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System for Accelerating Methods of Evaluating Toxicity and Hazardous Properties of Substances

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By studying a group of organophosphorus compounds (PhOC), a system of methods for developing models of chronic intoxication was approved, the purpose of which was to establish safe levels of chemical pollutants in the water. The magnitudes of threshold doses (TD) and ineffective doses (MID) were obtained by using MAC_{rr} (maximum admissible concentrations of substances in the air of industrial plants) and TLV [indices of admissible levels of substances in the air of industrial plants (USA standards)]. In compiling estimated and experimentally established MAC for 21 substances, variations were recorded within the range of 2-10 times. Forecasting on the basis of ED_{50}, E_{lim} and other measures during 5-20 day experiments made it possible to estimate threshold and inactive doses of PhOC during chronic intoxication, as well as to determine a cumulative coefficient and distribute these compounds into two series, according to the degree of their hazard.

This project made it possible to determine cumulative properties simultaneously and forecast the chronic toxicity of the PhOC group under investigation.

The present report is the result of studies carried out in the development and evaluation of various methods for predicting the toxicity and hazard from environmental chemical pollutants entering by mouth, for the purpose of establishing safe levels for their action on the organism.

On the basis of study of a group of organophosphoric compounds (PhOC), a system of methods was approved based on various models of chronic intoxication.

Mathematical Models

The method of mathematical modeling is one of the fastest and an accurate way of predicting the degree of toxicity of new chemical compounds. A series of computed equations was set up based on generalizations from quantitative indicators of the danger from PhOC, obtained in various areas of hygiene with different paths for introduction into the organism. The magnitudes of threshold doses (TD) and ineffective doses (MID) can be obtained with the greatest accuracy by utilizing: (1) values of maximum admissible concentrations of substances in the air of industrial plants (MAC_{rr}) [Eqs. (1)–(4), Table 1]; (2) indicators of admissible levels of substances in the air of industrial plants by U.S. standards (TLV) [Eq. (5), Table 1]; (3) the magnitudes of ineffective concentrations established during standardization of substances in atmospheric air (MIC) [Eq. (6), Table 1].

Less precise values of MID can be obtained by calculation of the magnitudes of acute toxicity of substances, i.e., of their average lethal doses (LD_{50}) [Eqs. (7)–(9), Table 1].

It should be noted that Eqs. (7)–(9) allow the parameters of chronic toxicity to be calculated for substances with weakly expressed cumulative properties. Thus, in carrying out computations for substances having a different degree of cumulativeness, it is necessary to introduce the following coefficient of correction (K) into the equation: for supercumulative ones it is -3; for highly cumulative ones, it is -2; for those with pronounced cumulative properties, -0.5; with poor cumulative properties, 0; for practically noncumulative ones, it is $+0.5$. In such a case, for example, Eq. (9) acquires the following form: $\log TD = 0.923 \log LD_{50} - 2.886 + K$.

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To verify the reliability of the proposed equations, two methods were used: the equations were tried out on those experimentally established standards for substances which had been worked out after investigating them; also, special chronic testing was done to determine the limits of disparity between computed and experimentally determined TD and MID (Table 2).

Analysis of differences between magnitudes obtained by the computation method and those determined experimentally has shown that for 15 substances the disparity does not exceed 2 to 5 times, while for eight substances, the disparity can be by a factor as much as 10. The results of a comparison of the data obtained from six chronic toxicological experiments with computed magnitudes confirmed that, for most substances, the difference in parameters between those computed and those experimentally determined does not exceed one order, i.e., it is found to be within the margin of error of the chronic experiments (*1*).

It should be emphasized that for organophosphorus compounds the disparity between threshold and ineffective doses, based on toxicological and other evidences of damage (organoleptic) is as a rule, great and consequently any possible error from mathematical determination of the MID is more than compensated for, while for the overwhelming majority of PhOC, the MID in water is determined by organoleptic evidence.

In evaluating the true possibility of applying mathematical models to obtain safe levels for the content of substances in water, it should be kept in mind that the information on the toxic properties of 300 substances accumulated in the field of industrial toxicology can already be utilized now for these purposes.

