Vinylidene chloride

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Protective Action of Diethyldithiocarbamate and Carbon Disulfide against Acute Toxicities Induced by 1,1-Dichloroethylene in Mice

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Protective Action of Diethyldithiocarbamate and Carbon Disulfide against Acute Toxicities Induced by 1,1-Dichloroethylene in Mice. MASUDA, Y., AND NAKAYAMA, N. (1983). *Toxicol. Appl. Pharmacol.* 71, 42–53. In male mice of ddY strain, a single dose of 1,1-dichloroethylene (1,1-DCE, 0.1 ml/kg, ip) produced severe renal damage at 24 hr, as evidenced by elevations in plasma urea nitrogen concentration and kidney calcium content and by massive renal tubular necrosis, while hepatic damage was less severe. A precipitous decrease in body temperature started as early as 30 min after administration of 1,1-DCE and lasted for 24 hr. Glutathione concentrations decreased in the liver and kidney, with a rebound increase seen in the former but not in the latter tissue. In carbon tetrachloride-poisoned mice, the renal toxicity of 1,1-DCE was markedly potentiated. Pretreatment with either diethyldithiocarbamate (DTC) or carbon disulfide (CS_2) blocked all of these 1,1-DCE-induced toxic manifestations in normal and carbon tetrachloride-poisoned mice. Both agents, however, did not prevent the hypothermia induced by monochloroacetic acid or chloroacetyl chloride, proposed active metabolites of 1,1-DCE. Since DTC and CS_2 inhibited hepatic and renal microsomal drug metabolizing enzyme activities (Masuda and Nakayama, 1982, 1983), it is probable that the protective action of DTC and CS_2 against renal and hepatic injury induced by 1,1-DCE may be due to an inhibition of the metabolic activation of 1,1-DCE to its proposed epoxide in each organ. The action of DTC given po may be mediated by CS_2 produced in the stomach. The hypothermia induced by 1,1-DCE may not result from a direct action of 1,1-DCE *per se*, but by its metabolites.

As previously reported (Masuda and Nakayama, 1982), diethyldithiocarbamate (DTC) and carbon disulfide (CS_2) protected mice against liver injury induced by various hepatotoxins such as carbon tetrachloride, chloroform, bromotrichloromethane, thioacetamide, furosemide, acetaminophen, bromobenzene, dimethylnitrosamine, and trichloroethylene, all of which require metabolic activation by the microsomal monooxygenase system. DTC and CS_2 also prevented chloroform-induced renal injury in mice (Masuda and Nakayama, 1983). Since both agents inhibited microsomal drug metabolizing enzyme activity in the liver and kidney in these studies, we suggested that the protective action might be due to an inhibition of bioactivation of these hepato- and nephrotoxins. It was also proposed that the protective action of DTC may be mediated through CS_2 particularly when administered po. Thus, as an extension of these studies, we examined the possible protective effects of these agents against 1,1-dichloroethylene (1,1-DCE), another hepatic and renal toxicant that requires bioactivation by the microsomal monooxygenase system (Bonse *et al.*, 1975; Bartsch *et al.*, 1975; Leibman and Ortiz, 1977; Jones and Hathway, 1978; Andersen *et al.*, 1980).

A protective action of DTC against the hepatotoxicity of 1,1-DCE in rats was reported

[1] To whom correspondence should be addressed.

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TOXICOLOGY AND APPLIED PHARMACOLOGY 68, 140–151 (1983)

Vinylidene chloride

The Uptake and Disposition of 1,1-Dichloroethylene in Rats during Inhalation Exposure[1,2]

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The Uptake and Disposition of 1,1-Dichloroethylene in Rats during Inhalation Exposure. DALLAS, C. E., WEIR, F. W., FELDMAN, S., PUTCHA, L., AND BRUCKNER, J. V. (1983). *Toxicol. Appl. Pharmacol.* 68, 140–151. The uptake, disposition, and respiratory elimination of 1,1-dichloroethylene (1,1-DCE) during inhalation exposure were evaluated to gain insight into the pharmacodynamics of the halocarbon. Anesthetized male Sprague–Dawley rats inhaled 25, 75, 150, or 300 ppm 1,1-DCE for 3 hr from an aluminized Mylar bag through a miniaturized one-way breathing valve inserted into the trachea. Periodic air samples were taken immediately adjacent to the valve from the separate inhaled air and exhaled breath streams concurrently with blood samples from a cannulated femoral vein and analyzed for 1,1-DCE content by gas chromatography. 1,1-DCE was absorbed very rapidly, in that substantial levels were present in the venous blood at the first sampling time (i.e., 2 min). Percentage systemic uptake decreased over time after initiation of exposure until equilibrium was established. Percentage uptake after reaching equilibrium varied inversely with the exposure concentration. 1,1-DCE venous whole-blood levels in animals exposed to 25, 75, and 150 ppm 1,1-DCE increased rapidly to near steady state within approximately 45 min, as did concentrations of 1,1-DCE in the exhaled breath and alveolar air. Calculation of the amount of 1,1-DCE taken up by the body over the course of the 3-hr exposures revealed that cumulative uptake of the inhaled chemical was statistically linear for the 25-, 75-, and 150-ppm exposures. Accumulation plots for 300-ppm exposed animals, however, were best fitted to a cubic curve form. Although trends toward the establishment of equilibrium were initially seen in the 300-ppm exposed animals, levels of 1,1-DCE in the blood and breath rose progressively during the latter hour of the 3-hr exposure period. Thus, despite increased exhalation of 1,1-DCE, these animals could not prevent systemic accumulation of the chemical.

