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**EFFECTS OF VINYL CHLORIDE EXPOSURE ALONE AND IN
COMBINATION WITH TRYPAN BLUE — APPLIED SYSTEMATICALLY
DURING ALL THIRDS OF PREGNANCY ON THE FETUSES OF CFY
RATS**

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SUMMARY

Vinyl chloride (VC) has been shown to be present in the fetal and maternal blood as well as in the amniotic fluid after the exposition of pregnant CFY rats to VC at an atmospheric concentration of 5500, 18 000 or 33 000 mg/m³ (~2000, 7000 or 12 000 ppm) for 2.5 h on the 18th day of pregnancy, indicating the permeability of the placenta to the agent.

Teratological investigation of the offspring of pregnant rats exposed continuously to VC at an atmospheric concentration of 4000 mg/m³ air (1500 ppm) during the first, second or last third of pregnancy has shown that VC has no teratological effect in the rat and has no embryotoxic effects either, when applied during the second or last third of pregnancy in the above concentration. Exposition to VC during the first third of pregnancy resulted in an increased fetal mortality and in the manifestation of embryotoxic effects. Fetal losses and induction of central nervous system malformation due to trypan blue administration were not potentiated by a combined exposure of pregnant rats to VC and the dye.

INTRODUCTION

VC is ranking 23rd from among the 50 most widely used industrial chemi-

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Abbreviation: VC vinyl chloride.

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cals. Carcinogenicity of the compound has been reported in rats, mice and hamsters [1-3]. In man the occurrence of hemangiosarcoma — a rare malignant neoplasm — of the liver has been brought into possible causal relationship with VC exposure [4-8]. VC or its metabolite, chloroethylene oxide, have been shown to induce mutagenic effects [9-14]. Chromosome aberrations have also been described in plant workers after VC exposure of various lengths [15-17]. However there are only a few data on the teratogenicity of VC. The results of the epidemiological studies are controversial. Infante [18] reported an increase in the incidence of congenital malformations of the central nervous system among people living near chemical plants working with VC. Further epidemiological study [19] did not confirm this finding. Infante et al. [20] found higher mortality in families where the father was subject to occupational exposure to VC. Paddle [21], however, questioned methodological aspects of this approach. The only study in experimental teratology with VC was conducted by John et al. [22]. They found no teratogenic effect after inhalation of VC at an atmospheric concentration of 50, 500 or 2500 ppm for 7 h daily, during organogenesis in S-D rats, CF-1 mice or N-Z rabbits.

The large scale production of VC and the wide usage of the polymer PVC, the great number of people exposed, the reported hazardous effects, mutagenicity and carcinogenicity of the compound as well as controversial data of epidemiologic studies of teratogenicity call for a detailed study of experimental teratology of the compound.

The present work was aimed to answer the following questions:

- (1) Does VC, inhaled by the pregnant animal, cross the placenta and result thus in a direct intrauterine exposure of the fetus?
- (2) Does VC in itself possess teratogenic or embryotoxic effects?
- (3) When given in combination with a known teratogenic agent does VC potentiate the teratogenic effect of the former compound?

MATERIALS AND METHODS

Female CFY rats of 240-280 g body wt. were mated in a harem system. The day of finding sperm in the vaginal smear was considered as the first day of gestation. The animals were kept on a standard rat pellet [LATI*, Gödöllő] and tap water ad lib. Body weights were recorded once a week.

Groups of 3 rats were exposed to VC for 2.5 h on the 18th day of gestation at 5500, 18 000, 33 000 mg/m³ (~2000, 7000, 12 000 ppm) atmospheric concentrations. At the end of the exposure the animals were sacrificed and maternal and fetal blood and amniotic fluid samples were collected for VC determination performed by the method of Lőrincz [23].

Allocation of other pregnant rats to experimental groups can be seen in Table I. Groups IA, IC, IIA and IIIA inhaled air in an inhalation chamber for 24 h/day on the days of pregnancy 1-9, 8-14 and 14-21, respectively. Groups IB, ID, IIB and IIIB were exposed to VC in an atmospheric concen-

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tration of 4000 mg/m^3 ($\sim 1500 \text{ ppm}$) for the same length during the same periods of gestation as their respective controls. The volumes of the inhalation chambers were 0.13 m^3 , the vertical flow rate of the air $2 \text{ m}^3/\text{h}$ at a regulated temperature of $24\text{--}25^\circ\text{C}$ and $50\text{--}55\%$ relative humidity. VC concentration in the inhalation chamber was determined by means of a type 5840 A Hewlett Packard digital gaschromatograph [24]. The rats in groups IC and ID were given subcutaneously 2 injections of 50 mg/kg body wt. trypan blue (1% solution) on the 7th and 8th day of gestation. Group IV was kept in the animal quarters during the whole period of gestation.

