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MONITORING FOR CHROMOSOMAL
DAMAGE IN EXPOSED INDUSTRIAL
POPULATIONS

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The Texas Division of Dow Chemical U.S.A. is located about 50 miles south of the city of Houston, Texas. While the area immediately around our 3500 acre site is predominantly rural and residential, we are part of a large industrial and petrochemical complex that extends along the northern coast of the Gulf of Mexico and the southeastern coast of the North American continent. The Texas Division has some 7000 full-time employees who work around or use a wide variety of chemicals (Table I). These chemicals have become the subjects of intense investigation in recent years because of their toxic potential. For many of these compounds, the potential for toxicity includes the possibility of genetic alteration in an individual's somatic or gametic cells.

The possibility of genetic damage, especially the association between an increased rate of chromosomal aberrations and carcinogenesis, is a matter of serious concern. One of the participants in this conference, Dr. James German (1972), has observed that many human cancers arise from a single cell and that this cell often has one or more chromosomes that differ in morphology from any in the complement of the noncancerous cells of the affected person. In addition, chromosomal instability, leading to an increased number of chromosomal abnormalities, mutation, and cancer is found in the several rare genetic disorders that feature increased chromosomal instability together with a greatly increased expectancy for cancer to develop. Certain agents - radiation, some chemicals, and certain viruses - have in common the ability to produce in-

TABLE I.--Dow Texas Division, Freeport, Texas.

7000 employees. Manufacturer or user of a variety of chemicals, including

Benzene	Toluene diisocyanate
Ethylenimine	Toluene diamine
Vinyl chloride	Phosgene
Vinylidene chloride	Carbon tetrachloride
Trichloroethylene	Chloroform
Epichlorohydrin	Hexachlorobenzene
Hexachlorobutadiene	

creased rates of cells with chromosomal abnormalities and to increase the risk of malignancy.

The comprehensive surveillance program established for Dow Texas Division workers is a good example of how cytogenetic monitoring can be integrated with a system of industrial monitoring. The surveillance program (Table II) incorporates four major areas of investigation: Periodic Evaluation, which includes a history and physical examination at preset intervals and appropriate laboratory tests; Cytogenetic Evaluation, done on a routine basis and in the event of accidental overexposure; Conventional Epidemiologic Studies, to determine the mortality and morbidity experience of exposed workers as com-

TABLE II. Vinyl Chloride (VC) Workers

Comprehensive surveillance program

Periodic evaluation

History
Physical
Lab work

Cytogenetic evaluation

Routine exposures
Over-exposures

Conventional epidemiology

Mortality
Morbidity

Special epidemiology

Reproductive study
(VC workers' wives)

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pared to a suitable control group; and Special Epidemiology, which, in a study underway now, will compare the reproductive patterns of the wives of vinyl chloride workers to the child-bearing experience of the wives of a matched control group.

The medical management team of Dow's Texas Division first became aware of the need for a program of cytogenetic monitoring of certain workers in the early 1960s when reports of the mutagenicity of ethylenimine (EI) in non-mammalian systems began to appear in the literature. Dow had started research on EI in 1958 and started to develop a commercial process in 1961. Actual production began in 1965. Because of the early reports of possible mutagenicity, cytogenetic monitoring of EI workers was made part of the medical surveillance program already in effect. When the cytogenetic program was started, the list of potential mutagens was short, and many compounds - such as vinyl chloride - were thought to be essentially harmless.

Looking back, we can see that our first efforts at cytogenetic analysis were relatively crude and our findings inconclusive. Continued work on the many technical problems, however, allowed us to become more confident in our results and to expand the cytogenetic surveillance program to a number of other worker groups, including those exposed to benzene and vinyl chloride.

Participation in a collaborative study of rat bone marrow cytogenetics was of particular help to us (Kilian *et al.*, 1977a, Kilian *et al.*, 1977b). Six cytogenetics laboratories, including our own, joined together, first, to devise methods to minimize interlaboratory differences and, second, to measure interlaboratory variation in results following the standardized administration of a known mutagen - triethylenemelamine - to rats from a common source. A preliminary workshop was held to resolve scoring differences, to develop a joint protocol and common glossary, and to reach agreement on uniform reporting methods. This study showed (Fig. 1) a good pattern of agreement among the laboratories, particularly for certain categories of aberration. It also (Table III) allowed us to evaluate the variability of individual scorers in our own laboratory.

