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Human Male Exposure to Vinyl Chloride and Possible Teratogenic and Mutagenic Risks: A Review

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Introduction

Vinyl chloride, also known as vinyl chloride monomer (VCM), chloroethylene, and chloroethene, was first synthesized in 1833.[1] Some years later in the mid 1930s, the polymerization process was discovered in Germany. The vinyl chloride related industries have developed rapidly in Europe, which accounts for about 50% of world production, and in the United States, which accounts for an estimated 25% of world production. Annual world production of vinyl chloride exceeds 10 billion kg.[2] Total world-wide employment in the VCM and polyvinyl chloride (PVC) producing industries is estimated to be well over 70 000 workers. The number of workers employed in industries using PVC as a basic element is estimated to total in the millions.

Data pertaining to the possible toxicity of vinyl chloride were published in the literature as early as 1930. At that time, Patty *et al.*[3] reported gross pathological changes in guinea pigs that died during exposure to vinyl chloride, including intense congestion and oedema of the lungs and a hyperemia of the kidneys and liver. Vinyl chloride may further be associated with reproductive related risks. Workplace policies, in fact, have been instituted prohibiting females from working in vinyl chloride polymerization areas, based on the belief that vinyl chloride may pose a health risk to females and to their unborn children. Lawsuits have subsequently been filed charging that such policies are discriminatory because vinyl chloride exposure may also adversely affect male reproductive functioning.[4–5]

A growing body of data indeed suggest that the exposure of human males to vinyl chloride in the workplace, and elsewhere, may be associated with various chromosomal aberrations in lymphocytes and sister chromatid exchanges. Paternal exposure to vinyl chloride may further be associated with various adverse effects on pregnancy and possibly spermatic alterations.

This review is intended to examine critically selected, available data concerning paternal exposure to vinyl chloride and possible reproductive related risks. The references included in the review date to 1983. It should be understood that these references were selected from among the best available published data known to the author.

Chromosomal aberrations

Data in the published literature are conflicting regarding possible chromosomal aberrations associated with the exposure of human males to vinyl chloride. Some investigators have reported negative findings for chromosomal aberrations in vinyl chloride exposed males. Fleig & Thiess[6] in 1978 reported data from chromosome analysis undertaken on lymphocyte cultures from male workers showing no symptoms of vinyl chloride illness. Compared with controls, there was no significant difference in the rate of chromosome aberrations; the frequency of aberrant metaphases totalled 3.1%, excluding gaps, and 7.5%, including gaps for exposed workers, corresponding values in the control group were 2.1 and 5.5%, respectively.

Several factors confound the analysis of the Fleig data. The sample population of workers was relatively small ($n = 10$). A relatively small sample size raises questions about statistical 'power', or an investigator reporting no association, when in fact one exists. In general, the smaller the sample size, the greater the chance that an association between an exposure and an effect will not be detected.[7] Precise data on level of vinyl chloride exposure were not presented for all the cases. Data concerning possible alcohol consumption were not presented. Although data were reportedly collected concerning age, occupational exposures, medical X-rays, recent viral diseases, drug consumption, and smoking habits, specific values were not given in the paper. Duration of exposure of cases ranged from 4 to 26 years. The age range of cases was 34 to 57. The controls were described as 'healthy' persons who were not exposed to vinyl chloride or to any other 'known' (not specified) chromosome damaging agent but worked in the same factory. This may raise the possibility that the controls were exposed to various clastogenic agents in the factory.

Fleig & Thiess further investigated 20 workers

showing symptoms of vinyl chloride illness. Compared with the same control group, there was a significant difference in the rate of chromosome aberrations. The rate of aberrant metaphases in cases was 5.2%, excluding gaps, and 11.2%, including gaps; in the control group, the corresponding values were 2.1 and 5.5%.

The further data are confounded by the fact that the 20 cases had an unknown rate of exposure. In addition, duration of exposure in the cases ranged from 4 to 30 years. Cases ranged in age from 32 to 64.

In 1980, Rossner *et al.*[8] reported data following the investigation of 31 males occupationally exposed to vinyl chloride monomer. A group of males from the same plant, but reportedly not exposed to vinyl chloride monomer, were chosen as matching controls. Several categories of chromosomal aberrations were analysed: chromatid and chromosome breaks, and chromatid and chromosome exchanges. Gaps were not included among aberrations. Two blood samplings were conducted, about a year apart. In all subjects, only breaks were detected. No significant differences were found between the cases and controls, or between the first and second sample taking.

Exact levels of vinyl chloride monomer exposure are not certain. Rosner *et al.* reported 'on average' the levels of vinyl chloride monomer concentration in the atmosphere of the workplace ranged below the Czechoslovak MAC level of 10 mg/m^3, and that the peak concentration did not exceed 30 mg/m^3. At the time of blood sampling, the workers being followed had been exposed to vinyl chloride monomer for 2 and 3 years. Cases ranged in age from 25 to 55. An analysis for drug and alcohol consumption habits was reportedly conducted for all subjects. Data for these parameters were not presented. There is no indication that subjects were questioned concerning smoking habits, or X-ray or other radiation exposure.