1,1-Dichloroethylene (1,1-DCE), also known as vinylidene chloride, is used widely as a monomer in the manufacture of a variety of plastic materials. In addition to the potential for exposure in the occupational environment, 1,1-DCE and other halocarbons are contaminants of drinking water supplies (U.S. EPA, 1975, 1977).

The pharmacokinetics and metabolic fate of 1,1-DCE are of considerable interest, in that both toxicity (Andersen and Jenkins, 1977; Andersen *et al.*, 1979a) and carcinogenicity (Maltoni *et al.*, 1977) are highly dose dependent. Maltoni *et al.* (1977), for example, saw no increase over controls in tumor incidence in male mice exposed to 10 ppm of 1,1-DCE vapor, but a significant incidence in 25-ppm exposed animals. McKenna *et al.* (1978a) reported that rats subjected to 10 ppm 1,1-DCE for 6 hr were capable of metabolizing about 98% of the systemically absorbed

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Table 1. *(continued)*

Chemical CAS No. NTP No.	NTP Report No. TR Report No. Peer Review Date	Use	Route/dose	Testing laboratory	NTP chemical manager	Results under the conditions of these tests[b,c]
Vinylidene chloride (1,1-dichloroethylene) 75-35-4 10109-A	NTP-80-082 TR-228 02/18/81	Intermediate for 1,1,1-trichloroethane, monomer for vinylidene copolymers	Gavage, 5 times/week: rats, 1 or 5 mg/kg; mice, 2 or 10 mg/kg	Gulf South Research Institute	Dr. R. S. Chhabra	Not carcinogenic for F344 rats or B6C3F$_1$ mice of either sex (increased incidence of liver necrosis in male mice 1/46, 3/46, 7/49*).
Zearalenone 17924-92-4 10770-C	NTP-81-054 TR-235 12/16/81	Nonsteroid estrogenic mycotoxin (anabolic steroid)	Feed: rats, 25 or 50 ppm; mice, 50 or 100 ppm	Southern Research Institute	Dr. D. Goldman	Not carcinogenic for F344 rats of either sex; should be considered carcinogenic for B6C3F$_1$ mice (male: pituitary adenoma 0/40, 4/45, 6/44*; female: pituitary adenoma 3/46, 2/43, 13/42*; hepatocellular adenoma 0/50, 2/49, 7/49*).
Ziram (Zinc dimethyldithiocarbamate) 137-3-4 10590-G	NTP-81-057 TR-238 12/16/81	Fungicide and accelerator in rubber vulcanization	Feed: rats, 300 or 600 ppm; mice, 600 or 1200 ppm	Southern Research Institute	Dr. D. Goldman	Carcinogenic for male F344 rats causing C-cell carcinomas of the thyroid 0/50, 2/49, 7/49*; not carcinogenic for female F344 rats or for male B6C3F$_1$ mice; interpretation of the increased number of female B6C3F$_1$ mice with alveolar/bronchiolar adenoma (2/50, 5/49, 10/50*) is complicated because of a concomitant Sendai viral infection (in all groups of mice).

[a]P = published.
[b]Incidence results are ordered by concurrent controls, low dose, and high dose groups, with the numerator being the number of tumor (or noneoplastic lesion) bearing animals and the denominator the number of animals examined.
[c]* = statistically significant, $p < 0.05$.

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Vinylidene chloride

Oncology 37: 136–141 (1980)

Long-Term Testing of Vinylidene Chloride and Chloroprene for Carcinogenicity in Rats

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Key Words. Vinylidene chloride · Chloroprene · Acute toxicity · Carcinogenicity in rats

Abstract. Vinylidene chloride (VDC) monomer dissolved in olive oil was given orally to female BD IV rats (150 mg/kg body weight) on the 17th day of gestation. Their offspring were treated weekly with 50 mg/kg body weight VDC by stomach tube from the time of weaning for life span. Liver and meningeal tumours were more frequently observed in treated than in untreated animals, but the total number of tumour-bearing animals was not significantly different between treated and untreated animals. Chloroprene (CP) monomer dissolved in olive oil was given orally to female BD IV rats (100 mg/kg body weight) on the 17th day of gestation and their offspring were treated weekly with 50 mg/kg body weight by stomach tube from the time of weaning for life span. Total incidence of tumours was similar in treated and untreated animals. The data presented provide limited evidence of the carcinogenicity of VDC and no evidence of the carcinogenicity of CP when given by the oral route to rats.

