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Study of the Combined Action of a Group of Chlorine Derivatives of Hydrocarbons Entering the Organism by Inhalation

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The combined action of substances having a munidirectional toxic effect on the organism was studied for several typical chlorine derivatives of hydrocarbons (1,2-dichloropropane, 1,2,3-trichloropropane, and perchloroethylene).

Since a correct assessment of the combined action of substances is based on the comparison of the effect of mixed compounds and the effects observed for each component alone, research was conducted on each individual substance on the basis of the "concentration–time" relationship. The action of several mixtures of substances was also studied at various concentration levels, from high to low concentration levels of substances which are actually present in the atmosphere.

Research results showed that the character of the mixture at both high and low levels of concentration was identical and approximate to the overall effects.

This report presents materials on experimental investigations on the subject of Soviet-American cooperation, development and improvement of methods for evaluating the combined effect of substances upon their simultaneous entry into the organism. The first portion of this study was reported at the joint symposium in Riga in 1974.

In studies carried out earlier (*1*), the nature of the combined effect of a group of chlorine derivatives of hydrocarbons, i.e., 1,2-dichloropropane (DChP), 1,2,3-trichloropropane (TChP), and perchloroethylene (PChE), was evaluated on the basis of acute experiments on animals within the parameters of their acute toxicity.

Subsequent investigations evaluated continuous inhalation to lower concentrations of DChP, TChP, and PChE.

The methodology of evaluating the nature of the combined action was based on determining the isoeffective concentrations of each of the components during their isolated action, along concentration–time dependence curves (*2, 3*), and included the following stages: (1) estimating dependency curves for the appearance of specific changes in indicators of animal life-activity from the action of various levels of concentrations, from high to low, actually present in atmospheric air; (2) establishing the time of appearance of equivalent effects during the combined action of substances; (3) calculating the coefficient of combined action.

The curves of time-concentration dependence was established on the basis of acute animal experiments (*1*) and experiments with constant continuous inhalation effect of each substance separately, utilizing indicators in line with the data in the literature on toxicodynamics of substances.

The investigations were carried out on white male rats weighing 200 to 240 g. The animals were subjected to dynamic poisoning with five or six concentrations (within a range from high to low) of DChP, TChP, and PChE. The state of the animals was evaluated by utilizing the following indicators: weight, enzyme activity and blood cholinesterase, the total threshold indicator (TTI), and the content in blood of erythrocytes, leukocytes, and hemoglobin.

By means of these indicators, the state of life-activity of the animals was assessed after 2, 4,

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12, and 24 hr from the start of intoxication and then after each 24-hr period up to 168 hr in experiments with a high concentration of the substances: DChP, 2.0, 1 mg/l.; TChP, 0.8, 0.35 mg/l.; PChE, 2.75 and 1.25 mg/l. Upon exposing animals with a combination of substances (DChP, 0.5 or 0.1 mg/l.; TChP, 0.1 or 0.02 mg/l.; PChE, 0.5 or 0.1 mg/l.) indicators were observed after 24 hr, and then after every 24 hr period until 360 hr. In the experiment with low concentrations (DChP, 0.009 or 0.0015 mg/l.; TChP, 0.0004 or 0.002 mg/l.; PChE, 0.019 or 0.0042 mg/l.), the investigations were done at later periods, starting with 25 days after the start of exposure and up to 86 days (after each 10 days).

At the end of the experiments a histological study was made of the lungs and liver; the content of the RNA and the activity of oxidizing enzymes in these organs were evaluated (SDH, NAD, and NADPH-diaphorase, DDG, T-6-PhDG), the ploidy of hepatocytes and the state of the cloudy-cell system of subcutaneous connective tissue; in the experiment with 1,2-DChP, an electron microscopic study of the lungs was made.

During the period of the experiment the weight gain of the animals was practically the same for both the experimental and the control groups. Differences in the content of erythrocytes, leukocytes and hemoglobin in the blood of experimental and control groups also showed no statistically significant differences.

The influence of DChP, TChP, and PChE on the central nervous system of animals was indicated by a change in the total threshold indicator, both with high concentrations and in some experiments with low concentrations. However, with a diminution in the concentration of substances, the period after which reliable changes in indicators appeared became longer. Thus, the changes in TTI ($p < 0.05$) from the action of TChP at a concentration of 100 mg/m³ appeared on the fifth day, similar to the results obtained for the action of the substance at a level of 2 mg/m³ on the 85th day after the start of the intoxication ($p < 0.05$).