Some published studies reporting negative findings for chromosomal aberrations in vinyl chloride exposed workers do not state the sex of cases and controls. Kilian & Picciano[9] reported preliminary data on 121 workers occupationally exposed to vinyl chloride and vinylidene chloride. Pre-employment examination records from 75 persons were used as control data. Compared with cytogenic data from the pre-employment examinations, the vinyl chloride workers showed less chromatid breakage, a greater frequency of dicentric chromosomes and a lower percentage of abnormal cells.

Data on the sex distribution of cases and controls are not presented by the investigators. The investigators stated that only about 50% of the vinyl chloride workers at the pertinent facility were included in the study. Cases and controls were not age-matched insofar as the pre-employment group contained only 2 persons over 50 years of age whereas the vinyl chloride group contained 30 persons more than 50-years-old.

Picciano *et al.*[10] reported cytogenic findings from 209 workers employed at a vinyl chloride plant. Control data were obtained from a group of 295 'pre-employment examinees' who had chromosome evaluation done as part of the routine pre-employment examination. Data from the two groups were scored and compared on the basis of chromatid breaks, chromosome breaks, rings, dicentrics, exchange figures and the proportion of abnormal cells. The investigators stated that results, expressed in terms of the mean percentage for these categories of aberrations, showed no major differences between cases and controls for any of the classifications.

Although Picciano *et al.* indicate that cases and controls were matched as much as possible for various factors, including sex, the investigators do not provide exact data on the sex distribution of cases and controls. Cases and controls were not age-matched. The average age of cases was 39.5 years (range of 18 to 67); the average age of controls was 25.1 years (range of 18 to 50 years). Duration of occupational exposure of cases, at the time of investigation, ranged from 1 to 332 months (average of 48.3 months).

Several studies have reported positive findings for chromosomal aberrations in human males exposed to vinyl chloride. In 1975 Ducatman *et al.*[11] presented data findings for 11 males exposed to vinyl chloride in a polyvinyl chloride polymerization plant. In comparison with 10 'healthy' male controls, without known vinyl chloride exposure, cases showed a non-significant increase in cells with simple aberrations, such as breaks and gaps, when using a *t*-test for the comparison of means, and marginally significant increases of such aberrations using a *F* ratio for the comparison of variance. The differences in cells with 'stable' chromosome aberrations, such as monosomies trisomies, deletions and exchanges, were also non-significant. However, cells with 'unstable' chromosome changes, such as fragments, dicentrics and rings, were observed significantly more frequently in the cultures from cases. The total of simple breaks, including those from multiaberrant cells, was increased but not significantly in the exposed workers. Complex breaking events were significantly more frequent in cases, and total breaking events were also significantly increased in cases.

The relatively small size of the sample population ($n = 11$) affects the statistical significance of the Ducatman *et al.* data. Exact data on levels of exposure of the workers are not provided. The investigators state that there is no record of ambient gas levels at the pertinent facility, but it was assumed that the levels must have exceeded 500 ppm at times, based on reports of dizziness, odour detection and headaches. Duration of vinyl chloride exposure of cases ranged

from 4 to 28 years (average of 15 years). Cases ranged in age from 25 to 61. Cases and controls were not age matched. The average age of cases was 40; the corresponding figure for controls was 27. Because chromosomal aberrations may be associated with various factors, it is vitally important to question subjects about prior and current exposure to known and suspected mutagens, X-ray and other radiation exposure, drug use, smoking history, alcohol consumption, and infectious diseases, particularly those of viral origin. Ducatman *et al.* did not provide data for cases and controls regarding these variables.

Funes-Cravioto *et al.*[12] in 1975 reported an increased frequency of chromosomal aberrations in seven males occupationally exposed to vinyl chloride. Three 'non-exposed' control subjects, from the same factory as the cases, were also studied. The frequency of abnormal cells in cases (9.52%) was significantly greater than in the controls ($P < 0.001$).

The small size of cases ($n = 7$) affects the epidemiologic significance of this study. Funes-Cravioto *et al.* do not provide precise data on levels of exposure to vinyl chloride. It is stated that the level of exposure to vinyl chloride had 'continuously decreased' during past years, and that in the weeks preceding blood sample collection for chromosomal analysis, the air concentration in the polymerization department was estimated to be 20–30 ppm. Duration of vinyl chloride exposure ranged from 9 to 29 years. A problem of relevant controls is raised. Since the three 'non-exposed' controls were employed in the same factory as the cases, there is the possibility that they may have been exposed to vinyl chloride. Also, data for the control subjects showed that 11/566 cells contained chromosomal aberrations. Funes-Cravioto *et al.* hypothesized that this finding might be associated with previous diagnostic X-ray exposure.

Also in 1975, Purchase *et al.*[13] reported findings for chromosomal aberrations in vinyl chloride-exposed workers. The investigators studied 80 workers. Fifty-six had been exposed to vinyl chloride monomer; the remaining workers worked in plants and laboratories where exposure to vinyl chloride reportedly did not occur. A comparison of chromosomal aberrations in the vinyl chloride exposed workers compared to controls not exposed to vinyl chloride showed a significantly increased ($P < 0.05$) percentage of B, Cu, and Cs cells (using the classification of Buckton & Pike, Journal of Radiation Biology 1964; **8**: 439–52) in the exposed workers.