Introduction

Vinylidene chloride (VDC), a chemical structurally related to vinyl chloride, is widely used in the manufacture of plastics, with a world production which probably was in excess of 150,000 t in 1976. In spite of its widespread production and use, relatively few (mostly inconclusive) studies are available on its possible acute and long-term effects in humans. One report on the long-term effects of VDC, involving 138 workers, indicated no statistically significant effects related to VDC exposure [1] but the number of individuals lost to follow-up in this study was high and the period of observation relatively short. In a preliminary mortality study on 629 workers from a VDC production and polymerization plant, where exposure to vinyl chloride and acrylonitrile also occurred, 7 of the 35 deaths reported were from malignant tumours, which was not greater than the expected value. Two bronchial carcinomas occurred in persons aged 35–39 whereas 0.08 were expected. However, no information was given on smoking habits [2; see also 15].

Experimentally, VDC has been shown to have a low toxicity in rats [3]. The results of a number of long-term carcinogenicity tests have recently become available, either as preliminary communications or as interim results of on-going studies [4–6]. The preliminary results of one study, in which VDC was given by inhalation, point to the induction of kidney carcinoma in mice and to an increased incidence of mammary tumours in rats, no carcinogenic effect was observed when VDC was administered by the oral route to rats or hamsters [4]. An increased incidence of lung, skin and liver cell tumours in mice and the induction of haemangiosarcomas in both mice and rats was reported in another inhalation study [7]. In addition, VDC has been shown to be mutagenic to *Salmonella typhimurium* [8].

Chloroprene (CP) is used as a chemical intermediate, mainly as the monomer in the manufacture of synthetic elastomers. World production of this compound in 1977 is estimated to have been 300,000 t.

Acute toxic effects in humans exposed to CP were reported to be depression of the central nervous system, various lesions of the lung, kidney and liver, hair loss

and irritation of skin and eyes [9]. The results of long-term studies of workers occupationally exposed to CP are contradictory. In one investigation, in which several inadequacies have been recognized (i.e. failure to distinguish prevalent from incident cases, no adjustment for effect of age and sex, no measure of the extent of exposure), excesses of lung and skin cancer were reported [10, 11]. In another study, which also had limitations (among which were no data on potentially confounding variables, incomplete follow-up and small number of persons/years of exposure) no excess rates of lung and skin cancer were reported in two cohorts of males engaged in the production of CP [12; see also 15]. Cytogenetic effects on human lymphocytes have been reported [13].

CP has been tested for carcinogenicity in rats by oral, subcutaneous and intratracheal administration and in mice by skin application [14]. No carcinogenic effect was detected in these studies. However, CP has been found to be mutagenic in *S. typhimurium* [8].

A comprehensive review of the data on VDC and CP is available [15].

We report here the results of a long-term carcinogenicity test in which VDC and CP were given by the oral route to rats.

Materials and Methods

Inbred BDIV rats, originally provided by Prof. *H. Druckrey* (Freiburg, FRG), were used in these experiments. The animals were kept in Makrolon N111 cages under normal laboratory conditions and were maintained on Charles River food pellets until January 1976 and then on Aliment Extralabo Biscuits (Pietrement). Water was available *ad libitum*. Samples of feed were analysed twice during the course of the experiments for the presence of nitrosamines (the analysis was carried out by Dr. *Preussmann* of the 'Deutsches Krebsforschungszentrum', whose collaboration is gratefully acknowledged): dimethylnitrosamine and diethylnitrosamine were present at levels of 0.2–0.6 ppb.

VDC (purity 99%, containing 0.03% 4-methoxyphenol) obtained from Merck-Schuchardt, Darmstadt, FRG, and CP (purity 99%, containing 0.8% 1-chlorobutadiene), provided by Distugil, Le-Pont-de-Claix, France, were dissolved in olive oil and administered to the animals by stomach tube. The same schedule of treatment was chosen for both experiments: single oral administration of the chemical to females on the 17th day of gestation and continuous weekly oral treatment of their offspring from time of weaning for life span.

24 female BDIV rats were given a single dose of 150 mg/kg body weight VDC in olive oil by stomach tube on the 17th day of pregnancy, and their progeny (89 males and 90 females) received weekly doses of 50 mg/kg body weight VDC in 0.3 ml olive oil.

17 female BDIV rats were administered a single oral dose of 100 mg/kg body weight CP in olive oil on the 17th day of pregnancy, and their progeny (81 males and 64 females) were treated weekly with 50 mg/kg body weight CP in 0.3 ml olive oil.

Controls for experiments with both VDC and CP were 14 female BD IV rats which received 0.3 ml olive oil on the 17th day of pregnancy and their progeny (53 males and 53 females), which were given 0.3 ml olive oil weekly for life beginning at weaning.