TChP at a level of 800 mg/m³ induced change in the TTI after 24 hr of inhalation, while DChP and PChE in concentrations exceeding the TChP concentration by 2.5 to 3 times induced a similar effect much later, after 48 hr. The same trend was likewise observed in comparing the action of lower concentrations of these compounds. Of the substances studied, TChP probably exerts the most pronounced action on the excitability of the nerve centers. Short-term inhalation of DChP, TChP, and PChE induced a rather rapid (4–12 hr) change in the catalase and cholinesterase of blood in the direction of a rise in their activity.

However, it should be noted that upon the action of relatively high concentrations, a phasic nature of changes was observed: in the course of the first two to three days of the action, enzyme activity increased, and then dropped. We did not observe any similar picture upon prolonged action of low levels of concentrations; in these experiments only a rise in enzyme activity took place. High concentration levels probably quickly exhaust the adaptive possibilities of the organism, hence a reduction in the level of activity of these enzymes apparently should be adjudged a damaging reaction.

Experimental data on the time of occurrence of the first statistically reliable changes in the TTI, cholinesterase, and catalase are shown in Table 1. As the concentration level of DChP, TChP and

Table 1. Dependence of period of changes in indicators on the concentration level during intoxication of white rats with DChP; 1_{TChP}, and PChE.

Substance	Concentration, mg/m³	Time T_1 to appearance of cholinesterase changes, hr	Degree of reliability of T_1	Time T_2 appearance of change in catalase, hr	Degree of reliability of T_2	Time T_3 to appearance of changes in total threshold indicator (TTI), hr	Degree of reliability of T_3
DChP	2000	4	2.62	4	3.23	48	2.85
	1000	—	2.90	12	2.48	72	2.57
	500	12	3.06	24	2.64	96	2.73
	100	72	2.92	120	3.89	360	2.42
	9	600	—	2040	1.19	2040	3.36
TChP	800	4	3.06	4	2.59	24	3.39
	350	12	2.93	12	3.21	72	3.01
	100	24	2.00	48	3.81	120	2.46
	20	120	2.72	144	2.42	360	3.06
	2	960	4.06	1690	2.46	2040	2.73
PChE	2750	12	2.59	4	2.41	48	3.89
	1250	24	2.88	12	2.47	96	4.13
	500	72	3.04	—	—	168	3.08
	100	240	2.75	240	3.13	360	2.48
	19	960	2.86	1320	3.14	2040	2.43

PChE declined, the period of time before appearance of the first reliable changes in indicators increased. This made it possible to express graphically the dependence of the time of appearance of certain effects on the level of concentration of each active substance. On a logarithmic scale chart this dependence is linear (Figs. 1–3). In order to obtain coefficients for the equations of these curves, the data shown in Table 1 were handled by the method of least squares (*4*).

Morphological study of the organs and tissues of rats that had been subjected to the separate action of the substances under subacute experimental conditions made it possible to establish the irreversible character of alterations in the biosystems being analyzed. They are characterized by symptoms of destruction of vascular permeability in the lungs, intensification of lung desquamoproliferative processes, and activation of the macrophage system.

Upon intoxication of animals with DChP in a concentration of 2.0 mg/l., TChP at 0.8 mg/l., and PChE at 2.75 mg/l., suppression of the activity of oxidizing enzymes in the lungs was observed; action of DChP at a concentration of 1 mg/l., TChP at 0.35 mg/l., and PChE at 1.25 mg/l. induced activity of bioelectric processes in lung tissue. Under the action of low concentrations of the substances in the lungs, a rise in the level of activity of oxidizing enzymes is observed on a background of reactive changes in the alveolar and bronchial epithelium and an increase in the number of microphages.

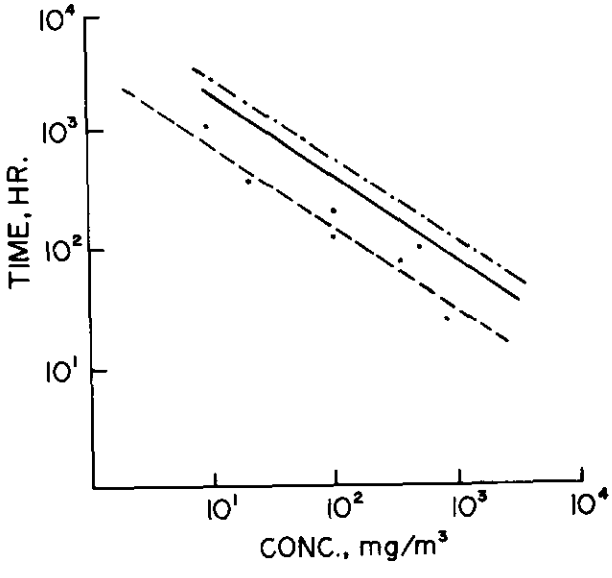

FIGURE 1. Dependence of time of appearance of changes in blood catalase activity of white rats on concentration levels during exposure with (——) DChP, (--) TChP, and (– · –) PChE.

