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Mortality and Cancer Rates among Workers in the Swedish PVC Processing Industry

by Gustavo Molina*, Bo Holmberg*, Stig Elofsson†, Lars Holmlund,‡ Rein Moosing,‡ and Peter Westerholm**

Personnel lists from four PVC-processing industries were collected on production of employees with at least three months of employment at the beginning of 1945 and the last day of employment December 31, 1974. Of 2073 persons, 103 could not be followed up, because they had moved abroad. The remaining persons comprise the cohort of 1970 individuals who were analyzed and compared with the national population with respect to mortality from various diseases and cancer morbidity.

The death risk from myocardial infarction is elevated in the cohort. This elevation is most clearly apparent in the subcohort which had at least two years of exposure time and where the analysis was directed at circumstances chronologically close to the time of exposure. The myocardial infarction risk related to vinyl chloride exposure is discussed in relation to earlier studies on the vascular effects of vinyl chloride. An indication of an elevated risk of morbidity and mortality from tumors in the digestive organs is also present. However, this is not statistically confirmed. A few future follow-ups of the present study are necessary in order to clarify any possible elevated risk of tumors in the PVC-processing industry.

Vinyl chloride has been shown to cause scleroderma, Raynaud's phenomenon, acroosterolysis, liver damage and liver cancer (hemangiosarcoma) (*1*) in workers exposed to vinyl chloride monomer (VCM). This has been shown in studies (*2*, *3*) performed at companies which fabricate poly(vinyl chloride) (PVC). In animal experimental studies it has been reported that inhalation of VCM causes malignant tumors in different organs in rodents (*4-6*).

In Sweden, in 1974, two cases of liver hemangiosarcoma were diagnosed in employees at a company engaged in the processing of VCM and PVC (*7*). Later another two cases occurred at the same factory.

Studies on other forms of cancer (*8*, *9*) suggest that VCM-exposed workers in the PVC fabricating industries may possibly run an elevated risk of contracting forms of cancer other than hemangiosarcoma in the liver. Earlier, an excess mortality from cardiovascular diseases was also observed (*10*) in employees in the PVC manufacturing industry.

The present retrospective cohort study was performed for the purpose of determining the pattern of morbidity and mortality in the PVC processing industry. The PVC processing industry, generally speaking, has had a lower level of exposure to VCM than the fabrication industry. In the Swedish PVC processing industry, at present, about 5000 persons are employed in production.

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Material and Methods

Information for the study was collected from four PVC processing companies. The four companies all used PVC which, after additions of various chemicals, is heat-treated for fabrication into floor covering, lace, pipes, and food packaging.

Data Collection

The following data were collected from the personnel lists at the companies: personal number, name, beginning and end of exposure (year and month) and class of exposure.

In order for a person to be included in the original cohort, at least three months of employment was required in the period beginning in 1945 and the ending December 13, 1974. The exposure was classified as follows: class 3 (high), work in the mixing department; class 2 (medium), heat treatment machines; and class 1 (low), other production departments.

The collected data were transferred to punched cards and magnetic tape for statistical processing. The magnetic tape was coordinated with the national total population and the so-called death tapes for the 1961-1976 period and checked against the cancer registry by the Central Bureau of Statistics (SCB). The personnel numbers which could not be recovered at this time were checked by the national taxation office. The original cohort included a total of 2,073 persons. Of these, 103 persons (5%) dropped out, 70 or whom had moved abroad, 5 were found in the missing persons register of the tax office, and 28 could not be traced.

Study Cohorts

For the statistical processing, the results of the cohort of 1970 persons were divided into a number of subcohorts (study cohorts): (1) all persons with at least three months of exposure (follow-up time from the beginning of exposure and through 1976); (2) all persons with at least six months of exposure excluding those who stopped before 1961 (follow-up time from beginning of exposure but no earlier than 1961 and through 1976); (3) all persons with at least six months of exposure and where the exposure began no earlier than 1961 (follow-up time from beginning of exposure and through 1976); (4) all persons with at least two years exposure (follow-up time from two years after beginning of exposure but no earlier than 1961 and through 1976 but no more than ten years after exposure stopped); (5) all persons with at least two years exposure (follow-up time from ten years after exposure began but no earlier than 1961 and through 1976).

The two latter named study cohorts were chosen in order to study whether any differences existed in the death cause pattern with respect to when the deaths occurred after the beginning of exposure. The first of the study cohorts was intended to shed light on possible causes of death which occur relatively early, e.g., accidents caused by the job. The second was intended to shed light on such death causes as occurred after a longer time had passed. Tumors caused by occupational exposure, for example, often have a long latency period, 5-10 yr or longer.

Results

The original cohort was relatively young at the beginning of exposure. The age distribution in the different exposure classes is given in Table 1. One finds various dissimilarities between the exposure classes. In class 1 (low), 41.7% were younger than 35 years at the beginning of exposure; in class 2 (average), 47.7%; and in class 3 (high), 50.6%. There

Table 1. Age distribution in original cohort at beginnin exposure

Age	% in each exposure class			
	1	2	3	1–3
< 19	1.6	2.0	8.9	2.1
20–24	7.5	14.6	14.3	9.2
25–29	13.5	15.7	21.4	14.4
30–34	19.1	15.4	17.0	18.3
35–39	17.0	13.2	10.7	15.9
40–44	13.7	12.6	9.8	13.3
45–49	11.1	12.6	8.9	11.3
50–54	8.0	7.8	5.4	7.8
55–59	5.1	3.9	1.8	4.7
60–64	2.6	2.0	1.8	2.4
> 65	0.7	0.3	0.0	0.6
No. of persons	100% (1501)	100% (357)	100% (112)	10?% (1970)

Table 2. Distribution of exposure time in the original cohort.

Months	% in each exposure class			
	1	2	3	1–3
< 5	13.1	0.3	0.0	10.1
6–23	38.4	8.4	8.9	31.3
24–59	25.0	17.4	10.7	22.8
60–119	15.3	45.7	15.2	20.8
> 120	8.2	28.3	65.2	15.1
	100% (M = 1510)	100% (M = 357)	100% (M = 112)	100% (M = 1970)

Table 3. Observed and anticipated number of deaths as of December 31, 1976.

Cohort	Number	No. of deaths		Ratio O/E	Approx. 95% confidence interval
		Observed	Expected		
Exp. class 1	1303	53	55.5	0.95	± 0.26
Exp. class 2	356	14	21.9	0.64	± 0.34
Exp. class 3	112	6	10.3	0.70	± 0.47
Exp. class 1–3	1171	73	87.8	0.84	± 0.19

Table 4. Observed and anticipated number of deaths from certain causes during the 1969–1976 period in those with at least six months of exposure including those who stopped before 1961.[a]

	Observed	Expected	Ratio O/E
Malignant tumors 140–209	17	14.0	1.21
Digestive organ tumors 150–159	8	4.9	1.63
Cardiovascular diseases VII	22	24.3	0.91
Myocardial infarction 410.90	15	20.0	1.49
Accidents, suicide, etc. XVII	13	9.2	1.42

[a]Risk calculated from the beginning of exposure but no earlier than 1961. Study cohort 2 (1771 persons).

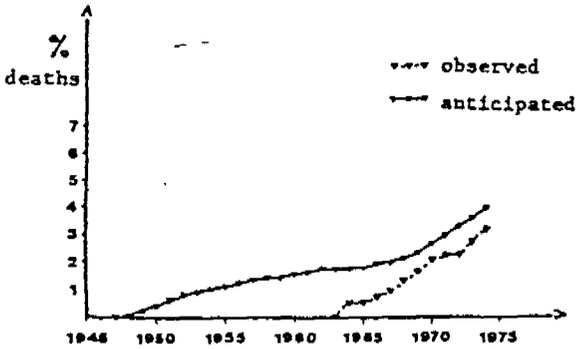

FIGURE 1. Cumulative deaths (in percent): (▽) observed; (▼) anticipated. Expected value calculated from beginning of exposure. Study cohort 1 (1970 persons). The percentage for a given year was calculated as 100 (number of persons dying through year in question divided by the number of persons beginning exposure up to and including the year in question).

were also dissimilarities in the length of exposure with respect to exposure class (Table 2). However, it should be noted that the table includes cases which were still under exposure at the final date for entrance into the cohort (December 31, 1974), for which reason, a certain bias toward short exposure times is found. Regardless of this, exposure class 3 has longer exposure times on the average.

The cohort as a whole reveals no noteworthy increase in the total risk compared with the national average, nor is there any indication of this in the subgroups making up the study cohort.

Study cohort 1, which includes everyone with at least six months of exposure and with calculation of the risk from the beginning of exposure, is somewhat remarkable in that the anticipated number of deaths is significantly higher than that observed up to 1964 (Fig. 1). This is commented on further in the discussion. Study cohort 2 (Tables 3 and 4; Fig. 2) includes persons with at least six months of exposure, excluding those who stopped before 1961. The risk calculation is made from the beginning of the exposure, but no earlier than 1961 and up to the end of the follow-up time (1976). The observed number of deaths is somewhat lower than expected, much lower in exposure class 2. Classes 2 and 3 are relatively small and are sensitive to random deviations in this type of analysis. In order for random deviations not to influence the results, the classes were combined. This is true of all study cohorts. This distribution with respect to the vari-

Table 5. Observed and anticipated number of deaths as of December 31, 1976 in those with at least six months of exposure beginning no earlier than 1961.[a]

Cohort	Number	No. of deaths		Ratio O/E	Approx. 95% confidence interval
		Observed	Expected		
Exp. class 1	1139	43	41.2	1.04	± 0.31
Exp. class 2	247	4	11.7	0.34	± 0.34
Exp. class 3	42	1	1.8		
Exp. class 1–3	1428	48	54.7	0.88	± 0.35

[a]Risk calculation from beginning of exposure. Study cohort 3 (1428 persons).

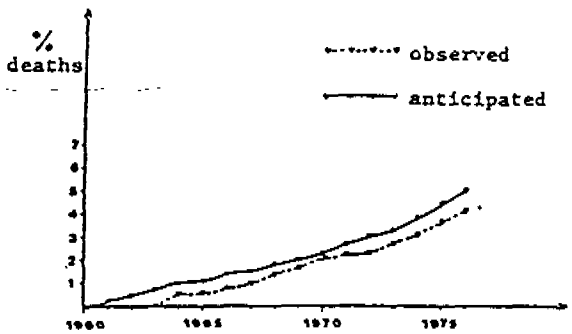

FIGURE 2. Cumulative deaths (in percent). Expected value calculated through 1961. Study cohort 2 (1771 persons). Percentage for a given year calculated as in Fig. 1.

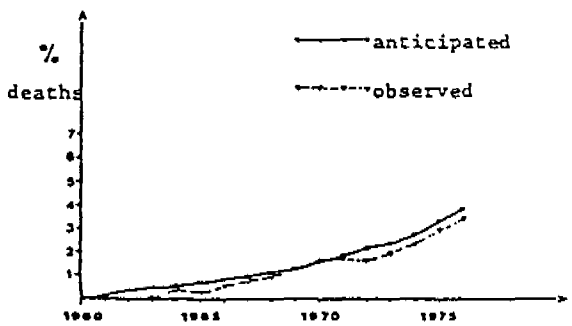

FIGURE 3. Cumulative deaths (in percent) of those who began exposure in 1960 or later. Study cohort 3 (1428 persons). Percentage for a given year calculated as in Fig. 1.

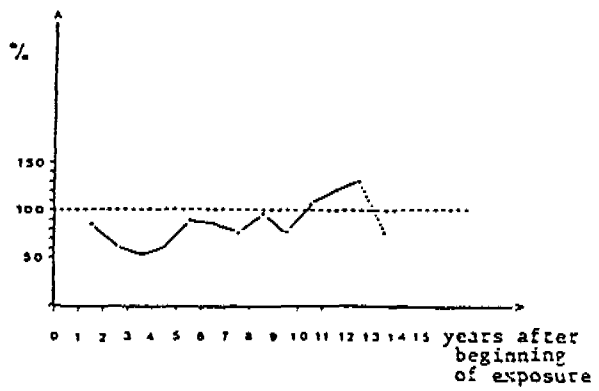

FIGURE 4. Observed death risk per year at different points of time after beginning of exposure expressed in percent of corresponding anticipated risk in those who began exposure in 1961 or later. Study cohort 3 (1428 persons).

Table 6. Observed and anticipated number of deaths from certain causes during the 1969–1976 among those with at least six months of exposure beginning in 1961 or later.[a]

	Observed	Expected	Ratio O/E
Malignant tumors 140–209	9	9.7	0.93
Digestive organ tumors 150–159	4	3.3	1
Cardiovascular diseases VII	16	16.2	0.
Myocardial infarction 410.90	14	11.2	1.25
Accidents, suicide, etc. XVII	11	7.3	1.51

[a]Risk calculated from the beginning of exposure. Study cohort 3 (1428 persons).

ous death causes is shown in Table 4. The observed and anticipated number of deaths during the 1961-1968 period is relatively small, only a few cases, and the death cause classification was modified as mentioned earlier in 1969, for which reason 1961-1968 period is not discussed separately. By and large, the picture is the same there as for the 1969-1976 period reported on. From Table 4, one sees that the observed number of deaths, especially those from tumors of the digestive tract, myocardial infarction and accidents, is somewhat higher than anticipated. However, the differences are not significant. Study cohort 3 (Tables 5 and 6; Figs. 3-5) which pertains to those who began working in 1961 or later but which otherwise satisfy the same criteria as study cohort 2, displays a similar picture.

An analysis of study cohort 3 according to formula B (Figs. 4 and 5) indicates that the annual risk during the first year of exposure is somewhat lower than the anticipated one, but that after about ten years, an increased risk occurs so that the observed risk becomes higher than the anticipated.

Table 7. Observed and anticipated number of deaths from certain causes during the 1969–1976 among those with at least two years of exposure.[a]

	Observed	Expected	Ratio O/E
Malignant tumors 140–209	5 (9)	7.4 (8.9)	0.86[b] (1.01)
Digestive organ tumors 150–159	2 (4)	2.6 (3.2)	0.78[b] (1.27)
Cardiovascular diseases VII	15 (16)	12.7 (15.8)	1.18[b] (1.01)
Myocardial infarction 410.90	11 (12)	5.4 (6.6)	2.03[b] (1.82)[b]
Accidents, suicide, etc. XVII	4 (5)	4.6 (5.1)	0.87[b] (0.97)

[a]Risk calculated from two years after beginning of exposure and no more than five years (10 years) after end of exposure. Stu cohort 4 (1155 persons).
[b]$p < 0.05$.

Table 8. Observed and anticipated number of deaths from certain causes during the 1969–1976 in those with at least two years of exposure.[a]

	Observed	Expected	Ratio O/E
Malignant tumors 140–209	9	6.0	1.51
Digestive organ tumors 150–159	4	2.2	1.85
Cardiovascular diseases VII	12	11.1	1.08
Myocardial infarction 410.90	8	4.5	1.77
Accidents, suicide, etc. XVII	2	2.5	0.79

[a]Risk calculated from 10 years after beginning of exposure. Study cohort 5 (680 persons).

Table 9. Observed and anticipated number of deaths from cancer during 1961–1976 in those with at least six months of exposure excluding those who stopped before 1961.[a]

	Observed	Expected	Ratio O/E
Malignant tumors (total)	51	44.6	1.14
Digestive organ tumors (150–159)	11	8.5	1.29

[a]Risk calculated from beginning of exposure but no earlier than 1961. Study cohort 2 (1771 persons).

In study cohort 4 (Table 7) which concerns time during ongoing exposure or a relatively short time after the end of exposure, i.e., "short-term perspective," one sees an increased death risk from myocardial infarction. Other causes are somewhat lower here than expected.

In study cohort 5 (Table 8), finally, one finds an indication of an increase in the death risk as regards tumors, but also for myocardial infarction. The differences between the observed and anticipated numbers are not, however, statistically confirmed at the 5% level.

The result with respect to mortality can be summarized as follows. In the study cohorts, overall, one finds no noticeable increase in mortality. On the other hand, there are indications of a shift in the death cause pattern compared with the national average. This shift is expressed primarily in the fact that the number of myocardial infarctions is noticeably higher during ongoing exposure or within a relatively short period of time after the end of exposure. There are also indications that the death from tumors can be elevated among persons with a long latency period (Tables 7 and 8).

In the question of cancer morbidity, there is no certain increase in study cohort 2 (Table 9 and Fig. 6). In the question of tumors of the digestive organs, in the same study cohort, 11 cases were observed as opposed to an anticipated 8.5. The

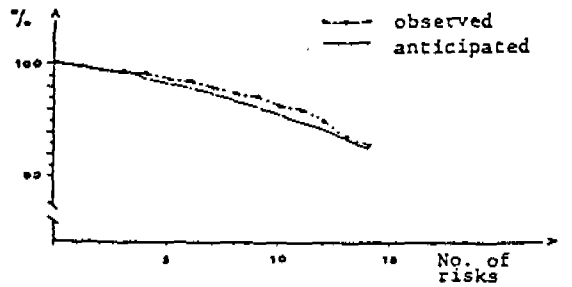

FIGURE 5. Cumulative survival probability (in percent) of those who began exposure in 1961 or later and have at least six months of exposure. Study cohort 3 (1428 persons).

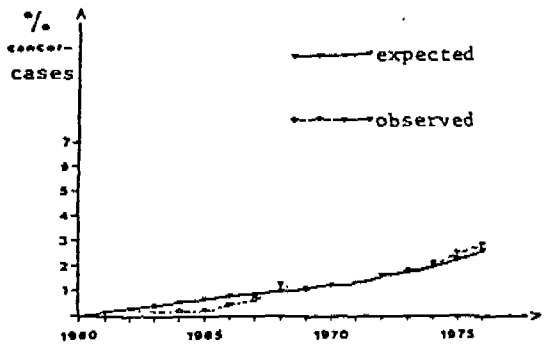

FIGURE 6. Cumulative rates of cases of all cancer (in percent). Study cohort 2 (1771 persons). The percentage for a given year was calculated as 100 (number of persons diagnosed through year in question divided by the number of persons beginning exposure through year in question).

difference is not statistically verified. One of these eleven tumors was liver cancer (ICD 155.0).

Discussion

A noteworthy finding which arises in the analysis of the total cohort mortality (Fig. 1) is that the number of deaths at the beginning of the observation period (1947-1964) is significantly lower than one would expect in relation to the national average. This difference is so great that one cannot directly consider it to be randomly conditioned, nor can it be entirely ascribed to the so-called healthy worker effect. Theoretically, of course, the possibility exists that the selected cohort, in the question of mortality and the factors which influence said mortality, deviates from the general population. A more credible possibility is, however, that the personnel register that was available at the company involved at the time of this study was incomplete in the matter of hirings during this early period. A personnel register which, in the mid-1960's, was purged

of persons who began employment before 1960, could lead to the difference mentioned above. The companies involved reported that such a purging did not occur, so far as they knew.

If such a purging (thinning out) nevertheless occurred, this would have resulted in the elimination of persons with a long observation time at the time of follow-up. In the present study, the risk calculations were limited to beginning no earlier than 1961. This means a limitation of the analysis to pertain to the group of employees who were living at the beginning of 1961 and where the risk of an elimination is positively eliminated. This limitation, however, signifies a weakening of the analysis, since parts of the cohort with long follow-up times are excluded. Basically, this weakening signifies a poorer possibility of discovering an elevated incidence of cancer if one exists.

The myocardial infarction mortality (ICD 410.90) is elevated in the cohort. This elevation occurs most clearly in the category of the total cohort which has at least two years of employment time and where the analysis was directed at the period of time following two years after the beginning of employment and extending to no more than five years after the beginning of employment. Therefore, this involves that fraction of the mortality from myocardial infarction which chronologically is relatively closely connected to the time of employment. It is impossible on the basis of such observations to draw conclusions that the elevation was caused by exposure to vinyl chloride. The observed increase in myocardial infarction mortality is, however, so striking that it, in combination with the known facts about the toxic properties of vinyl chloride, must be given consideration. There are no reasons to assume that varying diagnostics, standards or practices in filling out the death certificates alone could provide an explanation. A natural conclusion is, therefore, that if one disregards the possibility of a random local phenomenon, the increased frequency is to be ascribed either to selection of individuals susceptible to the risk or an outbreak of risk factors in the close environment of employees. A combination of these two circumstances is, of course, also possible theoretically.

