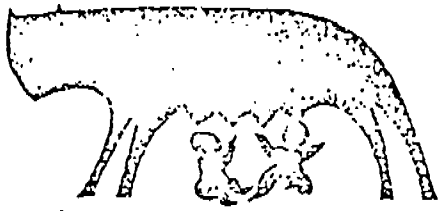
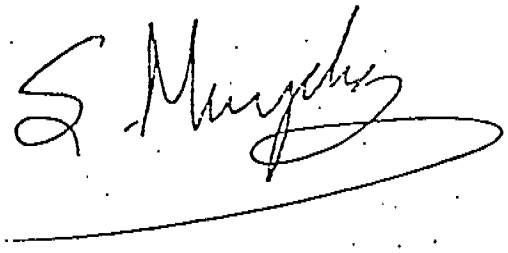






"Learn from the beasts the physic of the field"
Alexander Pope



# THE PHARMACOLOGIST

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718

INHALATION TOXICITY OF VINYL CHLORIDE (VC) OR VINYLIDENE CHLORIDE (VDC) IN RATS AND MICE. C. C. Lee, J. C. Rhinehart,* W. B. House,* P. J. Peters,* J. S. Woods, and R. L. Dixon. Midwest Research Institute, Kansas City, MO 64110, and Natl. Inst. of Environ. Health Sci., NIH, Research Triangle Park, NC 27709.

Exposure to VC at levels of 250 up to 30,000 ppm for 1 months was reported to cause tumors of the skin, lung, bo liver and/or mammary gland in rats or mice (Cancer Res. 2 516, 1971; Lincei-Rend. Sc. Fs. Mat. enat. 56:1, 1974). In the present study, rats of both sexes exposed to 50, 250, or 1,000 ppm of VC, 6 hr/day, 5 days/week, for up to 6 months did not show any significant adverse effects including weight gain, various clinical laboratory data, chromosome analysis, or pathologic lesions. In contrast, mice exposed to VC developed bronchiolar adenoma in 2 months and mammary gland carcinoma with pulmonary metastasis in 6 months. The carcinogenic effect appeared to be dose related. The bronchiolar adenoma was also observed in mice exposed to VDC at 55 ppm for 6 months. However, the carcinogenic effect of VDC was questionable. (Supported by Contract No. NIEHS-NO1-ES-2-2084.)

719

CONTINUOUS INHALATION OF 1,1-DICHLOROETHYLENE (DCE) BY RATS AND MICE DURING GESTATION. R. D. Short,* J. L. Minor,* W. B. House,* M. Marcus,* and C. C. Lee. Midwest Res. Inst. Kansas City, MO 64110 and Special Chemicals Branch, EPA, Washington, D.C. 20460.

CD rats and CD-1 mice were exposed to DCE for 23 hours a day for up to 10 days. In pregnant animals the $LC_{50}$ value was greater than 460 ppm for rats and 80 (43-119) ppm for mice. No mice survived 100 and 300 ppm of DCE. At 460 ppm 7/15 pregnant and 1/13 nonpregnant rats died. Rats and mice exposed to DCE from day 6 to 16 of gestation had both a reduced weight gain and feed consumption during treatment. These effects initially occurred between 15 and 55 ppm of DCE. DCE did not affect rat fetuses/litter at concentrations up to 460 ppm. No litters were produced in mice at 30 and 55 ppm of DCE. Rat fetal body weights were reduced at 300 and 460 ppm. Similar effects on development occurred in animals pair-fed to these groups. Therefore, these effects could not be directly attributed to DCE. No gross, soft-tissue, or skeletal malformations, which could be attributed to DCE, were observed in rats exposed up to 460 ppm or mice exposed to 15 ppm DCE from gestational day 6 to 16. (Supported by EPA Contract No. 68-01-3242.)

720

ALDRIN TOXICITY IN MAMMALIAN CELLS. D. P. DeVore*, H. A. Sheridan*, J. S. Pitman*, and T. B. Hutson* (SPON: R. I. Freudenthal). Battelle, Columbus Laboratories, Columbus, Ohio 43201.

Suspension cultures of HeLa cells were used to expand previous methodologies (J. Agr. Food Chem., 21:362, 1973) for the measurement of pesticide toxicity based on biochemical responses. Experiments were conducted to measure the time course of $^{14}$C-aldrin binding to HeLa cells at a single pesticide dose and as a function of filtrate dose as reflected by changes in protein synthesis ($^{3}$H-amino acid incorporation). Aldrin binding reached a maximum in 15 minutes, approximately 5 minutes later than complete inhibition of amino acid uptake. $^{14}$C-aldrin binding was dose dependent over a range of 0 to 400 ug/ml. At a survey dose of 302 ug of aldrin, 30.2 ug was bound to 2 x $10^6$ cells. Below 50 ug/ml little to no effect on amino acid incorporation was observed. Preliminary studies to examine mechanisms of pesticide toxicity were conducted by determining the effect of aldrin on ATP and cyclic AMP (cAMP) levels in exposed cells. ATP levels were grossly affected and decreased following aldrin incubation. Experiments to measure effects on cAMP levels are still in progress. These results provide basic information required for the development of an accurate-predictive cell culture screen for pesticides. (Supported by USPHS, NIEHS Grant ES00365).

