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The Distribution of 1,2-¹⁴C-Vinyl Chloride in Rat Tissue

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Summary. Rats have been pretreated with 6-nitro-1,2,3-benzothiadiazole which completely blocks metabolism of vinyl chloride. If the animals are exposed to atmospheric vinyl chloride, formation of an equilibrium between the compound in the gas phase and in the animal's organism is observed. Unmetabolized vinyl chloride is concentrated in adipose tissue. The distribution pattern of vinyl chloride in different organs of the rat is constant over the concentration range of 25 - 10,000 ppm of vinyl chloride in the exposure atmosphere. Distribution of metabolites of vinyl chloride contrasts to that of the original compound, metabolites primarily are concentrated in liver and in kidneys.

Key words: Vinyl chloride - Distribution in organs - Inhibition of vinyl chloride metabolism

Introduction

Since the time that it was demonstrated that vinyl chloride has to be categorized in the series of carcinogens, there has been an increased interest in questions of distribution and metabolism of vinyl chloride in the organism. Many authors showed (Hefner et al., 1975; Green & Hathway, 1975; Radwan & Henschler, 1975; Kappus et al., 1975; Watanabe et al., 1976 a,b; Norpoth et al., 1976) that in vivo and in vitro

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vinyl chloride metabolizes very quickly. The biotransformation leads to polar metabolites which leave the organism principally in the urine.

Hefner et al. (1975) was able to show that in rats the consumption of vinyl chloride from the atmosphere is inhibited by substances which inhibit the metabolism of vinyl chloride. This means that after the initial setting up of an equilibrium between vinyl chloride in the air and vinyl chloride in the organism the substance can only be absorbed to the degree that it is metabolized.

Recently we found that a pretreatment of rats using 6-nitro-1.2.3-benzothiadiazol, a potent inhibitor of oxidative drug metabolism, completely blocks the degradation of vinyl chloride in vivo (Bolt et al., 1976). This puts us in a position of being able to study the precise tissue distribution of nonmetabolized vinyl chloride under equilibrium conditions.

Knowledge concerning tissue distribution and metabolism of vinyl chloride in an animal test is of interest in the establishing of a technical guideline concentration for vinyl chloride as well as for the evaluation of the health risk with varying vinyl chloride exposures. With regard to lipophilic properties of vinyl chloride, known from chemistry, in the initial drafting of the trade union principles for occupational medicine, precautionary investigations, (section called) "danger due to vinyl chloride", as a precautionary measure overweight persons were designated as "not suited".

The present article now shows in an animal test that vinyl chloride has a very great affinity for fatty tissue. This contrasts with the behavior of metabolites which appear primarily in the liver and kidneys.

Experimental Section

Material

1,2-¹⁴C-vinyl chloride was synthesized by the radio chemistry department of Farbwerke Hoechst AG, Frankfurt. Financial support was cordially made possible by the Dynamit Nobel AG, Troisdorf.

(Inactive) vinyl chloride was cordially made available to us by the Wacker-Chemie Company, Köln.

6-nitro-1.2.3.-benzothiadiazol was made made available by Dr. C.F. Wilkinson, Cornell University, Ithaca, N.Y.

Exposing the Rats

Male Wistar rats (170-200g) were exposed to ¹⁴C-labeled vinyl chloride in a closed glass system (Bolt et al., 1976). Gas specimens could be taken at any desired time through a Teflon septum which were then investigated for their radioactivity. Using this method the consumption of vinyl chloride by the rats could be monitored.

Pretreatment

A complete blockage of the metabolism of vinyl chloride was achieved by means of a single injection (i.p.) of 50 mg/kg of 6-nitro-1.2.3.-benzo-

thiadiazol in 0.6 ml of DMSO which occurred one-half hour prior to the beginning of the exposure (Bolt et al., 1976).

Determination of the Radioactivity Concentration in the Organs

The animals were sacrificed immediately after the exposure. The various organs were taken out and homogenized in a fourfold volume of water. An aliquot of 0.25 ml homogenate was dissolved in 0.5 ml of hyamin-hydroxide and then counted in 10 ml of scintillator (Bray, 1960).