As the list of potential chemical mutagens grew longer, the need for well-controlled cytogenetic studies of human effects, using simplified, repeatable methods became more pressing. We have previously published details of our methods (Kilian and Picciano, 1976); I would like to review these and the conditions we find are necessary for meaningful results. The groups being monitored should be of significant size and relatively stable and healthy. Employee turnover rates should be low, there must be adequate technology for accurate monitoring of both chronic and acute exposures, and there has to

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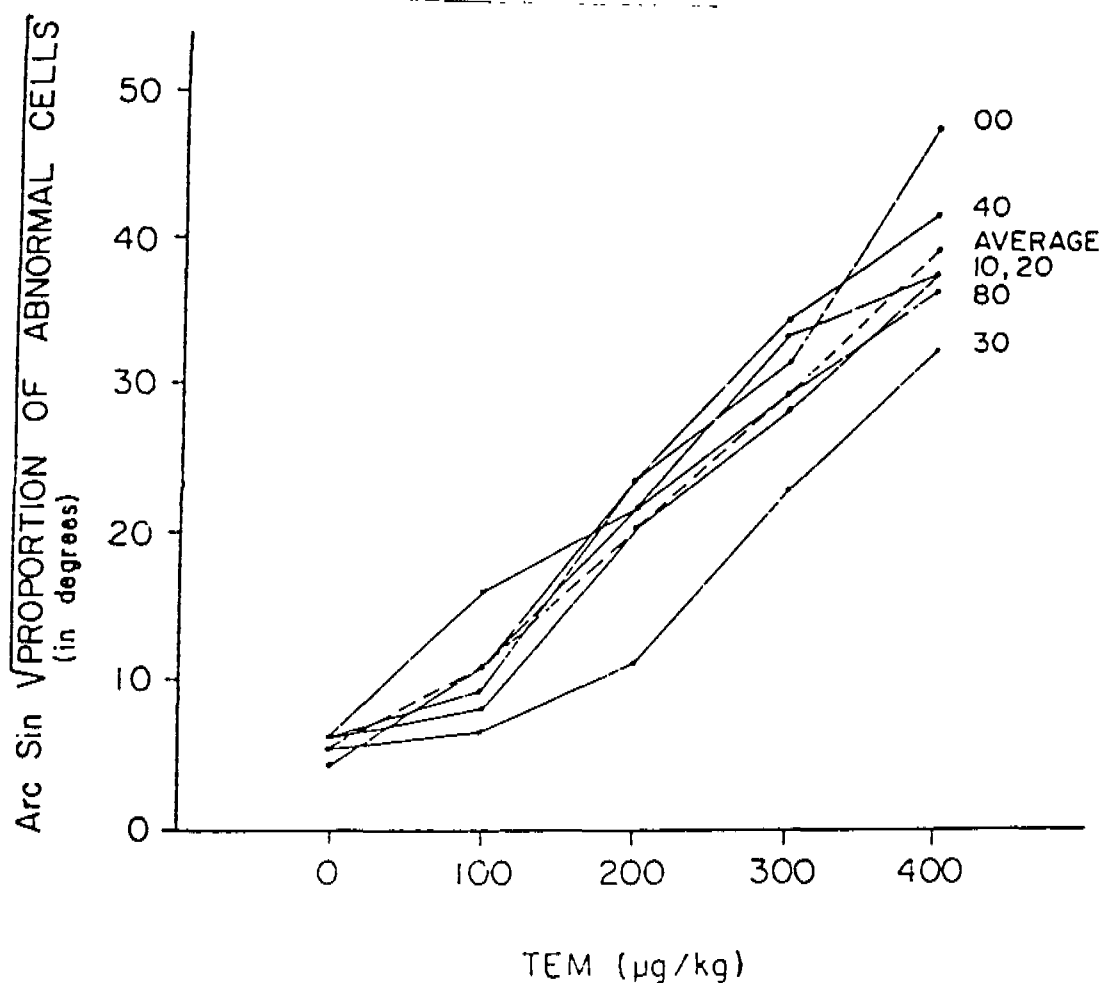


