I. Effect of SKF-525-A (β-diethylaminethyldiphenylpropyl-acetate) and AT (3-amino-1,2,4-triazole) on the Metabolism of Inhaled Vinyl Chloride Monomer (VCM) in Rats:

Further experiments have been completed to verify the effect of SKF-525-A on the metabolism of inhaled VCM in rats. The inhalation apparatus and methods described in our earlier paper, "Preliminary Studies of the Fate of Inhaled Vinyl Chloride Monomer (VCM) in Rats", were used in these experiments. Table 1 illustrates the experimental results. SKF-525-A pretreatment clearly inhibits VCM metabolism at VCM exposure concentrations of approximately 1000 ppm, but has little effect on the metabolism of VCM at exposure concentrations below 100 ppm.

1000 mg/kg AT has been shown to inhibit 90% of the liver catalase activity of rats following a 3 hour pretreatment. (Heim, Appleman and Pyfrom, 1955). Therefore, if an oxidation of 2-chloroethanol a postulated VCM intermediary metabolite occurred, via hydrogen peroxide and catalase, AT pretreatment should have an inhibitory effect. Pretreatment of rats with 1000 mg/kg AT 256 minutes prior to exposure to approximately 1000 ppm VCM for 60 minutes induced a 16.37% depression of VCM metabolism.

In a single preliminary experiment, a 180 minute pretreatment with 1000 mg/kg AT and a 50 minute pretreatment

with 75 mg/kg SKF-525-A, followed by exposure to approximately 1000 ppm VCM for 82.5 minutes, induced 20.69% depression of VCM metabolism. This degree of depression is somewhat greater than that induced by SKF-525-A or AT alone, and may indicate an additive effect of the two inhibitors. Thus one might conclude that oxidative pathways involving the peroxide of 2-chloroethanol and the epoxide of VCM may both operate simultaneously, when the metabolism of VCM via alcohol dehydrogenase is saturated by exposure to 1000 ppm VCM.

Table 1. Effect of SKF-525-A and/or AT on Metabolism of Inhaled VCM in Rats

Experiment Number	Vinyl Chloride Exposure		Pretreatment with Metabolic Inhibitors minutes prior to VCM Exposure		Percent Depression of VCM Metabolism
	ppm	minutes	SKF-525-A (75mg/kg)	AT (1000 mg/kg)	
1	970.3	90.0	35.0	none	8.9
	998.3	52.5	60.0	none	6.5
	1004.8	60.0	135.0	none	4.5
2	61.2	52.5	30.0	none	0.5
3	940.6	60.0	none	256	16.4
4	1021.1	82.5	50	180	20.7

II. Excretion and Tissue Levels of ^{14}C-Activity After Inhalation Exposure to Vinyl Chloride Monomer:

Studies on the elimination kinetics of 1,2-^{14}C vinyl chloride monomer (VCM) were conducted in rats exposed by inhalation to initial concentrations of 50, 850 and 7800 ppm for 60, 45 and 60 minutes, respectively. Four rats were concurrently exposed to the given concentration of VCM in a 4.7 ℓ recirculating inhalation system. Immediately after the exposure period the rats were placed individually in modified Roth-type glass metabolism cages for separate collection of urine, feces and expired air. The excreta were sampled at 12 hour intervals and analyzed by liquid scintillation techniques for ^{14}C radioactivity. After 3 days the animals were sacrificed and selected tissues as well as the remaining carcass were analyzed for ^{14}C activity. Data are expressed as percent of the total radioactivity recovered. The relative proportions of radioactivity excreted by the various routes did not differ significantly at the three exposure levels. Urine constituted the primary excretory route (64-67%) followed by expired CO_2 (14-19%), feces (2-3%), and expired VCM (0.02-0.45%). The radioactivity was readily excreted with 60-70% eliminated within the first 12-15 hours. The half-life ($t_{1/2}$) for the initial phase of urinary clearance was similar at all dose levels (approximately 6.0 hours) corresponding to

