Verh. Deutsch, Ges. Arbeitsmed. 18(1978)

Special issue from the annual report for 1978

Possibilities and limitations of biological monitoring.

Industrial Hygiene problems of the services trades.

Industrial Medicine colloquium

Report from the 18th annual meeting of the German Society for Industrial Medicine jointly with the Union of the technical professional societies. Frankfurt am Main-Höchst May 24 through 27 1978.

Pharmacokinetics and Carcinogenesis of Vinyl Chloride. Industrial Medicine risk evaluation.

A Butcher, H.M.Bolt*, J. Filser*, H.W. Goergens, R.J. Laib* and W. Bolt. Institute and Polyclinic for Industrial and Social Medicine from the University Of Cologne.
* Toxicology Institute from the University of Tübingen

The alkylation of nucleic acids by the vinyl chloride-metabolite and the ensuing damage to the base pairing mechanism are the likely explanations for the carcinogenesis of vinyl chloride((1,2,16,17); compare Laib,Bolt,Butcher,Kappus,Bolt, Verh .Dtsch.Ges.Arbaitsmed, 17 year, page 243-250,1977)

The pharmacokinetics of vinyl chloride on rats was already evaluated by our work team (4). Figure 1 presents the experimental devices and the pharmaco kinetic models used. Vinyl chloride is rapidly picked up from the atmosphere through the lungs (figure 2). There is an equilibrium between the vinyl chloride concentration in the exposure atmosphere and the organism which is expressed by the equilibrium constant

$$K = \frac{\text{amount of VC per g. of body weight}}{\text{amount of VC per ml of air}}$$

In the body the vinyl chloride is eliminated via metabolic processes. In order to evaluate the risk involved in the inhalation of vinyl chloride one needs to determinethe rate of vinyl chloride pick up and the rate rate of its metabolic elimination.

Figure 1: Pharmaco-kinetics of vinyl chloride by exposure in a closed system. Model by Bolt and coll(4) This model includes the metabolism of vinyl chloride. The constants were determined with experimental animals exposed to vinyl chloride initial concentrations below the 250 ppm level.

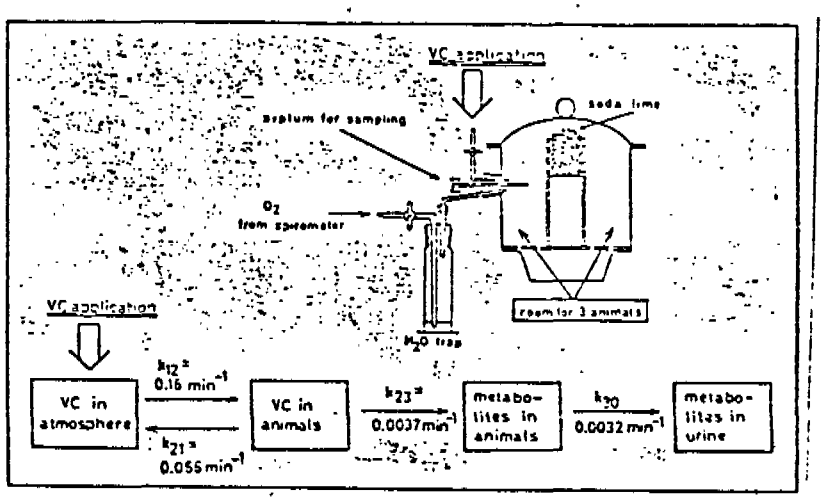
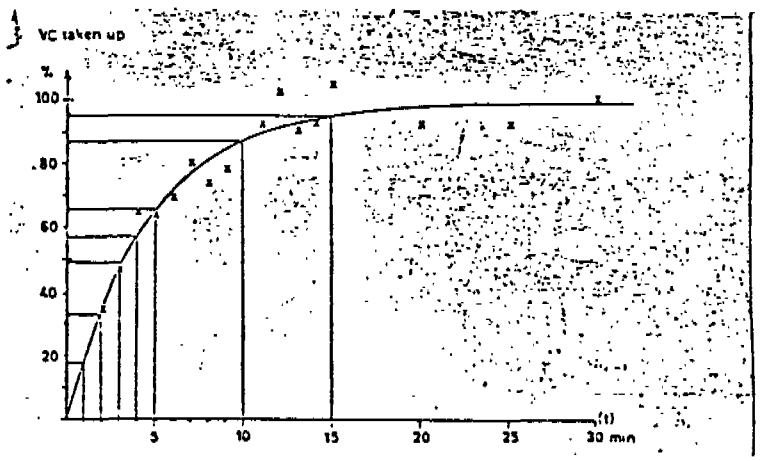