Electron microscopic study of the lungs after action of DChP allowed discovery of the reactivity of the macrophage system (Fig. 4), edema in the branches of type 1 alveolar cells, and accumulation of osmiophilic corpuscles in alveolar cells (Fig. 5). Reactive changes were also noted in the endothelium of capillaries (Fig. 6). Study of liver products showed that at high levels of action from

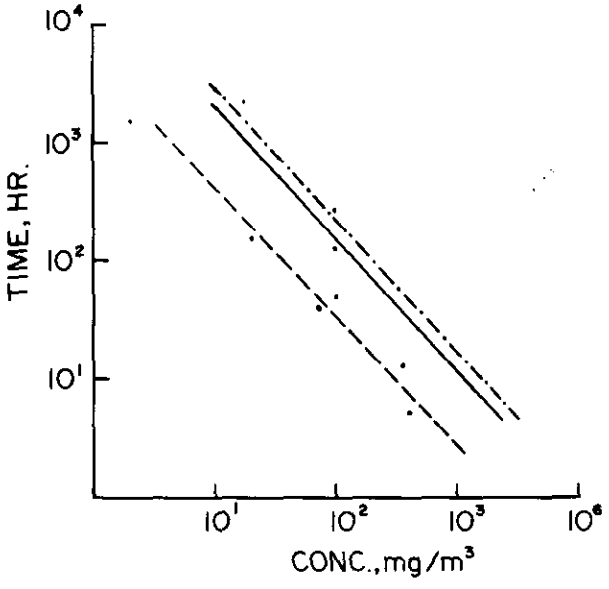

FIGURE 2. Dependence of time of appearance of change in TTI of white rats on the concentration level during exposure to (——) DChP, (--) TChP, and (– · –) PChE.

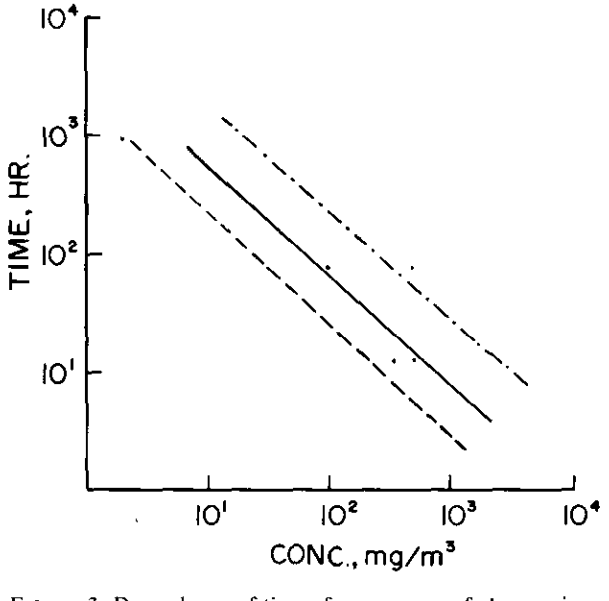

FIGURE 3. Dependence of time of appearance of changes in activity of rat blood acetylcholinesterase on the level of concentrations during exposure (——) DChP, (--) TChP, and (– · –) PChE.

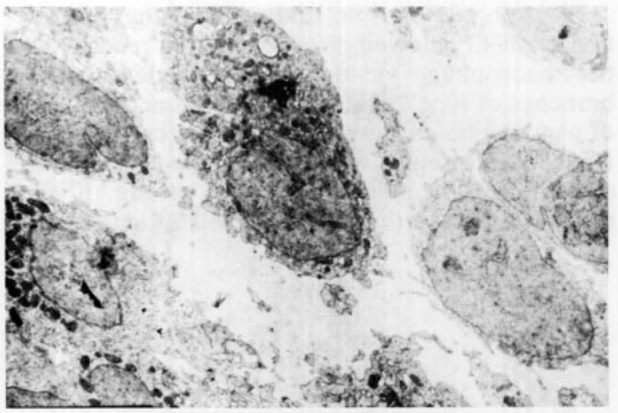

FIGURE 4. Macrophage reactions in lungs with exposure to DChP, 0.009 mg/l. 4000×.

