TAPPI

TOX No:

1977 PAPER SYNTHETICS CONFERENCE

September 26-28
The Drake
Chicago, Illinois

TECHNICAL ASSOCIATION OF THE
PULP AND PAPER INDUSTRY

One Dunwoody Park, Atlanta, Ga. 30338 U.S.A.
Telephone (404) 394-6130
TWX 810-757-0145

SL 040753

TOXICOLOGICAL AND PHARMACOKINETIC STUDIES ON INHALED AND INGESTED VINYLIDENE CHLORIDE IN LABORATORY ANIMALS

J. M. Norris
Associate Scientist
The Dow Chemical Company
P. O. Box 1706
Midland, Michigan 48640

ABSTRACT

The toxicity of vinylidene chloride (VDC) was evaluated in 90-day and 2-year studies incorporating VDC in the drinking water of rats or by inhalation of the vapors. The levels of VDC in the drinking water studies were 0, 60, 100 or 200 ppm (mg/ℓ) and in the inhalation studies 0, 25 or 75 ppm. The toxicity of inhaled and ingested VDC in rats exposed for 90 days was limited to minimal reversible liver changes at 25 and 75 ppm by inhalation and at 200 ppm only in the drinking water study. VDC was nontumorigenic in rats exposed for 2 years by either route. Pharmacokinetic studies on inhaled and ingested VDC indicated VDC is metabolized in the rat to a reactive metabolite(s) which is normally detoxified by conjugation with glutathione. At relatively high exposure levels of VDC, as liver glutathione becomes depleted, toxicity is elicited. Preliminary data on the metabolism of VDC in mice indicate increased production of the reactive metabolite over that in the rat rendering the mouse more susceptible to the toxic effect of VDC. Daily administration of VDC in peanut oil to dogs for 90 days caused no adverse effects at dosages of 0, 6.25, 12.5 or 25 mg/kg. The reproductivity of rats on a three generation study, incorporating 0, 60, 100 or 200 ppm VDC in the drinking water, was not adversely affected. VDC was not teratogenic in rats or rabbits exposed on gestation days 6-15 or 6-18, respectively, to vapor concentrations up to 160 ppm for 7 hours/day or in rats on drinking water containing 200 ppm. A cytogenetic study in rats inhaling 0, 25 or 75 ppm VDC for 6 hours/day, 5 days/week for 6 months revealed no chromosomal aberrations.

A multi-study program to investigate the toxicity of vinylidene chloride (VDC) in laboratory animals has been in progress since 1974 in the Toxicology Research Laboratory of The Dow Chemical Company. The program is being conducted under the auspices of the Manufacturing Chemists Association and sponsored by fifteen companies*.

*American Cyanamid Company, Asahi-Dow Ltd., BASF-Wyandotte Corporation, B. F. Goodrich Chemical Company, The Dow Chemical Company, E.I. duPont de Nemours & Company, W. R. Grace & Company, Kureha Chemical Industry Company, Monsanto Company, Morton Chemical Company, Olin Corporation, PPG Industries, Rohm and Haas Company, Staley Chemical, and Tennessee Eastman Company.

The various studies in this program are:

1) 90-Day Toxicological Study Incorporating VDC in the Drinking Water of Rats
2) 2-Year Toxicological Study in Conjunction With a 3-Generation Reproduction Study Incorporating VDC in the Drinking Water of Rats
3) 90-Day Toxicological Study on VDC in Peanut Oil Administered to Dogs
4) 90-Day VDC Vapor Inhalation Study in Rats
5) 2-Year VDC Vapor Inhalation Study in Rats
6) Teratology Studies on Ingested or Inhaled VDC in Rats and Rabbits
7) Pharmacokinetic Study on Ingested or Inhaled VDC in Rats
8) Cytogenetic Study on Bone Marrow Cells of Rats

The 90-day and 2-year toxicity studies, ingestion and inhalation, were supplemented by pharmacokinetic studies whereby the fate of vinylidene chloride in the body of the animal was determined following exposure by both routes at high and low dose levels. The cytogenetic study was conducted in conjunction with the long-term inhalation study.

