimental data on animals, indicative of a possible teratogenic effect (obtained on administering doses exceeding the actually encountered)

VIII. Embryotoxicity:

Selective embryotropism	manifested in doses not toxic to mother's organism
Moderate embryotropism	manifested along with other toxic effects

IX. Allergic properties:

Strong allergenes	cause allergic conditions in most people, even applied in small doses encountered in real life
Weak allergenes	cause allergic conditions in some individuals

The above classification was used by the World Health Organization (WHO) in drafting an international classification of pesticides based on their hazard rating (1975). The purpose of the WHO classification (1975) is to protect against harmful effects of pesticides those directly engaged in pesticide production and application, transport workers, and the population as a whole. The classification distinguishes between the toxicity and the hazard of pesticides, recognizing the fact that the hazard can be enhanced if the pesticide is in the liquid state. When preparing the draft classification WHO undertook a comparative evaluation of the pesticide classifications, based on their degree of toxicity, adopted in different countries. A new feature of the classification recommended by WHO is the different assessment of commercially available forms of pesticides, depending on the concentration of the active ingredient and the physical state of the agent (solid, liquid). It is considered necessary to exercise special care in the assessment of chemicals that cause special quantitatively irreversible effects, the chemicals whose inhalation toxicity hazard exceeds that observed when the chemical enters the organism through the mouth or the skin, or when the hazard for humans is known to be much higher than that for animals. The data on which the WHO classification is based are shown in Table 1. The classification proceeds from the acute peroral toxicity of pesticides to rats; however, if other animal species respond to a given pesticide or are known to have responses similar to man's, this fact must be taken into consideration. Also, a pesticide will be referred to a group of more hazardous substances if it causes irreversible damage to internal organs, cumulates intensively and provokes allergic reactions. There may be cases when

Table 1

CLASSIFICATION OF PESTICIDES BASED ON HAZARD RATING

AS PROPOSED BY W H O

		DL_{50} for rats, mg/kg			
		Peroral		Epicutaneous	
	Hazard rating	Solid substances	Liquid	Solid substances	Liquid[*]
Ia	**Extremely hazardous**	5 or less	20 or less	10 or less	40 or less
Ib	**Highly hazardous**	5-50	20-200	10-100	40-400
II	**Moderately hazardous**	50-500	200-2000	100-1000	400-4000
III	**Slightly hazardous**	more than 500	more than 2000	more than 1000	more than 4000

[*] **The terms** "solid substances" and "liquid" describe the physical state of the classified substance.

a substance will be grouped with less hazardous compounds in spite of its DL_{50} value. It is also possible for a pesticide to be included in a different group if man's sensitivity to it is known to differ from that of laboratory animals. The classification acknowledges the necessity of introducing unified principles for the labelling of pesticides.

In 1979 the classification recommended by WHO was improved and amended and, in particular, List 1a of extremely hazardous pesticidal products was compiled to include aldicarb (DL_{50} 0.93 mg/kg); chlorphenvinphos (10 mg/kg); demeton (1.7 mg/kg); dibromochlorpropane, dieldrin (10 mg/kg); dimefox (1 mg/kg); disulphoton (2.6 mg/kg); EPN (14 mg/kg); hexachlorbenzene (10 g/kg); leptophos (50 mg/kg); M-74, mercaptophos, metaphos, mevinphos (4 mg/kg); parathion (13 mg/kg); methylparathion (14 mg/kg); phenylmercuracetate (30 mg/kg); phorate (2 mg/kg); phosphamidon (17 mg/kg); schradan (9 mg/kg); sodium fluoroacetate (0.2 mg/kg); sulfatep (5 mg/kg); TPP (1.1 mg/kg), thiophos, thimet, and some other chemicals whose agricultural application is prohibited in the U.S.S.R. The notes, accompanying List 1a, state that hexachlorbenzene causes serious disturbances of porphyrinic exchange in humans, dibromochloropropane induces sterility in humans and has mutagenic and carcinogenic effect on animals, lethophos has a neurotoxic effect, small doses of phenylmercuracetate impair the kidney function in mammals and have a teratogenic effect on rats.

As the hazard of acute intoxication is associated not only with the toxicity of the primary dose but depends to a large extent on the preparative form of pesticides, this fact must be reflected in the classification. There are classifications that take into account both the primary dose and the toxicity of the preparatory form. Thus, according to the draft document worked out by the WHO European Regional Bureau, the 1st hazard rating includes: (a) all products, irrespective of concentration, containing ingredients with DL_{50} 25 mg/kg and less at peroral administration; (b) all other products with DL_{50} 200 mg/kg and less; (c) any product which, judging from its DL_{50} value for rats, must be grouped under hazard ratings 2-4, but may prove seriously hazardous to man.

Such an approach appears to be the most rational; a review of the data published in world literature shows, however, that most cases of acute intoxication have been caused by pesticides having DL_{50} values below 50 mg/kg.

Bearing in mind the WHO recommendations with respect to the differing hazard ratings of pesticides in the solid and the liquid form and the data on their long-term effects obtained in recent years, we suggest the following:

Hazard rating 1 (extremely hazardous substances) should include:

(a) all pesticides with primary DL_{50} value below 50 mg/kg (peroral administration) or 300 mg/kg (epicutaneous application), or with the potential inhalation toxicity index (PITI) - the ratio of saturation concentration to lethal concentration - equal to 300 and more;

(b) all liquid preparative forms with DL_{50} 200 mg/kg and less (peroral administration) or 500 mg/kg and less (epicutaneous application);

(c) all pesticides capable of causing long-term effects (carcinogenic, teratogenic) in humans or characterized by selective organotoxicity, selective embryotoxicity, strong mutagenic or allergenic properties proven in tests on animals.

Hazard rating 2 (highly hazardous substances) should include:

(a) all pesticides with a primary DL_{50} value from 50 to 200 mg/kg (peroral administration) or 300-1000 mg/kg (epicutaneous application), or those with PITI from 300 to 30;

(b) pesticides capable of causing unfavourable long-term effects of a moderate or slight degree in laboratory animals, but not proven by observations on humans.

Hazard rating 3 (moderately hazardous substances) should include:

pesticides with the toxicity of primary DL_{50} value from 200 to 1000 mg/kg (peroral administration) or causing the death of animals when applied epicutaneously in a dose higher than 1000 mg/kg, or with PITI from 30 to 3.

Hazard rating 4 (slightly hazardous substances) should include:

pesticides with the toxicity of primary DL_{50} value higher than 1000 mg/kg (peroral administration), not causing the death of animals when applied epicutaneously; or with PITI less than 3.

All other criteria (persistence, cumulation, etc.) remain in conformity to the hygienic classification applied in the U.S.S.R.

Pesticides under the 1st hazard rating are not to be used in agriculture.

Pesticides under the 2nd hazard rating may, whenever necessary, have a limited use; pesticides under the 3rd and 4th hazard rating may be used on a wide scale, provided all the necessary regulations and standards are adhered to.

The decision has to be made based on the limiting criterion, understood as that property of a substance on which the appearance of a pathological effect can primarily depend.

REFERENCES
(all in Russian)

Kagan Yu.S. On the quantitative criteria of the harmfulness of chemicals. In: "Hygiene and Toxicology of Pesticides and the Clinics of Poisonings". Kiev, "Zdorovye" Publ., 1965, p.46-60.

Kagan Yu.S. General Toxicology of Pesticides. Kiev, "Zdorovye" Publ., 1981, 176 p.

Lazarev N.V. Fundamentals of Industrial Toxicology. Leningrad, Medgiz, 1938. Leningrad section. 388 p.

Medved L.I., Kagan Yu.S., Spynu E.I. Pesticides and the problems of hygiene. Zhurn.Vsesoyuzn.khim.obshchestva im.Mendeleyeva, 1968, No.3, p.266-271.

Pravdin N.S. Minor Toxicology Techniques for Industrial Poisons. Moscow, Medgiz, 1947, 211 p.

Pinigin M.A., Krassovsky G.N. The problem of threshold values in the hygienic assessment of environmental factors. In: "The Problem of Threshold Values in Toxicology". Moscow, Publ. by Sysin Inst.of Gen.and Commun.Hygiene. 1979, p.7-10.

Sanotsky I.V., Ulanova I.P. Harmfulness Criteria in Hygiene and Toxicology. Moscow, Meditsina, 1975, 328 p.

Tolokontsev N.A. Dependence between the quantity of acting poison and the effect. In: "Quantitative Toxicology". Leningrad, "Meditsina" Leningrad section. 1973, p.23-46.

Cherkes A.I., Lugansky N.I., Rodionov P.V. (Ed.). Manual on Toxicology of Poisonous Substances. Kiev. "Zdorovye" Publ., 1964, 464 p.

EXPRESS AND CALCULATION METHODS FOR HYGIENIC REGULATION OF PESTICIDES

L. M. Sasinovich

In the complex of measures aimed at securing prophylaxis in pesticide poisoning, primary importance is attributed to problems of hygienic pesticide regulation in environmental objects.

Working out pesticide norms for an environmental object takes averagely about two years. A complex approach to hygienic pesticide regulation simultaneously for all media significantly reduces the time necessary for specifying hygienic norms and, consequently, cuts down the period elapsing from the synthesis of a pesticide till its application. However, experimental toxicological investigations aimed at establishing hygienic norms for new chemical substances are lagging behind their synthesis and, consequently, fail to meet fully the requirements of agriculture. This situation provided a stimulus for searching ways to reduce toxicological experiments aimed at regulation of the content chemicals in environmental objects. An analysis of literary data has shown that cutting down the time elapsing from the synthesis of a chemical substance till its introduction into practice is possible, on the one hand, by reducing the laboriousness of experimental researches into acute toxicity, cumulative properties, length of chronic experiment and, on the other hand, through prognostication of the parameters of the biological effect of the substances and of their hygienic norms. Thus, the work on the optimization of the number of experimental animals in a group has shown (Piruzyan, 1976; Majda Alexander, 1976) that in specifying DL_{50} (a lethal dose to 50% of the test animals) in an acute experiment it is sufficient to use four test animals(instead of six,as is universally accepted)for each of the doses studied. To increase the number of animals in a group is feasible only for more in-depth studies. It has been proposed (Loit, 1963) that in routine experiments simply the lethal dose to some of the animals rather than DL_{50} should be ascertained. A tentative idea of the cumulative properties of substances can be obtained from the experiment lasting either one month (A.O. Loit, 1963) or two months (L.M. Sasinovich, T.N. Pan'shina, 1976).

It would be labour-saving to prognosticate the DL_{50} values at various pathways of substances into an organism by the known results obtained in studying only one of the pathways (Golubev, 1970; Rumyantsev, Novikov, 1976; Sasinovich, 1979).

Introduction of mathematical methods into hygiene and toxicology has promoted the progress of researches into the laws and relationships between biological activity of pesticides, their structure and physico-chemical properties.

Calculation methods in the USSR toxicology were first introduced by Lazarev. In his book "Nonelectrolytes" he substantiated the possibility of using calculation methods to determine toxicity on the basis of physico-chemical properties of substances. Afterwards the Leningrad scientists, Lazarev's followers, were engaged in working out problems of the correlation between the structure, physico-chemical properties of chemical substances, on the one hand, and their biological activity, on the other. These investigations have been summarized in the monographs "Quantitative Toxicology" (1973) and "Principles of General Industrial Toxicology" (1976).

Today, the problem of this correlation is tackled both by Soviet scientists and by researchers in other countries. Thus, a correlation has been found to exist between the physical constants of volatile and low-volatile organic compounds and their toxicity indices (Lyublina, Golubev, 1963; Lisman, Lyublina, Rabotnikova, 1978); DL_{50} values were revealed to be dependent on the molecular mass for organophosphorous compounds with the molecular mass within 250-430 (Golubev, Lyublina, Tolokontsev, Filov, 1973); a linear dependence was found between the logarithm of the molecular mass of chemical compounds and the product of the animals death rate and their survival time (Vrovsky, 1974). Equations have been evolved for predicting toxicity parameters of volatile organic compounds of different chemical classes (Loit, 1963; Lyublina, Golubev, 1962, 1963; Zaugolnikov, Kochanov, Loit, 1974; Jounner, Purcell, 1976). Correlation between the biological activity and the structure, as well as physico-chemical constants, is observed to increase within certain homologous series. To determine a relationship between the toxic effect and the structure of the subsitutes in the benzene ring, the Hammett equation may be used, and for the derivatives of aliphatic compounds, the Taft equation is applicable. For some genetic series it is appropriate to use the Zagradnik equation linking up the biological activity of the i-th homologue with that of the ethyl derivative. Greater universality is inherent in the Hunch equation allowing for steric effects of the substitutes on the biological activity.

An original method has been developed (Labber, Franke, Ochme, 1976; Kier, 1976; Graig, 1974) for elucidating the relationship between the chemical structure and the biological activity of substances involving nonparametric analysis methods after Free and Wilson, 'sign recognition', and molecular orbital calculations.

Kagan et al. (1976) made use of the quantum-chemical approach to throw light on the relationship between the toxicity of organophosphorous pesticides and the constants of positive charge on phsophorus and the binding energy of the ether or thio-ether bond, the latter being one of the principal characteristics of the molecule determining the specifics of diverse properties of chemical substances (Korbakova, Timofievskaya, Nikitenko, 1967).

Cumulative properties of some organo-phosphorous compounds can be predicted by the values of the distribution coefficient in the system "oil-octanol-water", by the character of structural groups entering in the compound (Jounner, Purcell, 1976).

The chemical structure and physico-chemical properties are observed to be related not only to pesticide toxicity, but also to disorders in certain biochemical processes. Thus, a correlation has been found to exist between toxicity, cholinesterase activity and structural specifics of phosphonic acids ethers containing a trimethyl group (Tarakhovsky, Sanin, Borodach et al., 1976). The cholinesterase activity was also found to be dependent on Hammett's σ constants, molar refraction, as well as on the parameters characterizing the strength of the hydrogen bond, electrostatic charge, the nature of its transfer and steric differences between the compounds (Chiriac, Cuibotaru, Vilceanu, 1975).

Andreyeshcheva (1973, 1974) has proposed equations for estimating, by the chemical structure and the manifestation of the biological properties of substances, the thresholds of their reflectory activity.

Today attempts are being made to predict also the pharmacological activity of drugs by certain physico-chemical properties, particularly the distribution coefficient in the system "octanol-water" (Komsky, 1976), as well as by the electron properties of molecules (Hansch Corvin, 1976).

To reduce experimental procedure, studies are also carried out into the predicting the chronic effects by the results of short-term tests (Sidorov, Shapiro, 1976; Shigan, 1976). To calculate a tentative threshold of chronic effect an equation has been proposed based on the acute toxicity indices - the mean lethal concentration (CL_{50}) or the threshold of the inhalation acute effect (Lim_{ac}).

A most realistic possibility to cut down the period and to secure safety of introduction into practice of new chemicals proves to be the specification of tentative safe exposure levels (TSEL). An analysis of the methods for calculated regulation of chemical substances of different designations has revealed that they can be subdivided as follows:

1) substantiation of calculated norms (tentative safe exposure levels - TSEL) on the basis of physico-chemical properties of substances;

2) substantiation of TSEL on the basis of short-term

toxicological experiments;

3) specification of TSEL for a substance in a certain medium, provided norms for the other media have already been specified.

All of the proposed methods for calculated regulation are based on a common principle, namely, the elaboration of regression equations on the basis of the correlation analysis of the values of authorized MAC (maximum allowable concentrations), various physico-chemical constants and biological effect parameters. In some cases, physico-chemical constants are used to predict biological properties or less sophisticated biological indices are used to predict the more complex ones and the latter are employed to predict norms.

Lyublina (1958) observed a correlation between most accessible physico-chemical properties and MAC for a wide range of organic compounds. As a result, equations have been evolved, enabling estimation of tentative norms for production-room air for high-boiling organic compounds by their molecular mass.

It is worth noting that the majority of researchers (Sanotsky, 1969; Zayeva, 1970; Golubev, Lyublina, Tolokontsev, Filov, 1973; Rumyantsev, Novikov, 1976; Kagan, Sasinovich, 1976) have become convinced about the great reliability of TSEL calculated by the biological activity of substances while the TSEL values estimated by physico-chemical - only as approximate (Kagan, Sasinovich, Ovseenko, 1972, 1976).

We can use, as informational components in the TSEL calculation, the biological activity indices established by means of a full-scale toxicological investigation, and short-term experiments, as well as calculated mathematically. The basic condition for all the variants is the presence of a sufficiently strong correlation between MAC and each of the components used.

Equations have been elaborated for TSEL calculation for working-zone air by the known DL_{50}, CL_{50} (Lyublina, Golubev, 1963, 1967) involving also data obtained in a single intraperitoneal introduction (Rabotnikova et al., 1967), on the basis of data obtained in an acute or six-week tests (Speransky, 1965).

Sidorov and Shapiro (1976) have propsed that at first the acute toxicity data (CL_{50}) should be used to calculate the threshold concentration in a chronic experiment (Lim_{ch}) and the latter to be then used for TSEL estimation by using a special safety coefficient. The authors have recommended that inhalation toxicity data be used. This restricts the application of the evolved equations, since it is often impossible to determine the mean lethal concentrations for pesticides.

The method for TSEL calculation, proposed by Golubev (1970), takes account of the threshold of irritating effect. Since irritating properties are inherent in poisons to a varying degree, whilst the majority of pesticides are free from them altogether, this method cannot be widely used either. The same reasons underly a restricted application of the method based on a simultaneous use of threshold concentrations determined by the irritating and integral effect, as well as by DL_{50} and irritating effect threshold (Maksimov, 1969).

Information amassed (Kagan, Sasinovich, Ovseenko, 1971, 1972, 1973, 1975, 1989, 1982) opened up possibilities for applying the methods of correlation and regression analysis to find out the relationship between the various biological activity indices (DL_{50}, CL_{50}, Lim_{ac}, Lim_{ch}, cumulation coefficient) and authorized MAC for pesticides in working-zone air. We studied the possibility of predicting the hygienic pesticide norms in working-zone air, as well as the effects of their chronic action by the principal toxicity and cumulation parameters that can be obtained in short-term tests (DL_{50} when introduced into the stomach and applied onto skin, mean lethal and threshold concentrations - CL_{50}, Lim_{ac} under a acute inhalation exposure, cumulation coefficient), the latter being predicted by physicochemical constants (mol. mass, solubility, volatility, boiling, point, melting point, density).

The information was abundant enough to make calculations for organophosphorous (as well as separately for subgroups: derivatives of thiophosphoric and dithiophosphoric acids, esters of phosphoric, phosphorous, and pyrophosphorous acids), and organochlorine pesticides, for pesticides - the derivatives of carbamic, thio-, and dithiocarbamic acids. Regarding the fact that a significant number of pesticides, either being used or recommended for use, do not come within the above-described categories and at the same time they also need to be hygienically regulated, the same kind of work was carried out generally for all the pesticides subject to regulation.

The values of pair, multiple and partial correlation coefficients were indicative of the presence of strong correlation between pesticide MAC in working-zone air and the indices of toxicity (DL_{50} when introduced into the stomach and applied onto skin) and cumulation (cumulation coefficient) in organophosphorous and organochlorine pesticide groups. Correlation was found to be also strong among all the quantities studied for all the pesticides generally. In the pesticide groups comprising the derivatives of carbamic, thio-, and dithiocarbamic acids the correlation between the above-listed parameters was found to be weaker. As is known, the substances in the above group are in certain cases capable of producting remote effects, are endowed with allergenic properties, etc., and it was quite reasonable to determine a safety factor to allow for the aforementioned factors. Once the factors contributing to the higher safety factor for such substances in terms of remote effects have been determined, we could possibly calculate the TSEL value by toxicological criteria and thereafter bring it down in correspondance with the safety factor thus established. Correspondingly, we have evolved regression equations also for this group, so as to be able, firstly, to test their reliability in practice by using them to calculate TSEL for all regulated pesticides in this group, secondly, to be able to check our assumption about the possibility of TSEL calculation after toxicological criteria with its regular reduction later for substances with remote effects and other adverse properties and, thirdly, to be able to calculate an additional safety factor.

Proceeding from the above the paired and multiple regression equations were evolved enabling us to calculate TSEL for organophosphorous and organochlorine pesticides, derivatives of carbamic, thio-, and dithiocarbamic acids, as well as for all groups of pesticides at different stages of studying the preparations: exclusively from DL_{50} when introduced into the stomach; from DL_{50} when introduced into the stomach and applied onto skin; DL_{50} when introduced into the stomach and cumulation coefficient; from all the three parameters.

Given below are the equations to be used for calculating pesticide TSEL in the workin-zone air; where x_1 is DL_{50} mg/kg; x_2 is DL_{50} applied onto skin; x_3 is the cumulation coefficient; y is TSEL, mg/m^3:

1) Organophosphorous

$y = \exp(0.52 \ln x_1 - 3.93)$

$y = \exp(0.3 \ln x_1 + 0.25 \ln x_2 - 4.46)$

$y = \exp(0.48 \ln x_1 + 0.09\, x_3 - 4.35)$

$y = \exp(0.28 \ln x_1 + 0.24 \ln x_2 + 0.08\, x_3 - 4.81)$

2) Organochlorine

$y = \exp(0.97 \ln x_1 + 7.05)$

$y = \exp(0.74 \ln x_1 + 0.22 \ln x_2 - 7.21)$

$y = \exp(0.9 \ln x_1 + 0.14\, x_3 - 7.4)$

$y = \exp(0.77 \ln x_1 + 0.12 \ln x_2 + 0.14\, x_3 - 7.5)$

3) Derivatives of carbamic, thio-, and dithocarbamic acids

$y = \exp(0.2 \ln x_1 - 1.86)$

$y = \exp(0.01 \ln x_1 + 0.28 \ln x_2 - 2.73)$

$y = \exp(0.14 \ln x_1 + 0.05\, x_3 - 1.86)$

$y = \exp(0.12 \ln x_1 + 0.23 \ln x_2 + 0.03\, x_3 - 2.57)$

4) For pesticides in all groups generally

$y = \exp(0.58 \ln x_1 - 4.51)$

$y = \exp(0.47 \ln x_1 + 0.11 \ln x_2 - 4.66)$

$y = \exp(0.52 \ln x_1 + 0.1\, x_3 - 4.9)$

$y = \exp(0.46 \ln x_1 + 0.06 \ln x_2 + 0.1\, x_3 - 4.87)$

(For all the equations $P < 0.01$).

The equations furnished a basis for constructing nomograms enabling TSEL to be determined without special calculations.

Verification of validity of the equations for all experimentally regulated pesticides revealed a good agreement between calculated norms and the experimentally determined MAC. Full agreement (a full agreement of the norms was assumed to take place when TSEL and MAC differed not more than 1.5 times) between calculated norms and the maximum allowable concentrations was observed: in 54-65% of cases for organophosphorous compounds, 48-52% for organochlorine ones, 46-50% for pesticides in all groups generally. For the rest of the pesticides the TSEL values differed from MAC: in the organophosphorous pesticide group - not more than 2.5 times, organochlorine - not more than 5 times. Substances which did not come within the above groups were notable for great diversity with respect to structure, operating effect and designation. Approximately in 70% of cases (allowing for variations dependent on the equation) their TSEL differed from MAC less than 5 times. Least prominent were differences between TSEL and MAC for derivatives of urea, symm. triazines, derivatives of carbamic, thio-, and dithiocarbamic acids. With 1.5-3 times scattering it is possible to determine norms for compounds containing thiocyanogen, cyanogen, fluorine, as well as magnesium, calcium, zinc (inorganic compounds). TSEL in the case of persistent, organomercurial compounds (granosan, mercurbenzene, mercurhexane) differed from MAC by more than an order.

An analysis of the correspondence of TSEL computed with the use of all the recommended formulae with the experimentally determined MAC has revealed that the reliability of the calculations increases with the greater number of informative components used in the calculation, as well as when calculations are confined to individual chemical groups. The pair and partial correlation coefficients between MAC and each of the biological parameters introduced in the calculation, as well as the validity check of each of the equations on the experimentally regulated pesticides have shown that for organophosphorous pesticides the sufficiently reliable TSEL values can be calculated by the data on their acute toxicity and skin-resorptive effect, for organochlorine ones - by data on their acute toxicity and cumulative properties. TSEL should not be estimated for highly effective substances and highly toxic pesticides displaying pronounced cumulation and supercumulation, as well as for pesticides for which the limiting factors are those that cannot included into the equations (remote effects, high persistence in the environment, such as, for instance, in the case of organomercurial compounds, etc.). For these substances TSEL proves to be only a rough value which should be corrected in accordance with the manifestation of the above properties.

For organophosphorous pesticides (toxic, high-volatile) there have been evolved regression equations reflecting the dependence of TSEL upon inhalation toxicity parameters - $CL_{50}(x_1$; $Lim_{ac}(x_3)$; Lim_{ch}(calculated, x_4), as well as on the cumulation coefficient (x_2):

$$\ln y = 0.47 \ln x_1 - 3.13$$

$$\ln y = 0.38 \ln x_1 + 0.08 x_2 - 3.39$$

$$\ln y = 0.74 \ln x_3 - 1.7$$

$$\ln y = 0.015 \ln x_3 + 0.79 \ln x_4 - 1.6$$

TSEL calculations after CL_{50} were no more accurate then those with the use of DL_{50}. Once Lim_{ac} and Lim_{ch} (calculated) were used in calculations, they were strongly correlated with

MAC ($r = 0.83$; $R = 0.85$).

In validity checks on the equations evolved with the use of these components a good agreement between calculated norms and the experimentally determined MAC was observed. In this case Lim_{ch} is predictable with high accuracy ($R = 0.86$) as a derivative of the two parameters - Lim_{ac} and the cumulation coefficient:

$$\ln x_4 = 0.52 \ln x_3 + 0.21 x_2 - 2.03.$$

Calculations of Lim_{ch} after CL_{50} and the cumulation coefficient are less reliable ($R = 0.59$). Predicting chronic effects (Lim_{ch}) or cumulation by the acute toxicity data alone (DL_{50} orally, DL_{50} onto skin, CL_{50}, Lim_{ac}) does not appear possible, since no correlation was observed between these quantities.

Predicting hygienic norms by physico-chemical constants (mol. mass, solubility, volatility, density) is less reliable.

At present there have been elaborated recommendations on regulation of pesticides for agricultural use which take account of the specifics of agricultural production.

Pesticide TSEL in the working-zone air for agricultural production are, in effect, tentative concentrations which, given daily or regular work with pesticides over periods as long as is required by agricultural production conditions, throughout one's working life must not cause diseases or departures from one's good health, as detected by today's medical examination methods.

Pesticide TSEL in working-zone air are helpful to exercising a preliminary sanitary inspection over the introduction of pesticides in agriculture: routine sanitary inspection over the pesticide content at the workplaces in the case of their experimental-production application; determining permissible periods for allowing people's work in treated areas (by considering skin resorption).

TSEL are specified for pesticides for the period of their experimental-production application (3 years). After 3 years the TSEL values are to be revised with respect to the data having accumulated by that time (1) replaced for MAC; (2) authorized for a new period (this mainly applies to substances with a restricted use); (3) discarded if the pesticide has been banned for application.

Materials for substantiation of TSEL for pesticides with a possible allergenic effect (litherary data on the chemical concerned or its analogues) should likewise contain the results obtained in investigating the latter with the aid of express-methods.

Highly-effective poisonous chemicals and pesticides with possible remote effects (cancerogenic, mutagenic, teratogenic) are not liable to calculated regulation.

To obtain the necessary data experiments are to be conducted on a particularly sensitive animal species. Furthermore, considering that contact with pesticides being used in agriculture is short-term, investigations into cumulative properties of pesticides are allowed to be carried out during 2 months (rather than 4 months, as is the USSR practice when determining MAC for new substances in working-zone air) involving a daily introduction of the substance in a doze amounting to 1/10 DL_{50}.

Methods of calculated regulation are being worked out and successfully used at present also in other areas of hygiene, namely, the hygiene of atmospheric air, water, nutrition. In recent years equations for calculating tentative norms for soil have been worked out and recommended for use (Spynu, Sova, 1982).

Thus, the study of correlations between the thresholds of olefactory sensation, light sensitivity of the eye and bioelectrical activity of cerebral cortex, on the one hand, and the maximum one-time MAC in atmospheric air, on the other, served as a basis to evolve an equation (Krotov, 1971) enabling MAC in atmospheric air to be calculated with the above properties used as informative components.

There has been proposed an express-method for calculating tentative norms of chemicals in atmospheric air by their structure and physico-chemical properties; a method for determining the maximum one-time MAC - by the smell threshold. A method has been developed for predicting threshold concentrations and MAC of noxious substances in atmospheric air on the basis of physiological and biochemical data obtained in a short-term experiment (Shandala, Pazanych, Podloznyi, 1977).

Zaugol'nikov et al. (1975) evolved a number of equations which make it possible to determine tentative norms for atmospheric air and water reservoirs according to toxicity indices (DL_{50}, CL_{50}) and certain physico-chemical properties. The best agreement between the experimental and estimated norms was observed whenever inhalation toxicity indices were used (Zaugol'nikov, Kochanov, Liot, Stavchansky, 1978):

$$\lg MAC_{m.d.} = -3.16 + 1.72 \lg CL_{50} \qquad r = 0.66$$

$$\lg MAC_{m.d.} = -6.0 + 1.5 \lg DL_{50} \qquad r = 0.52$$

$$\lg MAC_{m.o.} = -2.08 + 1.02 \lg CL_{50} \qquad r = 0.55$$

$$\lg MAC_{m.o.} = -5.73 + 1.39 \lg DL_{50} \qquad r = 0.49$$

(m.d. - mean daily concentration; m.o. - maximum one-time) There have also been proposed (Krotov, 1972) equations to be used for determining mean daily MAC by taking account of toxicity indices (CL_{50}, DL_{50}).

Regression equations have been proposed for calculating the threshold and ineffective doses of chemicals in water reservoirs (Martyshchenko, Mironets, Shigan, 1976).

Krasovsky et al. (1971, 1974) have evolved equations to calculate the maximum ineffective dose (MID) by the results of studying the acute toxicity:

$\lg MID\ (mg/kg) = 0.45 \lg CL_{50} - 1.55$

$\lg MID\ (mg/kg) = 0.9 \lg DL_{50} - 3.60$

A good agreement between tentative norms for water reservoirs and the authorized MAC is observed with the use of the equations proposed by Zaugol'nikov, Kochanov, Loit, Stavchansky (1973, 1974):

$\lg MAC\ (mg/l) = -2.12 + 1.7 \lg CL_{50}$

$\lg MAC\ (mg/l) = -4.76 + 1.39 \lg DL_{50}$

By discovering a sufficiently strong correlation of the acute and chronic toxicity and cumulation with authorized allowable residue quantities (ARQ) of pesticides in foodstuffs has enabled (Kagan, Sasinovich, 1971, 1972) evolving equations for calculating tentative ARQ values for pesticides at different stages of studying the pesticides. It should be remembered that pesticide ARQ in experiment are not always determined on the basis of a toxicological criterion. Whenever the effect of pesticides on organoleptic properties of foodstuffs or their nutrition value is a constraining factor the proposed equations can only supply material for comparing the toxicological and other restricting criteria. The same is true for pesticides endowed with the capacity to produce remote effects.

The third path for predicting TSEL involves calculation in terms of known values of the MAC (or TSEL) for the other media. Tolokontsev (1967) discovered a correlation between the MAC of chemicals in the air of industrial establishments and the MAC in atmospheric air. Spynu and Ivanova (1969) have evolved an equation enabling a tentative mean-daily MAC of pesticides in atmospheric air to be calculated by the known norm for the working-zone air, as well as the maximum one-time concentration:

$$\lg MAC_{m.d.} = (0.88 \pm 0.16) \lg MAC_{o.c.} + (-2.16 \pm 0.28) \quad r = 0.69$$

$$\lg MAC_{m.o.} = (0.55 \pm 0.09) \lg MAC_{o.c.} + (-1.777 \pm 0.5) \quad r = 0.63$$

Loit, Kochanov, Zaugol'nikov (1971) have proposed an equation for calculating the mean daily MAC and maximum one-time concentrations of gases and vapours of organic compounds. Krotov (1972) has also evolved an equation for the calculation of the mean daily MAC of a substance in atmospheric air by the MAC in the working-zone air.

Golubev and Subbotin (1968) proposed equations to be used for calculation of the MAC for water reservoirs by the MAC in the working-zone air. Maximum ineffective concentration (MID) of chemicals in water reservoirs is recommended (Krasovsky et al., 1972, 1974) to be calculated by the following equation:

$$\lg MID\ (mg/kg) = 0.60 \lg MAC_{o.t.} - 1.31 \qquad r = 0.55$$

Summing all the above we would like to note that great headway has been made by now in the elaboration and introduction of methods for express regulation of pesticides in working-zone air, atmospheric air, in the water reservoirs; researches are under way on express regulation of pesticides in foodstuffs and soil. It is to be remarked that development of methods for calculated regulation is a dynamic process. As new information is amassed, calculation methods are liable to be revised and improved.

1. Urgent problems of industrial toxicology. Leningrad, Nauka Publishers, 1970. (In Russian).
2. Methods for determining toxicity and hazards of chemicals. Ed. I.V. Sanotsky, Moscow, Meditsina Publishers, 1970, (In Russian).
3. Principles and methods for specifying maximum allowable concentrations of noxious substances in production-room air. Moscow, Meditsina Publishers, 1970. (In Russian).
4. Quantitative toxicology. Leningrad, Nauka Publishers, 1973, (In Russian).
5. Principles of industrial toxicology. Leningrad, Nauka Publishers, 1976. Ed. N.A. Tolokontsev and V.A. Filov. (In Russian).
6. Application of mathematical methods for estimating and predicting the real danger of accumulation of pesticides in the environment and in the organism. Conference proceedings. Kiev, 1976. (In Russian).
7. Spynu E.I., Ivanova L.N. Mathematical prediction and prophylaxis of environmental pollution. Moscow, Meditsina Publishers, 1977. (In Russian).
8. Zaugol'nikov S.D., Kochanov M.M., Loit A.O., Stavchansky I.I. Express-methods for determining toxicity and hazards of chemicals. Leningrad, Nauka Publishers, 1978. (In Russian).

TOXICOLOGY OF THE MAJOR CLASSES OF PESTICIDES

T.N. Panshina

Pesticides, with their strong biological activity, can impair vital functions not only in their target living organisms but also in other off-target organisms including human beings and warm-blooded animals. Professor L.I. Medved has repeatedly stressed (1974, 1977) that pesticides passess some unique features as compared with the chemicals intended for other uses. Among them are their unavoidable circulation in the biosphere resulting in contacts of large groups of population with pesticides, their potential hazard for nature and man.

The most radical way to avert the harmful influence of pesticides is to discover such effective agents that would selectively affect crop pests with no damage done to man or the beneficial flora and fauna. Selective pesticidal action is, however, a theoretical problem of extreme complexity in modern biology and medicine. Therefore, the occupational hygiene of pesticide application acquires a great significance in the national economy.

The substances currently used in agriculture as pesticides belong to different classes of chemical compounds: organochlorine and organophosphorus compounds, derivatives of carbamic, thio- and dithiocarbamic acids, dinitrophenolic compounds; chlorine-containing derivatives of carboxylic acids, haloanilides of carboxylic acids; derivatives of urea, thiourea, sym-triazine, dipyridyl, coumarin, alkaloids, organomercury and other compounds.

Substances of the same class, while structurally similar, may have different pesticidal and toxic properties. Every substance has its own characteristic features. Despite that, have some different representatives of one and the same class of compounds common features and, not infrequently, the same primary mechanisms of action (L.I.Medved, 1974).

Organophosphorus compounds (OPCs) constitute a most essential class of presently used pesticides. They are most widely used as insecticides, acaricides, and defoliants. A hygienic advantage of OPCs is their relatively low persistence in the environment. Their major part decomposes in plants, soil and water within one month, however the insecticides and acaricides designed for use within plants (methylmercaptophos, anthio, phosphamide, kilval, octamethyl, sayphos, etc.) can persist longer, up to one year (N.N.Mel'nikov, 1974; Yu.S. Kagan, 1974). OPCs vary in terms of toxicity to warm-blooded animals. The highly toxic organophosphates are: methylmercaptophos, metaphos, DDVP, phosphamide, phthalophos, phosalon, cydial, hostaquick. The medium -toxic ones are: chlorophos, trichlorometaphos, methylnitrophos, methylacetophos. There are also low-toxic substances in the OPC group (sayphos, bromophos and gardona). The use of highly potent toxic organophosphates, such as thiophos, methylethylthiophos, and mercaptophos, is prohibited in the Soviet Union. By their volatility, most OPCs are low- (phencapton, chlorophos, phosphamide, metaphps) and medium - volatile (methylnitrophos, carbophos, octamethyl), and only a few are highly volatile (DDVP). But even among the medium-volatile OPCs there are some (methylmercaptophos, octamethyl) which are highly hazardous when inhaled as vapors, because their lethal and toxic concentrations are below the saturating ones (Yu.S.Kagan, 1974).