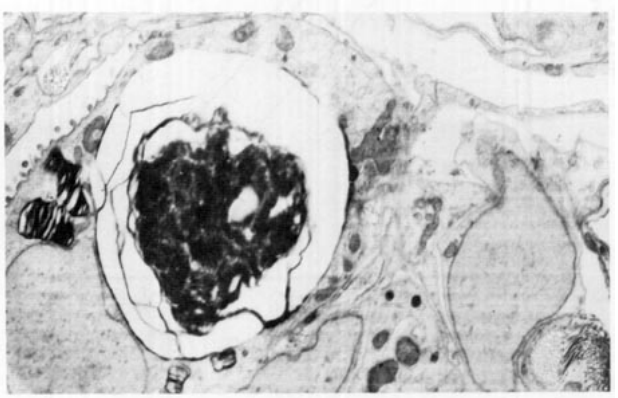

FIGURE 5. Typical osmiophilic corpuscle in Type II alveolar cells; exposure to DChP, 0.009 mg/l. 6000×.

the substances on a background of microcirculatory disruption, dystrophic alterations appear in the hepatocytes of central lobular portions of liver sections, with a reduction in the RNA they contain and suppression of oxidizing enzyme activity.

In the peripheral portions of liver sections the liver cells retain their structure and have a considerable RNA content and a high oxidizing enzyme activity. The glycogen content in the liver is noticeably reduced. From quantitative analysis of DNA it was established that DChP in concentrations of 1 and 2 mg/l. and TChP at 0.8 mg/l. produce changes in the short-term quantity of chromosome sets in the direction of increasing cells of higher ploidity. The same direction of action exists upon prolonged administration of the substances at low concentrations, but with a decline in the degree of prominence of the changes. Exposure to 0.0015 mg/l. DChP, 0.0004 mg/l. TChP, and 0.0042 mg/l. PChE results in a slight activation of the SDH and G-6FDG in the absence of structural changes. With an increase in the concentrations to 0.009 mg/l. DChP, 0.002 mg/l. TChP, and 0.019 mg/l. PChE, an insignificant decrease occurs in the RNA content and in the oxidizing enzyme activity in the central portions of liver sections, with a high enzymatic activity in the periphery (Fig. 7). Upon cytophotometry of DNA in the liver cells, an increase in ploidy is observed from the action of PChE at concentrations of 0.019 and 0.0043 mg/l., TChP at 0.002 mg/l. and DChP at 0.009 mg/l. Changes in the cloudy cell system as a result of high concentrations of the substances are characterized by statistically significant increases in the degranulation index (Fig. 8) and the appearance of degenerative forms breaking down into conglomerates (Fig. 9). Upon prolonged action of TChP at a concentration of 0.002 mg/l. and of PChE at 0.019 mg/l., statistically significant increases are also observed in the degranulation index of cloudy cells, but the degenerating structures are encountered considerably more rarely.

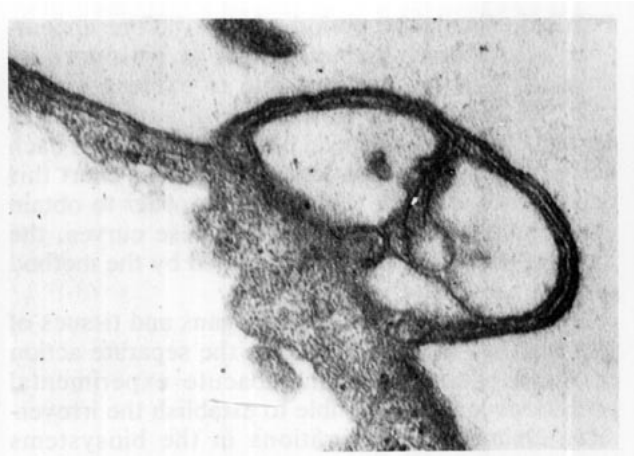

FIGURE 6. Swelling of cytoplasmic branching of endothelial cell in capillary; exposure to DChP, 0.009 mg/l. 32,000×.

Experiments evaluating the combined action of DChP, TChP, and PChE were performed on male white rats weighing 200-300 g. The conditions for exposure were the same as in the experiments studying the isolated action of each of the components. The experiments were carried out on six groups of animals and one control group (Table 2).