Figure 2: Pick up of gaseous vinyl chloride by rats which were previously treated with an inhibitor for the metabolism of vinyl chloride (6-nitro-1,2,3-benzothiadiazol, see Bolt and coll. (4)). The level of vinyl chloride which is attained at equilibrium between the vinyl chloride in the gas phase and in the experimental animals, is represented as 100%.



Comparative research of the vinyl chloride metabolism in experimental animals.

The extraordinary importance of the pharmaco-kinetics of vinyl chloride in humans led us to further experimental investigations on various animal species in order to improve the calculation of the vinyl chloride metabolism in humans and arrive to a better evaluation of the self-experiment. The following animals were employed in the investigation: 11 male white mice (Swiss mice) 380 g. altogether, 54 to 56 days old; 4 mongolic Rennmäuse (meriones unguiculatus), altogether 240 g;male rabbit, 3.2 kg. (The animlas came from Ivanovas,Kissleg,Allgäu)

Vinyl chloride was kindly supplied by the Dynamit Nobel AG, Troisdorf (Degree of purity according to data supplied by the firm, 99.995%)

The vinyl chloride concentration in the exposure atmosphere was determined by gas chromatography (Model no. 1440-1, Varian, Darmstadt). The samples were obtained with a disposable syringe and was injected into the gas chromatograph through a gas sampling valve provided with a 5 ml. sample loop. The column temperature was 150°C(steel column 3m long 1/8'', Poropak Q, 50-89 mesh), the temperature at the detector was 260°C. The hydrogen gas flow was 30 ml/min, 300 ml/min of synthetic air and 60 ml/min of nitrogen. The retention time for vinyl chloride was 2.15 min.

The addition of vinyl chloride was performed by way of a dosification sample loop through a septum in the exposure container. /Similarly to early experiments with rats, the corresponding initial concentration ranged about 50 ppm. The length of each experiment varied between 2.5 and 4 hours.

Table 1 shows the results of the experimental studies on the various animal species. The half-life time and the corresponding constant were determined for the "Invasion"(intoxication ?) for the initial time of the distribution of vinyl chloride in the organism until the establishment of the equilibrium. The elimination constant, the half-life time and the clerance were determined for the mtabolic processes of vinyl chloride. The clearance of vinyl chloride based on the body weight was first used in a comparison of the metabolizing rates of vinyl chloride for the different species. A further discussion together with the experimental results with humans. follows.

Investigation on the metabolism of vinyl chloride in humans.

Vinyl chloride is a carcinogenic material which will be used in the future for economical reasons. Therefore, it is the obligation of the Industrial Medicine to reduce the risk,on the involved worker, to a minimum, especially using early diagnostic methods for the identification of the Hämangio endothelum of the liver which is characteristic of tumors produced by vinyl chloride. The vinyl chloride limit concentration in the technique is the average yearly value for a work involving 8 hours per day and 40 hours per week, and the vinyl chloride concentrations allowed per day may increase as much as 3 times(using an hour as an average value). This leads to the main question of how to evaluate sporadic short-lived peak concentrations. Therefore, it will be of great significance that the technical limiting concentration, expressed as yearly average, allowed for sensibly higher short lived concentrations. The previously obtained data on experimental animals cannot be applied to humans unless they undergo previous treatment.

On the other hand, we cannot overlook the need for an exact evaluation of the risk involved. Thus, the following studies of the metabolism of vinyl chloride among humans in selfdeterminations were required. On the basis of the preceding studies on experimental animals the approximate order of magnitude was calculated for the expected for the vinyl chloride metabolism for humans. For these purposes one developed investigation devices which could provide extensive information on the distribution and rate of mtabolism of vinyl chloride in humans, without surpassing the limits of technical concentration and using the shortest possible exposure times. The studies were carried out according to the rules and regulations of the professional association for safety measures to protect the health in an environment containing vinyl chloride. They called for short-lived or incidental exposures to vinyl chloride which will "fail to produce effect". When there is an incident at a working place where there is a high exposure level to vinyl chloride, it may be expected to occur over the years in larger amounts.