FIGURE 1. Graph showing degree of agreement among six laboratories for average arc sin $\sqrt{\text{proportion of abnormal cells}}$ by laboratory and dose level of triethylenemelamine in cooperative study of rat bone marrow cytogenetics.

be medical and genetic expertise for the evaluation of whatever changes might be found in an individual. It is very helpful if exact levels of exposure to various compounds, alone or in combination, are known. The number of individuals available for study should be large enough to permit evaluation of matched control groups. In addition, the availability of findings from periodic medical examination and records from comprehensive health insurance plans - for the workers and their families - allows the development of cooperative study plans involving the industrial physician, epidemiologist, and cytogeneticist to evaluate not only the worker's medical status, but any reproductive consequences as well.

TABLE III. Proportion abnormal Cells by Scorer and Dose for Laboratory 00 - - - - -

Scorer number	Dose quantity					All doses
	000	100	200	300	400	
01	.00	.00	.10	.31	.41	.16
02	.00	.03	.19	.185	.66	.21
03	.01	.02	.29	.205	.28	.16
05	.03	.10	.20	.40	.72	.29
07	.01	.02	.08	.21	.53	.17
All scorers	.010	.034	.172	.260	.538	.20

A standardized medical history - including exploration of family and occupational background - is taken prior to pre-employment examination, and the worker's status in regard to those factors - such as exposure to chemicals, drugs, radiation, and virus infections - that are known to affect the chromosomes is recorded. The medical status of workers exposed to toxic chemicals is reassessed at periodic intervals thereafter. Standard culturing and harvesting procedures for peripheral blood are used.

Abnormalities are recorded on raw data sheets by cytogenetics laboratory technicians. The data are transferred to 80-column computer punch cards. Since one of our objectives is to set up longitudinal life-time studies to evaluate the consequences of chromosomal breakage, having the necessary information in computer language is very helpful. Scored cells are identified by vernier setting and slide number. Early in our cytogenetic experience, we scored 20 cells per individual and, later, increased this to 50 cells, whenever possible. At the present time, however, we are analyzing at least 200 cells per culture to ensure the validity of the test system.

As a general rule, results of cytogenetic evaluation of specific worker groups are compared to those found upon evaluation of preemployment examinees, that is, persons who have had chromosome analysis done as part of routine preemployment examination and who were judged, on the basis of the history taken at that time, to have no condition that would predispose them to increased aberration rates. In some cases, it is possible for the worker to serve as his or her own control. During the more than 12 years that the cytogenetics surveillance program has been in effect, more than 5000 cultures on more than 4000 individuals have been available for study.

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A description of our cytogenetic study of vinyl chloride-exposed workers will serve to illustrate the potential usefulness of our surveillance program. Our cytogenetic study group was composed of 209 currently employed persons who had worked in the vinyl chloride plant for periods ranging between one and 332 months - an average of 48 months - at the time of the study. Findings were compared to cytogenetic data from a group of 295 preemployment examinees. The records selected for inclusion in the control group were matched to those of the study group, insofar as possible, for sex, number of cells analyzed, and time period during which the culture was initiated.

A detailed evaluation of the results of our vinyl chloride cytogenetics study is now in press (Picciano *et al.*, 1977). The major finding of the study, however, can be summarized simply: that no cytogenetic differences of significance were found between the group of vinyl chloride-exposed workers and the preemployment control group. Table IV shows the comparison of the percentage findings for chromatid and chromosome breaks; rings, dicentrics, and exchanges; and the proportion of abnormal cells. Table V displays the results of Chi-square analysis for differences in the distribution of chromatid breaks between the two groups. Both groups were divided into those showing 0-to-5-percent aberrations and those showing greater-than-5-percent aberrations; the vinyl chloride workers were separated into those with estimated average exposure levels of less-than-one part per million (ppm), from 1-to-5 ppm, and greater-than-5 ppm. The same analysis was used to devaluate for differences in the distribution of chromosome breaks (Table VI) and the proportion of abnormal cells (Table VII). We would like to emphasize that our work-force has been exposed to relatively low levels of vinyl chloride and that we believe this has a definite bearing on our results. It is to be hoped that our demonstration of lack of significant