All survivors were killed at 120 weeks or when moribund. All animals were autopsied and major internal organs as well as those that showed gross abnormalities were examined histologically. Sections were routinely stained with haematoxylin-eosin; special stains were used when necessary.

Results

Vinylidene Chloride

The single oral LD_{50} level of VDC for the animals used in the experiment was established by giving groups of four rats single oral doses of VDC in olive oil and

Table I. Survival rates of BDIV rats treated with VDC and of controls

Group	Initial number of animals	Number of survivors according to duration of treatment (weeks)											
		10	20	30	40	50	60	70	80	90	100	110	120
Females given VDC	24	24	24	24	24	24	24	24	23	23	22	19	14
Progeny treated weekly with VDC													
Males	89	85	85	82	81	80	78	76	75	71	65	54	41
Females	90	86	85	83	81	81	80	80	79	75	72	61	56
Females given olive oil	14	14	14	14	14	14	14	14	14	14	12	11	11
Progeny treated weekly with olive oil													
Males	53	53	53	53	53	53	53	52	52	49	44	41	27
Females	53	52	52	52	52	52	51	50	49	47	40	34	26

Table II. Tumour incidence in female BDIV rats treated with VDC on day 17 of pregnancy, in their progeny treated weekly for life and in controls

Group	Effective number of rats[1]	Tumour-bearing rats		Number of tumours		Animals with more than one tumour		Distribution of tumours					
								meninges		oral cavity		stomach	
		n	%	total	per rat	n	%	n	%	n	%	n	%
Females given VDC	23	11	47.8	14	0.6	3	13	–	–	2	8.7	1	4.3
Progeny treated weekly with VDC													
Males	81	31	38.3	35	0.4	4	4.9	6	7.4	5	6.2	1	1.2
Females	80	53	66.3	64	0.8	11	13.8	–	–	1	1.3	2	2.5
Females given olive oil	14	5	35.7	7	0.5	2	14.3	–	–	1	7.1	–	–
Progeny treated weekly with olive oil													
Males	49	16	32.7	16	0.3	–	–	1	2.0	2	4.1	–	–
Females	47	24	51.1	29	0.6	5	10.6	–	–	1	2.1	1	2.1

The percentages and the number of tumours per rat are expressed in relation to the effective number of rats.

[1] Survivors at the time the first tumours were observed.

[2] Urinary bladder papilloma.

[3] 1 lymphoma, 4 pituitary adenomas; 3 adrenal cortical adenomas; 1 spleen haemangioma; 1 lung sarcoma, pleomorphic; 1 skin squamous cell carcinoma; 1 seminoma.

[4] 1 salivary gland carcinoma; 1 salivary gland adenoma; 1 lymphoma; 1 pituitary adenoma; 1 rectal adenomatous polyp; 1 uterine adenoma.

[5] Adrenal cortical adenoma.

[6] 1 osteosarcoma; 1 mediastinal sarcoma; 1 lung epidermoid carcinoma; 2 lymphomas; 1 spleen haemangioma; 2 pituitary adenomas; 1 adrenal cortical adenoma.

[7] 1 lymphoma; 1 uterine adenoma.

Table III. Survival rates of CP-treated BDIV rats and controls

Group	Initial number of animals	Number of survivors according to duration of treatment (weeks)											
		10	20	30	40	50	60	70	80	90	100	110	120
Females given CP	17	17	17	17	17	17	17	17	16	16	15	15	13
Progeny treated weekly with CP													
Males	81	77	76	70	66	66	66	66	66	64	60	48	40
Females	64	64	63	63	62	61	61	61	59	57	56	51	43
Females given olive oil	14	14	14	14	14	14	14	14	14	14	12	11	11
Progeny treated weekly with olive oil													
Males	53	53	53	53	53	53	53	52	52	49	44	41	27
Females	53	52	52	52	52	52	51	50	49	47	40	34	26

was calculated following the method described by *Weil* [16]; it was 1,800 mg/kg body weight for males and 1,500 mg/kg body weight for females.

Litter sizes and pre-weaning mortality were similar in VDC-treated and control groups (5%). Survival rates, summarized in table I, were similar in the two groups; and there was no difference in body weights between VDC and control animals.

liver		soft tissue		mammary gland		ovary		other		Liver hyperpl. nodules	
n	%	n	%	n	%	n	%	n	%	n	%
–	–	–	–	8	34.8	2	8.7	1[2]	4.3	2	8.7
1	1.2	9	11.1	1	1.2	–	–	12[3]	14.8	2	2.5
3	3.8	7	8.8	39	48.8	6	7.5	6[4]	7.5	6	7.5
–	–	1	7.1	4	28.6	–	–	1[5]	7.1	–	–
–	–	4	8.2	–	–	–	–	9[6]	18.4	–	–
–	–	–	–	22	46.8	3	6.4	2[7]	4.3	–	–

In rats weekly treated with VDC that died up to 30 weeks after the start of treatment, a pronounced congestion of lungs and kidney was detected. Later, at up to 80–90 weeks, haemorrhages and multiple lobular necrosis of the liver were observed. The animals which died after 90 weeks and some survivors killed at the age of 120 weeks, showed degenerative lesions of liver parenchymal cells, consisting of large, balloon cells with clear cytoplasm, often nodular with a distinct border towards normal parenchyma. No excessive storage of glycogen was detected by the appropriate histochemical method.