In this connection, it should be noted that many risk factors for myocardial infarction are environmentally conditioned in the fact that they constitute part of the lifestyle of the modern social environment in an industrialized country. Cigarette smoking, physical inactivity, overweight and high blood lipids constitute environmental factors which are related to social behavior. It is a well known fact that the risk of coronary vascular disease in the heart varies, inter alia, with the total load of risk factors. Among other risk factors, one can also name hereditary characteristics and high blood pressure. In this connection, there is reason to recollect that the causal network of coronary disease is multifactorial and that the disease has an environmental relationship in the broad sense. There is also reason to recall the aspect that the total risk increases when several risk factors, known or unknown, are allowed to collaborate (*11*, *12*).

It has not been possible to establish the distribution of such already known risk factors for coronary disease in the cohorts studied with respect to the national population in general. Therefore, no continued analysis of the matter of the causal relationship between close environment and heart disease morbidity can be made within the limits of this study.

Exposure classes 2 and 3 constitute subcohorts that are too small, in the present study, to allow a meaningful discussion of the myocardial infarction risks relative to the various exposure levels in the processing industry. In this connection, one should also consider the circumstance that the exposure classes in this study are based on interviews with the employees directed at the work environment at the time in question some 10 to 15 years Therefore, this involves an environment which subsequently undergone changes. Objective classification criteria in the matter of exposure, e.g., in the form of environmental measurements, do not exist. The distribution into exposure classes is, for this reason, fraught with uncertainty.

In animal experiments, it has been found that the toxicity picture in rodents chronically exposed to VCM involves the blood vessels. Besides hemangiosarcoma in the liver and the other organs (*4*, *5*) the inhalation of VCM is also believed to cause development of telangiectasis (*4*) in the liver of mice which can lead to death from hemocoele. Changes in the sinus cells have been observed in liver biopsies in VCM-exposed workers (*13*). Capillary changes in the skin of the fingers have also been observed (*14-16*), both in VCM-exposed workers with other vascular-involved diseases, such as acroosteolysis, Raynaud's phenomenon, and sclerodermia, and in VCM-exposed workers without such diseases. An over-representation of deaths from cardiovascular diseases has also been observed in a study on the PVC-fabricating industries (*10*). Animal experimental and previous medical studies of VCM-exposed populations therefore support the assumption that the increased risk of myocardial infarction observed in the present study could possibly be ascribed to VCM exposure.

As regards the mortality and morbidity f[illegible] tumors, the results are uncertain. There are certain

indications of an elevation, but the differences are not statistically confirmed. One can think of two possibilities here: (1) in reality, there is no increase in the risk of tumors; (2) there is indeed an increased risk of tumors. The results neither confirm nor refute this. Tumors do not occur until after a long latency period. The majority of the persons included in the study did not begin their exposure until the 60's and 70's and therefore could not be followed for a sufficiently long time. An accurate follow-up of the present cohort during the coming five-year period should bring greater clarity into this.

In the present connection, it is of interest that in a recently published mortality study (*17*) on almost 4300 deaths in the American PVC-processing industry, an overrepresentation in cancer mortality appears to exist (all cancer), especially gastrointestinal cancer in both sexes.

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Epidemiological Study of Pneumoconiosis in the Italian Poly(vinyl chloride) Industry

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Among 1216 workers employed in a poly(vinyl chloride) production factory, 20 cases of pneumoconiosis were found. None of these workers had had previous exposure to organic or inorganic dusts; 731 had been exposed to PVC dust (employed in drying, sacking and blending of polymer) and 485 had been exposed to monomer alone. Chest x-ray films were read by two independent physicians utilizing the ILO/UC Pneumoconiosis Classification, 1971. X-ray abnormalities were characterized by limited profusion, irregular type and low gravity; in a small percentage of cases these were associated with slight restrictive respiratory function impairments. All 20 workers with PVC-induced pneumoconiosis had been exposed to high PVC dust pollution for at least five years. Mild nonspecific alterations (profusion of 0/1 class) were found both in the group exposed to PVC dust and in the group exposed to VCM alone. Such changes (observed in 388 cases, 31.9% of the whole population), are related mainly to age and smoking habits, and the role of exposure is minor.

We examined the working population of plants producing poly(vinyl chloride) (PVC) in Porto Marghera, Italy; 1216 workers had no previous dust exposure. Of these 731 were exposed to PVC dust polymer alone while 485 were exposed to vinyl chloride monomer (VCM). In the drying, sacking and blending departments, PVC dust concentrations were over 10 mg/m^3 of total dust in about 60% of the samples, whereas in the polymerization departments no concentration over 10 mg/m^3 was found. In the samples taken, particles with diameters of 1 μm to 6 μm constituted 4.5 to 30.9% of total dust weight.

All the workers had chest x-rays according to ILO standards and a spirographic examination. Chest x-ray films were read by two independent physicians utilizing the ILO/UC pneumoconiosis classification. For statistical analysis, a consensus reading was used.

Table 1 shows that there are no significant differences in age and smoking habits, but the exposure duration is higher in the workers not exposed to dust.

Table 1.

Group	No. of subjects	% of smokers	Age, yr (mean ± SD)	Exposure, yr (mean ± SD)
Exposed to PVC dust	731	73.9	37.7 ± 8.8	6.1 ± 4.0
Not exposed to PVC dust	485	68.7	35.7 ± 8.5	8.6 ± 4.5

Table 2.

PVC dust exposure, yr	Age distribution of cases[a]			
	≤ 30 yr	31–40 yr	41–50 yr	> 50 yr
< 5	–	–	–	–
5–10	–	2(2.4)	5(8.3)	1(5.9)
> 10	–	2(3.8)	8(9.0)	2(9.5)

[a]Values in parentheses are percentages of subjects with PVC pneumoconiosis in each class of age and exposure.

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In 20 subjects we found chest x-ray changes of at least class 1 according to the ILO/UC classification. The mean age of this group was 44.9 ± 5.2 years and the mean length of exposure was 11.6 ± 5.4 years. Sixteen subjects (80%) were either smokers or ex-smokers.

Table 2 summarizes the distribution of cases in relation to age and length of exposure. In all age groups there is an increase of disease prevalence associated with the increasing length of exposure.

In the case of the x-ray changes, 16 subjects had class 1/0 profusion, 2 cases class 1/1, 1 case class 2/1, and 1 case class 2/2. Irregular opacities were prevalent: 10 were type s, three were type t, six were type p and one was type r. They were diffused, mainly over median lobe areas.

A chest x-ray of a worker exposed to PVC dust (Fig. 1) for 15 years shows a gross reticular pattern: profusion is class 2/2, type t. In the right hemithorax of the same subject (Fig. 2) there is a mottled reticular pattern. Figure 3 is an enhanced view of the right hemithorax showing the mottled reticular pattern more clearly.

Another case of PVC induced pneumoconiosis can be seen in Figure 4. The worker was exposed to PVC dust for 20 years. A fine, dense reticular micronodulation is evidenced. Profusion is class 2/1, type p-s. Figure 5 shows the right hemithorax of the same subject; pin-point opacities can be seen.

In spite of age and the considerable exposure, the majority of cases were in a low profusion category, indicating the slow evolution of the disease. All had worked in high air-borne dust level environments (mostly drying and sacking) for at least five years. None of the 20 subjects was in a group unexposed to dust and none had experienced previous occupational exposure to organic or inorganic dusts. We considered the alterations to be PVC pneumoconiosis.

In 388 subjects (31.9%) we found slight chest x-ray alterations consisting of linear or irregular vanishing opacities or both, classified as class 0/1; the remaining 808 subjects were class 0/0.

Table 3 reports the total population distribution excluding 20 subjects with PVC pneumoconiosis. Results are presented in a two-way table: each entry reports the number of observations. Samples are classified according to age and PVC dust exposure: PVC + represents presence, and PVC – absence of PVC; x-ray + indicates the group with

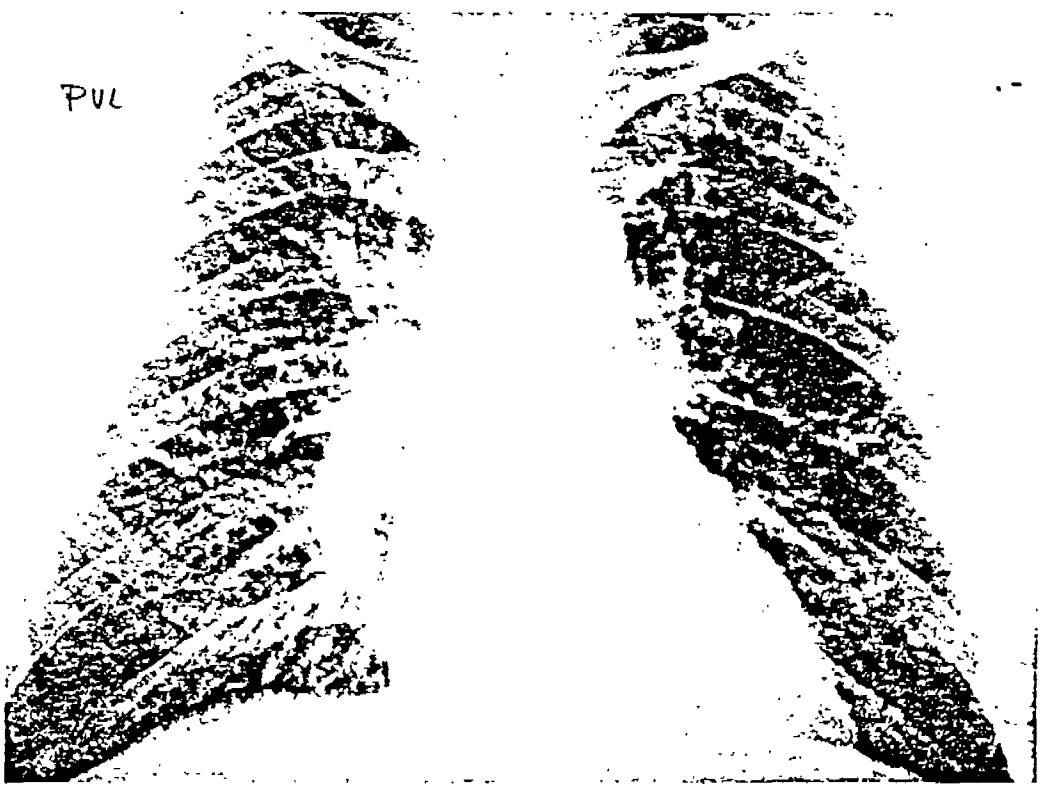

FIGURE 1. Chest x-ray of a worker exposed to PVC dust for 15 years. Profusion class 2/2; type t.

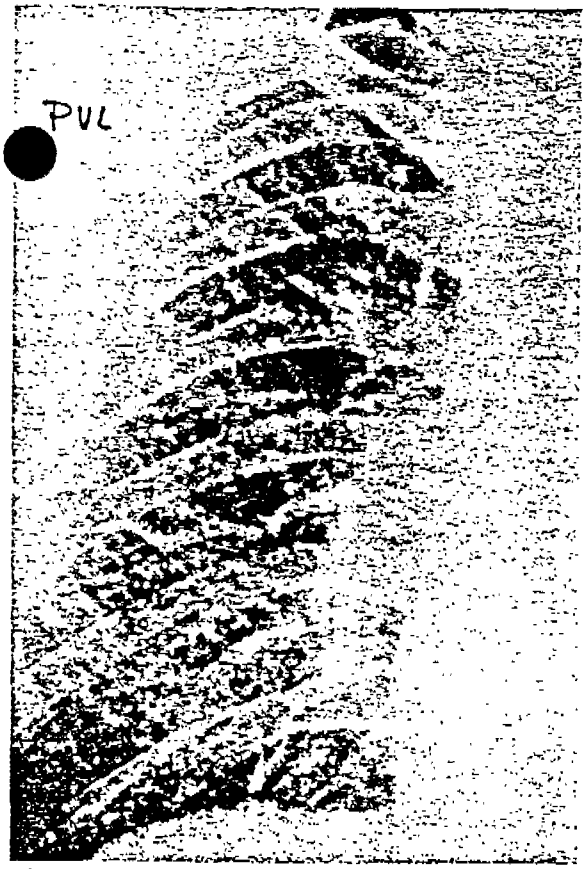

FIGURE 2. Right hemithorax of the same subject as in Fig. 1.

FIGURE 3. Enhanced view of the right hemithorax of Fig. 2, showing the mottled reticular pattern.

class 0/1 profusion. To assess the influence of both risk indicators we performed a two-way analysis of variance for proportions.

Table 4 shows that both age and exposure were significant factors influencing x-ray abnormalities. The square of a multiple partial association coefficient for qualitative data was calculated to measure the degree of association between the dependent variable (x-ray changes) and each of the two predictor variables (age and exposure). Age and exposure are alternately held constant. Age is a most important factor. When exposure is held constant, 33.2% of the x-ray changes are shown to depend upon age, when age is held constant, 6.5% are shown to depend upon exposure.

Table 5 summarizes the distribution of the cases according to smoking habits in workers exposed and not exposed to PVC dust.

Table 6 shows that chest abnormalities are significantly influenced by both risk indicators.

Table 3.

	Age distribution of subjects			
	≤ 30 yr	31–40 yr	41–50 yr	> 50 yr
PVC +				
X-ray +	20	78	123	35
X-ray –	133	197	108	17
PVC –				
X-ray +	17	45	55	15
X-ray –	119	158	67	9

Table 4.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Age	3	30.9713	10.3238	53.81[a]
PVC dust exposure	1	0.7843	0.7843	4.09[b]
Interaction	3	0.9882	0.3294	1.72
Error	1188	227.9403	0.1919	

[a] $p < 0.01$.
[b] $p < 0.05$.

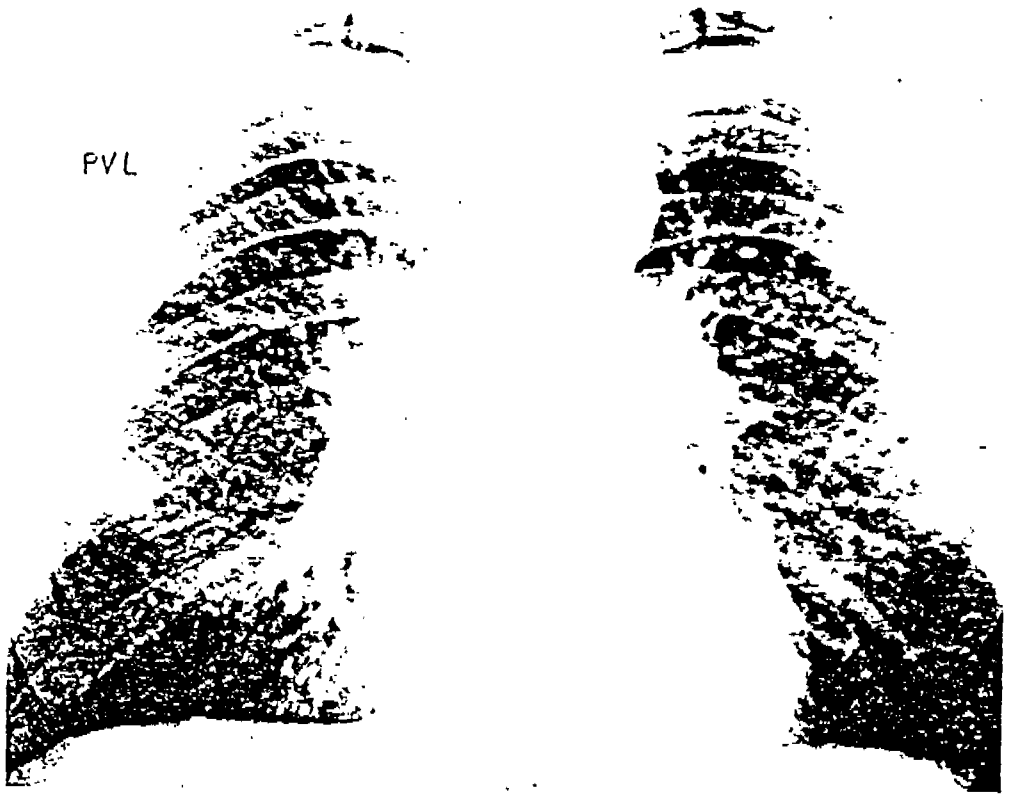

FIGURE 4. Chest x-ray of a worker exposed to PVC dust for 20 years. Profusion class 2/1; type p-s.

Table 5.

	Nonsmokers	Smokers
PVC +		
X-ray +	40	216
X-ray −	147	308
PVC −		
X-ray +	25	107
X-ray −	127	226

Table 6.

Source of Variation	Degrees of freedom	Sum of squares	Mean squares	F
Age	1	7.8850	7.8850	37.31[a]
PVC dust exposure	1	1.7847	1.7847	8.44[a]
Interaction	1	0.1023	0.1023	1.0
Error	1192	251.9125	0.2113	

[a] $p < 0.01$.

FIGURE 5. Right hemithorax of the same subject as in Fig. 4.

When exposure is held constant, habitual smoking is responsible for 17.1% of the changes. When smoking habits are held constant, exposure to PVC dust is shown to be responsible for 8.9% of the x-ray abnormalities.

This epidemiological study confirms experimental and pathological data already reported regarding the effects of PVC dust. Lung changes are directly related to PVC dust exposure, whereas VCM exposure alone fails to cause these changes. Therefore, we believe that pulmonary changes are not pathogenically similar to other vinyl chloride induced abnormalities, that is fibrosis of the liver, scleroderma-like skin changes, and peripheral vascular damage.

In our study there was only a 1.6% prevalence of pneumoconiosis in the total population, but in the workers exposed to effective risk of pneumoconiosis (731 subjects) the prevalence rose to 2.7%.

Apart from 20 cases of pneumoconiosis, mild nonspecific alterations (profusion of 0/1 class) were found in the group exposed to PVC dust and in the group exposed to VCM alone. Such changes are related mainly to age and smoking habits, and the role of exposure is minor.

Excess Lung Cancer Risk in a Synthetic Chemicals Plant

by Richard J. Waxweiler,* Allan H. Smith,† Henry Falk‡ and Herman A. Tyroler**

A standardized mortality ratio of 1.49 for respiratory system cancer (42 observed deaths versus 28.2 expected, $p < 0.01$) was observed among a cohort of 4806 males employed at a synthetic chemicals plant since its startup in 1942. Upon review of pathologic material, the excess was found to be limited to adenocarcinoma and large cell undifferentiated lung cancer. Many of the workers had been exposed to vinyl chloride, as well as to chlorinated solvents, poly(vinyl chloride) (PVC) dust, acrylates and acrylonitrile. To evaluate the association between lung cancer and occupational chemical exposures, detailed work histories for each cohort member were combined with exposure ratings for each of 19 chemicals for each job for each calendar year since 1942. A serially additive expected dose model was then constructed which compared the doses of the chemicals observed for the lung cancer cases to the doses expected based on subcohorts without lung cancer individually matched to the cases. PVC dust appeared to be the most likely etiologic agent ($p = 0.037$). Time trends of PVC dust exposure indicated a potential latent period of 5-16 years before death.

Introduction

Our previously published (*1*) retrospective cohort mortality study of workers exposed to vinyl chloride monomer (VCM) at a synthetic chemicals plant demonstrated an excess risk of death from respiratory system cancer in addition to the already recognized association between VCM exposure and angiosarcoma of the liver (*2-10*). Preliminary review of the lung cancer cases indicated they were all adenocarcinomas or large cell undifferentiated tumors—an unusual histologic distribution.

Although lung tumors have been induced experimentally by exposure to VCM (*2*), the epidemiologic data are suggestive but equivocal. Two cohort studies found no excess lung cancer risk (*4-6*). In six other studies, elevated lung cancer risks were found overall or among subcohorts; however, few of these excesses were statistically significant due to moderate excess risks and/or small numbers of cases (*7-9, 11-13*).