Determination of the Distribution of Unmodified Vinyl Chloride in the Rat Organs

Along with blocking of the metabolism due to the 6-nitro-1.2.3-benzothiadiazol, the distribution of ^{14}C -vinyl chloride was investigated with vinyl chloride concentrations in the air between 25 and 10,000 ppm.

In order to make the given concentration of vinyl chloride obvious, after each injection of a trace dosage of ^{14}C -vinyl chloride into the exposure system, the calculated amount of nonradioactive vinyl chloride was injected into the system.

After an exposure time of 5 hours the rats were sacrificed immediately after being removed from the closed system. The blood was collected in a vessel which contained heparin. Blood (1 ml) or organs (about 1 g) were then placed into a vessel with 5 ml toluene scintillator. The toluene scintillator contains 0.4% PPO and 0.01% POPOP in toluene. Homogenization, then centrifuging were conducted in a "Ultra-Turrax" and finally the toluene extract was put into a scintillation vessel.

Again 5 ml of toluene scintillator were added to the extracted tissue and mixing, centrifuging again occurred with the help of the "Ultra-furax" and this second extract was added to the first.

The radioactivity of the toluene extract was determined in a Berthold-BF 5000 liquid scintillation counter.

Results and Discussion

If the metabolism of vinyl chloride is blocked by 6-nitro-1.2.3-benzothiadiazol, then during exposure of a vinyl chloride-containing atmosphere, a stationary equilibrium sets up between vinyl chloride in the air and in the organism (Bolt et al., 1976).

Table 1. Equilibrium distribution of ^{14}C -vinyl chloride in various tissues of rats which were exposed to a ^{14}C -vinyl chloride atmosphere. The metabolism of vinyl chloride in this experiment was blocked by 6-nitro-1.2.3-benzothiadiazol (see experimental section).

The values show the equilibria quotients between vinyl chloride in the tissue and vinyl chloride in the atmosphere.

Tissue	$\frac{\text{nMol vinyl chloride in 1 g of tissue}}{\text{nMol vinyl chloride in 1 ml of air}}$	
Blood	0.65	± 0.13
Liver	0.62	± 0.10
Spleen	0.59	± 0.08
Kidneys	0.59	± 0.16
Muscle (M.psoas)	0.68	± 0.13
Fatty tissue	8.30	± 0.20

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The values indicate the average value $\pm$ standard deviation with 5 different atmospheric concentrations of vinyl chloride which were between 25 and 10,000 ppm (see also Figure 1).