Research Methodsfor vinyl chloride-metabolites in humans.

The studies were carried out in both, open and closed exposure systems. The closed system consisted in a "spirograph" with post addition of oxygen to achieve horiaontal stabilization.(Type 198,Dargatz, Hamburg). The volume of the system was determined using a standard gas. An initial concentration of 10 ppm of vinyl chloride resulted after the addition of a given defined amount of vinyl chloride. After the preceding test with an empty probe, the inhalative exposure began. The volume of the spirograph system was kept constant using the oxygen post addition. A small air probe was removed from the spirograph at every minute and its concentration in vinyl chloride was determined by the same method used in the studies with experiemental animals: gas chromatography,

(The condition of the measurements vary very little: Steel column 6m, 1/8", filled with 100/0 SE 30 out of Chromosorb WAW/DMCS,100/120 mesh, column temperature 40°C,Nitrogen 30ml/min synthetic air 300 ml/min and hydrogen 30 ml/min, retention time for vinyl chloride 2.65 min).

For the inhalation studies in the open system an apparatus was deviced to allow , for a constant inspiratory vinyl chloride concentration, the simultaneous measurement of the expiratory vinyl chloride concentration and the volume breathed per minute. Based on the system of the classic Respirations-Gasuhr (Max-planck-Institut, Dortmund) the in- and expiratory parts of the apparatus were separated by a mouth piece valve. The inspiratory apparatus consisted of a glass lined inlet and mixing part

for synthetic air and for a mixture of air and vinyl chloride. It also consisted of a gas bag (Linde, Unterschleissheim), and a respiratory bag with a capacity of 3 l.(Dräger,Lübeck). Synthetic air was supplied into the apparatus by a gas pump in a previously established quantities and in a continuous fashion. At the same time, the air-vinyl chloride mixture, contained in a gas bag, was added to the system using a previously calibrated pump (Auer-toximeter,Auer-Gesellschaft,Berlin). The mixture of air and vinyl chloride was obtained by evacuating the gas bag and then filling through a spirograph and additional introduction of vinyl chloride. The overall ratio of the mixture gave an inpiratory vinyl chloride concentration of about 2.55 ppm relative to the air. Attached to the expiratory part of the mouthpiece valve,there was a three necked flask (2 l. capacity) which functioned as mixing chamber. The size of the mixing chamber proved to be quite appropiate, because it guaranteed an extensive and thorough mixing of the expiratory air and allowed for a a rapid evaluation of concentration changes. The expiratory volume was registerd by a gas meter, and finally exhausted into a hood. The inhalation air samples were obtained from the mouth piece, the samples from the expiration gas were obtained from the mixing chamber, through a septum. The evaluation of the vinyl chloride concentration was carried out in the following manner.

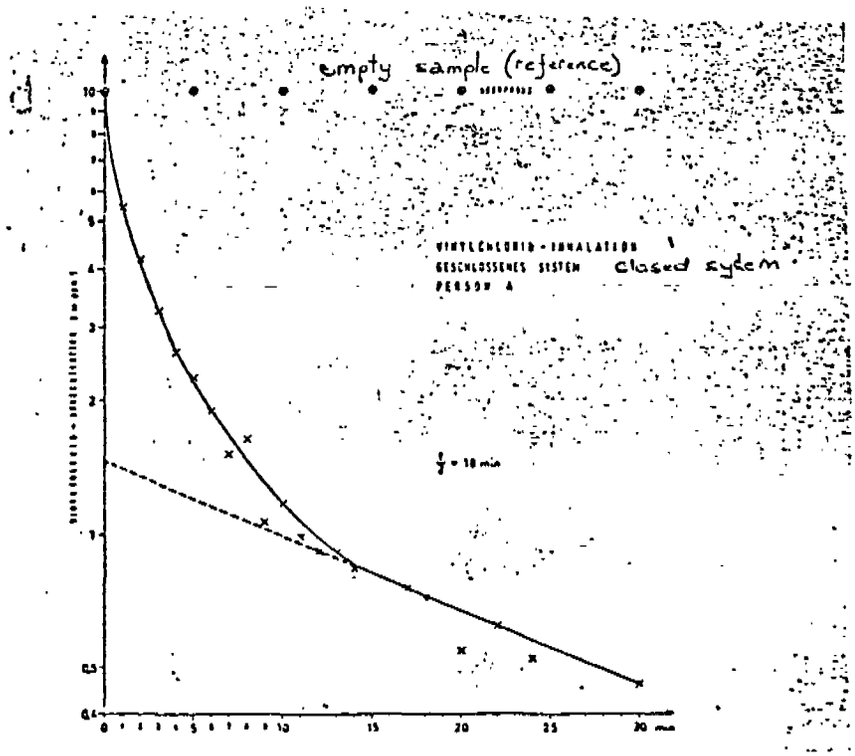
Kinetics of the distribution and metabolization of vinyl chloride in humans.