Tumours: data on tumour incidence are summarized in table II. There were minor differences in the percentages of tumour-bearing animals among VDC- and vehicle-treated animals, but these were not statistically significant.

A few tumours were observed among VDC-treated males that were not seen in vehicle-treated controls, notably, one squamous-cell carcinoma of the stomach, one liver cell carcinoma, one seminoma and one rectal adenomatous polyp. Meningiomas were also more frequent in VDC-treated males than in controls (6/81 vs. 1/49), but the difference was not significant ($p = 0.37$). In VDC-treated females, two liver cell carcinomas, one liver cell adenoma and one carcinoma and one adenoma of the salivary gland were observed, which were not seen among controls. Additionally, hyperplastic liver nodules were found in 2/23 females given a single VDC administration during pregnancy, and 2/81 males and 6/80 females among their progeny: no hyperplastic nodules were found in vehicle-treated controls. This difference was significant ($p = 0.04$).

Chloroprene

The oral LD_{50} of chloroprene in adult BDIV rats was determined as 900 mg/kg body weight. Litter sizes and pre-weaning mortality were not different in CP-treated animals from those in controls. Survival rates, summarized in table III, were similar in both treated and control groups of animals; and there was no difference in the body weights of CP-treated and control animals.

Animals treated weekly with CP that died within the first 23–35 weeks after the beginning of the treatment, showed severe congestion of lungs and kidneys. In some animals autopsied 80–90 weeks after the start of the treatment, multiple liver necroses were observed.

Tumours: data on tumour incidence are given in table IV. Although several tumours that were observed in males treated weekly with CP were not seen in vehicle-treated controls, and although subcutaneous fibromas were more numerous in CP-treated males than in controls, the total incidence of tumours was similar in CP-treated and control rats.

Discussion

The oral administration of VDC to rats did not produce a statistically significant increase in the total number of tumour-bearing animals, although an increased incidence of tumours at certain sites was observed. In particular, the incidence of liver tumours was increased in rats of both sexes and that of meningiomas in males. In addition, hyperplastic nodules of the liver were observed in both male and female rats; these were not seen in controls.

VDC was given to rats at a dose level which did not

Table IV. Tumour incidence in female BD IV rats treated with CP on day 17 of pregnancy, in their progeny treated weekly for life and in controls

Group	Effective number of rats[1]	Tumour-bearing rats		Number of tumours		Animals with more than one tumour		Distribution of tumours			
								oral cavity		mammary gland	
		n	%	total	per rat	n	%	n	%	n	%
Females given CP	16	9	56.2	14	0.9	5	31.3	1	6.3	6	37.5
Progeny treated weekly with CP											
Males	54	15	27.8	18	0.3	3	5.6	–	–	–	–
Females	62	33	53.2	37	0.6	4	6.5	–	–	25	40.3
Females given olive oil	14	5	35.7	7	0.5	2	14.3	1	7.1	4	28.6
Progeny treated weekly with olive oil											
Males	49	16	32.7	16	0.3	–	–	2	4.1	–	–
Females	47	24	51.1	29	0.6	5	10.6	1	2.1	22	46.8

The percentages and the number of tumours per rat are expressed in relation to the effective number of rats.

[1] Survivors at the time the first tumours were observed.

[2] 1 uterine squamous cell carcinoma; 1 lung reticulosarcoma; 1 forestomach papilloma; 1 sebaceous basal cell carcinoma.

[3] 1 intestinal leiomyosarcoma; 1 osteoma; 1 kidney mesenchymal tumour; 1 bone haemangioma; 1 neurinoma of the optic nerve; 1 adrenal cortical adenoma; 1 transitional-cell carcinoma of urinary bladder; 1 forestomach papilloma.

[4] Adrenal cortical adenoma.

[5] 2 lymphomas; 1 lung epidermoid carcinoma; 1 spleen haemangioma; 1 osteosarcoma; 1 mediastinal sarcoma; 1 meningioma; 1 adrenal cortical adenoma.

[6] 1 stomach fibrosarcoma; 1 lymphoma; 1 uterine adenoma.

produce any obvious toxic effects; it might therefore be inferred that the dose level used was not the maximum tolerated dose. While in keeping with the observations made by other authors [4, 7], the present results provide limited evidence of carcinogenicity of VDC in rodents. Additional evidence on which to base a final assessment of the carcinogenicity of VDC may be forthcoming from studies known to be underway [15].

