Letters to the Editor

PHENYLALANINE HYDROXYLATION IN CULTURED FIBROBLASTS FROM PATIENTS WITH PHENYLKETONURIA

SIR,—Estimation of the activity of phenylalanine hydroxylase in cultured human fibroblasts would permit characterisation of enzyme defects in variants of phenylketonuria (P.K.U.).[1-3] Furthermore, enzyme activity might also be detectable in cultured human amniotic cells, yielding a prenatal test for P.K.U. Differences in hydroxylation-rate between normal individuals and patients with P.K.U. should highlight differences in the expression of the gene coding for phenylalanine hydroxylase in human fibroblasts. We have found that the oxidation of phenylalanine to tyrosine in fibroblastic proteins from controls and patients with P.K.U. is in fact different. (The diagnosis of P.K.U. is based on clinical data only. Phenylalanine levels in serum exceeded 20 mg/dl, but further characterisation by loading tests[3] has not been done.)

Enzyme activity was tested in homogenates of fibroblasts cultured by standard methods. The reaction mixture (final volume 0·15 ml) contained the following components: 0·15 mg protein of homogenised fibroblasts, centrifuged at 45 000*g* for 30 min, "tris" buffer pH 7·44 75 mmol/l, magnesium chloride 3·0 mmol/l, D,L-5,6,7,8-tetrahydrobiopterin dihydrochloride 19 µmol/l (a gift from Dr W. Kapp and Dr S. Skunca, Hoffmann-La Roche, Grenzach-Wyhlen), catalase 0·03 mg, N.A.D.H 0·49 mmol/l, ^{14}C-phenylalanine 0·8 µCi/assay, specific activity 486 mCi/mmol (Amersham Buchler), ^{12}C+^{14}C phenylalanine 76 µmol/l. Incubation was performed at 37°C for 90 min. After chromatographic separation of ^{14}C-phenylalanine from its reaction products and liquid scintillation counting enzyme activity was determined by the amount of ^{14}C-tyrosine formed.

FORMATION OF TYROSINE FROM L-PHENYLALANINE BY PROTEINS FROM HUMAN FIBROBLASTS OF NORMAL PERSONS AND OF PATIENTS CLINICALLY DIAGNOSED AS CLASSICAL P.K.U.

Group	No. of determinations	Tyrosine formed (pmol/h/mg) (mean ± S.D.)
10 controls	15	45·0 ± 4·5
P.K.U.:	15	8·0 ± 6·9
A	6	0·0 ± 1·8
B	3	7·0 ± 5·7
C	3	8·1 ± 4·7
D	3	16·9 ± 6·0

We have compared the proteins of cultured fibroblasts of ten normal individuals of unknown state of heterozygosity matched in age and sex to four fibroblastic strains of patients with P.K.U. As can be seen from the table we observed a mean conversion of phenylalanine to tyrosine (given in pmol/h/mg fibroblastic protein) of 45·0 by normal individuals and 8·0 by patients with P.K.U.

Although three of the four patients oxidise a considerable amount of phenylalanine there is no overlapping in enzymatic activity within the two groups, at least in the range up to the third standard deviation. This result demonstrates in accordance with the values of the standard deviations obtained by testing fibroblastic strains of the same person for several times that P.K.U. can be diagnosed in cultured human fibroblasts.

Further studies will be pursued with incubation of the fibroblasts of these patients with tetrahydropterins as Milstien and Kaufman[4] observed a stimulation of enzyme activity in liver slices by this procedure. Thus cultured human fibroblasts will be an excellent system for testing therapeutic possibilities for patients with P.K.U.

In all cultured fibroblasts *Mycoplasma* infection was excluded.[5]

A detailed description of kinetic characteristics of the phenylalanine hydroxylase in normal individuals compared with patients with P.K.U. is in preparation

We thank Prof. E. Moench and Dr H. Bartsch (Universitätskinderklinik, Berlin), Dr S. Schlenker (Universitätskinderklinik, Goettingen), and Dr D. Feist and his colleagues (Universitätskinderklinik, Heidelberg) for the skin explants from patients with P.K.U., and Prof. V. Hay and Dr W. Noesgen and their collaborators (Allgemeines Krankenhaus, Hamburg-Harburg) for the skin biopsies from normal individuals. This work was supported by the Deutsche Forschungsgemeinschaft (Sch 175/2).

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1. Jervis, P., O'Flynn, M. E., Hsia, D. Y. *Lancet* 1967, i, 928.
2. Hsia, D. Y. *Prog. med. Genet.* 1970, 7, 29.
3. Kazazian, M. E., Schaeffler, G. E., Hsieh, S. *Arch. Dis. Childh.* 1974, 49, 835.
4. Milstien, S., Kaufman, S. *J. biol. Chem.* 1975, 250, 4737.
5. Russell, W. C., Newman, C., Williamson, D. H. *Nature*, 1971, 253, 461.

VINYL CHLORIDE AND MORTALITY?

SIR,—Excess cancer after exposure to vinyl chloride (v.c.) was demonstrated in animals by Viola et al.[6] and Maltoni and Lefemine[7] in Italy, and subsequently suggested by Monson et al.[8] and later definitively demonstrated by several investigations in man in the United States.[9-11] However, Duck et al.[12] of British Petroleum in the U.K. found no excess of cancer mortality—indeed, the longer workers were exposed to v.c., the healthier they seemed to be, as suggested by table II of their report, which shows a decreasing risk of death with an increasing duration of exposure.