OPCs are capable of penetrating into the body through intact skin without causing any local effect. This makes them extremely hazardous for the exposed workers as they may covertly trigger an acute intoxication. Some OPCs (methylmercaptophos, metaphos, M-81, DDVP, phosphamide) have a pronounced skin-absorptive toxicity (Yu.S.Kagan, 1968, 1974; Yu.I. Kundiev, 1975; T.N. Pan'shina, 1964; L.M. Sasinovich, 1967). For a majority of the organophosphates, their mechanism of toxic action works basically by inhibiting cholinesterase due to phosphorylation of its active centres. The pattern of human poisoning in exposure to different OPCs is similar. The differences involve primarily the severity of the excitation symptoms in the central and peripheral cholinereactive systems, the dominance of muscarine- and nicotine-like symptoms. Some of the OPC effects are caused by their direct impact upon the choline-reactive system and the influence upon the non-cholinergic systems. Some OPC poisonings (e.g, by triorthocresyl phosphate, chlorophos, phosphamide, etc.). result in the disfunction of the peripheral nervous system (neurites, phareses, paralyses of the extremities). Some data indicate the effect of OPCs on the functional state of the liver, morphological blood count, and redox systems of the body (Yu.S. Kagan, 1963, 1974, 1977).

In recent years there have been numerous reports on the ability some OPCs to cause unfavorable long-term effects. For example, selective embryotoxicity and teratogenicity were reported for phthalophos having a phthalimide group in its molecule (V.M.Voronina, 1971; V.M.Voronina, M.V. Pis'mennaya, 1973). The review of A.I. Kurinny and M.A. Pilinskaya (1974) cites evidence for a varying degree of mutagenicity in some OPC's. Blastomogenicity was noted for chlorophos and phosphamide. Yu.S. Kagan (1977), however, emphasizes that the long-term effects of OPC's are a complex issue. Definitive quantitative criteria are needed to assess the real hazard of these substances to human beings. Organochlorine compounds (OCCs) are widely used in agriculture as insecticides, acaricides and fumigants. OCCs represent chlorine derivatives of multi-ring hydrocarbons, cyclic paraffins, diene compounds, terpenes, benzene, and others.

The basic feature that distinguishes most hydrocarbon haloderivatives is their stability against the effect of various environmental factors - temperature, insolation, moisture, etc. In the hygienic classification, a number of OCCs are placed among very persistent and persistent pesticides (E.I.Spynu, 1974, 1977). A characteristic feature of several OCCs lies in their pronounced ability to accumulate not only in environmental objects but also in animal tissues and fat upon entry of even low OCC quantities.

Unlike OPCs, the majority of the organochlorine pesticides are categorized as medium-toxic compounds, and only a few of them, like the diene derivatives (aldrin and dieldrin), belong to the class of potent and very hazardous agents. The compounds of diene synthesis are characterized by their high intradermal toxicity. Also typical of many OCCs is their irritant effect on the skin and mucous membranes. Humans have been found more OCC-sensitive than warm-blooded animals (e.g. towards DDT, aldrin, and other OCCs) (E.I.Spynu, 1974).

A specific and highly pernicious OCC property is associated with the ability of some of them for cumulation, both material and functional. Though a few OCCs appear to be medium-toxic in a single entry into the body, chronic intoxication can be induced when they enter the body in low doses by various routes.

Organochlorine pesticides are referred to polytropic poisons, predominantly damaging the central nervous system and the parenchymatous organs, notably the liver. The functions of the endocrinal and cardiovascular systems, blood and kidneys are also impaired. This multifarious symptom-group is clearly manifested in the picture of an acute poisoning. A chronic poisoning causes lesions in the central and peripheral nervous systems, particularly with the characteristic development of hepatites, gastrites, and bronchites (E.I.Spynu, 1974, 1977).

Sensitizing properties have been established in a number of OCCs (DDT, HCCH, etc.), which may cause allergic responses on repeated exposure.

The mechanism of action of organochlorines upon the warm-blooded organism is not clear at present. The primary element in the mechanism promoting intoxication is the lesion of the parenchymatous organs, above all the liver, and functional impairment of the nervous system. The toxic effects of the OCCs are generally attributed to the changes in the activity of the enzymes of the respiratory chain and the impairment of tissue respiration (F.A.Onikienko, 1963; V.I.Osetrov, 1965; U.A. Kuzminskaya, 1973).

Several OCCs have been found to be gonadptoxic (DDT, HCCH, dieldrin), teratogenic (kelthane) and embryotoxic (polychlorocamphene, polychloropinene).

Because of their potential danger for man, a number of organochlorines are prohibited for agricultural uses in the USSR (DDT, aldrin, dieldrin) while the use of some others is stringently controlled (e.g., heptachlor, hexachlorobutadiene).

Derivatives of carbamic, thio- and dithiocarbamic acids have found extensive application in agriculture because of their high insecticidal, fungicidal, and nematocidal activity. Some of these substances are highly toxic to man and warm-blooded animals (baygon, pyrimor, carbathion). Others, like naphthyl (sevin, etc.) and cresyl (dicresyl, etc.), esters of methylcarbamic acid, are less toxic for warm-blooded organisms than the derivatives of methyl- and dimethylcarbamic acids, whose molecule contains heterocyclic radicals (G.A. Voitenko, 1974). The cumulative properties of the compounds in this group are only slightly pronounced. There is a considerable cumulative potential however, in the dithiocarbamic acid derivatives-ziram (K_{cum} = 1.2) and TMTD (K_{cum} = 2.8).

The carbamic acid derivatives exhibit a strong anticholinesterase activity which is believed to be the primary mechanism for their toxicity. But, unlike the organophosphorus cholinesterase inhibitors, the carbamyolated cholinesterase, formed under the effect of carbamic compounds, is readily hydrolyzable. Carbamates of the aliphatic series do not show any pronounced anticholinesterase activity. The impairment of endocrinal organs and interference with the oxidative processes and nucleic acid metabolism are of importance in the toxicodynamics of the aryl esters of carbamic acid (e.g. sevin). Similar alterations are also observed on exposure to the derivatives of thiocarbamic acid.

Of importance is the fact that under natural conditions destruction of dithiocarbamic acid derivatives is observed on food crops, in soil, and water. Their decomposition yields a number of very toxic volatile compounds (CS_2, H_2S, dimethylamine and methyl thioisocyanate) and several comparatively persistent products detectable in foods and water. For instance, tetramethylthiourea dimethylamine, a product of the destruction of TMTD and ziram; ethylene thiourea and ethylenthiuram disulfide, the conversion products of zineb, cuprocine, polycarbacine, etc. (G.A.Voitenko, 1977).

Some dithiocarbamates are allergenic (zineb, maneb, TMTD, and ziram) and cause dermatites, bronchial asthma and other disorders on repeated exposure.

The pesticidal derivatives of carbamic and dithiocarbamic acids can have long-term effects. Sevin, for example, was reported to have a marked influence on the reproductive function (G.A. Voitenko, 1977), bethanal was shown to be embryotoxic and teratogenic both upon oral and inhalation exposure (G.A.Voitenko, 1977), and so were zineb, ziram, and maneb (L.U.Martson, 1967, R.A.Ryazanova, 1967, 1968). Cytogenetic activity was discovered in TMTD, ziram, zineb and maneb from studies on bone-marrow cells and on chromosome aberrations in the lymphocyte culture (A.I.Kurinny, M.A. Pilinskaya, 1976). These findings were taken into account in the hygienic rating of these pesticidal agents. As a result, maneb and ziram were excluded from the list of the pesticides authorized for use in the USSR, and TMTD usage is permitted only as a seed desinfectant. For zineb, low permissible residual quantities have been permitted in vegetable foods, and none at all in milk and dairy products. Sevin applications are restricted.

Nitro and chlorine derivatives of phenol: dinitrophenol (DNP), dinitro-orthocresol (DNOC), dinitro-sec-butylphenol (DNBP), pentachlorophenol (PCP), sodium pentachlorophenolate, and acrex are wide-spectrum pesticides, designed for agricultural applications as insecticides, fungicides, herbicides and acaricides. Most of the substances are toxic to warm-blooded organisms. Their mechanism of action is primarily associated with the ability to interfere with the metabolism in the cell, particularly by uncoupling the oxidative phosphoryllation processes with the loss of energy-rich compounds like ATP and others.

The phenol chlorine derivatives are more volatile than nitroderivatives produce more obvious local irritant effect on skin and mucous membranes and easily penetrate through skin. In hot weather they pose a higher risk of intoxication because of intensified absorption and the specific stimulating effect they exert on heat production (E.N.Burkatskaya, 1977).

Organomercury compounds (OMCs) and their combinations with other chemicals are most effective seed disinfectants (granosan, mercurobenzene, mercurohexane). Upon entry into the body by various routes the organomercuries produce a strong toxic effect. Being patently cumulative, organomercury chemicals, besides acute poisoning, pose the risk of chronic poisonings. Experimental evidence and the data on occupational exposure show granosan to be the principal potential source of hazard for those handling organomercury pesticides at work (I.M.Trakhtenberg, V.E.Balashov, 1977).

Toxicity of OMCs is based on the fact that they are thiol poisons. As such, they react with the sulfhydryl groups of cell proteins and disrupt the activity of principal enzymatic systems which need free SH-groups to maintain normal functioning. This results in a series of diverse primary (largely, in the central nervous system) and mediated changes in the organism, specifically in the vegetative nervous system, peripheral neural formations, in the heart and vessels, hemopoietic organs and peripheral blood, liver, kidneys, and urinary tracts (L.I.Trakhtenberg, 1964; I.M. Medved, 1961). Extensive experimental evidence reveals the embryotoxicity and teratogenicity of mercury compounds in general and granosan in particular.

Organomercury agents are referred to potent toxic (or highly toxic) chemicals, strongly cumulative and very persistent. For this reason, mercury-free seed desinfectants are being introduced in agriculture in increasing amounts. They are: hexachlorobenzene, pentachloronitrobenzene, TMTD, anilate, vitavax, and the composite desinfectants (fenthiuram and hexathiuram).

In the total pesticides manufacture in the USSR and abroad there is now a clear-cut trend towards the predominant use of herbicides (M.S.Sokolov, 1971, N.N.Mel'nikov, 1973, 1974). The herbicides in agricultural use belong to different classes of chemical compounds. Among the most widely used are aryloxyalkyl carboxylic acids and their derivatives: 2,4-dichlorophenoxyacetic acid with its esters and salts 2M-4C, etc.

Derivatives of chlorophenoloxyacetic acid, 2,4-dichlorophenoxyacetic acid (2,4-D), 2-methyl-4-chlorophenoxyacetic acid (2M-4C) and their salts and esters are applied as weed killers on different crops, especially cereals. Used in low doses, they act as plant growth regulators. Amides, butyl, isopropyl and other esters are regarded as the most effective herbicides. Most of the chemicals in this group are medium-toxic agents, but have skin-absorptive toxicity. They can be retained in the organism of warm-blooded animals and induce chronic intoxications (E.N.Burkatskaya, T.V.Dyadicheva, 1977).

They impair the central nervous system, liver, kidneys, and endocrinal glands. Oxygen consumption by the tissues and the tension of the smooth muscles are reduced, and the body temperature descreases. For 2,4-D, its potential genetic risk for human and animal somatic cells, and embryotoxicity have been ascertained (A.I.Kurinny, M.A.Pilinskaya, 1976; T.A. Konstantinova, 1979).

<u>Derivatives of chlorophenoxybutyric acid</u>. This category covers herbicides 2,4-DM (2,4-dichlorophenoxybutyric acid) and 2M-4CM (2-methyl-4-chlorophenoxy-γ-butyric acid), selectively affecting weeds. They are medium-toxic compounds, weakly cumulative. By the mode of action on the body, the compounds resemble 2,4-D.

<u>Haloanilides of carboxylic acids</u> constitute a group of herbicides highly selective in their action on weeds. It includes propanide, ramrod, solan, suffix, menide, lasso, delachlor, karsil, etc. They are used on rice, vegetables, soya, corn, and other crops.

These herbicides are medium- and low-toxic; some of them possess strong cumulativity (propanide, dicryl, delachlor). An increase in ambient temperature raises the toxic effect of the chemicals by a factor of 1.5 to 2 (T.N. Pan'shina, 1977).

The ability of the haloanilides of carboxylic acids to influence the morphological composition of peripheral blood and redox enzymatic systems, formation of methemoglobin under the effect of the derivatives of the anilides of aliphatic carboxylic acids and, as a consequence of that, the development of hypoxia determines their principal mechanism of toxic action upon the organism of warm-blooded animals. Disturbances of protein metabolism are also included in that mechanism, as suggested by the changes induced by the herbicides in the amino-acid spectrum and the activity of reamination enzymes. The herbicidal toxic effect has been found to correlate with the chemical structure: the derivatives of the haloanilides of carboxylic acids, such as propanide, solan, menide and dicryl, trigger methemoglobin formation in the blood, with no such capability detected in the chloroacetanilides (ramrod, delachlor, and lasso). Structure-dependent differences were also noted for the effect of the herbicides on the metabolism of nicotine amidadenine dinucleotides. No pronounced embryotoxicity and teratogenicity have been discovered for propanide or ramrod (T.N. Pan'shina, 1977), however M.A. Pilinskaya and A.I.Kurinny (1976) established a mutagenic effect for ramrod, this being the reason for limiting the area of its use in future.

Urea and guanidine derivatives are extensively used to eradicate weeds in orchards, cotton fields, potatoes, legumes, sugar beet and other crops (dichloralurea, herban, fenuron, monuron, diuron, tenoran, kotoran, linuron, aresine, lenacyl, meturine, etc.).

Most of these chemicals are medium-to-low toxic, with moderate cumulativity. Signs of local irritant effects on the skin and mucous membranes of the eyes are possible. Characteristic of the compounds is their adverse effect on the thyroid gland (V.S.Buryi, 1977). Some herbicidal urea derivatives, notably monuron, were shown to be slightly blastomogenic (O.P.Chepinoga, 1970; B.L.Rubenchik, 1970).

Derivatives of sym-triazine (propazine, symazine, athrazine, prometryn, semeron, and others) are widely used in agriculture. Symtriazines are medium-to-low toxic, with poorly manifested cumulativity. They are, nevertheless, persistent in the environment and capable of causing untoward effects upon chronic exposure. Their proximity to pyrimidine bases attracts attention to them as prospective antimetabolites which would often require a long time to take effect (Yu.S.Kagan, 1981). Triazine poisonings are accompanied by impairments of the proteinforming and carbohydrate liver functions and alterations of blood morphological composition (E.N. Burkatskaya, T.V. Dyadicheva, 1977). Some triazines were reported to be weak blastomogens, e.g. symazine and 2,4,6-trioxo-trihydro-symm-tirazine (O.P.Chepinoga, 1970).

Today all industrialized countries are actively searching for chemicals effective against insects when used in small amounts and rapidly decomposable in the environment.

Synthetic pyrethroids, the analogs of natural pyrethrines, form a new group of pesticides highly effective against the insects resistant to the organophosphates, chlorines and carbamate insectoacaricides, and shows a good promise in future.

Pyrethroids are highly selective and are used at low application rates per hectar. Some of them, already in commercial manufacture, include permetryn (ambush, corsar, parmasect), cipermetryn (cimbush, ripcord, sherpa), decis (decametryn), bioresmetryn (isatryn, plactigrine), sumicidine (fenvalerat), rovicurt, and others.

Compounds of this group include both highly (decis) and low-toxic (neopinamine ambush) agents (O.A.Korotkova, V.K.Promonenkov, 1977, 1978; N.N.Mel'nikov and coworkers, 1980; T.N. Pan'shina, L.M. Sasinovich, 1981). Some of them have been noted to produce a pronounced irritant effect on the skin and eye mucosa (decis, cimbush). Cumulative effects are poorly pronounced.

The neural and hepatic toxicity of pyrethroids is important in their general mechanism of toxicity for the insects. In some pyrethroids (neopinamine and cimbush) no overt effect on embryogenesis has been discovered (L.V.Martson, et al., 1981; V.M. Voronina, 1982) and neither have any mutagenic properties been detected for neopinamine (A.I.Kurinny, et al., 1982). Thus, the new pesticidal group of synthetic pyrethroids can be favorably assessed from the hygienic point of view: most of them exhibit medium-to-low toxicity, short persistence in the environment and low volatility. On the other hand, quite a few aspects in their effects demand close attention in working out the regulations for their use. They manifest local-irritant (decis and cimbush) and skin-absorptive (decis) effects. Some pyrethroids must be considered with special reference to their high toxicity for the honeybees and fish.

Summing up, it should be emphasized that in the mechanism of action of various pesticides, intoxication caused by them, and the influence they exert on different body organs and systems, along with their polytropic affect, they also have specific points of attack. Analysis of data from literature and toxicological study of over 100 new pesticides from different chemical classes and their combinations have revealed (L.M.Sasinovich, T.N.Pan'shina, and S.S. Svetly, 1983) some specific patterns in the biological response of the body, depending on their individual chemical structure. Anticholinesterase activity has been the prevalent type of effect associated with the organophosphates and some carbamic acid derivatives (sevin), which agrees with the data available in literature (Yu.S.Kagan, 1974, 1977 et al.). A strong influence on the liver was ascertained by Sasinovich L.M. (1981) for organochlorine pesticides (DDT, lindane, polychloropinene, polychlorocamphene, etc.) and organophosphates, particularly those containing chlorine atoms in their molecule (chlorophos, DDVP, etc.). Severity of the hepatotoxic OCC effect is primarily determined by the duration of exposure and of the OPC effect by the administered dose. The hepatotoxic effect was somewhat less manifested for carbamates, urea derivatives, and the haloanilides of carboxylic acids. The blood system was seen to be affected by the derivatives of dithiocarbamic acid and haloanilides. The derivatives of dithiocarbamic acid (TMTD and zineb), urea and uracil typically affect the function of the thyroid gland. Exposure to combined chemicals, involving the derivatives of dithiocarbamic acid and chlorine - and copper-containing agents, induced more apparent disturbances (S.S.Svetly, 1981).

It should be noted that the activity of the monooxygenase liver system importantly involved in the metabolism and detoxication of xenobiotics, does not change unambiguously in response to differently structured pesticides as they gain access into the body (U.A.Kyz'minskaya, et al.,, 1977; Yu.S.Kagan, et al. 1981; L.M.Sasinovich, T.N.Pan'shina, and S.S.Svetlyi, 1983). This system responds by active and durable induction to the organochlorine pesticides and haloid anilides of carboxylic acids; a somewhat milder inductive effect is produced by organophosphates (phosalon, valexon, and phthalophos). By contrast, dithicarbamates (TMTD), some OPCs (heterophos,bromophos and chlorophos) and dipyridyls are inhibitors.

The specific mode of the effect of pesticides on the activity of the monooxygenase system determines in a number of ways the pattern of combined effect and the toxicity of pesticidal mixtures (U.A. Kuz'minskaya, et al., 1977; S.S.Svetlyi, 1981).

In conclusion, it should be stressed that pesticides, being biologically active, are potentially hazardous to animate nature and human beings. Only proper and strict compliance with scientifically substantiated hygienic standards and cautionary measures will ensure their safe application and afford protection against unfavourable health effects to the occupationally exposed and the population in general.

- - - -

REFERENCES

1. Medved L.I. Toxicology and hygiene of modern pesticides. Journal of the Mendeleyev All-Union Chemical Society, 19, N.2; 178-185 (1974) (in Russian).
2. Medved L.I. Hygienic criteria for a choice of pesticides. Improvement of choice. In: Application hygiene, toxicology of pesticides and clinical picture of poisonings. (Kiev: Urozhai, 1974 and 1977) (in Russian).
3. Manual on pesticides. (Ed. L.I. Medved). Application hygiene and toxicology. (Kiev: Urozhai, 1974 and 1977) (in Russian).
4. Manual on harmful compounds in industry. (Eds. N.V.Lazarev and I.D. Gadaskina) (Leningrad: Khimiya, 1977) (in Russian).
5. Manual on plant protection chemicals. N.N.Mel'nikov, K.V. Novozhilov, and T.N. Pylova. (Moscow: Khimiya, 1980), (in Russian).
6. Yu.S.Kagan, Toxicology of organophosphorus pesticides (Moscow: Meditzina, 1977) (in Russian).
7. Yu.S. Kagan, General toxicology of pesticides (Kiev: Zdorov'e, 1981) (in Russian).
8. Yu.I. Kundiev, Skin penetration of pesticides and prophylaxis of poisonings (Kiev: Zdorov'e, 1975) (in Russian).
9. A.I.Kurinnyi and M.A. Pilinskaya. Investigation of pesticides as mutagenic agents for the environment. (Kiev: Naukova Dumka, 1976) (in Russian).
10. T.N.Pan'shina, Hygienic aspects of agricultural application and toxicology of some herbicides. Gigiena i sanitariya, N.7: 38-41, 1976 (in Russian).

11. T.N.Pan'shina and L.M.Sasinovich, New synthetic pyrethroids: potential hazard and hygienic standardization: In: Problems of pesticide hygiene and toxicology. Part 2. (Kiev: 1981) (in Russian).

12. L.M.Sasinovich, T.N. Pan'shina, and S.S.Svetlyi, The effect of chemical structure of pesticides and their mixtures on the biological response. Gigiena i sanitariya, N.3: 30-34 (1983) (in Russian).

TOXICOLOGY OF ORGANOPHOSPHOROUS PESTICIDES

Prof. F.KALOYANOVA

Poisonings with organophosphorous (OP) insecticides take leading place in most of the countries. More attention is given recently in publications to specific problems. For example, of certain interest is the report made by Davies and all 1975 concerning poisoning with fat soluble OP - dichlorfenthion. The symptoms developed after a long latent period 40-48 days and persisted 5-48 days. The pesticide residues disappear in 54 days in the fatty tissue and in 75 days in blood.

More serious attention is given to impurities after the epidemic malathion intoxication in 1876 in 2800 Pakistan malaria workers associated with the use of formulations containing increased amounts of isomalathion, and poor work practices resulted in excessive percutaneous absorption (Baker and all 1978).

Attention is given to data that even in granular formulation a highly toxic insecticide as temic - provoked intoxication. In California - 38 cases occured for the period 1974-1976. (Peoples and all 1978).

The climatic factors could also effect the poisoning. High temperature 36-40°C potentiate the toxic effects of anthio in cotton farming workers (Demidenko and Mirgijazova,1974).

During spraying of a field crops from an aircraft a strong wind blew the insecticide 500 yards from the field. People working in the area developed symptoms of intoxication 2.5-6 h afte that (Kalas, 1978).

Chemical Structure and Use

Organophosphates (OPs) have similar chemical structures and show identical toxic effects due mainly to cholinesterase inhibition. They are, in fact, known as anticholinesterase substances or cholinesterase inhibitors.

Large numbers of OPs are in use, and they include different types, the most important of which are the phosphates, phosphorothioates, phosphorodithioates, phosphoroamides, phosphonates and pyrophosphates. OPs are used mainly as insecticides, but some of them are also used as fungicides, herbicides and rodenficides (Kagan, 1977). The most important types are shown at Table 1.

Table 1

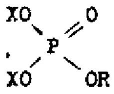
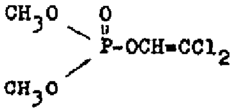
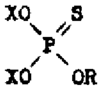
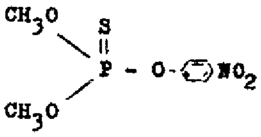
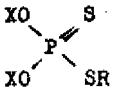
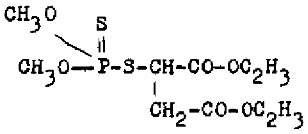
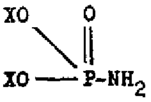
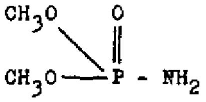
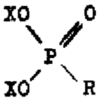
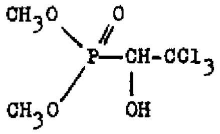
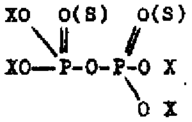
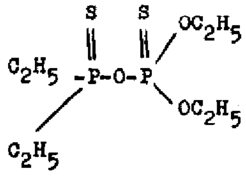
Type of compounds	General structure	Examples
Phosphates		 dichlorphos (DDVP)
Phosphorothioates		 methyl parathion

Table 1 (cont.)

1	2	3
Phosphorodithioates		 malathion
Phosphoroamidates		 Tamaron
Phosphonates		 Trichlorofon
Pyrophosphates		 Bladafum

Some of the used formulations are given at Table 2.

Table 2

Names	Chemical name	LD_{50} rat mg/kg	LD_{50} dermal mg/kg
1	2	3	4
Abate (temophos)	Tetramethyl thiodiphenylene phosphorothioate	8600	-
Actelic (pyrimiphos-methyl)	Dimethylamino methylpyrimidin dimethyl phosphorothioate	2000	-
Anthio (formathion)	Formylmethyl amino oxoethyl-dimethyl phosphorodithioate	365	1000 rat
Azinphos-ethyl (triazation)	Diethyloxpbenzotriazine-methyl diethyl phosphate	17.5	250
Azinphos-methyl (guthion)	Dimethyl oxobenzotriazine methyl-phosphorodithioate	13-16	220 rat
Azodrin (monocrotophos nuvacron)	Dimethyl phosphate hydroxy N-methyl-crotonamide	8-23	354 rabbit
Bromophos (Nexion)	Bromodichlorophenyl dimethyl phosphorothioate	3750	-

6300

Table 2 (cont.)

1	2	3	4
Carbophenothion trithion	p. Chlorophenylthiomethyl diethylphosphorothioate	32	-
Chloropyrophos (dursban)	Diethyl trichloro pyridyl phosphorothioate	135-155	2000 rabbit 202 rat
Cidial (phenthoate)	Dimethyl-ethoxy carbonyl benzyl phosphorodithioate	439	-
Curacron (polycron)	Bromochloro phenyl propyl-phosphorothioates	358	1610 rat
DDVP (Dichlorohos vapona, nuvan)	Dichlorovinyl-dimethyl phosphate	56-80	107 rat
Diazinon (Basudin)	Diethyl methyl (I-methyl ethyl) pyrimidinyl Phosphorothioate	800-850	2150 rat
Dicrotophos (Bidrin, carbicron)	Dimethyl (dimethylcarbamoyl methyl vinyl phosphate	22-15	225 rabbit
Dimecron (phosphamidon)	Dimethylchloro diethylcarbamoyl vinyl phosphate	17-30	
Dimethoate (fosfamid rogor)	Dimethyl-s(N-methilcarbamoylmethyl) phosphodithioate	320-380	650 guinea pig

Table 2 (cont.)

1	2	3	4
Fentrothion (Folithion sumithion)	Dimethyl-nitrotolyl phosphorothioate	500	1300
Gardona (tetra chlorvinphos)	Chlorotrichlorophenyl Vinyl dimethyl phosphate	4000	-
Hostathion	Phenyltriazoyl diethyl-thionophosphate	82	1100
Malathion (carbofos, mercapto-thion)	Diethyl (dimethoxyphosphinothioyl) butanedithioate	1000-1375	4100 rabbit
Menazon (azidothion sayfos)	Dimethyldiaminotriazinyl-methyl dithiophosphate	640-1950	-
Mephosfolan (cytrolane)	Diethoxyphosphinyl-imuno-methyl-dithiolane	8.9	9.7
Methylparathion (Meta-aphoswofatox)	Dimethyl-p-nitrophenyl-phosphorothioate	9-25	300-400
Mevinphos (phosdrin)	Methoxycarbonyl-I-dimethyl phosphate	3.7-6.1	4.2 4.7 rat

Table 2 (cont.)

1	2	3	4
Monitor (tamaron, methamidophos, acephatment)	Dimethyl phosphoramidothioate	18.3-21	
Parathion-methyl	Dimethylnitrophenyl phosphorothioate	24-14	67 rat 24-14 other species
Phospholan (cyolane)	Diethoxyphosphinyl-imino di-thiolane	8.9-12.1	23 rabbit 24 guinea pig
Primicide (pyrimifosethyl)	Diethyl aminomethyl pyrimidinyl diethyl phosphorothioate	132	1000-2000 rat
Supracid (methidathion)	Dimethyl phosphorodithioate ester with mercaptomethyl-methoxythiodiazolin	65	670
Thiometon (EKATIN, intrathion)	Ethyl thioethyl-dimethylphosphoro dithionate	120-130	1000 rat
Trichlorfon (dipterex, dylox, nequvon, chlorofos)	Dimethyl (trichloro- I hydroxy-ethyl) phosphonate	560-630	5000 rabbit

Table 2 (cont.)

1	2	3	4
Trichloromethaphos	Methylethyltrichlorphenylthiophosphate	150-500	
Zolone phosolone	Diethyl chloro exo benzoxozalin methyl phosphorodithioate	120	-
Volaton (Phoxim)	Phenylglyoxylonitrile oxime diethylphosphorothioate	1845	

Mechanisms of Action

The principal mechanism of action of the OPs is inhibition of activity of cholinesterase (ChE), the enzyme performing the hydrolysis of acetylcholine to choline and acetic acids. Fig. 1 specific ChE (acetylcholinesterase 3.1.1.7) is located in the nervous ganglionic synapses of neuromuscular structures and in erythrocytes, and nonspecific ChE (3.1.1.8.) occurs mainly in the plasma and liver. Organophosphorus insecticides generally inhibit both enzymes.

With the change of the membrane potential, acetylcholine (ACh) acts as a mediator of the nerve impulses. In the cytoplasm of the nerve end, before the synaptic membrane, special vesicules contain acetylcholine which is synthesized by the enzyme cholinacetylase (ChA) from acetyl CoA and choline.

The nerve impulse produces a discharge of ACh across the synaptic gap. The ACh contacts cholinergic receptor protein molecules of the postsynaptic membrane and changes its configuration, enabling Na and K cations to penetrate. The transfer of the nerve impulse continues. This process is very short, lasting about 1/500 s and is followed by hydrolysis of ACh by ChE.

The OPs act against this hydrolysis. They produce phosphorylation of the esteric binding side of ChE. Fig. 2. Phosphorylation inactivation of ChE stops the hydrolysis of ACh and causes accumulation of excessive quantities of ACh at peripheral ganglionic and central nerve endings (synapses) in effector organs and elevated concentrations in plasma and intestinal fluid. The symptoms are mainly of a cholinergic nature. Three effects of the intoxication are connected with the excitement of M- and N-choline receptors (present on nerve terminals of effector organs):

- muscarine effect due to excitement of M-choline receptors of post-ganglionic cholinergic nerve impulses to the lungs, gastrointestinal system, heart, kidneys, sweat glands, pupils and muscles;
- nicotinic effect on the receptors of ganglionic synapses and motoric plates, medular part of the glandular suprarenalis and carotic nodules; and
- central effect of ACh due to a direct impact on the choline receptors of the nerve cells or accumulation of ACh, with other enzymes also inhibited by OPs: lipase, holesterol esterase, proteinase, monoaminooxidase and other nonspecific esterases.

Clinical Picture of OP Intoxication

The OPs present a serious hazard of acute intoxication which varies considerably from compound to compound, with LD_{50}

varying from mg to g/kg body weight. Compounds containing a P = S nucleus, such as parathion, must be activated by the metabolic change of S by O, which is performed by mixed-function oxidase of the liver and intestinal wall. Such compounds are called indirect inhibitors of cholinesterase activity (ChEA). Symptoms of intoxication by OPs are given in Table 3. Death appears to be primarily asphyxial in some instances and cardiovascular in others. In many cases only a few of these symptoms are observed (Table 4). The intervals between exposure and onset of the symptoms may be as short as a few minutes but are usually 1-2 hours. Rarely, the interval exceeds 24 hours.

Other symptoms, such as fever, are also reported, but they are atypical. The respiratory failure results from a combination of blockage of the respiratory tract with excessive secretion from glands of the mouth and respiratory tract, by possible bronchocontraction and by paralysis of the respiratory areas of the brain stem. Unless exposure causes death, neurological effects dependent upon inhibitors of ChE are, in most cases,

Table 3

Signs and symptoms of anticholinesterase poisoning

(WHO TRS N 356, 1967)

Site of action	Signs and symptoms
1	2
	Following local exposure
Pupils	Miosis marked, usually maximal (pinpoint), sometimes unequal
Ciliary body	Frontal headache, eye pain on focusing,

Table 3 (cont.)

1	2
	slight dimness of vision, occasional nausea and vomiting
Conjunctivae	Hyperaemia
Mucous membranes	Rhinorrhoea, hyperaemia
Bronchial tree	Tightness in chest, sometimes with prolonged wheezing, expiration suggestive of bronchoconstriction or increased secretion, cough
Sweat glands	Sweating at the site of exposure to the liquid
Striated muscle	Fasciculations at site of exposure to the liquid
	Following systemic absorption
Bronchial tree	Tightness in chest, with prolonged wheezing, expiration suggestive of bronchoconstriction or increased secretion, dyspnoea, slight pain in chest, increased bronchial secretion, cough
Gastrointestinal system	Anorexia, nausea, vomiting, abdominal cramps, epigastric and substernal tightness (cardiospasm) with "heartburn" and eructation, diarrhoea, tenesmus, involuntary defecation

Table 3 (cont.)

1	2
Sweat glands	Increased sweating
Salivary glands	Increased salivation
Lacrimal glands	Increased lacrimation
Pupils	Slight miosis (occasionally unequal), later marked
Ciliary body	Blurring of vision
Bladder	Frequent or involuntary micturition
Striated muscle	Easy fatigue, mild weakness, muscular twitching, fasciculations, cramps, generalized weakness including muscles of respiration, with dyspnoea and cyanosis
Sympathetic ganglia	Pallor, occasional elevation of blood pressure
Central nervous system	Giddiness, tension, anxiety, jitteriness, restlessness, emotional lability, excessive dreaming, insomnia, nightmares, headache, tremor, apathy, withdrawal and depression, bursts of slow waves of elevated voltage in EEG especially on hyperventilation, drowsiness, difficulty in concentration, slowness of recall, confusion, slurred speech, ataxia, ge-

Table 3 (cont.)

1	2
	neralized weakness, coma with absence of reflexes, Cheyne-Stokes respiration, convulsions, depression of respiratory and circulatory centres with dyspnoea, cyanosis and fall in blood pressure
Circulatory system	Bradycardia, decreased cardiac output, cardiac arrest, paralysis of vasomotor centre

Table 4

Clinical symptoms at different levels of OP poisoning

(WHO TRS N 356, 1967)

Level of poisoning	Clinical symptoms
1	2
Mild	Weakness, headache, dizziness, diminished vision, salivation, lacrimation, nausea, vomiting, lack of appetite, stomach-ache, restlessness, miosis, moderate bronchial spasm, 60% reduction of ChEA Convalescence in 1-3 days
Moderate	Abruptly expressed general weakness, headache, visual disturbance, excess

Table 4 (cont.)

1	2
	salivation, sweating, vomiting, diarrhoea, bradycardia, hypertonia, stomach-ache, twitching of facial muscles, tremor of hands, head and other parts of the body, increasing excitement, disturbed gait and feelings of fear
	Miosis nystagmus, pain in the chest, difficult respiration, cyanosis of the mucous membrane, crepitations in the chest, 60-90% reduction of ChEA
	Convalescence in 1-2 weeks
Severe	Abrupt tremor, generalized convulsions, psychic disturbances, intensive cyanosis of the mucous membrane, oedema of the lung, coma, 90-100% reduction of ChEA
	Death from respiratory or cardiac failure

reversible. Local and less severe effects do not usually last more than a day. Miosis often disappears in less than a week, and most other symptoms diminish over the next 6 to 18 days. The duration of symptoms of poisoning depends in part on the rate of reactivation of the inhibited ChE and the rate at which the inhibitor itself is destroyed or removed from the tissue. Both these factors are related to the chemical structure of the compounds. A sponteneous reactivation depends on the chemical structure of the phosphoryl group attached to the enzyme and hence on the chemical structure of the insecticide. Thus, the phosphorylated enzyme produced when dimethyl phosphate esters inhibit ChE spontaneously reactivates more rapidly than that produced by diethyl phosphate esters. The reactivation of the inhibited enzyme can be considerably accelerated by special compounds (notably oximes) in vitro and in vivo. Several of these compounds have become important antidotes in the treatment of poisoning (Fig. 3).