There is a decrease in the vinyl chloride concentration in the spirograph at the beginning of the inhalation, due to the fact that the mixture of the vinyl chloride-containing air with the air remaining in the lungs of the subjectunder study. Similarly to the test for the functioningof lungs by the analysis of foreign gases, one is assuming that this thorough mixing step, lasts from two to 3 minutes in both subjects under research. Occurring at the same time but taking longer time, there is the phase of equilibration between the vinyl chloride in the exposure atmosphere and the vinyl chloride concentration in the organism of the subject. The metabolic process of vinyl chloride throughout the organism is also taking place. When one compares the present study with the previous research on experimental animals, the ratio between the volume of the exposure system and the body volume undergo a marked change. The body volume is now, about 10 times the volume of the exposure system.

Figures 3 and 4 show the decrease in the vinyl chloride concentration in the spirographs from persons A and B. After 1 minute the vinyl chloride concentration in the spirograph,for person A, is already 53% and it is 56% for person B, relative to the initial concentration.

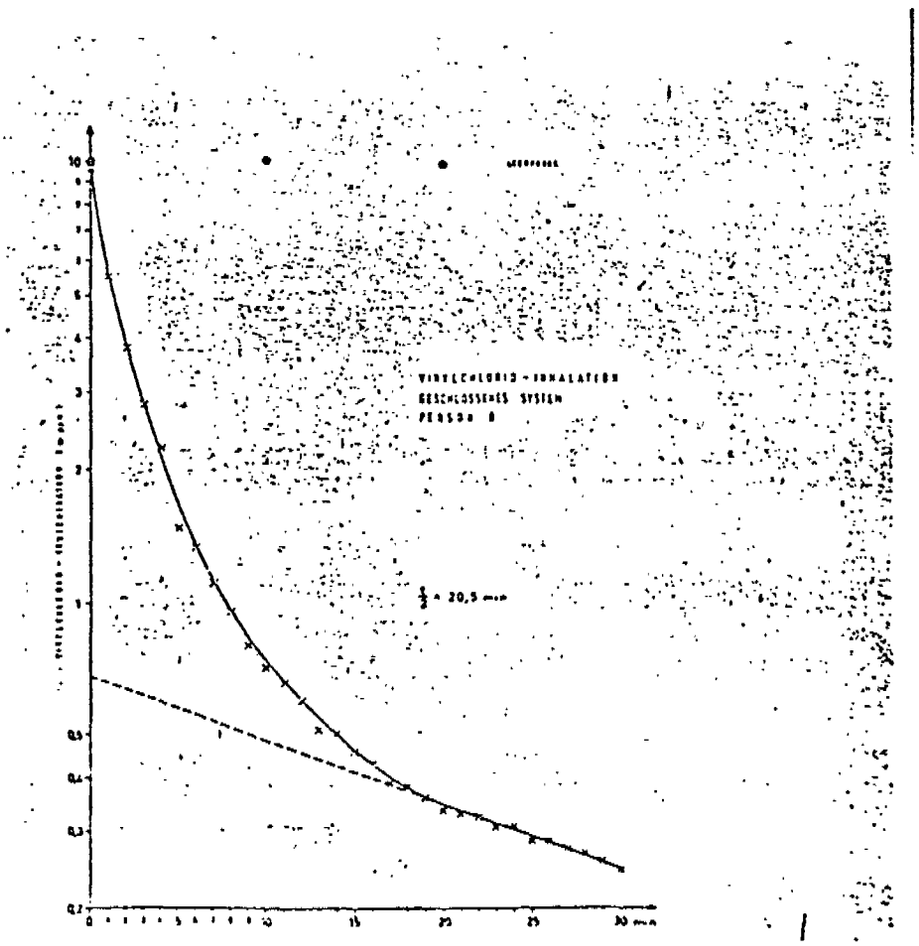
After 5 minutes the decrease brought the levels down to 21% and 15% of the initial concentration. If one plots the vinyl chloride concentration ina logarithmic scale and uses the linear scale for the time of exposure (figures 3 and 4), the curve describing the decrease in the vinyl chloride concentration in the spirograph system becomes a straight line after 15 minutes, for person A, and after 19 minutes for person B. The ensuing decrease in concentration takes place according to a first order kinetics. The decrease of the concentration with time is compatible with the assumption of an enzymatic metabolic process. Similarly to the previous studies on experimental animals regarding the pharmaco kinetics of vinyl chloride with or without inhibition of the oxidative metabolite, one can state that the decrease on the vinyl chloride concentration, as indicated above, can be attributed to a metabolic process of vinyl chloride throughout the organism below the saturation point. The half life for the rate of the metabolic process of vinyl chloride in the selected system amounted to 18 min (o.3 hr) for person A and 20.5 min (0.34 hr) for person B.