Purchase *et al.* did not state the sex of cases and controls. Data concerning levels of exposure and duration of exposure were also not provided. The mean age, and age range, of cases and controls were not given. Information about the smoking habits and alcohol consumption of cases and controls was also not included in the published study.

In 1976, Szentesi *et al*[14] reported data on chromosomal aberrations in 45 PVC workers (41 males). Forty-four 'industrial controls' (19 males) engaged in other chemical plants and reportedly not exposed to PVC but 'indirectly' exposed to 'other' (not specified) chemicals as well as 49 'normal' controls (34 males) with no reported occupational exposure to chemicals were further examined. The investigators concluded that the rate of chromosomal aberrations did not differ significantly between PVC workers and controls. However, the frequency of chromatid-type aberrations was higher in PVC workers compared to the two controls ($P < 0.001$). In addition, unstable chromosome-type aberrations were significantly higher in PVC workers ($P < 0.001$).

Several factors confound the analysis of the Szentesi *et al.* data. Duration of exposure of cases ranged from 0.5 to 12 years. The investigators do not provide data on the level of exposure of cases. A problem of relevant controls exists since the 44 'industrial controls' were 'indirectly' exposed to 'other' (unspecified) chemicals. Additionally, cases were not age matched with the industrial controls. The mean age of the industrial controls was 43.9 whereas the value for cases was 27.3. Data are not presented for such potential confounders as smoking history, alcohol use, exposure to X-rays and other radiation, possible drug use, and viral or other infectious diseases.

Purchase *et al.*[15] in 1976, reported data on chromosomal abnormalities in 80 workers, including exposed workers, non-exposed workers from the same industrial site, and non-exposed workers from a different environment. The population of exposed workers was further divided into groups, base on the type of work carried out. Autoclave operators and maintenance workers had higher percentages of B and C cells (classified according to Buckton & Pike, 1964), compared to those who worked in PVC production but not with autoclaves and the non-exposed control subjects from another environment. The difference between the combined results from the exposed groups and the control group was reported to be statistically significant.

Purchase *et al.* do not specify the sex of the cases and controls. The only information given concerning level of exposure to vinyl chloride was that the autoclave workers had the 'highest' exposure and workers employed in PVC production but not with autoclaves and maintenance workers in the same area had the 'lowest' exposure. The duration of exposure of cases is not given. Information about the age range of cases and controls is not presented. In addition, data were not given regarding smoking history, alcohol use and viral infections of cases and controls.

Some studies have presented cytogenetic data findings confounded by the fact that cases may have been exposed to vinyl chloride as well as other

potential mutagens. Heath *et al.*[16] in 1977, reported chromosomal damage in men occupationally exposed to vinyl chloride monomer and other chemicals. Cytogenetic analysis, primarily designed to measure frequencies of chromosomal breakages, were performed on peripheral blood lymphocytes of 35 males, in three different job categories: 14 in PVC polymerization, 4 in PVC processing, and 17 in rubber tyre manufacture (industry controls). When initial analysis showed no significant differences among the three industry groups, a further control group was selected, consisting of 4 males employed at the Center for Disease Control and reportedly 'not exposed directly' to any laboratory chemicals (CDC controls).

Levels of breakage in all three industry groups were increased significantly over levels in the CDC controls. However, no significant differences were observed between the three industry groups themselves. Chromatid gaps comprised the majority (86%) of observed aberrations.

It is difficult to interpret the data of Heath *et al.* Levels of exposure of cases and controls to vinyl chloride, and other potential mutagens, are not clear. The 14 PVC polymerization workers had 'presumed high' exposure to VCM, the 4 PVC processing workers had 'presumed low' VCM exposure, while the 17 workers in rubber tyre manufacture had 'presumed negligible' exposure to VCM. In the opinion of the investigators, the fact that overall breakage levels were similar in workers exposed heavily, lightly or negligibly to VCM may imply the presence of other clastogenic agents. Heath *et al.* concluded that, because of the wide range of chemicals to which rubber workers were exposed, primarily solvents, it was 'impossible' to relate any particular agents to the observed abnormalities. The average number of months worked for the 14 PVC polymerization workers ranged from 215 to 346, for the 17 industry controls the range was 114 to 357. The industry controls ranged in age from 31 to 58; the CDC controls had an age range of 37 to 51. Data were not given pertaining to smoking history, alcohol consumption, drug use, radiation exposure, or viral infections affecting cases and controls.

In the Heath *et al.* study, cells were cultured for 72 h in the presence of phytohaemagglutinin. The length of culture time may have affected the frequency of observed chromosomal aberrations. In general, cells will enter mitosis for the first time in about 48 h; in approximately 72 h, there will be a second division.[17] In the judgment of some investigators, the 48-h culture time may allow for a relatively more accurate indication of induced aberrations, because some changes may be lost or modified with each cell division. However, the 72 h culture may allow recovery of a greater number of cells for analysis.