The 3 generation reproduction study was con-

ducted to evaluate the possible effects of VDC on the reproductivity of the animals. The teratology studies were conducted to evaluate the possible effects on the unborn by exposing pregnant animals, by ingestion or by inhalation, to vinylidene chloride. The vinylidene chloride used in the various toxicity studies was sales grade material obtained from The Dow Chemical Company.

An epidemiology study which was not a part of the MCA program has been reported[1] by The Dow Chemical Company on 138 workers exposed to work atmospheres containing VDC starting in 1944. There were no findings statistically related or individually attributable to VDC exposure in this cohort. The average TWA, depending upon the job performed by the workers ranged from <5 to 70 ppm.

The design of the 90-day and 2-year studies incorporating VDC in the drinking water of rats is given in Table 1. Groups of male and female Sprague-Dawley rats, 6-7 weeks of age at the start of the study, were supplied drinking water containing 0, 60, 100 or 200 ppm (mg/ℓ), nominal concentrations, of VDC.

The various parameters monitored during the studies included nonprotein sulfhydryl content of the liver and kidneys of the animals on the 90-day study. The purpose of these analyses was to help elucidate the biochemical effects and possible mechanism of toxic action of vinylidene chloride. Parameters also included were clinical observations for changes in appearance and demeanor, body weight, food consumption, water consumption, periodic hematology determinations, clinical chemistries (liver and kidney enzyme studies) and urinalyses. The weight of the major organs was measured and complete gross and microscopic examinations of organs and tissues were made on those animals dying during the study, those sacrificed in moribund condition or after 90 days or those surviving until the termination of the 2-year study.

The highest concentration (200 ppm) of VDC incorporated in the drinking water in these studies was determined by the limit of solubility of VDC in water. The daily mg/kg body weight dosage to the animals on the 90-day and 2-year studies are given in Table 2.

All of the animals on the 90-day study survived. There was no evidence of a toxicological effect associated with the ingestion of VDC as monitored by the various parameters. Microscopic pathological examination showed that minimal liver changes had occurred at the 200 ppm concentration only. The microscopic findings at the two lower levels were comparable to those in the control animals.

Incorporation of VDC in the drinking water of rats for 2 years did not adversely affect body weight, food consumption or water consumption. There was a nondose related decrease in survival of male rats at 18 and 24 months. Gross pathological examination of those VDC-treated animals that died during the course of the study, those that were sacrificed in moribund condition or those surviving to the termination of the 2-year study showed no compound-related changes. Microscopic pathological examination of the tissues and organs from these animals revealed no compound-related tumorigenic changes. The only significant nontumorigenic change seen in the VDC-treated animals was a statistically significant greater incidence of fatty liver changes and hepatocellular hypertrophy (portal area) at all dose levels in the females and at the high dose level in the males, and central lobular atrophy at the high dose level in the females only.

The 90-day and 2-year VDC vapor inhalation studies in rats were initiated at the same time. Twenty rats/sex/dose level were used for the 90-day study and 100 rats/sex/dose level were used for the 2-year study. The exposures were 6 hours/day, 5 days/week. The design of the studies is given in Table 3. Initially the exposure levels used for these studies were 0, 10 and 40 ppm with the provision that if no adverse effects were seen among a group of animals sacrificed after 30 days, the levels would be increased to 25 and 75 ppm, respectively. Interim kills were conducted after 6 months and 12 months for the evaluation of possible pathologic compound-related changes. The parameters monitored were the same as in the drinking water study. A cytogenetic study to evaluate the possible mutagenic effect of vinylidene chloride was conducted on a group of animals exposed for 6 months.

No compound-related adverse effects from exposure to vinylidene chloride vapors were seen among the rats exposed to 10 or 40 ppm for 30 days. The only significant finding seen in the rats exposed for 30 or 90 days to 25 or 75 ppm of vinylidene chloride was minimal microscopic changes in the liver (characterized by a minimal increase in the degree of vacuolation in the cytoplasm of the hepatocytes). These minimal changes have been interpreted as being reversible. Subsequent interim kills at 6 and 12 months in the 2-year study continued to show minimal microscopic changes in the liver. The incidence of occurrence of these changes was greater in the animals exposed to 75 ppm vinylidene chloride after 90 days on the study. An increased incidence was not seen in the animals exposed to 25 ppm until the 12-month interim kill.