We did not produce a mixture of substances with a given concentration in the exposure chambers. The combination of substances, from relatively high levels to low ones, were formed at random. Even before the start of the experiment, the constancy of the mixture concentrations in each chamber was carefully worked out by means of special conditions (e.g., thermostatic control maintenance of the temperature of incoming air at a given level). The concentrations of DChP, TChP and PChE in the chambers were verified daily.

The results of the experimental investigations shown in Table 2 demonstrate that with exposure of

Table 2. Dependence of period of appearance of changes in cholinesterase, catalase, and TTI as a function of level of concentration in mixtures of substances.

	Substance		Cholinesterase	Catalase	TTI,
	Type	Concn, mg/m³	T_1, hr	T_2, hr	T_3, hr
I	DChP	680	—	4[a]	48[a]
	TChP	270			
	PChE	1200			
II	DChP	320	12[a]	12[a]	72[b]
	TChP	120			
	PChE	435			
III	DChP	190	24[a]	24[a]	120[b]
	TChP	35			
	PChE	210			
IV	DChP	35	120[a]	144[b]	360[a]
	TChP	70			
	PChE	50			
V	DChP	24	192[a]	360[a]	600[b]
	TChP	30			
	PChE	10			
VI	DChP	4.5	600[a]	1008[a]	1440[a]
	TChP	2.0			
	PChE	3.8			

[a] Significant, $p < 0.05$.
[b] Significant, $p < 0.01$.

animals to mixtures consisting of high concentrations of components (mixtures I-III in Table 2), shifts occurred rather quickly on the part of blood enzymes and TTI. It was considerably later that alterations appeared from the action of mixtures at low concentrations (mixtures IV, V, VI, Table 2). In the present investigations on the graphs of the concentration–time dependence (Figs. 1, 2, and 3), isoeffective concentrations were found; that is, concentrations which were found in the presence of separate action of 1,2-DChP, 1,2,3-TChP, and PChE induced a certain effect in the same time from beginning of the exposure as did the mixture of substances. This result was the same as that determined by the parameters of acute toxicity.

We illustrate the method by detailing the calculation of the indicator of total concentration of mixture I from a graph for the dependence of the time of change in catalase activity on the concentrations of DChP, TChP and PChE. From the graph of the concentration–time dependence (Fig. 1) we find the isoeffective concentrations of DChP, TChP, and PChE which resulted in a change in catalase activity under the separate action of each component 4 hr after the start of intoxication to be DChP, 2250 mg/m³; PChE, 2800 mg/m³; TChP, 920 mg/m³. We determine (by Finn's method) the relative proportions of each component of the mixture from the corresponding ones in the isoeffective ones. We define a series of content ratios C: 680 mg/m³ DChP/2250 mg/m³I, $C_{DChP} = 0.30$; 270 mg/m³ TChP/920 mg/m³I, $C_{TChP} = 0.29$; 1200 mg/m³ PChE/2800 mg/m³I, $C_{PChE} = 0.43$.

We calculate the coefficient of combined action C_{ca} by

$$C_{ca} = C_{DChP} + C_{TChP} + C_{PChE}$$
$$= 0.30 + 0.29 + 0.43 = 1.02$$

The coefficient of combined action (C_{ca}) which represents the total magnitude of relative concentrations of substances which, upon joint action of animals induce an effect 4 hr from the start of intoxication similar to the effect produced by separate action of the components is equal to 1.02.

Similar calculations were carried out for all mixtures of substances, and the coefficients of the combined action of mixtures (according to the indicators) are shown in Table 3.

It is well known that upon the combined action of several substances in the case of an additive effect, the coefficient of the combined action is equal to 2; in the case of intensification (potentiation) it is less than 2, and with weakening of the effect (antagonism) it is greater than 2.

As can be seen from the results of our investigations, the coefficient of the combined action of DChP, TChP and PChE is close to 1. Consequently, it seems, one may assume that in their simultaneous presence, the nature of the action of these substances is of the additive type. The results of morphological study have also shown that the action of a mixture of the substances at high concentrations does not lead to the appearance of theoretically new reactions and, by the strength of its action, it can be adjudged as an effect of combination.

