

TESTIMONY: "PHARMACOKINETICS OF VINYL CHLORIDE"

Hearing on the Environmental Protection
Agency's National Emission Standard
for Hazardous Air Pollutants
Proposed Standard for
Vinyl Chloride

R&S 100277

by

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DOW
EPA

EXECUTIVE SUMMARY

Studies have been conducted in the Toxicology Research Laboratory of The Dow Chemical Company which show that the dynamics for the fate of vinyl chloride in the body change as the level of the dose given either orally or by inhalation increases. In the body, two pathways for metabolism are operative. The primary or rapid pathway is overwhelmed as the dose is increased. This pathway is inhibited by the concomitant administration of ethanol. Both pathways produce reactive metabolites which are detoxified by reaction with glutathione; however, the reactive metabolites produced by the secondary or slower pathway are more likely to lead to toxicity.

A very important finding is that exposures of rats to concentrations of 150 ppm and greater vinyl chloride cause a depression of the glutathione content of the liver. At 50 ppm the depression is slight and inconsistent suggesting that this concentration lies in a threshold zone for this effect. Exposure to 10 ppm vinyl chloride produces no depression. Depression of this important detoxification system is likely to render the individual more susceptible to the untoward effects produced by reactive metabolites of vinyl chloride formed in the body. It is highly significant that the depression of

the hepatic glutathione content correlates with the induction of tumors. The equivocal depression of hepatic glutathione in rats exposed to 50 ppm coincides with an equivocal induction of tumors suggesting that a threshold for both activities is near this level of exposure.

Finally, it is strongly emphasized that because of changes in the dynamics of the fate of vinyl chloride and the depression of the hepatic glutathione levels, stochastic statistical projections of the untoward effects incurred with exposures to vinyl chloride exceeding 100 ppm to predict the incidence of untoward effects at lower doses are invalid. Indeed, they violate the a priori assumptions for such statistical projections.

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The purpose of my testimony is to inform you of studies being conducted in the Toxicology Research Laboratory of The Dow Chemical Company on the fate of vinyl chloride in the body and how the results of these studies relate to assessing the potential hazard of low level exposures to vinyl chloride. These studies have been supported in part by the Manufacturing Chemists Association as well as The Dow Chemical Company.

The studies I will describe are referred to technically as the "Pharmacokinetics of Vinyl Chloride". Pharmacokinetics is the study of the dynamic processes involved in the absorption, distribution, metabolism, and elimination of chemicals from the body.

Toxicity, including carcinogenesis, is caused by the presence of a specified amount of a chemical or its degradation product in tissues and cells; and subsequently reversible or irreversible reactions of these materials with cellular tissue components. In essence, toxicity is a measurement of what selected doses of a chemical or its degradation product do to the cell, a tissue or the body. Pharmacokinetics quantitates the presence of the chemical or its degradation product in the cell, a tissue or the body. Knowledge and consideration of both are inextricably associated in utilizing the results of toxicity evaluation of high doses to predict the hazard of low doses.

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To illustrate further my point, Slide 1 shows a typical dose-response curve for the percent of individuals (triangles) in a population responding adversely in some manner to selected doses of a chemical. The sigmoid curve represents a response of a population described by a normal or Gaussian distribution. These adverse responses are discernible over only a range of doses represented by the solid line because the investigator is limited by the number of individuals he can include in such a study.

It should be emphasized that in theory the tails of the sigmoid curve never reach zero on the low dose side and never 100% on the high dose side; i.e. the curve approaches 0 and 100% asymptotically. The adverse effect, cancer as well as other untoward effects, will occur in theory no matter how low the dose if the population is sufficiently large. However, for most adverse effects, experience leads us to conclude that there is a dose below which adverse effects will not occur; i.e. there is a threshold. Because drinking two 1/5's of alcohol over a short period of time would kill some people, we do not conclude that 1 drop of alcohol will kill some. A simplistic scenario is that for a faster runner to pass a slower one he needs to traverse one-half the distance between the two, then one-half that distance and so on; one mathematical theory suggests the faster runner can never pass the slower. However, definitive observation leads us to reject this simulation. Unfortunately, such definitive observations are nonexistent for carcinogenicity and I believe will remain so for a long time.

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In the case of carcinogenicity, stochastic, guess work by definition, statistical projections are made from the range of doses over which an increased incidence of cancer can be perceived to predict what percent of individuals may respond at lower doses. The a priori assumption for making such projections is that the chemical is handled the same by the body as the dose decreases. If the dynamics for the fate of the chemical change, such extrapolation is not valid. Conceptually, it is not surprising that the toxicity, including carcinogenicity of a chemical is expressed only after the detoxification processes of the body are overwhelmed. In such cases, the response of the population may be more accurately described by the other two dash lines in the lower left-hand corner of the figure.

Having provided the above background; I will proceed to describe the results of studies which show that the dynamics of the fate of vinyl chloride in the body change with dose and exposure levels. Initially, the motivation for these studies was the results of Maltoni which suggested strongly that the incidence of cancer in rats exposed to 50 ppm was less than that predicted from exposures to levels ranging from 250 ppm to 10000 ppm.

In the initial studies, we exposed 4 rats to varying initial concentrations of VCM in an apparatus like that shown in Slide 2. By monitoring the concentration of VCM in the system, that rate at which the rats took-up and chemically altered VCM was

determined, Slide 3. In this slide, it is apparent that the rate of removal of VCM from the chamber containing an initial concentration of approximately 75 ppm VCM was much faster than the rate of removal from the chamber when the initial concentration was approximately 1000 ppm VCM. The steeper the slope, the faster the rate of removal. In experiments like these, we demonstrated that the rate of removal between initial concentrations of 50 ppm and 105 ppm was the same having a half-life ($t_{1/2}$) of 86 minutes. Also between concentrations of 220 ppm and 1167 ppm VCM, the rate of removal was the same having a $t_{1/2}$ of 261 minutes. From the results of these studies, it was concluded that VCM is metabolized or degraded readily at low concentrations and that the primary pathway for degradation is swamped at concentrations exceeding 220 ppm.

Another objective means of demonstrating that the pathway for the metabolism or degradation of chemicals may be overwhelmed is to administer potential inhibitors of its metabolism. The next slide, Slide 4, shows that the administration of 5ml/kg 95% ethanol inhibits profoundly the metabolism of VCM by rats exposed to an initial concentration of 50 ppm ethanol but not of rats exposed to approximately 1000 ppm. The percent inhibition was 96 and 40% respectively. In other studies, we found that SKF-525A inhibited by approximately 20% the metabolism of VCM by rats exposed to initial concentrations of approximately 1000 ppm but caused no inhibition in rats exposed to initial concentrations of less than 100 ppm.