721

DIFFERENTIAL PROTECTION BY CERTAIN AGENTS AGAINST CARBON TETRACHLORIDE-INDUCED INCREASE IN BILE DUCT-PANCREATIC FLUID FLOW. T. Imamura*, J. M. Fujimoto, A. Klecker*, R. E. Peterson* and C. F. Ervin*. Med. Col. Wis., Milwaukee, Wis. 53233.

Since the mechanism by which $CCl_4$ (1 ml/kg i.p. in corn oil, 24 hr pretreatment) increases bile duct-pancreatic fluid flow (BDPF) is not known, the purpose was to see whether agents which protect against certain $CCl_4$ effects also protect against the increase in BDPF. Change in BDPF was assessed against increased SGPT, hepatic necrosis (HN), hepatic triglyceride (HTG), and decreased hepatic bile flow (HBF). Cysteine (1.9 g/kg, p.o.) administered 30 min before $CCl_4$ significantly protected against the increased BDPF, SGPT, HTG and HN but not the decreased HBF. Pyrazole (300 mg/kg, i.p.) administered 30 min before $CCl_4$ protected against the increased HN, HTG but not against the increased BDPF and SGPT. No protection against the decreased HBF was afforded. A small dose of $CCl_4$ (0.2 ml/kg, i.p.) 24 hr before the usual pretreatment with $CCl_4$ protected against all the effects including that on BDPF increase. Thus, with the combination of results, it is concluded that the increase in BDPF produced by $CCl_4$ is not correlated with the extent of liver necrosis, hepatic triglyceride accumulation or effects on hepatic bile flow. The source of the increased BDPF remains unknown. (Supported by USPHS Grant GM-16503.)

722

HEXACHLOROBENZENE (HCB) PORPHYRIA IN RATS: DIFFERENCE BETWEEN SEXES. G.D. Sweeney and K.C. Jones*, McMaster University, Hamilton, Ontario.

HCB, PCB's and tetrachlorodibenzo-(p)-doxin cause hepatic porphyria in laboratory animals. Characteristic features of this porphyria include a long latent period, excretion of porphyrins with 8, 7, 6, 5 and 4 carboxyl groups, and of isocoproporphyrins; δ-aminolevulinic acid synthase is elevated and there is induction of hepatic drug-metabolising enzyme system. An earlier report emphasized the greater susceptibility of female rats to develop porphyria; this study examined the phenomenon further. 0.2% practical grade HCB was added to the diet of female, castrated male and male Wistar rats. Porphyria appeared after 5, 7 and 10 weeks respectively in the three groups. Females excreted 20% of porphyrin as porphyrinogen, males 35-40%. Cytochrome P450 was highest (2.30nM/mg prot.) in the male group, intermediate (1.31) in the castrated and lowest (1.03) in the females. Ethyl isocyanide binding spectra with microsomes from the 3 groups at pH 7.4 were the same. The increased susceptibility of female rats to porphyria is associated with decreased induction of drug metabolising enzyme system; intact testes confer some protection. The sex dependence of the porphyrin/porphyrinogen ratio is of interest.
(Supported by the M.R.C. of Canada)

723

PERTURBATION BY ORGANOCHLORINE PESTICIDES OF $^{3}$H-TESTOSTERONE METABOLISM IN RODENT HEPATIC MICROSOMES AND PROSTATE GLAND IN VITRO. M.P. Donovan*, J.A. Thomas, L.G. Schein* and P.A. Klase*. W. Va. Univ. Med. Ctr., Morgantown, WV 26506.

Formation of $^{3}$H-dihydrotestosterone ($^{3}$H-DHT) from $^{3}$H-testosterone by rodent prostate gland in vitro was inhibited by organochlorine pesticides. Heptachlor was most potent, inhibiting $^{3}$H-DHT formation in rat tissue at $10^{-7}$M and in mouse at $10^{-6}$M. Chlordane ($10^{-5}$M) inhibited in tissue from mouse but not from rat. Tissue from either species was refractory to methoxychlor. Formation of $^{3}$H-hydroxytestosterone isomers by both mouse and rat hepatic microsomes was reduced by all three compounds at $10^{-5}$M. In addition, mouse enzymes were inhibited by $10^{-6}$M and $10^{-8}$M heptachlor. Interaction of these organochlorines with cytochrome P-450 produced Type I spectral changes in mouse liver microsomes. Spectral titrations indicated the presence of more than one type of binding site. (Supported by EPA 68-01-1926.)