a clearance rate constant of 0.12 hour^{-1}. ^{14}C-activity in the urine and feces was presumably non-volatile metabolites of VCM. The body burden after 3 days was 12-15% of the recovered dose. At this time, the liver showed the highest tissue levels followed by the kidney when expressed on a % dose/g tissue basis. Since a significant quantity of labeled CO_2 (14-19%) was produced, it is speculated that the radioactivity remaining in the body after 3 days may reflect catabolism of VCM to one carbon fragments and subsequent incorporation into native molecules. From the present investigation it is concluded that the rat rapidly excretes non-volatile VCM metabolites after short inhalation exposures of 50, 850 and 7800 ppm.

III. A) Isolation of Urinary Metabolites Following Inhalation Exposure to ^{14}C-VCM:

Four rats were exposed to 7854.5 ppm 1,2-^{14}C-VCM using the methods and inhalation apparatus described in our paper, "Preliminary Studies of the Fate of Inhaled Vinyl Chloride Monomer (VCM) in Rats". During the 62 minutes of exposure in the closed inhalation system the rats assimilated 424.3 ppm or 1.28 mg of VCM per rat, based on the kinetics of VCM metabolism and assuming equal distribution between the 4 rats. The urine collected during the twelve hours immediately following exposure was used for the experiments described herein.

Three milliliters of urine were applied to a Cellex D ion exchange column and eluted with a pH 8.0 to pH 5.5, 0.01M Tris HCl gradient. This resulted in the separation of two major bands of ^{14}C activity.

A fraction containing 86.9% of the ^{14}C activity, separated from the weakly basic column in the pH 8.0 fractions. A second fraction containing 13.1% of the ^{14}C activity separated in the acidic portion of the pH gradient. Thin layer chromatography revealed the presence of 3 distinct bands of ^{14}C activity, in the fraction containing 86.9% of the ^{14}C activity. Attempts to resolve these 3 bands using silica columns and various solvent systems have been unsuccessful to date. However, using 30% acetylated cellulose plates, the band containing the major amount of ^{14}C activity

has been well resolved from the other two and is currently being recovered for mass spectroscopic analysis.

The fraction containing 13.1% of the ^{14}C activity appeared to be homogeneous by thin layer chromatography. This fraction is currently being subjected to mass spectroscopic analysis.

III. B) Separation of Urinary Metabolites After Oral Administration of 1,2-^{14}C Vinyl Chloride Monomer in Rats:

^{14}C-VCM in corn oil was administered orally to 6 rats (20 mg/kg, 16 μCi/animal). Urine collected in the first 12 hour period was pooled and an aliquot (2-4 ml) subjected to ion exchange chromatography (Dowex 1x8, 200-400 mesh, carbonate form). The ion exchange column was eluted with 0.1N acetic and hydrochloric acids. The radioactivity was distributed in three fractions. 98% of the recovered activity resided in a fraction eluted with the acid eluants. Subsequent thin layer chromatography (TLC) of this primary fraction showed an additional 3 spots of radioactivity as detected by a TLC radio-scanner. The three spots of radioactivity were evident in two different TLC systems. In conclusion, there is evidence for at least 3 major and 2 minor urinary metabolites. Indication from the Dowex 1 ion exchange column suggest that the major metabolites have acidic functional groups; however, they are poorly extracted from acidified urine into diethyl ether. This would be characteristic of amphoteric molecules. Analytical techniques currently being tested to elucidate the VCM metabolites are derivatization, followed by gas liquid chromatography - mass spectrometry.