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The results of these studies led to the conclusion that VCM must be metabolized by at least two different pathways. The primary pathway for metabolism is overwhelmed as the exposure concentration is increased until at concentrations exceeding 220 ppm, the secondary pathway predominates. Slide 5 illustrates my point. As the flow into the barrel increases more escapes via the second slit. The results of the above studies as well as others yet to be discussed have been or are in the process of being reported in the literature. Copies of reprints will be conveyed to you for examination.

In subsequent studies, we were fortunate to have radioactively tagged Carbon 14 to follow the fate of VCM. This facilitated greatly following the disposition of the administered VCM.

Slide 6 shows the percent of ^{14}C activity eliminated via various routes following different single-oral doses of VCM in corn oil to rats. The ^{14}C activity expired consisted of $^{14}\text{CO}_2$ and ^{14}C -VCM. The ^{14}C activity found in urine, feces and carcass and tissues represents nonvolatile metabolites of VCM. The key item to note in this slide is that as the dose is increased from 0.05 and 0.10 mg/kg to 20 and 100 mg/kg, the percent expired as VCM increases markedly while the other parameters, particularly urinary excretion of ^{14}C activity, correspondingly decrease. Again, this demonstrates that the primary route for the elimination of VCM from the body is dose dependent; i.e. the primary route is overwhelmed and more begins to spill out via other routes, the upper slit in the barrel.

Since urinary and pulmonary excretion of VCM was dramatically altered as the dose increased from 1 to 100 mg/kg, the question is raised whether these processes of elimination may be a function of dose. This question can be examined via elucidating the rates of elimination. Slide 7 shows a plot of the logarithm of the ^{14}C activity eliminated via the urine as a function of time. Since the slopes or rates of elimination are unchanged, it must be concluded that urinary excretion of nonvolatile metabolites of VCM is unaltered by dose.

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Slide 8 shows similarly the expiration of VCM per se following various doses. Elimination following 0.05 or 0.1 mg/kg occurred in accordance with a first-order rate or mono-exponential process. When 100 mg/kg was given the elimination was biexponential. The rates or $t_{1/2}$ times for elimination are indicated in the figure; these correspond to those reported for blood by Withey (1975). These results are indicative of a material which is bound reversibly to some site in the body having a finite capacity. As the dose increases, the availability of these binding sites decreases and the chemical is free to find its way to other sites as well or to be eliminated. Thus it may be concluded that the expiration of VCM following different doses indicates that the pulmonary excretion of VCM is not a rate limiting step. Even more important, the data indicate that the state in which VCM exists in the body changes with dose.

Slide 9 summarizes the dose-dependent excretion of VCM via urinary excretion (solid line) and pulmonary elimination (broken line). The area demarcated by the rectangle represents that range of doses over which distributive or metabolic saturation occurs. It is noteworthy that in ongoing carcinogenicity studies, Maltoni has reported an angiosarcoma of the thymus in a rat given 50 mg/kg/day and one of the liver in a rat given 16.6 mg/kg/day. No tumors have, as yet, occurred in rats given 3.3 mg/kg/day.

Although the data collected from studies in rats given oral doses of VCM elucidate the dose-dependent fate of VCM, these hearings are more concerned with inhalation exposure. Slide 10 depicts the fate of ^{14}C -VCM in rats exposed for 6 hours to 10 or 1000 ppm VCM. Immediately following the exposure, the rats were placed in cages providing for collection of ^{14}C activity in the expired air, feces and urine over the subsequent 75 hours as can be seen. As in the experiments in which oral doses were given, the percent of ^{14}C activity expired as VCM increased as the exposure increased.

Also to be noted in this slide is that the percent ^{14}C activity found in the tissues and carcass increases slightly as the exposure is increased from 10 to 1000 ppm, although not statistically significant. This is remarkable because a much larger fraction was expired as VCM. This may mean that a larger fraction is being bound to the macromolecules of

tissues. This aspect is being investigated currently since such reactivity may explain carcinogenesis.

The two subsequent slides show the elimination of ^{14}C nonvolatile metabolites in the urine and VCM per se in the expired air, Slides 11 and 12. Again these results support the conclusions of previous studies, 1) that the fate of VCM changes with dose and 2) that this occurs because the primary pathway for the metabolism of VCM is saturated at high doses or exposures.

Since the metabolism of VCM appears to occur via, at least, two saturable pathways, a considerable effort has been made to elucidate the metabolites of VCM. Already, it had been demonstrated that a measurable amount of VCM was metabolized to the end-product of mammalian metabolism, CO_2 . Using high pressure liquid chromatography, three major metabolites have been isolated from urine. Two of the three have been identified by gas chromatography-mass spectroscopy. These are shown in Slide 13. Metabolite A is N-acetyl-S(2-hydroxyethyl)cysteine. Metabolite B is thiodiglycolic acid. Together these metabolites comprise 50 to 60% of the radioactivity found in urine. Both of these metabolites are likely formed from the compound shown at the bottom, 2-hydroxyethylcysteine. At one point in time, it appeared that the third major, metabolite in urine, comprising about 30% of the radioactivity, was this compound. Even though some analytical comparisons between the isolated metabolite and 2-hydroxyethylcysteine favored this conclusion, others failed to establish identity.

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The finding of these metabolites of VCM in urine indicates that VCM is transformed in the body to a reactive intermediate metabolite. This reactive metabolite is detoxified by reaction with glutathione, a tripeptide containing glutamic acid and glycine as well as cysteine. Subsequently, the glutamic acid and glycine moieties of the tripeptide are cleaved and the cysteine conjugate of the reactive metabolite of VCM is either acetylated or further oxidized and excreted as the aforementioned metabolites.

Before leaving the subject of metabolism it is important to state that the metabolites of VCM were not changed either qualitatively or quantitatively as the dose or exposure level was increased. Since evidence has been presented showing that there are at least two pathways for the metabolism of VCM, this indicates that both pathways produce reactive metabolites leading to the same end products. Our initial work and subsequently that of others (Kappus et al, 1975; Bolt et al, 1975 and Barbin et al, 1975) indicates that one pathway involves oxidation of VCM by microsomal enzymes to chloroethylene oxide. The other pathway, yet to be identified, is, we feel, the primary pathway at low doses or exposure levels; it is blocked by administration of ethanol and is very likely located in the soluble fraction of the cell. Reactive intermediates produced by the latter pathway are less likely to induce toxicity or cancer because critical macromolecules-protein, DNA, RNA--are less proximate to the

site of their formation. This concept, however, I concede is speculation at this time.