Spontaneous reactivation is not the only reaction that the inhibited enzyme may undergo. It may also be transformed into a state where no spontaneous reactivation occurs and also where oximes are no longer capable of reactivating it. This phenomenon is called "aging" and is characterized by removal of one of the alkyl groups from the phosphoryl groups attached to the enzymes. The rapidity of aging of inhibited ChE is dependent upon the chemical nature of the phosphorylating insecticides (WHO TRS N 356, 1967 and N 634, 1979).

Diagnosis of the intoxication can be difficult in mind cases when only miosis, nausea, vomiting, weakness, headache and giddiness are observed. In such cases, a good anamnesis and ChEA determination will help very much.

Chronic intoxications are almost rare because OPs are not highly cumulative. The same symptoms as in acute intoxications are found but are less pronounced: headache, giddiness, insomnia, weakness, increased sweating, nausea, loss of appetite, tremor and nystagmus. Other symptoms reported by different authors include changes in the liver function in connection with protein synthesis, troubles in the gastrointestinal tract, hypoacidity or achylia, occasional acidity, behavioural changes (Levin and Rodnitzky, 1976), neuritis, paralysis, ECG and EEG changes (Metcalf and Holmes, 1969, Aldridge and Johnson, 1971).

In epidemiological studies besides ChEA inhibition most frequently were reported liver, renal, skin, cardiovascular, hemopoetic and respiratory disturbances and aggravation of existing ill health conditions.

Changes in some biochemical parameters such as an increase of aminoacid levels and total serum protein and changes in albumin globulin index, and in serum enzyme activities of aldolase, alkaline phosphatase SGOT, SGPT, ornitincarbamyl-transferase have been attributed to the disturbances of the liver function. (Bogust, 1968). Liska and Tildova,1977,found in chronic exposure increased GPT and LDH isoenzyme activity (LDH_5).

Functional effects on the cardiovascular system have been found: bradicardia, hypotension, hypertension and electrocardiographic changes (Faerman, 1965).

From the five main blood dyscrasias - aplastic anemia, thrombocytopenia, leukopenia erythroid hypoplasia and hemolytic anaemia parathion was supposed to produce aplastic anemia (Christofor, 1969) and OP as a group - agranulicytose (Zaninovic, 1977).

Fibrinolysis, hypercoagulability in mevinphos intoxicated persons are reported by Holmes and all, 1974.

The neurotoxic effects resulting from exposure to OP insecticides can be classified as effects either directly related to ChE inhibition on delayed neurotoxic actions.

Delayed neurotoxic effects attributed to the OP insecticides have been associated with inhibition of the so-called neurotoxic esterases (Johnson M.V., 1969; Aldridge and Johnson, 1971) (Fig. 4). Chronic exposure to OP may result in peripheral neuropathy, myopathy, visual disturbance, cochlear and vestibular effects and behavioural changes. Cases of clinical paralysis in men have been described with leptopos, mypaphos and others. Phosphonate structure has positive correlation with delayed neurotoxicity (El Sebae and all, 1977). Both partial demyelization and an effect on the axon cylinders occur. Polyneuropathy developed 2-3 weeks later after acute intoxication with tamaron (Senanayka and Johnson, 1982). There are contradictory opinions concerning the diagnostic activity of the early motor impairments by electromyography.

Significant deviations from the norm were found in approximately 50% of the workers exposed to organophosphate insecticides (Jager and all, 1970).

A comparison of the maximum conduction velocity of motor nerve fibres in control and organophosphorous workers showed that the latter group had, on average, velocities 10% less than the control group (Roberts, 1976).

Recent studies didn't conform the conclusion that EMG is more sensitive parameter than ChEA inhibition. (Jusic and all, 1980).

Jager (1976) made extensive review of 218 papers to hold the light on the problem of neuropathy connected with OP exposure and the use of ENMG as a screening method for early detection of health impairments. He also reports his own results and summarises the changes found as follows:

- A decrease in the voltage of the action potential of the muscle. In non-exposed controls most values would be between 11 and 12 mV, in the exposed workers the range would be wider and values could occasionally be as low as 4 mV.

- A change in the pattern of the recorded electromyogram, indicating that not all muscle fibres react at the same time to the supramaximal stimulation.

- A decrease in the conduction velocity of the nerve fibres. Any signs and symptoms or overt disease have been related to the above findings, except such as fatigue, weakness, etc.

Jager's data on organophosphate workers indicate that the changes are reversible, but the literature review made by him shows that in more advanced stages which are accompanied by clinical signs and symptoms this may not always be so. Reversibility possibly depends upon both the magnitude and the duration of exposure.

Cholinesterase determinations and electroneuromyography both have their own place in the medical supervision of organophosphate workers. Which one gives the first indication of overexposure probably depends on the timing of the test in relation to the exposure and the degree and duration of exposure. ENMG-changes may be completely unrelated to cholinesterase depression.

Persistant central nervous system manifestations were first reported to include impaired memory, depression, impaired mental concentration, schizophrenic reactions and instability, lasting for 6-12 months in 16 subjects who had been exposed to organophosphorous insecticides for 18 months to 10 years (Gershon and Shaw, 1961).

Later on Dille and Smith (1964) described2 cases with mental disturbances. The study concerned pilots working mostly with OP. One developed depressive fobia, and the other fear and emotional instability. Schizophrenic reaction have been demonstrated after heavy acute intoxication as a sequel (Monov,1965). Neurastenic manifestations are communicated by Kovarik and Serel (1966) and West (1968) in survivors after acute intoxications. In the review of this problem prepared by Levin and all,1976, using their own and other publications the following conclusion is made.

Despite methodologic shortcomings in many of the published studies, investigators generally agree on the presence of several behavioural sequelae or organophosphate poisoning: (a) impaired vigilane and reduced concentration, (b) slowing information processing and psychomotor speed, (c) memory deficit, (d) linguistic disturbance, (e) depression and (f) anxiety and irritability.

The possible relationship between air craft incidents and effect of pesticides is discussed by Reich and Berher, 1968. They found from 12 accidents in 8 cases inhibition of ChEA in pilots - Wood and all, 1971, reported an accident in which pilot's disturbance in coordination and ability to regulate the speed of airplane have been found. Heat stress in hot climate conditions seems to play very important role in aircraft accident (Richter and all, 1980).

EEG investigations are used for defining characteristics of CNS disturbances in OP exposed workers. Duffy and all, 1979, stated that statistically significant group differences included increased beta activity, increased delta and theta slowing, decreasedalpha activity, and increased amounts of rapid eye movement sleep in the exposed population. The above findings represent an unexpected persistence of known short-term OP actions. It is also suggested that these results, when taken along with the reported long-term behavioural effects of OP exposure, provide parallel evidence that OP exposure can produce long-term changes in brain function.

Vision impairments in acute intoxication are well known. Long-term exposure to OP seems to produce visual impairment and eye abnormalities as well. Review of existing publications is made by Plestina and Piukovic - Plestina 1976. In this review, the authors have summarized most of the available literature data about eye and vision impairment attributed to the effect of anticholinesterases or pesticides in general. To this, the authors added a brief summary of their own incomplete investigation.

Visual field constriction, progressive myopia, astigmatism, aedema and atrophia of the optic nerve, lenticular changes catharacts, impaired sense of balance, slower reaction of papillary sphincter, and low dark adaptation ability, have been atributed to OP. An authors research revieled a mild constriction of peripheral visual fields and somewhat slower dark adaptation ability in exposed workers which might be connected with a possibly slower reaction of the pupillary sphincter.

Of interest is the communication made by Ishikawa, 1978, who had observed eye disturbances, severe myopia with corneal astigmatism, disturbance of the smooth pursuit eye movement, prolonged latency and reduced amplitude of the pupillary light reflex, and optic neuropathy in children living in an area where extensive use of OP was common practice. Minor neurological abnormalities were also noted and inhibition of ChEA in about one third of the cases.

Macular degeneration with visual impairment, night blindness, black dots in front of the eyes and blurring of vision is reported by Mistra and all,1982,in malaria control unit spraying OP and copper acetoarsenite.

Still now only experimental studies exist, showing teratogenicity, mutagenicity and cancerogenicity of some of OP pesticides. Suspected carcinogens are dimethoate, trichlorofon and tetrachlorphenvinphos. Mutagen-positive tests have resulted with methyl parathion, dichlorophos, oxydemetonmethyl, dicrotophos and monocrotophos. Compounds with teratogenic activity are parathion, methyl parathion, dichlorophos, diazinon, trichlorfon and phosmet. Embryotoxic effects have been found with trichlorfon, methyl mercaptophos, demeton, fenthion, phthalaphos and methyl parathion. Gonadotoxic effects have occurred with fenchlorphos.

Laboratory Methods for Diagnosis

Cholinesterase activity

As previously mentioned, OPs act principally by inhibiting the enzyme cholinesterase and pseudocholinesterase, which are responsible for hydrolyzing ACh at synaptic sites. The enzyme inhibition is almost irreversible. Reasonable correlation exists between ChEA and the clinical signs of acute poisoning. The ChEA of plasma decreases but is normalized more quickly than that of the cell. After a severe intoxication, the reduction of enzymes lasts up to 30 days in plasma and up to 100 days in erythrocytes. If the rate of ChE inhibition is rapid, correlation tends to be good between the inhibition of blood ChE and the severity of illness. If the rate of ChE inhibition is slow, the correlation with illness may be low or nonexistent. In fact, the blood enzyme levels may decrease markedly, resulting in very little activity, and without the occurrence of signs or symptoms because the body has adapted to the high levels of accumulated ACh. The clinically progressive decrease of ChEA is tolerated more easily than is the abrupt decrease. After continuous exposure, disturbances could be manifested only at inhibition at 75-85% of the level before the exposure of erythrocyte ChE.

Of special importance for protecting people continuously exposed to poisoning is the biological and health monitoring of the latter.

It is recommended both plasma/serum and RBC ChE activity to be studied because restoration of both is different, and inhibitory potential of various OP for serum and RBC ChE is not equal.

Fifth International Workshop of the Scientific Committee on Pesticides of the Int.Ass.Occup.Health on field exposure of pesticides made the following conclusions: There was no consensus on which types of cholinesterase should be measured. It appeared that there is still not enough knowledge about the biological significance of plasma ChE, whole blood ChE and erythrocyte ChE. This problem is particularly relevant in field work as one prefers to test only one type of ChE under field conditions (Tudor, 1980).

OP insecticdes are, as a rule, stronger inhibitors of plasma cholinesterase. The inhibition of plasma cholinesterase should be regarded as a very sensitive index of absorption and the inhibition of erythrocyte cholinesterase as a specific phenomenon of response, indicating the degree of adverse effect induced in the body (WHO TSR 571 Muller and Hundt, 1980; Levin and all, 1976).

Symptoms depend not only on the degree of inhibition but also on the rate at which it occurs. Studies in people have shown that, in the course of a prolonged exposure to relatively small dosages, a marked reduction, both in plasma and erythrocyte cholinesterase activity, can be observed without any symptoms. (WHO, TSR 571).

This is the reason that some authors failed to found correlation between subjective symptoms and ChEA levels. The recovery of blood cholinesterase takes about 2 weeks in patients with mild poisoning. However, the recovery of synaptic AChE appears to be very rapid, since signs and symptoms disappear within 24 hours in patients with mild or moderately severe poisoning. Complete recovery of plasma ChE requires 30 to 40 days, whereas recovery of the RBC ChE requires (as it occurs as red corpuscles are replaced in the circulation) 90 to 100 days following severe ChE inhibition.

Soliman and all, 1971, found in workers in contact with phospholan inhibition of RBC AChE, which is completely recovered in 3-4 weeks and more.

It was proposed as a hazard level inhibition of ChEA whole blood with 30% from preexposure level (Kaloyanova, 1959).

Later Gage (1976) proposed as a safe threshold the same per cent for ChEA both for plasma and erythrocytes.

The following are the hazard levels of cholinesterase depression suggested by the working group of WHO (TRS 571); seetylcholinesterase activity reduced by 30% of the pre-exposure value requires repeated determinations, after appropriate intervals, and appraisal of the general and individual work situation; acetylcholinesterase activity reduced by 50% of the pre-exposure value requires immediate action, including temporary removal from further exposure and appraisal of the work situation.

Many methods are available for ChEA determination. They could be used with success if correctly applied (Coppelstone, 1980).

Izmirova,1980, and Katayama and all, 1980, report on the advantages of paper test used in field conditions.

Kaloyanova,1976, evaluates the ChEA monitoring system for 10 years for occupationally exposed people. The percentage of persons with inhibited ChEA varies from 1.2% to 17%.

For 7 years period some results are given on Tables 5,6 and 7. Paper test developed in Bulgaria was used.

Table 5

ChEA in monitored workers before working season

Year	Number		%% inhib.
	Total	Inhib.	
1975	21 294	1 947	9.14
1976	26 761	2 894	10.80
1977	22 982	1 345	5.80
1978	31 376	2 616	8.33
1979	27 522	1 911	6.90
1980	28 941	3 360	11.60

Table 6

ChEA in monitored workers during spraying season

Year	Number		%% inhib.
	Total	Inhib.	
1975	8 033	589	7.33
1976	6 484	322	4.90
1977	9 345	405	4.30
1978	8 618	556	6.45
1979	10 079	388	3.84
1980	10 758	166	1.54
1981	8 458	992	11.70

Table 7

ChEA in monitored workers end of the working season

Year	Number		%% inhib.
	Total	Inhib.	
1975	1 176	269	22.87
1976	1 769	145	8.19
1977	2 019	153	7.57
1978	2 494	267	10.70
1979	2 227	127	5.70
1980	2 237	147	6.57
1981	2 869	140	4.87

Other laboratory investigations

Metabolites in urine, such as dimethyl and diethyl phosphates, can be measured as indicators of exposure (Hayes, 1980). Hyperglycemia found in acute intoxication correlates well with the symptoms. Leucocytosis (with neutrophilia and lymphocytopenia) and eosinopenia are found in acute intoxication, and leucopenia and anaemia with trombopenia are present in chronic intoxication. Tests for liver function should be used, and the renal function should also be examined (Tocci et al., 1969; Bogusz, 1968). In addition, tests for transaminase activity and changes in proteins (increased γ -globulines) can be used.

Preventive Measures

The organophosphorus pesticides have been responsible for more deaths than has any other group of compounds, and even those OP pesticides with low toxicity are occasionally dangerous. In Pakistan, where malathion was being used in 1975 in a malaria control programme, 2800 of 7500 sprayers became ill and five deaths resulted. These effects were unexpected because malathion, with an acute oral LD_{50} in the rat of 1375 mg/kg and an acute dermal LD_{50} in the rabbit of 4100 mg/kg, is one of the least toxic of the organophosphorus compounds. Isomalathion, a toxic isomerization product of malathion, together with poor practice in application, was responsible for the deaths. The World Health Organization has now changed the specifications for malathion, requiring that the toxic contaminant be below a certain percentage for use in malaria control (WHO TRS N 634, 1979).

Lately, the problem of impurities has become increasingly important as information indicates that they may considerably change the toxicity of a pesticide. Therefore, when registration of a pesticide preparation is made, an assessment of its purity should be carried out in parallel with the other indices.

Prophylactic measures are similar to those for all pesticides. In particular, the severe poisonings that result from the very rapid resorption of OPs by both the respiratory tract and the skin require special attention to be paid to personal protective means and personal hygiene. Attention should also be paid to limiting the use of pesticides with high acute toxicity and excluding those preparations which have long-term neurotoxic effects.

REFERENCES

Aldridge W.N., Johnson M.K. Side effects of organophosphorus compounds: delayed neurotoxicity. Bulletin of the World Health Organization, 1971, 44, p.259-263.

Baker E.L., Zack M., Miles J.W., Alderman L., Dobbin R.D., Miller S., Teeters W.R., Warren M. Epidemic malathion poisoning in Pakistan malaria workers. Lancet, 7 Jan. 1978, v.1, N 8054, p.31-34.

Bidstrup P. et al. Paralysis following poisoning by a new organic phosphorus insecticide (Mipafox). British medical journal, 1953, 1,p.1068.

Boguss M. Studies on the activity of certain enzymatic systems in agriculture workers exposed to the action of organophosphorous insecticides. Wiadonosci lekarskie, 1968, 23, p.786.

Chiistophers A.J. Hematological effects of pesticides. Annals New York Academy of Sciences, 1969, v.160, Art.2.

Copplestone J.F. Methods for field assessment of exposure to pesticides. In: Tordoir W.F., Heemstra-Lequin E.A.H., ed. Field worker exposure during pesticide application. Amsterdam, Elsevier North-Holland, 1980, p.17.

Demidenko N.M., Mirqijazova M.G. The combined biological effect of high air temperature and the pesticide anthio. Gigiena i sanitarija, 1974, 7, p.18-21.

Dille J., Smith P. Central nervous system effects of chronic exposure to organophosphorus insecticides.Aerospace Med., 1964, 9, p. 765.

Duffy F.H. et al. Long-term effects of an organophosphate upon the human electroencephalogram. Toxicol.Appl.Pharmacol., 1979, v.47, N 1, p.161-176.

El Sebae A.H. et al. Neurotoxicity of organophosphorous insecticides leptophos and EPN. J.Environm.Sci.Health, 1977, B12, N 4, p.269-288.

Faerman I. Some problems of clinics of intoxication by organophosphorous pesticides. In: Hygiene of application of toxic pesticides and clinics of intoxications. Kiev, 1965,p542.

Gage J. The significance of blood cholinesterase activity measurements. Residue review, v.18, p.159, 1967.

Gershon S., Shaw F. Psychiatric sequelae of chronic exposure to organophosphorus insecticides. Lancet, 1961, 1, p.371.

Hayes A.L. et al. Assessment of occupational exposure to organophosphates in pest control operators. Am.Indust.Hyg.Assoc. J., 1980, 41, p.568-575.

Holmes J.H. et al. Short-term toxicity of mevinphos in man. Arch.Environm.Health, 1974, v.29, N 2, p.84-89.

Ishikawa S. Ocular manifestations by chronic organosphate pesticide intoxication. Excerpta Medica.Int.Congr.Ser., 1978, 442, p.31.

Ismirova N. Methods for determination of exposure of agricultural workers to organophosphorus pesticides. In: Tordoir W.F. and Heemstra-Lequin E.A.H., ed. Field worker exposure during pesticide application. Amsterdam, Elsevier North-Holland, 1980, p.169.

Jager K.W. Organophosphate exposure from industrial usage, electroneuromyography in occupational medical supervision of exposed workers. In: Pesticides-induced delayed neurotoxicity. Proceedings of a Conference, 19-20 February 1976, Washington, DC. US Environmental Protection Agency, 1976. (Environmental Health Effects Research Series: EPA-600/1-76-025).

Johnson M.K. Delayed neurotoxic action of some organophosphorus compounds. Brit.med.bul., 1969, v.25, N 3, p.231-235.

Jusič A., Jurenic D., Milic S. Electromyographical neuromuscular synapse testing and neurological findings in workers exposed to organophosphorous pesticides. Arch.Env.Health, 1980, v.35, N 3, p.168-175.

Kagan J.S. Toxicology of organophosphorus pesticides. Moskaw, Medicina, 1977, p.296.

Kalas D. Light group poisoning by metathion E-50 inhalation when spraying by means of aircraft. Prac.Lek., 1978, v.30, N 4, p.145-147.

Kaloyanova F. Effects of certain organophosphorus insecticides upon cholinesterase activity in persons in conditions of agricultural labour. Sbornik trudove na HIOTPZ, 1959, 6, p.105-114.

Kaloyanova F. Cholinesterase activity as a biochemical indicator for monitoring exposure to certain pesticides. In: International Conference on Environmental Sensing and Assessment. New York, Institute of Electrical and Electronics Engineers, 1976, v.1.

Katayama G.T. et al. Evaluation of toxic hazards of organophosphorus and carbamate pesticides by the ACHOLEST method in an ethnic Japanese agricultural community (Atibaia). Revista brasileira de saude ocupacional. Apr.-May-June 1980, v.8, N 30, p.21-29.

Kovarik J., Sercl M. The influence of the organe phosphate insecticides on the nervous system of man. In: 15th Intern.Congr. Occup.Health (Vienna), 1966, Abstr.pap.6, p.205.

Levin H., Robnitzky R.L., Mick D.L. Anxiety associated with exposure to organophosphate compounds. Arch.Gen.Psychiatry, February 1976, v.33, p.225-228.

Levin H.S., Rodnitzky L. Behavioral effects of organophosphate pesticides in man. Clinical toxicology, 1976, v.9, N 3, p.391-405.

Liska D., Tildyova K. Study of the changes of the activity of certain enzymes in the blood of pilots and mechanics exposed to organophosphorus pesticides. Ceskoslovenska hygiena, Praha, Czechoslovakia, July 1974, v.19, N 6-7, p.289-295.

Metcalf D.R., Holmes J.H. EEG, psychological and neurological alterations in humans with organophosphorus exposure. Annals of the New York Academy of Sciences , 1969, 16, p.357-365.

Monov A. Some peculiarities in the clinic and treatment of parathion coma. Sovremena Medicina, 1965, v.16, N 3, p.146.

Muller F.O., Hundt H.K.L. Chronic organophosphate poisoning. S.Afr.Med.J., 1980, v.57, N 10, p.344-345.

Murphy S. Pesticides. In: Doull J. et al., ed. Toxicology, the basic science of poisons. New York, McMillan, 1980, p.357-407.

Peoples S.A., Maddy K.T., Smith C.R. Occupational exposure to Temik (aldicarb) as reported by California physicians for 1974-1975. Vet.Hum.Toxicol., 1978, v.20, N 5, p.321-324.

Plestina R., Piukovic-Plestina M. The effects of pesticides on the eyes and vision. Archiv za higijenu rada i toksikologiju, Zagreb, 1976, v.27, N 4, p.321-333.

Reich G., Berner W. Aerial application accidents. Arch. Environm.Hlth., 1968, v.17, N 5, p.776.

Richter E.D. et al. Heat stress in aerial spray pilots. In: Studies in Environmental Science 7. Field worker Exposure during pesticide application, 1980.

Roberts D.V. E.M.G. voltage and motor nerve conduction velocity in organophosphorus pesticide factory workers. Int.Arch. Occup.Environ.Hlth., 1976, 36, p.267-274.

Safe use of pesticides. 20th Report of WHO Expert. Committee on Insecticides. WHO, TRS, 1973, N 153.

Senanayak N., Johnson M.K. Acute polyneuropathy after poisoning by a new organophosphate insecticide. New England Journal of Medicine. Jan.1982, v.306, N 3, p.155-157.

Soliman S.A., El-Sebae A.H., El-Fiki S. Occupational effect of phosfolan insecticide on spraymen during field exposure. Journal of Environmental Science and Health, 1979, v. B14.

Tocci P.M. et al. Biochemical differences found in persons chronically exposed to high levels of pesticides. Industrial Medicine, 1969, 38, 6.

Tudoir W.E. Report of the Rapporteur on: Field worker exposure during pesticide application. St.in Env.Sc.7. Elsv.Sc. publ.S., 1980, p.18.

West J. Sequela of poisoning from phosphate ester pesticides. Int.Med.Surg., 1968, v.37, N 7, p.538.

WHO Technical Report Series, N 356, 1967 (Safe use of pesticides in public health: sixteenth report of the WHO Expert Committee on Insecticides).

WHO Technical Report Series, N 634, 1979 (Safe use of pesticides: third report of the WHO Expert Committee on Vector Biology and Control).

WHO Technical Report Series, N 571, 1975 (Early detection of health impairment in occupational exposure to health hazards: report of a WHO Study Group), p.63.

Zaminovic M. Agranulocytosis following use of insecticides. Archiv za higijenu rada i toksikologiju, Zagreb, 1977.

Binding of acetylchotine on cholinestarase

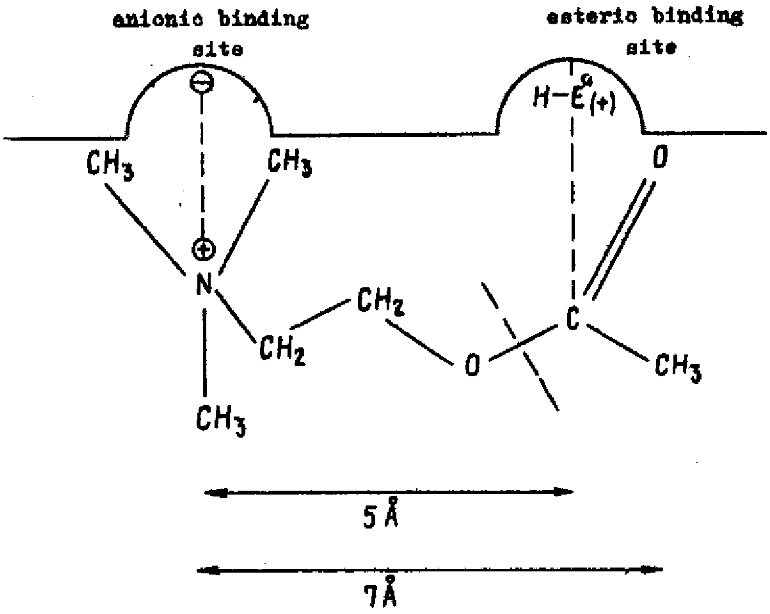


Fig. 1.

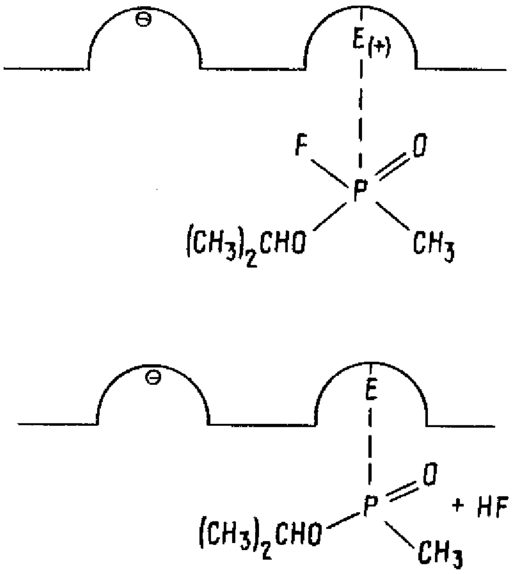
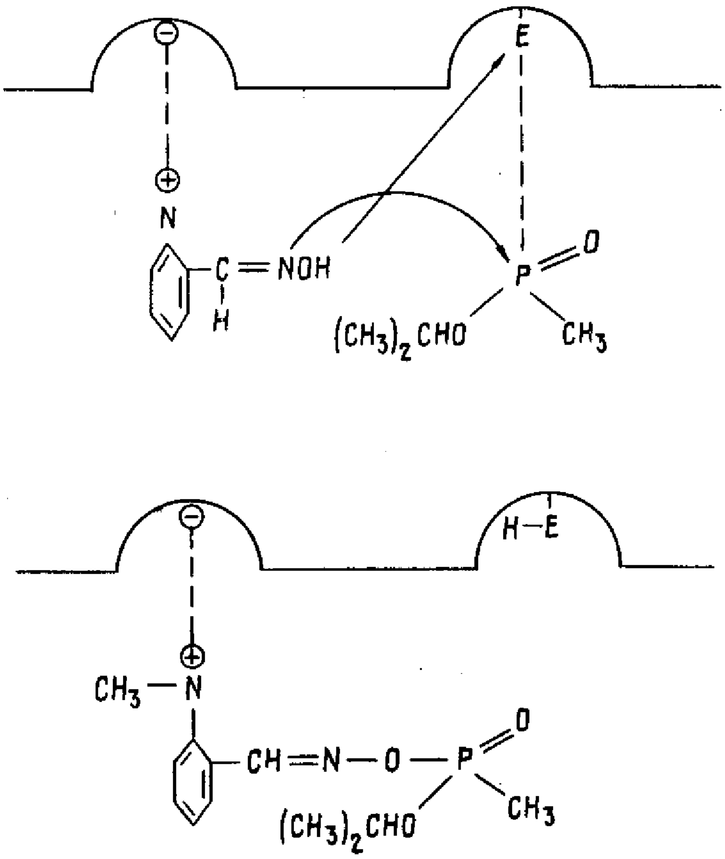


Fig. 2. Inactivation of cholinesterase by sarine

6300



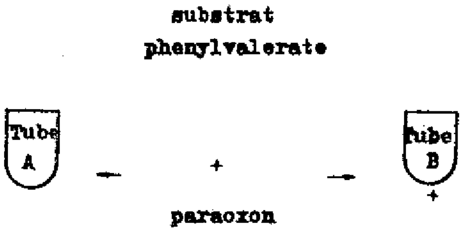
Reactivation of cholinesterase by PAM

Fig. 3.

NEUROTOXIC ESTERASE (NTE)

(Johnson 1969)

Inhibition of NTE is specific for OP with delayed neurotoxicity



Measurement of the rate of hydrolysis by phenol production

A - B = NTE

Fig. 4.

TOXICOLOGY AND APPLICATION HYGIENE OF ORGANOMERCURIAL PESTICIDES

I. M. Trakhtenberg

Among diverse chemicals being widely applied today as pesticides in agriculture an important place is held by organomercurial compounds.

Some of them are used as fungicides and bactericides in seed decontamination from some diseases (gummosis, fusarial wilt, helminthosporosis, various blights), others (in mixture with chlororganic compounds) are finding application as complex-effect preparations to give protection to agricultural crops both from fungous diseases and pest insects. A distinguishing feature of organomercurial pesticides is simplicity of their application and high efficiency at low consumption rates (1.5-2 kg to 1 ton of seeds).

Earlier the agriculture,as regards organic mercury derivatives, had been predominantly using chemicals based on ethylmercurphosphate and ethylmercurchloride. At present the pesticides in this group have significantly expanded in assortment, mainly due to the use of preparations containing as primary agents phenylmercuracetate (falisan 2.5, mordant P, leutosan, ruberon, ryogen), phenylmercurbromide (agronal), and methoxyethylmercuracetate (radosan). Furthermore, in recent years large-scale use has been made of mercuran and mercurhexane as complex-effect preparations, which, apart from organomercurial compounds, comprise chlororganic ones.

The present lecture provides data on toxicity of organomercurial pesticides and discusses basic problems of labour hygiene in their application. Special emphasis is laid on specific hygienic requirements and measures to be taken to secure prophylaxis against the harmful effect of organomercurial pesticides.

Physico-Chemical Properties of Principal Organomercurial Pesticides

GRANOSAN - its primary agent is ethylmercury chloride (C_2H_5HgCl). It is a crystalline powder with a specific odour, poorly soluble in water (0.7 mg to 1 ml at 25°C). Mol.weight - 265.13. Specific weight - 4.24, vapour pressure at 20°C - $3 \cdot 10^{-3}$ mm Hg. Granosan is used in the form of dust which contains 2 to 2.5% of ethylmercury chloride.

MERCURAN - a mechanical mixture of 2% of ethylmercury chloride and 12% of hexachlorcyclohexane gamma-isomer. Either talc or kaolin is used as a filler. This chemical preparation is of a light-grey colour and is applied as dust. Hexachlorcyclohexane gamma-isomer, similarly to ethylmercury chloride, is poorly soluble in water, but its volatility, as compared to ethylmercury chloride, is about seven times as high (vapour pressure at 20°C being $2.17 \cdot 10^{-2}$ mm mercury column).

MERCURHEXANE - a mechanical mixture of 1% of ethylmercury chloride, 14% of hexachlorcyclohexane gamma-isomer, and 20% of hexachlorbenzene. It is a powder of a light-yellow, white or grey colour and is applied as dust. Hexachlorbenzene is in the form of white crystals and is very poorly soluble in water. Its volatility is low (0.35 mg/m^3 at 20°C).

RADOSAN - its primary agent is methoxyethylmercury acetate ($CH_3OCH_2CH_2HgOCOCH_3$); it is a crystalline powder, poorly soluble in water, mol. weight - 318.66, vapour pressure at 20°C being $13 \cdot 10^{-6}$ mm mercury column. The chemical is applied as dust which contains up to 2.4% of methoxyethylmercuracetate, the rest of the substance being an inert filler.

AGRONAL - its primary agent is phenylmercurbromide (C_6H_5HgBr). It is a crystalline powder with a specific odour. Insoluble in water. Mol. weight - 357.63, melting point - 275°C, vapour pressure at 20°C being $6 \cdot 10^{-7}$ mm mercury column. It is applied as dust which contains 3.2% of phenylmercurbromide, the rest of the substance being an inert filler.

PHENYLMERCURACETATE (falisan 2.5, mordant P, leutosan, ruberon, ryogen). Its primary agent is phenylmercury acetate ($C_6H_5HgOCOCH_3$). The content of phenylmercury acetate in different preparations varies from 1.8 to 4%, the rest of the substance being an inert filler. A white crystalline substance, its melting point being 153°C, solubility in water at 25°C - 2 mg to 1 ml, specific weight - 2.88, molecular weight - 338.76, vapour pressure at 20°C - $0.7 \cdot 10^{-6}$ mm mercury column. All pesticides with phenylmercury acetate as their primary agent are applied as dusts.

GRANOSAN M (Dupon 1452 c, ceresan M, EMTS). Its primary agent is ethylmercuritoluenesulphanylide. This is a crystalline powder, insoluble in water. Mol. weight - 475.99. Granosan M is available as a 7.7%-dust and applied as an anticeptic and a fungicide in seed decontamination.

MERCURBENZENE - a mechanical mixture of ethylmercury chloride and hexachlorbenzene. It is available as 21%-wettable powder which contains 1% of ethylmercury chloride and 20% of hexachlorbenzene.

Toxicological characteristics. Experiments and production observations have revealed that the majority of organomercurial pesticides, once they penetrate into the organism via various routes (through respiratory organs, gastrointestinal tract, intact skin and mucous membranes), are bound to produce a specific toxic effect there.

In the course of acute intoxication we can identify three stages: I - manifested by brief excitations and increased motor activity; II - depressed states, adynamy; III - convulsions, parases, paralyses, and death. At the second stage, once adynamy has set in, there are observed disorders in the coordination of movements, in breathing rhythm, and fibrillar twitchings in some groups of muscles. Once granosan is introduced, this stage is likely to set in earlier compared to other organomercurial pesticides. Its length in the case of mercuran is 3 days, radosan - up to 5 days, agronal and phenylmercur acetate - up to 7-8 days and more. Death of the animals terminates stage III which is characterized by frequent attacks of clonic and tonic convulsions accompanied by the progress of paralyses and respiratory disorders.

A particularly prominent potential danger for people working with organomercurial pesticides is inherent in granosan. The complex-effect chemicals - mercuran and mercurhexane - are not as toxic as granosan. Their lower toxicity, compared to granosan, is due to the fact that the percentage of ethylmercurchloride in mercurhexane (1%) and, on many occasions, also in mercurane (2%) is inferior to that in granosan; moreover, the chlororganic components in these preparations (hexachlorcyclohexane and hexachlorbenzene gamma-isomer) being less toxic than ethylmercurchloride, they fail to make up for the lower toxic effect due to the lower content of ethylmercurchloride in the preparations. Furthermore, under short-term exposure of the organism to organomercurial and chlororganic compounds a certain antagonism between the two is observed and this may also account, to a certain degree, for the lower toxicity of mercuran and mercurohexane, as compared to granosan. At the same time, it should be noted that both mercuran and mercurohexane are endowed with sifficiently distinct cumulative properties.

Less toxic (2-3 times as low), compared to granosan, are radosan, phenylmercuracetate and agronal. The latter two feature low volatility and, since they arrive in the working-zone air predominantly as dust, rather than vapours, their penetration into workmen's organisms can be more easily prevented than would be the case with vapours. This as well as the fact that these chemicals are less toxic than granosan or radosan, make the use of phenylmercuracetate and agronal in agriculture more preferable, as regards labour hygiene, than would be a further expansion of the scope of application of granosan or other chemicals having ethylmercury compounds for their primary agents. The lower toxicity of phenylmercuracetate and agronal, compared to granosan and radosan, is dependent on differences in the chemical structure of those organic mercury derivatives which constitute the basis of these compounds.

Experimental and clinical evidence obtained in recent years has revealed phenylmercurial compounds to be less toxic to man and warm-blooded animals compared to ethylmercurial ones, while the lower toxicity of radosan compared to granosan in the latter group is apparently due to a difference in the acid radical.