On the 21st day of gestation the animals were anesthetised with ether. Abdominal wall and uterine horns were cut open and the number, position of fetuses living, dead or resorbed were noted. Fetuses and placentae were excised, weighed and macroscopic investigation was carried out. Half of the fetuses of each mother were put into Bouin's fixative and dissected after fixation under the stereomicroscope [25]. Organs with macroscopic abnormalities were embedded and hematoxylin-eosin stained sections were studied further. Histological investigation of representative other organs was also carried out. In order to investigate the skeletal system the other half of the fetuses were fixed in alcohol and stained with alizarin-red-S [26]. The mothers were dissected and their livers were processed in routine histology.

Arithmetic means and standard errors were calculated, Student's *t*-test was used for statistical comparison. The litter was regarded as the experimental teratological unit [27]; affected over total fetus ratios were calculated. Mann-Whitney U-test was used for the statistical comparison of the ratios obtained.

RESULTS

Considerably high VC concentrations were found in the blood of pregnant rats as well as their fetuses, when the mothers were exposed to VC at an atmospheric concentration of 5500 ; $18\,000$ or $33\,000 \text{ mg/m}^3$ (~ 2000 , 7000 , $12\,000 \text{ ppm}$) for 2.5 h on the 18th day of pregnancy. The presence of VC in the amniotic fluid was also detectable (Table II).

Maternal loss was not encountered in the experiments. No difference in the weight gain of pregnant rats expressed as percentage of the starting body weight was found with the exception of group IIIB exposed to VC during the third week of pregnancy. The weight gain in this group was lower, than in the other groups (Table I).

The maternal liver weight and liver weight/body weight ratio increased in response to trypan blue as well as to VC applied in the first or second week of pregnancy ($P < 0.01$ and $P < 0.05$, respectively) while no difference was seen in these parameters after VC exposure during the third week of pregnancy (Table I). No pathological change was observed in the liver of VC treated mothers at the light microscopic level. There was a marked periportal histiocytic reaction in the liver of trypan blue injected animals.

The number of resorbed fetuses as well as the fetal loss taken as percent-

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TABLE II

VINYL CHLORIDE LEVELS IN MATERNAL AND FETAL BLOOD AND AMNIOTIC FLUID OF CFY RATS ON THE 18TH DAY OF PREGNANCY AFTER 2.5 H OF EXPOSURE

Inhalation chamber (mg/m ³)	Maternal blood (μg/ml)	Fetal blood (μg/ml)	Amniotic fluid (μg/ml)
0	0 ± 0	0 ± 0	0 ± 0
5500 (~2000 ppm)	19.02 ± 1.70	12.80 ± 2.92	4.27 ± 0.42
18000 (~7000 ppm)	32.40 ± 2.12	22.67 ± 2.75	4.93 ± 0.18
33000 (~12000 ppm)	48.43 ± 1.95	30.52 ± 3.77	13.50 ± 2.99

±, S.E.M.

age of the total number of implants was significantly increased in the group exposed to VC during the first 9 days of pregnancy ($P \approx 0.05$); there was a tendency of increased resorption and fetal loss, though not significant in the group exposed to VC during the second week of pregnancy ($P < 0.1$) and no difference in the parameters was seen after an exposure to VC during the third week of pregnancy. There was an increase in the number of resorbed fetuses as well as fetal loss in the group injected with trypan blue ($P < 0.01$). Combined trypan blue administration and VC exposure was not more effective than the dye injection alone. The number of dead fetuses was increased by the trypan blue treatment (Table I).

A slight tendency of increase in the ratio of retarded fetuses was seen in the groups exposed to VC during the first 9 days of gestation or injected with trypan blue, but no significant change in the mean weight of fetuses or placentae and in the ratio of fetuses with weight retardation (less than 3.3 g) was seen no matter during which time of gestation the VC exposure occurred. Although the ratio of retarded fetuses was higher in the groups inhaling air, or VC in the inhalation chambers during the third week of gestation, this difference was not significant and probably may be due to the stressor effect of novel environment.

The findings of the dissection and skeletal investigation of fetuses are shown on Table III. None of the malformations or anomalies could be attributed to VC.

