NIEHS CONFERENCE NOTES MAY 9 1977

COMPARATIVE METABOLISM AND TOXICITY OF VINYL CHLORIDE
RELATED COMPOUNDS
Bethesda, Maryland
May 2, 1977

AGENDA: See attached Agenda

Dr. David P. Rall, Director, NIEHS was Chairman of the first portion of the morning session, May 2, 1977. After the break, Dr. Hans Falk took over the Chairmanship.

Manuscripts for summaries of the papers were not available at the meeting, and each author was requested to submit their manuscripts to NIEHS for eventual publication in Environmental Health Prospectives. Any request for papers should be addressed to the individual office rather than NIEHS.

Dr. Caesar Maltoni - Recent Findings on the Carcinogenicity of Chlorinated Olefins.

Dr. Maltoni discussed primarily the findings in the labs regarding carcinogenicity of vinyl chloride and vinylidene chloride. The following factors were used as an outline of his discussion:

1. Molecular Structure
2. Concentration
3. Length of Treatment
4. Route of Administration
5. Species
6. Strain
7. Age

Dr. Maltoni then discussed his comparative results of testing of vinyl chloride and vinylidene chloride. With vinyl chloride, Maltoni found multiple tumors in rats, mice and hamsters following inhalation exposure to vinyl chloride. In the case of vinylidene chloride, the only tumor Dr. Maltoni reported was adenocarcinomas of the kidneys. He did not find any tumors in the rats or in hamsters. The dosage tested of vinylidene chloride was 25 ppm.

With respect to concentration, Maltoni illustrated the vinyl chloride toxicity information using data for inhalation. The following was the table used:

Vinyl Chloride Concentration	Liver Angiomas	Nephure Blastomas
200	12	3
150	5	7
100	1	10
0	0	0

Dr. Maltoni used this illustration to show that the incidents of tumors is not always dose related, as illustrated by the Nephur Blastomas.

Maltoni then gave data, again using vinyl chloride inhalation exposure of 52, 17, 5 and 0 weeks exposure at two different concentrations, showing that the incidents of tumors is reduced with shorter periods of exposure.

With respect to routes of administration, Maltoni illustrated data comparing the level of toxicity in air as opposed to toxicity by oral administration. With vinyl chloride, the comparison was 10,000 ppm in air was roughly equivalent to 16 mg/kg body weight oral administration by gavage.

Strain - Maltoni indicated that the Sprague-Dawley strain was much more sensitive than the Wistar strain as it relates to vinyl chloride toxicity - carcinogenicity.

Sex - With the Swiss mice the males were much more sensitive than the females, i.e., the ratio of 24 to 1.

Maltoni then gave some toxicity values for vinylidene chloride for acute exposure, i.e., exposure four hours daily for two days at concentrations of 200 ppm. He showed that rats had no toxic effects of 200 ppm whereby mice there was toxic effects with the males being more sensitive than the females with each strain. Four strains of mice were tested with the following results:

STRAIN AND SEX	SURVIVORS
Swiss Males	9/60
Swiss Females	60/60
BALB/C Males	6/30
BALB/C Females	30/30
C3H Males	13/30
C3H Females	19/30
C57BL Males	23/30
C57BL Females	50/30

Maltoni then discussed comparative toxicity of vinyl chloride at various ages of animals showing how in the newborn there is no difference between sex whereas at a later age there is a development of sex differences. It is probable that the differences has to do with oxidative enzyme development in the females.

Dr. P. L. Viola - Carcinogenicity Studies on Vinylidene Chloride

Dr. Viola reported his studies of vinylidene chloride using the Wistar rat exposed at 200 ppm. The following is a summary of his results:

Treatment	Sex	Numbers	Tumor Bearing Animals	Tumor of Abdominal Cavity	Lung Tumor	Liver Tumor	Percent
200 ppm	M	51	8	7	0	1	16
200 ppm	F	23	9	8	1	0	39
Control	M	30	5	5	0	0	17
Control	F	30	10	0	1	0	33

On the Strague-Dawley rats the following was found:

Treatment	Sex	Numbers of Animals	Tumor Bearing Animals	Percent
100 ppm	M	30	3	10
100 ppm	F	30	17	57
75 ppm	M	16	1	6
75 ppm	F	20	9	43
Control	M	30	0	0
Control	F	30	15	50

It would appear that no conclusive results were obtained by Dr. Viola. It was unclear as to exactly what experimental protocol was used.