Purchase *et al.*[18] studied chromosomal morphology from cultured peripheral lymphocytes from 81 men. Fifty-seven of the workers (cases) were employed at plants manufacturing vinyl chloride or PVC, 19 of the workers were on-site controls, and the remaining 5 were off-site controls. The 57 cases were further divided into six groups. The on- and off-site controls were combined into a single control group, because no significant differences were observed between them. In a study published in 1978, Purchase *et al.* reported that the subgroup of cases consisting of autoclave workers had the greatest number of significantly increased values for chromosomal abnormalities. The values for total B cells, total Cu cells and total C cells (classified according to Buckton & Pike) were significantly higher than control values ($P < 0.01$). Purchase *et al.* further reported a significant correlation between smoking habits and total C cells.

Data pertaining to exposure levels in the Purchase *et al.* study were imprecise. Any experience in a specified one-year time period, 7/73–7/74, of exposure to vinyl chloride for short-term excursion which could be detected by smell was recorded. The group of cases who operated and cleaned the autoclaves were considered, by virtue of their jobs, as likely to be exposed to the highest average levels of vinyl chloride. The investigators gave figures for estimated average operator exposure to vinyl chloride on PVC plants, ranging from approximately 1000 ppm (1945–1955) to approximately 5 ppm (in 1975). Average duration of exposure to vinyl chloride ranged from 6.1 to 15.5 years, in the six groups of cases. There was an interval of about 18 months between the time of blood sampling, and the obtaining of smoking histories. No data were provided on alcoholic habits in cases and controls. However, subjects with recent X-ray exposure, 'prolonged' drug treatment, or recent viral infections were excluded from the study.

Some studies have presented data suggesting a possible relationship between a reduction in exposure to vinyl chloride and 'normalized' chromosomal breakage frequency. The blood sampling in the 1978 study by Purchase *et al.* was carried out in July 1974. Since that time, threshold limit values for vinyl chloride and plant exposure levels were reportedly reduced. In a study published in 1980, Anderson *et al.*[19] reported two further samples from the same population of workers analysed for chromosomal aberrations 18 and 42 months after the initial sampling.

The second sampling consisted of 21 workers, employed or formerly employed in plants manufacturing either vinyl chloride or PVC, re-examined in January 1976, together with 6 off-site controls. A third sampling, examined in January 1978, consisted of 23 workers employed as before together with 8 on-site controls. In both the second and third sampling, the populations were classified into four groups The investigators reported that, in comparing the results

between 1974 and 1976, the autoclave workers who had remained as such showed a statistically significantly ($P < 0.05$) larger increase than controls in B cells, total abnormalities and abnormalities excluding gaps (classified using an adaptation of the method of Buckton & Pike).

In the third sampling, the investigators reported a tendency to a decrease in the percentage of cells with chromosomal aberrations of various types in comparison with both prior samplings. When the results of the 1978 sampling were considered alone, Anderson *et al.* reported that the exposed workers had a similar percentage of all types of abnormalities compared to the control group, in contrast to the 1974 and 1976 samplings. The investigators further stated that the incidence of chromosomal aberrations had returned to control values during the time that levels of exposure had been reduced below 5 ppm. In the judgment of the investigators, it appeared that by reducing exposure to vinyl chloride, chromosomal abnormalities had returned to values similar to those of controls.

Lack of precise data concerning levels of exposure to vinyl chloride make it difficult to evaluate the possible 'normalization' occurrence reported by Anderson *et al.* The investigators state that although 'no accurate' data on exposure levels were available at the time of the 1974 sampling, 'marked' reductions in vinyl chloride levels had supposedly occurred since that time due to modifications and control measures. Subjects completed a questionnaire, including details of 'recent high' exposures, or exposures which could be detected by smelling. In the second sampling, it was reportedly not possible to exclude persons suffering from viral infections, as 11 sample members were suffering from colds. However, Anderson *et al.* stated that there was no evidence of increased aberrations in individuals with colds; this infection was not considered in the third sampling.

More data on the subject of normalized chromosome breakage frequency were published in a 1978 article by Hansteen *et al.*[20] Cytogenetic studies were carried out on 39 workers from a PVC plant in 1974. Fourteen of the workers were chosen at random, 13 were chosen because they had been 'heavily' exposed to vinyl chloride monomer for years; and 12 were workers whose first health screening revealed abnormalities but on clinical re-examination all laboratory tests proved normal. Sixteen healthy males, with no connection with the PVC plant, were chosen as controls. The cytogenetic study was repeated for 37 of the 39 workers 2 to 2.5 years later. The repeat study was done with 32 matched controls. In addition, 6 of the previous control group were re-examined. The exact sex distribution of cases and controls is not clear.