Now I want to return to a subject which is less speculative. As I indicated previously, the reactive metabolites of VCM are detoxified by reaction with glutathione. This is very important because it has been demonstrated that when high doses of some chemicals, for example bromobenzene and acetaminophen, are given the glutathione is used up at a faster rate than it can be produced by conjugation with the reactive intermediates. As the level of glutathione in the liver is progressively depleted, the reactive metabolites react with macromolecules such as protein, DNA, RNA--leading to toxicity (Gillette, 1974a and 1974b). Generally, it is accepted that at least one mechanism for chemical carcinogenesis occurs via such reactions.

To assess the effect exposures to VCM may have on hepatic glutathione levels, rats were exposed to concentrations of 10, 50, 150, 250, 1000 or 2000 ppm for durations of 1 to 7 hours. The results are shown in Slide 14. Exposure to 150, 250, 1000 or 2000 ppm VCM caused a progressive depression of the hepatic nonprotein sulfhydryl content; the hepatic nonprotein sulfhydryl content is primarily glutathione. Exposure to 50 ppm for 7 hours produced a small and inconsistent depression. No depression was observed in rats exposed to 10 ppm VCM. These

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results indicate that there is a measurable biological threshold for the depression of hepatic glutathione levels induced by exposures to vinyl chloride. Unequivocal depressions are produced by concentrations exceeding 50 ppm, while exposure to 50 ppm seem to be a transition zone and exposure to 10 ppm causes no depression.

How do these results relate to the carcinogenicity of VCM? In the studies of Maltoni and Lefemine (1975), the reported incidence of angiosarcoma of the liver in rats exposed 4 hours/day, 5 days/week to 2500 or 6000 ppm was 22%. The incidence in rats exposed to 500 and 250 ppm were, respectively, 12 and 7%. Reference to the figure indicates that the depression of the hepatic nonprotein sulfhydryl content observed after 4 hours of exposure coincides with the increased incidence of angiosarcoma.

In the study of Maltoni and Lefemine (1975) an incidence of 2% angiosarcoma of the liver occurred in rats exposed to 50 ppm VCM. As I indicated previously, exposure to 50 ppm for 7 hours caused a small and inconsistent depression of the hepatic nonprotein sulfhydryl content. I also indicated that this exposure appeared to be in the transition zone of the threshold for this biological effect. In a recent publication by Maltoni (Ambio, Vol. 5, 1975) not only the incidences of angiosarcomas of the liver were given but also the latency periods for their development were provided. The latency

periods were 64, 70, 78, 81, and 79 weeks in rats exposed to 10,000, 6000, 2500, 500, and 250 ppm, respectively. In rats exposed to 50 ppm, the latency period was 135 weeks. Indeed tumors were discovered in these aged rats when they were killed at the end of the study. Since the tumors in the former groups of rats were discovered as they died spontaneously, the discrepancy is even greater than the values indicate. The latencies for the development of other types of tumors showed the same discrepancy. Consideration of these results leads me to conclude that in rats exposure to 50 ppm VCM 4 hours/day is in the threshold transition zone for not only hepatic nonprotein free sulfhydryl depression but for tumor induction as well.

In summarizing the studies on the pharmacokinetics and metabolism of VCM, the data indicate that the fate of VCM in rats is dose-dependent following either single oral administration and after inhalation exposure. More importantly, it appears from the data available that a correlation exists between doses of VCM that cause tumors and those that saturate metabolic or detoxifying pathways.

The primary detoxification pathway for VCM, which appears saturable at high dose levels, involves conjugation of its reactive metabolites with nonprotein sulfhydryl groups. Therefore, it seems reasonable to postulate that as the

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nonprotein sulfhydryl groups are depleted, reactive metabolites may be free to react with other macromolecules (DNA, RNA, Protein, lipids) resulting in toxicity and carcinogenicity. Recent reports have demonstrated that in the presence of fortified microsomal enzyme preparations reactive metabolites of VCM are produced which covalently bind to rat liver microsomes (Kappus et al, 1975) protein sulfhydryl groups, RNA (Bolt et al, 1975) and adenosine of DNA (Barbin et al, 1975). Inclusion of glutathione in the system will decrease or preclude these reactions depending on the concentration. It is highly significant that exposure to 10 ppm VCM for 7 hours caused no depression of hepatic nonprotein sulfhydryl content. This indicates that there is a threshold of exposure in rats where the ability to replace sulfhydryl groups is not overwhelmed and physiologic defense mechanisms remain fully operative. Furthermore, this suggests that thresholds exist for toxic effects which are expressed with greater intensity as this protective mechanism is depressed. Studies currently in progress are designed to characterize the macromolecular binding of VCM to protein and Nucleic acids following exposure to various concentrations of ^{14}C -VCM.

Finally, I want to reemphasize strongly that stochastic statistical projections utilizing data collected from rats exposed to concentrations exceeding 100 ppm VCM are invalid for predicting the hazard of lower levels exposures. Such

projections violate the a priori assumption that the dynamics governing the fate of the compound are unaltered.

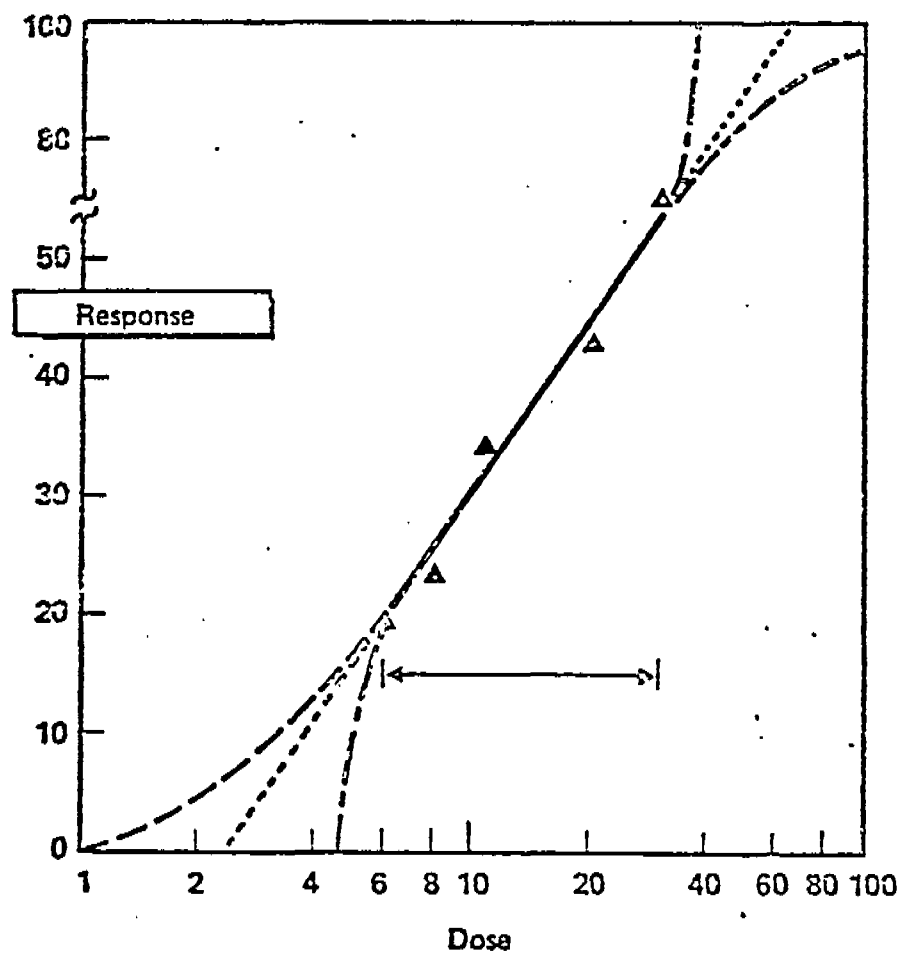
Our pharmacokinetic studies on the fate of VCM show that the dynamics for its disposition change as the dose or exposure level is increased. Also demonstrated was a threshold for the depression of hepatic glutathione. Finally, the discrepancy between the latent period for tumor development in rats exposed to 50 ppm VCM and the latent periods for those exposed to higher levels indicates a threshold for tumor development exists.