Dr. L. W. Rampy - Toxicity and Carcinogenicity Studies on Vinylidene Chloride.

Dr. Rampy reported the long term studies as sponsored by the Manufacturing Chemists Association at the Dow Chemical Laboratories. These studies include a two year drinking water study in rats and a two year inhalation study. The studies are not yet completed with respect to histopathological examination of all tissues, however all animals have been sacrificed and gross observations made. Dr. Rampy gave the purity of vinylidene chloride including any contaminant such as vinyl chloride. There was considerable variation in the vinyl chloride content of the vinylidene chloride with some values being in excess of 1,000 ppm.

The protocol for the study includes a companion 90 day study and a three generation reproduction study. The dosage concentration was 0, 60, 100 and 200 ppm in the drinking water, with 80 animals per sex in the control and 48 animals per sex in each of the treated groups. All drinking water was assayed, with an 8 to 10% overage of vinylidene chloride determined.

There was no effect on body weight or food intake due to the dietary treatment, excepting that at some intervals there was variation which may or may not be due to the experimental treatment. Mortality - there was little difference in mortality incidents at various intervals, however it was somewhat higher at some intervals in the treatment groups as opposed to the controls. Throughout the study there did not appear to be a significant decrease in body weight due to experimental treatment.

Dr. Rampy gave values on the tumor incidents, with no apparent increase due to experimental treatment of the tumors which were discussed. Overall there was no increase in tumor numbers or number of animals with tumors. The final report has not yet been prepared.

With respect to the inhalation study, this was conducted using the Sprague-Dawley rat with initial concentrations of 10 and 40 ppm. After completion of the subacute phase, the dosage was increased to 25 and 75 ppm. There was little effect on growth and mortality was slightly higher in the treated group.

The toxic effects were questionable reduction in testicular weight following 30 days of treatment, however this was not observed at later times. No other observation in the inhalation study was consistent.

Following the presentation of Dr. Rampy, there was a question and answer program. The first question had to do with the lowest concentration of vinyl chloride which would produce mammary tumors, the question was addressed to Dr. Maltoni. Dr. Maltoni said that he would not have the completion of his ongoing studies until mid-June, and at that time could report the results of low level inhalation exposure.

Dr. Rudolph Jaeger - from Harvard, then discussed Circadian Rhythms as it relates to possible detoxification mechanisms and influence of treatment timing upon detoxification. Dr. Jaeger said there was a ten to one hundred fold differences in the glutathion concentration in blood and liver, which was related to eating patterns. The lowest concentration being in the evening, where the animals would not be exposed to the inhalation work.

There also was discussion of mixed function oxidase, with the differences between the sexes probably being due to the concentration of these enzymes, i.e., the female has higher level than the male.

Dr. E. K. Weisburger - Carcinogenicity Studies of Halogenated Hydrocarbons

Dr. Weisburger discussed the contract with Hazleton Laboratories in which several halogenated hydrocarbons were evaluated in a carcinogenic bioassay program using the Osborne-Mendel rat and (C57BL X C3H)F_1 mice. She outlined the program from the standpoint of the acute dose ranging, using 10 small groups of animals, and determining the levels to be used in subacute studies. 8 week pilot programs were initiated using five males and 5 female rats in each group. All exposures were done by oral gavage using cottonseed or other edible oil as the carrier.

Carbon tetrachloride was used as a positive control in these studies. The duration of exposure was 78 weeks, followed by a 12 week post-treatment period in the mice and a 32 week post-treatment period in rats, for a total of 90 weeks mouse study and 110 weeks rat study.

Dr. Weisburger indicated that reports had been written on trichloroethylene chloroform and 1,1,1 trichloroethylene. She also said that a paper by Olson, et al regarding Ethylene dibromide and 1,2-dibromo-3-chloropropane has been published (J NCI 51 1993, 1973).

Dr. Weisburger reported the carcinogenicity of the TCE and Chloroform, and the fact that 1,1,1 Trichloroethylene had no effect on the rats or mice.