Because of the uncertainty in the literature regarding the association between VCM exposure and lung cancer and because of the diversity of occupational chemical exposures experienced by the lung cancer cases in our previous study (*1*), we decided to evaluate our cohort further to determine whether or not lung cancer was associated with VCM or with other chemical exposures. Thus, the following research had three objectives: (1) by using a retrospective cohort design, to determine if our previously published excess lung cancer risk among employees exposed to VCM at this synthetic plastics plant also existed for the total plant population (2) by using a case-comparand study, to determine whether an excess lung cancer risk of a particular histologic type was in force at the plant and (3) by using a serially additive expected dose model, to test whether one or more particular

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chemicals used at the plant were associated with either the excess risk of all or of a specific histologic type of lung cancer.

Methods and Results

Retrospective Cohort Study

The population at risk for the retrospective cohort study consisted of the 4806 males ever employed at this plant from its opening in 1942 until December 31, 1973. In the absence of individual data on race, everyone was assumed to be white because the company indicated that less than 2% of the population had been nonwhite. Date of birth and detailed work histories at this plant of all jobs and dates worked by each individual were coded. By follow-up of all study members from the first date hired at the plant through December 31, 1973, it was determined that 4174 were alive and 559 had died. The 73 (1.5%) persons lost to follow-up were considered alive throughout the study. The 16 (3%) deceased persons for whom no death certificates could be located were assumed dead, cause of death unknown. A modified life table analysis (NIOSH) was used to obtain person years at risk of dying by five year age and calendar time periods. United States white male death rates specific for five year age and calendar intervals were used to calculate the expected deaths and standardized mortality ratios (SMR's). SMR's were tested for statistical significance using the Poisson distribution (one-sided).

The cohort was young; by December 31, 1973, only 30% of the cohort, if alive, would have been over 54 years of age. However, 63% of the cohort had been hired before 1954 and thus had the opportunity to achieve 20 years' latency.

Two separate analyses of the cohort were made. Initially, all members were considered at risk from their first date of employment at the plant. This analysis yielded 556 observed and 550 expected deaths (Table 1). Risk of death due to malignant neoplasms of the central nervous system (SMR = 209) and respiratory system (SMR = 149) were both significantly elevated. A second "over ten-year latency" analysis was carried out by beginning person-years at risk only after an individual had achieved the tenth anniversary of his first date of employment at the plant. Results similar to those in the first cohort analysis were found but with slightly higher SMR's. Respiratory system cancer had an SMR of 156 based on 39 observed cases. Because our previously published analysis (*1*) of just the presumably VCM-exposed workers at this plant also found an SMR of 156 after 10 years latency, these results indicate an excess lung cancer risk not solely due to VCM exposure.

Table 1. Observed and expected deaths among chemical plant worker cohort.

Cause (ICDA–7 Code)	Observed	Expected	SMR
All causes	556[a]	550.2	101
All malignant neoplasms (140–205)	109	92.5	118
Digestive system (150–159)	24	25.6	94
Respiratory system (160–164)	42	28.2	149[b]
Central nervous system (193)	9	4.3	209[c]
Lymphatic and hematologic (200-205)	9	11.4	79
Other cancers	25	23.0	109

[a]Five persons, including three of the original 559 deaths, were not included in the cohort analysis because of missing work histories.
[b]$p < 0.01$.
[c]$p < 0.05$.

Case-Comparand Study

The second objective of this study was to determine whether an excess risk existed for a particular histologic type of lung cancer. Because no historical data exist on histology-specific lung cancer incidence or mortality rates and because histologic classification is somewhat variable between pathologists and over time, a case-comparand design was chosen to accomplish the second objective.

Medical records and pathology reports were obtained on all deceased members of the cohort whose death certificates mentioned cancer or respiratory disease. For 45 cohort members, primary or unspecified lung cancer was reported on at least one of these three records. A few persons who died after December 31, 1973, the cohort ending date, were included. Of the 45 cases, 42 were born in Kentucky or an adjacent state. Histologic material was requested. The worker case group consisted of the 27 of the 45 deceased individuals for whom histologic specimens were available.

As a comparison group, the lung cancer cases (comparands) most closely preceding and succeeding the chemical plant worker case in the chronologically ordered hospital pathology logs were selected that matched in age at diagnosis, sex, race, and county of residence. For four cases, only one matched comparand could be found.

Histologic material was reviewed by a panel of pathologists unaware of the employment history (case versus comparand status) of the deceased. The histologic type distributions, according to the

Veterans' Administration classification scheme (*14*), were compared between the cases and comparands.

The results of the majority opinion of the pathology panel are listed in Table 2. The panel found a significantly ($p < 0.05$, chi-square) higher percentage of large cell undifferentiated (type 4) cancers among the worker cases than among the community comparands (30% vs. 10%).

By using the SMR for respiratory system cancer of 149 in the retrospective cohort study and the observed and expected histologic distribution of lung cancer deaths based on the case-comparand study, Table 3 demonstrates the calculation of histology specific lung cancer SMR's (column C). If the 27 cases for which histologic specimens were available are considered representative of all 45 lung cancer cases, it appears the excess lung cancer risk appears limited to types 3 and 4, adenocarcinoma and large cell undifferentiated, with the greater risk due to the latter. Approximately 13.5 of the 14.8 excess lung cancers among the plant workers would be due to adenocarcinoma and large cell undifferentiated carcinoma.

It appears that there is a histology-specific excess lung cancer risk among workers at this plant and that this differential distribution of cell types is not an artifact of the geographic region nor of the pathologist's techniques. The fact that this risk occurs for adenocarcinomas and for large cell undifferentiated lung cancers makes it very unlikely that it is due to cigarette smoking.

Table 2. Case and matched comparand histologic distributions.

Histologic type	Veterans Administration classification code	Frequency: Worker cases	Frequency: Community comparand cases
Epidermoid	1	6 (22%)	15 (30%)
Small cell undifferentiated	2	6 (22%)	15 (30%)
Adenocarcinoma	3	7 (26%)	14 (28%)
Large cell undifferentiated	4	8 (30%)	5 (10%)
Other	–	0	1 (2%)
Total		27 (100%)	50 (100%)

Serially Additive Expected Dose Model

The third objective was to test whether any presumed occupational chemical exposures were associated with the excess lung cancer risk at the plant. It was decided to analyze the previously mentioned cohort data in a serially additive expected dose (SAED) model (*10*), obtaining for each lung cancer case the observed and expected doses of each chemical, conditional on certain characteristics. The purpose of the SAED model is to compare the observed exposure of each case in a study with the exposures of fellow workers close to the case in year of birth, and in age at commencement of work at the company. If the total work force in the plant under study is referred to as the cohort, then each case can be thought of as belonging to a subcohort of workers with approximately the same year of birth and age at commencement of work in the plant. In each year that a case worked at the plant, his exposure can be compared with that of the other members of his subcohort who were working in that year.

Company personnel compiled estimated exposures on a scale of 0 to 5 (5 being the highest exposure) for each of 19 chemicals (Table 4). Each job was assigned an exposure rank for each calendar year of the study (Table 5). These exposure data were then linked with work histories identifying the jobs each worker had in the plant and the calendar time involved. The analytical method is based on estimating exposure dose by multiplying the exposure level by the number of days worked at that level. These "doses" are accumulated over a calendar year for a case to yield the observed dose and for

Table 3. Histologic specific lung cancer risk among plant personnel.

	Histologic distribution: Observed (A)	Histologic distribution: Expected (B)	Histologic specific SMR $C = 149\ (A/B)$	Excess cases among those pathologically reviewed (D)[a]	Total excess cases (E)[b]
Total	100%	98%[c]	149	8.9[c]	14.8[c]
Epidermoid	22.2%	30%	110	0.6	0.9
Small cell	22.2%	30%	110	0.6	0.9
Adenocarcinoma	25.9%	28%	138	1.9	3.2
Large cell	29.6%	10%	441	6.2	10.3

[a] $D = (27\ A) - (27\ B \times 100/149)$.
[b] $E = (45\ A) - (45\ B \times 100/149)$.
[c] Specific cell types do not add up to total because of "other" type lung cancer among comparands.

his subcohort to yield an expected dose for each year that a case works. The methodology has previously been described in detail elsewhere (*10*).

In addition to testing the total-dose hypothesis, the SAED analysis facilitates examination of the observed and expected doses for each year of exposure before death. Exposures to 19 chemicals were assessed; seven of the chemicals were, however, excluded from the formal analysis because so few persons were exposed to them at levels 3, 4 and 5 (Table 6).

The SAED model analysis resulted in the observed minus expected cumulative dose differences per case in Table 7. The differences for PVC dust are striking in comparison with the other exposures. For the large cell undifferentiated cancer and adenocarcinomas combined and for large cell undifferentiated cancer by itself, the differences in exposure to PVC dust are three to four times as large as those estimated for vinylidene chloride, the

Table 4. Exposure ratings used to classify jobs.

Rating	Exposure
0	No exposure
1	Minimal exposure to low levels (chemical in building—not handled, low vapor pressure and dust level, probably works on different floor)
2	Moderate exposure (works around the chemical, but exposure is minimal)
3	Works in areas where subject to occasional high excursions (normally exposure is minimal but occasional spills, leaks, or dust exposure may occur)
4	Works in areas where level is high (exposure levels in the area are frequently high; might consider that some risk is involved if chemical is very toxic)
5	Intimate contact, skin or high inhalation (such as poly cleaners in earlier years handling slurry)

Table 5. Chemical exposure ratings specific for job identification number and calendar year for a given chemical.

Chemical #1, job identification number	Exposure ratings					
	1942	1943	1944	1945	1946	1973
1	0	0	0	2	2	2
2	5	5	5	5	5	5
3	2	2	1	1	0	0
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
84	4	4	4	1	1	1

Table 6. Frequency of job categories having at least one year of exposure greater than specific exposures levels.[a]

	No. at each exposure level				
	1	2	3	4	5
Acrylic acid	13	4	3	0	0
Acrylamides	14	4	4	0	0
Acrylonitrile	35	25	13	6	1
Acetylene	20	17	10	3	1
Acrylates	39	30	21	8	2
Bisphenol A	7	2	0	0	0
Butadiene	23	15	9	5	1
Caprylyl chloride	25	9	6	6	3
Chlorinated solvents	30	18	7	4	4
Chloroethyl vinyl ether	22	6	4	1	0
Diethyl maleate	14	6	0	0	0
Mercuric chloride	15	7	5	3	3
Methanol	30	21	14	2	2
Phenol	2	1	0	0	0
Toluene	2	0	0	0	0
Vinyl chloride	55	40	29	21	4
Vinylidene chloride	20	14	8	5	2
Vinyl acetate	26	18	10	0	0
PVC dust	56	32	19	9	6

[a]E.g., for acrylic acid, employees could have worked in 13 different job categories that had exposure levels of one or higher in at least one calendar year between 1942 and 1973.

Table 7. Observed minus expected cumulative dose differences per lung cancer case.

Chemical	All lung cancers	Pathologically reviewed cases	Adenocarcinoma and large cell	Large cell
Acrylonitrile	−652	−402	−446	−128
Acetylene	−353	−289	291	−167
Acrylates	−322	−251	−83	−339
Butadiene	−330	−207	−406	−622
Caprylyl chloride	−547	−258	−270	−1139
Chlorinated solvents	−868	−672	−150	749
Mercuric chloride	−507	−425	−211	−62
Methanol	−430	−618	−456	−1078
Vinyl chloride monomer	−1428	−795	26	907
Vinylidene chloride	−341	210	804	1525
Vinyl acetate	−707	−480	−217	328
PVC dust	763	2448	3225	4526

next most evident chemical. The significance levels of the larger of these differences are found in Table 8 for the total cumulative doses and also for cumulative doses until 10 years before death. Statistical significance was observed only for exposure to PVC dust.

Latency analysis for PVC dust (Fig. 1) shows a peak for the difference between observed minus expected dose during the period 5 to 16 years before death. As the diagnosis of the disease of interest becomes more narrowly defined as a pathologically homogeneous entity, the dose difference per case increases, yet the implied latent period stays constant.

Discussion

The first objective of this study was to determine if the excess lung cancer risk among employees exposed to VCM also existed for the total plant population, including those persons not exposed to VCM. The retrospective cohort mortality study showed that respiratory system cancers occurred approximately 50% more frequently among the employees of the entire plant than would have been expected based on age, sex, race, and calendar year specific United States death rates. A similar excess had been previously shown to exist among the subcohort of employees exposed to VCM (*1*). Thus, the excess lung cancer risk at the plant appeared to be independent of VCM exposure unless one hypothesized that it was due to extremely low levels of VCM that could have permeated throughout the plant.

Potentially confounding variables are age, sex, calendar year, race/ethnicity, migration, urban residence, cigarette smoking and socioeconomic status (SES). In the retrospective cohort study, age and calendar year cannot confound because they are adjusted in the results. All persons in the study are male and 98% are presumed white. White male specific rates were used for comparison to control those potential confounders by subject category restriction. Ethnicity and migration (both inter- and intracountry) effects are considered minimal for all three study designs because 42 of the 45 cases were born in Kentucky or an adjacent state. Urban residence is mainly associated with an excess of epidermoid and small cell undifferentiated lung cancer (*15*) rather than the histologic types of lung cancer found in excess in this study. Excess lung cancer risk is associated with low socioeconomic status (*16*, *17*), measured either by education (relative risk of 1.2 for persons with fewer than eight years of school) or by occupation (relative risk of 1.3 for laborers). Since the cohort consists of a mixture of socioeconomic status levels, there is probably insignificant confounding due to SES at the plant considered as a whole. Furthermore, recent infor-

Table 8. Probability value of paired *t*-tests of cumulative differences between observed and expected doses for lung cancer cases.

	Total (all years)[a]	10 or more years before death
All cases (N = 45)		
PVC dust	0.185	0.253
All pathologically reviewed cases (N = 27)		
PVC dust	0.026[b]	0.047[b]
Adenocarcinoma and large cell (N = 15)		
PVC dust	0.037[b]	0.061
Vinylidene chloride	0.267	0.333
Large cell (N = 8)		
PVC dust	0.068	0.177
Chlorinated solvents	0.360	0.435
Vinylidene chloride	0.201	0.322

[a]Twelve chemicals were tested for each lung cancer group, thus, under an assumption of independence of tests, one would expect one test to be significant at the 0.08 level.
[b]$p < 0.05$.

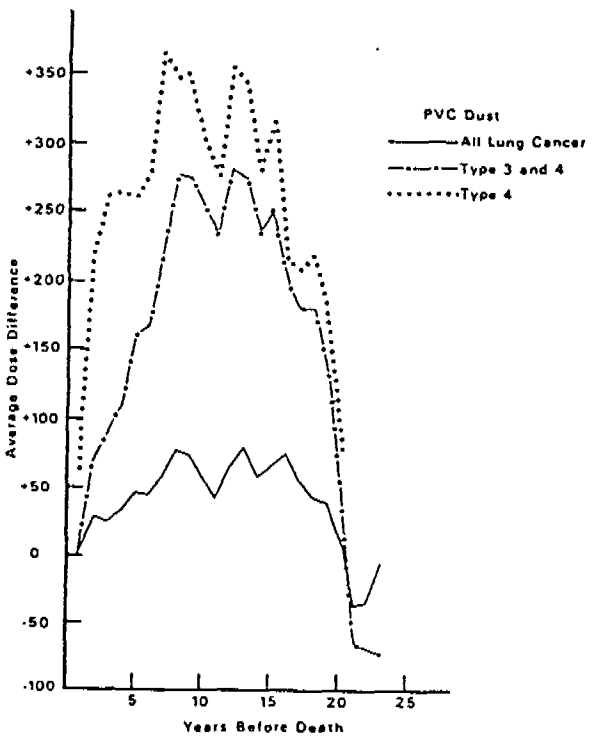

FIGURE 1. Observed minus expected dose differences per case among lung cancer cases for PVC dust.

mation indicates that these risk gradients may be partially due to smoking patterns (*18*, *19*), which would affect epidermoid and small cell undifferentiated lung cancers.

The second objective of this research was to determine whether an excess lung cancer risk of a particular histologic type existed. The case-comparand study showed that there was an excess of adenocarcinomas and large cell undifferentiated lung cancers among the cases occurring among plant employees compared to other lung cancer cases from the same community. Because the pathologists reviewed both sets of slides without knowing which were those of plant employees, it is unlikely diagnostic biases occurred.

Histologic specimens were more difficult to find among the cases which died earlier and which were older on the date of death. However, community comparands were matched with the cases on age and date of diagnosis and had to have specimens available themselves; hence a biased ascertainment by histology between cases and comparands would be unlikely. The choice of community comparands makes it unlikely that a community wide pollutant was responsible for the excess risk at the plant for types 3 and 4 lung cancer. Consequently, it can be inferred from Table 3 that the excess lung cancer risk in the cohort was limited to adenocarcinoma and large cell undifferentiated cancer. Adenocarcinomas, accounting for a minor proportion of this excess risk, have been shown to be weakly, if at all, related to cigarette smoking (*14*, *20-22*)

Large cell undifferentiated carcinoma of the lung is the only major histologic type that has appeared to be unrelated to cigarette smoking. Thus, cigarette smoking was probably not a major confounding variable. However, the role of smoking as a promoter or cocarcinogen cannot be ruled out. Nevertheless, the conclusion of this phase of the investigation was that an excess risk occurred among plant employees for types 3 and 4 lung cancer, especially type 4.

The third objective was to test whether one or more particular chemicals used at the plant were responsible for either the excess risk of all lung cancer or of the types 3 and 4 or just type 4 lung cancer. The SAED model was specifically developed for this objective.

The major hypothesis was tested for each chemical in the SAED model by using the one-sided *t*-test of the observed minus expected cumulative doses over all years before death and ten or more years before death (Table 8). PVC dust was the only chemical that was statistically significant. These results suggest that an excess of types 3 and 4 lung cancer exists at this plant and is related to PVC dust exposure.

The suggestion of PVC dust being a lung carcinogen is biologically plausible. Almost all PVC particles produced by the emulsion system, one of the systems at this plant, are in the respirable range (*23*). These particles could settle in the lungs and conceivably by themselves cause lung cancer. In fact, one case of supposedly PVC dust-induced pneumoconiosis has been found in a worker (*24*), and pulmonary granulomas (*25*, *26*) have been induced in animals exposed to PVC dust. In a large proportional mortality study of 4341 deaths that occurred among PVC fabricators, persons expected to be exposed to VCM and PVC dust demonstrated a slight (PMR = 117) excess lung cancer risk (*27*).

VCM gas is easily inhaled, and possibly would be in contact with tissue only a short time before being either exhaled or absorbed into the bloodstream. However, VCM becomes entrapped in the PVC dust and can be released slowly over time. Thus, PVC dust particles in the lung may slowly release VCM to small adjacent areas of the tissue, prolonging the contact time of that chemical to tissue. If this latter hypothesis is true, then it begs the question of why almost all of the liver angiosarcoma cases occurred among polymer reactor cleaners who received extremely high VCM doses while the lung cancer cases occurred frequently among the less heavily exposed workers. In fact, there was no relationship between VCM and lung cancer in this analysis; yet, one would expect the lung to be the major route of entry for VCM regardless of the cancer site.

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Review of Pulmonary Effects of Poly(vinyl Chloride) and Vinyl Chloride Exposure

by Ruth Lilis*

The contributions of several recent reports to the definition of pulmonary effects of PVC dust inhalation are reviewed. Granulomatous reaction, with inclusion of PVC particles in macrophages and histocytes, and associated interstitial pulmonary fibrosis have been found to lead to exertional dyspnoea, diffuse micronodular chest radiographic opacities and restrictive pulmonary dysfunction.

The effects of vinyl chloride (VC) monomer (gas) on proteins and the immunologic mechanisms triggered by the altered protein are possible mechanisms for the development in some cases of interstitial pulmonary fibrosis secondary to VC exposure.

Vinyl chloride, a confirmed carcinogen, has been associated with, among other malignant tumors, a significant increase in the incidence of lung cancer. The magnitude of this effect has not yet been completely evaluated.

Several recent reports (*1-3*) have contributed new observations on pulmonary disease in vinyl chloride (VC)- and poly(vinyl chloride) (PVC)-exposed patients.

Earlier reports, a few even preceding the identification in 1974 of vinyl chloride as a human carcinogen (with hemangiosarcoma of the liver the marker tumor, but most probably not the only tumor), had centered on the rather unexpected occurrence of pulmonary radiologic abnormalities (*4-8*) or pulmonary function impairment (*4, 9-12*) or had indicated dyspnea as a prominent symptom (*11, 13*) in VC and/or PVC exposed workers.