Manufacturing Chemists Association

Minutes of Meeting

MEDICAL SURVEILLANCE AND RECORDS SUBCOMMITTEE FOR VINYLIDENE CHLORIDE TECHNICAL PANEL

MCA Conference Room — Washington, D.C.

August 11, 1976



Dr. McDonough presided and convened the meeting at 12:00 p.m. The record of attendance is:

MEMBERS PRESENT:

| | |
|---|---|
| J. R. McDonough, Chairman | Tennessee Eastman Company |
| W. A. Fishbeck | The Dow Chemical Company |
| H. B. Lovejoy | PPG Industries, Inc. |
| M. Freifeld | MCA Staff |

MEMBERS ABSENT:

| | |
|---|---|
| Z. G. Bell, Jr. | PPG Industries, Inc. |
| M. N. Johnson | The B. F. Goodrich Company |

* * * * * * * * * * *

The purpose of the meeting was stated by Dr. McDonough as the preparation of the medical section of a document being prepared by the Work Practices Subcommittee. It was decided to start fresh rather than attempt to modify an earlier draft developed under the chairmanship of Dr. Ronayne, who is no longer on the Subcommittee. It was noted the finished work practices document would be distributed to supporting companies and possibly to customers using VDC as well as appropriate government agencies.

Dr. Fishbeck reviewed the favorable results of a Dow study of their employees exposed to VDC. It has been submitted for publication in the Journal of Occupational Medicine.

In the MCA-administered animal studies at Dow, Dr. Fishbeck said that both hepatic and kidney damage were noted.

A draft was then written for the medical section of the work practices document.

The meeting was adjourned at 3:00 p.m.

Milton Freifeld
Project Manager
Vinylidene Chloride Research

Minutes Subject to Approval

MF:ec

August 13, 1976

# EVALUATION OF THE ENVIRONMENTAL TOXICANTS VINYL CHLORIDE (VC) AND VINYLIDENE CHLORIDE (VDC)

by

Cheng-Chun Lee
Joseph M. Winston
Paul J. Peters
Jagdish C. Bhandari
John R. Hodgson
Jack H. Hagensen
Thomas W. Reddig
Ellen R. Ellis

PROGRESS REPORT NO. 9
1 January through 31 March 1976

Contract No. NO1-ES-2-2084
(Continuation of NIH-NIEHS-72-2-2084)

MRI Project No. 3612-B

For

National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709

Attn: James S. Woods

# EVALUATION OF THE ENVIRONMENTAL TOXICANTS VINYL CHLORIDE (VC) AND VINYLIDENE CHLORIDE (VDC)

(Progress Report No. 9)

## ABSTRACT

We have continued to study the inhalation toxicity of various levels of vinyl chloride (VC) and one level of vinylidene chloride (VDC) in rats and mice.

At the end of 6 months, rats of both sexes exposed to 50, 250, or 1,000 ppm of VC or 55 ppm of VDC, 6 hours a day, 5 days a week, did not show any significant adverse effects on various clinical laboratory data or cause any apparent gross or microscopic lesions. The weight gains of rats exposed to VDC were less than those of the controls.

Exposure to 50, 250, or 1,000 ppm of VC caused a number of deaths or early terminations in mice during the 21st to 26th week. The clinical signs in these mice included rough hair coat, lethargy, anorexia, and sudden weight loss. The high level caused changes in the peripheral blood elements including decreased hematocrit, hemoglobin concentration and/or erythrocyte count, and neutrophilia with a corresponding lymphopenia. The macrophage count in the lung washings was also elevated. All three levels caused acinar proliferation and/or bronchiolar adenoma; the middle and high level also caused angiosarcoma in the liver and/or mammary gland, ductular adenocarcinoma, and squamous cell and anaplastic carcinoma in the mammary gland. The squamous cell and anaplastic carcinomas metastasized to the lung. The incidence and severity of these tumors were in direct proportion to the levels of VC. In addition, malignant lymphoma occurred in the heart, lung, liver, spleen and kidney of one mouse exposed to the low level, and infiltrated the cervical tissue surrounding the trachea, blood vessels and esophagus of one mouse exposed to the high level. There was also a hemangioma in the connective tissue attached to the salivary gland of one mouse exposed to the high level. The tumors were responsible for deaths or early terminations of some mice. At 250 or 1,000 ppm of VC, nearly all mice that died or were terminated during the 21st to 26th weeks had tumors in one or more tissues. Exposure to the high level of VC also caused other lesions in the liver of some mice which were probably associated with angiosarcoma; these included infiltration of nucleated erythrocytes, focal degeneration with necrosis, and infiltration of inflammatory cells in the sinusoids and parenchyma.