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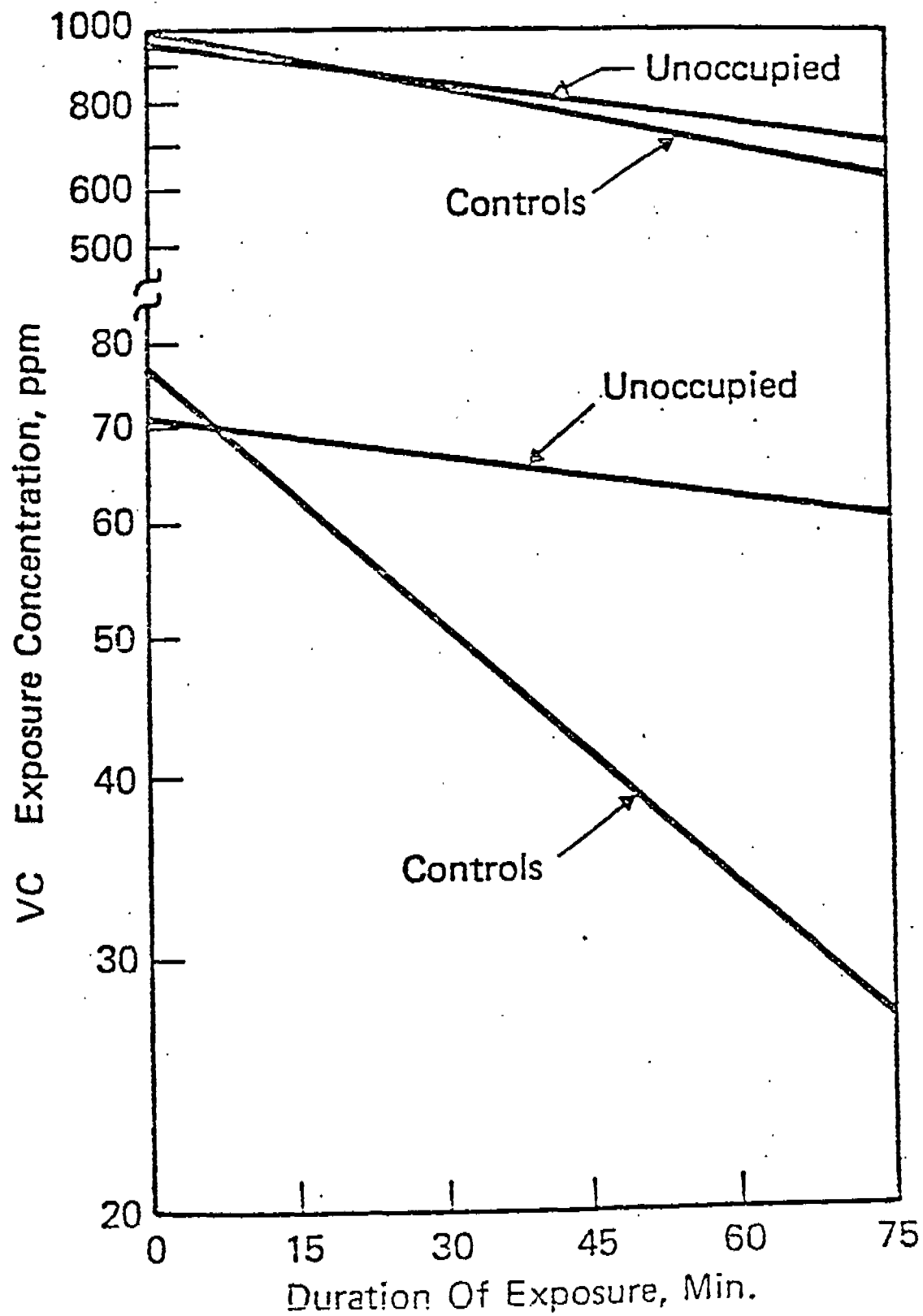
Slide I