Fig.3.- Decrease in the vinyl chloride concentration in a closed spirograph system by inhalative vinyl chloride pick-up, for person A.



In order to achieve a better comparison, the clearance for both persons was calculated (see table 1). When the straight line describing the decrease in vinyl chloride concentration, in figures 3 and 4, is extrapolated to time 0, one obtains the vinyl chloride concentration that would exist in the spirograph by momentary attainment of equilibrium (between the vinyl chloride in the exposure atmosphare and the vinyl chloride in the organism). Using this concentration, it was possible to calculate a total distribution volume of 57.6 with person A. After drawing off 7 l. from the volume of the spirograph and approximately 1 residual volume of the lungs, the claculation gave a distribution volume of 49.6 l for person A. The vinyl chloride clearance for person A amounted 1.83 l x min^{-1} and 1.74 l x h^{-1} x kg^{-1}, respectively.

Figure 4: Decrease in the Vinyl chloride concentration in a closed spirograph system by inhalative vinyl chloride pick-up in person B.

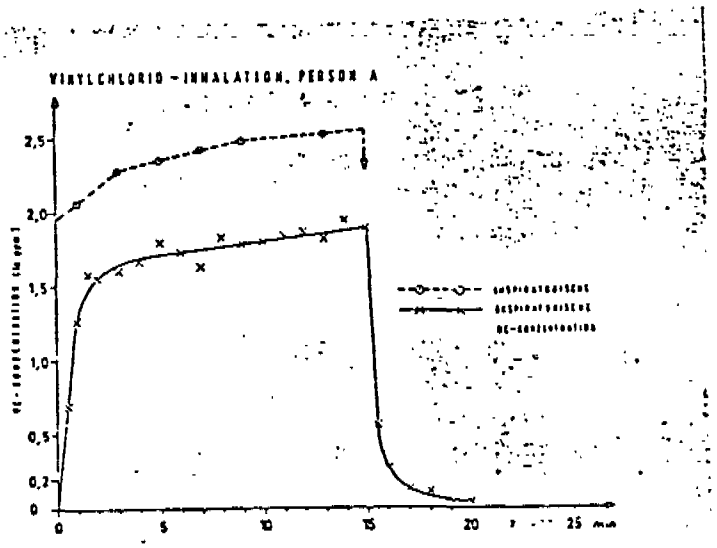


For person B, the total distribution volume amounted to 92.5 l and the corresponding distribution volume 84.5 l. The vinyl chloride clearance amounted to 3.36 l x min^{-1} and 2.43 l x h^{-1} x kg^{-1}, respectively.

In agreement with the requirements of the pharmaco kinetic model for vinyl chloride, the "equilibrium constants" were calculated. They amounted to 0.62 for person A and 1.1 for person B. For the initial phase of the vinyl chloride pick-up, the calculated half-life was 1 minute and 38 seconds for person A and one minute and 40 seconds for person B.