An analysis of the data on toxicity and the effect of arganomercurial pesticides now in use on an organism permits us to think that the said compounds must be classed under the fourth category in accordance with the pesticide classification, as recommended by the Committee on Professional Hygiene under the International Organization of Labour. As laid down in this classification, the above category is expected to comprise substances which are considered to be "... high-toxic and not intended for large-scale application". Nontheless, within this high-toxic group, a different approach should be taken to individual compounds, as regards their hygienic assessment, i.e., considering their potential danger to man's health that is likely to arise in the application of different organomercurial pesticides. On the whole, however, organomercurial compounds belong to high-toxic substances with district cumulative properties, these chemicals also display the skin-resorptive effect.

To conclude this part of the lecture I whould like to make a comparative assessment of potential danger inherent in various mercurial pesticides under the actual conditions of application by giving some information on their volatility.

Table 1

Toxicity of Organomercurial Pesticides

Preparation	Animal species	LD_{50}
Granosan (ethylmercurchloride)	white mice	30.0(25.7-36.3)
	white rats	50.0(36.4-74.3)
Mercuran (ethylmercurchloride and hexachlorcyclohexane gamma-isomer)	white mice	138.0(124.4-252.6)
	white rats	208.0(170.3-245.7)
Mercurhexane (ethylmercurchloride, HCCH and hexachlorbenzene)	white mice	350.0(262.4-397.6)
	white rats	560.0(437.6-682.4)
Radosan (methoxyethylmercuracetate)	white mice	60.0(53.5-66.5)
	white rats	70.0(52.3-88.8)
Agronal (phenylmercurbromide)	white mice	96.0(83.4-108.6)
	white rats	112.0(73.0-151.0)
Ruberon (phenylmercuracetate)	white mice	50.4(41.0-59.8)
	white rats	81.2(77.1-85.3)

According to our laboratory study results the volatility of ethylmercurchloride at 20°C, is 12 mg/m^3.

Ethylmercurchloride, hexachlorcyclohexane, and hexachlorbenzene, being the constituents of mercurhexane, have a differing volatility. Hexachlorcyclohexane gamma-isomer exceeds in this respect ethylmercurchloride as much as 7 times (its vapour pressure at 20°C being $2.17 \cdot 10^{-2}$ mm mercury column). At the same time, hexachlorbenzene volatility is by 30-40 times lower than of ehtylmercurchloride (0.35 mg/m^3).

The lowest volatility is displayed by argonal and phenylmercuracetate: the former being $6 \cdot 10^{-7}$ mm mercury column and the latter $0.7 \cdot 10^{-7}$ mm mercury column (International symposium on pesticides, Magdeburg, 1966).

Clinical picture of acute intoxication of man. Metallic taste in one's mouth, headaches, sickness, hypersalivation, vomiting, loss of consciousness, not infrequently abdomen pains, mucous diarrhea (often bloody diarrhea), great thirst, a sensation of burning in the mouth, gum swelling and stomatorrhagia. Later - unstable gait, trembling, limb paralysis, declining visual and auditory acuity, pangs in the joints, difficult swallowing, spontaneous urination and defecation, blindness. Urine is found to contain protein, peripheral blood - neutrophilic leukocytosis, accelerated ESR. The observed symptom complex is similar to the one observed in the case of toxic encephalopatia. In view of the distinct capacity of organomercurial compounds for cumulation, there appears, apart from an acute intoxication, a potential danger of the development of chronic intoxication.

Chronic intoxication of animals reveals disorders in the central nervous system (deranged coordination of movements, higher reflectory excitability, trembling, paralyses, convulsions); an erarly appearance of alterations in conditioned-reflex activity. Anemia, leukopenia and eosinopenia are making progress; lymphocytes decrease in number, toxic granularity is observed to appear in neutrophiles.

Young cells and mitoses in bone marrow decrease in number.

Chronic intoxication of man is accompanied by gradual development of emaciation, weakness, fatiguability, sleepiness; later - insomnia, headaches, dizziness, weakening of memory, thirst, occasionally metallic taste in one's mouth, hypersalivation. Patients find it difficult to move about and are compelled to support themselves against different objects. There are also observed distinct stomatic symptoms, often changing over into ulcerative gingivitis, the latter's progress with the attendant temperature rise and significant ESR acceleration. This is accompanied by sharply increasing weakness and syncopes. Some people during this period suffer from auditory and visual hallucinations, fearful dreams. All patients display sudden changes of mood, apathy, greater irritability, sensation of fear, tearfulness. Objective records show the lack of stability in the cardiovascular and vegetative nervous system; in some of the patients - trembling of hands, tongue, eyelids. The hemoglobin content in the patient's blood decreases and a relative lympho- and monocytosis is observed. The urine is found to contain mercury up to 0.6 mg/l. Cases of the poisoning of children with granosan have been recorded. Intoxication was developing in them at a quicker pace and its progress was much more painful than in adults and had a lethal outcome.

In the case of chronic poisoning the earliest complaints are about increased fatiguability, general weakness, headache (particularly towards the end of the working day), upleasant taste in one's mouth, disturbed sleep. Objective records show: stomatorrhagia, occasionally nasal bleedings, hand tremors, accelerated ESR, presence of mercury in urine (up to 0.03-0.05 mg/l and higher).

Both experimental data and production observations indicate that the greatest potential danger to people exposed to organomercurial pesticides comes from granosan.

Operating mechanism, toxicodynamics. Organic compounds of mercury, similarly to inorganic ones, belong to thiolic poisons. By reacting with the SH-groups of cellular proteins they are apt to disturb the activity of basic enzymic systems which cannot function normally unless free SH-groups are present. As a result a variety of different primary (principally, in the central nervous system) and indirect alterations in the organism appear, particularly in the vegetative system, in peripheral nervous formations, as well as in the heart and vessels, in the hemopoietic organs and peripheral blood, in the stomach, liver, kidneys, and in the urinary tracts, in the immunobiological state of the organism, etc.

The autopsy of the animals which had died of poisoning with mercury compounds revealed their blood being irregularly distributed in the organs (prominent plethora in the brain and in inner organs), degenerative alterations in the liver, kidneys (though without a typical nephrosis), particularly in the case of chronic poisoning, numerous focal hemorrhages and inflammatory foci (the latter being also observed in the mucous membranes of the stomach and intestine), degenerative alterations in the ganglion brain cells (these are particularly prominent), occasionally glia proliferation. In case mercury compounds have arrived in the organism through the respiratory tract the lung tissue is observed to be highly compact.

In the people working with organomercurial pesticides the Hg circulation in the organism is observed to be taking place during several weeks or months even upon termination of contact with them. Relatively small quantities of mercury were found to be present in the liver, kidneys, brain, blood, and bile, in certain cases - in gastric juice. The mercury content is significant in urine (up to 480-74 µg/day) and in feces (up to 245-6000 µg/day). In the people who had died from methyl mercury poisoning the largest quantity of mercury was found in the hypophysis and in the brain. The distribution pattern of mercury compounds is dependent on how they arrive in the organism. Thus, with ethylmercurchloride introduced into animals through respiratory tracts the largest quantity of mercury was found to be present in the lungs, then in the brain and in spinal cord and kidneys, the liver and the heart containing lower quantities; arriving through the alimentary tract, large quantities of mercury are found to be present in the kidneys, then (nearly in similar quantities) in the brain and in the spinal cord, the lungs containing much less mercury. The mercury content in the central nervous system, in the case of poisoning with organic compounds, is many times as high as in the case of poisoning with mercuric chloride.

Organic mercury compounds will be eliminated from the organism with urine and feces; they are capable of penetrating into the milk of nursing mothers.

Variations in the mercury quantities in urine are not directly related to the degree of intoxication. A more definite relationship is often observed to exist between the mercury content in feces and the gravity of poisoning.

Mercury compounds show distinct cardiotoxic properties. Persons intoxicated with mercury compounds suffer from disorders in the cardiac activity. Alterations in the auricular complex were often observed to be combined with bradycardia, arterial hypotonia and other vagotonia manifestations. T-wave alterations were often accompanied by a retarded intraventicular conduction and a displacement of the T interval. The variability pattern of the T-wave fails to be affected either by physical loading or atropine. The latter indicates that the above displacement is due to a derangement of the exchange processes in the myocardium.

Experimental researches have confirmed that under exposure to mercury compounds the alterations result from the disordered extracardiac regulation of the cardiac activity and a direct toxic effect on the myocardium in which distrophic alterations are usually observed to take place. Organomercurial pesticides - granosan, merfuran, radosan, etc. - are observed to affect the generative function and to be endowed with a distinct embryotoxic property.

In case of a repeated arrival in the organism of warm-blooded animals they are known to affect the progeny, this being manifested in delayed physical development, peripheral blood alterations and quantitative shifts in the content of sulfhydryl groups of proteins in the blood and in the inner organ tissues in the newborns. Mercuran or mercurohexane arriving in the organism of pregnant females are likely to cause significant disorders in the development of the fetus, even its resorption. Mercury compounds have been found to be effective inhibitors of mitosis in both vegetal and animal cells.

Clinical observations carried out by some researchers have revealed acute and subacute intoxication with granosan, capable of interfering with the normal progress of pregnancy and thus affecting the progeny. In pregnant women intoxication with granosan proceeds more gravely and is accompanied by mental disorders.

Children born to mothers who had suffered from acute or subacute granosan intoxication not infrequently turned out to be sickly and lacked vitality. In many of them there were recorded encephalopathia phenomena, cases of the paresis of the skull-brain nerves, an inadequate development of the brain accompanied by serious disorders in some of its structures. The milk of nursing mothers who had been in contact with mercury compounds was found to contain as much as up to 0.04 mg/l of mercury.

Investigations into the effect of mercury compounds on the functional state of gonads have revealed both female and male sexual glands to be highly sensitive to the effect of poison.

The effect of granosan was observed to significantly bring down fertility. The threshold of the gonad-toxic effect was found to be 5-10 times as low as the general toxic one. In Japan there was described a case of a disordered nervous system in 26 babies as a result of the intrauterine intoxication caused by the mothers' feeding on the fish contaminated with alkylmercurial compounds.

There are indications to mercury compounds producing an allergizing effect. Researchers both in the Soviet Union and in other countries observed an enhanced sensitivity to mercury compounds in persons working with decontaminated seeds, as manifested in dermatitides, allergic edemas, etc.

As was experimentally proved, a reaction between albumine and mercury compounds gives rise to a complex compound showing the properties of a complex antigene. The complex antigene thus formed (mercury albuminate) will affect, in turn, the immune systems, bringing them into an intricate pathogenic process.

The immunocompetent cells will synthetize antibodies which prove specific for the given complex. Development of these phenomena can be regarded as both normergic and hyperergic reactions.

THE SPECIFICS OF WORKING CONDITIONS IN APPLICATION OF ORGANOMERCURIAL PESTICIDES AND PROPHYLACTIC MEASURES

The use of organomercurial pesticides in agriculture involves potential intoxication hazards at various handling stages, such as transportation of toxic chemicals to work areas, during the storage period of toxic chemicals and their delivery from the storage places, during the decontamination of the grain and afterwards during its storage, while transporting decontaminated grain to the place of sowing and during the sowing proper. At all of these stages the organomercurial compounds may enter the breathing zone of the people at work, find their way onto mucous membranes and skin, thereby penetrating into the organism and causing acute and chronic intoxication.

Investigations into working conditions in warehouses for short-term storage of toxic chemical have made it clear that not infrequently vapours of organomercurial compounds are found to be present in the breathing zone of the people working there. The highest concentrations were recorded at the places where granosan and mercuran were being stored and handed out for use, while the content of ethylmercurchloride vapours varies from 0.015 to 0.7 mg/m^3.

The presence of such great quantities of ethylmercurchloride vapours in the storage rooms may be attributed to the high volatility of this compound. Lowbr concentrations (0.008-0.03 mg/m^3) were recorded in the storerooms being used for the storage of mercurhexane containing 2-2.5 times (1%) as little ethylmercurchloride as does granosan (2.5%). A relatively low concentration of an organomercurial compound (methoxyethylmercuracetate) was recorded in the air of the storage rooms keeping radosan: within 0-015 mg/m^3. The lowest concentrations of organomercurial compounds were observed in the breathing zone of the storekeepers workings on the storage grounds of phenylmercuracetate and agronal, i.e., preparations possessing insignificant volatility. Their content in storage-room air was found to be within the maximum allowable concentration as specified for the most high-toxic compound, i.e., ethylmercurchloride (0.005 mg/m^3), and was within 0.01-0.0045 mg/m^3.

Special researches have made it clear that organomercurial compounds are apt to be present in significant quantities on the surface of the packages being used for pesticide transportation and storage (from 0.6 to 0.9 mg/cm^2), in structural elements of a storeroom, in work clothing, in individual protective devices, if kept in the same rooms where toxic chemicals are being stored (from 0.5 to 0.82 mg). A particularly high content of organomercurial pesticides was recorded on the package surface and on the work clothing, their quantity being less on building materials. However, significant pesticide quantities were found in cement coating and in plastering (0.1–0.6 mg/100 g), as well as in wooden structures (0.08–0.6 mg/100 g), their quantity being less in brick (0.06–0.08 mg) and insignificant in ceramic tile (0.001–0.002 mg/100 g). Maximum danger of intoxication with organomercurial compounds arises whenever they are stored in open packages.

Prophylactic measures in the use of organomercurial pesticides in agriculture should cover every stage of the production process where the workman's organism is likely to be exposed to the effect of toxic substances. The process of seed decontamination proper emerges as particularly important in this respect. The system of measures for ensuring healthy working conditions in pesticide treatment of grain, should be aimed, above all, at the maximum possible diminishment of the concentration level of toxic chemical in the working-zone air. The crucial factor here is the application of those organomercurial pesticides which display insignificant volatility (phenylmercuracetate, agronal, etc.).

Seed decontamination should be done only in special decontaminating machines, in a specially allocated area not closer that 200 m from the living quarters, utility structures, water supply sources and nutrition centres, or in closed rooms with a ufficiently effective general-exchange ventilation.

It should be also stressed that organomercurial pesticides are capable of spreading out in significant quantities to 250 m from the decontamination area. This dictates the necessity of setting up seed decontamination places at still greater distances. Experience has shown that closed rooms should be used

630C

for seed decontamination only in bad weather or in winter time, i.e., when the use of open-air places is precluded. Outsiders, i.e., people taking no part in decontamination work or in inspection procedures, are not to be admitted to the seed decontamination places. Nor will be admitted persons with medical counterindications, who have failed to pass medical tests, teenagers below 18 years old, pregnant or nursing women.

Seed decontamination in hot seasons is to be carried out in morning hours at minimum air temperatures, care being taken to keep grain movement to minimum velocities.

It is recommended that seed decontamination should be done with moistening, since this more adequately meets hygienic requirements as compared to dry decontamination. Whether one or the other method is used in seed decontamination with the use of organomercurial pesticides, care should be taken to protect respiratory tracts, skin and mucous membranes from contact with toxic chemicals. To this end, use is made of special respirators, overalls, gloves, special footwear. The length of the working day in seed decontamination with the use of organomercurial pesticides should not exceed 4 hours, the rest of the time being spent on jobs unrelated to pesticides handling.

A timely and effective desinfection of the working clothes, individual safety devices, packages, transport facilities and production equipment proves to be an important element in the complex of prophylactic measures aimed at securing prophylaxis of acute and chronic intoxication with organomercurial pesticides.

In the Soviet Union the use of high-toxic organomercurial pesticides in presently limited to the application of granosan and mercurbenzene. The sanitary standards for the allowable ethylmercurchloride content in the working-zone air are: 0.005 mg/m^3, in the atmospheric air of settlement zones - 0,0009 mg/m^3 (single maximum), and 0.0001 mg/m^3 (average daily), in water reservoirs for utility-drinking and cultural-domestic use - 0.0001 mg/l.

Organomercurial pesticides, predominantly used as fungicides and bactericides, happen to be high-toxic preparations endowed with a distinct neurotropic effect and a significant cumulative effect. Ethylmercurchloride, methoxyethylmercuracetate, phenylmercuracetate, phenylmercurbromide and others, making up the basis of such pesticide preparations as granosan, radosan, falisan, agronal, etc., belong to the category of thiolic poisons, the operating mechanism of which predominantly involves inactivation of SH-groups of cellular proteins. Efforts should be made to restrict their wide application in today's farming production and to replace the pesticides of this group with less toxic ones. Care should be taken rigorously to adhere to the authorized sanitary standards, hygienic requirements and regulations and to carry out the demercurization and sanitary prophylactic measures.

TOXICOLOGY OF BIORATIONAL PESTICIDES

E.A. Melnikova

Creating and culturing resistant crop varieties and high agricultural technology are decisive factors in the protection of plants from detrimental organisms, but there are situations when it is impossible to eliminate crop losses with no application of specific procedures. Chemical means of plant protection (chemical pesticides) are now the main tool for fighting detrimental organisms in plant growing. However, a widespread and long use of chemical pesticides has resulted in a pollution of the environment and food-stuffs almost everywhere, which presents a serious hazard not only for useful species of the fauna and the flora, but also for health of the people.

Among the measures to control environmental pollution with chemical pesticides, an ever growing importance is gained by the use of biologic objects and preparations in systems for integral protection of farm crops and forest plantations. For this purpose three basic approaches are now used:

(1) species of insects and birds that can fight pests;

(2) biorational pesticides based on microorganisms and their metabolic products;

(3) biologically active substances such as insect (pheromones) or plant hormones (antifeeding compounds).

The present lecture is devoted to a consideration of medical and hygienic aspects of using the microorganism-based biorational pesticides (viruses, bacteria, fungi) and their metabolic products (antibiotics).

1. General Properties of Microbiologic Means of Plant Protection

Microbiologic means of plant protection form a fundamentally new pesticide class whose basic feature is that a living microorganism or its metabolic product serves as an active ingredient of a pesticide preparation.

The microbiologic method for combatting crop pests proposes using viruses, bacteria, microscopic fungi, rickettsiae, protozoa, as well as their metabolic products such as toxins or antibiotics.

Studies of Russian microbiologists I.I.Mechnikov and I.M. Krasilshchik had promoted development of the microbiologic method for plant protection. I.I. Mechnikov who studied diseases of cereal beetle in 1878 to 1879 isolated several species of bacterial, fungous and nematodic agents of insect diseases and prepared a plan of biolaboratory for propagation of entomopathogens to use them in practice. I.M. Krasilshchik has implemented this plan by organising in 1883 the world's first biolaboratory.

As to their purpose the microbiologic preparations can be used as insecticides (they induce infection disease epizooties in pest population and lower the pest number to an economically safe level), fungicides (they inhabit activity of phytopathogenic microflora) and herbocides (they exhibit selective phytotoxicity).

(a) Viral Insecticides

In contrast to other means of controlling detrimental insects, particularly chemical means or even such microbiologic means as fungi or bacteria, entomopathogenic viruses exhibit a narrow specificity, i.e. an ability to affect certain insect species with no pathogeneity for insects of closely related species. Moreover, insect viruses do not accumulate in the environment since they are not capable to develop outside living cells. About 260 viruses pathogenic for insects are known at present.

Viral insecticides are standardised by the content of polyhedrons or granules (crystallized protein bodies-inclusions formed by the virus inside the damaged cell). The titer of commercial viral compositions is about 1 to 2 billion viral inclusions per 1 g (ml) of the preparation. Viral insecticides are applied similarly to chemical insecticides (spraying plants with a suspension). the application rate being between 150 and 250 g per hectare.

(b) <u>Bacterial Insecticides</u>

Bacterial insecticide compositions for plant protection are produced mainly using various strains of Bacillus thuringiensis. At present 18 varieties of Bacillus thuringiensis belonging to 12 serotypes have been isolated and identified. Using different strains of this microorganisms the following compositions are produced commercially: Agritrol, Bactospeine, Bakthane, Biotrol, Dipel, Thuricide, etc. (USA), Bactospeine (France), Bathurine (Czechoslovakia), Bactucal (Yugoslavia), Gommeline, Bitoxibacillin, Entobacterin, Dendrobacillin, etc. (USSR). Spores and endotoxin crystals or the spore-crystal complex serve as the active substance of these preparations. Studies aimed at isolating other bacterium types from sick insects have been carried out during the recent years. For example, using entomopathogenic Pseudomonas as a basis a composition Carnecine was developed in the USSR.

Bacterial insecticides are applied similarly to chemical insecticides (dusting, spraying of solution, etc.) the application rate being from 3 to 5 kg per hectare;

(c) Fungal Insecticides

Microscopic fungi hold an important place among insect disease agents. Infesting insects with entomopathogenic fungi proceeds through gastrointestinal tract or cuticle. Pathogenic action of fungal insecticides on insects is caused by physical damage of the pest body by germinating spores or by the effect of toxins.

In the USSR there is a commercially produced fungal insecticide sold under the trade name of Boverin. Boverin contains spores and entomopathogenic toxins of fungus Beauveria bassiana which is capable of killing about 190 insect species of various orders. Boverin is applied by spraying soil or plants in agriculture or forestry. To accelerate the effect a fungal insecticide is applied in combination with a chemical pesticide which is used at a rate lowered 10 fold as compared to using it alone. The application norm for Boverin is 2 to 3 kg per hectare.

Laboratory and field studies are now under way to use fungi of Aschersonia, Entomophtora, Pecilomyces, Fusarium, Cephalosporum and Aspergillus genera as insecticides.

Many fungi during their growth produce toxic metabolites that lower the fertility of pests and cause development of abnormal insects.

(d) Compositions for Control of Plant Diseases

Studies of competitive antagonism between different species of the microflora formed the basis for using various microbiologic means for fighting diseases of crops and trees.

The use of antagonists or antibiotics for protection of plants against phtopathogenic microflora has become recently a form of microbiologic control of harmful organisms. Commercial production of antagonist-action composition such as Trichodermin (fungal composition) or Mycolitin (bacterial composition) is being implemented now using the live microorganisms as a basis. Technical efficiency of such compositions is under testing in various climatic and geographic zones under field and greenhouse conditions.

The isolation of antagonistic bacteria or fungi from soil and testing them for control of plant disease agents is of less importance than using antibiotics for this purpose. Japan holds the first place in the world by the scale of production and application of fungicide antibiotics. According to P. Cunt more than 30 thousand tons of antibiotics is produced annually in Japan for plant growing applications. Antibiotic mixtures for plant protection are manufactured in a number of countries under various trade names: Agrimicin-100 (15% streptomycin sulphate and 1.5% terramycin), Agrimycin-500 (17.55% streptomycin sulphate, 0.175% terramycin and 42.4% copper, Agri-Strep (37% streptomycin sulphate), Phytomycin (20% streptomycin nitrate, 0.176% terramycin and 42.2% copper, Phyto-Strep (15% mixture of streptomycin and oxystreptomycin at a ratio of 10:1), Kasubacid (a mixture of Kasumin and Rubocid), and others. Antibiotics of fungicidic action such as Griseofulvin, Antimycin, Acti-dione, Cellocidin, Validacin, and others are used too.

In the recent years, antibiotics intended exceptionally for plant protection are looked for.

Antibiotics Phytobacteriomycin and Trichotecin are produced in the USSR especially for plant growing purposes.

According to its chemical nature Phytobacteriomycin belongs to the group of streptotricine compounds.

All the studied antibiotics of the streptotricine group have a common moiety in their structure and are, actually, mixtures of components X,A,B,C,D,E and F. Streptotricine C and streptotricine D are the main components of Phytobacteriomicin.

Phytobacteriomycin is manufactured in the form of 2% or 5% dust or 10% wettable powder and composition Phytolavin which is applied as 33% dust. This antibiotic has a broad antibacterial, fungicidic and antiviral action. It is applied for the control of diseases of leguminous crops or forest plants.

Trichotecin has mostly a broad antifungal activity. This antibiotic is a isocrotyl ester of trichotecolon keto-alcohol. It exhibits a fungicide effect against agents of cereal root rot, corn fusarium wilt, sugarbeet root fire, pine seedling lodging and other plant diseases caused by phytopathogenic fungi. Trichotecin is used mainly for the control of cucumber mildew in greenhouse. The preparation is delivered in the form of 10% dust or 10% wettable powder.

2. Infection and Toxicologic Properties of Microbiologic Means for Plant Protection

Experimental assessment of pathogenicity of microbiologic means for plant protection (bacterial, fungal, viral, antibiotic, etc.) has procedural features determined by properties of their active ingredients. However, it is expedient in all cases to ob-

6300

tain, by tests using animals of different species, data on the nature of interaction between the macroorganism and the active ingredient, perform testing of the commercial form, and obtain information on the composition behavior in the environment.

(a) Viral Insecticides

Considering a special role of viruses in the pathology of biologic species, the studies for assessing safety of entomopathogenic viruses are to be performed with an exceptional care. By means of tests with various simulation models (laboratory animals, mammal tissue cultures, chicken embrio, etc.) one should explore the virus ability to develop in warm-blooded organisms, determine whether these viruses will exhibit an infection, toxic or allergenic effect, as well as whether they will be able to produce a remote negative effect (such as embriotoxic, carcinogenic or teratogenic).

By present, safety studies have been performed with at least 50 entomopathogenic viruses a major part of which belongs to the Baculovirus family. Determination of the possible detrimental effect of entomopathogenic viruses was performed using 20 vertebrate species including fishes, amphibians, mammals, primates and man.

The studies show that the baculoviruses investigated and their compositions exhibit no toxicity for warm-blooded organisms, and it is impossible to determine average lethal doses for laboratory animals since viral compositions have very low toxicity. Entomopathogenic viruses did not cause any negative phenomenon a like infection or toxicity after administration of polyhedrons, virions or viral DNA to the organisms by various routes such as intragastric, intravenous, intracerebrial, subcutaneous, intrapulmonic or intraperitoneal in single exposure, as well as under long feeding conditions. An application of viruses or their compositions on healthy or scarified skin of animals or on the eye mucosa did not cause any irritation or other pathology.

The corn earworm nucleopolyhedrosis virus is the most studied in relation to safety for non-target organisms. Negative results of multiple experiments aimed at revealing a possible detrimental effect of this pathogen on various vertebrate species allowed to conduct experiments on human. Polyhedrons of the cabbage moth polyhedrosis virus in gelatine capsules were administered intragastrically at a single dose of 1 billion units for 5 days (total dosage of 5.82 billion units) to 10 human volunteers, men and women, aged 21 to 60. The same gelatine capsules containing equivalent amount of moth caterpillar tissue protein were administered orally to a control group of volunteers. Individuals under test were examined one day before the experiment and then on day 10 and day 30. No differences in health state between individuals of both groups were found. Two years later all individuals of experimental and control groups remained healthy.

Until recently there was a view that baculoviruses do not penetrate from the gastrointestinal tract into animal organs. However, recent studies have demonstrated that viral antigen can be detected by the immunofluorscence diagnostics in lymphatic or parenchymatuos organs of animals infected. But the virus reproduction does not proceed in this case either and the antigen is fradually removed from the organism. It was established also that circulation of the entomopathogenic virus antigen in the organism of animals gives rise to a change of the immunologic status and to the development of delayed allergy.

In examining people having an occupational contact with baculoviruses specific antiviral antibodies and signs of sensitisation were detected in one third of individuals studied.

When viral insecticides were used for application in practice, examination of individuals handling the compositions did not reveal any pathologic states or disturbances of their state of health related to the exposure.

When viral insecticides were used for plant spraying by aircraft or tractor-mounted application systems a viral aerosol having a concentration of 1.8 10^3 to 10^4 polyhedrons per cubic meter of air was formed over the field for the first minutes, the pollution level lowering by one or two order of magnitude three hours later and less than 10^1 polyhedrons per 1 m^3 containdd in air three days later. Residual quantities of viral insecticides on plants are as low. Initially after application about 10^3 tp 10^4 polyhedrons per square centimeter are detected, while three days later the pollution level lowers to 10^1 - 10^2 polyhedrons per square centimeter.

(b) Bacterial Compositions

Most bacterial compositions for plant protection are produced in all countries using entomogenic Bacillus thuringiensis. Studies of possible pathogenic effect of these bacterial relative to warm-blooded animals were carried out since the first years of the production and testing of the compositions.

The pathogenic effect of crystal-forming Bacillus thuringiensis was tested using 10 species of mammals (mice, white rats, guinea-pigs, rabbits, cows, sheep, pigs, chipmunks, young bulls and humans - 20 volunteers), 7 bird species (pheasants, sparrows, ducks) and 5 fish species (trout, perch, young salmon, gambusia and some aquarium fishes).

In all tests Bacillus Thuringiensis did not induce infection diseases or propagate in warm-blood organism. However, it was demonstrated that bacteria can penetrate from the gastrointestinal tract into the blood flow and internal organs. With no propagation in the organisms they are excreted through lymphatic system and kidneys, which results in complete removal from the organism 5-10 days later. Average lethal doses of commercial strains of this group bactwria for laboratory animals (mice, rats, rabbits) are dozens of billion bacterial cells per animal.

Commercial forms of formulations based on entomopathogenic bacteria and proposed for plant protection against pest insects or diseases are no more virulent for animals used under laboratory conditions. Data on toxicity for bacterial and some fungal formulations are presented in Table 1.

Commercial forms of bacterial formulations can produce a slight irritation effect on the mucosa of upper respiratory system and eyes as a result of physical damage of the epithelium. Working suspensions of commercial formulations usually show no irritation effect. Bacterial spore compositions are shown to have a slight allergenic activity.

Many Bacillus thuringiensis serotypes produce a thermostable exotoxin exhibiting a teratogenic action on susceptible insects. This urged researchers studying the safety of the Bacillus thuringiensis compositions to carry out experiments with exotoxin. It was found that the beta-exotoxin of Bacillus thuringiensis acts as an ATP competitor. Numerous experiments performed with various types of tissue cultures and bacteria allowed to determine that the exotoxin inhibits a DNA-dependent RNA-polymerase and RNA synthesis. Toxicity (DL_{50}) of the pure exotoxin for mice is about 400 mg per kg body weight. As for the possibility of teratogenic action of beta-exotoxin on warm-blooded organisms, this question has no definite solution. Investigations performed with rats and rabbits revealed no teratogenic effect.

Residual quantities of bacterial formulations on crop products after their treatment are safe for humans. Immediately upon applying the bacterial insecticides on plants there are up to 10^7 - 10^9 spores per kg of plant weight (apple-trees, strawberries, mushrooms, grass, leaves of forest plants, potato and tomatoes). The bacterial spore level lowers by 2-3 order of the magnitude under influence of sunlight, wind and moisture in the course of 2-3 days. Bacillus thuringiensis spore concentration of 10^4 - 10^5 microbial cells per 1 gram of soil is produced in the surface soil layer immediately after application. The soil spore content lowers by 2-3 order of magnitude 3-7 days later. Bacillus thuringiensis spore concentration over a treated field is 10^5 - 10^6 cells/m^3, while 5 days later it is below the natural background.

(c) Fungal Formulations

Safety of entomopathogenic fungi and the corresponding formulations for warm-blooded animals was studied using mice, rats, rabbits and guinea-pigs as test animals. It has been proved on mice and rats that fungal formulations (Boverin, Aschersonia and Pecilomyces) fail to propagate in tissues of organs and spores leave the animals' organisms within 5 to 7 days.

The toxicity of commercial fungal pesticide formulations is very low. Average lethal doses of certain fungal formulations for laboratory animals are presented in Table 1.

Experiments with guinea-pigs showed that spores of Beauveria bassiana and Trichoderma lignorum are potent allergenes. Antibodies to fungal antigenes and allergic responses were found in humans too. Allergic responses manifested in the form of fatigue, asthenia, headache, giddiness, allergic rash and rhinitis. Allergy to fungi develops usually in the case of significant spore dissemination of the working zone air, which occurs mainly in the course of manufacturing the preparations. In the agricultural use of fungal formulations no cases of allergy were detected.

When an orchard was treated with 0.2% Boverin suspension the formulation was detected in the working zone air at the concentration of 10.4 mg/m^3 and on apples, at 0.08 mg/kg. These amounts are considered safe.

(d) Antibiotic Compositions

Among antibiotics recommended for plant protection there are highly toxic, moderately toxic, low toxic and practically non-toxic substances. The average lethal doses for certain fungicide antibiotics are presented in Table 2.

Table 1

Toxicity of Bacterial or Fungal Formulations

for Laboratory Animals

Formulation	Active ingredients	DL$_{50}$		Class of the hazard
		mice	rats	
		Intragastral administration		
Bitoxibacillin	bacteria	9.0 ±0.7	over 40.0	5
Insectin (liquid)	bacteria	32.0 ±7.3	9.7 ±2.0	5
Carnecin	bacteria	10.2 ±3.6	over 32.0	5
Lipidocide	bacteria	over 12.0		5
Mycolitin	bacteria	4.2 ±1.5		5
Toxobacterin	bacteria	15.3 ±1.3		5
Trichodermin	fungi	6.2 ±0.6	7.5 ±0.9	5
Entobacterin	bacteria	over 20.0	over 20.0	5
		Intraperitoneal administration		
Boverin	fungi		0.63 ±0.14	4
Carnecin	bacteria	0.08 ±0.03	0.11 ±0.03	3
Mycolitin	bacteria	0.05 ±0.005	0.08 ±0.03	3
Paecilomin	fungi	0.92 ±0.08	0.52 ±0.07	4
Trichodermin	fungi		0.27 ±0.02	3
Entobacterin	bacteria		0.15 ±0.02	3

NOTE: Class of the hazard: 1 - extremely active poisonous substance

2 - highly toxic substance

3 - moderately toxic substance

4 - low toxic substance

5 - essentially non-toxic substance

Table 2

Toxicity of Antibiotics Proposed for Control of Plant Diseases

Antibiotic	DL$_{50}$, mg/kg		Class of the hazard
	mice	rats	
	Intragastral administration		
Acti-Dione		320 ±70	3
Antimycin		80 ±2	2
Blasticidin, 5% dust		35.6 ±6.0	1
Imbricin	475 ±35	3500 ±500	4
Kasumin	over 2000		5
Trichotecin	560 ±58	260 ±43	3
Phytobacteriomycin	320 ±73	550 ±45	3
Phytolavin	1500 ±324		4
	Parenteral administration		
Imbricin (intravenous)		28 ±5.0	2
Blasticidin, 4% dust (intraperitoneal)		1.4 ±0.2	1
Polyoxin (intravenous)	210 ±50		5
Trichotecin (inhalation)		over 100 mg/m^3	4
Phytobacteriomycin (inhalation)		4.8 ±0.4 mg/m^3	2

Some antibiotics are able to accumulate in organs and tissues when the exposure is long enough. For example, Antimycin is highly cumulative, cumulation coefficient for Acti-Dione is $K_{cum} = 0.5$ and that for Phytobacteriomycin is $K_{cum} = 21$. Trichotecin is not practically cumulated by the organism (K_{cum} = over 7).

Many antibiotics produce an irritating action on skin and mucosa. The irritating action is characteristic of Trichotecin, Phytobacteriomycin, Imbricin, Polymycin.

An ability to disturb the function of natural microflora inhabiting the skin, mucosa and certain cavitary organs is an important feature of antibiotic action. The disturbance in the function of natural microflora of the gastrointestinal tract can result in disbacteriosis and disorder of the intestine function. It should be noted that antibiotics change the immunologic reactivity of the organism and are able to sensitize a microorganism. Accordingly the allergy to antibiotics can develop in individuals who handle them for a long time.

All the antibiotics are known to penetrate into plants wherein they are subjected to the deactivating action of enzymes. Antibiotics Kasumin, Blasticidin, Acti-Dione, Antimycin, Polymycin, Phytobacteriomycin and Trichotecin are quickly destroyed in vegetating fruit crops and not detected in the fruits 2-3 days after the treatment.

Spraying on plants may create very high antibiotic concentrations in the air. Concentrations of moderately toxic antibiotics approaching several tenths of milligram per cubic metre of air should be considered as hazardous.

Industrial production of antibiotics may also contaminate the working zone air with hazardous concentrations of antibiotics.

REFERENCES

1. Processes for Production and Properties of Bacterial Entomopathogenic Formulations, Survey, Series 6, Issue 1, Moscow, Flavmikrobioprom, 1979.
2. Alyoshina O.A., Kononova E.V., Pokidova N.V., Biologic Features of Entomopathogenic Fungi and Feasibility of Commercial Production of Boverin, in book: Microbiologic Means for Plant Protection and the Bacterial Formulations, Moscow, Glavmikrobioprom, 1978, pp 40-44.
3. Petrukhina M.T., Burtsevich L.A., Production and Application of Antibiotics for Plant Protection (Foreign Formulations), Survey, Moscow, Glavmikrobioprom, 1972.
4. Gural A.L., Vasilyeva V.L., Dyachenko M.A., Trusov V.I., Tonkal T.E., Experimental Data for Assessment of Insect Virus Pathogenoity to Vertebrate Animals, in book: Molecular Biology, 1979, pp 84-94.
5. Murza V.I., Toxicologo-Hygienic Properties of Insecticide Compositions Based on Spore-Producing Bacteria of Bacillus Thuringiensis Species, Cand.Sci. Thesis, Kiev, 1977, 136 pp
6. Melnikova E.A., Hygienic Study of Antibiotics and Microbial Compositions for Agricultural Use, Doct.Sci. Thesis, Kiev, 1977, 340 pp.