The mean breakage frequency for the 39 workers was 3.7% in the initial study. Because of different factors, such as radiation and drugs, which may influence breakage frequency, 4 workers were omitted. The mean breakage frequency for the remaining 35 workers was then 3.41%, compared with a control value of 1.79%. The mean breakage frequency for the heavily exposed workers (3.79%), was higher than the value for the rest of the workers (3.15%). All tests for cases compared to controls showed that workers scored significantly higher on a 2.5% significance level in the initial study. However, in the repeat cytogenetic study, no significant differences were found between chromosome breakage frequencies in cases versus matched controls. The chromosome breakage frequencies for the three groups of workers were back to normal. No significant difference was found between the control group in 1974 and the matched controls. Hansteen *et al.* concluded that the repeat study demonstrated that the chromosome breakage frequency was back to normal after a 'significant' reduction in exposure for 2 years.

Imprecise data concerning vinyl chloride exposure levels affects the epidemiologic significance of the Hansteen *et al.* study. Exact measurements of exposure in the PVC plant were provided for 1974 (25 ppm). However, for other years, the air concentration of vinyl chloride monomer in the PVC plant was estimated, based on the level of production and the number and types of autoclaves used in a particular time period. The estimated values were quite wide-ranging (from 2000 ppm, for 1950–1954 to 1 ppm, for 1975). Employment in years ranged from 1 to 18.5 in the workers chosen at random, 4 to 21 in the workers examined on clinical grounds, and from 10.5 to 24 in the heavily exposed workers. The age range in the cases and controls was considerable: 23 to 66 in the randomly chosen workers; 26 to 67 in the workers examined on clinical grounds; 39 to 63 in the workers heavily exposed; and 27 to 66 in controls. Hansteen *et al.* reported that matching for age between cases and controls was attempted, but was 'not entirely' successful. Although a medical history, including viral infections, radiation exposure and drug intake, was reportedly obtained for all participants in both studies, no specific data are presented regarding drug use, smoking history, alcohol use, radiation exposure, or viral infections.

Chromosomal changes may be reversible. Based on data from the Anderson *et al* and Hansteen *et al.* studies, a reduction in chromosomal aberrations may occur after lowering of exposure to vinyl chloride. A failure to take repeated samples from persons exposed to varying levels of vinyl chloride may be a limitation of studies examining possible chromosomal aberrations in vinyl chloride exposed workers. The process of replacement of aberrant cells by normal cells may occur at the rate of 1 to 2% per year.[21] Thus, if very high levels of aberrant cells are observed

Table 1 Summary: chromosomal aberrations in human males exposed to vinyl chloride

Reference	*No. of cases*	*Average duration of exposure (years)*	*Dose of exposure*	*No. of controls*	*Major findings*	*Study limitations*
Ducatman *et al.*[11]	11 males	15	Inexact	10	Significant increase in total breaking and complex breaking events and 'unstable' chromosomal changes; non-significant increase in simple and 'stable' aberrations	Small sample size; inexact exposure data; no age matching
Funes-Cravioto[12]	7 males	16.5	Inexact	3	Significant increase in abnormal cells	Small sample size, inexact exposure data
Kilian & Picciano[9]	121	Not given	Varying	75	Less chromatid breakage: lower % of abnormal cells: greater frequency of dicentric chromosomes	Sex distribution of cases/controls not given
Purchase *et al.*[13]	56	Not given	Not given	24	Significant increase in B, Cu and Cs cells	Sex of cases/controls not given; no exposure data
Purchase *et al.*[15]	56	Not given	Not given	24	Significant mutagenic effect	Sex of cases/controls not given; no exposure data
Szentesi *et al* [14]	41 males, 4 other	Not given; but range of 0.5–12 years	Not given	44 'industrial' 49 'normal'	Higher frequency of chromatid-type aberrations, significantly higher 'unstable' aberrations	No dose data; inexact duration data, some controls 'indirectly' exposed to 'other' chemicals
Heath *et al.*[16]	18 males	Varying	Inexact	17 'industry' 4 'CDC'	Significant increase in breakage levels compared to CDC controls	Small sample size; inexact exposure data
Picciano *et al.*[10]	209	48.3 months	Inexact	295	No major differences reported for mutagenic effects	Exact sex distribution of cases/controls not given; Inexact exposure data
Fleig & Thiess[6]	10 males 20	15.4 12.4	Inexact Not known	10 20	No significant difference in rate of chromosomal aberrations; Significant difference in rate of chromosomal aberrations	Small sample size, inexact exposure data Unknown exposure rate
Hansteen *et al.*[20]	39 (initial) 37 (repeat)	Varying	Inexact	16 (initial) 32 + 6 (repeat)	Significant mutagenic effect; No significant difference in chromosomal breakage frequencies	Exact sex distribution of cases/controls unclear, inexact exposure data, no specific data on drug use, smoking or alcohol use
Purchase *et al.*[18]	57 males	Varying	Inexact	24	Significant increase in chromosomal abnormalities; significant correlation between smoking and total C cells	Inexact exposure data, about 18 month gap between blood sample and taking of smoking history
Anderson *et al.*[19]	21 23	Varying	Inexact, but 'reduced' over time	6 8	Tendency to a decrease in chromosomal aberrations, compared to prior samplings	Inexact exposure data; small sample size
Rossner *et al.*[8]	31 males	Varying	Inexact	35	No significant mutagenic effect	Inexact exposure data

initially, the return to normal levels may take quite some time.