The radiologic pattern first described in 1975 (*5*) was essentially that of reticular-linear and/or nodular (small rounded) opacities, involving both lungs, predominantly in the lower zones.

Pulmonary function abnormalities reported have been both restrictive and obstructive dysfunction with diffusion defects and arterial desaturation in some cases.

Three recent reports, one a case report (*1*), the other two epidemiologic surveys (*2, 3*), seem to identify PVC dust as the etiologic agent in a peculiar type of pulmonary fibrosis associated with a granulomatous reaction.

Exertional dyspnea, diffuse micronodular chest radiographic abnormalities and restrictive pulmonary dysfunction, were the main characteristics in the case of PVC pulmonary fibrosis associated with granulomatous lesions (*1*).

Electron microscopic examination of lung tissue showed giant multinucleated cells containing a nonhomogeneous material in their cytoplasm, which was identified to be PVC (*1*). A similar pattern was reproduced by incubation of human macrophages obtained by bronchial lavage, with PVC powder: absorption of PVC particles in the cytoplasm was rapid, with thinly granular lysosomal material deposited against the PVC particles.

Similar histologic lesions had been previously described in a human case (*4*) and in an experimental study in guinea pigs and rats (*14*). In another experimental study, intratracheal administration of PVC dust in rats (*15*) has been shown to result in an increase in the activity of lysosomal enzymes, interstitial fibrosis and granulomatous lesions surrounded by fibroblasts, reticulin and collagen fibers.

An epidemiologic study of a large group of workers exposed to PVC and VC (*2*) detected 20 cases of "typical pneumoconiosis," i.e., chest x-ray changes consisting in irregular opacities or micronodular shadows of at least class 1 profusion,

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according to the ILO U/C Classification. All these cases were found among PVC exposed employees. The pattern of radiologic abnormalities described is very similar to that reported in the case in which the lung biopsy revealed fibrosis and granulomatous reaction, with inclusion of PVC particles.

The same study reported the presence of less marked radiologic abnormalities, of the linear-reticular type, in a much larger proportion (32%) of the population examined; these changes were present both in VC monomer exposed and in PVC exposed employees. While the prevalence was higher in smokers than nonsmokers, 65 of the 388 x-rays with linear-reticular opacities were found in persons who had never smoked.

In another large epidemiologic study (*3*), exposure to respirable PVC dust was found to be associated in a proportion of exposed workers with the presence of small rounded opacities on the chest radiograph and a decline in mean ventilatory capacity.

The question of pulmonary effects due to vinyl chloride monomer continues to be of great interest. The multi-organ effects of vinyl chloride include the peculiar syndrome of acroosteolysis, scleroderma-like skin changes, vascular changes affecting the arteries, arterioles and capillaries of hands and fingers, liver and spleen capsular fibrosis, liver fibrosis, abnormalities of the sinusoidal vessels in the liver, and portal hypertension. Ward (*16*) investigated the immunologic status of 58 workers from a VC polymerization plant. The findings included hyperimmunoglobulinemia, cryoglobulinemia, cryofibrinogenemia, *in vivo* complement activation via the classic pathway, with C_4 and C_3 conversion and an increase in the B cell lymphocyte population. Immunofluorescent examination of skin, muscle and lung biopsy specimens revealed the presence of circulating immune complexes, with deposition on the vascular endothelium and occlusion of small vessels. Immunoglobulin, complement and fibrinogen deposition in the subintimal regions of the vessel were found in areas with subintimal fibrosis and luminal occlusion.

Grainger et al. (*17*) have accumulated evidence suggesting the following mechanism: the vinyl chloride metabolite cyclic chlorethylene epoxide, an alkylating agent with high biological activity binds to IgG producing structural conformational changes that promote aggregation of IgG molecules. The modified IgG may also become antigenic. The IgG aggregates are cryoprecipitable and may initiate complement activation. Precipitation of IgG aggregates by cold leads to complement activation, platelet aggregation and conversion of fibrinogen to fibrin with polymerization. Occlusion of small vessels results, and ischemia stimulates new collagen biosynthesis.

Similar abnormalities of the immunologic status were found in another study of 22 workers exposed to vinyl chloride, with Raynaud's syndrome and, in some cases, acroosteolysis. Latent cryoglobulinemia was detected in 18 cases, with increases of immunoglobulins, IgA and IgG (*18*).

Circulating cryoimmunoglobulins are a prominent feature of idiopathic pulmonary fibrosis (*19*) and increased IgG levels have been shown to be characteristic for bronchoalveolar lavage fluid of such patients.

Interstitial pulmonary fibrosis is a possible effect of vinyl chloride exposure. The occurrence of more dramatic and specific abnormalities in other organ systems—liver, spleen and peripheral circulation—has probably prevented more focused attention on pulmonary effects of vinyl chloride in the past.

Long-term effects of vinyl chloride include well documented carcinogenicity. Lung cancer has been found to occur with an increased incidence in several mortality studies (*20-22*). Abnormalities in sputum cytology tests have been found to be more frequent in VC/PVC-exposed workers than in other chemical industry employees and in smokers (*23*). In experiments on mice, Suzuki (*24*) has described hyperplastic changes of the alveolar lining cells and pulmonary tumors in the majority of exposed animals. The ultrastructure was thought to indicate that the tumors originated in type II alveolar cells. Alveologenic tumors were also described in several other experimental studies (*25-27*). Interestingly, other known carcinogens, such as polycyclic aromatic hydrocarbons, nitrogen mustard and chromates, produce pulmonary tumors in experimental animals similarly, originating in the type II alveolar cell.

The effects of vinyl chloride-poly(vinyl chloride) exposure on the respiratory system of exposed workers seem to indicate two patterns of nonmalignant effects: a granulomatous reaction to PVC dust, with inclusion of PVC particles in macrophages and histocytes and associated interstitial fibrosis, and an interstitial pulmonary fibrosis due to vinyl chloride monomer effects on protein molecules and the immunologic mechanisms triggered by the altered protein.

The long-term carcinogenic effect, with a significant increase in the incidence of lung cancer, also is of concern, although the magnitude of this effect has not yet been completely evaluated.

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Vinyl Chloride: Inhalation Teratology Study in Mice, Rats and Rabbits

by J. A. John,* F. A. Smith* and B. A. Schwetz*

These studies evaluated the effects of inhaled vinyl chloride monomer (VCM) on mouse, rat and rabbit embryonal and fetal development. Groups of pregnant CF-1 mice, Sprague-Dawley rats and New Zealand white rabbits were exposed to 500 ppm VCM for 7 hr daily during the period of major organogenesis. Subsequently, other groups of mice were similarly exposed to 50 ppm VCM, and rats and rabbits were exposed to 2500 ppm. While maternal toxicity was observed, exposure to VCM did not cause significant embryonal or fetal toxicity and was not teratogenic in any of the three species at the concentrations tested. Simultaneous exposure of some of the pregnant animals to VCM by inhalation plus 15% ethanol in the drinking water resulted in toxic effects greater than those associated with exposure to VCM alone in the three species. The fetal effects observed were similar to those reported for these three species following administration of ethanol without VCM exposure.

Introduction

Inhalation exposure to vinyl chloride monomer (VCM) has been shown to be carcinogenic in laboratory animals (*1*, *2*) and in humans (*3*). The carcinogenic potential of inhaled VCM following *in utero* exposure has been reported (*2*), but observations to determine the teratogenic potential in laboratory animals were not made. Epidemiologic studies (*4-6*) of incidence rates for malformations of the central nervous system among families of employees or residents in the vicinity of vinyl chloride polymerization facilities have not supported any evidence that VCM is teratogenic in humans. A series of studies were conducted in our laboratory to assess the hazard associated with exposure and to investigate the mechanism by which VCM might exert its toxic effects. The purpose of the studies described in this report was to assess the embryotoxic and teratogenic potential of inhaled VCM in mice, rats and rabbits.

The exposure levels tested in the teratology studies are presented in Table 1. In an initial experiment, groups of mice, rats and rabbits were exposed to 500 ppm of VCM, 7 hr daily on days 6-15 (mice and rats) or 6-18 (rabbits) of gestation. Subsequently, additional groups of rats and rabbits were exposed to 2500 ppm. For each concentration tested, concurrent control groups of mice, rats and rabbits were sham-exposed to filtered room air. Since previous studies in this laboratory indicated that the primary metabolic pathway for VCM is blocked by ethanol (*7*), it was considered possible that simultaneous administration of ethanol in the drinking water of animals exposed to VCM might alter its metabolism in a manner which would enhance its toxic or teratogenic potential. Thus, in this experiment some of the VCM-exposed animals were given 15% (v/v) ethanol in their drinking water during the same period of gestation.

Table 1. Teratology studies with vinyl chloride: levels of exposure.

Species	VCM concn, ppm	Ethanol concn, %
Mice	500	0
	500	15
	50	0
	50	15
Rats, rabbits	2,500	0
	2,500	15
	500	0
Mice, rats, rabbits	0	0

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The teratogenic potential of imbibed ethanol in mice, rats, and rabbits was previously studied in our laboratory and reported by Schwetz et al. (*8*). No teratogenic effects were observed when 15% ethanol was given in the drinking water to mice and rats on days 6-15 of gestation or to rabbits on days 6-18 of gestation, although retarded fetal growth and development were observed in mice and rats.

Methods

Female CF-1 mice (Carworth, Portage, Michigan) weighing 25 to 30 g, Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, Michigan) weighing approximately 250 g and New Zealand white rabbits (Langshaws Rabbitry, Augusta, Michigan) weighing 3.5-4.5 kg were used in this study. The day on which a vaginal plug was observed or the day on which sperm were seen in a vaginal smear was considered day zero of pregnancy for mice and rats, respectively. The day of natural mating was considered day zero for rabbits. Between daily exposures, animals were housed in wire-bottom cages in a room controlled for temperature, humidity and light cycle. Commercial laboratory animal food (Ralston Purina Co., St. Louis, Missouri) and tap water or tap water containing ethanol were available during the periods between exposure to VCM. Food consumption was measured at 3-day intervals for mice and rats and at 2-day intervals for rabbits. All animals were deprived of food and water during the 7-hr exposure period each day.

Exposure of bred animals was conducted in stainless steel chambers of 3.7 m^3 volume under dynamic conditions. The atmosphere of VCM was generated by diluting gaseous VCM with filtered room air at a rate calculated to give the desired concentration. Vinyl chloride monomer (chloroethylene) obtained from Matheson Gas Products, Joliet, Illinois was used for the exposures. The actual concentration was measured with an infrared spectrophotometer (Perkin Elmer 12A or Miran I) with a multipath gas cell.

All animals were observed daily throughout pregnancy and maternal body weights were recorded at several intervals during gestation. Pregnant mice and rats were sacrificed by carbon dioxide inhalation on day 18 and 21 of gestation, respectively. Pregnant rabbits were sacrificed on day 29 of gestation. The uterine horns were exteriorized through a midline incision in the abdominal wall and the number and position of live, dead and resorbed fetuses were noted. After being weighed, measured (crown-rump length) and sexed (mice and rats), the fetuses were examined for external anomalies. One-third of each litter was immediately examined for evidence of soft tissue anomalies by dissection under a low power microscope (*9*). The heads of those fetuses (mice and rats only) were preserved in Bouin's solution and examined for soft tissue but not skeletal anomalies (*10*). Rabbit fetuses were sexed on the basis of examination of internal genitalia. All fetuses were then eviscerated, preserved in alcohol and subsequently cleared and stained with Alizarin Red-S for examination for skeletal anomalies (*11*).

The Fisher exact probability test (*12*) was used to evaluate the incidence of resorptions among litters. Maternal and fetal body weights and body measurements and maternal liver weights were analyzed statistically, by an analysis of variance and Dunnett's test (*13*). The incidence of fetal anomalies was analyzed by the Wilcoxon test as modified by Haseman and Hoel (*14*). The group of animals which was exposed only to vinyl chloride served as the control for those animals which were exposed to vinyl chloride in combination with 15% ethanol in the drinking water. The controls for animals exposed to vinyl chloride and maintained on tap water without ethanol were exposed to filtered room air in exposure chambers which were similar to th[illegible] used for exposure to vinyl chloride.

Results and Discussion

Maternal Toxicity

Exposure to 500 ppm VCM was maternally toxic to mice; deaths (5 of 29 bred females), decreases in the amount of weight gained during gestation, in food consumption and in absolute liver weight as compared to the air only controls were observed. Evidence of toxicity was not apparent among mice exposed to 50 ppm of VCM. The combination of VCM exposure with 15% ethanol in the drinking water significantly enhanced the toxicity as compared to VCM alone for both concentrations, though no deaths occurred in the 50 ppm VCM plus ethanol group of mice.

Rats exposed to 500 ppm VCM gained less weight than controls during gestation, but no other evidence of toxicity was observed at this level. One maternal death among 17 bred females, decreased food consumption and an increase in liver weight were observed at 2500 ppm VCM in rats. Ethanol given in combination with 2500 ppm VCM was more maternally toxic than exposure to VCM alone; the amount of body weight gained by pregnant ra[illegible] during gestation and food consumption were s[illegible] nificantly decreased in the group given the combi-

nation. The liver weight relative to body weight was increased in this group as compared to the rats exposed only to VCM, but no deaths were observed as a result of treatment with the combination. An ethanol group was not included among the rats exposed to 500 ppm of VCM.

Some deaths were observed among pregnant rabbits exposed to 2500 ppm VCM alone (one of seven bred females) or in combination with ethanol (3 of 19 bred females); however, other evidence of toxicity in rabbits consisted only of decreases in food consumption among those exposed to 500 ppm VCM alone and among rabbits given the combination of 2500 ppm plus 15% ethanol in the drinking water.

Among mice, rats and rabbits given 15% ethanol in the drinking water during gestation, Schwetz et al. (*8*) reported that maternal toxicity, as evidenced by decreased body weights, occurred in all three species.

Observations at the Time of Cesarean Section

Among litters of mice exposed to 500 ppm of VCM, the incidence of resorptions was increased; 13% of the implants were resorbed versus 7% in the concurrent air controls (Table 2). Historical control data from 801 litters of CF-1 mice in our laboratory show the mean percentage of implantations resorbed to be 11% with a range of 6-22%. Thus, both of these values are within the range observed for control groups of this strain. Litter size and fetal body weight were decreased at 500 ppm. These effects may have been secondary to the toxicity observed among pregnant dams at this exposure level. Toxic effects on the embryo or fetus were not observed among litters of mice exposed to 50 ppm VCM.

Ethanol in combination with VCM produced greater fetotoxicity than exposure to VCM alone at both levels. In the 500 ppm VCM plus ethanol group, a decrease in the percentage of pregnant mice was observed. This resulted in only seven litters, two of which contained only implantations which were resorbed. Fetal body measurements were decreased among the ethanol groups when compared to groups given either 50 or 500 ppm VCM alone.

No adverse effects on the percentage of pregnant dams or the incidence of implantations resorbed were observed among rats (Table 3). Exposure to

Table 2. Mice: observations at the time of cesarean section.

	No VCM, no ethanol	50 ppm VCM		No VCM, no ethanol	500 ppm VCM	
		no ethanol	15% ethanol		no ethanol	15% ethanol
Number of litters	21	20	16	26	19	7
Live fetuses/litter[a]	10 ± 4	11 ± 4	10 ± 4	12 ± 2	11 ± 2[b]	8 ± 6[c]
% Implantations resorbed	15(40/261)	8(18/238)	11(19/172)	7(26/351)	13(33/248)[b]	19(13/69)
Fetal body weight, g[a]	1.00 ± 0.11	1.02 ± 0.10	0.84 ± 0.14[c]	1.07 ± 0.06	0.99 ± 0.11[b]	0.78 ± 0.15[c]
Fetal crown-rump length, mm[a]	23.0 ± 1.9	24.2 ± 0.8[b]	22.4 ± 1.5[c]	23.7 ± 1.2	23.6 ± 1.0	21.2 ± 1.5[c]
% Pregnancy	57(21/37)	74(20/27)	57(16/28)	88(28/32)	72(21/29)	31(9/29)[c]

[a]Mean ± S.D.
[b]Significantly different from air-exposed control, $p < 0.05$.
[c]Significantly different from VCM-exposed group, $p < 0.05$.

Table 3. Rats: observations at the time of cesarean section.

	No VCM, no ethanol	500 ppm VCM, no ethanol	No VCM, no ethanol	2500 ppm VCM	
				No ethanol	15% ethanol
Number of litters	28	31	19	16	16
Live fetuses/litter[a]	12 ± 2	12 ± 2	12 ± 2	13 ± 2	12 ± 2
% Implantations resorbed	1(4/342)	3(11/398)	4(9/238)	3(6/220)	4(7/195)
Fetal body weight, g[a]	5.67 ± 0.29	5.44 ± 0.38[b]	5.59 ± 0.27	5.62 ± 0.29	5.34 ± 0.32[c]
Fetal crown-rump length, mm[a]	42.6 ± 1.2	43.6 ± 0.8[b]	43.6 ± 1.5	43.3 ± 1.1	42.4 ± 0.9[c]
% Pregnancy	96(28/29)	94(31/33)	95(19/20)	100(17/17)	94(16/17)

[a]Mean ± S.D.
[b]Significantly different from air-exposed control, $p < 0.05$.
[c]Significantly different from VCM-exposed group, $p < 0.05$.

2500 ppm VCM plus ethanol resulted in fetal body weights and crown-rump lengths which were lower than fetal body measurements among litters from rats exposed only to 2500 ppm VCM. In the group exposed to 500 ppm of VCM alone, fetal body weights were decreased as compared to concurrent air controls, though fetal crown-rump lengths were significantly increased.

In rabbits, the incidence of resorptions was significantly increased among litters given 2500 ppm VCM plus ethanol, where 53% of the implantations showed evidence of resorption versus 24% among

Table 4. Rabbits: observations at the time of cesarean section.

	No VCM, no ethanol	500 ppm VCM, no ethanol	No VCM, no ethanol	2500 ppm VCM	
				No ethanol	15% ethanol
Number of litters	18	19	11	5	16
Live fetuses/litter[a]	8 ± 1	7 ± 2[b]	6 ± 3	6 ± 4	4 ± 4
% Implantations resorbed	6(10/162)	9(14/150)	22(19/88)	24(10/42)	53(79/149)[c]
Fetal body weight, g[a]	35.23 ± 4.82	34.13 ± 4.17	36.46 ± 4.82	33.77 ± 4.48	32.48 ± 5.88
Fetal crown-rump length, mm[a]	91.0 ± 4.2	92.6 ± 5.0	92.6 ± 4.7	87.1 ± 5.2	87.7 ± 6.3
% Pregnancy	100(18/18)	95(19/20)	100(11/11)	86(6/7)	95(18/19)

[a]Mean ± S.D.
[b]Significantly different from air-exposed control, $p < 0.05$.
[c]Significantly different from VCM-exposed group, $p < 0.05$.

Table 5A. Mice: incidence of fetal anomalies.

	No. fetuses (no. litters) examined					
	No VCM, no ethanol	50 ppm VCM		No VCM, no ethanol	500 ppm VCM	
		No ethanol	15% ethanol		No ethanol	15% ethanol
External examination	221(20)	220(20)	153(14)	325(26)	215(19)	56(5)
Soft tissue examination	74(20)	75(20)	50(14)	107(26)	73(19)	29(5)
Skeletal examination	221(20)	220(20)	153(14)	325(26)	215(19)	56(5)
Bones of the skull	147(20)	145(20)	103(14)	217(26)	142(19)	37(5)

Table 5B. Mice: incidence of fetal anomalies.