DISCUSSION

Continuous exposure of rats to VC results in a permanent elevation of its blood level [28]. A permanent increase in blood level and the low molecular weight of VC facilitate a rapid extravascular distribution of the chemical [28]. One can assume, that during a continuous exposure of pregnant rats

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to VC the fetuses like the mothers are also permanently exposed. Our detection of VC in the amniotic fluid and in the blood of fetuses of pregnant rats exposed on the 18th day of gestation (Table II) justify this assumption.

In order to study the possible teratogenic and embryotoxic effects of the compound, rats were exposed to VC at 4000 mg/m³ (1500 ppm) atmospheric concentration continuously during the first, second or third week of pregnancy. In spite of the exposure at this very high level of concentration no obvious alteration in the behaviour of experimental animals was observed; their food and water consumption and weight gain did not differ from that of the controls and activation of self protective mechanisms [29] was not seen either.

Although our study of the great number of fetuses gave essentially negative results, some tendencies found — not reaching the level of significance — might deserve further attention.

There was an increase in the number of resorbed fetuses in the groups subjected to VC during the first and second week of pregnancy. Though the increase in the number of implantations and higher birth rate might explain this difference in the group exposed during the second week; no increase in the number of implants was seen in the rats exposed during the first week. Thus it is most probable that the toxic effects of VC might explain the increased fetal loss close to the level of significance ($P \approx 0.05$). This is all the more probable, because toxic agents independent of their chemical nature have been shown to result in similar embryotoxic effects, when applied during the first third of pregnancy [30].

Among the offspring of mothers exposed to VC during the first week of pregnancy one case of microphthalmia and an other case of anophthalmia occurred. In spite of the fact that this was not consistent with a significant increase in the incidence of congenital malformations, these cases deserve further attention for the following reasons. None of these malformations was observed in the group exposed to air in the chamber, or in the untreated controls. Both malformations are related to the central nervous system, and an increased incidence of congenital malformations of the central nervous system have been brought into causal relationship with VC exposure by Infante [18].

On the basis of our results VC exposure in itself has no teratogenic effect in CFY rats, but an embryotoxic effect of VC exposure during the early stages of pregnancy at high atmospheric concentrations should be taken into consideration.

A similar lack of teratogenic effect of VC has been reported by John et al. [22]. Their conclusion is based on studies of the effect of VC applied during arbitrarily chosen short periods of organogenesis. One could emphasize here that it is a minimal requirement of experimental studies aimed to reveal the teratogenic effect of any particular chemical that the pregnant mothers are exposed to the chemical in such a way as to provide continuous exposure of the fetuses during the whole period of organogenesis. The fulfilment of this

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requirement is particularly important with VC and other toxic agents taken up by inhalation and exhaled rapidly. In our view the teratogenic effect of a chemical cannot be excluded in studies using shorter exposure times than the whole period of organogenesis.

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We are not aware of any data on the effect of VC in early pregnancy. Even if the results of our animal experiments cannot be applied directly to the human and considering that an exposure to VC at 250 ppm atmospheric concentration would result in the saturation of the metabolizing capacity of VC [31], the hazards of an occupational exposure of women in the fertile age requires thorough consideration.

Infante [18] claims that the high incidence of congenital malformations of the central nervous system in the neighbourhood of PVC producing plants is due to VC. Edmonds et al. [19] were not able to confirm this view. In this context we studied the possibility that even if VC has no teratogenic effect to induce malformations of the central nervous system it might potentiate a teratogenic effect of other agents. Trypan blue has been reported to be embryotoxic and bring about malformations of the nervous system [32-34]. Negative results have been obtained in this respect; a concomittant exposure to VC did not affect either the teratogenic or the embryotoxic effects of trypan blue, as no higher incidence of congenital neural malformations (exencephaly, anophthalmia, microphthalmia, aplasia of the orbit) or higher fetal losses were encountered in the group with combined VC and trypan blue treatment.

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Chromosomal Damage in Men Occupationally Exposed to Vinyl Chloride Monomer and Other Chemicals

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INTRODUCTION

Data from various sources suggest that the biologic effects of vinyl chloride monomer (VCM) include mutagenicity as well as oncogenicity. Pertinent studies include observations concerning the mutagenic effects of VCM in bacterial test systems (Rannug *et al.*, 1974; Bartsch *et al.*, 1975), mutagenicity of VCM metabolites in mammalian cells (Huberman *et al.*, 1975), and cytogenetic studies of polyvinyl chloride (PVC) polymerization workers (Ducatman *et al.*, 1975; Funes-Cravioto, *et al.*, 1975; Kilian *et al.*, 1975; Purchase *et al.*, 1975). Also relevant may be observations suggesting increased fetal loss in families of PVC workers (Infante *et al.*, 1976). Among the several cytogenetic studies reported, three have suggested increased chromosomal breakage among polymerization workers (Ducatman *et al.*, 1975; Funes-Cravioto *et al.*, 1975; Purchase *et al.*, 1975), and one has not (Kilian *et al.*, 1975). This report concerns cytogenetic analyses conducted on workers employed at a rubber and plastics plant, some exposed to VCM and others not.