During the inhalation of a constant vinyl chloride concentration in an open system (figures 5 and 6), a constant expiratory vinyl chloride concentration was obtained after about 5 minutes for person A and after 7 minutes for person B. Already after 1 minute, the expiratory concentration attained 3/4 of the value measured later, when the expiratory equilibirium is attained. After the initial distribution of vinyl chloride in the organism, a constant percent of the vinyl chloride present in the inhalation is eliminated through the organism. The figures are, 26% for person A and 28% for person B. The vinyl chloride clearance amounted to 2.4 l/min for person A and 2.5 l/min for person B. On the basis of the preceding data on experimental animals, one can deduce that the constant difference between the inspiratory and expiratory vinyl chloride concentration, which exists after the first distribution phase of the elimination process, can be attributed to metabolic changes.

Figure 5.- Inspiratory and expiratory vinyl chloride concentration in person A. Inhalative vinyl chloride exposure in a open system.

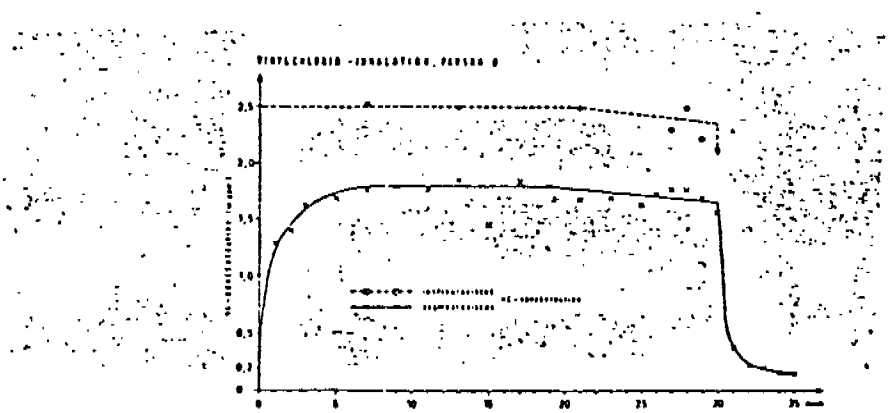


Besides, figures 5 and 6 show that the inhalative pick-up of vinyl chloride at the beginning, and the elimination of vinyl chloride at the end of the exposure, take place in a very rapid fashion. After one minute exposure to vinyl chloride, 74 to 70% of the expiratory vinyl chloride concentration was reached. A minute after the end of the vinyl chloride exposure, and breathing the room air, only 15 to 22 % of the previous expiratory vinyl chloride concentration was detectable.in the expiratory breathing gases.

Discussion of the pharmaco kinetics of vinyl chloride in humans and in experimental animals.

The basic results obtained in the studies on the metabolization in various animal species and among humans are comp iled in table 1. The data on rats is obtained from our previous studies, already published (3,4). The studies on the various animal species and on humans were performed in closed exposure system using a definite initial concentration of vinyl chloride. In the last column of the table appears the research results with humans using the inspiration of a constant amount of vinyl chloride. The selected vinyl chloride concentrations correspond to actual situations in the working place for PVC polymerization and are within the limits of the presently enforced limit concentration for vinyl chloride.

Figure 6.- Inspiratory and expiratory vinyl chloride concentration for person B. Inhalative vinyl chloride exposure in an open system.



The initial phase of the distribution of vinyl chloride in the organism is defined by the establishment of a physical equilibrium between the vinyl chloride concentration in the organism, referred to the body weight, and the vinyl chloride concentration in the exposure atmosphere.

Said "equilibrium constant" amounted to 2.86 in rats, 0.91 in rabbits, 0.62 in person A and 1.1 in person B. The differences between the various animal species and various persons can be attributed to a different content in fatty tissue. As we have previously shown (6), after the physical equilibrium is established, the concentration of vinyl chloride is ten times higher in the fatty tissue than in blood or in other organs. When studying comparable persons, not suffering from overweight, and after the establishment of the physical equilibrium, one can assume as standard value the figure of 80%, that is, 80% of the vinyl chloride concentration in the air, will be found in the organism, expressed relative to the body weight. Both in animals as well as in humans, the establishment of the physical equilibrium takes only a few minutes.

In the studies in closed systems with rats and rabbits, the half-life value for the rate of "invasion" amounted to approximately 3 minutes and for men about 1.5 to 2 minutes. This means that in humans, 2 minutes after the exposure the organism has, already, one half of the expected vinyl chloride concentration that would result when the equilibrium is reached. Accordingly, the distribution phase, for constant inspiratory vinyl chloride concentration, was completed after 5 minutes for person A and after 7 minutes for person B.