In those exposed for less than 10 years, the standardised mortality from all causes was 112, but it fell to 107 for those exposed between 10 and 14 years and to 61 for those exposed for more than 15 years. Several interpretations of these findings are possible: (1) the formulated v.c. as received by B.P. is uniquely non-toxic, (2) v.c. as polymerised or processed at B.P. is uniquely safe, (3) workers at B.P. have a particular genetic endowment which decreases their likelihood for v.c.-induced cancer, or conversely, other working populations[13-14] have a unique susceptibility to v.c., or (4) certain dietary factors unique to the workers at B.P. may scavenge free-radical v.c. (e.g., some have advocated eating lots of onions or garlic containing free sulphydryl groups.[15]) Before venturing any interpretation in biological, occupational, or technological terms, however, a closer consideration of the B.P. data seems wise, especially in view of studies[16-17] which demonstrated that the S.M.R. for total mortality increases with an increased duration of employment, due to elimination of the "healthy worker" effect. If in a follow-up study one selects, for example, a subgroup of workers by the fact that they have achieved at least 15 years' exposure, then none of these workers could have died before the 15th anniversary, so information on risk of dying can only come from the number of man-years at risk and the number of deaths after 15 years. Of course these same men, provided they are properly regrouped together with those dying or coming to the end of the follow-up between, for example, 10 and 14 years can provide similar information for this time-interval—and so on for all previous time-intervals. This

6. Viola, P. L., Bigotti, A., Caputo, A. *Cancer Res.* 1971, 31, 516.
7. Maltoni, C., Lefemine, G. *Ann. N.Y. Acad. Sci.* 1975, 246, 195.
8. Monson, R. R., Peters, J. M., Johnson, M. N. *Lancet*, 1974, ii, 397.
9. Waxweiler, R. J., Stringer, W., Wagoner, J. K., Jones, J., Falk, H., Carter, C. *Ann. N.Y. Acad. Sci.* 1976, 271, 40.
10. Nicholson, W. J., Hammond, E. C., Seidman, H., Selikoff, I. J. *Ibid.* 1975, 246, 225.
11. Tabershaw, I. R., Gaffey, W. R. *J. occup. Med.* 1974, 16, 509.
12. Duck, B. W., Carter, J. T., Coombes, E. J. *Lancet*, 1975, ii, 1197.
13. Stukinger, H. E. *Proc. R. Soc. Med.* 1976, 69, 283.
14. McMichael, A. J., Haynes, S. G., Tyroler, H. A. *J. occup. Med.* 1975, 17, 150.
15. Boyland, D. L., Dement, J., Wagoner, J. K., Bleier, H. P. *Ann. N.Y. Acad. Sci.* 1976, 271, 324.

RE-ANALYSIS OF DATA BY DUCK ET AL. SHOWING [illegible] OBSERVED VERSUS ESTIMATED NUMBERS OF EXPECTED DEATHS AND S.M.R.'s BY DURATION OF EXPOSURE AND CAUSE OF DEATH

Duration of exposure (yr)	Cause of death								
	All causes					Total cancers			
	O	E		S.M.R.		O	E		S.M.R.
		Duck et al	RE	Duck et al	RE		Duck et al	RE	Duck et al	RE
<10	23	74·01	105·46	112	79	23	18·65	26·62	123	86
10–14	28	26·91	23·49	104	119	4	5·23	5·23	58	76
15+	25	41·30	7·09	61	353	5	10·19	1·87	73	428
	Digestive system cancer					Lung cancer				
<10	8	5·04	3·04	196	73	10	7·76	11·06	129	90
10–14	3	2·15	1·62	47	62	3	2·97	2·25	101	133
15+	4	5·31	0·57	121	702	3	4·60	0·82	62	366

O=Observed. E=Expected. RE=Recalculated estimates.

is the principle of the life-table method, which Duck et al. seem to have ignored.

Their table II puts the man-years at risk for the 336 workers who had 15+ years duration of exposure at 6084. Our maximum estimate for this same category is only 4032. We assumed that all 336 workers began employment on Jan. 1, 1948 (the first day of the study period) and had continuous employment until Jan. 1, 1975, and that the 25 deaths in this group occurred on Jan. 1, 1975, thus estimating maximum man-years at risk. Since these workers belonged to the exposure group that completed 15+ years of employment, they would begin to accumulate man-years at risk of dying on Jan. 1, 1963, so by Jan. 1, 1975, the maximum estimate for man-years at risk is 336 men × 12 years of observation (=4032). Duck et al. would seem to have started accumulating man-years of observations for these workers immediately when employment began rather than after 15 years of employment, thus diluting the estimate of the risk of death after 15 years of exposure. We have redistributed the man-years at risk shown in table II of the original paper and recalculated the expected numbers of deaths, and the S.M.R.s. These data are shown in the accompanying table, alongside those reported in the original paper.