MATERIALS AND METHODS

The work described here was part of a cross-sectional study designed to provide multiphasic medical screening data on the health status of men employed at a large rubber and plastics plant. The overall study was conducted jointly by the Center for Disease Control (CDC) (National Institute for Occupational Safety and Health and Bureau of Epidemiology) and the Occupational Health Studies Group of the University of North Carolina in cooperation with the Firestone Corporation and the United Rubber Workers. Results of the entire study, together with a detailed description of the study's design and methodology, will be published separately.

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Cytogenetic analyses, primarily designed to measure frequencies of chromosomal breakage, were performed on peripheral blood lymphocytes from 35 men; specimens were obtained from 18 men in October 1974 and 19 in January 1975 (2 on both occasions). Subjects were restricted to men primarily or exclusively employed for 10 years or longer, in 3 different employment categories: 14 in PVC polymerization (presumed high exposure to VCM, intermittent or sustained), 4 in PVC processing (presumed low exposure to VCM), and 17 in rubber tire manufacture (industry controls; presumed negligible exposure to VCM). Work history details were obtained by interview and confirmed by review of company records.

Initially, the study was designed to compare breakage frequencies in workers exposed to VCM with frequencies in other workers. However, when initial analyses showed no significant differences among worker groups, a control group was selected (April 1975) consisting of four male employees at CDC not exposed directly to any laboratory chemicals. Material for cytogenetic analysis was prepared in the same manner with the same reagents as the earlier material from workers at the plant. To assess comparability in microscopic reading of slides between April 1975 and the earlier dates, previously read slides from the high VCM and industry control groups were blindly interspersed among the CDC worker slides. Breakage frequency recorded on the second reading of high VCM/industry control slides (6.0% of 150 scored metaphases) did not differ significantly from the first reading. Cytogenetic material was processed using standard procedures (Moorhead *et al.*, 1960). Cells were cultured for 72 hours in the presence of phytohemagglutinin. Giemsa staining was used without banding.

RESULTS

Frequencies of chromosomal breakage in each group are shown in Table 1. Levels of breakage in all three industry groups, whether exposed to VCM or not, were significantly increased over the CDC control level, the high VCM group at the $P < 0.01$ level, the low VCM and industry control groups at the $P < 0.05$ level. At the same time, no significant differences were noted between the three industry groups themselves. Groups were comparable in terms of age and number of months worked. Breakage frequencies for individual subjects within groups did not vary significantly one from another.

An effort was made to relate levels of breakage to duration of employment and hence extent of potential toxic exposure (Table 2). While a significant gradient was seen for industry controls, interpretation is uncertain because of small numbers and because the mean age of subjects increased with employment duration. No gradient was seen for the high VCM group, whether in terms of total employment or of employment in contact with VCM. The low VCM exposure group was too small to permit analysis by exposure duration.

Chromatid gaps comprised the majority (86%) of aberrations seen (Table 3). Similar types of aberrations were seen with similar frequencies among all four groups.

DISCUSSION

When reviewed in terms of comparisons between worker and nonworker

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groups, the present observations are not inconsistent with prior studies suggesting that industrial exposure to VCM is associated with an approximately twofold increase in levels of chromosome breakage as measured in culture of peripheral blood lymphocytes. In contrast with at least one prior report (Huberman *et al.*, 1975), however, breakage consisted mostly of simple chromatid gaps rather than more complex forms.

The fact that overall breakage levels were similar in workers exposed heavily, lightly, or negligibly to VCM may imply, in this particular work setting at least, the presence of agents other than VCM capable of inducing chromosome breaks. Because of the wide range of chemicals to which rubber workers at this plant were exposed (primarily solvents of various kinds), it was impossible to relate any particular agents to the abnormal effects observed. No clear-cut pattern was seen to relate degree of breakage to duration of exposure.

SUMMARY

Measurements of chromosomal breakage were made in peripheral blood lymphocytes from workers exposed heavily, lightly, or negligibly to VCM at a large rubber/plastics plant. Breakage levels in all three groups were significantly increased over levels in nonindustrial controls. Breakage consisted mostly of simple chromatid gaps. The results suggest that other agents, in addition to VCM, may cause cytogenetic damage in workers employed in the rubber/plastics industry.

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