TABLE IV. Cytogenetic Study of 209 Workers Exposed to Vinyl Chloride

	Workers	Controls
Number of cultures	209	295
Number of cells	10,483	14,761
Chromatid breaks	2.4%	3.6%
Chromosome breaks	1.0%	1.1%
Rings, dicentrics, and exchanges	0.4%	0.2%
Abnormal cells	3.7%	4.5%

TABLE V. Distribution of Chromatid Aberrations Related to Vinyl Chloride (VC) Exposure

	Number in group	Percentage of group with 0-5% aberrations	Percentage of group with >5% aberrations
Exposure to VC ¹	209		
<1 ppm	70	90	10
1-5 ppm	98	77	23
>5 ppm	41	80	20
Controls	295	75	25
$\chi^2_{(3)} = 7.75 \quad (p \sim 0.06)$			

¹Exposure levels are estimates based on calculations for specific job classifications; prior exposure for individuals may have been higher or lower; exposure levels for individuals are known to vary within job classification

TABLE VI. Distribution of Chromosome Aberrations Related to Vinyl Chloride (VC) Exposure

	Number in group	Percentage of group with 0-5% aberrations	Percentage of group with >5% aberrations
Exposure to VC	209		
<1 ppm	70	96	4
1-5 ppm	98	94	6
>5 ppm	41	95	5
Controls	295	94	6
$\chi^2_{(3)} = 0.36 \quad (P \sim 0.95)$			

effect at these levels will be useful in establishing levels of exposure that are genetically safe.

In 1974-75, a group of five reports appeared in the literature concerning chromosome aberrations in vinyl chloride and polyvinyl chloride workers (Table VIIa). Three of the study teams concluded that an increased rate of chromosomal aberrations was associated with vinyl chloride exposure (Ducatman *et al.*, 1975; Funes-Cravioto *et al.*, 1975; Purchase *et al.*, 1975), while two studies - one of them a preliminary investi-

TABLE VII. Distribution of Abnormal Cells Related to Vinyl Chloride (VC)-Exposure

	Number in group	Percentage of group with 0-5% aberrations	Percentage of group with >5% aberrations
Exposure to VC	209		
<1 ppm	70	84	16
1-5 ppm	98	71	29
>5 ppm	41	73	27
Controls	295	70	30
$\chi^2 = 5.97$ ($P \sim 0.12$)			

TABLE VIIla. Published Cytogenetic Studies of Workers Exposed to Vinyl Chloride

Authors	Cases reported		Exposures		Effect
	Workers	Controls	Type	Magnitude	
Ducatman et al.	11	10	PVC	Estimated to exceed 500 ppm	Positive
Funes-Cravioto et al.	7	3	PVC	Decreased in recent years to 20-30 ppm	Positive
Purchase et al.	56	24	PVC	Not given	Positive
Kilian and Picciano	203	108	VC	1969-74: average 5 ppm; 1974-75: 1-2 ppm	Negative
Fleig and Thiess	10	4	PVC	Until 1974: >100 ppm; 1975: 10- 25 ppm	Negative

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TABLE VIIIB. Conditions (Neoplastic or with Neoplastic Association) Displaying Chromosome Anomalies¹

Chronic Myelocytic Leukemia	Ataxia-Telangiectasia
Acute Myelocytic Leukemia	Fanconi's Anemia
Chronic Lymphatic Leukemia	Bloom's Syndrome
Polycythemia Vera	Kostmann's Infantile
Myeloid Metaplasia	Agranulocytosis
Erythroleukemia	Glutathione Reductase
Sideroblastic Anemia	Deficiency
Meningioma	Down's Syndrome
Burkitt's Lymphoma	Turner's Syndrome
	Klinefelter's Syndrome
	Wilms' Tumor-Aniridia Syndrome
Retinoblastoma in D-Deletion Syndrome	

¹Source: Mulvihill, 1975

gation from our laboratory - appeared to show no differences of statistical significance between exposed and control groups (Fleig and Thiess, 1974; Kilian *et al.*, 1975). These studies tend to indicate a dose-related response since the exposure levels of polyvinyl chloride workers to the vinyl chloride monomer are, generally, much higher than the exposure of vinyl chloride manufacturing workers (Occupational Safety and Health Administration, 1974). The indication of a dose-related effect was reaffirmed by further study of vinyl chloride workers at Imperial Chemical Industries in which increased aberration rates were significantly correlated to length and intensity of exposure (Purchase *et al.*, 1976).