The oral administration of CP to rats resulted in an incidence of tumours that was no different from that observed in vehicle-treated controls. Results from further investigations, including the use of different species and, possibly, administration by inhalation, are necessary before the carcinogenicity of CP can be assessed.

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References

1 Ott, M.; Fishbeck, W.; Townsend, J., and Schneider, E.: A health study of employees exposed to vinylidene chloride. J. occup. Med. *18:* 735–738 (1976).

2 Thiess, A.M.; Frentzel-Beyme, R., and Penning, E.: Mortality study of vinylidene chloride exposed persons in the BASF; in Proc. Vth Medichem Congr., San Francisco 1977 (in press 1979).

3 Jenkins, L.J.; Trabulus, M.J., and Murphy, S.D.: Biochemical effects of 1,1-dichloroethylene in rats: comparison with carbon tetrachloride and 1,2-dichloroethylene. Toxicol. appl. Pharmacol. *23:* 501–510 (1972).

4 Maltoni, C.; Cotti, G.; Morisi, L., and Chieco, P.: Carcinogenicity bioassays of vinylidene chloride. Medna. Lav. *61:* 241–262 (1977).

5 Rampy, L.W.; Quast, J.F.; Humiston, C.G.; Balmer, M.F., and Schwetz, B.A.: Interim results of two-year toxicological studies in rats of vinylidene chloride incorporated in the drinking water or administered by repeated inhalation. Environ. Health Perspect. *21:* 33–43 (1977).

6 Viola, P.L. and Caputo, A. Carcinogenicity studies on vinylidene chloride. Environ. Health Perspect. *21:* 45–47 (1977).

7 Lee, C.C.; Bhandari, J.C.; Winston, J.M.; House, W.B.; Peters, P.J.; Dixon, R.L., and Woods, J.S.: Inhalation toxicity of vinyl chloride and vinylidene chloride. Environ. Health Perspect. *21:* 25–32 (1977).

8 Bartsch, H.; Malaveille, C.; Montesano, R., and Tomatis, L.: Tissue-mediated mutagenicity of vinylidene chloride and 2-chloro-

ovary		thyroid		soft tissue		pituitary		other	
n	%	n	%	n	%	n	%	n	%
2	12.5	–	–	–	–	1	6.3	4[2]	25.0
–	–	1	1.9	7	13.0	2	3.7	8[3]	14.8
9	14.5	1	1.6	–	–	2	3.2	–	–
–	–	–	–	1	7.1	–	–	1[4]	7.1
–	–	–	–	4	8.2	2	4.1	8[5]	16.3
3	6.4	–	–	–	–	–	–	3[6]	6.4

butadiene in *Salmonella typhimurium*. Nature, Lond. *225:* 641–643 (1975).
9 Oettingen, W. von: 2-Chlorobutadiene: its toxicity and pathology and the mechanisms of its action. J. ind. Hyg. Toxicol. *18:* 271–272 (1936).
10 Khachatryan, E. A.: Lung cancer morbidity among persons working with chloroprene. Vop. Onkol. *18:* 85–86 (1972).
11 Khachatryan, E. A.: The role of chloroprene compounds in the process of skin neoplasm formation. Gig. Truda, prof. Zabol. *18:* 54–55 (1972).
12 Pell, S.: Mortality of workers exposed to chloroprene. J. occup. Med. *20:* 21–29 (1978).
13 Sanotskii, I.V.: Aspects of the toxicology of chloroprene: immediate and long-term effects. Environ. Health Perspect. *17:* 85–93 (1976).
14 Zilfyan, V.; Fichidzhyan, B.; Garibyan, D., and Pogosova, A.: Experimental study of chloroprene for carcinogenicity. Vop. Onkol. *23:* 61–65 (1977).
15 IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Monomers, plastics and synthetic elastomers, vol. 19 (International Agency for Research on Cancer, Lyon 1979).
16 Weil, C.S.: Tables for convenient calculation of median effective dose (LD_{50} or ED_{50}) and instructions in their use. Biometrics *8:* 249–263 (1952).

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Vinylidene chloride

FOLLOW-UP STUDY ON THE CARCINOGENICITY OF VINYL CHLORIDE AND VINYLIDENE CHLORIDE IN RATS AND MICE: TUMOR INCIDENCE AND MORTALITY SUBSEQUENT TO EXPOSURE

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Carcinogenic and other toxic effects in rats and mice were examined during a 12-mo period following exposure to vinyl chloride (VC) or vinylidene chloride (VDC). Exposure of male and female mice to 50, 250, or 1000 ppm VC for 6 h/d, 5 d/wk, for 1, 3, or 6 mo resulted in increased numbers of deaths and increased moribundity at all dose levels during the exposure and postexposure periods, as compared with air-exposed controls. Similar observations were made with rats after 1, 3, 6, or 10 mo exposure to VC. Cumulative tumor incidence at various organ sites also increased in both species during the postexposure period in proportion to dose or duration of exposure at higher dose levels. However, except for mammary gland tumors in female mice, no significant increase in cumulative tumor incidence occurred in either species at 50 ppm VC or 55 ppm VDC, regardless of duration of exposure. These results suggest that exposure to vinyl halides at dose levels lower than those that elicit a significant increase in cancer incidence during the lifetime of the animal may, nonetheless, increase the risk of early death or moribundity from toxic pre- or subcarcinogenic effects. At dose levels higher than those consistent with the physiological defense or repair capabilities of the cell, ultimate tumor incidence becomes proportionate to length of exposure and may reflect the number of carcinogenic events elicited during the exposure period.