	% fetuses (% litters) affected					
	No VCM, no ethanol	50 ppm VCM		No VCM, no ethanol	500 ppm VCM	
		No ethanol	15% ethanol		No ethanol	15% ethanol
External examination						
Cleft palate	1(10)	1(10)	2(21)	0	1(5)	6(40)
Anopthalmia	0	0	0	0	0	2(20)
Exencephaly	0	0	0	1(8)	1(10)	2(20)
Soft tissue examination						
Thymus	0	0	4(7)	0	0	0
Skeletal examination						
Skull bones, unfused	0	0.7(5)	24(50)[a]	1(12)	5(21)	11(20)
Skull, delayed ossification	9(35)	8(37)	40(100)[a]	13(54)	30(58)[b]	70(100)[a]
Sternebrae, unfused	3(20)	3(25)	13(57)[a]	2(19)	9(42)[b]	34(80)[a]
Sternebrae, delayed ossification	7(50)	4(35)	44(100)[a]	1(12)	6(42)[b]	43(100)[a]
Vertebrae-lumbar spurs	4(35)	5(40)	2(21)	4(31)	3(21)	14(80)[a]
Vertebrae, forked atlas	0.4(5)	1(10)	4(36)[a]	0	0	4(20)
Vertebrae, delayed ossification	0	0	1(14)	0	0	5(40)[a]

[a]Significantly different from VCM-exposed group, $p < 0.05$.
[b]Significantly different from air-exposed control, $p < 0.05$.

those exposed only to VCM (Table 4). Only five litters were available for examination in the latter group. Litter size was decreased as compared to concurrent air controls among litters of rabbits exposed to the lower level of 500 ppm, but no effect on litter size resulted from exposure to 2500 ppm of VCM. The decreased mean litter size at 500 ppm most likely occurred because the rabbits in this group released fewer ova; the number of corpora lutea observed on the ovaries was lower among animals in this group.

In the studies by Schwetz et al. (*8*), a slight increase in the incidence of resorptions was observed among litters of rabbits, but not among litters of mice or rats given 15% ethanol in their drinking water during gestation. Decreases in fetal body measurements were observed by Schwetz et al. (*8*) among litters from both mice and rats maintained on drinking water containing ethanol. In the present study, decreases in fetal body measurements were more pronounced when ethanol was given in combination with VCM in these two species; however it is not clear if this apparent synergistic effect was mediated via metabolic interference in the maternal animal.

Incidence of Fetal Anomalies

Among litters of mice, no external or soft tissue anomalies were observed at a significantly higher incidence than the respective controls for any of the exposed groups (Table 5). Cleft palate was observed in 40%, or two of the five litters examined, at 500 ppm VCM plus 15% ethanol. This incidence is not statistically increased as compared to the group exposed to 500 ppm VCM alone. Due to the low percentage of pregnancy in the females, only five litters were available for examination in the 500 ppm VCM plus ethanol group. Among litters of mice exposed to 500 ppm VCM without ethanol in the drinking water, increased incidences of three skeletal variants indicative of delayed skeletal development were observed. Ethanol, when given in combination with VCM, caused a significant increase in the occurrence of a number of these skeletal variants at both levels of VCM exposure. Thus, the occurrence of delayed skeletal development coincided with those treatment regiments which were maternally toxic and resulted in decreased fetal body measurements.

Among rats exposed to 2500 ppm of VCM, the

Table 6A. Rats: incidence of fetal anomalies.

	No. fetuses (no. litters) examined				
				2500 ppm VCM	
	No VCM, no ethanol	500 ppm VCM, no ethanol	No VCM, no ethanol	No ethanol	15% ethanol
External examination	339(28)	387(31)	229(19)	214(16)	188(16)
Soft tissue examination	113(28)	129(31)	76(19)	73(16)	63(16)
Skeletal examination	337(28)	387(31)	229(19)	214(16)	188(16)
Bones of the skull	225(28)	259(31)	153(19)	141(16)	125(16)

Table 6B. Rats: incidence of fetal anomalies.

	% fetuses (% litters) affected				
				2500 ppm VCM	
	No VCM, no ethanol	500 ppm VCM, no ethanol	No VCM, no ethanol	No ethanol	15% ethanol
External examination					
Omphalocele	0	1(3)	0.4(5)	0	0.5(6)
Soft tissue examination					
Microphthalmia	0	0	0	0	2(6)
Dilated ureter	2(7)	2(6)	5(10)	27(50)[a]	5(19)[b]
Small kidney	0	0	0	0	2(6)
Skeletal examination					
Vertebrae, lumbar spurs	1(4)	9(52)[b]	14(68)	12(69)	35(69)[b]
Vertebrae, delayed ossification	0.3(4)	2(16)	7(53)	4(50)	21(81)[a]

[a]Significantly different from air-exposed control, $p < 0.05$.
[b]Significantly different from VCM-exposed group, $p < 0.05$.

incidence of a single anomaly, dilated ureter was significantly higher than controls (Table 6). Ethanol did not further increase the incidence of this anomaly. The incidence of dilated ureter was significantly lower in the ethanol group as compared to the VCM exposed group. Only minor skeletal variants were observed at an increased incidence among the exposed rats. Lumbar spurs occurred more often than controls among litters at 500 ppm, but not among those exposed to 2500 ppm VCM alone. This variant was again observed at an increased incidence among the litters of rats treated with the combination. The incidence of delayed ossification of vertebral centra was also increased in this group and is indicative of a slight delay in skeletal development. As in mice, these skeletal changes occurred in those treatment groups where the combination of high exposure levels of VCM and ethanol produced evidence of maternal toxicity and decreased fetal body measurements.

Among rabbits, external and soft tissue anomalies were observed at a low incidence in those groups exposed to 2500 ppm of VCM alone or in combination with ethanol (Table 7). These included a single fetus with a dilated cerebral ventricle at 2500 ppm and a single fetus with cleft palate from the group exposed to 2500 ppm plus 15% ethanol. A dilated renal pelvis was observed in two fetuses from a single litter in the latter group. Two additional fetuses from this group exhibited an enlarged atrium of the heart. Ossification of the fifth sternebra was delayed at the 500 ppm level, but not at the 2500 ppm level of exposure. Only four litters were available for examination at the 2500 ppm of VCM exposure level.

These data show that the combination of VCM exposure with ethanol in the drinking water was more toxic to the developing fetus, as it was in the maternal animal, than exposure to VCM alone. However, neither treatment regimen was teratogenic in the species tested. Fetal effects consisted of increased incidences of minor skeletal variants indicative of a delay in development in mice and rats. Similar skeletal changes (delayed ossification of sternebrae or vertebral centra and unfused sternebrae or bones of the skull) were observed by Schwetz et al. (*8*) in these two species given 15% ethanol in drinking water. Thus, the exposure to high concentrations of VCM in combination with ethanol in the drinking water produced toxic effects in the developing embryo or fetus which were similar to those produced by ethanol alone.

A similar lack of teratogenicity of VCM in mice and of VCM alone or in combination with ethanol in rats was reported recently by Ungvary et al. [illegible] Several experiments were conducted: Exposure to 1500 ppm for 24 hr/day during organogenesis was reported to have no teratogenic or fetal effects

Table 7A. Rabbits: incidence of fetal anomalies.

	No. fetuses (no. litters) examined				
	No VCM, no ethanol	500 ppm VCM, no ethanol	No VCM, no ethanol	2500 ppm VCM	
				No ethanol	15% ethanol
External examination	152(18)	136(18)	69(9)	32(4)	70(9)
Soft tissue examination	50(18)	47(18)	24(9)	10(4)	25(9)
Skeletal examination	152(28)	136(18)	69(9)	32(4)	70(9)

Table 7B. Rabbits: incidence of fetal anomalies.

	% fetuses (% litters) affected				
	No VCM no ethanol	500 ppm VCM, no ethanol	No VCM, no ethanol	2500 ppm VCM	
				No ethanol	15% ethanol
External examination					
Cleft palate	0	0	0	0	1(11)
Soft tissue examination					
Dilated renal pelvis	0	0	0	0	8(11)
Dilated cerebral ventricle	0	0	0	10(25)	0
Enlarged atrium, heart	0	0	0	0	8(11)
Skeletal examination					
Sternebrae, delayed ossification	[illegible]3(77)	38(94)[a]	20(44)	16(75)	24(67)

[a]Significantly different from air-exposed control, $p < 0.05$.

apart from an increase in fetal liver weight. Exposure during the third part of pregnancy produced no deleterious effects, whereas exposure during the early days of gestation increased fetal mortality and resulted in decreased fetal body weights. Exposure to vinyl chloride and simultaneous maintenance on a liquid alcoholic diet during the neurulation period produced evidence of skeletal retardation, but no malformations in rats. Though the exact days of gestation during which the different treatment regimens were employed were not stated by the author, the reported results are in apparent agreement with those from our laboratory.

A report by Mirkova et al. (*16*) summarized the fetal and postnatal effects following exposure of pregnant rats to 6.15 mg/m^3 of VCM by inhalation throughout the entire gestation period. An increase in early embryo deaths (immediately after blastocyst implantation) and a decrease in fetal body weight were reported by these authors. Observed anomalies in the offspring included generalized hematomas, (8-fold increase over controls) internal hydrocephalus (54.5% of the fetuses), encephalocele (2.53%), and variations of sternebral ossification (2.8% of the fetuses). Several postnatal effects indicative of hepatotoxicity and disturbances in the hepatobiliary system in the progeny following *in utero* exposure were also reported. The 6.15 mg/m^3 level of exposure is equal to only 2.5 ppm of VCM. The exact length of exposure periods and details of the testing methods employed, especially as pertains to vapor generation and analyses, were not reported by Mirkova et al.; thus no explanation for the differences in observed effects of VCM is apparent. The reported results are markedly inconsistent with those from our laboratory where a thousandfold increase in exposure level 2500 ppm was not embryolethal in rats, and similar fetal anomalies were not observed.

In summary, exposure of pregnant mice, rats or rabbits to VCM by inhalation at concentrations sufficiently high to cause maternal toxicity was not teratogenic in any of these species. Fetal effects consisted of delayed skeletal development in mice at 500 ppm, an exposure level which was maternally toxic, and an increase in the incidence of dilated ureter in rats following maternal exposure to 2500 ppm. In mice exposed to 500 ppm of VCM, the incidence of fetal resorptions was increased over concurrent air controls. The incidence of resorptions observed in this group was at the high end of the range for historical control groups in our laboratory.

Ingestion of 15% ethanol in the drinking water enhanced the toxicity of inhaled VCM. Fetal body measurements were decreased among mice and rats given the combination and increases in the occurrence of skeletal variants indicative of delayed development were observed in both species. The fetal effects observed were similar to those reported for these test species following administration of ethanol without VCM exposure. The incidence of resorptions was increased in rabbits given ethanol in combination with VCM, and maternal toxicity was enhanced by ingestion of ethanol in all three species.

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Prenatal Susceptibility to Carcinogenesis by Xenobiotic Substances Including Vinyl Chloride

by Jerry M. Rice*

The carcinogenicity of vinyl chloride for experimental animals when administered transplacentally is reviewed in comparison with known transplacental carcinogens, including those that, like vinyl chloride, are dependent on enzyme-mediated metabolic conversion to a reactive intermediate in maternal or fetal tissues. Vinyl chloride is converted by mixed-function oxidases to the reactive metabolite chlorooxirane, the carcinogenicity of which is also reviewed. Vinyl chloride is unequivocally a transplacental carcinogen for the rat. No evidence exists, however, to support the hypothesis that exposure of male rats to vinyl chloride or any other carcinogen confers an increased risk of tumor development on their progeny. Many structural analogs of vinyl chloride, i.e., substituted ethylenes, are also carcinogenic for adult animals, and can with confidence likewise be predicted to be effective transplacental carcinogens.

Introduction

The carcinogenicity of vinyl chloride and its production for the hepatic blood vessels of both experimental animals and man is now well recognized, and the substance is rightly regarded as a serious hazard in the workplace. Moreover, the demonstration by Maltoni (*1*) that vinyl chloride is not only carcinogenic for adult rodents but is a transplacental carcinogen for the rat as well, has raised concern over a possible risk of carcinogenesis in children born to mothers who had been employed in vinyl chloride manufacturing during their pregnancy. In the United States this has also led to questions of whether it is reasonable to single out women in the workplace as individuals especially at risk and whether to do so unfairly infringes upon their rights to equality in employment. It has been asked whether men may not equally be at risk in terms of the potential of workplace exposure to vinyl chloride and related compounds to cause genetic damage in workers so exposed, and in this indirect way to contribute to an increased risk of cancer in their offspring. These are important questions, not all of which can be fully answered at the present time. Our laboratory has not been engaged in research on vinyl chloride and therefore can contribute no new data to what has been presented during the course of this conference. However, we have been engaged for many years in studying the phenomena of prenatal carcinogenesis by a variety of chemical carcinogens and in species as diverse as the mouse and subhuman primates, and it is the purpose of this presentation to provide a context of current knowledge about the phenomena of prenatal carcinogenesis within which the risk of prenatal exposure to this agent and related compounds can be evaluated.

Mechanisms of Chemical Carcinogenesis

When one tests a substance or a mixture of substances for carcinogenic activity in rodents, a positive result commonly takes one or more of the following three forms: (1) a higher incidence of tumors of one or more organ systems is observed

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among treated animals in comparison with controls; if several different doses have been administered, test animals receiving higher dosage levels (except at very high, toxic levels) have a higher incidence of tumors than test animals receiving lower dosage levels; (2) a higher multiplicity of tumors occurs in one or more organ systems in treated versus control animals; if more than one dosage level is given to different groups of test animals, multiplicity, like tumor incidence, also varies in rough proportion to dose, and animals that receive the highest nontoxic dosage regimen develop the highest multiplicity of tumors; (3) the latency for tumor development is shortened in test animals that develop tumors in comparison with control animals that develop the same tumors; this effect also may be proportional to dose, so that animals that receive the highest dosage of test compound develop tumors after the shortest period of time from the beginning of treatment. The results of a bioassay by itself can only assert that a substance indeed is carcinogenic, in the purely phenomenological sense of the term, by one or more of the above criteria. A bioassay says nothing about the mechanism by which carcinogenesis has been effected. This is a significant point, as evidence is steadily accumulating that not all of the extremely diverse agents capable of inducing tumors in animals or man act by the same mechanism.

There exist a wide variety of chemicals that by these criteria have been shown to have the capacity to induce tumors in animals. These agents are extremely diverse in their chemical features, and include certain heavy metal cations; mineral fibers such as asbestos; and organic polymers, both soluble (iron–dextran; DEAE–dextran) and insoluble, such as plastic films. By far the greatest number of known substances carcinogenic for man or animals, however, are small organic molecules. For the overwhelming majority of these carcinogens there is a common mechanism of action. Agents belonging to this class either react chemically, or are transformed in the course of metabolism to products that react chemically with intracellular nucleophiles, including nucleic acids, to form covalent bonds. Such reactions are irreversible, and proceed through the intermediate formation of an electropositive, or electrophilic, intermediate (*2*). Agents of this sort react with DNA to induce mutations and can be detected by their capacity to induce nonscheduled DNA repair synthesis in nondividing cells; the term "genotoxic" is becoming accepted as a descriptive term for agents of this class, to which vinyl chloride belongs.

Most genotoxic carcinogens are not themselves chemically reactive and require metabolic transformation to a chemically reactive metabolite or ultimate carcinogen. Although there exist a variety of mechanisms involving different enzyme systems by which such changes can be effected, the most common route, which is applicable to vinyl chloride, involves the mixed function oxidases (*3*). This is a class of cytochrome-containing enzymes, dependent on molecular oxygen and reduced nicotinamide-adenine dinucleotide phosphate (NADPH), which have very broad substrate specificities and are capable of catalyzing a variety of reactions, including the epoxidation of carbon-carbon double bonds, the oxidation of aromatic compounds through the formation of arene oxides, and the oxidative dealkylation of compounds such as nitrosamines by hydroxylation of carbon atoms alpha to a nitrogen or oxygen atom. The capacity of these enzyme systems to metabolize different classes of foreign substances, including chemical carcinogens, varies from individual to individual and from tissue to tissue, and is in part responsible for variation among individuals and from one organ system to another in susceptibility to different types of chemical carcinogens.

Transplacental Carcinogenesis in Experimental Animals

To demonstrate transplacental carcinogenesis, experiments must be designed to eliminate the possibility of exposure to a chemical carcinogen by any route other than across the placenta. For rats and mice, in which species most such experiments have been carried out, this is usually accomplished by allowing the carcinogen-treated, timed pregnant female to deliver her young on top of a wire mesh screen so that the pups, as they are delivered, fall through the mesh into a cage below which is inhabited by a lactating female. With luck, the latter will gather up the newborns and care for them as her own, raising them to maturity without their having come postnatally into contact with their carcinogen-contaminated natural mother. For mice, such screens are generally made of 1/2 in. hardware cloth; for rats, poultry fencing generally proves adequate.

General features of experimental transplacental carcinogenesis have been reviewed (*4-6*). The periods of susceptibility to different types of transplacental toxic effects in rodents by chemical carcinogens are strictly related to stages of prenatal development. Exposure during the interval between conception and implantation of the blastocyst will either be without effect or will be embryocidal. Exposure of rats and mice to the same agent

between approximately days 7 and 10, or between implantation of the blastocyst and development of the true placenta, when embryogenesis is occurring with great rapidity, may be lethal to the conceptus; at nonlethal doses there may be severe developmental abnormalities. At lower dosage levels, in the range of exposures that are carcinogenic during later periods of development, tumors are generally not induced in offspring of animals exposed to carcinogens during this period even though their life expectancy is not significantly shorter than that of untreated offspring. After day 12, however, and usually with increasing efficiency thereafter until termination of pregnancy, exposure of a gravid female to a carcinogen will cause tumors to develop in her offspring. Tumors develop in different organ systems in different species in response to a given agent, but are generally at least in part morphologically and anatomically similar to those inducible by postnatal exposure to the same agent. A given agent may not affect a given tissue or organ similarly in all species, and the tumor spectrum seen as a consequence of prenatal exposure may vary markedly from one species to another.

The most potent transplacental carcinogens are direct-acting alkylating agents, which, like methylnitrosourea (Fig. 1), decompose to reactive intermediates without enzymatic catalysis. The next higher homolog of this compound, ethylnitrosourea (ENU), is extremely active transplacentally and has been extensively studied in both rodent and nonrodent species. When this compound is administered as a single injection to a pregnant rat on one of the first 11 days of gestation, high doses result in devastating teratogenic effects or in death of the embryo, but not in tumorigenesis in surviving offspring. Beginning on day 12 and with increasing efficiency thereafter the offspring, which appear normal at birth if dosage is kept at a level below the acutely toxic range, subsequently develop tumors of the central and peripheral nervous system, with overt signs of disease developing 2 months to 2 years after birth (*7*). All three of the parameters previously mentioned as indices of carcinogenicity can be observed in experiments with ENU; the incidence of tumors and the multiplicity of tumors of the nervous system in treated offspring are both directly proportional to the dose administered to the mother, while latency is inversely proportional to dose. Despite the fact that ENU is a direct acting agent and is distributed nearly uniformly throughout the tissues of the rat fetus, tumors rarely appear in organ systems other than the nervous system. Occasionally, tumors of the kidney are seen, and an occasional offspring may develop leukemia. Epithelial tumors of the liver and the lung, for example, are virtually never encountered in rats transplacentally exposed to ENU.

When one compares the dose-response relationships for offspring of rats treated on day 15 of gestation with the response of adult rats to the same agent, it is found that the offspring are approximately 50-fold more susceptible than adults to the carcinogenic effects of this agent: the dose necessary to induce one or more neurogenic tumors in 50% of exposed adult rats is on the order of 150 mg/kg, but the corresponding dose for transplacentally exposed offspring is approximately 3 mg/kg (calculated on the basis of the mother's total body weight).

If exactly the same sort of experiment is conducted in the mouse, however, the results are qualitatively much different. Tumors of the nervous system are relatively rare in mice following transplacental exposure to ENU. As in rats, however, transplacentally treated offspring exposed on or after day 12 of gestation develop tumors. In contrast to rats, mice exposed transplacentally to ENU develop epithelial tumors of the lung and hepatocellular tumors of the liver in high incidence and multiplicity (*8*). In both species, the fetus is quantitatively more susceptible than the adult: susceptibility is greatest during the second half of the period of gestation, and increases as gestation proceeds towards parturition. Adult animals are less sensitive, by one to two decimal orders of magnitude, to the carcinogenic effects of ENU. This much higher prenatal susceptibility is one of the principal reasons for concern that transplacental exposure to carcinogens in the mother's workplace or environment may be of significant risk for the human fetus.