LONG-TERM EFFECTS OF PESTICIDES ON THE ORGANISM

V. I. Vashakidze

Under the present conditions - in the age when chemicals are widely used in the national economy a host of new medical problems arise, including the impact of chemical agents on specific functions of the body and on the progeny.

Although numerous clinical experimental data are available that characterize pathologies of a chemical nature, some relevant issues still get inadequate attention. In particular, we do not understand well enough the effects of pesticides on the sexual function of human beings and their progeny.

There has been a long debate on the influence that exogénous factors exert on the sexual glands of parents and their progeny. According to some researchers, the most sensitive, mature sex cells of the parents are killed by exposure to harmful factors while those with low sensitivity survive it undamaged.

The advocates of this concept maintained that the sex cell is destroyed by a chromosome lesion and, if not destroyed as in the case of mild exposure, it is rendered incapable of reproduction. If reproduction does occur, then the lesion must have been so minor as to allow complete recovery.

These investigators, however, failed to pay attention to the data of some authors on the birth of defective progeny resulting from chemical exposure of the organism prior to conception.

Numerous present-day studies indicate that partial injury of the sex cells is possible. If so, the affected cells still get fertilized but the birth of inadequate progeny is not unlikely.

Integral and specific functions of the body are investigated to ascertain the specific action of pesticides.

The ovaries are known to be most sensitive to various exogenous factors at the follicule maturation stage, i.e. the period of relative functional dormancy. This accounts for selective injury of mainly mature follicules caused by pesticide exposure.

Following the modified ovogenesis, the course of the estrous cycle becomes irregular both in qualitative and quantitative terms.

Medium and small doses (1/50, 1/100 and 1/500 DL_{50}) of dinitrocresol, sevin, phthalan, captan, morocide, etc. impair the estrous cycle by extending the passive stages (di- and meta-estrus) and substantially reducing the active phases (pro- and estrus).

The percentage of females in estrus is thus decreased and normal cycles among the test animals are less frequently observed (2.8 against 4.2 in the control group) as the cycle length increases with longer exposure to the agents.

The severity of disturbances in the estrous cycle is directly dose- and time-dependent, just as the reversibility of the process itself varies directly with the dose rather than the length of the exposure.

The toxic response to organomercuric pesticides, besides interference with the estrous cycle of the kind mentioned above, involves a manifestation of permanent estrus suggesting an injury in the endocrinal regulation of the ovaries which renders the affected animals incapable of fertilization.

Generally, pesticides produce adverse effects on the gonadal structure during prolonged exposures and in threshold or subthreshold systemic toxic doses.

Spermagonia and spermatocytes are the most sensitive cells in the spermatogenic epithelium to pesticide exposures.

Along with the spermatogenic epithelium, the Sertoli cells also diminish in number on exposure to pesticides (BMC, sevin, captan, etc.), giving evidence of a damage to the normal trophism of the testis. The pesticides of the phthalimide (phthalan) and phenol-containing series (morocide) mainly affect the Leidig cells by bringing down their quantity. This is looked upon as resulting from an altered hormonal function of the gonads.

The effect of pesticides increases the number of tubules with the spermatogenic epithelium in the 12th stage of meiosis, especially after prolonged exposures to chemical factors. Consequently, the pesticides (BMC, captan, etc.) characteristically produce a cytostatic effect for they act at this very stage.

Moreover, pesticides cause desquamation of the seminiferous epithelium or detachment of their cells from the basal membrane, when present in small doses.

Impairment of the male gonads by the pesticides examined shows up not only as morphological changes, but also as their dysfunction.

Pesticides induce sterility in most animals. When the fertilizing ability of the intact females mated with poisoned animals persisted, one noteworthy development was the prevalence of underdeveloped embryos or fetal death.

For several pesticides their injurious action reaches maximum during the stage of spermatogonia (BMC, captan, phthalan) while for other pesticides (morocide) this occurs at the stage of spermatocytes and mature forms.

Pesticides can trigger subtle changes in the chromosome machinery in the form of chromatid bridges and fragments.

Their frequency reaches the peak 12 hours after the exposure to a pesticide.

The pesticides of the carbamate category are more often responsible for chromosome sticking-together.

Though the latter's outcome is still unclear, it is known to result in gross impairments such as the shortage or excess of a substantial part of the cells. Typically, they result in the death of the cells, this being the reason for the highest rates of non-implanted and dead embryos after pesticide exposures, especially to organomercuries and those of the carbamate category. The above changes point to potential hazard of the birth of inadequate offspring for the persons contacting pesticides.

The mechanism by which the pesticides affect the specific functions of the body is not adequately known.

It has been established, however, that dysfunctions of the nervous, endocrinal and enzymatic systems, along with those of several vital organs are prominent in the pesticidal injury of the body.

6300

When the pesticide-induced effect on the organism combines with the systemic toxic effect the histochemical structure of the hypothalamus-hypophysis-adrenal cortex system is impaired; the ribonucleoproteid and glycoproteid metabolism of the hypothalamus and hypophysis is disrupted; and the function of the adrenal cortex is debilitated. Again , the severity of the disturbances depends on the dose, the type of the chemical involved, and the duration of the exposure.

The impact of pesticide is characterized by copious deposition in the adenohypophysis of glycogen granulations that are not normally present. This is indicative of major irregularities in the carbohydrate metabolism of the adenohypophysis, largely in response to the substances of the carbamate and phenol-containing groups.

The most sensitive indicator of the pesticide-induced lesion of the organism is the function of the adrenal cortex which is inhibited by pesticides used in minimum doses. The state of the adrenal cortex is, therefore, involved in the mechanism responsible for the injury of the gonadal function in a pesticide-exposed organism.

Pesticides promote mainly accumulation of gonadotropic hormones in the hypophysis, evidenced by a three-fold or higher increase in uterine and ovarian mass in infantile animals following the injection of a hypophysis suspension from the animals poisoned by various pesticides, as compared with the control infantile animals.

Despite the lesion of the sexual glands, the mucous membrane of the vagina continues to function normally.

A folliculin injection provokes estrus in such animals. Thus, it is the sexual glands that are affected by pesticides whereas an enhanced gonadotropic function is the organism's response to the hypofunction of the gonads.

That the altered gonadotropic activity of the hypophysis does not cause any gonadal dysfunction is evident from the data concerning a greater gonadotropic activity of the hypophysis on exposure to various doses of pesticides after the gonadal function has been lesioned.

There are reports on the increasing content of the luteinizing hormone in the hypophysis as a result of ovariectomy (Swartz, 1965).

The argument in favour of a selective lesion of the gonads by most pesticides is also supported by the experimental evidence obtained from transplantations of normal ovaries to poisoned non-cycling females.

Resumption of the estrous cycle in the poisoned animals following the transplant operation shows that pesticides injure primarily the function of the ovaries.

Large doses of pesticides produce severe chromosome lesions that cause the cells to die. With minor doses, cells appear with gene mutations and adequate viability to reach the stage of mature cells when they divide to form new cells with certain defects.

Documented evidence of the injurious effects of pesticides on animal brain cells gives reason to believe that germinative human cells will probably sustain a far greater damage under similar conditions.

Pesticides have deleterious effects on the progress of embryogenesis via pregestational exposure, but, in addition and more, so during pregnancy.

The effect of pesticides is most harmful either in the first half of the pregnancy, and especially during implantation (organomercuries, carbamates, phthalimides, etc.), or during organogenesis (organochlorines and phosphates, phenol-containing agents, etc.).

In the event of a pesticide exposure during the whole period of pregnancy, it is chiefly responsible for embryo death and impaired organogenesis. This is due to the fact that the administration of pesticides since the very first day of pregnancy leaves them little time for accumulation before gastrulation and vigorous organogenesis begins, with the result that their injurious effect shows up primarily as systemic intoxication of the embryo leading to its death (A.P. Dyban, 1968).

It is known nevertheless that teratogenesis involves, over and above serious anatomical developmental abnormalities, unassayable lesions of histogenesis and functional inadequacy of various systems.

The validity of this conclusion is proved by further evidence of developmental anomalies among the progeny with normal appearance and body weight. They are to be seen later, postpartum, and consist of retarded body weight increment, late puberty, lesion of the gonads, and impaired activity of some enzymes such as transferases, phosphatases, etc.

Nevertheless the embryo survival and underdevelopment are a characteristic indicator of general teratogenicity and emryotoxicity, by which one can judge the real risk of damage to embryogenesis following pesticide exposure.

It is known that the placenta builds no absolute barrier to many chemicals, notably mercury, which crosses it to pass into the fetal organism. Nor is it impossible for pesticides to affect the fetus indirectly, by augmenting the sensitivity of the maternal body, consistent with its physiological status (pregnancy).

There is a certain theory about the mechanism of the embryo's high susceptibility to various pathogenic factors.

One probable cause for disturbed embryogenesis is irregular patterns of carbohydrate, fat and protein metabolism after exposure to organomercuric and organophosphorus pesticides, the substances of the phthalimide series, and other agents.

A frequent combination of embryo death with the atrophy of corpora lutea in pesticide intoxications suggests the involvement of the atrophy of corpora lutea in embryo mortality, besides other causes.

Embryos' sensitivity to the effect of pesticides depends on the dose and the degree of the embryos development. They are more vulnerable in the first period of pregnancy than in the second one. On the other hand, embryonic damage appears to be more severe during implantation than during organogenesis. It is thus assumed that exposure to pesticides makes implantation a critical period of embryonic development.

The disruption of the reproductive function among producers by exposure to various exogenous factors prior to pregnancy is commonly due to three types of injuries, i.e. dominant lethals are responsible for death before birth, semilethals cause death between birth and the reproductive age, and mutations cripple viability and only on occasion cause death (Russel, 1954).

Some pesticides upset the ratio between sexes in the progeny by inducing the predominant birth of females (captan) or males (granosan).

Some authors explain the changes of the sex ratio leading to male prevalence in the progeny after exposure of the producer organisms to exogenous factors by the manifestation of lethal mutations coalesced with sex, i.e. those localized in the x-chromosome, or by the transfer of paternal chromosomes to the daughters only.

Consequently, the effect of the dominant mutations that occur in the daughter organisms is expected to reduce the number of daughters and relatively increase the number of sons.

In recent years, extensive factual evidence has become available that strongly implicated an impairment of the chromosome machinery in the pathogenesis of defective progeny.

One argument to support the involvement of chromosome restructurings is frequent non-disjunction of chromosomes and developmental anomalies in the offspring.

The fact that the increased number of sterile animals occurs in combination with a larger percentage of the tubules with spermatogenic epithelium in the 12th stage of meiosis after pesticide exposure favors the above assumption.

In addition, the greater percentage of sterile progeny, female and male alike, in all three generations, especially on exposure to organomercuric pesticides, should be attributed to disturbances in the course of ovo- and spermatogenesis, or to fragmentation and division of the chromosomes which cause male sterility, or to the injury of x-chromosomes in the ova or spermatozoa.

The inhibition of incremental body weight in the test progeny is observed mainly during the period of vigorous movements when the food requirement is the highest. An impairment of metabolic processes at that period can considerably affect the body weight.

Later on, the lagging body weight becomes increasingly obvious and reaches maximum during pubescence, the period when the body achieves the highest rate of metabolic processes and activation of the endocrinal glands. Once the processes and the functions of the endocrinal glands are affected, the general development of the progeny is affected.

Pubescence in the progeny of pesticide-poisoned producers is delayed by two to three months.

The distruption of ovo- and spermatogenesis has been recorded mainly for the progeny of the producers poisoned with organomercuric and phenol-containing pesticides and the chemicals of the carbamate and phthalimide series.

These changes of the sexual function make most animals sterile and considerably shorten the period of sexual activity which is generally regarded as the pretimely advent of senescence.

The producers affected by threshold doses of some pesticides give birth to progeny whose pesticide sensitivity is increasing from generation to generation, i.e. the parameters of pesticide toxicity keep declining.

Disproteinemia due to the expansion of gamma-globulins, decline of toxicity parameters and functional inhibition of the hypothalamus - hypophysis-adrenal cortex system suggest that the test progeny develops sensitization of the organism to pesticide exposure.

The above data reveal a major general developmental anomaly in the progeny of the producers that were poisoned by toxic chemicals.

An objective of prime importance for the prevention and treatment of the gonadal lesion, as well as for the elucidation of some aspects of the intoxication mechanism, is their early diagnosis, even before symptoms of the lesion in the reproductive function of the body become clear.

In this regard, it becomes essential to identify bodily alterations among the persons exposed to pesticides but exhibiting no obvious symptoms of intoxication.

Toxicologic assessment and hygienic standardization of pesticides in the air of the working zone require investigations to better understand long-term health effects due to exposure to chemical compounds - gonadotropic, mutagenic, embryotropic, and teratogenic.

Hence the extreme importance which is now attached to the study of chemical mutagenesis in mammals and the cytogenetic study of human tissue cultures.

THE PROBLEM OF MUTAGENESIS IN THE HYGIENE OF PESTICIDES APPLICATION - BASIC RESULTS OF GENETIC AND HYGIENIC INVESTIGATIONS

M.A. Pilinskaya

One of the leading aspects of the problem of the so-called "late sequences" of an unfavourable action of pesticides is the evaluation of their possible contribution to induced mutation process in man, resulting in increased incidence of pathology with a genetic component.

The most urgent and promising trends in studies of biological effects of pesticides are, therefore, genetic and hygienic investigations aimed at providing a scientific basis to prognoses, and preventing mutagenic hazard of pesticides to the health of the present and future generations.

At present genetic and hygienic investigations of pesticides are conducted in the following principal directions:

- experimental testing of pesticides and their metabolites for mutagenicity;
- cytogenetic examination of persons exposed to occupational contact with pesticides;
- cytogenetic monitoring of human subpopulations for which pesticides are the prevailing factor of environmental pollution;
- biological detection of mutagenic pesticides in the environment.

The most advanced direction among these is the experimental evaluation of pesticide mutagenic potential for various biological objects. Thanks to experimental studies, we now have information on the mutagenicity of over 400 pesticides, among them about 50 per cent are mutagens.

Since no universal test-system is presently known for detecting in a chronic experiment on mammals all types of mutations induced by the studied substance in doses met with in practice, use is made of a set of conventional methods, supplementing each other, for experimental estimation of mutagenic activity of pesticides.

The ability of pesticides to induce genic mutations is tested mainly on microorganisms and yeast (the Ames salmonella) microsome test, the method of scoring direct and reverse mutations in Escherichnia coli and yeast, the host-mediated assay, the fluctuation test on bacteria, the assay of mitotic gene conversion and mitotic crossingover in Saccharomycetes, etc.). Of lesser use are animal tissue cultures (detection of mutations of resistance to 6-thioguanine and oubaine in Chinese hamster, CHO and V79 cell clutures, as well as in the mouse lymphoma L5178Y cell culture); the method of detecting mammalian genic mutations in vivo: mouse hair coloration mutation in somatic (spot-test) and germ cells (specific locus test) was applied in very few works.

The induction of chromosome mutations (aberrations) is, as a rule, detected by cytogenetic methods making use of somatic cells of man in vitro (peripheral blood lymphocyte culture) and of experimental animals in vivo (bone marrow cells); by micronuclear test on mice (this allows one to reveal not only structural lesions, but also nondisjunction of chromosomes which results in aneuploidy); by the method of dominant lethal mutations on mice and the method of recessive sexlinked lethal mutations on Drosophila (which reveal chromosome mutations in germ cells). A limited number of studies have employed the analysis of meiotic chromosomes directly in germ cells (mainly in spermatocytes), of laboratory animals, the hereditary translocation test in mice and the method of recording abnormal spermatocytes in rats. Very few studies have been devoted to the effect of pesticides on the unscheduled (repair) synthesis of DNA in vitro and induction on sister chromatid exchanges (SCE) in mammalian cell cultures.

Experimental studies of the mutagenic effect of pesticides are not different from those aimed at detecting mutagens in other chemicals polluting the environment. They represent either the results of testing a small number of compounds by various test systems or an account of a large number of compounds tested by one system (usually microbial). Works of this kind are usually of a statement-like character: they indicate the presence or absence of mutagenic activity in pesticides tested by a particular system, but contain no assessments of the degree of this activity and the magnitude of hazard posed by a chemical; also they do not give any concrete hygienic recommendations, which makes practical utilization of the results rather difficult.

Very few investigations have been devoted to mutagenic effects of pesticides applied in complex or combinations to various biological objects. Practically unstudied remains the problem of possible modifications in the genetic effect when the organism of mammals receive simultaneously or in succession several pesticides that differently affect the activity of microsomal enzymes essential both for activating promutagens and in-activating mutagens. A very young, though promising, branch of research concerns pesticides'possible effect (including doses that may actually exist) on the mutability of different organisms, and their sensitivity to other mutagens.

Of considerable interest are the recent experimental studies into the mutagenicity of metabolities of some pesticides that may appear in the environment and the body of mammals from biodegradation and biotransformation processes. Besides studies on the mutagenic effects of chemically pure metabolites or using the traditional system of metabolic activation of substances by microsomal fraction of the liver of laboratory animals (chiefly in experiments on salmonellae), several authors have determined the genetic activity of pesticides after nitrozation (which is believed to take place in the human stomach when pesticides react with nitrites contained in food or when environment pesticides react with nitrogen-containing mineral fertilizers) and also after activation by tissues of their target plants. A significant modification has been noticed in the genetic activity of certain substances, for example, nitrozation of carbaryl and activation of atrasin with plant homogenates considerably enhanced the mutagenic effect of the original pesticides. Experiments of this kind make it possible to simulate a more real situation, and enable one to judge about the genetic activity of not only the original, but also of the expected end products of pesticide transformation, the products with which a man is likely to come in direct contact.

The whole body of accumulated experimental evidence warrants the conclusion that certain pesticides and their metabolites are mutagenic chemical pollutants of the environment and present a potential mutagenic hazard for man.

At the same time, no matter how perfect are the experimental techniques of studying pesticides genetic activity, the results obtained cannot be considered as final, because extrapolation of the data on mutagenic activity and chemical hazards from models to man (particularly in the quantitative aspect) is yet one of the most difficult problems.

Therefore, of much significance for genetic-and-hygienic evaluation of pesticides are the data on possible mutagenic effect of pesticides on man, obtained particularly in cytogenetic examination of occupational groups that can be regarded as critical by their intensity of contact with pesticides. Unfortunately, such examinations have been yet carried out on a rather limited scale, not because of methodological and organizational difficulties, but apparently, due to the fact that we do not fully realize the possible genetic hazards of pesticides for those who come in contact with them.

Thus, the analysis and generalization of our own data and those from the literature suggest that chromosome aberrations in the peripheral blood lyphocytes have been studied in persons exposed to occupational contact with only 16 pesticides out of 400 investigated for mutagenicity: derivatives of dithiocarbamino- and carbamic acid - mupe ciram, cinebom, thyram, benomyl and pyrimicarb; organochlorine pesticides - DDT, gamma-HCH, polychlorinated terpenes, kamfechlor, dibromchlorpropane and hexachlorbutadien; organophosphorus compounds - trichlorphon, phosmet and diacinon; carbonic acids derivatives - chlorinol, buvinol, and 2,4-D. No cytogenetic effect was noted in persons contacting benomyl, gamma-HCH, dibromchlorpropane, chlorinol, buvinol and 2,4-D. Persons working with DDT, hexachlorbutadiene and organophosphorus pesticides showed a tendency to increased chromosomal damage; contact with other pesticides led to a significat increase in frequency of aberrant metaphases in comparison with the control.

Several studies have been devoted to cytogenetic investigations of agricultural and hothouse workers contacting a group of pesticides. In only one group of workers of an experimental hothouse, the level of chromosome aberrations did not significantly differ from the spontaneous one, this being apparently connected with the effective sanitary measures and individual protective means. Other groups showed a statistically significant increase in the rate of chromosome aberrations or a tendency towards them in comparison with the control.

It is quite likely that the cytogenetic effect observed in these groups of professional workers was due to occupational contact with pesticides adverse in their evironment.

In the majority of aforementioned studies it has been shown that the induced effect is highly variable within each group, which is apparently due to their different sensitivities to chemical mutagens. Despite significant person-to-person variations, many pesticides studies were characterized by a specific "saturation limit", maximum rate of aberrant metaphases which did no increase with prolonged exposure to pesticides under permanent working conditions. This phenomenon was particulerly apparent from repeated examination (at one year's interval) of the same persons engaged in cineb fungicide production: their frequency of chromosome mutation remained at essentially the same level.

Some studies have shown that after the termination of occupational contact with pesticides (for example, after seasonal contact of agricultural workers with a number of agents, or in case of intermittent contact with pesticides in hothouses) the chromosome aberration rate gradually returns to its normal.

Of certain interest are the results of investigators who observed a distinct cytogenetic effect in persons who has sustained an accidental acute intoxication with some pesticides: organochlorine compounds - kamfechlor and chlorinated terpenes; organophosphorus compounds-mupe Malathion, paration-methyl, trichlorphon, dichlorophos, diacinon and demetoate. In some of the studies an attempt has been made at investigating the dynamics of the mutagenic effect in time. It has been demonstrated that in case of organochlorine intoxication an increased chromosome aberrations level persists for 10 to 12 months after the primary examination. When exposed to Malathion the cytogenetic effect is noticed immediately after the poisoning, with paration-methyl and trichlophon - this effect is observed in one month, and disappears in six months after the intoxication. In the rest of the cases the nonavailability of the required samplings hampers the interpretation of data concerning dynamics of the mutagenic effect.

A few works concern the study of sperm of persons exposed to pesticides. The incidence of anomalous spermatozoa heads in 50 males engaged in the manufacture and application of carbaryl, and of anaeuploidy by Y-chromosome in spermatozoa of 18 workers having occupational contact with dibromchlorpropane has been studied. Positive results are indicative of the disturbance of meiosis in persons exposed to the aforementioned pesticides.

A promising approach in occupational genetic monitoring, particularly in estimating the effect of a number of substances, is the testing of workers' urine for the presence of mutagenic metabolites (by Ames or any other in test system vitro). Some investigators believe that genetic activity of urine can serve as an integral biological index of the mutagenic effect of chemical hazards in the body. With regard to pesticides, studies along this line are at the stage of approbation.

Thus, thanks to genetic studies of occupational groups, information has been obtained, confirming the possibility of mutagenic effect in somatic cells of man in contact with pesticides. Such information is of scientific and practical value in several aspects: supplementing and confirming the experimental data is necessary for the development of investigations on comparative mutagenesis; it is of individual significance in industrial genetic monitoring of pesticides; it is the source of information on interindividual variations of the response of genetic apparatus to identical mutagenic action; it makes possible the detection of "increased risk" groups, and can serve as a serious indication of possible genetic hazard for population if the environment is polluted with particular mutagenic pesticides.

Total contribution of pesticides to the mutagenic process in the population can be evaluated on the basis of dynamic observations over the mutation rates in human populations directly, in combination with continuous monitoring of the actual environment pollution with pesticides.

Practical implementation of this programme entails great difficulties, although some prerequisites have been created. Methodological difficulties are primarily related to the absence of a sufficiently developed programme of genetic monitoring of human populations, with regard to both selecting the most informative and easily analysable mutability indices, and establishing a relationship between the mutational process intensity and the action of a particular factor or a group of factors of environmental pollution.

As to the programme of investigations, N.P. Bochkov believes that a possible version can be offered by integral monitoring of genetic-related pathology. The programme includes, in particular, cytogenetic examination of all outcomes of pregnancy (spontaneous miscarriages, perinatal mortality, children with inborn developmental abnormalities and healthy newborns). A good supplement to monitoring based on progeny effects may be the measuring of the rate of chromosomal mutations in somatic cells (peripheral blood lyphocytes) of the representative groups of population, which, at the present stage, is more feasible, both methodologically and organizationally. With regard to pesticides, only one study has been performed in this direction to date (M.A. Pilinskaya, 1977, 1978). The study was aimed at obtaining objective initial information on the level of spontaneous chromosome aberrations in the population of areas with intense and limited application of pesticides, and hence with different residual pesticide content in environmental objects. Cytogenetic investigations of several groups of practically healthy teenagers of both sexes denying any deliberate contact with pesticides or any other known or expected mutagens have established that with increased use of pesticides the level of spontaneous chromosome aberrations in the somatic cells of those examined increases. This confirms the hypothesis that a complex of pesticides contained in environmental objects can affect the process of spontaneous mutagenesis in man.

Since pesticides are not the only mutagenic environmental pollutants, a new promising line of genetic-and-hygienic research related to pesticides,the beginning of which has been marked in the works of A.I. Kurinny (1980), is the development of methodological approaches to regulations of using mutagenic pesticides, making allowance for the already existing mutagenic background.

As an initial step in this direction, a system for bioindication of effects of mutagen complexes upon natural ecosystems and components thereof was developed. With the method of determining pollen sterility in a number of plant species, as well as of scoring chromosome aberrations in seedlings a significant difference has been shown to exist between mutational backgrounds of two environmental preserves (where practically no pesticides were used) and the adjoining to them regions where intense use was made of pesticides. The results of the study suggest that pesticides are the major cause of the difference in the mutagenic background of the zones under study.

These are the main directions of genetic-and-hygienic research related to pesticides.

The problem becomes much more complicated when it concerns evaluation of the mutagenic risk from pesticides and the use of the results in practical areas, such as setting hygienic standards and regulations making allowance for pesticide-induced mutagenesis.

According to Academician N.P. Bochkov: "Evaluation of genetic consequences resulting from exposure of man to chemical pollutants of the environment is yet in the development stage;

the objectives have been defined in general and the ways to achieve these goals and the difficulties to be encountered are known approximately". Nevertheless, in some studies of the recent years attempts have been made to estimate the populational risk, caused by the use of certain mutagenic pesticides (for example, captan and atrazin), on the basis of the approaches proposed for estimation of genetic hazards of strong chemical pollutants as compared to the action of ionizing radiation.

M.A. Pilinskaya and A.I. Kurinny (1978) cling to the opinion that evaluation of potential mutagenic hazard of pesticides should rest on approaches differing from those applied for standard chemical mutagens and be determined by the features which distinguish pesticides among other genetically active environmental factors.

Our own results, as well as the literature data suggest that, although mutagenic pesticides are placed in different groups of chemical compounds and differ in physico-chemical properties, biological activity, toxicity, mechanism of action, biotransformation pathways, etc. their majority has one common feature: the ability to induce a relatively weak mutagenic effect when applied at a rather narrow range of high doses and concentrations, and can thus be referred on the whole to mutagenic factors of low intensity, compared to other known mutagens. At the same time, the inevitable contact of virtually the whole population with pesticides considerably aggravates the prognosis of their possible genetic hazard which for any mutagenic factor is determined, in the first,place, by the number of reproductive age persons in contact with this factor. Therefore, pesticides, weak mutagens as they are, can present considerable genetic haard to man.

Taking into account the above said, an acceptable approach to initial evaluation of pesticide mutagenic hazard seems to be the one offered by A.I. Kurinny (1978) consists in evaluating comparatively mutagenic potential of pesticides from three parameters:

1) degree of expression of mutagenic action, determined by the significant rise in the maximum induced mutation effect over the control or its multiple level;

2) effective dose (concentration) level defined as the minimum active dose (concentration) in units of weight, taking into account its toxicity (fraction of DL_{50});

3) university of mutagenic activity, that is the ability to induce mutations in as many test systems as possible.

The first two parameters may be used for initial characterization of pesticides based on the results obtained by one test system only, in particular, when determining cytogenetic activity on experimental animals. A combination of two parameters (maximum induced effect and minimum effective dose level) allows one to classify the compounds in question into several conditional groups: mutagens having different mutagenic activity (4 groups) dubious mutagens (which show cytogenetic activity only at toxic doses), and non-mutagens. Classification of this kind allows one to give tentative recommendations as to whether the pesticide concerned can be applied at all or/and at what scale, what hygienic norms and regulations should be used, and as to whether observations of occupational groups in contact with the most mutagenic pesticides are necessary.

The extent of potential mutagenic hazard of pesticides should be taken into account as one of the criteria in grouping pesticides to a definite class of substances, according to the hygienic classification.

The mentioned evaluation scheme was approbated by us (M.A. Pilinskaya et al., 1980, A.I. Kyrinny et al., 1981) by analyzing over 50 widely used and prospective pesticides belonging to 17 classes of chemicals. On the basis of this analysis, a preliminary conclusion was drawn on the extent of the potential genetic hazard of the studied substances and appropriate practical recommendations were given; this may be regarded as a solution to the problem in first approximation.

Further development of genetic-and-hygienic investigations of pesticides and improvement of methods of evaluating their mutagenic hazard shall enable us to stipulate such permissible levels of environmental pollution with pesticides (if their use is necessary and inevitable) that would be safe for health and human well-being of man.

References

Bochkov N.P. Genetic Effects of Exposure to Environmental Factors. Vestn. Acad. Med. Nauk USSR, 1981, No. 3, 13-19 (in Russian).

Bridges B.A. The Mutagenecity of Captan and Related Fungicides. Mutat. Res., 1975, 32, No. 1 3-34.

Brusick D. Value of Short-Term Mutagenicity Tests in Human Population Monitoring. - "Progress in Mutational Research" (Ed. by K. Bora et al.), 1982, 3, 125-135.

Drake J. et al. Environmental Mutagen Hazards.-Science, 1975, 187, No. 2, 503-514.

Dubinin N.F., Pashin Yu.V. Mutagenez i okruzhayuschchaya sreda (Mutagenesis and the Environment). Nauka Publishers, Moscow, 1978, 130 pp.

Kuleshov N.P., Shram R. Genetic Monitoring of Human Populations in Connection with Pollution of the Environment. - In: Perspektivy meditzinskoy genetiki (Prospects of Medical Genetics) (Ed. Bochkov N.P.). Meditsina Publishers, Moscow, 1982, 123-163.

Kurinny A.I., Pilinskaya M.A. Issledovanie pestitsidov kak mutagenov vneschney sredy (Study of Pesticides as Environmental Mutagens). Naukova Dumka Publishers, Kiev, 1976, 114 pp.

Kurinny A.I. Concerning the Genetic and Hygienic Regulations for Pesticides Application with Consideration to Their Potential Mutagenic Hazard. Tsitologia i genetica, 1978, 12, No. 4, 353-358 (in Russian)

Kurinny A.I. et al. Implementation of a Programme for Cytogenetic Screening of Pesticides; Initial Estimation of Pesticides. Tsitologia i genetica, 1982, 16, No. 1, 45-50 (in Russian).

Kurinny A.I. Indication of Pollution of the Environment with Pesticides-Mutagens by Their Gametocidal Effect on Plants. Tsitologiya i genetica, 1983, 17, No. 4, 32-35 (in Russian).

Loprieno N. and Adler I.D. Comparative Programme of the European Economic Community of Short-Term Assays for Mutagenicity. IARC Sci. Publ., 1980, No. 27, 331-341.

Pilinskaya M.A. and L'vova T.S. Results of Cytogenetic Examination of Populations Exposed to Limited and Intensive Use of Pesticides. Tsitologiya i genetica, 1979, 13, No. 3, 229-231 (in Russian).

Pilinskaya M.A. et al. Initial Estimation of Cytogenetic Activity and Potential Mutagenic Hazard of 22 Pesticides. Tsitologiya i genetica, 1980, 14, No. 6, 41-47 (in Russian).

Pilinskaya M.A. Significance of Cytogenetic Examination of Occupational Groups for Hygienic Evaluation of Pesticides Gigiyena Truda i Professionalnye Zabolevaniya, 1982, No. 4, 28-32 (in Russian).

Sankaranarayanan K. Determination and Evaluation of Genetic Risk to Humans from Exposure to Chemicals. In "Chemical Mutagenesis, Human Population Monitoring and Genetic Risk Assessment (Ed.

by K. Bora et al.), Elsevier Biomedical Press, Amsterdam, 1982, 289-323.

Sorsa M, Hemminli K. and Vainio H. Biologic Monitoring of Exposure to Chemical Mutagens in the Occupational Environment. Teratogenesis, Carcinogenesis and Mutagenesis, 1982, 2, 137-150.

Waters M, et al. An Overview of Short-Term Tests for the Mutagenic and Carcinogenic Potential of Pesticides. - J. Environ. Sc. Health, 1970, B.15, 867-906.

METHODS OF DETECTING PESTICIDES IN FOOD PRODUCTS AND OBJECTS IN THE ENVIRONMENT

M.A.Klisenko

Entering the human habitat, many pesticides can stay there for a long time, migrating from one object of the environment to another and changing into more toxic compounds.

It is quite evident that in the absence of reliable methods of the quantitative detection of pesticides in different media the theoretical and practical tasks of preventing pesticides' adverse impact on man and the biosphere will never be resolved.

Organic compounds constitute the overwhelming majority of pesticides. Their structure, physical and chemical properties and reaction capacity have determined the basic trends and difficulties in the development of a new field of analytical chemistry, notably the analytical chemistry of pesticide microquantities.

The principal difficulty in detecting the residual amounts of pesticides is that sensitive identification methods are required that would meet stringent requirements dictated by the specific biological effect of pesticides and their low hygienic thresholds. As a rule, one has to detect fractions of a microgramme of the substance analyzed in a sample. The methods employed should be selective, since a sample may contain several pesticides of different structure and biological activity, and there is a possibility of pesticides transforming into more toxic derivatives than the original substances.

In an analysis, significant difficulties are involved in separating pesticides from the samples and ridding them of admixtures surpassing them many times in both quantity and concentration.

Considering the brief periods of farming operations involving the use of pesticides, the fast perishing of certain food products and the large number of analysis performed, the laboratory control methods must be rapid and simple.

Several hundreds methods are being used in the world to determine the content of pesticides of different chemical composition in various media. They are based on different principles differing in analysis procedure and metrological characteristics.

In this connection, many countries have set up methodological centres for testing methods of pesticide detection and recommending their introduction at production facilities. In the USSR, the priority methods of detecting the residual amounts of pesticides in food products and environmental objects are tested by an interdepartmental group of experts and approved by the USSR Ministry of Public Health. There are annually published bulletins with methodology recommendations on detecting residual amounts to be employed by sanitation and epidemiological centres, agrochemical and other laboratories.

The broadening of the pesticides' assortment, multiplication of their uses and the increasing need for laboratory control of their contents gave rise to the need for standard methods of pesticide detection. This question assumed particular importance due to expansion of international trade in food products and the establishment of computer-based control systems. While taking into account sanitary control requirements, a standard methodology must ensure idendification and quantitative determination of individual pesticides or groups thereof, similar in chemical structure and physical and chemical properties, as well as of toxic metabolites of derivatives of the original preparations in food products, water, soil and air if hygienic standards have been set in respect of them. Requirements as to the metrological characteristics of the methods concerned are determined by the hygienic standards regulating the contents of pesticides in objects analyzed. The lower limits of detection (mg/kg; mg/l and mg/m^3), with the exception of specified cases, must not exceed the maximum permissible level or concentration (MPC). Under the USSR State Standard 12.1.005-76 an analysis method must ensure detection of the contents of the substance analyzed in an air sample at a level of 0.5 MPC with the duration of aspiration not exceeding 30 minutes. The overall error in this case must not exceed 25 per cent. Under the USSR State Standard 17.0.0.02-79, methods of detecting pesticides' content in water reservoirs or rivers used as sources of drinking water or as fisheries must have a lower limit of no less than 0.5 MPC for measuring analyzed substances.

FAO and WHO experts regard as satisfactory methods allowing some 80 to 110 per cent of the pesticide analyzed to be detected in food products with a relative error of ±10 per cent. Whenever the average values are less than the figures given, a correction factor is introduced in the calculations.

Quantitative detection may rely of physical-chemical or physical methods, including photometry, spectrophotometry, chromatography (gas, thin-layer, liquid and other), polarography or other instrumental methods. If they fail to yield satisfactory results, one can switch over to ferment or biological methods.

Standard methods must ensure selective detection of pesticides, similar in their chemical structure and field of uses.

To enhance its identification reliability, the standard methodology must ensure the detection of pesticides using methods based on different pronciples, for instance, polarographic and chromatographic, photometric and thin-layer-chromatographic, gas-liquid chromatography, etc.

Since the availability of reagents and equipment differs from laboratory to laboratory, CMEA countries' specialists deem it useful to include in standard methodologies some "back-up" methodologies of comparable accuracy, which contribute to a better identification and allow the available equipment and reagents to be used.