Studies on rats have shown (3,4) that the saturation point for the elimination of vinyl chloride from an exposure atmosphere is about 250 ppm with healthy animals. It is assumed that there is a saturation of the interested enzymatic systems (13). With isolated, perfused rats livers, on the contrary, no saturation point was found for the enzymatic system. (14). An unequivocal interpretation for the divergence of these findings cannot be given at the present time. The present studies were carried out for air-vinyl chloride concentrations in the neighborhood, or better within the range of 0.25 to 0.50 ppm. It is then assumed that the elimination of vinyl chloride from the exposure atmosphere took place below the saturation point of the enzymatic system relative to vinyl chloride. The metabolization rate of vinyl chloride showed different values for the individual animal species and for humans. The values for experimental animals and for humans, can also be compared using different research devices, via the calculation of the clearance for vinyl chloride per kg of body weight.

The greatest clearance for vinyl chloride was shown by mice with 25.6 $l \times h^{-1} \times kh^{-1}$, followed by the desert mouse with half that amount.

The clearance value for vinyl chloride for rats amounted to 8.4 l xh^{-1} x kg^{-1}, for rabbits it was 2.74 of the same units. Among humans there was good agreement between the data obtained from various study devices used with both persons. The mean value of two studies on both persons gave a clearance for vinyl chloride of 2.02 l x h^{-1} x kg^{-1}. This means that vinyl chloride metabolizes the slowest in humans. The vinyl chloride clearance for rats, in which most of the studies on the pharmaco kinetics of vinyl chloride were performed, is about 4.3 times the value for humans. On this regard one must consider, that the liver in rats (male Wistar-rats) exhibits a content in enzyme cytochrom P-450 which is twice as high as in humans. Based on the studies of induction and inhibition of the vinyl chloride metabolite in rats, it is possible to deduct that vinyl chloride is metabolized through the cytochrome P-450 system.

The toxicity of vinyl chloride is due to the poisoning produced by its metabolic transformation in the organism. Also, the amount of metabolized vinyl chloride depends upon the amount contained in the dosis. Therefore, it is convenient to run the exposure to vinyl chloride at very low concentration over long periods of time. According to Henschler (14) this approach has a better chance for an enzymatic detoxication, inactivation by coupling onto glutathion, than that consisting of intermitent exposure to short and strongly increased vinyl chloride concentrations. The safety provisions for the protection of health in a vinyl chloride environment, as allowed by the professiona associations of the chemical industry, in force since July 1st, 1977, do establish that an automatic alarm should be sounded, at the latest, 5 minutes after a vinyl chloride concentration of 40 ppm has been surpassed. The people concerned, for example, must immediately leave the autoclave room or carry protective respiratory devices. This regulation is apparently originated from the premise that the pick up of vinyl chloride by the organism and its subsequent distribution require a longer time. However, the studies that we carried on the pharmaco kinetics of vinyl chloride in humans show that, after 5 minutes, the distribution phase of the inhalated vinyl chloride is virtually completed. The release of a warning for the concerned individual, five minutes after the appearance of a high vinyl chloride concentration, comes from the assumption the assumption that peak concentrations are dangerous, a concept maintained for a long time. On the basis of new findings from studies with humans, it is recommended to limit to one minute the effective time for a peak vinyl chloride concentration. For the daily practice it means that , immediately after the recording of a higher than normal vinyl chloride concentration in a measuring device, a warning must be given to the co-workers because....

Table I.- Studies on the pharmaco kinetics of vinyl chloride on experimental animals and on humans.

Columns

Rats Rabbits Mice desert mice / persons: mean value

Rows: Exposure system
Initial concentration of vinyl chloride in ppm
End concentration of vinyl chloride in ppm

Distribution phase:
Duration (min)
Half-life(min)
Equilibrium constant K

Metabolization:
Half-life (min)
Elimination constant $k_{23}(h^{-1})$
Clearance: 1 x min^{-1}
1 x h^{-1}
1 x h^{-1} x kg^{-1}