No treatment-related adverse effects on demeanor or appearance, hematologic parameters or clinical chemistry values were observed in the animals on the 2-year study. There was a decrease in body weight gain in male rats only exposed to both concentrations during months 8-12 or 13 of the study. There was a slight increase in mortality in the female rats at both dose levels after 12 months on the study and in the males in the last months of the study. Gross pathological examination of animals dying during the study, those sacrificed in moribund condition or at interim kills or those surviving until the termination of the study showed no compound-related changes in the liver, urinary system, respiratory system or lymphoreticular system. A final statement on the pathological

examination must await the completion of the microscopic examination of the tissues and organs from these animals.

Results of the cytogenetic study in rats exposed for 6 hrs/day, 5 days/week for 6 months to 0 (control), 25 or 75 ppm VDC revealed no chromosomal aberrations in control or VDC-exposed animals.

The study in which VDC was administered daily to beagle dogs for 90 days showed no compound-related effect at dose levels of 6.25, 12.5, or 25 mg/kg as judged by clinical observations of demeanor and appearance, body weights, food consumption, hematologic or clinical chemistry determinations, organ weights and gross and microscopic pathological examinations of tissues and organs.

The three generation reproduction study was started in groups of rats on the 2-year study after they had been exposed to the various concentrations of VDC in the drinking water for 90 days (refer to Table 1). Each group of VDC exposed animals used in the reproduction study consisted of 10 males and 20 females, with 15 males and 30 females used for controls. The females of the first mating (F_0 dams) were remated because of decreased fertility in all groups including the controls. The pups in the F_{1B} litter were mated to produce the 2nd generation. The pups from the F_2 litter were mated to produce the F_{3A}, B and C litters. The parameters monitored in this study were the litter size and sex ratio at birth and weaning, the growth and survival of the neonate, toxicity prior to mating, fertility, length of gestation, delivery of live litters and gross and microscopic pathological examination of tissues and organs from dams and pups. No clear cut effect due to VDC in the drinking water was observed in this reproduction study. There was a slight, but noncompound-related effect, on pup survival in the F_2 litters and a marked effect at all dose levels in the F_{3A} litters but not in the F_{1A}, F_{1B}, F_{3B} or F_{3C} litters.

The teratology study on ingested VDC in rats was conducted at the highest possible concentration of VDC in the drinking water, 200 ppm. The teratology study on inhaled VDC was conducted in rats and rabbits at vapor concentration of 0, 20, 80 and 160 ppm for 7 hours/day. The rats in both studies were exposed to VDC on days 6-15 of gestation and the rabbits on days 6-18 of gestation.

Vinylidene chloride was not teratogenic in rats or rabbits inhaling up to 160 ppm of the compound for 7 hrs/day or in rats given water containing 200 ppm of the compound. Some evidence of embryotoxicity or fetotoxicity was observed in both rats and rabbits exposed to vinylidene chloride by inhalation; these effects were associated with maternally toxic levels of exposure. At concentrations of vinylidene chloride which had little or no effect on the dam, no adverse effect on embryonal or fetal development was discerned in either species.

Pharmacokinetic studies were conducted as a part of this multi-study program to ascertain the fate of vinylidene chloride in the body of the rat and to give perspective to the toxicological findings. Using radioactive VDC, the rates at which VDC was absorbed, distributed and ultimately cleared from the body of the rat via metabolism and/or excretion were determined for high and low dose levels. Thus the pharmacokinetic profile was obtained for a single oral dosage of 1 or 50 mg of radioactive VDC/kg body weight of the rats and after a single 6-hour inhalation exposure to 10 ppm or 200 ppm of the radioactive material. The elimination of the radioactivity was followed for 72 hours in both fed and fasted rats. This involved analysis for radioactivity of expired air, urine and feces. Subsequently the distribution or residual radioactivity in tissues and carcass was determined and an attempt was made to identify urinary metabolites. These studies show that the fate of VDC following oral administration or inhalation exposure of rats is dependent upon the dose administered and the nutritional state of the animals. As the dose level is increased from 1 to 50 mg/kg oral or from 10 to 200 ppm inhalation, the metabolic pathway becomes saturated so that, percentage-wise, less of the dose administered is metabolized and more is eliminated via the lungs as VDC. At the 1 mg/kg oral dose and the 10 ppm inhalation dose there was no difference in elimination by fed vs. fasted rats. However, at relatively high levels of 50 mg/kg oral or 200 ppm inhalation there was a significant increase in the excretion of VDC via the lungs and decrease in the urinary excretion of radioactivity in fed vs. fasted rats. The studies indicated that VDC is biotransformed to a reactive metabolite(s) in the liver. At low levels, 1 mg/kg or 10 ppm, the reactive metabolite(s) is detoxified by conjugation with glutathione in the liver. However, when the liver glutathione levels are lowered or depleted i.e., in fasted animals or at high doses of VDC, 50 mg/kg or 200 ppm, VDC or the reactive metabolite(s) are free to react with tissue macromolecules and a toxic response is elicited. Two of the four major urinary metabolites that have been identified give evidence of the conjugation with glutathione. These are thiodiglycolic acid and N-acetyl-S-(2-hydroxyethyl)cysteine.