The close correlation between the MAC of

Table 1. Equations for predicting thresholds doses (TD) and ineffective doses (MID).

Eq. no.	Equation	n	$r \pm m$	t
(1)[a]	$\log MID = 0.47 \log MAC_{rv} - 1.398$	140	0.79 ± 0.035	13.6
(2)[a]	$\log TD = 1.055 \log MAC_{rv} + 0.014$	74	0.51 ± 0.18	4.67
(3)	$\log MID = 1.099 \log MAC_{rv} + 0.599$	14	0.68 ± 0.21	9.05
(4)	$\log TD = 0.99 \log MAC_{rv} + 0.602$	17	0.65 ± 0.23	8.45
(5)	$\log MID = 0.99 \log TLV - 1.061$	9	0.99 ± 0.13	44.32
(6)	$\log MID = 1.25 \log MIC + 0.885$	8	0.766 ± 0.2	2.06
(7)	$\log MID = 0.75 \log LD_{50} - 3.66$	24	0.35 ± 0.09	1.73
(8)	$\log TD = 0.91 \log LD_{50} - 2.0$	25	0.31 ± 0.11	1.46
(9)[a]	$\log TD = 0.923 \log LD_{50} - 2.886$	286	0.53 ± 0.21	4.22

[a] *Equations used for substances for various chemical groups.*

Table 2. Maximum ineffective doses of chronic toxicity of substances (MID).

Substance	MID, mg/kg: Determined experimentally	MID, mg/kg: Computed by means of regression equations	Factor by which calculated magnitude differs from experimental	Factor by which MID exceeds threshold by organoleptic indicator (smell)
Metaphos	0.025	0.02	No difference	25
Tiphos	0.01	0.009	No difference	60
NDVF	0.05	0.043	No difference	−1.5
Trichlorometaphos	0.06[a]	0.06	No difference	3
Saiphos	0.1	0.06	No difference	20
Methylacetophos	0.25[a]	0.02	No difference	16
Phthalophos	0.2	0.1	2	20
Isophos-2	0.06[a]	0.03	2	24
Methylnitrophos	0.04[a]	0.05	1.2	8
Intration	0.01	0.05	5	143
Phosalon	0.05	0.2	4	1000
Mercaptophos	0.015	0.0034	4	30
Methylmercaptophos	0.1	0.02	5	200
Sulfide phos	0.001[a]	0.0002	5	20
Ricide	0.1[a]	0.02	5	133
Phosphamide	0.02	0.2	10	13
Acetophos	0.05	0.004	11	33
Koral	0.08	0.004	20	3
Carbophos	1.0	0.12	8	400
Hydrolyzed butyl-aircraft	2.5	0.22	11	50000
Phosbutyl	1.5	0.2	7	1000

[a] Results of our own investigations.

harmful substances in waters of ponds, developed and determined in the USSR (magnitude MID) and the standards for admissible contamination in the air of industrial plants in the USA open prospects for mutual utilization of the results of toxicological research in the two countries as an initial informational base for predicting the toxicity of substances.

Rapid Experimental Method of Prediction

The most reliable approach for forecasting parameters of chronic toxicity is an experimental method based on study of the substance in a short-term experiment. The main problem in carrying out such investigations is the determination of the cumulative properties of the substances and allowing for the regularities of the development of toxicodynamic processes by the magnitudes of isoeffective doses (ED_{50}, ED_{lim}, ED_{min} and others) on the basis of the dose-effect dependence (*2*). The results of such experiments are expressed in the form of a curve of isoeffective doses for each day of the study. The developed curve typifies adequately the cumulative properties of the substances. Of course the fullest impression of the cumulative properties of chemical compounds can be obtained after carrying out prolonged experiments with determination of the "zone of chronic action" according to the ratio LD_{50}/MID (*3*). However, the growing quantity of chemical pollution in the environment requires an intensification of investigations for evaluating their dangerous and safe levels.

For this purpose, forecasting methods based on study of cumulative properties of substances may be useful, on condition that strictly quantitative and reliable parameters are established for evaluating their cumulativeness in the rapid experiment.