Selection of samples is one of the most important stages in detecting pesticide residual amounts in food products and environmental objects.

Errors in sample selection may result in a wrong hygienic evaluation of the sample, reducing to naught the work of a chemical analyst even when sensitive and selective analysis methods are used.

Sample taking is limited by time and financial inputs comprising the cost of the producte studied and reagents, instruments depreciation costs as well as remuneration of experts for selecting, transporting and analysing a given quantity of samples.

Considering the high analysis costs, it would appear sensible to achieve the required accuracy through selecting a larger number of sampes and taking a lesser number of parallel samples.

There is extensive literature on procedures for extracting pesticides from food products and other media. A procedure is only considered to be satisfactory if it ensures extraction of a pesticide, enhances the detection sensitivity through concentration of a substance and improves the method's selectivity.

The choice of the procedure is determined by many factors, such as:

- the possibility of a chemical transformation of the pesticide under certain chosen conditions of extraction;
- the possibility of the pesticide's transformation in the substrate analyzed, accompanied by the formation of products sharply differing from the original preparation in their physical and and chemical properties;
- the possibility of the pesticide's chemical reaction with the substrate, for instance, with fructose, protein, folic and humic acids, with the formation of conjugates that cannot be extracted by ordinary methods. In evaluating the threat of the conjugates, one ought to bear in mind the capacity of some of them for self-destruction with the formation of original pesticides. It has been proven experimentally that the "ageing" of samples makes pesticide extraction more difficult. This can probably be explained by the chemisorption of pesticides, for instance by the lipides of animal and vegetation tissues.

The extraction of fat-soluble, especially chloride-organic pesticides, from milk samples is a special problem. Organochloric pesticides are contained in the fat of the milk. Milk's fat in surrounded by a strong protein-phospholipide film that is so dense that solvents capable of dissolving fat fail to extract pesticide, if fat globules have not preliminarily been subjected to chemical treatment by alcohol, alkali, acid or a surfactant.

Thus, in assessing the effectiveness of extraction processes with respect to pesticides, one needs above all to have a clear idea of the pesticide's stability and capacity for reaction under the given conditions.

The extraction procedure must assure maximum extraction of the preparation under study without destroying its structure.

Ordinarily, following the concentration, an extract contains, in addition to pesticides, a large amount of (mostly organic) substances, which, when detected by different physical and chemical methods, can lead to analysis errors.

For extract cleaning, use is made of column, paper and thin layer chromatography, re-distribution between non-mixing solvents, vacuum sublimation, helium filtration and other techniques. The choice of the purification technique depends on the nature of the extracts. When adsorption methods of extracts purification are used, reproducibility of results may strongly fluctuate, chiefly because of the differences in the sorption properties of absorbents used.

The thin-layer chromatography is used much more frequently for extracts purification. In this case the effectiveness of separating admixtures from given pesticides in comparable to that achieved through paper chromatography, but the process takes much less time, and pesticide desorption is much easier to achieve by the use of benzene, hexane, ethylacetate or acetone. Silica gel is used most frequently as a sorbent for extract purification in thin-layer chromatography.

At a temperature of 80-90°C and pressure of 0.002 mm Hg, many pesticides may undergo sublimation. This property provides the basis for a method of ridding water extracts of different extractive substances many of which, under these conditions, have a lower vapour resilience and are incapable of sublimation.

Distribution of non-mixing solvents in the systems is broadly used for separating pesticides from fats and oils. In this case most frequent use is made of acetonitrile-hexane or dimethyl-formamide-hexane systems. In the process, pesticides pass over into acetonitrile or dimethyl-formamide, while pollutants stay in p-hexane.

To rid pesticide extracts of fats and oils, successful use is made of gel-penetrating chromatography of gel filtration. It has been found that 98 per cent of fats and oils in the extract is eluted from the gel-filtrating columns before the elution of pesticides. The gel-filtration processes can easily be automated. The degree of extract purification reached in the process surpasses that achieved through distribution in the systems with non-mixing solvents.

Successful detection of residual amounts of pesticides in foodstuffs and in the natural environment depends largely on advances in chromatographic methodology. Chromatography as a method of separating and identifying organic substances was discovered by M.S.Tsvetov - a prominent Russian scientist.

Gas and thin-layer chromatography are methods used most frequently for identification and quantitative determination of pesticide residue. Effectiveness of gas chromatography (GC) in determined by the detector's type and operation regime, by the nature of the dividing column, the properties of the combinations chromatographed and the equipment used.

Only few of the detectors employed in gas chromatography found application in analyzing pesticide microamounts. This is due, on the one hand, to insufficient sensitivity of many of the detectors, and, on the other, to insufficient discrimination with respect to the combinations analyzed. Broadly used at present for pesticide analysis are such detectors as the electronic-trapping (ETD), the detector of permanent recombination speed (PRD) which is a modification of the former, and the highly selective and sensitive ones like the thermo-ion (TID), microcoulimbometric and the flame-photometric (FRD) detectors. These detectors feature a heightened sensitivity to substances including such elements as phosphorous, sulphur, haloid and nitrogen. Often selective detectors do not require prolonged preliminary cleaning of samples analysed.

A chromatographic column must enable the sorbent it contains to be fully effective. For pesticide analysis, use is made of glass columns only , since the preparations tend to disintegrate in metal ones. Most effective are the stable phases based on dimethylpolysiloxan (DS-200, SE-30, DS-11, SE-301 and others); fluorinated silicone (QS-1) based on a copolymer of epichlorydrine and hiphenols (epicot 1001); nitrilesilicons (XE-60); polyethylene glycols (PEG-6000; PEG-20000; carbowax 20 M). OV series stable phases, containing different quantities of phenil- and methyl-siloxanes, have been widely used in recent years. The choice of the phase to achieve a particular separation task requires large experience, and there can be no universal recipe to suit all cases. As a first attempt at separation, one may use non-polar phases SE-300, DS-200 and others).

The USSR produces chromatographs designed specifically for pesticide analysis: "Tsvet", "Gazokhrom" and others.

The thin-layer chromatography method (TLC) is one of the simplest methods for determining microquantities of pesticides. Soviet scientists N.A. Izmailov and M.S. Shreiber were the first to describe and use the method, in 1938, for separating drug alkaloids in a thin layer of aluminium oxide. TLC is one of the types of liquid chromatography and, depending on how the sorption surface retains the components separated, can be based on the principles of adsorption, distribution, ion-exchange, molecular-sieve and affinity. TLC's main qualitative characteristic is the R_f value, which is the speed of the given substance's movement across the plate. In assessing R_f values mentioned in publications one should bear in mind that they characterize the effectiveness of separation in a given chromatographic system, and that their reproducibility depends on many factors, including the nature of the sorbent, the layer thickness and the mode of its preparation, the degree of the chromatographic chamber's saturation with solvent vapours, the distance covered by the solvent front, the distance from the solvent's start to its level in the chamber, the sample quantity applied, the solvent in which the sample substance was applied to the chromatographic plate, and the purity of solvents used as the mobile phase.

Comparison of factors determining the resolving capacity of the sorbent indicates that R_f is more affected by the sorbent's graining than its temperature or humidity.

An analysis' sensitivity is primarily determined, and its selectivity enhanced by the choice of the processing reagent. In determining pesticides use is made of over 50 different processing reagents. Iodine, permanganic acid potassium and potassium bichromate solutions are the most common ones. For a detailed description of the techniques of preparing processing reagents, and examples of their uses in pesticide detection, the reader may be referred to specialized studies. It should be, however, stressed that the use of reagents with different reactive capacity enhances the possibilities of identifying both the original combinations and their transformation products. Separation effectiveness may be increased by the use of two-dimensional chromatography and multi-stage elution in unidimensional chromatography, or by the use of plates with sandwich layers of different sorbents. For instance, the use of layers consisting of a silica gel and kieselguhr has made possible successful separation of herbicides which are derivatives of phenoxyalkancarboxylic acids.

With thin-layers chromatograms, the principles of pesticide quantitative detection are the same as those for other combinations. Visual comparison of the size of the spot and the intensity if its colouring with standard solutions is most widespread. The findings reliability is enhanced by the use of scanning densitometers and fluorimeters. Errors in the use of the densitometric method in TLC are usually within the 5 to 8 per cent range, and depend on the sorbent layer's thickness and humidity, the presence of foreign substances, etc.

At present, a new trend is developing in thin-layer chromatography, notably high-efficiency thin-layer chromatography (HETLC). This method is characterized by a high resolution capacity and speed. Its high effectiveness is achieved by the use of sorbent with optimized characteristics, and of new chromatographic techniques. Micro-thin-layer chromatography (with sorbent particle diameter of 3 to 5 micromillimeters) has been used to detect DDT, its metabolic products and polychlorpinene, when present in combination.

Comparison of the effectiveness of TLC and GC has revealed a number of TLC advantages, since the latter is capable of discovering combinations unidentified by GC. This shows the promise of using TLC in combination with other methods for identifying samples substances and their metabolic products.

TLC is a promising method for detecting the presence of pesticide biopreparations in harvested crops and the environment. Studies using TLC for detecting pesticide residues in different objects increase in number from year to year.

The development and utilization of photometric methods was a significant contribution to the development of pesticide control. The main drawback of the photometric technique is its limited selectiveness, labour-intensity and insufficient sensitivity. Comparison of the photometric technique and GC in analyzing water, soil and vegetables containing residual herbicide amounts, revealed the advantages of the latter.

Atomic-absorption spectrometry is used mainly for detecting Hg-containing pesticides in food products and biological materials.

The area of polarography's utilization was substantially broadened with the emergence of oscillographic polarography. One merit of polarography is the possibility of detection regardless of the colour and turbidity of the solution, which means a significant simplification of sample preparation for quantitative detection. To determine certain derivatives of ethylenebiscithiocarbamic and dialkyldithiocarbamic acids and of their metabolic products, as well as of certain organophosphorus pesticides use is made of catalitic kinetic techniques characterized by high sensitivity and specificity.

In the USSR, standard methods, approved by the USSR Ministry of Public Health, are used for pesticide residue control. A brief description of the principles underlying the techniques for detection of the most broadly used pesticide groups is given below.

The standard methodology for organophosphorus pesticides identification in water, soil and food products of plant and animal origin is based on pesticide extraction by organic solvents (acetone, chloroform and others), extract purification by column chromatography or by re-distribution in a hexane-acetone systems. Fats and waxes precipitate from cold acetone. To rid thermally resistant pesticides (methylparathion, phenitrothion, dimetoate and others) of co-extractive substances, use is made of microsublimation in vacuum. Quantitative detection and identification of pesticide microquantities in performed by using the GC and TLC techniques. The job is done by selective detectors and columns of different polarity. In case of the TLC technique different detecting reagents are employed or use is made of the chromato-enzyme methods. The average determination value is 86-92 per cent for water, 72-108 per cent for plants, and 90 per cent and more - for animal-origin samples. The relative error in case of maximum permissible levels varies, depending on the properties of the preparation and the object analyzed: from 2 to 10 per cent limits for food products in the case of GLC; from 5 to 20 per cent fot TLC; from 3 to 5 per cent for water in the case of GLC, and from 3 to 12 per cent for TLC; from 6 to 14 per cent for soil in the case of GLC, and from 7 to 18 per cent for TLC.

The standard methodology for detecting organochloric pesticides (DDH, HCCH, celtan, heptachloride, methoxychlor, etc.) in food products, fodder and environmental objects is based on extracting preparation with hexane and acetone; extract purification by using NSA silica gel or with sulphuric acid with subsequent determination by the GLC technique using an electronic trapping detector on 5 per cent SE-30 columns, or by the TLC technique on plates with a layer of aluminium oxide or silica gel. The processing reagent is nitrate of silver. With the GLC technique, the lower determination limits are 0.0007-0.004 mg/kg and mg/l, and with the TLC methods, 0.05-0.5 mg/kg.

Detailed descriptions of methods for detecting residual pesticide amounts in food products and environmental objects can be found in methodological reference books.

References

1. "Methods for Determining Microquantities of Pesticides in Food-stuffs, Fodder and in the Natural Environment". Ed. by M.A.Klisenko, Moscow, Kolos Publishers, 1977, 868 pp. (in Russian).

2. M.A.Klisenko, L.G.Alexandrova "Determining Residual Quantities of Pesticides". K. Zdorovie, 1983, 248 pp.

A System of Post-Graduate Advanced Courses in the Toxicology and Hygiene of Pesticides

N.A. Popovich

Pesticides are employed to kill insects, mites, rodents, bacteria, viruses, fungi and weeds. Being highly potent biologically these chemicals may impair the vital activity not only of the organisms they are intended to control but, of warm-blooded animals and humans, as well. This can be regarded as the first specific feature of pesticides.

Their second peculiarity is that they are environmental pollutants whose impact cannot be effectively controlled; some of them circulate in the biosphere.

Concentrations of pesticides used in agriculture for killing pests sometimes turn out to be toxic for workmen, too. Toxic concentrations in the air are a necessity:lower concentrations will not kill pests. This is the third feature of pesticides.

Their fourth feature is associated with the exposure of large groups of people to pesticides owing to their circulation in the biosphere and the presence of their residues in foodstuffs and other media. Tee above-mentioned features of pesticides should be taken into account when exercising sanitary control over the application of pesticides and taking measures to protect human health and environment.

One of the main services supervising the application of pesticides is the public health service and, in particular, bodies of the sanitary epidemiologic service. All the activities related to control of pesticides and mineral fertilisers application can be divided into two stages, namely, preventive and current sanitary supervision.

Since the curricula of medical colleges and university departments of biological sciences do not provide adequate information on these questions, our aim is to organize postgraduate courses for specialists working in the public health system whose main line of duty lies in the supervision of pesticides utilization.

These courses are organized within the framework of the Kiev Institute for Advanced Training of Physicians and are run by the department of Pesticide Hygiene and Toxicology attached to the All-Union Research Institute for the Hygiene and Toxicology of Pesticides, Polymers and Plastics.

The curriculum of the "Toxicology and Hygiene of Pesticides" envisages three independent courses, namely, the hygiene of application and toxicology of pesticides, the analytical chemistry of pesticides, and the clinical toxicology of pesticides. The duration of instruction in each course is two months.

The course in the hygiene and toxicology of pesticides is intended mainly for training hygienists and toxicologists effecting control over commercial uses of pesticides.

A course in analytical chemistry of pesticides is intended for training analytical chemists and laboratory physicians engaged primarily in the public health system whose prime duty is to determine residual amounts of pesticides in various environments.

A course in clinical toxicology of pesticides is designed for training therapeutists, shop physicians, and physicians practicing in rural areas. This course is aimed at training in diagnosis and treatment of patients with acute and chronic pathology caused by different groups of pesticides.

Lectures, seminars, practical and laboratory classes are the principal methods of training. Every course ends with a final examination.

Curriculum of a course in toxicology and hygiene of pesticides consists of five sections, namely,

1. General toxicology of pesticides
2. Fundamentals of general hygiene of pesticides application
3. Specific problems of the toxicology and hygiene of pesticides application
4. Fundamentals of the analytical chemistry of pesticides
5. The clinical picture and treatment of pesticide poisoning.

1. General toxicology of pesticides

It outlines the goals and tasks of agricultural toxicology in relation to pesticides and describes the principal concepts such as toxicity, toxicity criteria used for pesticide evaluation. The trainees became acquainted with the main toxicometric methods, the metabolism of pesticides in the body of animals and humans.

6300 The training program covers in detail the combined effect of pesticides in farming and gives examples of antagonism and potentiation, describes methods of quantitative assessment of these effects and determination of the cumulative properties of combined preparations.

The trainees become familiarised with the methods used for the hygienic assessment of pesticides with long-term effects - teratogenic, blastomogenic, embryotoxic, gonadotoxic, mutagenic - as well as with the techniques for identifying these effects. The program envisages visits to laboratories where these effects are studied.

2. Fundamentals of the general hygienic principles of pesticides application

The program provides the characteristics and history of pesticides application as one of the new sections of hygiene, covering its tasks and methods, familiarises the trainees with various pesticide classifications, namely, occupational, hygienic, chemical, by long-term effects, as well as with the classifications suggested by foreign authors. The students learn methods of the correct application of hygienic classifications in their practical work.

It illuminates the importance of practical knowledge of the impacts of pesticides circulating in the biosphere on man's health, including, a possibility of some pesticides to form more toxic (has compared to the original) substances in the soil causing human poisoning.

The course discusses at lenght the principles of pesticide hygienic standardization and provides examples of calculations of the permissible daily doses of pesticides which pose no hazard for human health. It outlines the principles of establishing safe concentrations of pesticides in foodstuffs, the air of the working zone, the ambient air, water, and soil. The program describes the principle of establishing standards for the combined uses of pesticides. The trainees learn how to determine tentative safe exposure levels and their use in practice.

The trainees familiarise themselves with the documents used for monitoring pesticides application, the structure of the services engaged in utilizing pesticides, sanitary rules of pesticide storage and transport as well as with methods of supervising various units in the medical service. The trainees are supposed to exchange experience in work, and learn methods of propagation of health education.

3. Specific questions of toxicology and hygiene of pesticide application

This section of the course familiarises the students with the methods and ways of pesticides application. It discusses in detail spraying, dusting, use of aerosols, and seed treatment from the viewpoint of hygiene, i.e. possible effects on humans and the environment. It describes the machinery and apparatus, for various uses involving pesticides and the procedure of sanitary supervision over the above equipment.

Aerial spraying, its advantages and disadvantages, namely, the organisation and conducting of sanitary supervision in the preparatory period, in the period of spraying, and at the final stage, is given a special emphasis. The trainees learn the hygienic requirements which airfields and aricrafts used for aerial spraying must meet. An opportunity is given to examine airfield designs from the point of view of sanitary hygiene and to bocemo acquainted with the activities of a toxicological laboratory within the medical service of the USSR civil aviation.

The trainees are also to study protection of water bodies against pollution with pesticides, their pathways into water bodies, and aftereffects. Sanitary-hygienic, engineering and agrotechnical methods are suggested to protect water bodies from pesticide pollution. Attention is focused on the characteristics of water protection in conditions of irrigated farming.

In practical studies, the trainees go through the rules of collecting samples from open and underground water sources for analyzing pesticide content.

Sanitary protection of soils against pesticide contamination is dealt with. The pesticide pathways to the soil are defined and the laws governing migration of chemicals in the soil as a constituent of biosphere are presented. The trainees are supposed to master soil sampling methods under various conditions.

The course covers problems of monitoring the air pollution of the working zone and the ambient air. It considers the specific methods of air sampling for the analysis of pesticides, and the relevant equipment. It also describes the procedure of monitoring the contamination of workers' skin and protective clothing with pesticides and the measures, aimed at preventing pesticide pollution of the air.

The program analyses at lenght the causes of foodstuffs pesticide contamination and the counter-measures. The trainees learn the rules of foodstuff sampling, aimed at determining the residual quantities of pesticides. Practical situations concerning the utilization of products contaminated with pesticides over the permissible levels are discussed.

The program covers the characteristic features of the chemical treatment of forests and describes the hygienic measures, aimed at protecting the health of workers and holiday-makers and at preventing contamination of mushrooms, berries as well as grass used as fodder. The trainees become familiarised with the methods of determining the time when the pesticide-treated forests become safe for foresters, holiday-makers, and cattle grazing.

Similarly, the program deals with the problem of applying pesticides indoors. The trainees familiarise themselves with various types of greenhouses, labour conditions of green-house personnel, features of pesticide application under these conditions, analysis of disease incidence in green-house personnel, control over the safe "(back-to-work") concentrations following pesticide application, and workmen's individual protective devices. The program envisages a visit to a greenhouse complex in Kiev and its health centre.

The course covers at lenght the questions pertaining to the hygiene and toxicology of pesticides used in cattle breeding and poultry farming. It introduces the trainees to the hygienic standards of pesticides used to protect animals, the hygienic requirements to be met in treating livestock barns, sheds and poultry houses, and the measures aimed at preventing and reducing pesticide-caused contamination of products of cattle breading and poultry farming.

The program considers the hygiene and toxicology of disinfectants and provides the toxicological characteristics of major chemicals employed for seed treatment; discussed are the hygienic characteristics of working conditions while treating seeds, their storage and sowing; the trainees are familiarized with the seed-treating apparatus, visit the chemicalization facilities where they inspect seed treatment sites and storehouses for treated grain.

Besides, the program covers the practical aspects of the hygiene and toxicology of pesticides used for disinfection in public health. The trainees learn the rules of personal and public safety measures to be observed in disinfection. A visit to a disinfection station in Kiev is envisaged.

This section provides toxicological and hygienic characteristics of certain groups of pesticides according to the following scheme:

- general characteristic of a group, its purpose;
- chemical classification;
- physicochemical properties;
- residues in the environment;
- toxicity for the warm-blooded animals and man with different pathways of penetration;
- cumulative properties;
- toxicity in case of long-term exposure, late effects;

- mechanism of a toxic effect, metabolism;
- a clinical picture of poisoning and therapy principles;
- prevention of poisoning and other pathology.

According to the above scheme the material is presented as follows: organochlorine pesticides, organophosphorus pesticides, organomercuric compounds, inorganic pesticides (copper-, arsenic-, fluorine-, zinc-containing chemicals) herbicides (various chemical groups), mineral fertilizers, fumigants, biological preparations, and plant growth regulators.

Attention is given to pesticides detoxication. The trainees familiarise themselves with the rules governing detoxication of packing material, containers, workers' protective clothing, transport vehicles, machinery and apparatus intended for pesticide use. The requirements to the design of toxic chemicals burial grounds and activities related to pesticides dumping and bruning are discussed at lenght. Durint visits to chemicals-handling facilities the trainees become acquainted with the lay-out of sites where containers, equipment and motor vehicles are detoxicated.

A penetrating insight into the current methods of protecting workmen against pesticides is given. The program outlines the hygienic requirements for protective clothing and other individual protective devices, norms and order of their supply. Situation problems, related to the choice of protective devices, depending on the type of work to be performed and pesticide, are solved.

The section of the analytical chemistry of pesticides introduces the trainees to the principles of the current methods of analysis of the residual quantities of pesticides, namely, thin-layer chromatography, gas-liquid chromatography, photometry, polarography, and other methods. The trainees study the principles of operation of the main types of analytical equipment.

In the section, devoted to the clinical picture and treatment of pesticide poisonings the trainees become acquainted with the clinical picture of poisonings caused by the major groups of pesticides, the pathogenesis of poisonings, and a possible present-day antidote therapy. Pesticide intoxication cases are demonstrated.

A course in the analytical chemistry of pesticides

The trainees should master the methods employed to determine the microamounts of pesticides in the ambient media, namely, the air, soil, water, and foodstuffs.

The trainees are introduced to the theoretical foundations of the chemical analysis of pesticides, in particular, thin-layer, gas-liquid, column, paper and ion-exchange chromatography, photometry, and polarography. In the laboratory, the students master the methods of determining pesticides and solve situation problems in identification of pesticides.

The participants of the course become familiarised with the structure and operation of laboratories engaged in identifying the residual quantities of pesticides, as well as with instruction manuals.

A special section of the course presents the fundamentals of the hygiene and toxicology of pesticides with in the scope required for analytical chemists.

A course in the clinical toxicology of pesticides

This two-month course is intended for therapeutists working predominantly in rural areas as well as for other specialists. The program includes a course of lectures, practical classes, and seminars.

The lecture course presents current clinical data on intoxication caused by the effects of a variety of pesticide groups and covers the mechanisms responsibly for the effects of a particular pesticide upon a human body, the clinical picture of acute poisoning and chronic intoxication, differential diagnosis, laboratory diagnosis, combined treatment, preventive measures and working ability tests.

The program outlines the problems pertaining to pesticide toxicology and application hygiene, namely, general information on poisons, characteristics of their action upon the body, pesticide physicochemical properties and prophylactic measures against poisoning.

Practical studies include following pesticide poisoning cases; mastering the patients's examination procedure with due regard to peculiar lesions caused by chemicals; clinical examination and making rounds in the wards; pathologico-anatomic conferences; and visiting patients at home in response to toxicologic emergency calls.

Practical studies in laboratory diagnosis provide trainees with the essential methods of diagnosing functional insufficiency of internal organs, caused by pesticide intoxication; methods employed to determine blood cholinesterase content, investigation of pesticide levels in the body substrates; and the clinical interpretation of findings of these investigations.

The study course envisages seminars where the trainees discuss complex clinical cases and attend conferences to hear the reports of other students on topics closely related to the program and supplementing it.

Experience has shown that public health bodies encounter certain difficulties in making optimum hygienic decisions as regards the employment of pesticides in agriculture and whenever a necessary arises to identify pesticide-caused pathology in humans. The feed-back information, received by the advanced studies department from specialists who have completed their training course, indicates that the knowledge they gained helps them in finding more competent and efficient solutions of these problems.

In this respect special emphasis is placed on practical skills to be mastered by the trainees. A list of minimum skills on each study course is given below:

The toxicology and hygiene of pesticides

- Keeping of control documentation on pesticide applications;
- Analysis and examination of designation of designs of various chemicalization facilities;
- Methods of inspecting pesticide storehouses, sprayer sites, airfields, and other chemicalization facilities;

- Calculation of permissible daily doses of pesticides for humans;
- Calculation of tentative safe levels of exposure to airborne and waterborne pesticides;
- Methods of studiung labour conditions during chemical treatment of plants, seeds treatment, fumigation and other chemical operations;
- Methods of assessing foodstuffs contaminated with pesticides;
- Methods of collecting samples from the air, water, soil, and foodstuffs to test them for the residual quantities of pesticides;
- Selection of individual protective devices for persons handling pesticides;
- Supervision of dumping and burial of pesticides;
- Calculation of pesticide toxicity parameters;
- Carrying out of toxicological experiments;
- Medical aid in cases of pesticide poisoning.

The analytical chemistry of pesticides

- Methods of preparing sorption masses;
- Purification of pesticide-containing extracts;
- Applying samples on chromatographic plates;
- Spectrophotometric determination of triazines;
- Thin-lzyer chromatographic determination of organophosphorus pesticides, and other chromatographic methods;
- Determination of organochlorine pesticides in a variety of media;

- Methods of obtaining chemically pure pesticides from commercial products;
- Analysis of organomercuric pesticides;
- Thiocarbamate analysis;
- Methylthiocarbamate analysis;
- Dinitro compound analysis.

<u>The clinical toxicology of pesticides</u>

- Collection of occupational "chemical" and "allergological" histories;
- Mastering the methods of rendering urgent medical aid in cases of pesticide poisonings, namely, recovery of the respiratory and cardiovascular system functions, augmented diuresis, gastric lavage for the unconscious patient;
- Intracardiac injection of medicines;
- Collection and assessment of scarified and intracutaneous diagnostic samples using exogenous and endogenous allergens;
- Clinical interpretation of blood counts in order to make a differential diagnosis of various intoxications;
- Clinical interpretation of urinalysis in order to make a differential diagnosis of various intoxications;
- Clinical interpretation of hepatic function tests in case of pesticide intoxications;
- Determination of cholinesterase in the blood.

Accumulation of the latest scientific data and changes in the assortment of pesticides utilised in the country necessitate revision of the curricula of postgraduate study courses in toxicology and hygiene of pesticides every five years.

PREVENTIVE TOXICOLOGY OF PESTICIDES IN HOT CLIMATE CONDITIONS AS RELATED TO COTTON-GROWING

T.I. Iskandarov

In contrast to the application of pesticides in areas with a temperate climate of the Soviet Union, their utilization in the cotton-growing areas of Uzbekistan is influenced by the following specific features: hot climate, location of population centers in the vicinity of cotton fields, irrigated farming, water supply in rural areas primarily from open water bodies, a frequent rate of treatment, a wide range of chemicals used, etc.

From April through September the operations on cotton plantations in the open air are carried out under the conditions of high temperature, low humidity and strong solar radiation.

Great amounts of heat and sunshine, in combination with a variety of soil types and irrigation, provide favourable conditions for cotton growing but, on the other hand, they are also favourable for reproduction of various pests which cause a great deal of harm to cotton plants.

Throughout the year cotton fields are repeatedly treated with pesticides, and fertilizers are put into the soil. As compared to other agricultural plants, the production of this most valuable technical crop requires the greatest utilization of pesticides.

The initial step in the entire package of agrotechnical operations, related to the application of chemical protection agents, involves controlling pathogens, cotton seed and sprout parts by presowing treatment.

Dry, semihumid and humid (suspension application) methods may be employed to treat cotton seeds. A method to use depends on the chemical composition of disinfectants, seed preparation (linters or delinters), degree of disease and pathogen development, and capacities of seed-treating machines.

The persons who permanently work with disinfectants are to be examined once a year by a therapeutists, neuropathologist, ophthalmologist and given laboratory tests (roentgenology, blood count, and urine test). Those who have passed medical examination are given an appropriate certificate.

Children and teenagers younger than 18, pregnant and lactating women, persons having contraindications, and men older than 55 and women older than 50 are not to be admitted to this job.

To control cotton pests, seed treatment is followed by tractor or aerial spraying of pesticides. In the tractor method a variety of sprayers and dusters are used, namely, OTH-8-16, OBT-1, OBX-14 and OBX-28.

We have conducted a comparative test on the hygienic aspects of application of phosphamide, formathion, butyphos and methyl mercaptophos in the tractor and aerial techniques used within two periods; viz. April through May with an air temperature of 15-25°C, and July through August with a higher air temperature of 27-30°C.

It has been found that application of all the above-mentioned pesticides, whether it is a tractor or aerial spraying, whether the air temperature is optimal or high, gives rise to contamination of the air in the working zone, the skin, preventive clothing and such environmental media as the air, soil, and vegetation.

A variety of factors are responsible for the degree of contamination. Due to their volatility, methyl mercaptophos and butyphos contribute more than other chemicals to the environmental pollution.

However, a comparative investigation of the degree of air pollution in case of the aerial and ground methods has shown that an aerial spraying contributes more to the pollution, both in terms of its intensity and the distance involved. On the day of treatment the concentrations of chemicals in the air at a distance of 700-1,000 m exceed the one-time maximum permissible concentration.

It should be noted that an aerial spraying results in smaller levels of pesticides in the soil, cotton plant leaves as compared with the treatment by tractor. This is due to the fact that during an aerial spraying a considerable amount of the chemical used never reaches the cotton plants and enters the ambient air.

Proceeding from the foregoing, we believe that from the viewpoint of general hygiene and with due regard for a specific nature of pesticide application in conditions of Uzbekistan, an aerial spraying of organophosphorous pesticides is less acceptable than the treatment by tractor. Besides, it is prohibited to use organophosphorous chemicals (OPC) in densely populated areas.

Comparisons of the results obtained at the optimal and higher temperatures shows that a high air temperature on the day of treatment gives rise to increased pollution of the environmental media (the air of the working zone, ambient air, water, etc.) but on the other hand the levels of chemicals in the environment drop rapidly. Therefore, a negative feature of high temperature is a stronger pollution of the environment, the ambient air in particular, while its positive feature is associated with a quick breakdown of the chemical thereby decreasing the "waiting time".

On the basis of the results obtained, we built concentration curves of the chemicals under study as a function of the air temperature and a distance from the treated field. Based on these curves, a chemical concentration under certain parameters may be determined as follows:

$$\Psi = A \frac{t}{l} + C,$$

where:

t - temperature, °C;

l - distance from the treated field, meters;

A - proportionality factor;

C - transient term of the equation

We have determined a mathematical dependence of concentration of methyl mercaptophos, butyphos, phosphamide, formathion in the air depending on the mode of application, time of treatment, air temperature, and a distance from the treated field. Table 1 lists the formulae derived.

These formulae are of a certain theoretical and practical significance, for they have enabled us to draw up nomograms. Using these nomograms, no preliminary calculation is needed to predict the air pollution under specific conditions, viz. the application technique, treatment time, air temperature, and a distance from the treated field.

Based on this formulae, we have identified valid sanitary-protection zones for methyl mercaptophos, butyphos, phosphamide, formathion, their values being dependent on the application method and air temperature. We have performed calculations in the following way.

Knowing that a mathematical empirical dependence of concentration of butyphos on the day of a tractor treatment on the air temperature and a distance from the treated fields, is expressed as $\Psi = 2.302 \frac{t}{l} - 0.035$, while the maximum one-time permissible air concentration of butyphos at the temperature of $20 \pm 2°C$ equals 0.01 mg/m^3, one can determine an estimated sanitary-protection zone (SPZ):

$$SPZ = \frac{2.302 \cdot t}{MAC + 0.035} = \frac{2.302 \cdot 20}{0.01 + 0.035} = 1.000 \text{ m}$$

Using this method, we have calculated a sanitary protection zone for MMP, phosphamide and formathion both at the air temperature of 20 and 30°C. Our experimental and field findings suggest that pesticides may be applied provided the air temperature does not exceed 30°C, for in a reverse case an increased pesticide toxicity and adverse changes in man's thermoregulation may take place.

An analysis of the data related to SPZ, indicates that in conditions of Uzbekistan, when a tractor is used to apply organophosphorous pesticides, a sanitary protection zone ranges from 1,000 to 1,500 m, whereas in case of an aerial spraying it is 3,000-6,000 m. These findings are another testimony to the fact that an aerial spraying contributes to the air pollution much more than a tractor treatment.

We believe that a strict compliance with the sanitary protection zones, depending on the method of application and the chemical used, is essential to the protection of the air in populated area where pesticides are extensively utilized.

We believe that tractors alone should be used in cotton-growing to apply OPC pesticides. To this end, the tractor driver's labour conditions should be improved.

In a hot climate, the only way to ensure favourable working conditions for the tractor driver is to provide him with an air-conditioned pressurized cabin, free of dust and pesticides.

The inadequacy of present available preventive working clothing is an outstanding problem in protecting the health of those who work with pesticides in conditions of a hot climate. The fact that the available preventive clothings are not used properly accounts for a large proportion of acute poisonings of farmers.

In cooperation with the Tashkent branch of the Central Tailoring Industry Research Institute and Tashkent Polytechnical Institute we have developed the following types of preventive clothing:

6300

Table 1

Mathematical Empirical Dependence of Pesticides Concentration (mg/m^3) in the Air Upon the Treatment Time, Air Temperature (t), a distance from the Treated Field (1) and an Application Method

Chemical	Treatment time			
	1st treatment day	2d treatment day	3d treatment day	4th treatment day
	Aerial spraying			
1. Methylmercaptophos	$4.560 - \frac{t}{l} - 0.021$	$2.730 - \frac{t}{l} - 0.034$	$1.388 - \frac{t}{l} - 0.014$	$0.563 - \frac{t}{l} - 0.006$
2. Butyphos	$4.090 - \frac{t}{l} - 0.021$	$2.120 - \frac{t}{l} - 0.016$	$1.105 - \frac{t}{l} - 0.016$	$0.680 - \frac{t}{l} - 0.021$
3. Anthio	$3.021 - \frac{t}{l} - 0.034$	$1.537 - \frac{t}{l} - 0.017$	$0.902 - \frac{t}{l} - 0.018$	$0.416 - \frac{t}{l} - 0.016$
4. Rogor	$2.930 - \frac{t}{l} - 0.027$	$2.041 - \frac{t}{l} - 0.025$	$0.642 - \frac{t}{l} - 0.007$	$0.213 - \frac{t}{l} - 0.009$
	Treatment by tractor			
1. Methylmercaptophos	$4.200 - \frac{t}{l} - 0.126$	$0.860 - \frac{t}{l} - 0.006$	$0.985 - \frac{t}{l} - 0.037$	
2. Butyphos	$2.302 - \frac{t}{l} - 0.035$	$0.712 - \frac{t}{l} - 0.006$	$0.313 - \frac{t}{l} - 0.002$	
3. Anthio	$1.725 - \frac{t}{l} - 0.030$	$0.872 - \frac{t}{l} - 0.009$	$0.557 - \frac{t}{l} - 0.011$	
4. Rogor	$2.504 - \frac{t}{l} - 0.063$	$1.213 - \frac{t}{l} - 0.032$	$0.350 - \frac{t}{l} - 0.004$	

Table 2

Sanitary-Protection Zone During Pesticide Applications As a Function of Air Temperature, Mode of Application and Type of Chemical

Chemical	Treatment by tractor		Treatment by aircraft	
	20°C	30°C	20°C	30°C
Butyphos	1,000	1,530	2,630	3,960
Phosphamide	760	1,140	1,950	2,950
Formathion	770	1,150	1,200	1,820
Methylmercaptophos	1,260	1,600	4,200	6,300

1. An insulated pneumatic overall fed with a cooled dust- and gas-free air; it is intended for those who work with highly toxic and volatile pesticides (methyl mercaptophos, thiophos, butyphos, etc.) for servicemen in particular.
2. Preventive clothing (overalls with a helmet, cope and underwear) for people exposed to pesticides, viz. tractor drivers, signalers, cottonseed treatment workers, toxic chemical storage personnel, etc.).
3. A set of preventive clothing (trousers, a blouse, hat and underwear) intended for those farmers who are indirectly exposed to pesticides, namely, agronomists, vehicle drivers, transporting pesticides, agronomist-entomologists, spriklers, mechanics, technicians, choppers, watchmen, forecasters, etc.