It is known that increased rates of chromosomal breakage, as have been demonstrated in cases of exposure to high levels of vinyl chloride, have occurred following x-ray treatment of ankylosing spondylitis (Buckton *et al.*, 1962), in atomic bomb survivors (Bloom *et al.*, 1970), radium dial painters (Vaughan, 1962), persons exposed to Thorotrast (Fischer *et al.*, 1966), and workers exposed to lead (Garza Chapa *et al.*, 1976), benzene (Vigliani and Forni, 1976), uranium dust, and plutonium. Increased rates of chromosomal breakage are also seen in a variety of diseases (Table VIIIB) in which an increased risk of cancer is also evident (Mulvihill, 1975).

Evidence of a relationship between chromosomal abnormalities and increased risk of neoplasia has continued to accumulate, although the exact nature of this relationship is far from clear. It is because of this evidence that we believe cytogenetic monitoring can be an extremely valuable tool in the detection of those environmental situations that may be

associated with an increased risk of cancer and, perhaps, reproductive problems for the chemically exposed worker as well.

A cytogenetic program and periodic medical evaluation, however, are not enough to ensure comprehensive surveillance of worker groups at risk. Epidemiology programs, both conventional and special, are also essential. Regarding our epidemiology efforts, we are fortunate to be reasonably close to the University of Texas Medical Branch at Galveston. Under the direction of University of Texas epidemiologist, Dr. Patricia Buffler, a unique epidemiology program was started a few years ago; the heart of this program is the cooperative efforts put forth by both our own industrial concern and academia. University of Texas employees actually work at the plant site on a continuous basis to identify and process necessary records for later analysis at the medical school. Complete confidentiality is maintained through the use of various coding systems.

Epidemiologic analysis of our vinyl chloride workforce showed that the employees, past and present, fell into one of three major assignment areas (Table IX): the vinyl chloride plant itself, the maintenance units, and the developmental laboratory. Dozens of specific job classifications were identified, and we were able to arrive at fairly firm estimates of actual or potential exposure for most of them. The estimates were based on both personnel and area monitoring; Table X shows a few of them. It needs to be noted that actual exposures will vary from person to person within the same job classifications and that accidental, short-term exposures of some workers to concentrations in excess of the mandatory standard probably occur from time to time. Documentation of such incidents is usually difficult. The current U.S. standard for

TABLE IX. Vinyl Chloride (VC) Cohort for Historical-Pro-
spective Study of Mortality Experience. Worker Classifications

VC production plant
Operators
Loaders
Supervisors
Quality control laboratory
Maintenance workers
Electricians
Boiler-makers
Pipefitters
Others
Developmental laboratory

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TABLE X. Estimated Exposure to Vinyl Chloride (VC) for Vinyl Chloride-Related Job-Classifications

Job classification	Estimated exposure in parts per million, as time-weighted-average		
	1973-1974	1960-1972	Before 1960
R&D engineer	1.7	4.4	8.2
Production engineer	1.7	4.4	8.2
Quality control laboratory	8.7	11.4	15.2
Instrument man	1.3	4.0	7.8
Painter	0.7	0.7	0.7
Machinist	2.4	5.1	8.9
Material handler	0.5	0.5	0.5
Production foreman	1.3	4.0	7.8
Control A OP. (Vinyl)	2.8	5.5	9.3
Control B OP. (Oxy & Chloride)	0.7	0.7	0.7
Control C OP. (Chlorination)	2.6	5.3	9.1