The 336 workers in the 15+ years exposure category had passed through the <10 and the 10–14 years exposure categories so we calculated the man-years at risk for the 15+ years exposure category by subtracting 336×10 years (<10 year period) and 336×5 years (10–14 years period) from 6084 to give 1044. The 3360 and 1680 man-years were then redistributed to the <10 year and 10–14 year exposure categories, respectively, and the process was repeated for the 10–14 year exposure category, and these excess years were also redistributed back to the <10 year exposure category. We then calculated the ratio of our man-years to those reported by Duck et al. and applied these ratios to the expected death figures in their report. For example, in the 15+ year exposure category, our estimate for total expected deaths is: 1044/6084 (=0·1716) × 41·30 (expected deaths reported by Duck et al.) = 7·09 (our expected). As shown in the table, there were (25 deaths observed/7·09) × 100=353 as our S.M.R. This is contrasted with an S.M.R. of 61, as reported by Duck et al. These same procedures were carried out for other causes of death using ratios of 0·7615 and 1·4294 for the 10–14 years and <10 years duration of exposure categories, respectively. Since the exact distribution for worker exposure is unknown to us, we recognise that our estimates only approximate the numbers of man-years at risk. However, conditional to this approximation, and contrary to what was stated by Duck et al.[12] ("there was no suggestion of an increased frequency of deaths from the most common malignant diseases"), a definite pattern of excessive site-specific cancer mortality associated with increasing duration of v.c. exposure clearly emerges.

As shown in the table and as expected from previous reports,[9 10 14 15] the S.M.R.'s for total mortality and cancer deaths tend to increase with an increased duration of occupational exposure. This example of misallocation of person-years at risk, the resultant erroneous conclusions, and subsequent reference to these conclusions[16 17] demonstrate the need for standardisation of data-handling techniques for cohort mortality studies, as suggested during a workshop sponsored by the International Agency for Research on Cancer in January, 1975, in Lyon, France.[18]

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*** This letter has been shown to the B.P. workers, whose reply follows.—ED. L.

SIR,—Dr Joan Davis (personal communication) has also pointed out the value of an additional tabulation of deaths more than fifteen years after first exposure in those exposed for at least fifteen years, and this has been calculated. We have just received these results (see table). The only major discrepancy between observed and expected deaths is in cancers of the digestive system. Two of these deaths were from stomach cancer and two from carcinoma of the colon. No firm conclusions can be drawn from such a small amount of data, and only the passage of time will establish whether there is any real excess of cancer mortality in this group.

MORTALITY OCCURRING AFTER FIFTEEN YEARS' EXPOSURE

—	Observed	Expected	S.M.R.
All causes	25	24·13	104
All cancer	8	6·51	123
Digestive system cancer	4	1·98	(202)
Lung cancer	3	2·96	(101)

We would like to point out the methodological errors which led Wagoner et al. to their gross underestimates of expected deaths. An age-standardised death-rate can be calculated only if the age structure of the population is known. The simplistic application of rates derived from a young population to one which is older—as a consequence of excluding the first fifteen years of exposure—will produce a considerable underestimate of expected deaths and hence inflate any mortality ratios which are calculated in this manner. The number of man-years in the long-exposure group will also be in excess of the presumed figure, since exposure records started in 1948, but death-rates have been studied for the period 1955–75. This design makes a life-table analysis for the whole study population impossible.

We investigated mortality during the period 1955–75 for three groups of workers in which each individual was classified by his total duration of exposure. Inevitably the low mortality throughout this period for the men with over fifteen years' exposure will reflect the long period of survival required to enter this group.

A mortality study with a detailed cohort analysis covering the whole U.K. polyvinyl chloride production industry is to be published shortly and the results will certainly be of interest.[19 20]

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16. Baxter, P. J., Fox, A. J. *Lancet*, 1976, i, 243.
17. International Agency for Research on Cancer, Lyon, France. I.A.R.C. internal technical report no. 75/001. January, 1975.
18. Kissin, P. *Ann. N.Y. Acad. Sci.* 1976, 271, 22.
19. Fox, A. J., Collier, P. F. *Br. J. ind. Med.* (in the press).
20. Fox, A. J., Collier, P. F. *Br. J. prev. soc. Med.* (in the press).

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HAEMOPEXIN LEVELS IN 27 RECOGNISED CARRIERS OF DUCHENNE MUSCULAR DYSTROPHY

Status	Haemopexin (mg/dl)	C.P.K. >50 mU/ml
Definite carriers	69·62	—
	113·30	+
	75·44	+
	104·73	+
	101·39	+
	92·05	+
	74·29	+
	141·81	+
	78·62	[illegible]
Probable carriers	93·13	[illegible]
	201·[illegible]	[illegible]
	71·36	[illegible]
	101·39	[illegible]
	106·18	[illegible]
	37·22	—
	106·16	—
Possible carriers with abnormal C.P.K.	111·34	—
	119·46	[illegible]
	116·33	[illegible]
	79·00	[illegible]
	81·25	[illegible]
	92·05	—
	122·92	—
	87·90	—
	1[illegible]·24	[illegible]
	1[illegible]·39	—
	106·75	[illegible]

[illegible] ple mean of the carrier group) should be regarded as suspect in presumed carriers with normal C.P.K. values. The accompanying table gives the individual values of haemopexin in our series of 27 recognised carriers (5 new cases have lately been added), along with the results of the C.P.K. test. The detection rate, calculated on the group of definite and probable carriers, is 55% for haemopexin if we assume a threshold of [illegible] mg/dl. C.P.K. detection rate in the same series is 75% (against about 70% reported in the literature). The concomitant use of the two techniques gives a detection rate of 87%.