The authors thank Mr. J. L. Minor for his statistical analysis of the tumor data; Mr. J. H. Hagensen and Mrs. K. J. Smith for their assistance with inhalation, chamber monitoring, and animal care operations; and Mrs. E. R. Ellis for her supervision of histology preparation.

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Vinylidene chloride NTP draft report

and 50 B6C3F1 mice of either sex for 103 weeks. Groups of 50 untreated rats and mice of either sex served as controls.

After week 20 in mice and week 40 in rats, mean body weights of high-dose females were lower than those of the untreated controls. No compound-related clinical signs or effects on survival were observed. Feed consumption by dosed rats and dosed mice of either sex was lower than that of the controls. No tumors were observed in either species at incidences that were considered to be related to administration of guar gum.

Hepatocellular carcinomas occurred in dosed male mice at incidences significantly lower than that in the controls. The combined incidence of male mice with either hepatocellular adenomas or carcinomas was also significantly lower in the high-dose group than in the controls.

Under conditions considered as adequate for the carcinogenesis bioassay of a test material, guar gum was not carcinogenic for F344 rats or B6C3F1 mice of either sex.

GUM ARABIC

A bioassay for carcinogenicity of gum arabic, a widely used food stabilizer, was conducted by feeding diets containing 25,000 or 50,000 ppm of the test substance to 50 F344 rats and 50 B6C3F1 mice of either sex for 103 weeks. Groups of untreated rats and mice of either sex served as controls.

Throughout most of the study, mean body weights of dosed mice of either sex and of dosed male rats were comparable with those of the controls; mean body weights of the dosed female rats were slightly lower than those of the controls. No other compound-related clinical signs or effects on survival were observed. Mean daily feed consumption by high-dose rats and mice of either sex was 86%-88% that of the controls. No compound-related lesions were found in mice or rats of either sex.

Under the conditions of this bioasay, gum arabic was not carcinogenic for F344 rats or B6C3F1 mice of either sex.

TARA GUM

A bioassay for carcinogenicity of tara gum, a potential stabilizer for cosmetics and foods, was conducted by feeding diets containing 25,000 or 50,000 ppm of the test substance to 50 F344 rats and 50 B6C3F1 mice of either sex for 103 weeks. Groups of 50 untreated rats and mice of either sex served as controls.

In the chronic bioassay, mean body weights of dosed and control rats of either sex were comparable over the course of the study; feed consumption by high-dose male rats was comparable with that of the controls and feed consumption by high-dose female rats was 79% that of the controls. Mean body weights of high-dose mice of either sex were lower than those of controls; feed consumption by dosed mice was comparable with that of controls. Interstitial-cell tumors of the testis occurred at significantly higher incidences in dosed male rats than in controls (40/48, 46/46, 48/48); however, this tumor is common in male F344 rats and thus the biological significance of these findings is reduced. No other tumors were observed in increased incidences that were considered to be related to administration of tara gum to either species. Although the rats and mice might have been able to tolerate higher doses, 50,000 ppm (5%) is the recommended maximum concentration of a test substance mixed in feed, according to the guidelines of the Bioassay Program.

A significant negative trend was observed in the number of male rats with pancreatic islet cell adenoma, in the numbers of female mice with alveolar/bronchiolar adenomas, and in the numbers of female mice with hepatocellular adenomas.

Under conditons considered adequate for the carcinogenesis bioassay of a test material, no evidence was found that tara gum was clearly carcinogenic for F344 rats of B6C3F1 mice of either sex.

VINYLIDENE CHLORIDE

A subchronic and a chronic study of vinylidene chloride, a widely used chemical intermediate and monomer, was conducted in F344 rats and B6C3F1 mice. In subchronic studies, groups of 10 rats and 10 mice of either sex were administered vinylidene chloride in corn oil by gavage five times per week at 0, 5, 15, 40, 100, or 250 mg/kg body weight for 13 weeks. At the end of this study, all surviving animals were killed and representative tissues from these animals were subjected to histopathological examination. The liver was identified as a target organ for vinylidene chloride toxicity.