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Slide 3

FIGURE 32

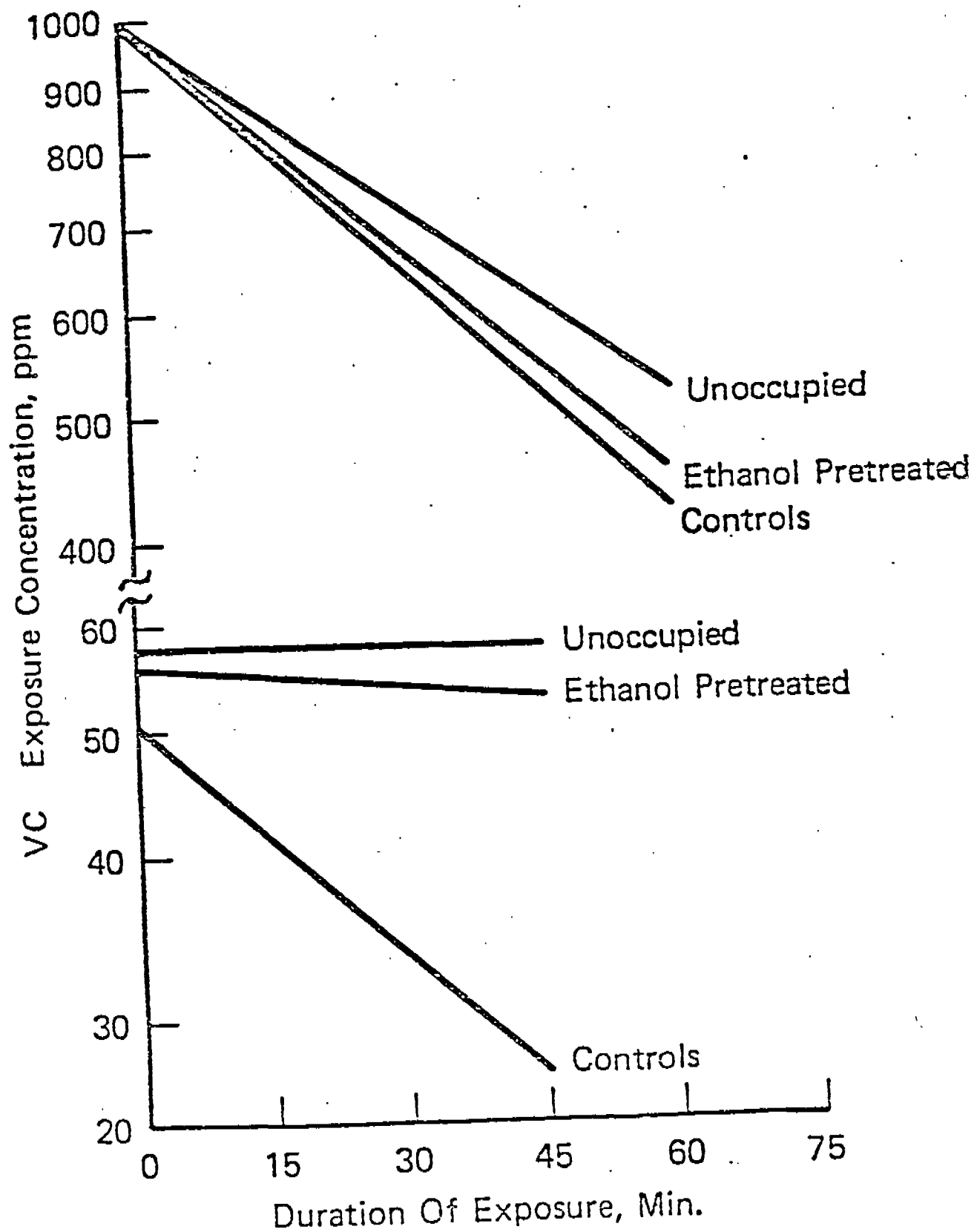


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Slide 4

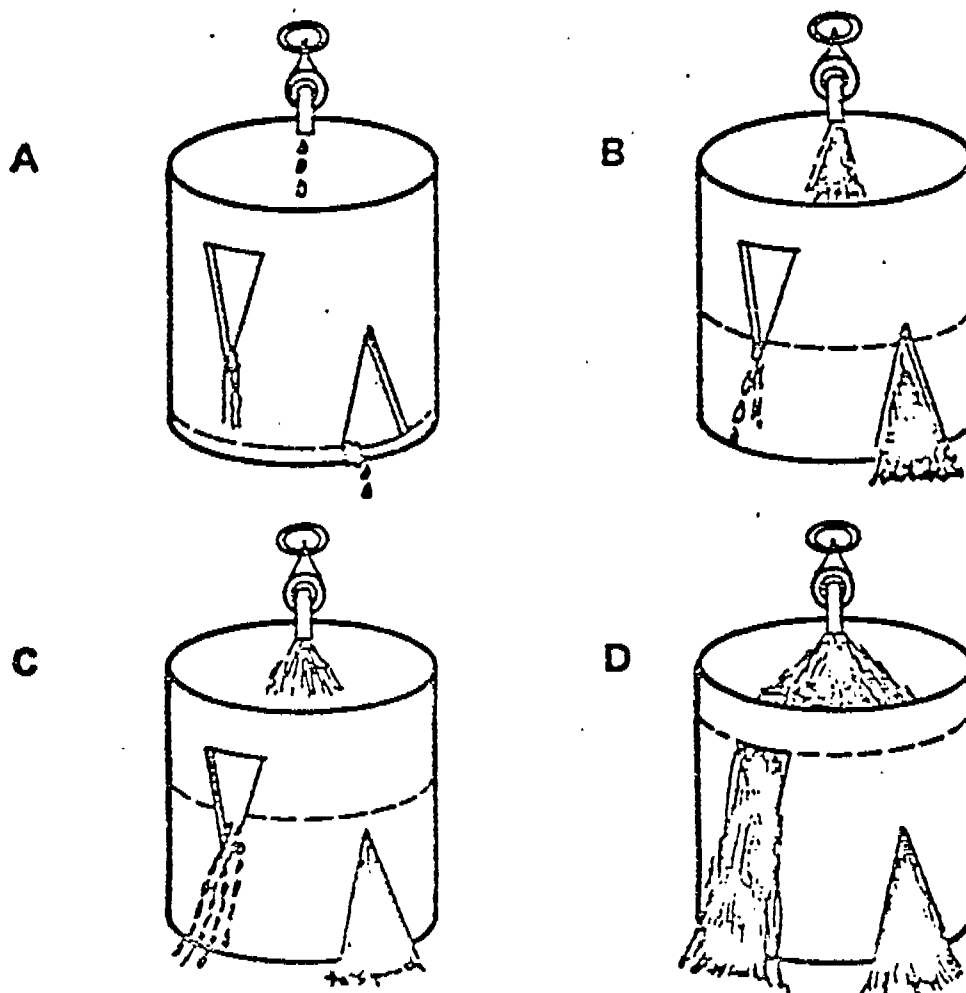
FIGURE 33

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Slide 5

FIGURE 7



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Slide 6

PERCENT OF ADMINISTERED ^{14}C -ACTIVITY ELIMINATED
FOLLOWING A SINGLE ORAL DOSE OF VINYL CHLORIDE

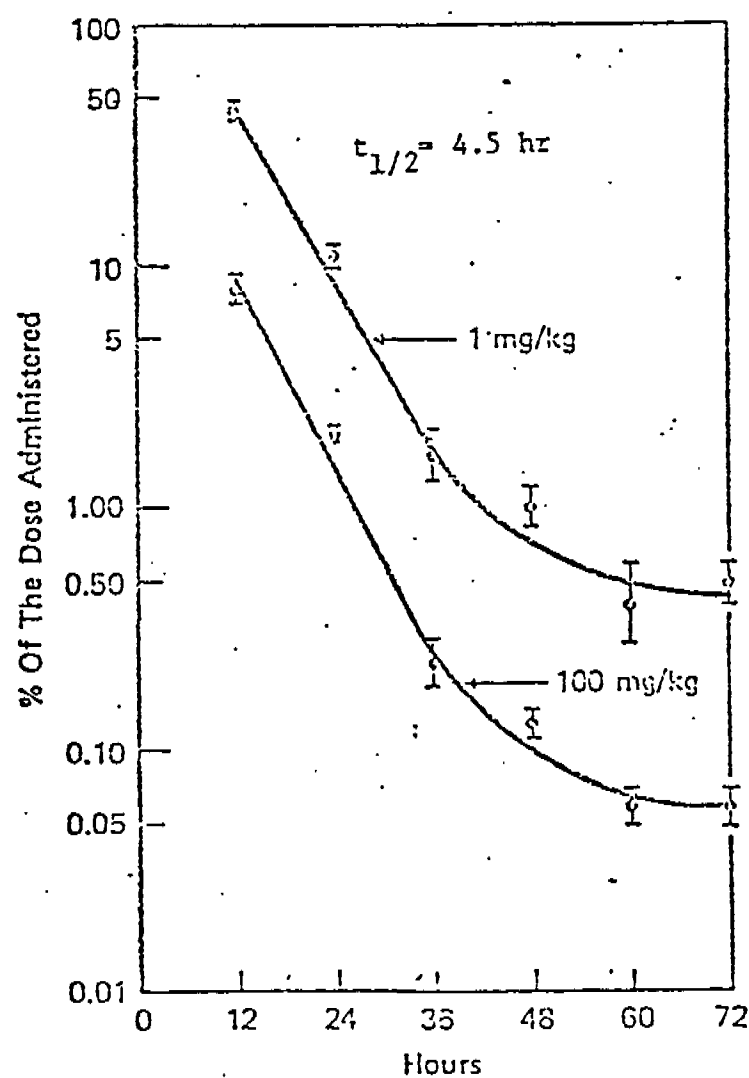