TABLE XI. Vinyl Chloride (VC) Workers: U.S. and Dow Standards for Exposure

1948-1958	Dow	No specified standard; exposure levels unknown
1959	Dow	50 ppm, averaged concentration, established as air quality goal
1959-1967	Dow, Texas Division	Estimated average exposures: below 50 ppm
1968-1973	Dow, Texas Division	Estimated average exposure: below 5 ppm
Before 1974	Other U.S. VC producers	No specified standard; exposure levels unknown; some very high
1974	Other U.S. VC producers	Threshold limit value of 500 ppm recommended by ACGIH ¹ as industry guideline; voluntary compliance
1974	Dow, Texas Division	Estimated average exposure; 1-2 ppm
1975	Dow, Texas Division	Estimated average exposure: below 1 ppm

¹American Conference of Governmental Industrial Hygienists

TABLE XII. Vinyl Chloride (VC) Cohort for Historical-Prospective Study of Mortality Experience

Cohort membership:	On Dow payroll
	Actual exposure, or potential exposure
	Exposed at least 4 consecutive work-weeks
Types of exposure:	Continuous
	Intermittent

TABLE XIII. Vinyl Chloride (VC) Cohort for Historical-Prospective Study of Mortality Experience. Cohort Membership

Currently employed	
Formerly with VC unit	
Presently with VC unit	
Present status determined	100%
Formerly employed	
Retired	
Terminated	
Disabled	
Dead	
Present status determined	100%

vinyl chloride is 1 ppm as a time-weighted-average over an 8-hour day. Table XI illustrates the changes in the standard for vinyl chloride that have occurred over the years.

In defining our vinyl chloride cohort for epidemiologic study, it was decided to include (Table XII) only those on the Dow payroll with documented exposure, or significant potential for exposure, to vinyl chloride for at least a four-week-consecutive period. We were able to identify a total of 533 persons meeting these criteria; this group is composed of persons who are working now, or who have worked previously, in the vinyl chloride plant and those who worked with vinyl chloride in the past, but are no longer employed by Dow in any department. The present status of our currently employed workers (Table XIII) was relatively easy to ascertain, but it took considerable effort to arrive at the 100% follow-up figure for former employees. It is our belief, however, that close to 100% follow-up is necessary for proper assessment of the risks that may be faced by any group of chemical workers.

The vinyl chloride cytogenetics study is one of several involving Texas Division employees. A special epidemiologic

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TABLE XIV. Reproductive Consequences of Vinyl Chloride Exposure: Survey of Workmen's wives

	Participation	
	Number of women	Percentage of group
Study group	205	88%
Control group	144	81%

TABLE XV. Comprehensive Surveillance Program for All Workers Exposed to Toxic Chemicals

Periodic Evaluations
History and Physical Examination
Laboratory Tests
Computerize Findings
Mutagenic Evaluation
Cytogenetic Monitoring
Routine Exposures
Accidental Over-Exposures
Other Mutagenicity Tests
Conventional Epidemiology
Mortality
Morbidity
Special Epidemiology
Reproductive Studies
Hospital Records
Vital Statistics

study is now underway to investigate the possibility that increased rates of fetal loss or birth defects in offspring are experienced by wives of vinyl chloride workmen. We have had a gratifying response from the women - both in the study group and a control group - who were asked to participate. The study group was composed of women married to currently employed Dow workmen identified as having had at least two consecutive months of exposure to vinyl chloride. The comparison group was made up of wives of currently employed workmen who did not work in production, a laboratory, or the maintenance department. As shown in Table XIV, almost 350 women, 88 per cent of the study group, and 81 per cent of the control group, agreed to discuss these personal matters with the trained, University

of Texas interviewers. The interviewing is complete, the data are being analyzed, and we hope to have a report of the final results in the literature soon.

Our major contribution to this conference is to demonstrate the feasibility of studying the whole person and that person's family through coordinated, long-term investigations (Table XV). Life-disease processes will be recognized and recorded by medical monitoring and insurance programs, mutagenic exposures and somatic consequences will be recognized by appropriate test methods, and conventional and special epidemiologic studies will be used to evaluate occupational disease and germinal mutation problems. It is only through such a coordinated effort that the hazards of chemical exposure to the human species can be accurately evaluated. Such a program not only benefits the individual worker and family, but society as a whole.

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