A pharmacokinetic study of inhaled VDC in Swiss mice was started in Dow's Toxicology Research Laboratory after recent reports by Maltoni[2] and Lee[3] of tumorigenic findings in mice exposed to VDC. Maltoni qualified the finding of kidney tumors in Swiss mice exposed to 25 ppm, but not 10 ppm, indicating that (1) 25 ppm was near the acutely lethal dose (50 ppm) for Swiss mice; (2) 25 ppm caused toxic changes in the kidney early in the study, (3) Swiss mice have a high incidence of kidney disease which could have enhanced the susceptibility to the tumorigenic changes, and, most significant, (4) the Swiss mice were far more susceptible to VDC-induced toxicity and/or lethality than the rat or man.

Lee reported that CD-1 mice exposed for one year to 55 ppm VDC, a lethal level for the Swiss mouse, showed hepatomas, angiosarcoma of the liver and pulmonary adenomas. Preliminary pharmacokinetic data the Dow study suggest that production of reactive metabolite(s) is greater in the mouse than in the rat, thus rendering the mouse more susceptible to the toxic effects of VDC. Comprehensive interpretation of the toxic and tumorigenic effects seen in the mice must await the completion of this study. However, it is sufficiently evident that the use of mouse data for human hazard evaluation of exposure to VDC is inappropriate.

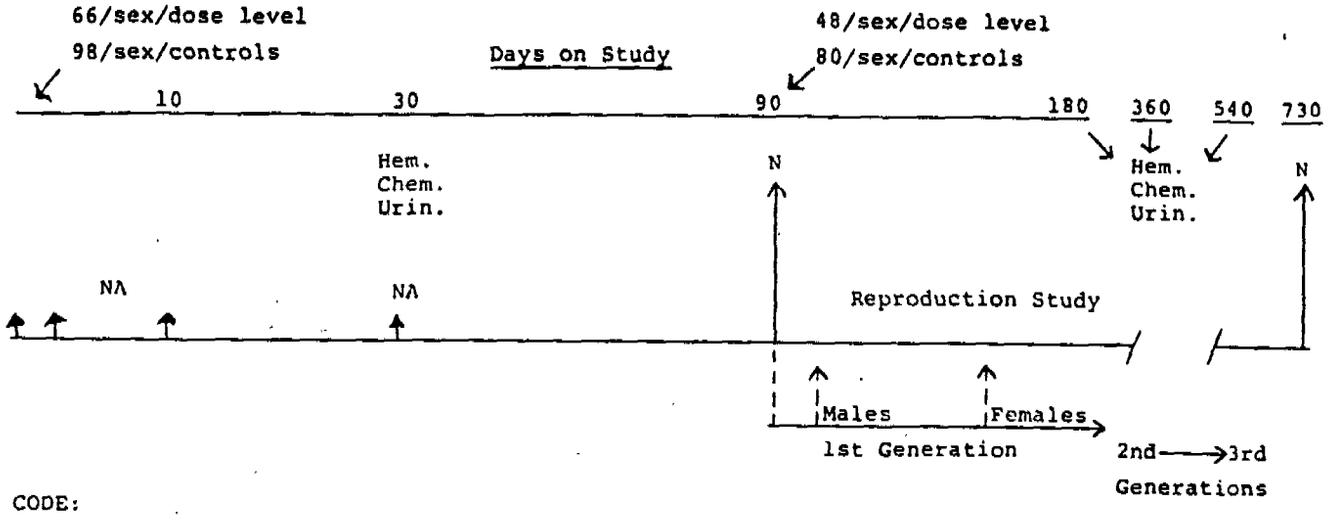
To summarize - ingestion of drinking water containing VDC by rats produced minimal liver changes interpreted as being reversible in nature in a 90-day study at the highest concentration administered, 200 ppm, which was equivalent to daily dosage of 19-26 mg/kg body weight. The results of the pharmacokinetic study in rats, indicating saturation of metabolic pathways at relatively high levels, tie in with the minimal toxicological findings at the highest level in the 90-day ingestion study. There was no grossly observable evidence of progression of this effect on the liver or an effect on any other organ in the animals in the 2-year ingestion study. Dose related toxic changes were seen on microscopic pathological examination of liver tissue at all dose levels in the females and only at the top dose level in the males. Ingestion and/or inhalation of VDC by rats on teratology, cytogenetic or reproduction studies showed the compound to be neither a teratogen, mutagen or one adversely affecting reproductivity. Dogs administered daily doses as high as 25 mg/kg body weight for 90 days showed no compound-related adverse effects.