Table 1 summarizes major data findings and information from the foregoing studies on chromosomal aberrations.

Sister chromatid exchanges

In addition to examination of gross chromosome aberrations, the investigation of sister chromatid exchanges (SCEs) may provide a promising means of biological monitoring in instances of exposure to vinyl chloride, or other known or suspected mutagens. SCEs reflect intrachromosomal rearrangements of the DNA helices.[22] Crossing over between the sister chromatids of a single chromosome is a phenomenon that was recognized after the development of a special technique.[23] Cultured cells are permitted to replicate twice in bromodeoxyuridine (BUdR), allowing incorporation of BUdR into newly synthesized DNA in place of thymine. BUdR affects the staining properties of chromatids. If a SCE has occurred, this may be recognized by the fluorescence patterns along the chromatids.

The molecular mechanism of SCE induction is not clear. However, SCEs may be a sensitive indicator of DNA damage, particularly directly following the exposure in question. Gross morphological changes in chromosomes may persist for several years whereas the SCE frequency in lymphocytes may remain at a high level for 4 to 16 weeks following exposure.[22] The time lag between exposure and analysis, as well as the type and duration of mutagen exposure, may thus affect the relative suitability of gross chromosome aberrations and SCEs as means of biological monitoring.

Data are available from several studies pertaining to SCEs in industrial populations exposed to vinyl chloride. Anderson *et al.*[24] in 1981, reported data from a study designed to assess whether the SCE technique may offer advantages over conventional chromosomal analysis in monitoring human populations expose to mutagens. The sample population comprised of 21 male workers and 6 controls. The cases and controls represented a second sampling from a group of vinyl chloride exposed workers and controls first examined 18 months earlier and described in the 1978 study by Purchase *et al.*[18] Sample members were examined for the presence of chromosomal aberrations or SCEs, in their peripheral lymphocytes. In all exposed groups significant increases were reported for most types of chromosomal abnormalities. The number of SCEs per cell was slightly increased in the exposed groups, with the highest value in the 10 cases who were autoclave workers. However, the increases were not statistically significant.

The investigators postulated possible mechanisms for the data results. It was hypothesized that the exposure levels of vinyl chloride which the workforce were exposed to were not high enough to induce SCEs. Anderson *et al.* concluded that the analysis of SCEs may be of limited value after very low chronic chemical exposure or if some time has elapsed after acute exposure. However, analysis of SCEs may be of value during high chronic chemical exposure and during the first few days immediately following acute exposure.

Some investigators have reported significantly increased levels of SCEs in workers occupationally exposed to vinyl chloride compared to control values. Kucerova *et al.*,[25] in 1979, reported data from an analysis of three blood samples from each of 9 workers occupationally exposed to vinyl chloride monomer. In the third blood sample series, the investigators also used the SCE technique and compared the frequency of observed chromosomal aberrations with the number of SCEs in each sample. Blood samples from 8 healthy persons were used as controls. The sex distribution on cases and controls is not stated. The investigators reported an increase in the frequency of chromosomal aberrations and the number of SCEs per cell in 7 blood samples in cases compared to controls. The level of significance for the frequency of SCEs in cases compared to controls was $P < 0.001$, according to the *t*-test. It was the opinion of the investigators that evaluation of the frequency of chromosomal aberrations and the number of SCEs may be equally

Table 2 Summary: sister chromatid exchanges in human males exposed to vinyl chloride

Reference	*No. of cases*	*Average duration of exposure*	*Dose of exposure (avg ppm)*	*No of controls*	*Major findings*	*Study limitations*
Kucerova *et al.*[25]	9	Not given but range of 10–27 years	Inexact	8	Increased frequency of chromosomal aberrations and SCEs	Sex distribution of cases/controls not given; small sample size; inexact exposure data
Anderson *et al.*[24]	21 males	Varying	Inexact	6	Significant mutagenic effect; 'slightly increased' SCEs	Inexact exposure data

suitable methods for testing high-dose vinyl chloride mutagenicity *in vivo*.

The relatively small size of the sample population in the Kucerova *et al.* study (9 cases, and 8 controls) affects the statistical significance of the study. The 9 cases had been exposed to 'relatively high' mean annual doses of vinyl chloride monomer, estimated to be about 20—150 ppm of air. The controls were reportedly not exposed to 'known' (not specified) mutagens, during the 3 months before the time of blood collection. Duration of exposure for the 9 cases ranged from 10 to 27 years. Controls were matched for age and sex. Several of the cases and controls were smokers and/or used alcohol. Six of the cases used 1 or more drugs. Precise data for these variables are not presented. However, the investigators stated that the smoking and other habits of workers appeared to have no effect on the frequency of any chromosomal changes.

A summary of information concerning human male exposure to vinyl chloride and SCEs is presented in Table 2.

Effects on pregnancy

The exposure of human males to vinyl chloride may be associated with various adverse pregnancy outcomes. Published data on this subject are inconclusive. Some of the difficulties potentially involved in reaching definitive conclusions in this area are mentioned in a review piece by Clemmesen.[26]

In 1978, Edmonds *et al.*[27] reported data from an epidemiologic investigation undertaken in Kanawha County (Charleston), West Virginia. The investigation was undertaken because of concern that high rates of congenital central nervous system (CNS) defects observed in Kanawha County might be associated with environmental exposure to vinyl chloride monomer.