Percutaneous Absorption of Vinyl Chloride (VCM) Gas in the Rhesus Monkey:

The experiments described herein were designed to determine the degree to which vinyl chloride monomer (VCM) gas (Matheson Gas Products, Joliet, Ill., 99.9% purity) may be percutaneously absorbed. A male Rhesus monkey (Macaca mulatta) weighing 5 kg was used in the experiment. Prior to exposure, 30 mg/kg sodium pentobarbital was administered intravenously. An endotracheal catheter with an inflatable collar was inserted into and the trachea connected to a Harvard respiratory pump adjusted to deliver a volume of 20 ml of air at a rate of 28 to 38 cycles per minute. A catheter was placed in the saphenous vein of the left leg so that additional sodium pentobarbital could be administered during exposure. The body of the monkey was placed in a 191.5L plexiglas chamber with the head protruding from the chamber through a silastic rubber membrane. After the hair around the neck had been carefully removed with clippers the membrane was fitted around the neck and secured to the skin with tape, to provide an adequate seal. The body of the monkey rested on 3 large glass bell jars in the chamber. A slight vacuum was pulled on the chamber, and 1.5L of VCM gas was metered in to establish a VCM concentration of approximately 7000 ppm in the chamber. Chamber concentrations were periodically monitored by infrared analysis (10.9 μ).

To trap VCM in the expired air, a polyethylene tube filled with 0.5g of carbon made from saran beads was placed in the exhaust port of the respiratory pump. A similar tube placed on the intake port filtered any VCM from the air pumped into the monkey. The expired air traps were changed at 1/2 or 1 hour intervals while the intake traps were changed every 2 hours. The VCM from these traps was eluted with carbon disulfide at dry ice temperature and analyzed by gas chromatography. Additionally, a sample of control saran carbon was checked for VCM.

It was found that the VCM concentration in the exposure chamber declined more rapidly when a monkey was present than if empty, however, it was not possible to attribute this difference solely to percutaneous absorption. Comparison would be valid only if identical chamber conditions could be obtained. This is not possible since an empty chamber could not be made to simulate the leakage characteristics, adsorption to fur, and thermal mixing characteristics in the test solution.

A monkey was exposed to approximately 7000 ppm ^{14}C-VCM (specific activity 0.180 μCi/mg) for 2 hours. Immediately post-exposure the monkey was sacrificed and tissues (brain, heart, lung, liver, muscle, stomach, duodemum, thymus, spleen, mesenteric fat, testes, kidney) were sampled and homogenized. Urine was taken from the urinary bladder and bile

from the gall bladder. Aliquots of the tissue homogenates were combusted and the ^{14}C-CO_2 trapped in 2-methoxyethanol-monoethanolamine solution. The resulting CO_2-2-methoxyethanol-monoethanolamine solutions, as well as aliquots of urine and bile were counted in scintillation cocktails using a Mark II liquid scintillation counter. ^{14}C activity in excess of twice background was detected in only the bile, kidneys, and liver. 1792 DPM of ^{14}C activity was in the bile, 11,352 DPM in the kidneys, and 105,821 DPM in the liver, for a total of 118,965 DPM. Expired air samples contained a total of 182,709 DPM for the 2 hour exposure period, thus a total of 314,818 DPM or 0.024% of the VCM (3.28 g or 1,316,987,916 DPM) in the chamber was absorbed percutaneously over the 2 hour period. Of the total dose absorbed percutaneously, 39.4% was in the tissue, while 60.6% was in the expired air. The aforementioned experimental results demonstrate that small amounts of VCM are percutaneously absorbed by a monkey maintained under the described experimental conditions.

Assuming that the percutaneous absorption of VCM is proportional to the surface area of the body, extrapolating the amount absorbed by the monkey under the specified conditions to that which would be absorbed by a 6 ft, 90 kg

man yields 4.76 mg of VCM. Possible influence of fur and clothing was not determined and, therefore, not considered in making this extrapolation. However, this data indicates that the amount of VCM a man would absorb through the skin in an atmosphere containing 7000 ppm in two hours would be equivalent to the man breathing an atmosphere containing 0.186 ppm VCM for an eight hour working day. This theoretical calculation assumes that a 10 m^3 volume of air was breathed over eight hours and all VCM was assimilated.