Inhalation of 25 or 75 ppm VDC by rats for 90 days produced the same kind of minimal liver changes seen at the highest dose level in the ingestion study. The results of the inhalation pharmacokinetic study, indicating diminished ability to metabolize VDC at a concentration in excess of 10 ppm, also tie in with the minimal toxic effects seen at 25 or 75 ppm. There was no grossly observable evidence of progression of the effect on the liver or an effect on any other organ in the animals on the 2-year inhalation study.

1. Ott, M. G., Fishbeck, W. A., Townsend, J. C. and Schneider, E. J., J. Occup. Med. 18(11):735-738 (November, 1976).

2. Maltoni, Cesare, "Carcinogenicity Bioassays of Vinylidene Chloride" paper presented at TAPPI International PVDC Seminar, Hamburg, Germany; January 24-26, 1977.

3. Lee, Cheng-Chun, "Evaluation of the Environmental Toxicants Vinyl Chloride (VC) and Vinylidene Chloride (VDC), Progress Report No. 10, MRI Project No. 3612-B for National Institute of Environmental Health Sciences (1977).



CODE:

N = Necropsy (study terminated)
NA = Necropsy for nonprotein bound SH determination
HEM = Hematology determinations
Chem.= Clinical Chemistry determinations
Urin.= Urinalysis

TABLE 1

Design of 90-Day and 2-Year Toxicology Studies and Three Generation Reproduction Study on Vinylidene Chloride Incorporated into the Drinking Water of Rats

Concentration in Water, ppm	Calculated Equivalent Dose (mg/kg/day)	
	90-Day Study	2-Year Study
0 (controls)	0	0
200-230[1]	6-8[2]	16-40[3]
100-120	10-13	8-20
60-70	19-26	5-12

[1] Maximum concentration based on water solubility.

[2] Range based on body weight of 305-487 grams for male rats and 213-313 grams female rats.

[3] Range based on body weight of 200-500 grams for male rats on 2-year study. Female dose range falls within range given.

TABLE 2

90-Day and 2-Year Studies Incorporating Vinylidene Chloride in the Drinking Water of Rats

	Days on Study					
	90-Day Study		2-Year Study			
Monitored Parameter	30	90	30	180	365	735
Clinical Observation	X	X	X	X	X	X
Body Weights	X	X		X	X	X
Hematology		X		X	X	X
Urinalyses		X		X	X	X
Clinical Chemistries	X	X	X	X	X	X
Organ Weights		X	X	X	X	X
Cytogenetics				X		
Nonprotein Bound Sulfhydryl		X				
Gross Pathologic Exam	X	X	X	X	X	X
Microscopic Pathologic Exam	X	X	X	X	X	X

TABLE 3

Design of the 90-Day and 2-Year Vapor Inhalation Studies on Vinylidene Chloride in Rats