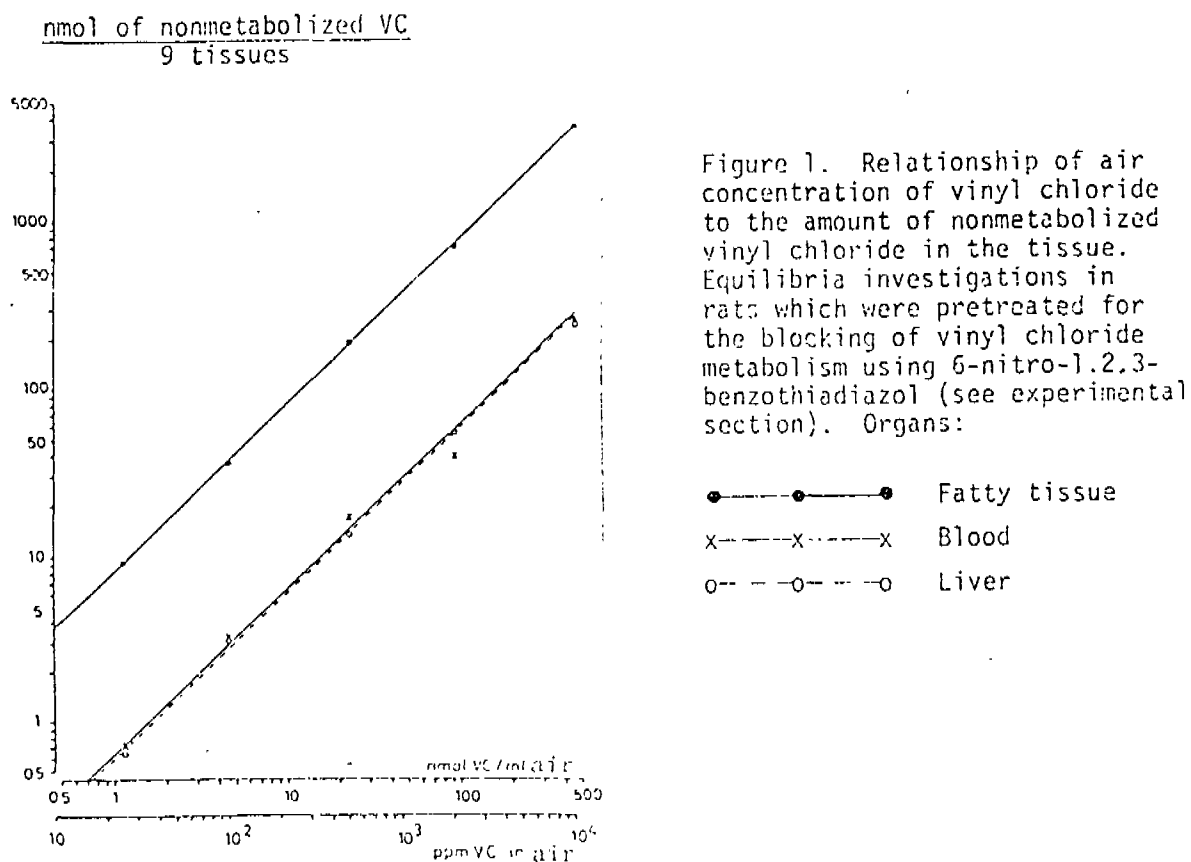


Table 1 shows the situation of the equilibrium for various organs: in most organs vinyl chloride is found in an equilibrium with about equal amounts. The concentration (per g) of vinyl chloride is roughly 1/2 to 2/3 of the air concentration of vinyl chloride (per ml). Fatty tissue in which about 10 times higher concentrations of vinyl chloride were

found behaves completely differently. Figure 1 shows that this ratio throughout all of the investigated concentration range of 25 to 10,000 ppm of vinyl chloride in the air remains constant.

Table 2 contrasts the results which were obtained with equilibria conditions with the exclusion of metabolism and the contrast here is with the distribution of radioactive metabolites of ^{14}C -vinyl chloride as they are normally found (in intact metabolism) (see also Bolt et al., 1976). Here large amounts of radioactivity appear in the liver as the main metabolism organ of vinyl chloride as well as in the kidneys which excrete the polar metabolite.

In contrast to this, the amount of radioactivity which is found in fatty tissue is very low. It must be taken into consideration here, however, that in the intact metabolism the concentration of vinyl chloride in the closed system used (Bolt et al., 1976) after about 5 hours of exposure has dropped to 1/10 of the initial value since the half-life of vinyl chloride in our system amounts to about 1 hour. The concentration of non-metabolized ^{14}C -vinyl chloride present after 5 hours in the system, since it is in an equilibrium with the fatty tissue, in the present case must amount to about 3 to 4 nMol/g. The table shows that this is actually the case. The fatty tissue, therefore, even with intact metabolism, does not contain significant amounts of vinyl chloride metabolites, rather non-metabolized vinyl chloride.

Table 2. Amounts of radioactivity in the organs of rats which had been exposed for 5 hours in a sealed system to a 1.2-¹⁴C-vinyl chloride-containing atmosphere (initial vinyl chloride concentration = 100 ppm).

The values are indicated as nMol of vinyl chloride or vinyl chloride metabolites per g of moist tissue.

Organ	Rats after pretreatment with 50 mg/kg of 6-nitro-1.2.3-benzothiadiazol (average value from 2 tests)	Rats without pre-treatment (average value of 3 tests)
Liver	3.5	25.4
Spleen	3.5	5.0
Kidneys	2.5	29.2
Muscle (M.psoas)	3.1	4.4
Fatty tissue	37.2	3.0

With all due caution and skepticism concerning the transferring of animal-experimental findings to human conditions, on the basis of the present animal-experimental investigation results as well as on the basis of the physical-chemical lipophilic properties of vinyl chloride it must be taken into account that even in human beings an accumulation of nonmetabolized vinyl chloride can take place as a function of exposure. Along the lines of preventative steps in occupational medicine it must, therefore, be maintained that persons who are heavily overweight are not suited for employment where there is vinyl chloride exposure.

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