	<u>0.05 MG/KG</u>	<u>1.0 MG/KG</u>	<u>20 MG/KG</u>	<u>100 MG/KG</u>
EXPIRED AS:				
VC	1.4	2.1	41.6	55.6
CO ₂	9.0	13.3	4.8	2.5
URINE	68.3	59.3	22.6	10.8
FECES	2.4	2.2	1.0	0.5
CARCASS AND TISSUES	10.1	11.1	11.0	1.8
TOTAL RECOVERY	91.3	88.8	81.0	22.3

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Slide 7

(2)

PERCENT OF ADMINISTERED ^{14}C -ACTIVITY EXCRETED IN THE
URINE FOLLOWING SINGLE ORAL DOSE OF VINYL CHLORIDE



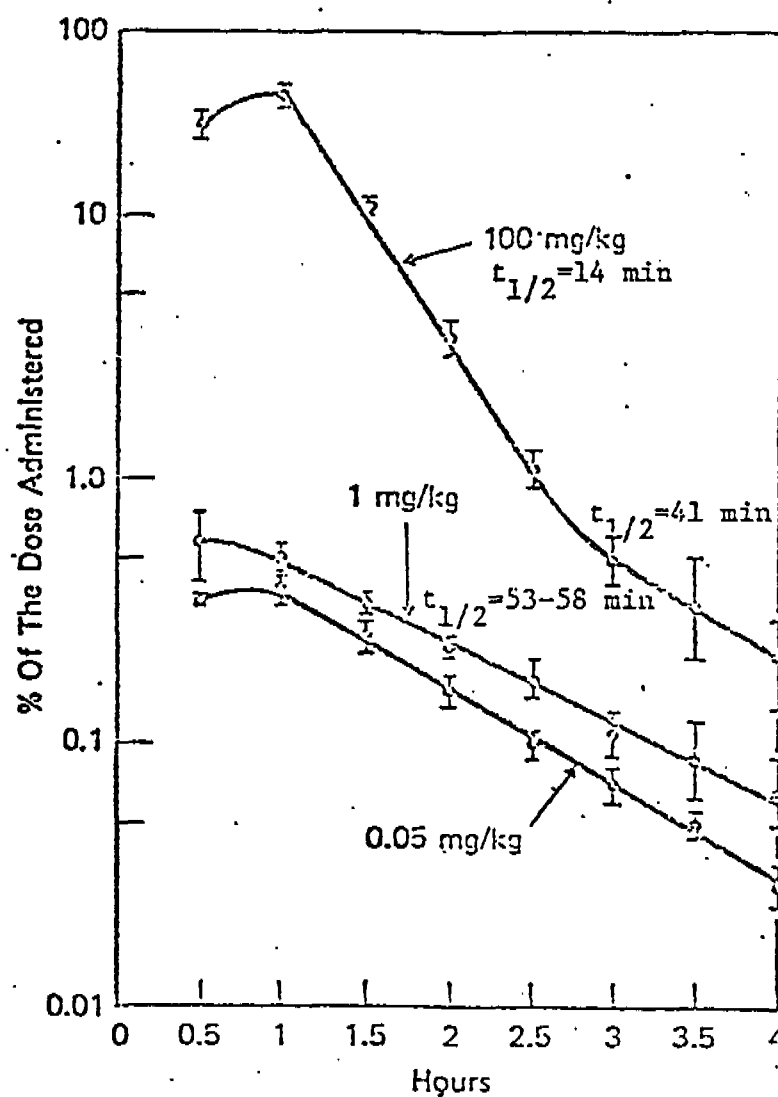
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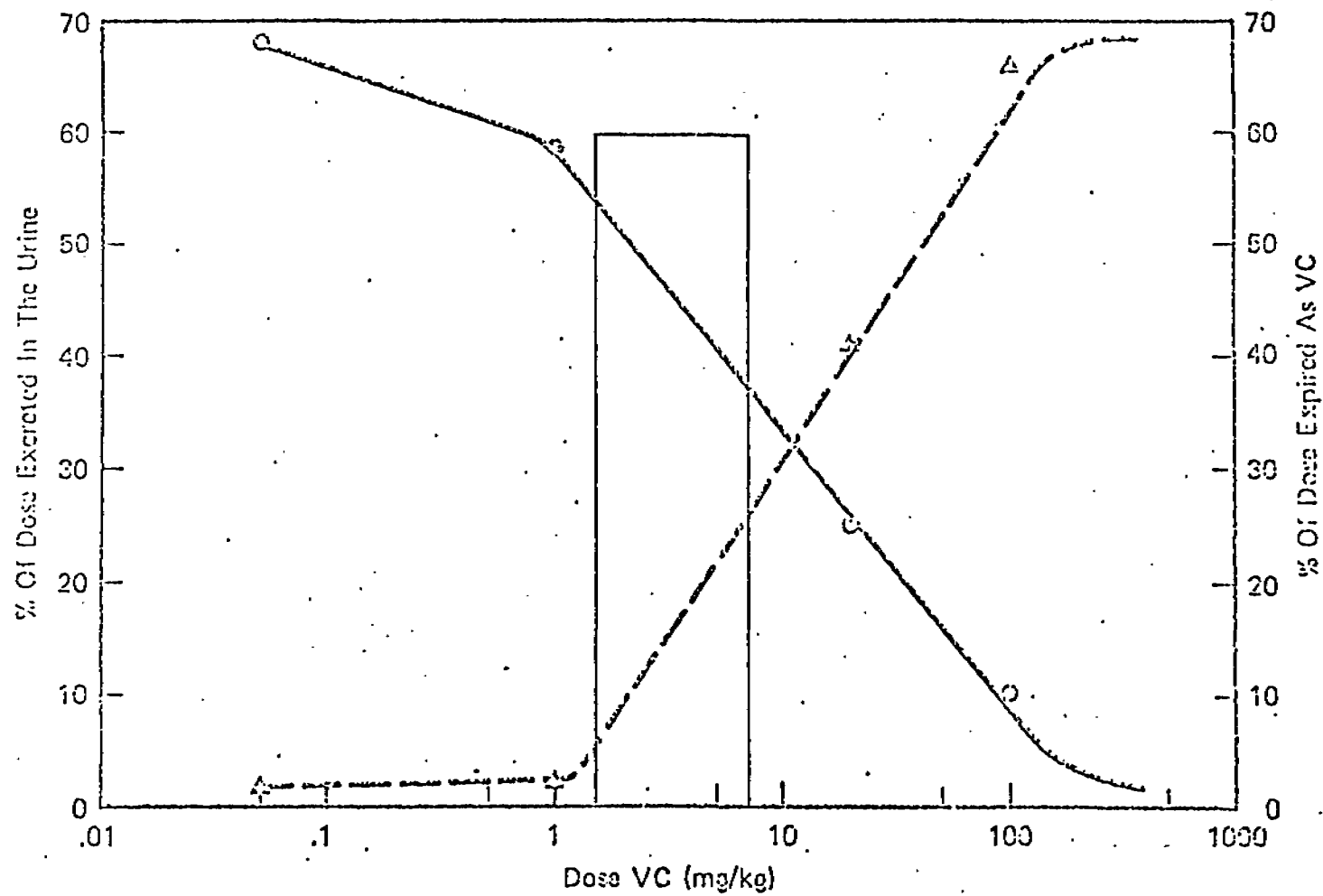
Slide 8