If the effect of the action is analyzed according to the levels of the acting mixtures, then it should be

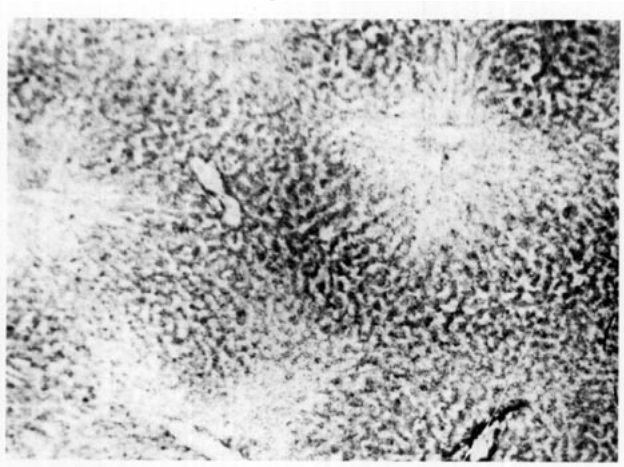

FIGURE 7. Decrease in activity of SD2 in central-lobular portions of liver during maintenance of high enzyme activity in portal zone; exposure to DChP, 0.002 mg/l.; Nakhlas method; 150×.

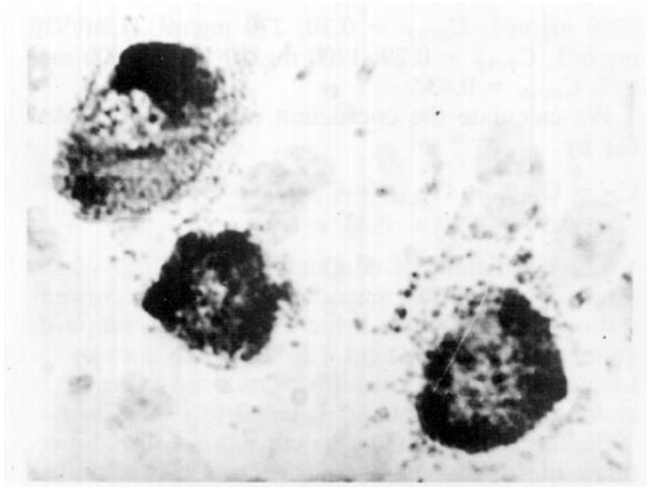

FIGURE 8. Degranulation of cloudy cells; exposure to TChP, 0.002 mg/l. Toluidine blue, 1350×.

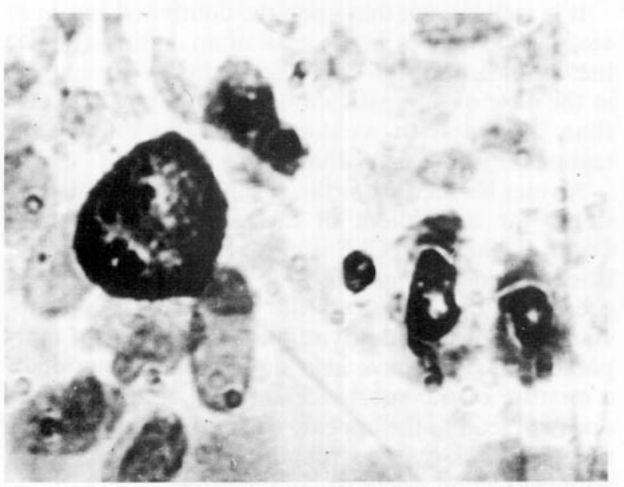

FIGURE 9. Degenerative changes in cloudy cells; exposure to TChP, 0.002 mg/l. Toluidine blue, 1350×.

noted that at the level of high concentrations (mixtures 1 and 2) the action of the mixture on the TTI was expressed somewhat more weakly than in experiments with lower concentrations. The coefficients of combined action are about the same, when computed by the changes in blood catalase and cholinesterase activity.

Conclusions

Evaluation of the effects of continuous inhalation of DChP, TChP and PChE in experimental animals results in a concentration–time dependence which is linear when plotted logarithmically.

For a correct evaluation of the nature of the combined action, the appearance of isoeffective concentrations of components is necessary; this may be achieved by means of graphs of the concentration–time dependence.

The nature of the combined effect of DChP, TChP, and PChE takes place in accordance with an additive effect.

The effects of the action of mixtures at a level of high and low concentrations do not differ theoretically; this is probably correct for chemical compounds with a unidirectional toxic action on the organism.

To extrapolate the effect of combined action from large concentrations to low ones, accumulation of experimental data on other groups of chemical compounds is necessary, to the extent that the possibility of forecasting on the basis of short-period experiments will accelerate evaluation of the combined action of atmospheric pollutants.

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