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VINYL CHLORIDE AND MORTALITY

SIR,—Wagoner et al.[1] have drawn attention to the misleading presentation of results by Duck et al.[2] who have ignored a basic principle of the "subject-years at risk" method and have accumulated risk in various subgroups over periods when, by definition of these subgroups, deaths could not be occurring. For example, in calculating the subject-years at risk for those whose duration of exposure exceeded 15 years, they included these 15 years in the total subject-years at risk. Thus their observed and expected deaths are not comparable.

In reply, instead of performing the correct analysis, Duck and Carter[3] concentrated on criticising the way Wagoner et al. scaled the expected deaths for the recalculated years at risk without taking into account the important effect of age. It is not difficult to find weaknesses in the approximations used by Wagoner et al., but the full data were not available to them, and it would obviously have been a waste of their effort to refine their approximations, when the correct figures could easily be produced from the data by Duck et al.

The fact that Wagoner et al.'s criticism was fully justified can be seen by comparing the expected deaths in the group with over 15 years' exposure, as given in the paper by Duck et al., and in the reply by Duck and Carter:

Comparison of expected deaths given by Duck et al. and Duck and Carter for duration of exposure greater than 15 years

	Expected deaths A	Expected deaths B	Observed
All causes	41·3	24·2	25
All cancer	13·9	6·5	8
Digestive-system cancer	3·5	2·0	4
Lung cancer	4·8	3·0	3

A: from Duck et al. B: from Duck and Carter.

The point of the criticism is that it is only valid to compare the observed deaths with those expected in column B, and not those in column A, which was done by Duck et al.

Using all the figures given in the paper[2] and the correspondence,[3,4] and applying what appears to us to be reasonable guesstimates, gives the following comparison of observed and expected mortality.

Reanalysis of data by Duck et al. by duration of exposure and cause of death.

	<10 y O	<10 y E	<10 y S.M.R.	10–14 y O	10–14 y E	10–14 y S.M.R.	15+ y O	15+ y E	15+ y S.M.R.
All causes	83	94·0	88	28	24·1	116	25	24·2	103
All cancer	21	21·7	97	4	6·2	65	8	6·5	123
Digestive-system cancer	6	7·2	83	1	1·9	53	4	2·0	200
Lung cancer	10	10·0	100	3	2·6	115	3	3·0	100

O: observed. E: expected.

The figures for the less than 15 years' exposure categories are of course unsatisfactory alternatives to the correct figures calculated from the data, which Duck and Carter appear reluctant to supply. However, there is little evidence of excess mortality, either overall or from cancer, or of a trend with increasing duration of exposure. This is in contrast to the inverse trend given by Duck et al. and the exaggerated trend given by the overcorrection of Wagoner et al.

The "subject-years at risk" method of comparing observed and expected mortality, first set out by Case and Lea[1] over 20 years ago, is an extremely powerful analytical tool, but in common with all statistical and epidemiological methods requires attention to detail in execution.

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SIR,—Duck et al.[2] described the results of a mortality study of men exposed to vinyl-chloride monomer in the manufacture of P.V.C. Weaknesses in the analysis were pointed out by Wagoner et al.,[3] and were corrected by Duck and Carter.[4] Measurement of the effect of increased duration of exposure on the death-rates of groups of workers is, however, not as simple as was indicated, and two further weaknesses in the analysis remain. [illegible]

[illegible] Men exposed for "more than 15 years" should not have been included in the comparison of exposure groups. Such comparisons should be based on the mortality of men who have left the employment of interest. Enterline[5] has pointed out that dose-response relationships established from studies for which the exposure period and the follow-up period overlap are of limited value because a long (continuing) exposure is incompatible with death.

The accompanying figure illustrating the follow-up periods [illegible]

1. Wagoner, J. K., Infante, P. F., Saracci, R. Lancet, July 24, 1976, p. 194.
2. Duck, B. W., Carter, J. T., Coombes, E. J. ibid. 1975, ii, 1197.
3. Duck, B. W., Carter, J. T. ibid. July 24, 1976, p. 195.

1. Case, R. A. M., Lea, A. J. Br. J. prev. soc. Med. 1955, 9, 62.
2. Duck, B. W., Carter, J. T., Coombes, E. J. Lancet, 1975, ii, 1197.
3. Wagoner, J. K., Infante, P. F., Saracci, R. ibid. July 24, 1976, p. 194.
4. Duck, B. W., Carter, J. T. ibid. p. 195.
5. Enterline, P. E. J. occup. Med. 1976, 18, 150.