Dr. Weisburger also discussed the results of 1,2-dichloroethane, 1,1-dichloroethane, 1,2-trichloroethane, hexachloroethane, 3-chloropropane and tetrachloroethylene. All of these compounds provoked tumors in the mouse excepting 3-chloropropane.

Dr. B. L. Van Duuren - Chemical Structure, Reactivity and Carcinogenicity of Halohydrocarbons.

Dr. Van Duuren discussed possible mechanisms for activation of the halogenated hydrocarbons, using Trichloroethylene as an example. He discussed the formation of peroxides or free radicals as potential mechanisms. Dr. Van Duuren discussed possible substitutes for trichloroethylene, indicating that methalene chloride had been substituted for trichloroethylene in the manufacture of Sanica. He was concerned about the possibility of carcinogenicity of methylene chloride and said it "should be looked at". Dr. Van Duuren then discussed metabolic studies on several compounds, including perchlcroethylene and tetrachloroethylene, including potential metabolites such as epichlorhydrin and glycydaldehyde.

Van Duuren also discussed his attempts to trap intermediates, and using mercaptan derivatives. He had been unsuccessful in those attempts.

Dr. Kociba - Chronic Toxicity and Reproduction Studies with Rats Given Hexachlorobutadiene.

Dr. Kociba reported results of reproduction studies using 0,0.2,2, and 20 mg/kg of Hexachlorobutadiene orally.

Using adult animals, he found toxicity at 20 mg/kg, lesser effects at 2 mg/kg, and no effect at the 0.2 mg/kg. In the long term toxicity studies he observed at 20 mg/kg kidney nodules, kidney neoplasia and kidney hyperplasia. At 2 mg/kg kidney hyperplasia and 0.2 mg/kg there was no effects.

With respect to the kidney neoplasia the following was observed:

	DOSAGE			
	0	0.2	2.0	20.0
Males				
Renal Adenomas	1/90	0/40	0/40	2/39
Renal Adenoma Carcinomas	0/90 1/90	0/40 0/40	0/40 0/40	7/39 9/39
Females				
Renal Adenomas	0/90	0/40	0/40	3/40
Renal Adenoma Carcinomas	0/90 0/90	0/40 0/40	0/40 0/40	3/40 6/40

It was concluded that there was a dosage effect upon tumor incidents of hexachlorobutadiene.

Dr. C. C. Lee - Toxicity and Carcinogenicity of Vinyl Chloride Compared to Vinylidene Chloride

Dr. Lee conducted his work at the Mid-West Research Institute, Kansas City, MO. under the contract from NIEHS.

The protocol used was inhalation exposure, with rats and mice, with 36 animals per sex per group. With vinyl chloride the concentrations were 50, 250 and 1,000 ppm with vinylidene chloride the concentrations were 55 ppm.

Inhalation exposure was 6 hours per day, 5 days per week. There were intermediate terminations of animals at 1, 2, 3, 6 and 9 months, and the study was terminated at 12 months.

With respect to the vinylidene chloride, Dr. Lee found 7 mice exposed to 55 ppm vinylidene chloride had bronchial alveolar adenomas, as compared to one mouse in the control group. Dr. Lee also reported Hepatic angiosacoma in females exposed to the 55 ppm vinylidene chloride. With respect to malignant lymphoma he found it in the vinyl chloride but not in vinylidene chloride. Lee found the rats more sensitive to vinyl chloride than mice.

Dr. Maltoni questioned Dr. Lee as to the strain of mice. The answer was CD1. Dr. Maltoni said that the dosages used by Lee is much higher than could be tolerated in the four strains of rats tested in Italy. Maltoni also pointed out that there was no angiosacoma reported in the Italian studies. There was a good deal of discussion as it relates to the method of administration of the vinyl chloride and vinylidene chloride in the inhalation chambers, and the possibility of carry-over in that the same chambers were used for both compounds. The measurement of the gases in the chamber was by gas chromatograph using a carbontetrachloride standard. Dr. Lee was not certain as to whether the same columns were used for both gases, and whether the separation between the vinyl chloride and vinylidene chloride was such that there could be a determination of cross contamination.

Dr. Lee also reported the results of acute studies of vinyl chloride and in vinylidene chloride found acute hepatitis in 5 days after exposure to vinylidene chloride at sublethal doses.

WAO/lr