(3)

PERCENT OF ADMINISTERED VINYL CHLORIDE EXPIRED BY
THE LUNGS FOLLOWING SINGLE ORAL ADMINISTRATION

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R&S 100301

4

Slide 9

Slide 10

TABLE 1

Percent ^{14}C -Activity Eliminated During 72 Hr Following
Inhalation Exposure to ^{14}C -Vinyl Chloride for 6 Hr^a

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Exposure Concentration: Expired:	Percent ^{14}C -Activity	
	<u>10 ppm</u>	<u>1000 ppm</u>
As VC	1.61±0.16 ^b (4) ^c	12.26±0.96 ^b (814) ^c
As CO ₂	12.09±0.43 (30)	12.30±0.63 (817)
Urine	67.97±1.71 (169)	56.29±1.96 (3739)
Feces	4.45±0.22 (11)	4.21±1.05 (280)
Carcass and Tissues	13.84±1.16 (34)	14.48±0.52 (977)
Cage Wash ^d	0.15±0.08 (<1)	0.23±0.09 (15)
Total µg equivalents VC recovered	(248)	(6642)

^aExpressed as percent of the total ^{14}C -activity recovered.

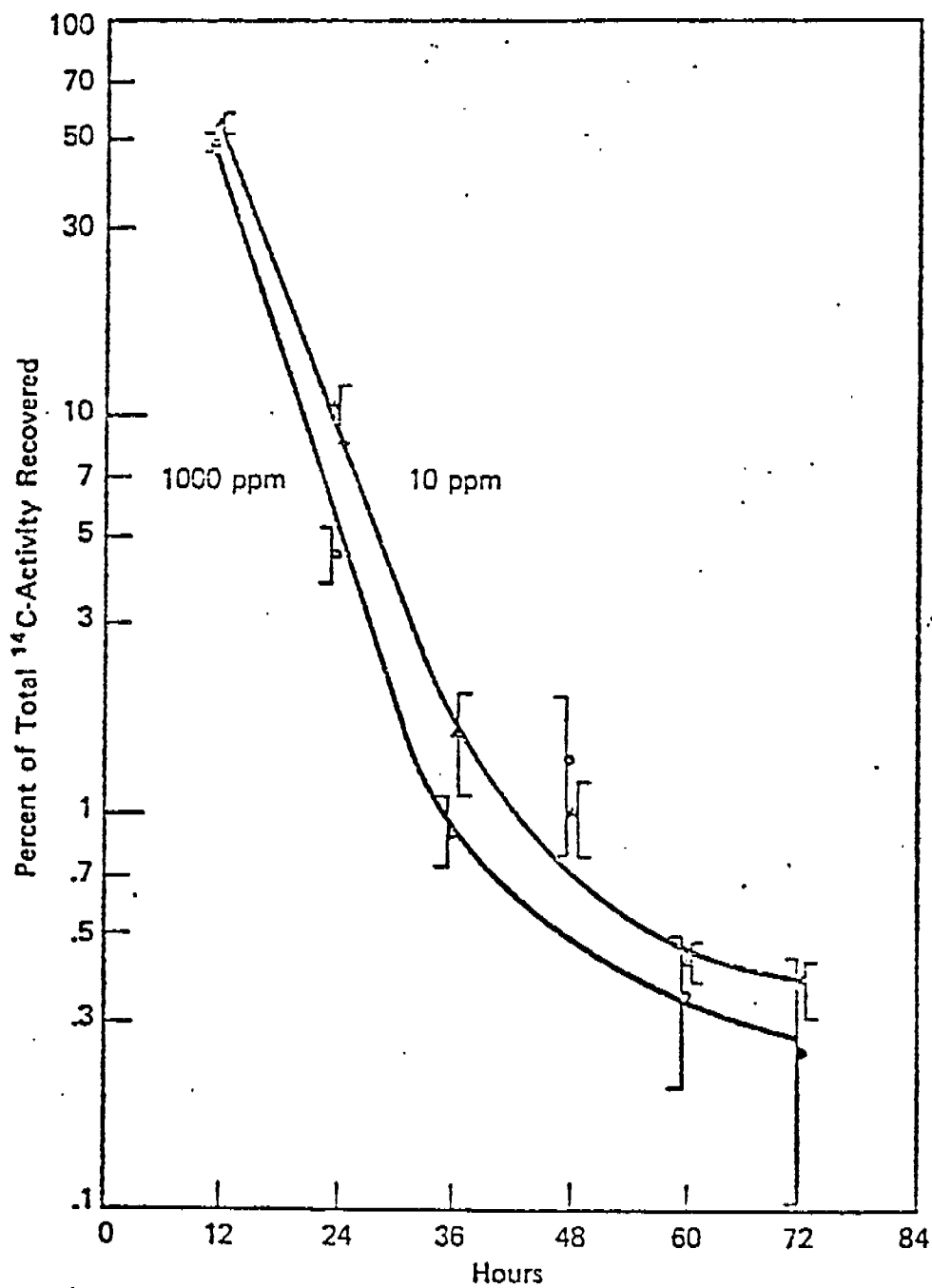
^bMean ± standard error from 4 rats.