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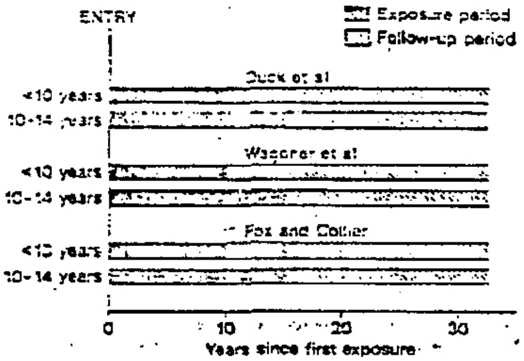
considered by various authors[2,3,4] concentrates on men exposed for less than 15 years. By calculating expected deaths from the date of entry Duck et al. ignored information implicit in the definition of longer exposure—i.e., that men exposed for more than 10 years could not have died within 10 years of entering the industry. Wagoner et al. pointed out this error, but overlooked a second, more subtle bias. Men who because of ill health left this industry within 10 years of entering will automatically be included among men exposed for less than 10 years. This group may therefore be found to have a higher death-rate than that including men exposed for more than 10 years. This is part of the complex process of selection and survival described[8] as the "healthy worker" effect. The implications on death-rates of fit people entering an industry and those that subsequently become unfit leaving it were described at length in 1885.[9]

Mr Collier and I[6,7] recognised these two weaknesses when we analysed the deaths for all men who had been employed in this industry in Great Britain. We concluded that an unbiased measure of the effect of duration of exposure could only be obtained from studies of men who had left the industry within a fixed period and were still alive at the end of that period. We in fact considered a period of 15 years and compared the mortality of men who had been exposed for less than 5 years with that for those who had been exposed for 5–9 years and that for those who had been exposed for 10–14 years. Follow-up for all three groups began 15 years after entry.



Follow-up periods for men exposed for less than 15 years.

It has since been pointed out to us that those men who had left the year before the follow-up might include more men who left because of ill-health than those who had left many years before and survived to follow-up. Although we have not measured this effect it is unlikely to be important for more than one or two years.

Despite these refinements to the analysis we found little evidence to support the suggestion of an excess risk of lung and brain cancer associated with vinyl-chloride exposure in Great Britain. The study did, however, demonstrate an excess of deaths due to cancer of the liver, in particular angiosarcoma.

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6. Fox, A. J., Collier, P. F. Br. J. ind. Med. (in the press).
7. Fox, A. J., Collier, P. F. Br. J. prev. soc. Med. (in the press).
8. McMichael, A. J., Haynes, S. G., Tyroler, H. A. J. occup. Med. 1975, 17, 128.
9. Ogle, W. Supplement to 45th Annual Report of the Registrar General, 1885, pxxiii.

K CELLS AT ISSUE

SIR,—We wish to reply to Dr Mikulski's comments (July 3, p. 44) of our comments[1] on the paper by Cochrane et al.[2]

Dr Mikulski states that Cochrane et al. did not show that only complement-receptor-bearing cells were cytotoxic for hepatocytes. However, Cochrane et al. themselves concluded that the loss of cytotoxicity after removing cells forming EAC-rosettes further supports the assumption that the effector cells are unlikely to be T cells—"Since it suggests that the effector cells have complement receptors." We do not, therefore, imply anything that Cochrane et al. did not conclude, although Dr Mikulski appears to disagree with their conclusions.

We stated that the cytotoxicity system was not antibody dependent, when perhaps we should have stated that their system was not shown to be antibody dependent. Dr Mikulski, however, seems to have interpreted our statement too strongly by attributing to us a categorical exclusion of the antibody-dependent nature of cell mediated cytotoxicity. He presumably meant this incorrect reference to our comment to apply only to the system under debate, since we would hardly dispute the existence of antibody-dependent cellular cytotoxicity (A.D.C.C.) in general.

A.D.C.C. may be mediated by more than one cell, but rather than stress the possible heterogeneity, as Dr Mikulski does, we would emphasise that the common denominators of A.D.C.C. as it is currently understood are the presence of antibody in the system (inherent in the definition) and the presence of a receptor for Fc IgG on the effector cells. K cell designation for the effector cells would be more universally acceptable had Fc IgG receptors been detected rather than receptors for complement. Although receptors for complement have been described in some systems, this receptor is not considered to be the hallmark of this effector cell.

Although we agree with Dr Mikulski that the paper by Cochrane et al. is challenging we would dispute his implication that this renders it exempt from (constructive) criticism.

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ACNE AND TESTICULAR FEMINISATION

SIR,—Shuster[1] suggests that, because of its persistence in "non-hairy mammals", acne could very well confer some biological advantage. He suggests that the sebaceous glands may be a source of precursors for vitamin D synthesis in the epidermis, or perhaps augment and maintain the capacity for delayed immune hypersensitivity.

It would seem that the natural model for testing this hypothesis is individuals with the syndrome of testicular feminisation. These are XY individuals with a female phenotype due to an inborn error of metabolism in which there is end-organ unresponsiveness to androgens. The development and activity of the sebaceous glands in the face and upper trunk is androgen-dependent. Thus, XY individuals with testicular feminisation are not affected by acne, presumably because their sebaceous glands are unresponsive to androgenic stimulation. However, we are unaware of any study relating to the size and activity of their sebaceous glands. Nor has hypovitaminosis D or impaired immunity been described in these people. On the other hand, we are not aware of anyone looking critically at these parameters in patients with testicular feminisation.

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1. Miller, D. J., Dwyer, J. M. Lancet, 1976, i, 909.
2. Cochrane, A. M. G., Moussouros, A., Thomson, A. D., Eddleston, A. L. W. F., Williams, R. ibid. p. 441.
1. Shuster, S. Lancet, 1976, i, 1328.