	I Ratten 0.25 kg	II Kaninchen 3.2 kg	III Maus 0.30 kg	IV Wüsten-maus 0.24kg	Person A 63 kg	Person B 83 kg	Person A	Person B	Mittel-wert A u.B
Expositionssystem (l)	10.3	58.5	10.2	10.2	7.0	7.0	offenes Syst.		
VC-Anfangskonzentrat. (ppm)	50	50	50	50	10	10	2.5	2.5	
VC-Endkonzentration (ppm)	1.5	25	2	20	0.5	0.25	2.5	2.5	
Verteilungsphase :									
Dauer (min)	15	30	30	15	15	19	5	7	
Halbwertszeit (min)	3.2	3.4			1.6	1.7			
Gleichgewichtskonstante K	2.86	0.91			0.62	1.1			
Metabolisierung :									
Halbwertszeit (min)	67.8	277	44	143	18	20.5			
Eliminationskonstante $k_{23}(h^{-1})$	0.61	0.15	0.945	0.29	2.31	2.03			
Clearance : (l min^{-1})					1.83	3.36	2.8	2.25	2.46
(l h^{-1})	6.3	8.78	9.73	2.936	109.5	201.7	144	135	148
(l h^{-1} . kg^{-1})	8.8	2.74	25.6	12.48	1.74	2.43	2.29	1.63	2.02

[15] *Kappus, H., H. M. Bolt, A. Buchter, W. Bolt:* Rat liver microsomes catalyse covalent binding of ^{14}C-vinyl chloride to macromolecules. Nature 257, 134–135 (1975).

[16] *Kappus, H., H. M. Bolt, A. Buchter, W. Bolt:* Liver microsomal uptake of ^{14}C-vinyl chloride and transformation to protein alkylating metabolites in vitro. Toxicol. Appl. Pharmacol. 37, 461–471 (1976).

[17] *Laib, R. J., H. M. Bolt:* Alkylation of RNA by vinyl chloride metabolites in vitro and in vivo: Formation of 1-N^6-ethenoadenosine. Toxicology, im Druck.

[18] *Leibman, K. C.:* Current literature: Metabolism of vinyl chloride. Drug Metab. Disposition 5, 93–94 (1977).

[19] *Müller, G., M. Spassowski, D. Henschler:* Metabolism of trichloroethylene in man. II. Pharmacokinetics of metabolites. Arch. Toxicol. 32, 283–295 (1974).

[20] *Norpoth, K., G. Müller, U. Witting, D. Gottschalk, J. Gottschalk:* Untersuchungen über den Stoffwechsel des Vinylchlorid und über Wirkungen der Vinylchlorid-inhalation auf Regulationsmechanismen des Leberstoffwechsels. Verh. Dtsch. Ges. Arbeitsmed. 16, 187–194 (1976), (A. W. Gentner Verlag, Stuttgart).

[21] *Potter, H. R.:* Articles of general interest: Vinyl chloride Fd. Cosmet. Toxicol. 14, 347–349 (part I), 498–501 (part II) (1976).

[22] *Reynolds, E. S., U. T. Moslen, S. Szabo, R. J. Jaeger, S. D. Murphy:* Hepatotoxicity of vinyl chloride and 1,1-dichloroethylene. Am J. Pathol. 81, 219–236 (1975).

[23] *Van Duuren, B. L.:* On the possible mechanism of carcinogenic action of vinyl chloride. Ann. N. Y. Acad. Sci. 246, 258–267 (1975).

[24] *Watanabe, P. G., G. R. McGowan, P. J. Gehring:* Fate of ^{14}C-vinyl chloride after single oral administration in rats. Toxicol. Appl. Pharmacol. 36, 339–352 (1976).

[25] *Watanabe, P. G., J. D. Young, P. J. Gehring:* The importance of non-linear (dose-dependent) pharmacokinetics in hazard assessment. J. Env. Pathol. Tox. 1, in press.

[26] *Withey, J. R.:* Pharmacodynamics and uptake of vinyl chloride monomer administered by various routes to rats, J. Toxicol. Env. Hlth. 1, 381–394 (1976).

References of the author:
Dr. A Buchter M.D., Dr in industrial medicine
Institute and polyclinic for industrial and
Social medicine of the University of Cologne
Joseph-Stelzman Str. 9,5000,Köln(Cologne)41.

Anschrift der Verfasser:
Dr. med. A. Buchter, Arzt für Arbeitsmedizin, Institut und Poliklinik für Arbeits- und Sozialmedizin der Universität zu Köln, Joseph-Stelzmann-Str. 9, 5000 Köln 41