^cMicrogram equivalents vinyl chloride

^dWater, acetone wash of the metabolism cage at termination
of the experiment.

Slide 11

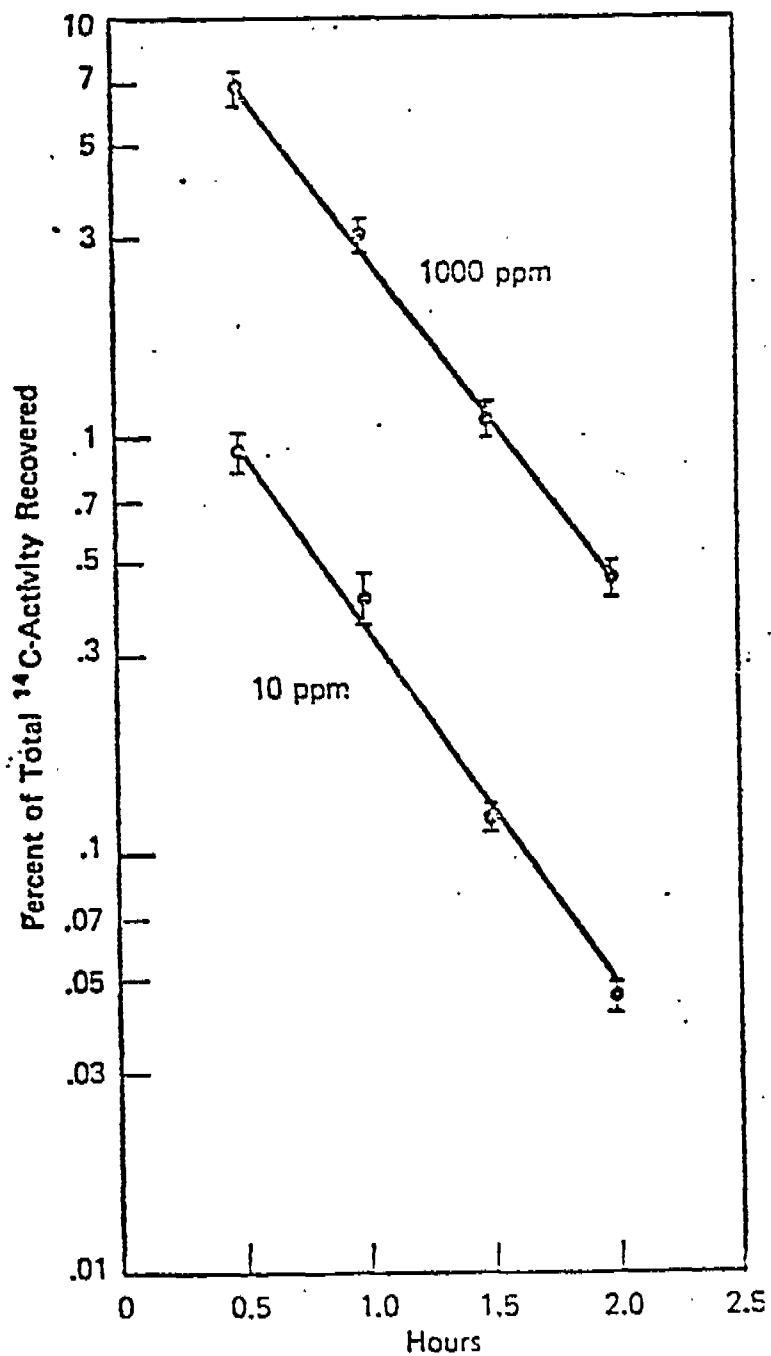
FIGURE 2



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Slide 12

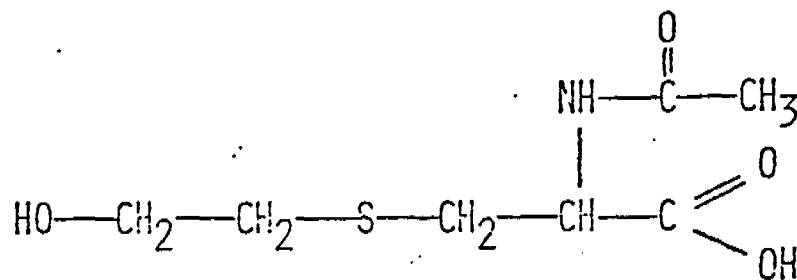
FIGURE 1



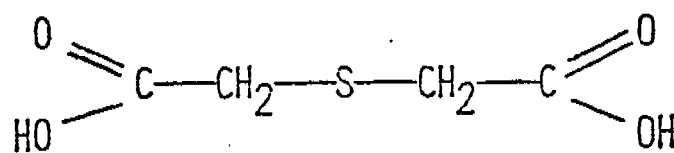
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Slide 13

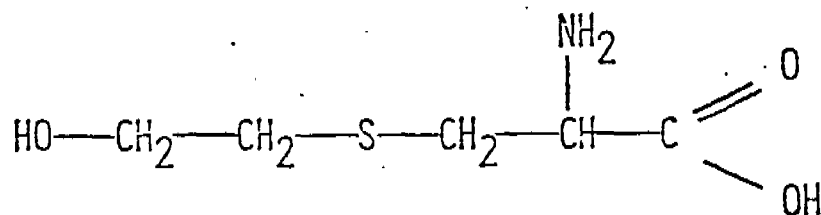
URINARY METABOLITES OF VINYL CHLORIDE



(A) N-ACETYL(S-2-HYDROXYETHYL)CYSTEINE



(B) THIODIGLYCOLIC ACID



S-(2-HYDROXYETHYL)CYSTEINE

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Slide 14