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Mutation Research, 41 (1976) 131—142

CARCINOGENIC, MUTAGENIC AND TERATOGENIC RISKS ASSOCIATED WITH VINYL CHLORIDE

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(Received May 13th, 1976)

Summary

The data presented demonstrate clearly that vinyl chloride (VC) is related to a significant excess of mortality from cancer of the liver, lung and brain among workers occupationally exposed to VC. The risk of dying from cancer of the lymphatic and hematopoietic system also appears to increase with an increase in latency. These cancer sites could have been predicted by the animal bioassay conducted by Maltoni. With regard to the liver, even the histopathologic type of cancer (angiosarcoma) was observed first in experimental animals. A study of cancer mortality among populations residing proximate to VC polymerization facilities also demonstrated an increased risk of dying from CNS and lymphatic cancer. These latter findings raise cause for concern about out-plant emissions of VC, but without further study these cancers obviously cannot be interpreted as being related to out-plant exposure to VC.

Various test systems now have elicited a positive mutagenic response to VC. Thus, our observations of a significant excess of fetal mortality among the wives of males, who were occupationally exposed to VC, raise public health concern that VC may be mutagenic in humas.

With regard to the teratogenicity of VC, observations of a significant excess of children born with birth defects were reported among populations residing proximate to VC polymerization facilities. Additional epidemiologic study is needed to determine whether a repeated pattern of excessive numbers of children born with birth defects can be observed in other communities with VC polymerization facilities.

Introduction

In 1930, the first adverse health effects of vinyl chloride (VC) were reported [22]. Since then, numerous investigators have reported the toxic effects of VC on the central nervous system, the liver, the bones of the fingers and the lungs

[2,5,9–12,18,20,28]. More recently, the study of VC toxicity has broadened to assess the spectrum of carcinogenesis [2,15,16,21,26,29,30], mutagenesis [1,3,4,6,13,14,23,24]. and teratogenesis [8]. These efforts were stimulated by the work of Viola et al. [29] who, in 1971, reported the induction of tumors of the skin, lungs and bones in rats exposed by inhalation to 30,000 ppm of VC over a period of twelve months. Widespread concern for the carcinogenic activity of VC, however, did not occur until early 1974, when Creech and Johnson [2] reported four deaths from angiosarcoma of the liver among workers employed in the manufacture of polyvinyl chloride (PVC) resins. It was later learned that Maltoni had previously demonstrated VC-induced hepatic angiosarcomas, lung adenomas, brain neuroblastomas, lymphomas and various other tumors in mice, rats and hamsters [15,16].

With regard to mutagenicity, several investigators have induced mutations via microbial test systems [1,14,24]. Also, VC metabolites have induced mutations in mammalian cells [6]. In addition, reports from several countries have demonstrated significant excesses of chromosomal aberrations among workers exposed to VC as contrasted with those not exposed [3,4,13,23]. Because of these observations and of the widespread exposure to VC among both workers and the general population, the National Institute for Occupational Safety and Health (NIOSH) in the U.S.A. undertook an epidemiologic program to evaluate the magnitude and spectrum of VC toxicity to humans. This program was 4-fold in nature and sought to evaluate: (1) The site-specific risk of cancer among workers exposed to VC [30]. (2) The risk of some cancers among populations residing proximate to VC polymerization facilities [8]. (3) The site specific risk of congenital anomalies among populations residing proximate to VC polymerization facilities [8]. (4) The risk of fetal wastage among the wives of workers occupationally exposed to VC [7].

Cancer risk among workers exposed to VC

To assess the neoplastic risk of workers exposed to VC, a population consisting of employes from four VC polymerizing facilitates was selected for cohort mortality study [30]. Since occupationally induced cancers often take many years to become manifest, the study cohort was restricted to workers who had achieved five or more years of employment and for whom at least 10 years had lapsed since initial employment. Thus, the exposure period was five or more years and the latency period was 10 or more years. Follow-up for study cohort members was greater than 99%.

Table I shows the total mortality experience among the study cohort. The expected numbers are based on United States mortality data applied to NIOSH's modified life-table method. For all malignant neoplasms, there were 35 observed vs 23.5 expected, the SMR was 149. This excess was significant at the $P < 0.05$ level of confidence. For total mortality, 136 deaths were observed vs 126.3 expected. Selecting a cohort with at least five years work experience and 10 years latency eliminated the "healthy worker effect" [19] and so there were more total deaths than expected and this was mostly at the expense of cancer mortality.

Table II shows cancer mortality experience by greater than 10 and 15 year latency periods. At the greater than 10 year latency period, only biliary and

TABLE I

MORTALITY EXPERIENCE AMONG COHORT WORKERS EXPOSED TO VINYL CHLORIDE

Cause of death	ICD code [a]	Observed	Expected	SMR [b]
All malignant neoplasms	(140–205)	35	23.5	149 [c]
Heart	(400–443)	57	54.7	104
Non-malignant respiratory diseases	(470–527)	6	3.4	176
Cirrhosis	(581)	2	4.0	50
All other causes		36	40.7	88

[a] ICD, International Classification of Diseases, 7th revision.
[b] SMR, standardized mortality ratio.
[c] Significant at $P < 0.05$.

liver cancer deaths were significantly in excess; however, when the sub-cohort with 15 years of latency since initial employment was used, deaths from three categories of cancer were significantly in excess. The excess in mortality from cancer of the lymphatic and hematopoietic systems was not significant; however, the SMR increased from 159 to 176 with an increase in latency. These comparisons show the importance of latency when looking for occupationally-induced cancers.