Exposure to 55 ppm of VDC for up to 6 months in mice did not cause any adverse signs, including changes in laboratory data or macrophage counts in the lung washings. At the end of 6 months, one male of the eight mice which were terminated had a mild bronchiolar adenoma and two other males had mild acinar proliferation in the lung.

## IV. DISCUSSION AND CONCLUSIONS

### A. Rats

Exposure of rats of both sexes at 6 hours/day and 5 days/week for up to 6 months to 50, 250, or 1,000 ppm of VC or 55 ppm of VDC did not cause any serious adverse effects. The weight gains of the female rats exposed to 55 ppm of VDC were less than those of the controls during the 6th month. The laboratory results, including hematology, clinical blood chemistry, serum immunoglobins, serum proteins, and macrophage counts in lung washings were not apparently affected. The cytogenetic effects as measured by chromosomal changes in bone marrow cultures are under evaluation. Collagen content in the liver and lung of these rats was not apparently altered. Exposure to VC or VDC did not cause any apparent changes in organ weights nor cause any lesions.

### B. Mice

#### 1. Vinyl Chloride

Exposure to various levels of VC caused a number of deaths in mice between the 21st and 26th weeks. There were two deaths in the group exposed to 50 ppm, three early terminations in the group exposed to 250 ppm, and four deaths in the group exposed to 1,000 ppm. Clinical signs in these mice included rough hair coat, lethargy, anorexia, and sudden weight loss. One female exposed to the middle level and three females exposed to the high level had tumors in the mammary gland which were from white to gray to dark red in color.

Exposure to the high level of VC caused some changes in the peripheral blood elements. There were decreases in hematocrit, hemoglobin concentration and/or erythrocyte count. Neutrophilia with a corresponding lymphopenia occurred. These changes were not seen in the control mice or in mice exposed to 1,000 ppm of VC for 1, 2, or 3 months.[2/] The macrophage count in the lung washings of mice of both sexes exposed to the high level was elevated. Since macrophage counts were not performed in mice exposed to the low or middle level, the possibility of a dose response relationship cannot be assessed. Exposure to various levels of VC did not cause any other apparent changes in peripheral blood elements or clinical blood chemistry tests. As for rats, the cytogenetic effects as measured by chromosmal changes in the bone marrow cultures are under evaluation.

Gross and microscopic examinations revealed that 50, 250, or 1,000 ppm of VC caused acinar proliferation and/or bronchiolar adenoma in the lung. The middle and high level also caused angiosarcoma in the liver and various tumors in the mammary gland. The angiosarcoma was also found in the mammary

gland of two females and hemangioma in the connective tissue adjacent to the salivary gland of one male exposed to the high level. The pathogenesis or sequence of changes for the various tumors in the mammary gland is complex. Whether the tumors started at first as ductular adenocarcinoma and then underwent a metaplastic change to squamous cell and anaplastic carcinomas, or all tumors started simultaneously is questionable. However, the squamous cell and anaplastic carcinomas did metastasize to the lung, suggesting a strong malignancy of the neoplasms. In addition, a marked, diffuse malignant lymphoma was found in the heart, lung, liver, spleen and kidney of one mouse exposed to the low level of VC and a malignant lymphoma infiltrated the cervical tissue surrounding the trachea, blood vessels and esophagus of one mouse exposed to the high level. Between the 5th and 6th months the various tumors occurred in five of 10 mice necropsied at 50 ppm, 10 of 11 mice necropsied at 250 ppm, and all 12 mice necropsied at 1,000 ppm. The severity of these tumors in the lung, liver, and mammary gland was also in direct proportion to the exposure level of VC. These tumors were responsible for the various deaths and early terminations of some mice.

Exposure to the high level of VC also caused other lesions, probably associated with angiosarcoma, in the liver of some mice. These lesions were infiltration of nucleated erythrocytes, focal degeneration and necrosis, and infiltration of inflammatory cells in the sinusoids and parenchyma. Intranuclear inclusions increased in the hepatic cells of two mice exposed to the high level of VC. The significance of this increase as related to the treatment is questionable.

2. Vinylidene Chloride

Mice exposed to 55 ppm of VDC for up to 6 months did not show any adverse signs, any changes in laboratory data or macrophage counts in the lung washings. Cytogenetic effects are under evaluation. One male of eight mice terminated at the end of 6 months had a mild bronchiolar adenoma. Two other males had mild acinar proliferation in the lung. These three and another male also had increased intranuclear inclusions in the hepatic cells. The clinical significance of this increase is not understood.