During the pertinent time period, 1/1/70–31/12/74, 47 residents of Kanawha County at time of birth had confirmed CNS defects. All cases were white. For each confirmed case, 2 controls were selected from birth certificate records. The controls were live born infants whose parents lived in Kanawha County and whose birth certificates immediately preceded and followed that of an infant with a CNS defect and gave no indication of congenital malformation. The families of affected infants and matched controls were interviewed by telephone. The investigators reported that 'close' case control matching was achieved for the following variables: paternal education, maternal age, and Hollingshead Index. Edmonds *et al.* further stated that cases and controls were 'comparable' with respect to maternal education, proportion of previous fetal deaths and other children with congenital anomalies.

The investigators reported initially high, albeit declining, rates of CNS defects. Defect rates for 1970–1972, particularly rates for anencephaly, were 1.5–2 times higher than those recorded for white births in surveillance systems using similar data sources in Florida, Nebraska and Georgia. However, the rate of total CNS defects declined from 1970 to 1974. In 1970, 14 cases of CNS defects were confirmed (including 9 cases of anencephaly, 4 cases of spina bifida, and 1 'other' CNS defect). However, in 1974, only 1 case (of spina bifida) was confirmed. The investigators stated that the decrease was not unique to Kanawha County insofar as a similar, though less marked, trend was evident in available data for other parts of the country.

The Edmonds *et al.* data do not provide a clear basis for evaluating the possible relationship between parental exposure to vinyl chloride and other possible atmospheric pollutants and the rate of various congenital CNS defects. Edmonds *et al.* reported no differences between cases and controls concerning parents' possible exposure to vinyl chloride at the time of, or for 5 years before, their child's conception, either by direct occupational exposure or by location of work place with respect to the PVC polymerization plant in Kanawha County. The investigators therefore concluded that, on that basis, there was no evidence that the observed higher CNS defect rates could be related to parental exposure to vinyl chloride at the place of occupation.

If there is an association between community exposure to atmospheric vinyl chloride and observed rates of CNS birth defects, a decrease in atmospheric vinyl chloride levels might reasonably be followed, after an approximate 9 month time lag, by a corresponding decline in rates for CNS malformations. However, Edmonds *et al.* reported that the greatest decrease in rates occurred in 1973, and preceded the decline in levels of vinyl chloride emissions. Data on vinyl chloride emissions from the Kanawha County PVC plant showed that the annual mean vinyl chloride emissions (lb/h) decreased from 265 in 1973 to 180 in 1974 and 76 in 1975. Edmonds *et al.* further indicated that there are seven major chemical plants and several smaller ones in the Kanawha River Valley. Thus, to conclude that vinyl chloride alone may be associated with high rates of CNS malformations disregards over 100 other compounds emitted from Kanawha Valley area plants.

Studies pursued in communities containing chemical industrial facilities, producing or using multiple known or suspected toxins, in general may be confounded by difficulties in determining whether one or more of the substances, either independently or in an additive or synergistic manner, may be associated with observed levels of abnormalities.

Some investigators have reported a significant

excess of fetal loss among wives of workers following exposure to vinyl chloride. In 1976, Infante *et al.*[28] reported data from a study of pregnancy outcome among the wives of workers exposed to vinyl chloride monomer. Data for the wives of vinyl chloride polymerization workers were contrasted with data for the wives of PVC fabrication workers and rubber workers. A total of 95 vinyl chloride polymerization workers and 158 rubber and PVC fabrication workers were interviewed.

Because fetal loss is known to increase with increasing parental age, the fetal death rates for the vinyl chloride polymerization workers were age adjusted to the control group. Infante *et al.* found that mong pregnancies occurring prior to exposure, fetal death rates were 6.9% for controls versus 6.1% (age adjusted) for the vinyl chloride polymerization workers. These rates were not significantly different by Mantel-Haenszel chi square testing. However, among pregnancies occurring subsequent to the husband's exposure, the difference in the frequency of fetal deaths between cases and controls was significant, at $P < 0.05$. The investigators stated that the significant difference subsequent to exposure reflected the relatively greater fetal mortality rate associated with younger aged husbands in the group of polymerization workers. For pregnancies occurring after exposure, the fetal mortality rates associated with husbands 30 and older were 13.0% for the polymerization workers and 12% for the control group. However, for husbands less than 30, fetal mortality rates were 20.0% for the polymerization workers but only 5.3% for the control group.

Since one spontaneous abortion may be associated with a 66% increase in the risk of a subsequent abortion, it is possible that part of an observed increased loss occurring in the wives of men exposed to vinyl chloride may be associated with a high percentage of spouses with a history of previous abortions.[7] To determine whether women who had chronically experienced abortions might have weighted the results in the current study, in favour of a higher fetal death rate in the group of polymerization workers subsequent to the husband's exposure, the investigators stated that pregnancies of women who had more than two abortions were eliminated from analysis. Infante *et al.* reported that with this adjustment the trend was maintained. Prior to exposure, the fetal death rates for controls and polymerization workers were 6.9 and 3.1% (age adjusted), respectively. After exposure, the rates were 6.8 and 10.8% respectively.