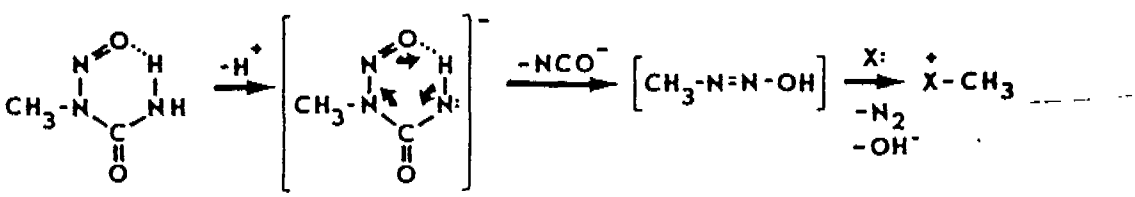

FIGURE 1. Decomposition of methylnitrosourea to yield a reactive alkylating diazonium hydroxide product. The initial step is pH-dependent, but does not require enzyme-mediated catalysis.

Recent studies carried out with ENU in our laboratories have shown, however, that two significant differences from the patterns of response seen in rodents occur when transplacental carcinogenesis experiments are carried out in a nonhuman primate. Different tissues are affected by the carcinogen, and the period of greatest prenatal susceptibility is early rather than late in gestation. Experiments on transplacental carcinogenesis in the Old World monkey, *Erythrocebus patas*, whose gestation period averages 170 days, have clearly shown that ENU is a transplacental carcinogen for this primate (*9*), but that it causes tumors principally of the vascular connective tissues and to a lesser extent the liver, kidney and brain, a pattern of response different from either the rat or the mouse. Tumor incidence is higher and latency is shorter in offspring than in pregnant or nonpregnant treated adults; in that respect the pattern resembles that seen in both rodent species, but neither the rat or the mouse yields a spectrum of tumors predictive of the results of exposing monkeys to the same agent. In our experiments pregnant patas monkeys were exposed to ENU during either the first half or the second half of gestation, or throughout pregnancy. It has become strikingly apparent that animals exposed for the first time at day 30 are at much higher risk for tumor development than animals given comparable exposure but beginning even 30 days later in gestation. This marks a significant departure from the patterns seen in rodents and suggests that the period of maximum intrinsic susceptibility to chemical carcinogens in other primates, including man, may well be during the first trimester of pregnancy and in that respect may resemble the period of greatest susceptibility to teratogens. Obviously, this includes the early fraction of gestation during which, at the time of possibly greatest vulnerability of her conceptus, a woman may not know for certain that she is pregnant and may therefore not be warned to take special precautions to prevent exposure to noxious agents.

In other important respects, such as the greater susceptibility of the fetus, experience in the patas monkey is comparable to that in the rat and mouse and suggests that the phenomenon of high fetal susceptibility to carcinogens is a general one. The fact that the organ systems principally affected by ENU in this species are different from those of the rodent species further emphasizes that one should be extremely cautious in extrapolating from any other species to man in predicting the site of action of a chemical carcinogen, even a direct acting one. The one common pattern valid across species lines in the matter of prenatal organ specificity is that the nervous system and the kidneys appear to be susceptible to at least some extent to this agent in all species tested, an observation which brings to mind the fact that tumors of the kidney and nervous system predominate among solid tumors of childhood.

Transplacental Carcinogenesis by Metabolism-Dependent Carcinogens

The vast majority of genotoxic chemical carcinogens are not direct-acting, but, as indicated previously, require metabolic conversion to a chemically reactive ultimate carcinogen in order to effect carcinogenesis. It has been shown that in rodents, the mixed function oxidase enzymes principally involved in activation of chemical carcinogens are present at low or virtually undetectable levels in fetal tissues until immediately prior to parturition, and even then are present at levels that are minuscule in comparison with those in adult tissues (*10*). The picture is complicated by the fact that these enzymes are inducible, and their levels in tissues such as the liver may be significantly altered by exposure to chemical agents that are substrates for these enzymes, including carcinogens such as methylcholanthrene (*3*). The role of enzyme induction in modifying fetal susceptibility to transplacental carcinogens has not yet been well studied and remains conjectural. However, the low levels of enzymes present in noninduced fetal rodent tissues result in extremely inefficient conversion of most substances to reactive ultimate carcinogenic metabolites. Thus, when the chemically reactive metabolite is very unstable, i.e., has an extremely short half-life under physiologic conditions, it cannot be effectively generated in maternal tissues and transported via maternal and fetal bloodstreams to fetal tissues. Very short-lived reactive metabolites must be generated in situ in any fetal tissue in which carcinogenesis is to occur. Agents whose carcinogenicity is mediated by such metabolites are extremely poor transplacental carcinogens. An example is dimethylnitrosamine (DMN; Fig. 2). DNM is metabolized by mixed function oxidases by the *N*-dealkylation mechanism, generating an intermediate methyl(hydroxymethyl)-nitrosamine which is far too unstable to demonstrate even spectroscopically. It has never been synthesized. This is an excellent example of an agent which presumably must be formed by fetal enzymes. When the parent compound, DMN, was tested for transplacental carcinogenic activity it was found as expected to be much less efficient in offspring than in their mothers in the induction of tumors of the kidney (*11*).

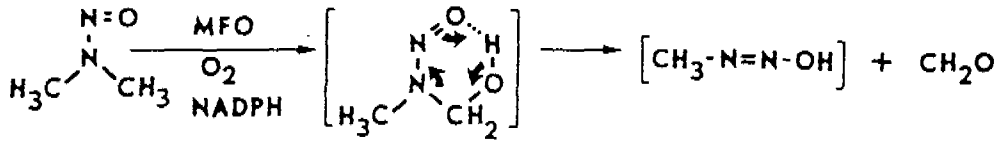

FIGURE 2. Metabolic activation of dimethylnitrosamine by a mixed-function oxidase (MFO) to a reactive intermediate formally equivalent to that generated nonenzymatically from methylnitrosourea as diagrammed in Figure 1. The enzyme catalyzed *N*-demethylation reaction requires molecular oxygen and NADPH, and yields a product too unstable and short-lived to isolate or synthesize.

From the example of dimethylnitrosamine, one might be tempted to infer that the transplacental route of exposure is insignificant for metabolism-dependent carcinogens and, since this encompasses the vast majority of genotoxic carcinogens, is not a significant route of human exposure. Such a prediction is probably wrong. Proximate and ultimate carcinogenic metabolites of many metabolism dependent carcinogens are much more stable than methyl-(hydroxymethyl)nitrosamine. An excellent example is afforded by the polynuclear aromatic hydrocarbons, especially 7,12-dimethylbenz[a]anthracene (DMBA, Fig. 3) which in common with many of the other carcinogenic substances belonging to this chemical class can undergo metabolism to a variety of chemically reactive, arene oxide metabolites which are all ultimate carcinogens of varying potency. The most carcinogenic metabolites of this type of compound are the bay region diolepoxides (*12*), formed by three sequential metabolic steps as indicated in Figure 3. The arene oxides, dihydrodiol, and diolepoxide (Fig. 3) have all been synthesized and are sufficiently stable for not only synthesis and characterization, but for direct testing of carcinogenic potency in experimental animals (*13*, *14*). Accordingly, it is quite possible that metabolites of this sort may be formed in maternal tissues and may succeed in traversing the maternal bloodstream and placenta to reach the fetus. When the parent hydrocarbon DMBA was tested for transplacental carcinogenicity in rats by using a foster nursing procedure (*15*), it was found to be extremely potent. The experiment was terminated at 52 weeks with only 20% of the offspring still surviving; large numbers of tumors were seen in the central and peripheral nervous systems, the kidneys, and blood vessels, as well as in other sites. Thus, not only is this particular metabolism dependent agent a potent transplacental carcinogen, but it actually affects a broader spectrum of organ systems in the fetus of the rat than the extremely potent direct acting carcinogen ENU.

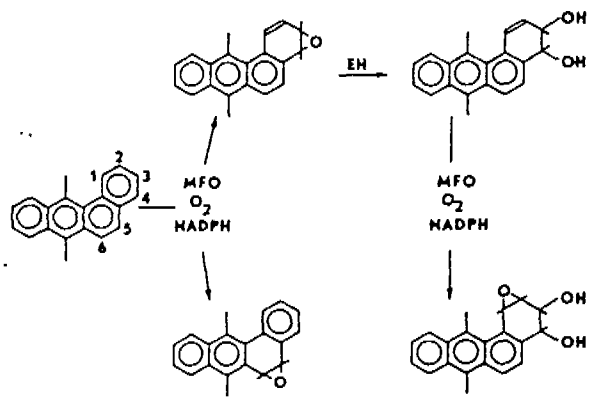

FIGURE 3. Some mixed-function oxidase reactions of the polynuclear aromatic hydrocarbon 7,12-dimethylbenz[a]anthracene (DMBA), yielding various mutagenic arene oxide products. These may subsequently be hydrolyzed by epoxide hydratase (EH) to dihydrodiols, which in turn may serve as substrates for MFO reactions that yield diolepoxides. Although carcinogenic and very reactive, diolepoxides can be synthesized.

Metabolism and Carcinogenicity of Vinyl Chloride

The carcinogenicity of vinyl chloride for rats, mice, and hamsters over an extremely wide range of doses as well as its transplacental carcinogenic effects in rats, are presented by Maltoni (*16*); an earlier version of his data for rats (*1*) is summarized in Table 1. It can be seen that on inhalation of vinyl chloride, tumors are induced in dose-dependent fashion in adult rats in the liver, nasal cavity, kidneys, Zymbal's gland, and most importantly and consistently, in the blood vessels, especially those of the liver. Transplacental exposure for a period of one week, between the 12th and 18th days of pregnancy, generated tumors in three of these tissues, the kidney, blood vessels, and Zymbal's gland, in the offspring (Table 2). Although no controls were included specifically in the transplacental study, the very large series of historical control animals carefully examined in that laboratory are convincing proof that the elevated incidence of tumors of these three tissues is real, and that vinyl chloride is effectively a transplacental carcinogen in the rat. It is noteworthy that the sites and kinds of tumors in-

duced by vinyl chloride are different in part from those that resulted from transplacental exposure to any of the agents discussed previously, none of which affected the Zymbal's glands. The tissues and organs affected in offspring exposed transplacentally to vinyl chloride included some but not all those in which tumors developed in adults subjected to much more prolonged exposures (cf. Tables 1 and 2), and in the transplacental study, significant numbers of tumors were induced in the offspring but not in the mothers.

Vinyl chloride is a metabolism-dependent carcinogen, dependent for its carcinogenicity on mixed function oxidases (*17*) which convert vinyl chloride to its epoxide derivative chloroethylene oxide (chlorooxirane, Fig. 4). The carcinogencity of chloroethylene oxide and of its rearrangement product, chloroacetaldehyde, were recently investigated by researchers at the International Agency for Research on Cancer in Lyon, France, who tested both compounds by subcutaneous injection and by skin painting, the latter followed by phorbol ester promotion, in mice. Chloroethylene oxide proved to be an effective carcinogen, inducing both papillomas and carcinomas in the skin and giving rise to sarcomas on injection (Table 3). Its rearrangement product, chloroacetaldehyde, was toxic, but not demonstrably carcinogenic (*18*). It was noted by the IARC investigators is that the half-life of chloroethylene oxide for hydrolysis at 37° C is on the order of 0.9 min. This is sufficient time for chloroethylene oxide formed in maternal tissues to reach the fetus by way of the placenta, and provides strong suggestive evidence that maternal metabolism contributes to transplacental carcinogenicity of vinyl chloride. To date, however, chloroethylene oxide has not itself been tested for transplacental carcinogencity.

Analogs of Vinyl Chloride

It is important to note that vinyl chloride is by no means unique with respect to chemical structure, and that a wide variety of compounds are in current use in large volumes in industrial processes which differ from vinyl chloride only by further substitution of the vinyl chloride molecule. A partial list of such substances is given in Table 4. Furthermore

Table 1. Tumors in Sprague-Dawley rats after inhalation exposure to vinyl chloride, 4 hr daily and 5 days weekly for 52 weeks. Partial results after 135 weeks.[a]

Vinyl chloride, ppm	Rats at risk (both sexes)	Rats with tumors					
		Zymbal's gland carcinoma	Nephroblastoma	Angiosarcoma		Liver cell tumors	Nasal cavity[b]
				Liver	Other		
30,000	60	35	0	18	1	1	1
10,000	69	16	5	9	3	1	7
6,000	72	7	4	13	3	1	3
2,500	74	2	6	13	3	2	5
500	67	4	4	7	2	3	0
250	67	0	6	4	2	0	0
50	64	0	1	1	1	0	0
None	68	0	0	0	0	0	0

[a]Data from Maltoni (*1*).
[b]Originally reported (*1*) as neuroblastoma of the brain.

Table 2. Tumors in female Sprague-Dawley rats exposed to vinyl chloride by inhalation 4 hr daily from day 12 through day 18 of gestation, and in their offspring.[a]

Generation	Vinyl chloride concentration (ppm)	Rats exposed		Rats with tumors after 115 weeks			
		Total	Survivors	Zymbal's gland carcinoma	Nephroblastoma	Angiosarcoma	
						Liver	Other
Parents	10,000	30	30	1	0	0	1
	6,000	30	30	0	0	0	0
Offspring	10,000	54	12	3	1	0	2
	6,000	32	8	1	0	0	2

[a]Data from Maltoni (*1*).

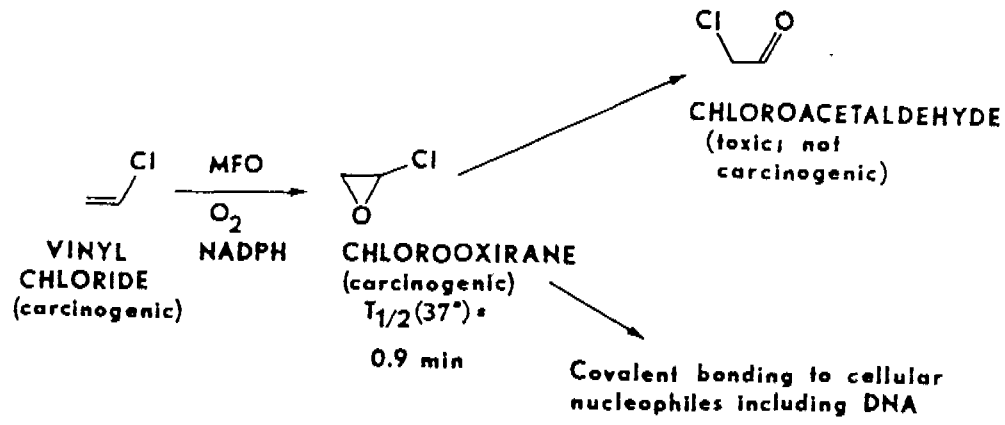

FIGURE 4. Metabolism of vinyl chloride by mixed function oxidases to chlorooxirane, a carcinogenic substance that is stable enough to synthesize.

Table 3. Carcinogenicity of chloroethylene oxide in adult mice.[a]

Effective number	Route	Tumor-bearing animals, %	Duration of experiment, days
28 M	32 SC injections	15 (54)	549
24 F		12 (50)	549
Control			
30 M		0 (0)	549
28 M	1 mg initiation (skin); TPA promotion	26 (93), papillomas 5 (18), carcinomas	590
Control			
28 M		4 (15), papillomas 0, carcinomas	590

[a]Data from Zajdela et al. (*18*).

Table 4. Carcinogenicity in adult rodents of structural analogs of vinyl chloride reviewed in the IARC Monograph Series.

Compound	Structure	Reference	Site of lesions	
			Rats	Mice
Vinyl chloride	$CH_2=CHCl$	(*22a*)	Angiosarcoma Zymbal's gland CNS Kidney	Angiosarcoma Lung Mammary
Vinylidene chloride	$CH_2=CCl_2$	(*22b*)	Similar to vinyl chloride, but incomplete in 1979	
Trichlorethylene	$CHCl=CCl_2$	(*23a*)	(Inadequate)	Lung, liver
Tetrachlorethylene	$CCl_2=CCl_2$	(*23b*)	Negative	Liver
Chloroprene	$CH_2=CH\text{-}CCl=CH_2$	(*22c*)	Inadequate	–

there is an even larger list of substances that resemble vinyl chloride in that they are substituted derivatives of ethylene, but differ in that they lack a chlorine atom. A very brief list of important derivatives of this sort is given in Table 5. A much longer list is given in the review by Posner and Falk (*19*). It is reasonable to postulate that metabolism via reactive epoxides is one route of metabolism to be expected for all these compounds, and mutagenic metabolites of some of them have been demonstrated (*20, 21*). If mutagenic epoxides are formed in significant quantities in maternal tissues and are chemically reactive, yet stable enough to reach the fetus, it is probable that they too would have some measure of transplacental carcinogenic activity. Some of the substances listed in Tables 4 and 5 have been reviewed for risk of carcinogenicity to man in the IARC monograph series (*22, 23*). Other compounds

Table 5. Compounds structurally similar to vinyl chloride that may have similar carcinogenic activity.

Compound	Structure	Reference	Site of lesion	
			Rats	Mice
Vinyl bromide	$CH_2=CHBr$	Not yet reviewed		
Styrene	$Ch_2=CHC_6H_5$	(*22d*)	–	Lung (?)
Acrylonitrile	$CH_2=CHCN$	(*22e*)	Brain Forestomach Zymbal's gland	
Acrylamide	$CH_2=CHCONH_2$	Not yet reviewed		
Ethyl acrylate	$CH_2=CHCOOC_2H_5$	Not yet reviewed		

listed here have been tested in the United States by the National Cancer Institute-National Toxicology Program. As a significant number have been found to be carcinogenic in at least one rodent species, it is reasonable to regard the entire class with suspicion as possible potential carcinogens and transplacental carcinogens pending the acquisition of further data.

Effects of Transplacental Carcinogens on Subsequent Generations: Lack of Male Parental Risk for Prenatal Carcinogenesis

In the context of our present knowledge, prenatal carcinogenesis is virtually synonymous with transplacental carcinogenesis. That is, there is very little evidence to indicate that exposure of either male or female parents to a chemical carcinogen prior to conception confers an increased risk of carcinogenesis on their offspring. It should be pointed out that carcinogenic risk is not the only risk, and that exposure of males to toxic agents may cause sterility or reduce fertility. In rodents it has also been shown that dominant lethal mutations may be induced in this manner, the consequence of which is death of the conceptus (*24*). These hazards however are different from carcinogenic risk for surviving offspring, which has not been shown to be a consequence of exposure of adult males or of adult females prior to conception.

The experimental data which are relevant to this question generally concern what has been termed the "second generation effect" in prenatal carcinogenesis, which has been reviewed by Tomatis (*25*). The general design of experiments of this sort is as follows: The female of a parental generation, P, is given a chemical carcinogen during pregnancy. Male and female offspring of the treated female, constituting the F_1 generation, are then at risk for transplacental carcinogenesis. On attaining sexual maturity, and before tumors resulting from transplacental exposure appear, F_1 males and females are mated to produce an F_2 generation. Both parents of the F_2 generation had been exposed to carcinogen during prenatal life, but at no time were the F_2 animals exposed. In addition, males of the prenatally exposed F_1 generation were bred with untreated females to produce offspring designated F_2M, whose male parents only had been subjected to chemical carcinogens. Likewise, the F_1 females were bred with untreated control males to produce a F_2 F generation of which only female parents had experienced exposure to chemical carcinogens. Similar experiments have been continued to the third and higher generations in attempts to demonstrate a higher incidence of tumors in comparison with untreated controls. Such experiments have been carried out with polynuclear aromatic hydrocarbons, direct acting nitrosourea carcinogens, and with ethyl carbamate, in three laboratories. They have consistently demonstrated that a small, often statistically insignificant but nevertheless reproducible and persistent excess risk of carcinogenesis is present in F_2 and F_2F generations. F_2M offspring in general have shown no excess risk. The numbers of tumors observed in the second generations in experiments of this sort have invariably been extremely small, leaving much to be desired in terms of statistical significance. However, these studies constitute the only experimental evidence for a chemically induced enhanced susceptibility to cancer mediated by damage to the germ cells. Present evidence indicates that if this effect is real, it is most significant for the female germ cells, which are undergoing rapid mitotic division during the final week of intrauterine development in the female rat or mouse fetus.