In a 104-week chronic exposure study which was conducted primarily to determine possible carcinogenic potential of vinylidene chloride, the 50 F344 rats and 50 B6C3F1 mice of either sex were gavaged with vinylidene chloride suspended in corn oil at dose levels of 1 or 5 mg/kg in rats and 2 or 10 mg/kg in mice. Groups of 50 rats and 50 mice of either sex received corn oil alone and served as vehicle controls. Throughout most of the study, mean body weights of the dosed rats of either sex and high-dose female mice were comparable with those of the corresponding controls; the mean body weights of dosed male and low-dose female mice were slightly lower than those of the controls. The results of histopathological examination indicated an increased incidence of necrosis of the liver in high-dose male mice and chronic renal inflammation in high-dose rats of either sex. The carcinogenic effects of the chemical were analyzed statistically and, under the conditions of this bioassay, vinylidene chloride was not carcinogenic for F344 rats or B6C3F1 mice of either sex.

EPA FINAL RULES WITH DEFERRED EFFECTIVE DATES

46 FR 11972, Feb. 12, 1981

ENVIRONMENTAL PROTECTION AGENCY

[AS-FRL 1752-3]

40 CFR Parts 6, 52, 56, 162, 230, 403, 413, and 429; Deferral of Effective Dates

AGENCY: Environmental Protection Agency.

ACTION: Notice of deferral of effective dates.

SUMMARY: This notice defers until March 30, 1981, the effective date of all the regulations listed below. This action is taken pursuant to the President's order of January 29, 1981, requiring postponement of the effective date of pending regulations for 60 days.

EFFECTIVE DATE: The new effective date of all the regulations listed below will be March 30, 1981.

FOR FURTHER INFORMATION CONTACT: Faith Halter, Special Assistant to the General Counsel, Environmental Protection Agency, 401 M Street, SW., Washington, D.C. 20460, 202-755-0709.

SUPPLEMENTARY INFORMATION: The following is a list of all the regulations whose effective date is deferred by this notice.

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Effects of Vinylidene Chloride on DNA Synthesis and DNA Repair in the Rat and Mouse: A Comparative Study with Dimethylnitrosamine[1]

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Effects of Vinylidene Chloride on DNA Synthesis and DNA Repair in the Rat and Mouse: A Comparative Study with Dimethylnitrosamine. REITZ, R. H., WATANABE, P. G., MCKENNA, M. J., QUAST, J. F., AND GEHRING, P. J. (1980). *Toxicol. Appl. Pharmacol.* 52, 357–370. Exposure to vinylidene chloride (VDC) vapor has been reported to induce tumors in mice, but rats are apparently insensitive to this effect of VDC. This species difference has been correlated with the greater capacity of mice to activate VDC to a reactive electrophile which can react with macromolecules. To increase our understanding of the molecular events associated with this species difference, we have investigated the potential of VDC to cause DNA alkylation, DNA repair, and DNA replication in the liver and kidneys of rats and mice. For comparative purposes, the potent carcinogen dimethylnitrosamine (DMN) was also studied. Male Sprague–Dawley rats and CD-1 mice were exposed to 10 and 50 ppm VDC for 6 hr. DNA alkylation after 50 ppm [^{14}C]VDC was minimal in liver and kidney of both rats and mice (one or two orders of magnitude less than reported for DMN in rats). Similarly, DNA repair in the kidney of mice exposed to 50 ppm VDC was only 38% higher than control values, while DNA repair in the liver of mice injected with 20 mg/kg DMN was elevated 637%. However, tissue damage and increased DNA replication (25-fold) were seen in the kidneys of mice exposed to 50 and 10 ppm VDC. Comparable effects were not seen in the liver of mice exposed to VDC (50 or 10 ppm) or in the liver or kidneys of rats exposed to 10 ppm VDC. Thus an important distinction between DMN and VDC has been demonstrated. Tumorigenic doses of DMN produced relatively little tissue damage, but were associated with a high degree of DNA alkylation and DNA repair synthesis. In contrast, exposure to tumorigenic doses of VDC resulted in massive tissue damage but induced minimal DNA alkylation or DNA repair synthesis. This suggests that the tumors observed in mice exposed to VDC arise primarily through effects of the chemical on nongenetic components of the cells. Consequently protection of humans from levels of VDC sufficient to cause tissue damage should also serve to preclude any carcinogenic activity of VDC.

Vinylidene chloride (1,1-dichloroethylene, VDC) is an intermediate in the synthesis of many commercially important plastics. Although carcinogenicity of this material has not been observed in several long-term studies with rats (Viola and Caputo, 1977; Rampy *et al.*, 1977; Maltoni *et al.*, 1977), VDC-related tumors have been reported in mice (Maltoni *et al.*, 1977). Maltoni reported that male mice were fare more sensitive to VDC than females, that the target organ in male mice was the kidney, and that the tumorigenicity of VDC was associated with significant injury to kidney tissue in male mice (Maltoni *et al.*, 1977).

Studies by McKenna *et al.* (1977) have shown that the metabolism of VDC to electrophilic species which bind to cellular

[1] This study was funded by the companies supporting the vinylidene chloride projects being administered by the Manufacturing Chemists Association, Washington, D.C.

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