To develop the methodological design for carrying out a rapid determination meeting the stated needs, short-term and prolonged experiments were done with six PhOC and one sodium compound: ricide, methylnitrophos, sulfidephos, isophos-2, methylacetophos, trichlorometaphos, and *m*-dinitrobenzene. The experiments were carried out on white rats; observation for intoxication was done allowing for the period of maximum change in the controllable functions of the organism (activity of the choline esterase of blood and methemoglobin content in blood). Graphic analysis of the isoeffective curves showed that a monotypical development occurs in the first phase of intoxication. However, some differences in their course enable one to distinguish three most characteristic types: curves with a predominance of intoxication processes; curves with a predominance of "adaptive" reaction processes; curves with a rapid occurrence of equilibrium among these processes.

Predictions on the Basis of Magnitudes of ED_{50}

From the results obtained (Table 3 and Figs. 1–3), it follows that the differences between the magnitude of ED_{50} on days 5-20 of investigation (the minimum dose was used in the analysis during a month of research) and a similar dose of chronic effect did not exceed two. Analysis of data in the literature showed that these differences can reach ten. For all the compounds studied, the ratio ED_{50} (5-20 days)/MID did not exceed 100. The coefficient established makes it possible to calculate the threshold and ineffective doses of chronic PhOC intoxication on the basis of rapid test results.

Predictions on the Basis of Cumulative Properties of Substances

The methodological design for carrying out short-term tests enables one to determine the coefficient of cumulation CC of each substance:

$$CC = ED_{50}\ (5 \text{ to } 20 \text{ days})/ED_{50}\ (1 \text{ day})$$

As is evident from Table 3, there is a certain correlation between the magnitudes of coefficients of correlation and the relationship of ED_{50} (5-20 days)

Table 3. Correlation coefficients and various parameters.

Substance	ED_{50} (5-10 days)	ED_{50} (6-20 mo.)	TD	MID	Ratio ED_{50} (5-20 d) / MID	CC	LD_{50}/MID
Methylnitrophos	2.5	2.0	0.1	0.04	62	0.15	12.000
Ricide	7.0	5.0	1.5	0.1	70	0.06	2.900
Sulfide phos	0.17	0.16	0.06	0.001	100	0.03	230.000
Isophos-2	5.6	3.7	1.0	0.06	93	0.13	4.800
Methylacetophos	1.6	0.9	1.0	0.025	64	1.0	5.100
Trichlorometaphos	7.6	4.4	0.6	0.06	127	0.025	25.000

to the MID, which enabled us to propose the recommendations below:

At CC = 0.01:
ED_{50} (5-20 days)/MID = 500
At CC = 0.01-0.1
ED_{50} (5-20 days)/MID = 100–200
At CC = 0.1-1.0
ED_{50} (5-20 days)/MID = 50–90

In calculating the MID magnitude it is necessary to start with the smallest magnitude of ED_{50} established in the rapid test.

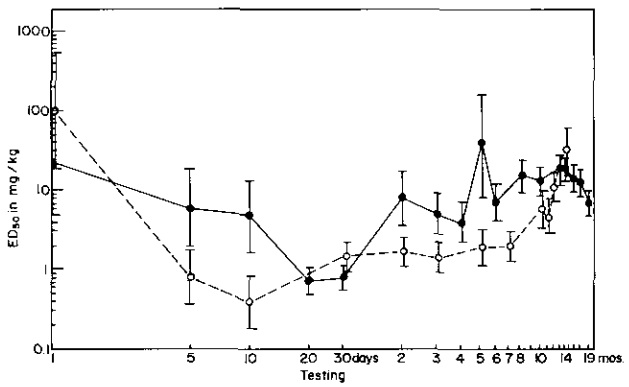

FIGURE 1. Toxicodynamics of isophos and trichlorometaphos: (——) isophos; (– –) trichlorometaphos.

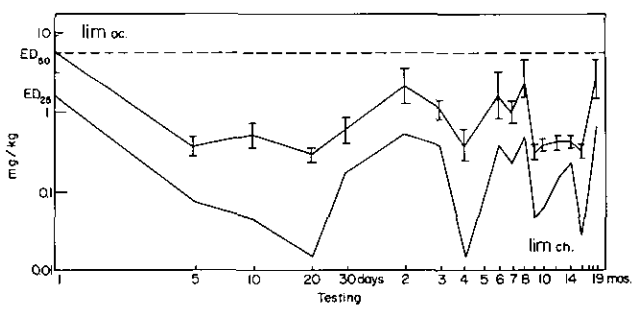

FIGURE 2. Toxicodynamics of sulfide organophosphorus compounds.