On the basis of present information, therefore, it is clear that prenatal carcinogenesis by chemicals is principally a direct effect of chemicals or their fetal or maternal metabolites upon fetal tissues, rather than upon the germ cells of the parents of either

sex; if an effect mediated via damage to parental germ cells exists, that damage with its attendant increased risk of carcinogenesis in the offspring appears greatest when the female parent is exposed to a carcinogen; and that the fetus is at risk on account of maternal exposures to potential carcinogens, not only because it is inevitably exposed to any agent that may find its way into the maternal bloodstream, but also because carcinogenic or toxic metabolites which may be even more dangerous than the environmental precursor may be generated by maternal tissues and transferred via the placenta to the fetus.

The incidence of tumors that will develop in a given organ system following transplacental exposure to a carcinogen is not necessarily the maximum incidence possible. Cocarcinogenic phenomena, in which exposure to a second, noncarcinogenic agent accelerates the development and increases the yield of tumors resulting from a previous carcinogenic exposure, are well demonstrated in transplacental carcinogenesis (*26*), especially in the mouse where they have been documented for the skin and for the liver. Potential tumor cells may remain quiescent for prolonged periods after exposure to the inducing agent, and in evaluating the significance of transplacental exposure one must bear in mind that subsequent, postnatal exposure to noncarcinogenic promoting agents may act in a strongly synergistic fashion with prenatal exposure to a carcinogen and may greatly increase the risk of carcinogenesis.

Finally, all agents known to be prenatal—that is, transplacental—carcinogens are also carcinogenic to some extent during postnatal life in one or more species. To some extent, this is a consequence of the manner in which the science of transplacental carcinogenesis has developed; known carcinogens have been selected for testing for transplacental effects. Nonetheless, the generalization holds that there is no known purely transplacental carcinogen.

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Mutagenicity Studies of Vinyl Chloride

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Mutagenicity studies in both man and in test organisms clearly demonstrate positive mutagenic activity of vinyl chloride. In terms of the mutagenicity studies using a variety of *in vitro* procedures covering both eukaryotes and prokaryotes, positive effects were found. Cytogenetic *in vivo* studies in animals and in humans indicate not only somatic mutations, but also germinal effects with this chemical.

The importance of mutagenicity studies for the evaluation of the genotoxicity of a chemical lies principally in the transmission of that genetic mutation to the offspring. Therefore, in order to evaluate the reproductive hazards to man of a potential carcinogen or mutagen, one must look at the results of mutagenicity testing in short-term tests. Today, a number of test systems are available in different organisms including bacteria, Neurospora, Drosophila, mammalian cells in culture and *in vivo* tests in rodents. Since a number of agents, including viruses and chemicals, can cause heritable mutations, it is important to identify these agents and to understand the different kinds of genetic diseases which they cause.

Table 1. Genetic disease and incidence of anomalies per million live births.

Disease classification	Current incidence per 10^6 live births
Dominant traits	10,000
X-chromosome	400
Recessive traits	1,500
Chromosomal anomalies	
Unbalanced rearrangements	1,000
Aneuploidy	4,000
Congenital anomalies	15,000
Anomalies expressed later	10,000
Constitutional and degenerative diseases	15,000
Total	56,000

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The kinds of genetic disease and the incidence of these anomalies per million live births are listed in Table 1. The most frequent of these diseases concern congenital anomalies and constitutional and degenerative diseases which occur at a frequency of 15,000 per million births. Chromosomal rearrangements, such as unbalanced translocations, occur at a rate of 1 per 1000 while dominant inheritance occurs at 1 per 100. In all, a total of approximately 5.6 per 100 neonates are affected by some kind of genetic anomaly. This constitutes a very large number of children and certainly represents a significant portion of the population (approximately 1/20 births). It is even more surprising to consider that many of the genetically unfit fetuses simply do not survive to term and are aborted spontaneously. It is estimated that as many as one in two conceptuses do not implant or are aborted during early gestation (*1*, *2*), many of these occurring so early that the mother is not even aware of the pregnancy. In fact, even though the overall rate of chromosomal anomalies among live births is 1 in 20, the actual rate of genetic anomalies is probably tenfold higher, and, in this context the role of environmental chemicals which act as mutagens becomes extremely important.

A significant decrease in infant mortality rates results from the control of infectious diseases seen during the past years (from approximately 30% in 1915 to 3% in 1965) (Fig. 1). The contribution of genetic anomalies to infant mortality, however, has increased during the same period from 5% in 1915 to 15% in 1965 (Fig. 1). An environmental component may be involved in the present incidence of birth defects and may be responsible for the increase in genetic aberrations.

With the advance of medical care of the newborn, individuals with genetic abnormalities that would have normally expired at birth are now surviving. An excellent example of this is the individual with Down's Syndrome (trisomy 21) who a decade ago would not have been expected to live much past the age of 12. Today, however, due to the advances in the care of the newborn and in the control of infectious diseases, children with Down's Syndrome may survive well into their forties, and some of the females have even reproduced. One can, therefore, conclude that natural selection has been somewhat neutralized in the past few years. This factor is coupled with the increasing number of both environmental and industrial chemicals which have been only recently identified that can induce genetic damage. Thus, the utilization of natural selection, as well as the increasing amount of chemical exposure, may both be viewed as important factors leading to the increase in genetic abnormalities (congenital anomalies) which have been observed in man in recent years.

An important social measure of the consequences of birth defects is indicated by life years lost, and as seen in Figure 2, this is much greater than that attributable to cancer, stroke or heart disease (2).

We can, therefore, ask: what are the consequences of mutations in germ cells? These include a number of events including an increase in spontaneous abortions, infant mortality, and congenital and developmental birth defects. Developmental defects may appear as children with abnormal puberty, neurological disease, higher mortality or as inborn errors of metabolism.

A measure of these consequences obtained from the National Institute of General Medical Science is that 33% of all admissions to hospital pediatric wards are directly related to genetic defects. It has been estimated that each apparently normal individual carries 5 to 8 deleterious genes (possibly lethal) which are heritable and that each couple stands a 3% risk of bearing a genetically defective child. Furthermore, it has been suggested that approximately 80% of the clinical mental retardation in the country arises from genetic causes, and that many types of genetic damage resulting in brain dysfunction go undetected. We also see that 35% of all spontaneous abortions (more than 100,000 cases per year) have a gross chromosomal defect, many of these due to environmental causes.

The types of point mutations which can be induced by mutagens include transitions (in which an AT pair may be replaced by a CG pair), transversions (in which AT may be replaced by TA), insertions (a GC pair may be inserted) or deletions (AT pair may be deleted). These events are seen in Figure 3, which illustrates some possible genetic effects of radiation or chemical mutagens (i.e., vinyl chloride). Changes in the nucleotide sequence of the DNA molucule may occur and subsequently affect single base pairs, causing transversions or even deletions. In some instances, where long segments of DNA can be affected, inversions, deletions or translocations may result.

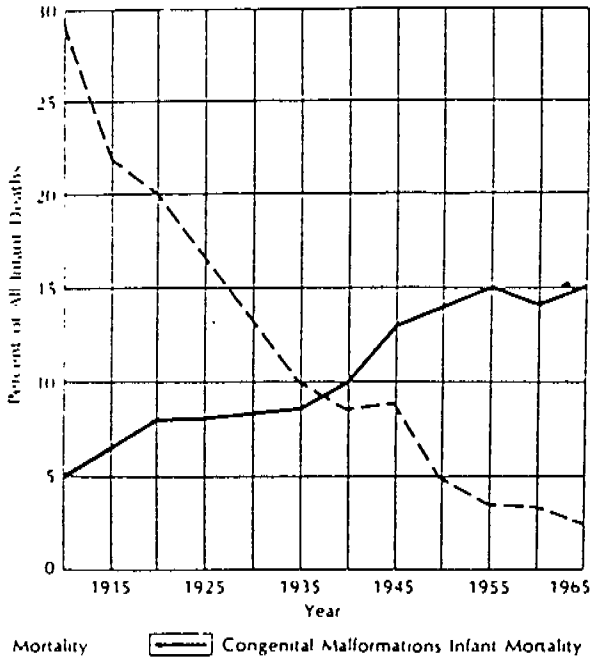

FIGURE 1. Infant mortality rates due (---) to infectious diseases and (——) to congenital malformations.

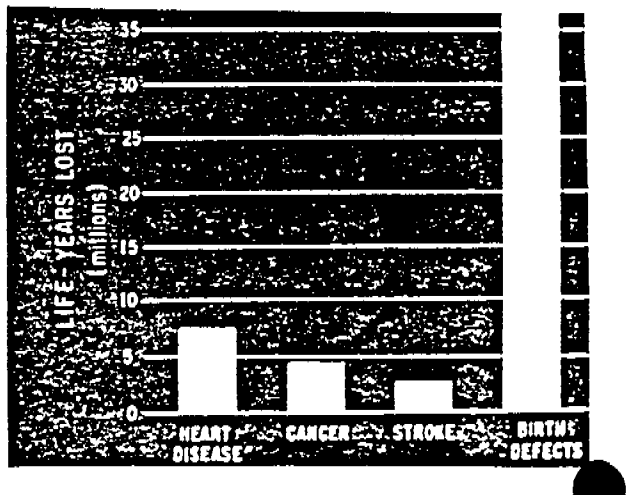

FIGURE 2. Life years lost due to heart disease, cancer, stroke and birth defects.

In addition, triplets can be affected, causing deletions or insertions of bases, all of which may lead to mutations which may be heritable if occurring in germ cells or nonheritable if occurring in somatic tissue. The large number of short-term tests for mutagenicity presently provides a diversity of assays for the testing of these possible genetic lesions. Furthermore, these tests provide the capability for reproducibility of test results and for quantitative measurements.

The simplest of the short term tests for mutagenicity is the Salmonella/mammalian microsome assay, also called the Ames test. The direct mutagenic ability of vinyl chloride in strain TA1530 is seen in Table 2. With a concentration of vinyl chloride as low as 2%, an increase in revertant colonies was observed when compared to the nontreated controls (7 versus 33). In an atmosphere of 20% vinyl chloride, over 100 colonies were observed, while over 200 colonies were observed in an atmosphere of 2% following S9 activation. However, an increase to 500 colonies was observed in plates which were pretreated with Aroclor 1254 and S9-activated (as compared to a spontaneous rate of 12) (Table 3).

A listing of the different test systems as well as the mutagenic activity of vinyl chloride is seen in Table 4. Positive results were reported for vinyl chloride in three bacterial systems. McCann et al. (*4*), Bargin et al. (*5*), Rannug et al. (*6*), and Barstch et al. (*7*), all reported an increase in total number of revertant colonies in Salmonella similar to that seen in Table 2. Greim et al. (*8*) in 1975 reported a positive response in *E. coli*, and Shahin and coworkers (*10*) as well as Loprieno (*11*) showed mutagenicity in yeast both in the gene conversion and in the gene mutation assays.

Similarly, Vogel and Sobels (*13*) as well as Magnusson and Ramel (*14*) reported positive results in the recessive lethal assay in Drosophila. In the Magnusson study, Drosophila were exposed to varying concentrations of vinyl chloride ranging from 1 to 20%. Increased frequencies of both complete and mosaic recessive lethals were seen. They also showed that following pretreatment of phenobarbital for 24 hr an even greater mutagenic effect from vinyl chloride was observed, which indicated a mixed function oxygenase system can be induced in flies which is similar to that reported in mammals.

A number of mammalian cell studies have been completed during the past few years. In one of these studies, increased metabolites were observed in Chinese hamster V79 cells (*15*). However, in a study by Anderson et al. (*16*), an increase in murine embryonic mortality was not seen in females mated to male mice who had received vinyl chloride prior to mating. These authors did report decreased fertility in the males which had received the highest

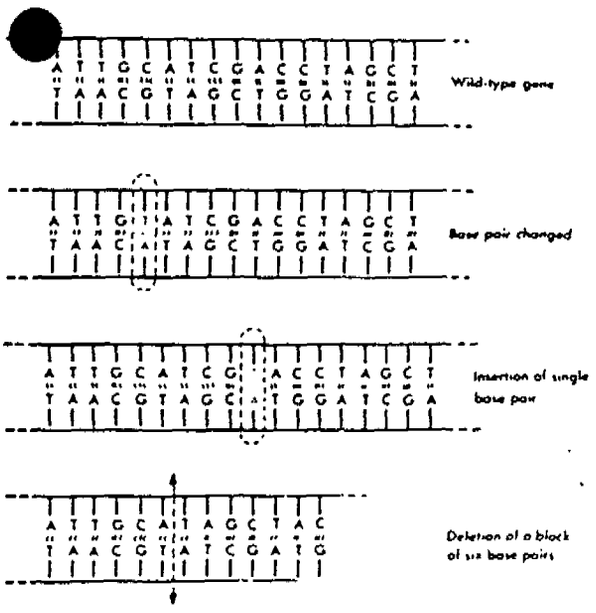

FIGURE 3. Some possible effects of radiation or chemical mutagens.

Table 2. Direct mutagenic activity of VCM towards TA 1530.

VCM concn in atmosphere, %	No. histidine revertants per plate[a]	Spontaneous revertant rate
2	33 ± 6	7
7	48 ± 5	10
12	54 ± 9	7
20	101 ± 16	10

[a]Mean values ± SEM of two assays performed in triplicate.

Table 3. Mutagenicity of VCM towards TA 1530 as observed in simultaneously incubated plates containing either bacteria alone or bacteria and the S9 mix obtained either from control mice or from mice pretreated with Aroclor 1254; VCM concentration in the atmosphere, 2%.[a]

	Control		Aroclor 1254 pretreatment	
	With S9 fraction	Without S9 fraction	With S9 fraction	Without S9 fraction
Number of histidine revertants per plate[b]	206 ± 18	52 ± 9	562 ± 71	102 ± 8
Spontaneous reversion rate	14	16	12	15

[a]Data of deMeester et al. (*3*).
[b]Mean values ± SEM of two assays performed in triplicate.

Table 4. Mutagenicity of vinyl chloride in different test systems.

Test system	Mutagenic activity	References
Salmonella typhimurium	+	McCann et al. (*4*)
(strain TA 1530)	+	Barbin et al. (*5*)
	+	Rannug et al. (*6*)
	+	Bartsch et al. (*7*)
E. coli (K12)	+	Greim et al. (*8*)
Lambda prophage induction	–	Speck et al. (*9*)
Saccharomyces cerevisiae		
Gene conversion/mutation	+	Shahin et al. (*10*)
Gene conversion/mutation	+	Loprieno et al. (*11*)
Neurospora crassa	–	Drozdowicz and Huang, (*12*)
Drosophila recessive lethal	+	Vogel and Sobels (*13*)
assay	+	Magnusson and Ramel (*14*)
Mammalian cell systems		
Chinese hamster V79 cells	+	Drevon et al. (*15*)
Dominant lethal (mice)	–	Anderson et al. (*16*)
Dominant lethal (rats)	–	Short et al. (*17*)
Human studies		
Chromosomal aberrations	+	Ducatman et al. (*18*)
in workers	+	Fumes-Cravioto et al. (*19*)
Chronic lymphocyte	+	Kucerova (*20*)
cultures	+	Szentesi et al. (*21*)
	+	Hansteen et al. (*22*)
	+	Purchase et al. (*23*)
Acute lymphocyte cultures	–	Hansteen et al. (*22*)
Excessive miscarriages in wives of male workers	+	Infante et al. (*24*)

dosage of vinyl chloride, or 50 ppm for 6 hr/day for 5 days. Surprisingly, however, these authors did not regard this fertility decline as "proven" even though 96% of the controls successfully bred while only 55% of the treated animals proved to be successful.

In recent years, chromosomal morphology from cultured peripheral lymphocytes has been studied by a number of investigators in workers exposed to vinyl chloride. In these reports (*18-23*, *25*), increased frequencies of both chromatid and chromosomal damage have been reported. These increases are found to be correlated with the length of exposure as well as with a history of exposure to excursion levels of vinyl chloride during the year prior to sampling (*23*). This study showed 3.18% abnormal cells in the autoclave workers while only 1.08% of the cells sampled from the controls had aberrations. A total of 57 workers were studied.

Ducatman (*18*), as well as Funes-Cravioto (*19*) and their co-workers, reported a similar increase in chromosomal aberrations in 20 workers exposed to vinyl chloride. The most common type of cytogenetic damage reported in these studies were breaks and gaps (*22*), although all types of chromosomal and chromatid danage were seen. Similar results were also reported by Szentesi (*21*), Kucerova (*20*) and Heath and co-workers (*25*). A review of birth defects, and fetal wastage caused by vinyl chloride prepared by Downs et al. (*26*) for The Society of Plastic Industries, Inc. questioned the validity of some of the positive reports with human subjects. Although some of these studies can individually be questioned in terms of experimental design and conclusions drawn, the preponderance of evidence taken as a whole clearly indicate the mutagenic activity of vinyl chloride.

In an important follow-up study on vinyl chloride workers who had initially showed increased frequencies of chromosomal aberrations and who were later removed from that site in the plant to a less exposed area when re-examined two to two-and-one-half years later, showed no differences in chromosomal aberration frequency when compared to controls either in the first study or in the follow-up study (*22*). The original breakage rates of these workers were approximately 3.4%, but upon retesting, only 1.9% of the cells examined were found to have aberrations. These data are extremely important, in that they clearly demonstrate increased cytogenetic aberrations in vinyl chlori[illegible] workers as well as provide documentation for a relationship between a reduction in exposure to vinyl chloride and normalized chromosomal breakage frequency.

Although the majority of mutagenicity studies in vinyl chloride have been found to be positive, there are, nonetheless, others which show no response (Table 3). These include one study using two different strains of Neurospora (*12*) a lambda prophage induction test (*9*), and dominant lethal tests in both mice (*16*) and rats (*17*). In addition, a cytogenetic study in man was also found to be negative in which both sister chromatid exchange frequencies, as well as chromosomal breakage, were studied following one five minute acute exposure to vinyl chloride (*22*). However, the few studies which show no effect are certainly not persuasive in light of the volume and diversity of the other assays in which positive results are seen.

In man, there are also some epidemiological data which document an increase in spontaneous abortions in the wives of workers exposed to vinyl chloride (*24*). Only husbands (workers) were interviewed in this study, and results showed increased spontaneous abortions in the wives of workers. However, had this report interviewed the wives of the workers, the percent of the observed frequency of spontaneous abortions would have been ev[illegible] more dramatic; therefore the results of this stud[illegible] should be considered conservative.

In summary, there are now sufficient data from mutagenicity studies in man and in test organisms to clearly demonstrate positive mutagenic activity of vinyl chloride. In humans, there are data to indicate not only somatic mutations, which are seen as increased frequencies of chromosomal aberrations in lymphocytes of workers, but also increased spontaneous abortions in the wives of vinyl chloride workers, probably due to mutations occurring in the germ cells of the husbands. There are probably an even greater number of spontaneous abortions which had occurred in these people than those reported and which were simply not detected.

Thus, the hazard of vinyl chloride in reproductive studies is indeed considerable. There is a need for more epidemiological studies to determine the extent of the danger, but that certainly does not detract from the immediate need for an assessment and for public health intervention for exposure of both males and females to vinyl chloride and to structural analogs, using vinyl chloride as a biological model.

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Power Considerations in Studies of Reproductive Effects of Vinyl Chloride and Some Structural Analogs

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We review the evidence examining the relation of reproductive function and exposure to vinyl chloride and selected structural analogs. Investigation of these compounds for possible reproductive effects has focused on paternal exposure, a much less well studied route than maternal exposure. Drawing on animal models, we discuss what is known about the possible reproductive consequences of exposure to the father as well as to the mother. In evaluating the studies of reproductive outcome in relation to vinyl chloride or analogs, we consider what biologic model may have been tested and whether there was statistical power to detect moderate increases in risk. Parameters influencing statistical power are reviewed, and recommended sample sizes are set out which would insure sufficient power, in future studies, to detect adverse effects.

As a setting for research on the relations between exposures and adverse reproductive events, the workplace has both strengths and limitations. A first advantage is that exposures in the occupational setting are usually at higher dose levels than those in the general environment. Since higher levels of exposure are often associated with greater risks, studies of occupationally exposed individuals may facilitate the detection of modest effects. A second advantage is that it is usually possible to distinguish which parent is exposed, since most parents do not share a common work environment. A limitation of studies set in the workplace is that frequently the number of exposed subjects is too few to yield a valid test of the association being sought.

This problem of small numbers revolves around the question of power, the statistic that guards against the observer reporting no association, when in fact one does exist. The smaller the study population, the greater the chance that an association between an exposure and an effect will not be detected. False negative results can lead, in turn, to erroneous inferences about the safety of the workplace.