The question of the combined effects of a high temperature and pesticides is of great theoretical and practical significance, for these effects may induce reactions which would be somewhat different from those observed in the body in case of isolated effects.

We have studied combined effects of a high air temperature and certain pesticides when the chemicals were absorbed by ingestion.

1,310 white mice and 714 albino rats were used to determine pesticide toxicity parameters at an optimal (20°C) and high (35°C) temperatures. V. Prozorovsky's (1962) test technique was used to calculate the mean lethal dose.

To determine toxicity parameters at a high temperature, intragastric administration of the chemical was followed by placing the test animals, for 14 days, 4 hours a day, in a thermal chamber, its air temperature being 35 ±1°C and a total volume of 750 l. The air warmed up by an air heater was fed into the chamber. In an acute experiment the animals were kept in a thermal chamber for four hours, while in a chronic experiment, for two hours.

With reference to methylmercatophos, butyphos, kilval, formathion, milbex, HCCH gamma-isomer, monuron, and sodium fluosilicate, the acute toxicity parameters were determined for white mice and albino rats at optimal and high temperatures (Table 3). Comparisons of the DL_{50} for each chemical at the above-mentioned temperatures reveals that at a high temperature their toxic effects increase 1.5 - 2-fold for white mice and albino rats alike.

Apart from the acute toxicity parameters at optimal and high air temperature for a number of pesticides, we also studied some aspects of the mechanism of combined effects of a high air temperature and certain organophosphorous pesticides, viz. methylmercaptophos, butyphos, and formathion, at a variety of doses in acute and chronic experiments.

A four-month chronic experiment, involving 28 groups of rabbits, was conducted to study the combined effect of a high temperature (30, 35 and 40°C) and a some pesticides (methylmercaptophos, butyphos, and formathion) in doses of 0.1 and 1.0 mg/kg. Following daily intragastric formathion administrations the rabbits were placed in a thermal chamber (20, 30, 35 and 40°C) for a 2-hour exposure. We investigated the indicators available before the exposure, those obtained in dynamics following 10, 20, 40, 60, 80 and 100 administrations of the chemical, and those resultant one month after the exposure completion (recovery period).

There we studied intoxication symptoms, body weight, rectal temperature, activity of the enzyme of cholinesterase of the whole blood, erythrocytes, serum and internal organs according to Khestrin; total protein levels and percentage of blood protein fractions; blood morphological composition; transminase activity of catalase; potassium and sodium levels in plasma, erythrocytes and in such organs as muscles, skin, liver, and kidneys; calcium and potassium levels in plasma; inorganic phosphorus levels in the blood plasma; chloride levels in plasma; total water in tissues, and butyphos contents in organs.

An analysis of the findings reveals: at a high temperature the chemicals raise body and rectal temperatures; moisture losses

Table 3

Comparative Toxicity (DL_{50}) at Optimal and High Temperatures of Some Pesticides for Albino Rats and Mice During a Single Administration (By Active Principle in mg/kg)

Nos	Chemical	Optimal temperature	High temperature
		Albino rats	
1.	Methylmercaptophos	164 (142 + 186)	91 (71 + 111)
2.	Butyphos	288 (196 + 260)	113 (87 + 139)
3.	Kilval	114 (102 + 127)	79 (63 + 95)
4.	Formathion	310 (266 + 354)	193 (151 + 235)
5.	Milbex	1,200 (1,050+1,350)	680 (508 + 852)
6.	HCCH gamma-isomer	290 (244 + 336)	168 (136 + 200)
7.	Monuron	2,350 (2,086+2,604)	1,410(1,098+1,722)
8.	Sodium fluosilicate	158 (132 + 184)	104 (84 + 124)
		White mice	
1.	Methylmercaptophos	76 (70 + 82)	53 (47 + 59)
2.	Butyphos	145 (127 + 163)	81 (69 + 93)
3.	Kilval	42 (43 + 49)	39 (25 + 53)
4.	Formathion	195 (172 + 218)	93 (77 + 109)
5.	Milbex	950 (787 + 1,113)	445 (369 + 521)
6.	HCCH gamma-isomer	330 (274 + 386)	205 (175 + 235)
7.	Monuron	2,500(2,405+2,595)	1,240(1,116 + 1,364)
8.	Sodium fluosilicate	50 (32 + 68)	37 (31 + 43)

increase; the cholinesterase activity is not markedly affected; a deficiency of potassium in erythrocytes develops with an increase in its level in plasma; the ratio of erythrocyte potassium to plasma potassium sharply decreases. The latter suggests that intracellular potassium enters the extracellular space, which seems to result from the impaired permeability of the cell membranes.

A high temperature contributes to the development of the cholinesterase enzyme activity, while formathion and butyphos inhibit it; the combined effect brings about inconsiderable alterations in the enzyme activity.

Such organs as the liver, brain, lungs, and kidneys exhibit greater amounts of butyphos against those observed under the conditions of an optimal temperature. This can be accounted for by an increased vessel permeability and circulation due to a high temperature as well as by lipoidotropism of organophosphorous pesticides.

The mineral composition of the blood and tissues of the water-salt-storing organs show significant alterations. At an optimal temperature some shifts have been reported at high doses, whereas at a higher temperature, these changes resulted with all test doses. The muscles, liver, and skin have exhibited greatly reduced potassium and increased sodium. Since potassium is vital to the neuromuscular system functions and contributes to the transfer of stimulation through the nerve, its deficiency in the muscles points to some pathological deviations in the body.

Potassium ions contribute both to the formation of a mediator, acetylcholine on the nerve endings, and to the formation of the inverted tissue appropriate response to the mediator action. The increased activity of cholinesterase in response to a high temperature may be accounted for by potassium deficiency in the body, which gives rise to decreased acetylcholine. Therefore, the combined effect of a high temperature and butyphos triggers an antagonistic impact on the cholinesterase activity, namely, butyphos inhibits it whereas a high temperature increases it, i.e. decreases the level of acetylcholine.

In a chronic experiment we studied the combined effect of a high temperature (30, 35, 40°) and certain pesticides (methyl mercaptophos, butyphos, formathion) in the doses of 0.1 and 1.0 mg/kg. In so doing we investigated the same indicators as in the acute experiment.

At a high temperature, rabbits and rats exhibited an increased body temperature. Rats and rabbits took 30 and 40 days respectively to adapt to a high temperature. The electrolytic composition of the blood and tissues revealed changes characteristic of the expedient redistribution, essential to the formation of adaptation processes. The influence of a high temperature on the cholinesterase activity in a chronic experiment is different from the one observed in acute exposure. At the initial stage of the experiment the cholinesterase activity increased, whereas by the ene of the experiment it was drastically inhibited.

An analysis of the data related to the effects of a high temperature on some indicators of the water-salt exchange and biochemical tests has revealed that the changes observed are dependent on temperature. The higher the temperature, the more pronounced are the changes.

A combined exposure to pesticides (methylmercaptophos, butyphos, formathion) and a high temperature have brought about more pronounced shifts in cholinesterase inhibition and the electrolytic composition of the biosubstrates analyzed than those observed at the same dose under optimal temperature conditions.

An analysis of the findings has shown that a combined exposure to the temperature of 30°C and the chemical in a dose of 0.1 mg/kg is responsible for the most minimal changes. Methylmercaptophos and butyphos have been found to be the most toxic compounds.

The results of investigating the test animals' blood electrolytic composition have demonstrated that the serum exhibits hyperkalemia in combination with potassium deficiency in erythrocytes, the effects being dependent on chemical, dose, and temperature value. A combined exposure has revealed earlier and more marked changes against exposure to chemicals at an optimal temperature. Such potassium redistribution seems to be indicative of the increasing permeability of the erythrocyte membranes.

Being abundant in erythrocytes, potassium actively participates in their respiratory function. Potassium is present in erythrocytes as a salt of oxyhemoglobin. Owing to a low partial pressure of oxygen in the capillaries, this salt dissociates into hemoglobin, oxygen, and potassium. Oxygen enters the cells and takes part in oxidative processes, while a liberated potass-

6300

ium cation reacts with a carbonic acid anion to form bicarbonate. Therefore, the deficiency of potassium in erythrocytes is undoubtedly a pathological sign.

Changes have been found in the levels of sodium in the blood plasma and erythrocytes: the blood plasma has shown reduced amounts of the chemical, while increased levels have been found in the erythrocytes.

The plasma also exhibits changes in the levels of magnesium. A more pronounced increase in magnesium is observed under high temperature conditions. Magnesium largely participates in the body metabolical and immunobiological reactions to adapt to new conditions. Increased magnesium in the blood plasma of the butyphos-exposed animals evidently points to the participation of the chemical in the body immunogenesis and adaptation reactions.

Examination of the electrolytic composition of the tissues of the water-salt-storing organs (liver, muscles, kidneys, skin) has disclosed that exposure to the analyzed chemicals gives rise to calcium deficiency in the muscles, liver, and, to a lesser degree, in the kidneys and skin. The joint effect of the chemicals and a high temperature is associated with a stable progressing deficiency of potassium in the organs throughout the experiment. The levels of sodium have been found to be quite high against the background of a considerable deficiency of potassium. Decreased potassium in its vital storing organs and erythrocytes in combination with its increased levels in the blood plasma suggest a loss of intracellular potassium and its entry into the blood, from where it is intensely eliminated by the kidneys.

Potassium is a cortical and parasympathetic stimulant. It takes part in the synthesis of acetylcholine and destruction of cholinesterase and affects the transfer of the nervous impulse through potassium polarization and depolarization.

Therefore, a high temperature is capable of altering the status of the body as a biological entity, especially following the impairment of the central nervous system regulatory mechanism. Considerable functional changes are, sometimes, due to new conditions for the resorption, distribution and elimination of the pesticide and its effect proper. In other words, environmental temperature conditions do not immediately influence pesticides action but first induce certain functional changes and, consequently, turn into internal conditions of this action, as it were. The effects of overheating on the body sensitivity to pesticides and the effects of intoxication on its resistance to the effect of a high temperature have, in the final analysis, been found to be equally involved.

TOXICOLOGY OF POLYMER MATERIALS

V.B. Danilov

Progress in science and engineering involves constant development and improvement of new materials and equipment which have to meet increasingly stringent standards.

Many natural materials, including metals and alloys, gradually give way to synthetic materials, for they no longer satisfy demands of modern production. This has stimulated a rapid growth of the production of polymers and plastics and their broad introduction into all shperes of human activity.

As other chemical compounds, polymer materials and pastics, are biologically active and, therefore the problem of their possible adverse effects on the human being calls for preventive measures to ensure optimum industrial and domestic conditions.

Synthesis of new polymer materials, the development of corresponding technological processes and equipment and their use in different fields of national economy call for a comprehensive toxicological assessment of the product from the laboratory stage to that of its practical application.

Polymers are high-molecular compounds whose molecules consist of numerous identical chemical groups. Plastics are obtained from synthetic resins; they may have simple composition when they include only resin or a complicated one. Complex plastics consist of a resin base and binders, filters (sawdust, carbon black, cloth, andesite, cellulose, asbestos), which enchance mechanical strength, plasticizers which represent organic liquids imparting high plasticity to the material (oils, dibutylphthalate), dyes, stabilizers, and other compounds. Plastics obtained from phenolmelamine, ureaformaldehyde, furan, and other resins are classified as complex ones.

Several classifications of plastics that were suggested are based on different principles (similar properties of composition, methods of preparation, and so on).

Exposure of workers both to the final polymer products and to diverse chemical compounds released from them may, under certain conditions, result in acute poisoning and occupational diseases. Latent period and severity of poisoning depend on the degree of toxicity, mechanism of the biological effect and nature of the combined effect, as well as on the dose of substances entering the body.

The up-to-date knowledge indicates conclusively that standartization of technological processes, use of similar equipment and, consequently, in the case of polymers of the same group, identical sources of chemical compounds release, lead to a relatively constant (qualitative and quantitative) composition of vapour-gase and vapour-gas-aerosol mixtures in conditions of polymer production and their application. This makes it possible to classify such mixtures and to substantiate their hygienic ratings.

Soviet toxicologists have suggested to divide the mixtures most often found in the air of the working zone, into 4 groups.

The first group comprises mixtures consisting of the initial, intermediate, and final products, i.e. mixtures whose composition at different technological stages of the same industrial production remains largely unchanged but the quantitative compositions of the components vary greatly.

The second group includes mixtures containing compounds which contaminate the initial raw materials, non-reacted initial and side-products.

The third group contains mixtures of compounds formed either upon hydrolytic decomposition of the main product or as a result of chemical reactions in the air between the released compounds. The group also includes vapour-gas-aerosol mixtures formed during thermooxidative destruction of synthetic materials.

Mixtures of the fourth group are composed of products similar in chemical composition (such as gasoline, kerosene, alcohols, and the like).

Depending on the physical and chemical properties of synthetic products (including polymers and plastics) as well as on the conditions of their production and application, complex vapour-gas and vapour-gas-aerosol mixtures (to a smaller extent) are largely included in the first three groups, and therefore hygienic ratings of their content in the air of the working zone has its own features in each specific case. Hence, to prevent an adverse effect of chemical compounds on the workers in conditions of production and application of synthetic materials, a comprehensive study of their combined effect is required.

At present the mathematical methods of arriving at hygienic ratings of complex vapour-gas and vapour-gas-aerosol mixtures have been developed so as to take into account concrete manner of combinations of chemical compounds, chemical and physico-chemical interaction between them in the air as well as the combined effect of poisons on the basis of their biological effects and quantitative characteristics.

Thus, for instance, to regulate air pollution with chemimical mixtures of a relatively stable but not completely studied composition, it is recommended to establish hygienic standards with respect to at least two components: the one which determines the clinical picture of intoxication and the other pointing to the source of release of the whole mixture. At the same time the maximum permissible concentrations, recommended for individual effects of each of the components, must be adjusted (with due regard for their combined action) with other mixture composites.

Such a procedure is used for regulating the content in the air of the working zone of volatile components and products of thermooxidative destruction of polymer materials and inorganic and synthetic lubricants, combustion products of organic compounds, vapour-gas-air mixtures containing initial, intermediate, and final products (the first group of pollutants), as well as the content of mixtures of compounds formed when by-products and impurities of the final product (the second group of pollutants) pollute the air of the working zone.

The most important stage is the assessment of the combined effect of compounds and the choice of the main component. Sometimes, for the rating of complex vapour-gas-air mixtures and for the choice of the main ingradients, it is suggested to resort to multiple correlation analysis, to establish the presence of absence of correlations between the concentrations of substances in the mixture and their toxic effect. It is recommended to accept such components of the compositions under study as the leading ones in which the density of the mentioned correlations is the greatest.

The method of partial regression which characterizes the combined joint effect of multicomponent mixtures at different exposure levels and helps in the objective identification of the most hazardous components is rather promising for the assessment of complex steam-gas-air mixtures.

The use of this method makes it possible to control air pollution with complex mixtures not only by the character, but also by most haradous component whose absolute toxicity (determined by the regression coefficient value) proves to be the greatest in comparison with the other composition ingredients.

Studies of sensitizing properties of synthetic materials and their components is a pressing problem of toxicology, since, as has been shown, in some cases this pathology the leading in exposure to polymers and plastics.

For many years the experimental and clinical investigations in this field concentrated on solving the principle problem whether the allergenic effect of chemical compounds could be classified as a threshold one or whether the role of individual sensitivity towards them was so high that the threshold allergenic effect (Lim_{al}) could not be found.

Studies of the incidence and the express on degree of allergic reactions in quinea pigs administered various chemical compounds to them epucutaneously and by inhalation have shown that allergenic effect could be referred to the threshold one. Minimum doses do not cause any allergic processes.

Sanitary and chemical study of a polymer material is an important stage in the assessment of its hygienic properties. These studies characterize qualitatively and quantitatively the chemical mixtures released into the air from the polymer. The results obtained usually help the researcher to plan further investigations and sometimes show whether the material can be permitted to be used in industry.

The data of sanitary and chemical studies of polymer materials can be expressed as three main indices:

1) milligrams of the substance in 1 m^3 of air (mg/m^3);

2) milligrams of the substance released from 1 m^2 of the material surface for 1 hour, $mg/m^2/hr$ the amount characterizing the intensity or release rate of hazardous substances;

3) milligrams of the substance released in the processing of polymer material per kg of the material being processed.

The indices are used depending on the aims of the investigation. The first one finds application in theoretical investigations, for instance, in studying the release of harmful substances from polymer materials under the action of various factors. The second index is required for hygienic assessments of polymer materials which are to be used in building. The third index is suitable for hygienic assessments of polymer materials processed into articles (items) under industrial conditions in order to find the released amount of hazardous substances depending on the efficiency function of the equipment.

The indices are interrelated since concentration, as a rule, is a function of the intensity of the harmful substance release. In some cases, however, the relationship between these two parameters is more complicated.

The list of hazardous substances capable of being released from the polymer under study is drawn up on the basis of the formulation of the polymer material and technology of its preparation.

Sanitary and chemical assessment of polymers has to be preceeded by a thorough investigation of physico-chemical properties and, if necessary, the development of methods of determining chemical substances in the air. Besides, the technological process and the conditions of application in industry must also be considered, since the choice of tactics in sanitary and chemical investigation can largely depend on them (the volume of the generator chamber, temperature, pressure, the weighted sample, surface area of the polymer, and so on).

Performing sanitary and chemical investigations, one should take into account the time elapsed after polymers preparation. Laboratory studies of polymers encounter considerable difficulties: it is not always possible to simulate industrial conditions of plastics processing (high pressure, high temperature, and others) and also to design special experimental equipment.

The problem is also complicated by the fact that, when polymers are processed at high temperature and pressure, not only the initial and composite volatile compounds can be released into the air but also desturction products sometimes of unknown composition. Nevertheless, sanitary and chemical investigations allow to prognosticate the release of chemical compounds into the air of the working zone under the production conditions, to choose compounds for toxicological assessment, and, in some cases, to introduce polymers into industry without special hygienic and toxicological tests. Besides, the data of these investigations may serve as a starting point in designing v ntilation systems, estimating the space of industrial premises and other parameters.

The hazard and toxicity of polymer materials introduced into industry must be assessed in two stages.

The first stage is a preliminary assessment of a possibility of introducing the particular synthetic material in industry. It has to include:

1. The assessment of hazard and toxicity after a single intragastric administration and epicutaneous application to experimental animals and, if according to sanitary and chemical research there is a leading volatile component, after an inhalation exposure.

2. The performance of subacute and acute experiments to reveal cutaneous-resorptive effect and cumulative properties of the compound regarding it as an integral chemical substance.

3. Hygienic testing under conditions of semi-industrial production and application.

The second stage is a final assessment largely depending on the results of preliminary evatuation. If the sanitary and chemical investigations show the mixture of air-released volatile components to contain substances of known hazard and toxicity it is apparently sufficient to study only the character of their combined effect on experimental animals.

If the volatile components contain substances of unknown hazard and toxicity, investigations are called for to substantiate the maximum permissible concentrations in the air of the working zone for each of them, and then to study their combined effect with consideration to the possible long-term effects on the body.

A possibility of long-term after-effects resulting from exposure to various chemical compounds attracts ever more growing attention of medical scientists. Of special importance are problems related to various disturbances of the reproductive function. Researchers' attention has been drawn to increased incidence of sterile marriages and to the birth of maldeveloped children.

Pertinent literature data show the problem of a possible effect of chemical agents on heredity to shift ever more from pure academical interest to practical field. Scientists all over the world call for an urgent study of possible effects of all newly synthesized chemicals on the reproductive function, and for testing the known, especially the widespread, ones.

It becomes clear from the above-said that, in considering the effect of industrial poisons on the specific female body functions, especially on the reproductive function, complex hygienic, clinical, statistical, and experimental investigations with a thorough analysis and comparison of the results are necessary.

Among the compounds exerting teratogenic and embryotoxic effects there are various chemicals such as benzene, phenol and its derivatives, formaldehyde, organotin, and other compounds entering into the composition of polymers.

Chemical mutagens contained in polymer materials include: acrolein, hexamethylenediamine, isopropylbenzene hydroperoxide, dimethylamine, dimethylformamide, diethylamine, cresol propylene oxide propyleneglycol, urethane, phenol, formamide, formaldehyde, epichlorohydrin, ethyleneglycol, ethyleneamine. A high degree of correlation has been established between mutagenic and allergenic properties of chemical compounds. In some cases toxic properties are known to be directly opposite to mutagenic and modification ones pointing to the individual mechanisms of action.

Study of mutagenic effects may sometimes help in uncovering the causes for embryonic malformations.

Blastomogenic effect of chemicals is of great hygienic importance. The following carcinogenic compounds enter into the composition of polymeric materials: polycyclic hydrocarbons, oxycompounds (in particular, peroxides and hydroperoxides) epoxides, diethyleneglycol, and others.

Toxicological evaluation of the combined effect of gases and aerosols is a complex problem. Literature on the subject has accumulated a few references concerning the body responses to the combined impact of various gas and aerosol combinations. This, however, allows so far to map out only the tentative approaches to prognostication of the toxic effect of such compositions.

Thus, standardization of chemical compounds which are or may be toxic and cause long-term after-effects is complicated by various factors affecting people both at home and outdoors. They include compounds which are released from building polymeric materials in new houses, plastics water mains, as well as released from clothes, shoes, various households pastes, toys, packing materials for foodstuffs, and so on. The majority of researchers believe that hygienic assessment of polymeric materials must be based on the following aspects.

The polymeric materials components which do not migrate into the environment are not hazardous. The components released from polymeric materials are harmful only when their hazard has been confirmed by toxicological investigations. If no toxicity of a substance has been revealed, it must be considered safe. Experiments on the release of chemical compounds from polymeric materials must simulate the most hazardous conditions of operation.

It should be noted that with increasing concentrations many chemicals released from polymers may change the organoleptic properties of air, water, and foodstuffs, altering their taste and smell. Hence, a change in organoleptic properties may be used as a criterion of the acceptability of polymeric materials.

Permissible levels (PL) of harmful substances released into air and water should serve as one of the main indices of acceptability of polymers. These levels have to be constantly reviewed as new data on biological effect of polymers accumulate and production technology improves.

PL of harmful substances released from construction materials are such concentrations of chemicals which cause neither pathological changes and diseases nor physiological changes of compensatatory-adaptive shifts at life-long daily around-the-clock exposure.

In conclusion we wish to emphasize that combined investigations of technologists and hygienists brought about favourable results: a number of effective principles and methods have been developed to reduce toxicity of synthetic polymers materials. If physico-chemical properties of raw materials and their biological effect are taken into account and optimum production procedure is used, the creation of polymers with specified properties becomes a reality.

PREVENTIVE TOXICOLOGY OF ECOSYSTEMS

J.H.KOEMAN

Department of Toxicology, Agricultural University,
The Netherlands

INTRODUCTION

The use of pesticides in agriculture and vector control may give rise to undesirable effects on non-target organisms. Whether undesirable side-effects are likely to occur depends on a large number of factors such as the type of pesticide, dose-rate, formulation and application techniques used, geohydrological conditions, climate and the local ecology of animals and plants. Accordingly the hazards a certain pesticide may pose to the environment will vary from one situation to another. For instance there will be much more contamination of non-target areas when relative high dosages are applied by aeriel application than when relative small dosages of the same compound are selectively applied by hand spraying. The degradation of a pesticide may be much faster in a rich organic soil under tropical conditions than in a sandy soil in temperate regions. In one place there may occur animals or plants which are especially vulnerable while these do not occur in other areas where the same chemical is applied. It is therefore extremely difficult not to say impossible to extrapolate ecotoxicological data from one situation to another.

With regard to the types of ecological hazards effects can be classified into several categories, as follows.

(1) Interference with productive functions

A depressive effect on non-target organisms of economic importance will give rise to a decrease of the productivity of natural resources. For instance when the use of an insecticide gives rise to mass mortality of fish and shrimps.

(2) Interference with regulatory functions

Pesticides may disturb regulatory processes such as the mineralisation of organic matter (e.g. biological purification). Effects on entomophagous insects may seriosly interfere with pest management. The latter may occur when the entomophagous ensects are directly affected, but also indirectly when alterations in biotopes disturn the life-cycle of these insects (e.f. through a loss of host plants following certain usages of herbicides).

Interference with carrier functions

Effects on certain species may result in a loss of certain amenities which are not related to productive or regulatory functions. One may think about recreational functions (sportfishing) and information functions (loss of species of possible future interest). Under this heading one should also comprise the aspect of ethical values ('the moral right for survival of a species').

It depends on local judgement and politics whether a certain degree of ecological damage by pesticides is considered to be acceptable or not. As the socio-economic situation and views on nature conservation vary from country to country the outcome of a cost/benefit analysis with regard to the use of a certain pesticide may also vary from country to country. In certain countries some damage to fish populations is found acceptable while other countries may come to the opposite conclusion. This of course depends on the emphasis lead on the importance of local fisheries.

ASSESSMENT OF THE ENVIRONMENTAL HAZARD OR RISK BY PESTICIDES

It should be noted that hazard is not synonymous with risk. Hazard can be defined as the presence of a danger (exposure to something potentially dangerous), while risk is interpreted more narrowly to mean the probability or chance of an effect.

Hazard or risk evaluations can be made if it is possible to compare exposure with adequate data on the toxicity of a chemical. When the time course of exposure and the level of exposure of a population of a certain species are known as well as data on the toxicity of the compound for the species the likelihood of an effect on the population may be assessed, e.g. a certain percentage of mortality. One may come to the conclusion that individuals of the species are at risk. However, the evaluation of effects on a higher level of organization, for example on population, community or ecosystem level in a given geographical area and the evaluation of the reversibility of effects, is another matter.

For this part of the evaluation ecological information is required. Hence, ecological hazard and risk evaluations should proceed stepwise at two levels:

- toxicological (comparison of exposure or exposure potential and toxicity for different species);
- ecological (consideration of population dynamics, life cycle biology, community and ecosystem structure, baseline data).

In hazard or risk evaluations a distinction should be made between a new compound and an existing one. For the former the evaluation is essentially predictive and reliance must be placed upon laboratory data and rarely some data may be available from limited field studies (e.g. field enclosure studies). For the latter a retrospective approach is possible. Here exposure and effect data actually collected in the field can be used. From existing experience it can be concluded that predictive evaluations may provide an assessment of hazard but only rarely of risk. The latter may be possible in retrospective studies.

THE PREDICTION OF ENVIRONMENTAL EXPOSURE

For a prediction of the exposure of a species or ecosystem to a pesticide, information is required on the release and behaviour of the pesticide in the environment. With respect to the release it is important to know the amounts, application techniques and frequency, and time and place of the application.

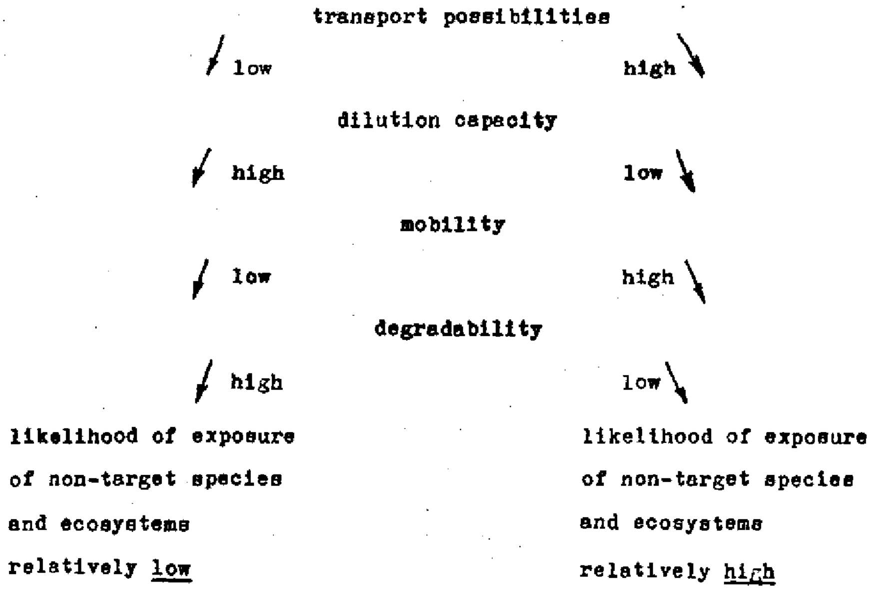
The behaviour of a pesticide in the environment determines its distribution and fate. A pesticide can be transported geographically and into different biota and may be chemically transformed. The pathways available for transport in the environment are determined by physical and chemical properties of the pesticide, like solubility in water, partitioning between polar and non-polar media, volatilization, leaching characteristics, degradation and formation of chemical complexes. Key properties of ecotoxic substances are environmental mobility and persistence.

The exposure of a species or ecosystem depends on the likelihood that a pesticide or its conversion products will actually reach the system and its availability for the biotic components. For the assessment of the ecological vulnerability of an area with regard to an intended use of pesticides, factors regarding behaviour and exposure should be considered in relation to the ecological characteristics of the area.

The assessment of exposure of the environment to pesticides

In Fig. 1 four successive levels of judgement are indicated which may allow for a preliminary assessment of the likelihood of exposure of non-target species and ecosystems. The first two levels, transport possibilities and dilution capacity, are mainly determined by the use pattern and characteristics of the environment concerned, while the other two, mobility and persistence, are mainly determined by the properties of the compound. The procedure in Fig. 1 gives an indication on the likelihood of exposure of non-target species and ecosystems being high, moderate or low.

Fig. 1. Levels of judgement for the assessment of the likelihood of exposure of desirable species to pesticides.



Transport possibilities

Transport possibilities relate to the probability that the pesticides used move away from the target area as a consequence of (1) the use pattern chosen and (2) prevailing physical and climatic characteristics. Transport possibilities are high, for instance when application gives rise to considerable drift of the pesticides into adjacent territory, or when pesticides are washed away by surface run-off after rains. Especially in sandy soils leaching into neighbouring habitats may occur after rainfall or groundwater movements. High temperatures cause high evaporation of the compound that may be transported and subsequently fall out in precipitation elsewhere.

Dilution capacity

The biotic and abiotic circumstances which influence the concentration of a pesticide within as well as outside the target area are referred to as dilution capacity. An important dilution factor is formed by the possible volatilization or codestillation of the compound as determined by climatic conditions. When a pesticide washes out from a piece of arable land into a relatively large fast flowing river, the ultimate concentration in the water will generally be much lower than when the same amount of material enters in a small tributary with low waterflow. Dilution may also occur when the pesticide is intercepted by a large surface of dense tropical forest vegetation. In this case being spread out over a large surface may imply that concentrations to which non-target species are exposed, are no longer meaningful. A low dilution capacity exists in a type of vegetation with a relatively low total surface area, like for example in a dense but narrow gallery forest ecosystem.

In this connection a relation between the total amount of biomass and the likelihood of exposure of desirable species and ecosystems may be assumed. If the total biomass of natural plant and animal species is relatively large compared to the economic species to be protected, the likelihood of critical exposure is less than in the situation of a large crop biomass in relation to the biomass of non-target species living in the area.

Mobility

The mobility of a pesticide is largely determined by its physico-chemical characteristics. In general it can be said that chemicals that are soluble in water and at the same time show a low tendency to adsorption on organic matter or mineral particles will move away much faster by (ground) water movement than substances with a low water solubility and a high adsorption to soil components. If a chemical, which is sprayed on leafy vegetation remains on the leaf surface, it can be washed or blown away, but when it is absorbed by the plants, it is less likely to disappear from the sprayed area. Furthermore, the vapour pressure of the compound determines the likelihood of volatilization of the material.

Degradability

The degradability of a compound depends largely on its chemical structure (e.g. hydrolysis, photodecomposition, biodegradation). Besides, the physical and microbial conditions of the environment have a marked influence. It will be obvious that a pesticide of a relatively persistent structure will be present for a longer period of time and will have a higher probability of reaching non-target areas than a pesticide which is easily degradable.

In many countries baseline data on mobility and degradation are required before a pesticide can be registered. In this context guidelines have been devised to provide the appropriate information. Therefore this type of information becomes available for an increasing number of compounds. For general information on the subject the reader is referred to the Test Guidelines of the OECD (Reports of OECD Expert Groups on Physical Chemistry and on Degradation and Accumulation, 1981) and to the Second Expert Consultation on Environmental Criteria for the Registration of Pesticides (FAO, 1981). For a number of pesticides additional information is available from field observations.

THE PREDICTION OF ENVIRONMENTAL EFFECTS

A considerable amount of effort has been put into the development and improvement of test methods on the basis of which adequate predictions can be made about possible ecological hazards that the use or disposal of chemicals may pose. At the international level a lot of valuable work has been carried out by a number of OECD working groups in connection with the Chemical Testing Programme of this organization and by the FAO Expert Consultation on Environmental Criteria for the Registration of Pesticides referred to in the previous section.

Test methods have been proposed which may enable the user to identify a number of potential properties of chemicals indicating a hazard for functionally important organisms, such as a relatively high toxicity for fish or invertebrates. In essence the majority of these tests are laboratory procedures. At present it seems obvious that for the judgement of ecological hazards by authorities involved in pesticide registration or premanufacturing notification procedures will be largely based on the outcome of this type of tests. In certain cases when sufficient information is available on the ecology of an area and on the possible levels of exposure of the biota it is imaginable that adequate predictions can be made. However, considering the tremendous variability among the world's ecosystems with respect to factors like species composition, population interactions, life-cycle phenomena and climatic and other ambient conditions, there will also exist situations in which adequate predictions cannot be made on the basis of laboratory data alone.

It was also recognized by the OECD Expert Group on Ecotoxicology that "where appreciable environmental concentrations of the chemicals are likely to be involved and/or some indication of environmental hazards exists, it may be necessary to assess the effect in experimental systems more closely approaching natural conditions, especially with regard to interspecific relations and the functioning of multispecies systems". In this connection the FAO Guidelines (1981) propose limited field enclosure studies or model-ecosystems and limited field trials to simulate conditions that may arise when the pesticides concerned are used in practice. The special value of these studies is that they allow observations of the recovery of affected populations which can

6300

be carried out realistically only under field conditions. Moreover environmental factors like suspended matter, mineral content and temperature of natural waters can have a profound effect on the results obtained, but cannot be reproduced readily in the laboratory. In addition, such studies have the advantage that the effects of inter-relationships between the various components of the system are reflected in the results.

After a pesticide has been registered for certain uses it is desirable to be able to confirm that the predictions made on environmental effects at the time of registration are valid. If doubts arise about the validity of the predictions, field surveillance and monitoring studies may have to be carried out both on the occurrence of residues and on possible biological effects. If the surveys indicate suspected or harmful effects, additional laboratory or other work may be necessary for reconsideration of the registered uses; on the other hand absence of effects is reassuring and can be valuable in considering extension of registered uses. Whenever there is a substantial change in the proposed use pattern, for example, additional crops or different methods of application or a marked increase in the quantity used, then the implications of these changes should be considered in the light of the available data. Additional work may be required to extend the initial predictions to the new use pattern. For more details on methodology the reader is referred to FAO and OECD Guidelines.

Adequate hazard predictions and assessments can be made properly only when (eco)toxicologists and ecologists cooperate closely. The contribution of ecologists in the ecotoxicological risk evaluation should be to help to identify key factors in the relevant ecosystems which are important with respect to the natural regulation of numbers and which may indicate the period in which species are particularly vulnerable.

In the introduction the concept of ecosystem functions was discussed. The performance level of the regulation, carrier and production functions of an ecosystem determines the distribution and degradation of a chemical in the environment. Therefore, an eco-toxicological valuation model may contain these elements, characterized by parameters as given in Table 1. The system considers both exposure and effects. When this approach is followed it will become obvious that the environmental hazards of pesticides will vary regionally and that one may have to conclude that a pesticide which is acceptable in one place may be for ecological reasons unacceptable in another. Similarly a use of a pesticide may be acceptable in a certain place in one season but not in another.

Table 1. Ecotoxicological Valuation System

Element	Parameter
Degradation capacity	Soil conditions and climate
Dilution capacity	Pathways, scale, and seasonality of water flow
Types of disturbance cycles	Scale and frequency of flood, drought, climate, and agricultural cycles
Key regular species	Life-cycle characteristics of economic species (fish, shrimp, fish food organisms, predators, parasites, soil microflora, etc.)
Special features	Importance for protected species and migratory species

Source: Koeman, 1982.