Infante *et al.* concluded that fetal loss was significantly more among wives of workers following exposure to vinyl chloride. Several possible mechanisms for the observed fetal loss were suggested. It was hypothesized that either fetal or maternal toxicity or germ cell mutagenesis in the mother associated with indirect vinyl chloride exposure from the father might be considered. However, in their view, these mechanisms seemed unlikely because of the highly volatile nature of vinyl chloride. Based on the study findings, taken in conjunction with the prior demonstration of a mutagenic response in microbial systems and the observations of significant excesses of chromosomal aberrations in workers exposed to vinyl chloride, it was the authors' opinion that the leading possibility is germ cell damage in the father through direct vinyl chloride exposure.

Several variables affect the analysis of the data of Infante *et al.* The investigators do not provide precise data on the level of exposure to vinyl chloride. The rubber workers were selected from work areas described as being 'relatively free' from known (unspecified) toxic materials. Data on duration of exposure were not provided. Significant risk factors for spontaneous abortion may include a maternal age of 30 years of more, excessive cigarette smoking and alcohol use during pregnancy. Infante *et al.* did not obtain data concerning maternal age, except indirectly through paternal age. Data concerning the smoking habits, alcohol use, drug use, radiation exposure, or infections in cases and controls are not presented. Although some investigators suspect that women may be more accurate reporters of reproductive history than their mates, Infante *et al.* did not conduct any interviews with workers' wives.[7]

The study by Infante *et al.* has been critically examined in the literature. In a letter published in the *Lancet*, Paddle[29] stated that the assertion by Infante *et al.* of a significant excess of fetal loss among wives of workers following exposure should be supported by a specification of the methods of data collection and a tabulation of the raw data before analysis. It was Paddle's view that the use of a questionnaire, and the ensuing low response rates, where the subject is spontaneous abortion, must detract from the study's accuracy. It is further observed that the Infante *et al.* data are presented after an age adjustment procedure which behaves 'very misleadingly'. For instance, a crude percentage of 10.1 for a sample whose average age is 26.4 was adjusted down to 6.1, while a figure of 16.5 for a sample of average age 30.2 was reduced only marginally, to 15.8.

In a responding letter, subsequently published in the *Lancet* Infante *et al.* stated that the questions about pregnancy outcome were contained in a questionnaire which was part of a cross-sectional health survey.[30] The results of the survey reportedly showed no consistent bias towards a higher prevalence for the indices measured in the study or control group. With regard to age adjustment, they explained that the study group rate became 6.1% after direct age adjustment, as a result of a younger paternal age for pregnancies in the control group. Additionally, with

direct age adjustment, the 16.5% figure was reduced to 15.8%. They stated that age adjustment for the subsequent to exposure comparisons resulted in little change in the rates because the age distributions for pregnancies in both the study and control groups after exposure were similar.

It is difficult to determine precisely the causes of observed adverse reproductive outcomes. Spontaneous abortions may result from the embryotoxic effects of various environmental agents and from various other factors, including maternal endocrine disturbances, abnormalities of the placenta, faulty embryo development, intrauterine infections, and severe maternal trauma.[31] Congenital malformations may be associated with various toxic chemical agents. A number of toxins may be associated with impaired fertility in men. Future studies intended to document a possible relationship between impaired pregnancy outcome and paternal exposure to vinyl chloride must carefully validate information on pregnancies and take into account various confounding variables.

Spermatic alterations

A 1980 study by Sanotsky *et al.*[32] raises the possibility that human male exposure to vinyl chloride may be associated with spermatic alterations. The paper was not published in English. However, an English language abstract states that reproductive functioning was studied in men occupationally exposed to either chloroprene, vinyl chloride or antimonite ore dust with reproductive functioning assessment being based on 'indirect' evidence and from analyses of ejaculates. The abstract states further that 'pathologic changes' were detected in the ejaculates.

This study is the only published literature known to the author pertaining to human male exposure to vinyl chloride and possible resultant adverse effects on sperm. A salient research need may thus exist for extended investigation of this aspect of paternal vinyl chloride exposure, including study of possible effects on sperm count, motility and morphology.

Conclusion

On the basis of selected, available data, the exposure of human males to vinyl chloride may be associated with various chromosomal aberrations in lymphocytes as well as increased levels of sister chromatid exchanges. Paternal exposure to vinyl chloride may further be associated with adverse reproductive outcomes. The possibility of adverse effects on sperm associated with human male exposure to vinyl chloride must also be considered. Data pertaining to the possible biological effects associated with paternal vinyl chloride exposure are relatively sparse, conflicting and inconclusive. The mechanisms for the possible reproductive related risks posed by paternal exposure to vinyl chloride are not certain. Extensive, carefully designed studies should be undertaken to further elucidate the biological effects, and associated mechanisms, possibly associated with paternal exposure to vinyl chloride.

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