In this evaluation of the studies where exposure to vinyl chloride and structural analogs has been examined in relation to adverse reproductive outcomes emphasis will be placed on considering whether the statistical power in studies reporting negative results was sufficient to justify strong inferences from the findings. Conversely, we will also evaluate whether results reported as demonstrating an association truly support this conclusion.

The paper is divided into three sections. First we briefly consider the types of effects which may follow on either exposure to the mother or to the father. Second, we outline the parameters which influence statistical power. Third, we review the evidence with a view to summarizing current knowledge of the relation of vinyl chloride exposure to reproduction.

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Types of Reproductive Effects

The birth of a child with malformations is only one of many outcomes that may follow on exposure to a reproductive hazard. So too, maternal exposure during pregnancy is just one of the routes through which an agent may affect reproduction (*1*). We consider below, several outcomes and routes of exposure.

Paternal Exposure

The route of exposure may be through the father, in which case possible reproductive effects include: sterility, infertility, reduced sperm production or mobility, alterations in sperm morphology and genetic damage to the germ cell. Work in the laboratory lays the necessary foundation for our thinking about these processes, but it is unfortunately often less precise, particularly in descriptions of outcomes, than we would now wish. Thus we would ask not only that experimental work distinguish between types of agent, dose, age at administration and duration of exposure, and the supposed action on spermatogonia, spermatocytes, spermatids and sperm; but also that outcomes be distinguished in the offspring, in terms of chromosome structure and function, as well as morphology and morbidity.

With few exceptions, such specificity is available on almost none of the exposures with which we are concerned in the workplace. Given that there are known interspecies variations in tolerance levels which must be considered to act not only in absolute terms, but also in terms of stage of development and tissue affected, it is unlikely in any event that studies in animals can substitute fully for studies in man.

When the route to the conceptus is through the father, exposures prior to conception must be considered. The interval between exposure and conception that is relevant in regard to potential effects of exposure is not known, and it may vary with the type of exposure and mechanism. The period is sometimes specified as three months, roughly corresponding to the 75-80 days it takes for sperm to regenerate. However, it may be that some agents act not on the spermatids, spermatocytes and spermatozoa, but rather on the spermatogonia, which give rise to the sperm. In that case, judging from the experimental work, all subsequent populations of sperm might be affected, and not simply the generation present at the time of the exposure. Such an example is found in mice, where paternal irradiation exposure is associated with an excess of mutations in all litters conceived after exposure (*2*): we do not know of a similar example in humans.

There is some evidence (*3-5*) that various drugs may be carried in the human semen. If this is indeed the case, then the developing conceptus may also be affected by exposures to the male parent during gestation. Certainly Naeye (*6*) has now presented evidence suggesting that intercourse during late pregnancy can cause amniotic fluid infection and abruptio placentae.

Maternal Exposure

When the mother is exposed, events occurring during pregnancy as well as prior to conception can influence the outcome. In the female the germ cells are present at birth; thus any postnatal exposure, and possibly exposures encountered when the mother-to-be is still an embryo, may affect the germ cells. There is some evidence that the germ cells are more vulnerable to exposures at some stages (perhaps during follicular development) than at others.

Adverse outcomes from maternal exposure before conception include infertility, and conception of a zygote with anomalies in either chromosome number or structure and/or with a gene mutation. Exposure to the mother during pregnancy can result in anatomic malformations in the conceptus (a teratogenic effect), it may lead to disability in the conceptus but without patent malformation (a fetotoxic effect), or it may lead to premature expulsion of a normal conceptus (an abortifacient effect). A carcinogenic effect on the offspring is also possible (*7, 8*).

Experimental models to distinguish these effects in mice are elegantly displayed in the work of Maudlin and Fraser (*9*).

Tentative though our understanding may be, at this stage, of the processes involved, we would still argue for researchers to spell out, at the outset of their investigations, the likely hypothetical model they are testing. In the discussion of statistical issues and in the critique of papers that follows, we have had in most cases to superimpose the model that we assumed was the one being investigated. By so doing, we may sometimes have been less than just to the investigator, and we will point up this type of problem when it arises.

Issues Relating to Statistical Power

Although studies evaluating the effects of occupational exposure often permit specification of the parent exposed and the timing of exposure, [illegible] answers to important questions about effects on the

fetus may be impossible to give, because of the small number of individuals exposed. Sample size affects the power of the test. Formally, power can be defined as the probability that, in any study, a raised risk of a specific size will be detected, if it is present (*10*). Other determinants of power are: the research design, the test statistic, the level of statistical significance established, the size of the increase in risk, and the prevalence of the condition under study in the unexposed population. Some of these relationships are illustrated in the next two tables.

Table 1 illustrates the relation between the prevalence of a condition in an unexposed sample and sample size, fixing the relative risk to be detected, the statistical power, and the significance level. In the first column, we set out the frequencies of an outcome among the unexposed; these vary from 0.1% to 45%. In the second column we show a doubling in the risk. (Throughout, we define a relative risk of 2, or a doubling, as a nontrivial effect that one would wish to detect.) In the third column we set out the sample size needed in each study group in order to have 80% power to detect the doubling in relative risk (at $\alpha = 0.05$, two-tailed). It is obvious that the rarer the outcome in the unexposed population, the larger the sample needed to detect a doubling in risk among the exposed. The need to use large samples when studying rare outcomes relates to the fact that a doubling in a rare event—which may result from an additional handful of cases—is far more likely to arise by chance than a doubling of a more common event.

Table 2 illustrates the relation between statistical power and the size of effect (relative risk), fixing the sample size of the two study groups and the frequency of the outcome among the unexposed. In the example, there are 100 individuals in each cohort and a prevalence of 15% for the outcome among the unexposed. It is obvious that statistical power increases with increases in the relative risk. The greater the size of the effect, the smaller the sample required to detect it.

The moral of these two tables is that not all studies with negative results are equal. Some negative studies are more equal than others. In order to interpret a negative finding, we need to determine the probability that a particular increase in risk would have been detected, if present. It is with this in mind that we evaluate the studies which have examined exposure to vinyl chloride and structural analogs, in relation to reproductive outcomes.

We have grouped the studies by outcome. For each outcome, we consider whether those studies which appear to produce conflicting results were designed to detect effects of the same magnitude.

Table 1. Relation of prevalence to sample size requirements.[a]

Probability of outcome among unexposed group	Probability of outcome among exposed group	Samples sizes of exposed and unexposed groups[b]
0.001	0.002	22,403
0.01	0.02	2,243
0.10	0.20	197
0.15	0.30	123
0.25	0.50	65
0.35	0.70	41
0.45	0.90	28

[a]Illustrated by the need to detect a doubling in relative risk (RR) with 80% statistical power.
[b]Sample sizes were calculated for $\alpha = 0.05$, two-tailed test.

Table 2. Relation of relative risk and statistical power.[a]

Probability of outcome in unexposed group	Probability of outcome in exposed group	Relative risk	Statistical power to detect increase in risk
0.15	0.19	1.3	0.11
0.15	0.23	1.5	0.29
0.15	0.27	1.8	0.56
0.15	0.32	2.1	0.81
0.15	0.37	2.5	0.95

[a]Illustrated among 100 exposed and 100 unexposed subjects when prevalence among the unexposed is 15%. Power calculated for $\alpha = 0.05$, two-tailed test.

A Review of the Evidence

Birth Defects

The initial suggestion that vinyl chloride might pose a risk to human reproduction came from a study of birth defects in three Ohio communities housing vinyl chloride production facilities (*11*). This was an ecological study, comparing malformation rates in the index communities with the statewide rates. Attention focused on the finding of a significant excess of central nervous system malformations, especially prominent in one of the cities, where the risk of neural tube defects relative to the state as a whole was 5.8.

Ecological studies always raise knotty statistical issues so that some biostatisticians and epidemiologists shun them utterly. It was therefore entirely appropriate that, following this first report, the Center for Disease Control (CDC) undertook a case-control study to see if, individually, the cases in this city could be linked to the vinyl chloride facility (*12*). Occupation and residence data from hospital records were used to explore, first, whether the parents of cases had had direct occupational exposure to vinyl chloride and, second, whether

their homes were located closer to the plant than the homes of controls. No differences in work place exposure or in proximity to the plant were found between the two groups.

In this sample, comprising 15 cases and 30 unaffected controls, the chance of detecting a doubling in the proportion of residents living close to the plant compared to controls was about 70%. It seems certain that power was ample to detect a sixfold relative risk, even a twofold risk, but not a more modest effect.

More recently, CDC reported a second study examining the relation of neural tube defects to parental exposure to vinyl chloride (*13*). Data from the Birth Defects Monitoring Program were reviewed for other locales with poly(vinyl chloride) facilities, and an intensive investigation was launched in Kanawha County, West Virginia, where rates of CNS defects had also been observed to be significantly higher than in reference populations. In this study, unaffected births (controls) were matched to affected births (cases) on several factors (seasonality, race, social class and maternal age) which may relate to CNS malformations. Reproductive, residential and occupational histories were obtained by telephone interviews with the parents of affected and unaffected births; a matched-pair analysis was performed to test whether the frequency distribution of distances from the plant were similar for cases and controls (Table 3).

The power of this study of 46 matched pairs was the same as in the earlier CDC study; that is, there was 80% power to detect a 2.3 increase in the proportion of cases living close to the plant compared with controls, but only 70% power to detect a doubling in this proportion. Once again, no association was found between either working in or living near a poly(vinyl chloride) plant and central nervous system defects. In fact, the proportion of parents employed at the plant was equivalent (4%) in the case and the control group, and the percentage living close to the plant was similar within a radius of either one or three miles. When the addresses of the two groups were plotted on a map, the direction of the residences with respect to the plant did differ, with families of affected births living more to the northeast and families of unaffected births living to the south of the plant. Emission and meteorologic data were explored to see if exposure levels varied with direction, but the results were ambiguous.

Table 3. Vinyl chloride neural tube defects.

Study		
Study 1	Ecological analysis	RR of CNS defects in community with PVC plant = 5.8
Study 2	Case-control study 15 cases 30 controls	Power to detect a doubling = 70%; RR detectable with 80% power = 2.3
Study 3	Case control study 46 matched pairs	Power to detect a doubling = 70%; RR detectable with 80% power = 2.3

In summary, if there is an association between parental exposure to vinyl chloride and CNS defects in offspring, these two case-control studies suggest it is likely to be smaller than the moderate effect exemplified by an odds ratio of 2.3.

The route of exposure was never explicitly specified in these studies of neural tube defects. However, malformations in offspring are often considered to implicate the mother rather than the father as the source of exposure. Hence the study examining effects of vinyl chloride inhalation in pregnant female animals, described elsewhere in this volume, is of interest (*14*). Since power considerations are as pertinent to the laboratory as to population studies, we calculated, from the published report, the power of the most sensitive test available in this experiment. That comparison could only achieve 8[illegible] power if the effect on the treated animals was lar[illegible] that is, only an increase of more than 4-fold in the incidence of anomaly was likely to have been detected. Reporting such findings as negative is to disregard considerations of power, which are not species-specific.

Spontaneous Abortion

A later investigation into the reproductive effects of vinyl chloride explicitly proposed the father as the route of exposure and fetal loss as the outcome (*15*). Using fetal loss data obtained by interview with the fathers, rates of loss were compared in the wives of exposed and unexposed workers, in both the time periods before and after exposure to vinyl chloride. In the time period subsequent to exposure, when mean paternal ages were equivalent (and, by inference, maternal age, a known risk factor for spontaneous abortion), the rate of fetal loss among the wives of exposed workers was 16.5%, and that among wives of unexposed workers was 8.8%, yielding an unadjusted relative risk for fetal loss of 1.8 (Table 4).

A comparison of age-adjusted rates of loss for exposed and unexposed men was carried out by the authors, taking the number of pregnancies to the two groups (412 in total) as the sample size. Fr[illegible] this chi-square analysis it was concluded that th[illegible]

Table 4. Vinyl chloride/fetal loss: comparison of fetal loss rates in wives of exposed and unexposed workers.

	Prevalence in unexposed	N per group	Power
Study 1	0.088	62 exposed 113 unexposed	Power to detect doubling = 31%; RR detectable with 80% power = 2.9
Study 2	0.15	205 exposed 144 unexposed	Power to detect 1.8 RR = 79% RR detectable with 80% power = 1.81
	0.12	205 exposed 144 unexposed	Power to detect 1.8 RR = 65% RR detectable with 80% power = 1.96

was a statistically significant difference between the two groups in the rate of fetal loss. However, there is evidence that women with multiple spontaneous abortions were concentrated in the group which later became exposed (*16*). Since one spontaneous abortion is associated with a 66% increase in the risk of a subsequent abortion, it is possible that some of the seemingly excessive loss occurring in the wives of men exposed to vinyl chloride is owed to the increased proportion among them of women experiencing previous abortions prior to exposure. Thus the subsequent abortions cannot be considered independent events, and the analysis does not satisfy the assumption which underlies the chi-square statistic, that all observations are independent. If the rates are compared basing sample size on the 62 wives of exposed workers and the 113 wives of unexposed men, then the difference in fetal loss rates is not statistically significant (t = 1.43, 173 df). However, the power of this test to detect a doubling in the frequency of abortion is only 31%. Thus a negative finding in this analysis does not rule out the possibility of a moderate effect.

The statistic that is appropriate here depends essentially on the explicit model that is being tested. We have noted the chi-square is incorrect, because a woman's first pregnancy and her subsequent pregnancies cannot be considered independent. It could be argued, however, that, if the model to be invoked involves an effect of vinyl chloride on the spermatocyte II layer of the father, then pregnancies that followed within a given period after exposure, and only those, would be affected. In such a case, provided that there was some way of controlling for other risk factors for abortion (like maternal age and previous spontaneous abortion), then the test statistic might legitimately be based on pregnancies rather than on women (although some statisticians will still balk at this procedure).

A new investigation may shed some light. Fetal loss is one of the endpoints currently being evaluated as part of a study of vinyl chloride workers conducted at the University of Texas (*17*). The design of this study has been fully described, though results relating to reproduction have not yet been published. Fetal loss data in this investigation will be based on telephone interviews with the wives of workers; interviewers will be blind to the husband's exposure status. Information will also be collected on potentially confounding variables such as cigarette smoking and prior reproductive history.

The Texas group has thus far interviewed 205 wives of exposed men and 144 wives of unexposed men. Does this sample of 349 wives yield sufficient statistical power to detect an effect of the magnitude suggested by the prior study (RR ~ 1.8)? The statistical power will depend on the rate of abortion in the unexposed wives. If our suspicion that women are more accurate reporters of reproductive history than their mates is correct, then we can expect that the baseline frequency of abortion in this study may be somewhat higher than that of the earlier study based on reports from male workers only. In Table 4 we have computed power based on two different estimates of abortion frequency in the unexposed sample: 15% and 12%. The rate reported will depend partly on the definition of fetal loss and partly on the distribution of risk factors in the population observed. Then if the prevalence of spontaneous abortion among the unexposed is 15%, there will be an adequate test of whether or not paternal exposure to vinyl chloride is associated with a 1.8 relative risk of spontaneous abortion. If, on the other hand, the frequency of abortion among the unexposed is 12%, there is only a 65% chance of detecting this increase in risk.

Infertility

Also bearing on the question of reproductive risk are investigations of effects on fertility in workers exposed to ethylene dibromide (EDB) and epichlorohydrin (ECH), structural analogs of vinyl chloride shown in animal studies to interfere with spermatogenesis. In studying these compounds, the attempt has been made to demonstrate exposure

effects directly in the male, by examining semen and hormone samples, as well as indirectly, using outcome of pregnancy in wives. Wong and colleagues assessed the fertility of male married workers exposed to EDB, by comparing the number of livebirths to their wives with age-parity-race-calendar year-specific birth probabilities for all U.S. women (*18*). Effects on single workers were not evaluated, nor was this method able to control for regional differences in fertility rates.

In reporting their negative findings, the authors claimed to have power of 90% to detect a 20% increase in infertility. However, this computation used the number of person-years observed rather than the number of persons as the sample size. Again, whether "men" or "person years" is the correct number to use in the computation depends on the explicit model that is being tested, which is not here spelled out.

Sperm Counts

The relationship of semen quality to infertility and/or outcome of pregnancy is still imperfectly understood. Men with sperm counts less than ten million have, for instance, been shown capable of impregnating (*19*), but follow-up studies have not been done to see whether the frequency of adverse pregnancy outcome is greater than among men with normal counts. Kapp et al. have found increased aneuploidy in the sperm of 18 dibromochloropropane-exposed workers investigated (*20*). Although an increased number of sperm with two Y chromosomes suggests there might be an increased risk of 47-XYY offspring, this outcome has not been demonstrated.

Milby and Whorton, in a recent paper (*21*), have summarized results of studies they have conducted on epichlorohydrin in two occupational cohorts. Also Venable and colleagues have recently reported a study of glycerine workers with multiple chlorinated hydrocarbon exposures, including epichlorohydrin (*22*). Sperm count distribution has been the major focus in these studies comparing semen quality in exposed male volunteers and unexposed controls, although it has been argued that sperm morphology provides a more stable and predictable parameter (*23*).

These studies vary in the detail with which they have been reported, and in methodology. The focus in the present paper is on issues relating to statistical power; however, several of the methodologic problems in the studies of sperm are important and call into question the value of considering the data in this way. (For instance, in the Milby and Whorton ECH studies, the participation rate among eligible workers was 36% in one cohort and 45% in the other, raising the possibility that the samples were not representative of the exposed populations.)

Negative findings for an association between exposure to ECH and decreased sperm count have been reported for all three cohorts, although the Venable study does note a suggestive reduction in sperm concentration in a subgroup of those exposed (Table 5). For each study, we calculated the size of the relative risk which could be excluded, with a 20% probability of falsely concluding that there was no association between exposure and sperm count;

Table 5. ECH/sperm concentration: semen analyses of exposed and unexposed volunteers.

	Prevalence in unexposed	*N* per group	Power
Study 1	0.055	44 exposed	Power to detect doubling = 19%
		90 unexposed	RR detectable with 80% power = 4.1
Study 2	0.055	84 exposed	Power to detect doubling = 38%
		90 unexposed	RR detectable with 80% power = 3.5
Study 3	0.095	64 exposed	Power to detect doubling = 35%
		63 unexposed	RR detectable with 80% power = 3.0

Table 6. Recommended sample sizes for future studies.

Outcome	Prevalence in unexposed population	Relative risk to be detected with 80% power[a]	Number required in each study group
Neural tube defects	0.001/livebirths	6.0	1862 livebirths
Spontaneous	0.15/pregnancies	1.8	174 mothers
abortions	0.12/pregnancies	1.8	240 mothers
Sperm counts < 20 million	0.07/males		2.0

[a]Power calculated for $\alpha = 0.05$, two-tailed test.

the proportion of men with sperm counts less than 20 million was the index evaluated.

In the first cohort observed by Milby and Whorton, there was an 80% chance of detecting a 4-fold increase over the 5.5% baseline rate observed in the control group; in the second cohort there was an 80% chance of detecting a relative risk of 3.5. The Venable study which observed a 9.5% rate of low sperm count in its control group had power of 80% to detect a threefold increase in risk.

None of these studies had sufficient power to detect a doubling. If we agree that a doubling in the frequency of sperm count depression is not a trivial effect, then these studies are not sufficient to lay fears to rest concerning a possible effect of ECH on sperm count in exposed males.

Conclusions

What, then, is it possible to infer at this time? At present, there are no data which point unambiguously to a relation between vinyl chloride or analogs and reproductive outcome. On the other hand, there are several studies which report no association where the statistical power to detect a modest association between exposure and outcome, if it should be present, is either insufficient or not able to be calculated from the published data. Certainly there is no evidence to indicate that the fetus is at greater risk from maternal exposure than from exposure to the father. Altogether, one must point to the need for carefully designed and executed studies, where the association of vinyl chloride with reproductive outcome can be examined. In Table 6, we set out some estimates of the sample sizes that would be needed, depending on the outcome under study and its prevalence in an unexposed sample. For different outcomes, we have required that the sample be sufficient to detect effects of the size observed in previous studies. Certainly, we stand a better chance of understanding whether and how vinyl chloride affects reproduction if future studies are designed with the ability to detect these adverse effects.

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R&S 133752