REFERENCES

FAO Plant Production and Protection Paper 28, Second Expert Consultation on Environmental Criteria for Registration of Pesticides, FAO, Rome, 1981.

Koeman, J.H. Ecotoxicological evaluation: The eco-side of the problem. Ecotoxicol.Environ.Safety, 1982, 6, 358-362.

CHEMICALS AND HOT ENVIRONMENT

P.Schmidt

In the discussion of the health implications due to simulesneous action of several environmental factors on the organism, particular attention has always been devoted to the combined exposure of chemical substances and high ambient temperature. Numerous publications in occupational medicine, in industrial toxicology and in pharmacology bear evidence to this great interest. Some important results from field studies and experimental investigations are summarized in the Tables 1-3. The data are interpreted by most investigators in such a manner that high ambient temperature increases the toxicity of chemical substances (reviews in 1,2,3,4). Therefore, some authors recommended correction factors for the MAC values in chemical substances are used at hot work places (Table 4). No comments from WHO expert committees or other international authorities on these proposals are hitherto published.

Reductions in the MAC values, likewise as the introduction of many other additional measures in the field of occupational hygiene and safety, entail considerable technological and economical implications. Hence, their justification must do justice to great demands. The statement that increased temperature enhances the toxicity of chemical substances is obviously insufficient to this end and doesn't permit the foundation of effective and technically and economically feasible measures of health protection and of industrial safety. The toxicological and hygienic evaluation of the simultaneous action of chemical substances and high ambient temperature is rather connected with a differentiated analysis of those phenomena and conditions in the environment and in the body which result at last in the biological response of the organism to the combination of factors. In this context especially the following possible target points of high ambient temperature are to discuss in detail - the action on

- Exposure conditions
- Route of entry (by inhalation, percutaneously)
- Toxicokinetic
- Toxicodynamic
- Toxicometric parameters

Questions which must be answered in this context by occupational medicine and occupational hygiene concern in industrial hygiene:

- validity of MAC values;
- validity of biological exposure limits;
- air ventilation at work places;
- personal protective equipment;
- overall cleanliness;

in industrial medicine:

- frequency of medical examinations.

1. Exposure conditions

Intoxications are to be expected the sooner , the greater than amount of substances is which are certained in the environment. The most important temperature-dependent physico-chemical property of a substance in this content is the vapour pressure. High ambient temperature can clearly increased the quantity of a substance in the air of a room. For example, in the solvents mentioned in Table 5 this quantity can potentially be increased by 2.3 to 3.3 times with an increase in temperature from 20° to 40°C.

Table 1: **Effect of high ambient temperature on toxicity - results from field studies**

Substances	Temperature °C	Effect	Ref.
Carbon Monoxide 0.01-0.1 $mg \cdot l^{-1}$ (7-10% COHb)	22-25 winter 40-50 summer	more CNS, cardiocirculat.syst.disord.	Korenevskaja (5)
Benzene 0.05-1.2 $mg \cdot l^{-1}$	20-28 winter 28-42 summer	more blood disord. (moderate anemia, leuko- a.thrombopenia)	Kozlova (6)
Mercury 0.01-0.06 $mg \cdot m^{-3}$	"normal"	No differences between the groups	Trachtenberg (7)
0.001-0.02 $mg \cdot m^{-3}$	28-30		
-Methylstyrene	34-42	more disord. (headache, vertigo, insomnia, stenocardia, mental irritability)	Kepkaev, Suchenova (8)
Hot vulcanization (Styrene, oil mist, formaldehyde, acrylonitrile, acrolein)	33-35	more CNS, digestive tract, respiratory tract, blood disord.	Volkova et al (9)
Phenol-formaldehyde resins	40-46	more CNS, PNS disord.(encephalopathy, polyneuropathy, neurasthenia)	Karapetjan (10)

Table 2: Effect of high ambient temperature on toxicity - some experimental investigations in the range of high doses

Substances (application	Species	Temperature (°C) length of exposure[1]	Effect	Ref.
Benzine, Benzene Aniline (inh., 2 hrs)	mice	40 2 hrs, simult. with substance	mortality 100%	Navrockij, Dubasinskaja (
50 Substances (ip.)	rats	36 72 hrs	LD_{50} [2] 2-17 times	Keplinger et al. (12)
Dinitroglycol Trichloroethylene Carbon Disulphide (sc.)	mice	35 7 hrs	LD_{50} 1.2-4.9 times	Zanardi et al. (
1.2-Dichloroethane (oral)	rats	40 4 hrs	LD_{50} 2.7 times	Michajlove (
Carbon Tetrachloride Allyl Alcohol (oral)	rats	35 4 hrs	LD_{50} 1.3-2.7 times	Schmidt (3)
Digoxin (ip.)	rats	35 72 hrs	LD_{50} 3.6 times	Hovevey-Sion, Kaplanski (15)

[1] immediately after substance application; [2] = decrease of the value (-times)

6300

62-I

Table 3: Effect of high ambient temperature on toxicity - some experimental investigations in the range of low concentrations or doses

Substances (application)	Species	Temperature (°C) length of exposure	Effect	Ref.
1	2	3	4	5
Benzene 0.1, 0.3 or $0.6\ mg \cdot l^{-1}$ 2 hrs/die, 3 mo.	rabbits	30 or 40 simult.[1]	more disord. in CNS, blood picture	Kozlova (6)
Mercury $0.1-0.12\ mg \cdot m^{-3}$ 4 hrs/die, 10 or 50 days	rabbits	39/40 simult.	changes in b.w., plasma protein fractions, SH-group content in serum ($0.03-0.4\ mg \cdot m^{-3}$ no effect)	Sachbanzjan, Savickij (16)
Intrathion $0.5\ mg \cdot m^{-3}$ 4 hrs/die, 1 mo.	rats	34/38 simult.	more disord. in b.w., blood picture, cholinesterase activity in blood	Jakubov (17)
Mercury $0.01-0.03\ mg \cdot m^{-3}$ 4-6 hrs/die, 1-2 mo.	rats rabbits	38/40 simult.	more changes in plasma protein fractions, SH-groups in tissues	Trachtenberg et al. (7)

[1] simultaneously with substance

Table 4: MAC-values for combined exposure to chemicals and hot climate at work - correction factors (CF) derived from animal experiments[1)]

Substances	Species	Temperature in the experiments °C	Correction factors	Ref.
Heavy Metals (Co, Cd, Pb, Hg)	rats mice	30-52	0.5	Savickij (20)
Iodine	rats	33-34	0.3-0.5	(21)
1,2-Dichloroethane	rats	35	0.5	Michailova (14)
Hepatotropic Compounds (Carbon Tetrachloride, Allyl Alcohol a.o.)	rats	35	0.5	Schmidt (3)

[1)] $(\text{MAC} - \text{value}_{\text{normal temperature}}) \cdot \text{CF} - \text{MAC-value}_{\text{high temperature}}$

Table 5: Temperature and vapour pressure

Substances	Vapour pressure (mm Hg) 20°C	40°C	-fold increase
Benzene	75	181	2.4
Acetone	186	425	2.3
Ethanol	44	133	3.0
Isopropanol	32	107	3.3
Ethyl Acetate	73	186	2.6
Carbon Tetrachloride	90	211	2.3

It is also possible to assume qualitative changes in the chemical nature of the substances, for instance, by an accelerated hydrolysis in moist, warm air.

How far the adhesion or solution of air pollution particles or vapours on moist skin deteriorates the exposure conditions, has not been investigated in detail. But such an effect can indeed be assumed.

In all field studies concerning the problem of a combined action of high ambient temperature and of chemical substances, sufficient data are lacking concerning the extent of change in the exposure conditions in consequence of the action of temperature, that is, a comprehensive characterization of the working conditions.

2. Route of entry

6300

2.1. The uptake by inhalation of substances is not essentially influenced by high temperature, most probably. A relevant additional uptake of substances would be possible, if the ventilated volume per minute, similarly as for instance due to physical activity, increased clearly in high ambient temperature. In experimental studies under conditions of rest and extrme temperature no comparable hyperventilation could be observed (22). The same behaviour can be assumed during work in hot environment (23). Only in an increase of the rectal temperature above 39°C does the quotient from ventilation and oxygen consumption increase (24).

2.2. Contrary to the pulmonary uptake of substances, the percutaneous resorption is significantly influenced by temperature increases relevant to occupational hygiene (Table 6). Warming and moistening of the skin lead to an essential acceleration of percutaneous resorption.

Table 6: Q_{10}-values for the percutaneous asorption velocity of some substances (values from different authors, see 22)

Substances	Q_{10}-values
Helium, Argon	2.9
Nitrogen	3.2
Carbon Dioxide	4.2
Sarin	2-3
Parathion	2
Acetylsalicylic Acid	2.7-3.4
Methyl Salicylate	2.0-2.2
Carbon Disulfide	2.4-2.8
Water	2.0-2.4

3. Susceptibility to chemical substances in hot environment

Only after excluding a temperature-dependent additional uptake of substances a more intensive biological response after combined action of high ambient temperature and chemicals can be interpreted as a consequence of a change in an organism's susceptibility. It is beyond the scope of this lecture to regard all possible causes of an increased susceptibility in detail. Only the most important viewpoints can be outlined here.

3.1. High ambient temperature leads to physiological responses whose knowledge is necessary for an interpretation of combined effects.

If high ambient temperature acts on organism which are not temperature adapted, this leads to a peripheral vasodilatation followed by perspiration. The peripheral vasodilatation is concomitant with a displacement of great blood volumes from the core to the shell of the body. By compensation, the heart rate and the heat volume increase. Besides, plasma is diluted and the urine flow is decreased, and also the excretion of sodium- and of chloride-ions in urine. The better flushing of the periphery leads to a limited supply of blood for major parenchymatous organs (23,25,26). Thus the hepatic venous oxygen content fell and in the arterial blood the lactate content is increased (27).

The temperature stress started adaptation reactions which lead to a more economical temperature regulation. The secretion of sweat is increased and begins on a lower level of high ambient temperature. The adaptation of the circulatory system is stabilized by ensuring the transport of heat from the core of the body to the shell and by guaranteeing a sufficient blood supply to internal organs. The best production of the body decreases. At the same level of high ambient temperature the core temperature and the pulse rate are lower. The efficiency of adaptation to hot climate depends decisively on the air humidity (1,26).

3.2. Toxicokinetics

Very probably, the mentioned physiological reactions can influence the toxicokinetic behaviour of chemical substadces (metabolites). The changed distribution of blood and the initial dilution of the plasma change the volume of distribution of the substances (25). Swartz and Sidell (28) proved this for pralidoxim and for p-aminohippuric acid. However, informations are absent for substances of interest to industrial toxicology.

Probably, an acceleration of the circulatory rate can cause a more rapid increase in the content of substances in the target organs, as can be inferred from combined effects (especially shortening of LT_{50} values) in experiments on animals (Table 7).

The shortening of the LT_{50}, which is not at all reflected in simultaneously determined LD_{50} or LC_{50}-values, was also ascertained in our laboratory in experiments with carbon tetrachloride (Table 8).

Qualitatively sufficient data are lacking on the content of substances in target organs (32,33,34, Table 9) so as to contribute to elucidating this problem.

The shortening of the LT_{50} ("acceleration of the toxic process" /2/) is not full explainable with changes in the toxicokinetic of the substances. Probably, in the formation of the effect some changes in the susceptibility of the CNS also participate (2).

Table 7: Effect of high ambient temperature on the mean lethal time after application of chemical substances

Substance (application)	Species	Temperature °C	Mean lethal time	Ref.
Parathion	mice	22.8	11.1 hr	Baetjer,
16.5 $mg \cdot kg^{-1}$,ip.		35.6	2.3 hr	Smith (29)
Nitrogen Oxides	mice	20	50 min	Paribok,
0.4-0.5 $mg \cdot l^{-1}$		35	20 min	Ivanova (30)
Dinitro-o-cresol	mice	20	51.3 min	Tesic
22 $mg \cdot kg^{-1}$,sc.		40	18.8 min	et al. (31)

Table 8: Influence of high ambient temperature on CCl_4-toxicity - LD_{50} and LT_{50}; 35°C, 50% rel.hum., 4 hrs immediately after ip.-application of different single CCl_4-doses, male mice

LD_{50} ($mg \cdot kg^{-1}$ b.w.)	CCl_4 (1)	CCl_4 + temp. (2)	$\frac{(1)}{(2)}$ [1]
after 24 hrs	3390 (3070-4120)	2330 (2090-2800)	1.49
after 48 hrs	2810 (2310-3160)	2090 (1930-2320)	1.34
LT_{50} (hrs)			
2370 $mg \cdot kg^{-1}$ b.w.	-	31.4 (22.6-44.9)	
2590 -"-	59 (47 -86)	9.2 (6.5-11.9)	6.94
2840 -"-	67 (55 -86)	5.5 (3.0- 7.6)	11.6
3400 -"-	12.6 (10.1-15.8)	-	

[1] all differences are significant (p 0.05)

Table 9: High ambient temperature - influence on substance content in tissues

Substances (application)	Species	Temperature °C Exposure conditions	Organ	Substance content under temperature influence	Ref.
2-Amino-4-arsenoso-phenol, iv.	rabbits	41.5 (core temp.) for 3.5 hrs	different	increased (significant)	Stokinger (35)
100 mg Aniline $\cdot kg^{-1}$ b.w., iv.	rabbits	35 for 2 hrs (after that p-Amino-phenole excretion in the 24 hrs-urine)	blood urine	increased decreased (significant)	Volkova (36)
30 mg Pentobarbital$\cdot kg^{-1}$ b.w., ip.	guin. pigs	37 for 1, 2 or 3 hrs after that determination	liver brain	increased increased (significant)	Sotaniemi (34)
Pb-acetate 1% in food 6 weeks	rats	continuously 23 (I) or 33 (II), intermittently heated to 42 (core temp.)	different	in I increased (liver, cardiac muscle), decreased (adrenals) or no differences between I and II	Wright, Lessler (37)
30 mg Me-HgCl$\cdot kg^{-1}$ b.w., oral	mice	39 for 56 hrs, after that determination	brain	no diff.	Nomiyama et al. (38)

In general, there are only few data available on the concentration of substances in organs from temperature and substance-exposed animals. Table 9 contains findings from experimental studies. They show no essential changes, although occasionally some significant differences were observed. In an experiment with carbon tetrachloride we also saw no significant differences (Table 10, unpublished results).

Table 10: Carbon tetrachloride in rat liver (nmol $CCl_4 \cdot g^{-1}$ liver) after a single oral CCl_4-dose (5.2 $mmol \cdot kg^{-1}$ b.w.) and high ambient temperature (35°C, 50% rel.hum., = 4 hrs)

Time of CCl_4-determination (decapitation) after oral application (hrs)	CCl_4 (1)	CCl_4+temp. (2)	$\frac{(2)}{(1)}$ 1)
0.25	312	330	1.06
0.5	291	247	0.85
2.0	163	140	0.86
4.0	151	162	1.07
8.0	125	154	1.23
24.0	20	20	1.00

1) All differences are insignificant

The reduced blood flow in the
temperature in this organ probably lead
mostly decreased capacity for biotransformation
(for instance aniline ---- p-aminophenol (38), cyclophosphamide ---- active metabolites (39)). In rats treated with CCl_4 and high ambient temperature we found exhalation of

$CHCl_3$ (Fig. 1). We interpret this in an immediate consequence due to a reduced oxygen content in the liver (40,41,42).

The excretion of substances or of metabolites via the lungs, the kidney, sweat or bile is certainly influenced by the temperature-induced physiological changes in the organism. Data are lacking however for a concluding judgement. We would like to indicate here once more the decrease in the excretion of CCl_4 via the lungs as demonstrated in Fig. 1. Such a decrease in the elimination was also observed after the application of chloroform.

3.3. Toxicodynamics

High ambient temperature with a great probability does not lead to qualitative changes in the course of intoxication. Individuals exposed to combined factors display pictures of symptoms which are characteristic for the substances involved. In the range of submaximal doses or concentrations, such effects in the picture of intoxication are obviously modified by temperature, whose releasing is connected to temperature-sensitive functional processes. Thus, in the case of hepatotropic substances, it is possible to prove combined effects for instance in serum enzyme activities, in the volume of the bile or in the content of glycogen in the liver. But obviously, also more intensive reactions of individual parameters do not exceed the limits of that picture of effects which is characteristic for the substance involved. Probably, the intensity of the effects of a combined substance-temperature-exposure is much more determined by the present concentration of substances and metabolites in the target organs or target tissues than by temperature-dependent changes in the susceptibility.

3.4. Adaptation to high ambient temperature

Almost all investigations in experiments on animals regarding the influence of temperature on the action of substances were carried out on animals not adapted to temperature. Certainly, this fact is important which is also advocated by the LD_{50}-values presented in Table 11. The data were obtained on animals naive to the experiment and on animals which were adapted already. Systematic investigation of this fact did not take place.[1] The influence of handling was also not separated.

Acclimatisation to high ambient temperature leads in the animal and in humans to an essentially more economical manner of response to further temperature loads. This explains the less intensive response of adapted animals after the application of substances. With all due caution therefore the accumption is expressed that an adaptation to temperature reduces the importance of high ambient temperature as a factor which changes the toxicity. This is also advocated by the fact that from geographical zones with a hot climate there are available no reports about generally increased effects of drugs or of industrial substances (as has been pointed out by Weihe (1)). To be excepted from this are such substances which impair the temperature regulation (for instance atropine).

As an implication there results the necessity of a more

---

[1] Methods to proof the course of heat acclimation are for instance measurement of food and water intake (44), voluntary physical activity (44), body temperature under temperature load (45) body weight, organ-to-body weight-ratios (45), glucose-6-phosphat content, glucose-6-phosphatase activity and glycogen content of liver (46,47).

Table 11: Influence of high ambient temperature on toxicity in temperature - adapted animals

Substance	Species	Temperature °C			Ref.
		normal	high, non- adapted	high, adapted	
			mortality rate		
Benzene 22 mg.l^{-1}, 2 hrs	mice	25[1]/0%[2]	40/100%	40/40%	Navrockij, Dubasinskaja (11)
Aniline 1.79 mg·l^{-1}, 2 hrs	mice	25 /0%	40/100%	40/0%	
			LD$_{50}$ (mg·kg^{-1})		
Digoxin ip.	rats	22 /32[3]	35/8.8	35/	
Benzene ip.	mice (rats	22 / 118 26 /1150	- 36/225)[4]	38/115	
Toluene ip.	mice (rats	22 / 126 26 / 800	- 36/225)[4]		

1) Temperature 2) Mortality rate 3) LD$_{50}$ 4) Keplinger et al. (12)

frequent medical examination of workers exposed to substances and rapid changes of temperature. This has already been pointed out by Savickij (48).

It is necessary of course to refer here to the possibility, that the above-mentioned lack of data also depends on insufficient medical surveillance in many tropical countries and from the fact, that the industrialization is still in early stages in these regions of the world.

4. Toxicometric parameters

The full importance of temperature-induced changes in toxicity for health protection and for industrial safety becomes clear if they are presented as statistically significant differences of toxicometric parameters. In this context not the changes in LD_{50}- or ED_{50}-values, but the behaviour of threshold doses or of threshold concentrations has the most practical, hygienic importance.

Data available in the literature concerning the behaviour of these threshold values in high ambient temperature allow to make the following remarks:

. threshold doses or threshold concentrations were not derived from dose-effect relationships, but were at most obtained in less extensive experiments (from effective, little effective or uneffective doses);

. in the comparisons of threshold doses (substance to substance plus temperature), no adequate statistical tests had been performed;

. quotients from intensities of effects were often equated with the dose quotients, which is inadmissible.

In our own experiments, threshold doses of some hepatotropic substances after a single dose with (ST) or without temperature exposure (S) were derived from dose-effect relationships for many parameters and dose quotients were determined. In nonsignificant dose differences, the quotients (ST/S) were in the range between 0.71 and 1.38 with confidence intervals at most between 0.24 and 4.26. Significant dose differences had values for quotients from 0.35 to 0.50. They were only seen, when carbon tetrachloride was applied.

It was possible to find the behaviour of threshold doses (or concentrations) present in Fig. 2 and Fig.3. Obviously, high ambient temperature rather causes a greater slope of the dose-effect relationship than a change of the threshold dose.

The discussion of the importance of a temperature lead for the position of the threshold doses must not be limited to these considerations.

In an ordering of the examined parameters according to the magnitude of the determined threshold doses involved, there resulted for instance for the case of an oral application of CCl_4 (Table 12) that the triglyceride content of the liver responds to a very low dose of the substance, but is not influenced by temperature. Significant shifts of threshold doses only occurred in the range of higher doses. The morphological findings were parallel to this.

With all due caution it may be inferred from this that temperature leads to a shift of thresholds the less, the lower the dose of the substance in the intoxication-specific dose range is to which the tested parameter just responds.

Table 12: Influence of high ambient temperature on threshold doses for CCl_4-induced effects. Male rats, treatment with different single oral CCl_4-doses and immediately after that 35°C, 50% rel. hum. for 4 hrs; 24 hrs after substance application determination of the effect intensity for each parameter; calculation of dose-effect relationships (own unpublished results)

Parameter	CCl_4 (1) threshold doses	CCl_4 + temp. (2) (mmol·kg^{-1} b.w.)	$\frac{(2)}{(1)}$ dose-ratio (confid.interv.)
Triglyceride content of liver	0.29	0.29	1.00 (0.45-1.87)
S-GPT	1.01	0.91	0.87 (0.39-1.99)
Hexobarbital sleeping time	1.87	1.52	0.81 (0.52-1.26)
Aminophen. N-demethyl. 9.000xg supernat. of liver	2.09	1.65	0.79 (0.24-1.54)
S-LAP	2.28	0.91	0.40 (0.05-0.75)
Ascorbic acid excretion in urine	2.29	3.16	1.38 (0.84-3.99)
BSP-elimination from plasma	2.90	1.44	0.50 (0.27-0.89)

Although the derivation of dose quotients from dose-effect-relationships because of logistic problems could not be demonstrated in long-term experiments, no principal objection is seen against transferring the conclusions to chronic combined exposure of substances and high ambient temperature. In chronic experiments with low doses of 4.4-diaminodiphenylmethane (49) or 1.1.2.2-tetrachloroethane (50) and temperature load we found no or only mild changes of the intensities of effects. Hence, it would not be necessary to correct MAC-values for working in hot climate.

To sum up, for the questions posed initially, the following answers and conclusions are possible:

. High ambient temperature can clearly enhance the potential risk in handling chemical substances. Therefore the economic costs or/and the technical demands for the compliance of the working conditions with hygienic standards (adequate ventilation, overall cleanliness, personal protective equipment) are higher in such situations.

. With increasing ambient temperature, the velocity of percutaneous resorption increases by a hygienic important extent whereas a pulmonary additional uptake has no comparable importance.

. High ambient temperature can lead to changes in the susceptibility of the organism to substances. If this is the case, then combined effects can be expected in the range of higher doses (concentrations). This has importance especially for situations with abrupt changes of exposure conditions, for instance, in agriculture on very hot days.

. The organism which is not adapted to temperature responds to combined actions of substances and temperature in a more sensitive manner than the adapted one. Therefore abrupt climatic changes at workplaces with chemical exposure require more frequent substance-specific medical examinations.

. There are no convincing data for a decrease of threshold concentrations which are compelling to make corrections in MAC values for work in hot environment.

. Not clear is the validity of biological exposure limits for the evaluation of the chemical risk at workplaces with combined substance-temperature-exposures.

Further research is needed in the field of combined action of chemical substances and high ambient temperature. The most important gaps in knowledge concern the following topics:

. Toxicokinetic of industrial substances in the temperature-exposed and working man (biological exposure limits).

. Combined acute effects of neurotoxic substances and high ambient temperature in man (for instance experimental studies with psychological methods).

. Epidemiological data in order to confirm the above-presented conclusions, mostly derived from animal experiments.

Literature

1. Weihe W.H. The effect of temperature on the action of drugs. In: Lomax P. & Schonbaum E., ed. The Pharmacology of Thermoregulation. Basel, Karger, 1973, p.409-425.

2. Kustov V.V., Tiunov L.A., Vasil'ev G.A. Kombinirovannoe dejstvie promyslennych jadov (Combined action of industrial toxic substances). Moskva. Medicina, 1975.

3. Schmidt P. Zur kombinierten Wirkung chemischer Substanzen und erhöhter Umgebungstemperaturen (Combined action of chemical substances and high ambient temperature). Zeitschift für die gesamte Hygiene und ihre Grenzgebiete, 1977, 23, 9, p.617-619.

4. Health effects of combined exposures in the work environment. Report of a WHO Expert Committee. World Health Organization Technical Report Series 662, Geneva 1981.

5. Korenevskaja E.I. K voprosu o vlijanii vysokoj temperatury vozducha na toksiceskoe dejstvie okisi ugleroda (About the influence of high air temperature on the toxic action of carbon monoxide). Gigiena i sanitarija, 1955, 20, 9, p.19-24.

6. Kozlova T.A. K voprosu o sovmestnom dejstvii na organizm benzola i vysokoj temperatury vozducha (About combined action of benzene and high air temperature on organism). Gigiena i sanitarija, 1957, 22, 4, 18-25.

7. Trachtenberg I.M., Saviokij I.V., Sterengare R.Ja. O vlijanii na organizm malych koncentracii rtuti (About the action of low mercury concentrations on the organism). Gigiena tuda i professional'nye zabolevanija, 1965, 9, 12, p.7-12.

8. Kapkaev E.A., Suchanova V.A. O vlijanii -metilstirola na organizm v uslovijach provysernoj temperatury (About

the action of -methylstyrene and high temperature on organism). Gigiena truda i professional'nye zabolevanija, 1968, 12, 5, p.11-16.

9. Volkova Z.A., Milkov L.E., Lopuchova K.A., Maljar L. Makarenko Ju.L., Sachova T.K. Uslovija truda i sostojanie zdorov'ja rabocich zanjatych na processach gorjacej vulkanizacii obuvnych rezin (Working conditions and the health state of workers engaged in hot vulcanization of synthetic materials for shoes). Gigiena truda i professional'nye zabolevanija, 1970, 14, 1, p.31-34.

10. Karapetjan S.O. Porazenie nervnoij sistemy u rabocich ecmiadzinskogo zavoda plastmess (Alteration of the nervous system in workers of the factory for plastics in Etschmiadschinsk). Zurnal eksperimental'noj i kliniceskoj mediciny, 1971, 11, 2, p.100-103.

11. Navrockij V.K., Dubasinskaja S.M. Dejstvie promyslennych jadov na organizm zivotnych v uslovijach vysokoj temperatury vozducha (Action of industrial toxic substances and high air temperature on animal organism). Gigiena i sanitarija, 1951, 16, 8, p.22-28.

12. Keplinger M.L., Lancer C.R., Eichman W.D. Effects of environmental temperature on the approximate lethal doses of compounds administered intraperitoneally to albino rats. Toxicology and applied pharmacology, 1959, 1, 1, p.156-161.

13. Zanardi S., Strambe B.M., Giglio M. Tossicita e temperatura ambientale (Toxicity and environmental temperature). Lavoro e medicina, 1966, 20, 6, p.285-305.

14. Michajlova A. Combined exposure of 1.2-dichloroethane and high ambient temperature on some liver functions. Avtoreferat dissertacii. Sofia, 1975.

15. Hovevey-Sion, D., Kaplenski J. Toxicity of digoxin in acutely and chronically heat-exposed rats. Research communications in chemical pathology and pharmacology, 1979, 25, 3, p.517-524.

16. Sachbazjan G.Ch., Savickij I.V. Kombinirovannoe dejstvie tiolovych jadov i vysokoj temperatury vozdusnoj sredy na organizm (Combined action of SH-poisons and high air temperature on organism). Vestnik akademii medicinskich nauk SSSR, 1963, 18, 2, p.38-42.

17. Jakubov A.Ja. Nekotorye dannye o dejstvii fosfororganiceskogo insekticida M-81 na organizm eksperimental'nych zivotnych pri vysokoj temperature (Some data about the combined action of the phosphororganic insecticide M-81 and high temperature on the organism of experimental animals). In: Gigiena i toksikologija pesticidov i klinika otravlenij. Kiev, Zdorov'e, 1965, p.291-296.

18. Mel'nikova S.K. K voprosu o chroniceskom dejstvii metilmerkaptofosa na organizm zivotnych pri vysokoj temperature (About chronic combined action of methylmercaptophos and high temperature on animal organism). In: Kommunal'naja gigiena, gigiena pitanija, primenenie i toksikologija pesticidov, Alma-Ata, 1970, v.2, p.271-273.

19. Grigorowa R., Binnewies S. Über die kombinierte Wirkung von phosphororganischen Pestiziden und erhöhter Umgebungstemperatur in inhalatorischen Kurzversuchen an Ratten (Combined action of phosphororganic pesticides and high ambient temperature on rats in acute inhalation experiments). Internationales Archiv für Arbeitsmedizin, 1973, 31, 4 p.295-307.

- - - -

20. Savickij I.V. Vlijanie vysokoj temperatury vozdusnoj sredy na tecenie intoksikacii tiolovymi jadami (Influence of high air temperature on intoxications with SH-poisons), Gigiena truda i professional'nye zabolevanija, 1967, 11, 10, p.30-35.

21. Begisev A. Materialy po charakteristike toksicnosti parov joda v uslovijach povysennoj temperatury vozducha (Data about the toxicity of iodine in the presence of high air temperature). Gigiena truda i professional'nye zabolevanija, 1975, 19, 6, p.38-41.

22. Schmidt P. Zur arbeitshygienischen Bewertung der gemeinsamen Einwirkung chemischer Substanzen und erhöhter Umgebungstemperatur auf den Organismus (Hygienic evaluation of the combined action of chemical substances and high ambient temperature on the organism). Zeitschrift für die gesamte Hygiene und ihre Grenzgebiete, 1980, 26, 6, p.393-398.

23. Robinson S. Cardiovascular and respiratory reactions to heat. In: Yousef M.K., Horvath S.M., Bullard R., ed. Physiological adaptations desert and mountain. New York and London, Academic Press, 1972, p.77-97.

24. Ts'ao C.H., Meyer F.R., Epperson B.E., Holgarsen C.C. The influence of temperature on respiration in exercising men. Physiologist, 1962, 5, 2, p.222-234.

25. Ballard B.E. Pharmacokinetics and temperature. Journal of pharmaceutical sciences, 1974, 63, 9, 1345-1358.

26. Ingram D.L., Mount L.E. Man and animals in hot environments. Berlin Heidelberg New York. Springer, 1975.

27. Rowell L.B., Brengelmann G.L., Blackmon J.R., Twiss R.D., Kusumi F. Splanchnic blood flow and metabolism in heat-stressed man. Journal of applied physiology, 1968, 24, 4, p. 475-484.

28. Swartz R.D., Sidell F.R. Effects of heat and exercise on the elimination of pralidoxime in man. Clinical pharmacology and therapeutics, 1972, 14, 1, p.84-89.

29. Baetjer A.M., Smith R. Effect of environmental temperature on reaction of mice to parathion, an anticholinesterase agent. The American journal of physiology, 1956, 186, p.39-46.

30. Paribok V.P., Ivanova F.A. Vlijanie temperatury vozducha na toksiceskoe dejstvie okislov azota (The influence of air temperature on the toxic action of nitrogen oxides). Gigiena truda i professional'nye zabolevanija, 1965, 9, 7, p.22-26.

31. Tesic D., Terzic L.J., Dimitrijevic B., Zivanov D., Slavic M. Experimental investigation of the effect of environmental temperature and some medicaments on the toxic effect of dinitroorthocresol. Acta Vet.Beograd, 1972, 22, 2, p.45-52.

32. Herr F., Borsi J., Pataku G. Wirkung der Umwelttemperatur auf die Toxizität der Analgetika (The action of environmental temperature on the toxicity of analgetics). Archiv für experimentelle Pathologie und Pharmakologie, 1954, 223, 1, p.56-63.

33. Setnikar J., Temelcou O. Effect of temperature on toxicity and distribution of pentobarbital and barbital in rats and dogs. Journal of pharmacology and experimental therapeutics, 1962, 135, 2, p.213-222.

34. Sontaniemi E. The influence of environmental temperature on the concentration of pentobarbital and barbital in the liver and brain. Acta pharmacologica et toxicologica, 1967, 25, Suppl.5, p.1-63.

35. Stokinger H.E., Dorn F.L., Boak R.A., Carpenter C.M. The effect of fever on the distribution of arsenic in the tissues of rabbits injected intravenously with mapharsen.

American journal of syphilis, gonorrhea and venereal diseases, 1944, 28, 4, p.460-470.

36. Volkova Z.A. Vlijanie temperatury vozducha na processy prevrassenija i obezvrezivanija anilina v organizme zivotnych (The influence of air temperature on metabolism and detoxification of aniline in the organism of animals). Gigiena truda i professional'nye zabolevanija, 1958, 2, 4, p.30-36.

37. Wright G.L., Lessler M.A. Interaction of lead and high ambient temperatures in the rat. Environmental research, 1979, 18, p.377-384.

38. Nomiyama M., Matsui K., Nomiyama H. Effects of temperature and other factors on the toxicity of methylmercury in mice. Toxicology and applied pharmacology, 1980, 56, 3, p.392-398.

39. Clawson R.E., Egorin M.J., Fox B.M., Ross L.A., Bachur N.R. Hyperthermic modification of cyclophosphamide metabolism in rat hepatic microsomes and liver slices. Life sciences, 1980, 10, p.1133-1137.

40. Reiner O., Athanassopoulos S., Hellmer K.H., Murray, R. ., Uehleke H. Bildung von Chloroform aus Tetrachlorkohlenstoff in Lebermikrosomen, Lipidperoxidation und Zerstörung von Cytochrom P 450 (Generation of trichloromethane from carbon tetrachloride in liver microsomes, lipid peroxidation and destruction of cytochorme P 450). Archiv für Toxikologie, 1972, 29, 3, p.219-233.

41. Kieczka ., Remmer H., Kappus H. Influence of oxygen on the inhibition of liver microsomal activation of carbon tetrachloride by the catechol 2-hydroxyestradiol-17 . Biochemical pharmacology, 1981, 30, 4, p.319-324.

42. Hanzlik R.P. Reactivity and toxicity among halogenated methanes and related compounds. Biochemical pharmacology, 1981, 30, 22, p.3027-3030.

43. Nomiyama K., Matsui K., Nomiayma H. Environmental temperature, a factor modifying the acute toxicity of organic solvents, heavy metals, and agricultural chemicals. Toxicology letters, 1980, 6, p.67-70.

44. Kerr G.S., Squibb R.L., Grankel H.M. Effect of heat acclimation (32°C) on rat liver and brain substrate levels. International journal of biochemistry, 1975, 6, p.191-195.

45. Cassuto Y., Chaffee R.R.J. Effects of prolonged heat exposure on the cellular metabolism of the hamster. American journal of physiology, 1966, 210, 2, p.423-426.

46. Chayoth R., Cassuto Y. Carbohydrate metabolism of heat-acclimated hamsters. I. Control glycogenesis in the liver. American journal of physiology, 1971, 220, 4, p.1067-1070.

47. Chayoth R., Cassuto Y. Carbohydrate metabolism of heat-acclimated hamsters. II. Regulatory mechanisms of the intact animal. American journal of physiology, 1971, 220, 4, p.1071-1073.

48. Savickij I.V. Znacenie teplovogo faktora pri otravlenijach, vyzvannych professional'nymi jadami iz gruppy tjazelych metallov (Significance of high temperature for the development of intoxications with occupational used heavy metals). Vracebnoe delo, 1971, 3, p.138-142.

49. Schmidt P., Burck D., Bürger A., Gohlke R., Jäger H., Just A., Rothe R. Zum Verlauf der 4.4'-Diaminodiphenylmethan-Intoxikation bei Belastung des Organismus mit erhöhter Umgebungstemperatur (The course of the intoxication with 4.4'-diaminodiphenylmethane under the action of high ambient temperature on the

organism). Zeitschrift für die gesamte Hygiene und ihre Grenzgebiete, 1981, 27, 1, p.51-57.

50. Gohlke R., Schmidt P., Bahmann H. 1.1.2.2.-Tetrachlorethan mit Hitzebelastung im Tierexperiment - morphologische Ergebnisse (1.1.2.2.-tetrachloroethane and high temperature in animal experiments - morphological data). Zeitschrift für die gesamte Hygiene und ihre Grenzgebiete, 1977, 23, 5, p.278-282.