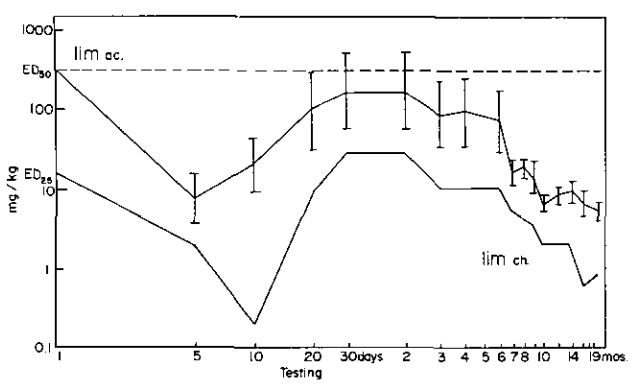

FIGURE 3. Toxicodynamics of ricide.

Relationship of Toxicity Parameters and Danger from PhOC

Regularities in the development of toxicodynamic processes in the short-term experiment and the direct connection established between the CC of substances and their "zone of chronic effect," expressed by the ratio LD_{50}/MID (Fig. 4), also makes it possible to utilize the results of these experiments for determining magnitudes of admissible doses under prolonged action of chemical compounds. Evaluation of substances according to their degree of hazard, on the basis of CC and LD_{50}/MID values, enables one to describe studied compounds into the two series: (1) sulfide phos, trichlorometaphos, methylnitrophos, methylacetophos, isophos-2, and ricide, and (2) trichlorometaphos, sulfide phos, ricide, isophos-2, methylnitrophos, and methylacetophos.

In comparing the strength of the cumulative action of substances on the basis of the two methods used, close results are obtained. The most dangerous compounds from the viewpoint of possible development of chronic intoxication are sulfide phos and trichlorometaphos.

On applying all the requirements of the methodological design for carrying out the rapid test, the toxicodynamics of one of the representatives of the methemoglobin developers—m-dinitrobenzene was studied. Development of intoxication processes from the action of this substance is analogous to processes in poisoning by PhOC. However, further accumulation of data is needed for this group of compounds in order to discover the corresponding regularities for the purpose of establishing methods of forecasting safe doses.

Chronic tests over a period of 1 to 2 years on the six organophosphorus compounds mentioned above

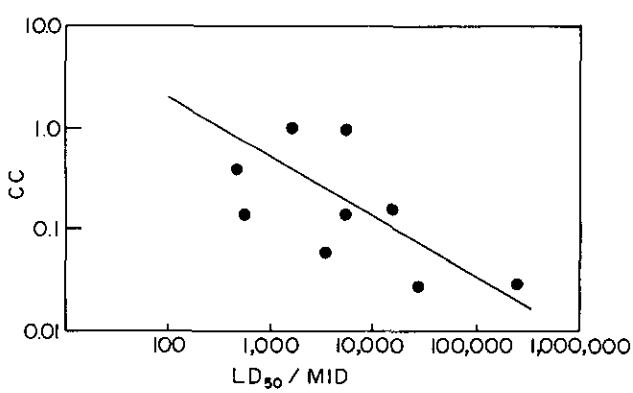

FIGURE 4. Correlation of coefficient of cumulation (CC) of compounds with range of chronic action (LD_{50}/MID).

showed sufficient reliability in the proposed methods for predicting the parameters of chronic toxicity, to the extent that the predicted MID differed from that established experimentally by no more than a factor of 5.

Thus, the results of the investigations carried out enabled these conclusions to be drawn. (1) The possibility is demonstrated of reliably utilizing mathematical models for predicting toxicity of and danger from PhOC. (2) A rapid test design developed permits simultaneous determination of the cumulative properties of chemical compounds and establishment of quantitative methods for predicting the parameters of the chronic toxicity of substances.

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