TABLE II

CANCER MORTALITY BY INTERVAL SINCE INITIAL EXPOSURE AMONG WORKERS EXPOSED TO VINYL CHLORIDE

Site of malignancy		10+ years		15+ years
All malignant neoplasms	Obs. [a]	35		31
	Exp. [b]	23.5		16.9
	SMR [c]	149 [d]	<	184 [e]
Brain and CNS cancer	Obs.	3		3
	Exp.	0.9		0.6
	SMR	329	<	498 [d]
Respiratory system cancer	Obs.	12		11
	Exp.	7.7		5.7
	SMR	156	<	194 [d]
Biliary and liver cancer	Obs.	7		7
	Exp.	0.6		0.4
	SMR	1155 [e]	<	1606 [e]
Lymphatic and hematopoietic system cancer	Obs.	4		3
	Exp.	2.5		1.7
	SMR	159	<	176
All other malignant neoplasms	Obs.	9		7
	Exp.	11.7		8.4
	SMR	77	<	83

[a] Obs., observed.
[b] Exp., expected.
[c] SMR, standardized mortality ratio.
[d] Significant at $P < 0.05$.
[e] Significant at $P < 0.01$.

In laboratory studies, Maltoni has induced hepatic angiosarcomas, lung adenomas, brain neuroblastomas and lymphomas [16]. Therefore, the predictive value of animal bioassay studies can be demonstrated, not only by the observation of a significant excess of angiosarcoma of the liver among VC workers, but also by significant excesses of lung and brain tumors.

Cancer risks in populations residing near VC polymerization facilities

The risk of some cancers among adult populations residing in the only three Ohio communities with VC polymerization facilities was assessed [8]. All three communities had at least one facility in operation by 1954 and Painesville had a second plant in operation by 1967. The community population sizes varied from 12,000—24,000. Between 1960—70 the population had remained stable in Painesville and Ashtabula, but had increased by 30% in Avon Lake. As reported previously, for the three communities taken as a whole, there were no apparent differences in racial origin or family income as compared to the average for the State [8].

As a result of previous findings [26], cancers of the central nervous system, lymphatic and hematopoietic systems, were selected for study. Because of possible error in death certificate data in terms of metastases from other primary sites, data for lung and liver cancers were not analyzed.

Table III shows data for observed versus expected CNS cancer deaths in the white population by sex for the period 1958—73. North Ridgeville is the only community shown which does not have a PVC polymerization facility. It was included in the analyses of the cancer data because it is located contiguous to Avon Lake and because it had a high incidence of children born with birth defects [8]. If North Ridgeville had not been included in the analyses of cancer mortality, little difference in the results would have been observed. Expected values are based on the occurrence in the balance of the counties over the same period of time. As shown in Table III, there was a significant excess of CNS cancer deaths in males. The excess was greatest in Painesville and North Ridgeville. With all communities combined, there were 27 CNS cancer deaths observed in males versus 14.1 expected; the SMR was 191. The difference was significant at $P < 0.01$.

In females, with all groups combined, there was only a slight excess. With sex groups combined, the excess was significant at $P < 0.01$. It may be noteworthy that one father-daughter combination for CNS tumor deaths occurred in Painesville. The daughter died of a papillary ependymoma in 1963 at the age of 16. The father died two years later of a glioblastoma multiforme at the age of 58. It would be difficult to determine whether this observation is the result of genetic or environmental factors.

Table III also shows deaths from lymphomas. Although the differences between observed and expected deaths in males were not significant, there was a consistent excess in each community. In females, there were excess lymphoma deaths in two of the four communities. With sex groups combined, a significant excess of lymphoma deaths was observed in Ashtabula. With sex groups and communities combined, the number of observed deaths was 61 versus 48.7 expected. The SMR was 125. This was significant at

TABLE III

OBSERVED AND EXPECTED DEATHS FOR THREE TYPES OF CANCER FOR RESIDENTS 45 YEARS AND OLDER IN THE OHIO COMMUNITIES WITH VINYL CHLORIDE POLYMERIZATION FACILITIES, 1958–73

Expected numbers of cancer deaths based on occurrence over the same period of time in the balance of the counties in which the communities are located.

	Males		Females		Sexes combined	
	Obs./Exp.	SMR	Obs./Exp.	SMR	Obs./Exp.	SMR
	CNS cancer (191–192)[a]					
Ashtabula	7/ 6.1	115	6/ 3.8	158	13/ 9.9	131
Painesville	12/ 3.8	316 [d]	2/ 2.8	71	14/ 6.6	212 [b]
Avon Lake	2/ 2.3	87	1/ 1.8	56	3/ 4.1	73
N. Ridgeville	6/ 1.9	316 [b]	2/ 1.5	133	8/ 3.4	235 [b]
Communities Combined	27/14.1	191 [c]	11/ 9.9	111	38/24.0	158 [c]
	Leukemia and aleukemia (204–207)[a]					
Ashtabula	13/14.7	88	11/ 8.6	128	24/23.3	103
Painesville	8/ 7.1	113	7/ 6.8	103	15/13.9	108
Avon Lake	1/ 3.6	28	5/ 2.2	227	6/ 5.8	103
N. Ridgeville	2/ 3.2	63	0/ 1.9	0	2/ 5.1	39
Communities Combined	24/28.6	84	23/19.5	118	47/48.1	98
	Lymphomas (200–203)[a]					
Ashtabula	18/12.6	143	14/ 5.8	241 [c]	32/18.4	174 [c]
Painesville	12/10.4	115	4/ 8.3	48	16/18.7	86
Avon Lake	4/ 3.3	121	5/ 2.9	172	9/ 6.2	145
N. Ridgeville	3/ 2.8	107	1/ 2.6	38	4/ 5.4	74
Communities Combined	37/29.1	127	24/19.6	122	61/48.7	125

[a] International Classification of Diseases, 8th revision codes.
[b] $P < 0.05$.
[c] $P < 0.01$.
[d] $P < 0.002$.

$0.05 < P < 0.10$. Data were then analyzed for leukemia and aleukemia mortality. As shown in Table III, the observed versus expected mortality was virtually identical.

Birth defects among populations residing near VC polymerization facilities

The occurrence of birth defects was studied [8] for the same three Ohio communities with VC polymerization facilities. Birth data for residents of North Ridgeville were not combined with data for the three index communities in the initial analyses (Table IV) because the high incidence of birth defects in North Ridgeville, which lies contiguous to Avon Lake, was identified in subsequent analyses [8]. Data for North Ridgeville, however, were included in subsequent analyses (Table V) for specific birth malformations. The observations among community residents were compared to both the occurrence in

TABLE IV

RESIDENT BIRTHS, MALFORMATION RATE PER 1000 LIVE BIRTHS IN OHIO AND IN THREE SELECTED COMMUNITIES AND OBSERVED VERSUS EXPECTED NUMBERS OF MALFORMATIONS IN EACH CITY, YEARS COMBINED, 1970—73

Malformations are based on codes 740—759 of the International Classification of Diseases, 8th revision, 1968.

Area	Births	Malformations			
		Rate/10^3	Number observed	Number expected [a]	χ^2
Entire state of Ohio	719, 287	10.1	7295	—	—
Ashtabula city	1900	17.4	33	19.3	9.78 [b]
Painesville city	1381	18.1	25	14.0	10.29 [b]
Avon Lake city	738	20.3	15	7.5	7.56 [b]
All three communities combined	4019	18.2	73	40.8	27.13 [c]

[a] Expected numbers are based on state rate per 1000 live births.
[b] $P < 0.01$.
[c] $P < 0.001$.

the balance of the counties in which the communities are located and to the occurrence in the State for the period 1970—73. Between 1970—73, the rate for birth defects changed from 9.3 to 11.3 per 1000 live births for the balance of the counties, from 9.6 to 10.8 for the entire state, whereas, the rate for the three index cities combined changed from 17.0 to 22.5. Thus, the incidence of birth defects for children born in the index cities was almost twice as great as the incidence in the balance of the counties or in the entire State, and the differences appear to be increasing with time.

Table IV shows data for malformation rates in each index community versus the rate for the entire State. These differences were all highly significant. The rates ranged from 17.47 in Ashtabula to 20.33 in Avon Lake, as compared to 10.14 for the entire State. When the community experience was compared to the occurrence in the balance of the counties in which the communities were

TABLE V

OBSERVED, EXPECTED AND RELATIVE RISK FOR SPECIFIC CONGENITAL ANOMALIES IN INDEX AREAS INCLUDING N. RIDGEVILLE, 1970—73 [a]

Defect category	Number of defects		RR [b]
	Observed	Expected	
All defects (740—756, 758, 759) [c]	109	56.0	1.95
Central nervous system (740—749)	17	5.6	3.02
Cleft palate and lip (749)	10	6.5	1.53
Genital organs (752)	16	8.4	1.90
Clubfoot (754)	23	8.2	2.79
All other defects	43	27.2	1.58

[a] Excludes skin, hair and nails (757).
[b] RR, relative risk (observed/expected).
[c] International Classification of Disease codes, 8th revision, are shown in parentheses.

located, the differences remained significant. Therefore, whether you compare the occurrence in the communities to the expected, based on the average for the State, or for the balance of the counties, the excess of children with congenital anomalies in the communities appears to be significant.

Table V shows observed versus expected numbers and the relative risk for total and selected malformations. Although an increase in most organ systems was observed, the greatest excess of severe defects included malformations of the CNS, cleft lip and palate, club foot and genital organs. These observations have been reported previously in more detail [8].

Fetal mortality among wives of workers exposed to VC

Since VC had elicited a positive mutagenic response via microbial test systems [1,6,14,24] and also had been associated with significant excesses of chromosomal aberrations in the lymphocytes of workers occupationally exposed to VC [3,4,13,23], concern was expressed that VC may induce germinal mutations. Thus, a questionnaire-interview survey was conducted to ascertain the incidence of fetal loss (defined as any product of conception not born alive) among wives of workers exposed to VC [7a]. All current VC polymerization and polyvinyl chloride (PVC) fabrication workers were included for study together with a similar number of current rubber workers selected from work areas relatively free from known toxic materials and matched as a group to the VC workers by age. No interviews were conducted with workers' wives and no data were obtained concerning maternal age, except indirectly through paternal age. Group participation rates ranged from 62—77 percent. Data for the wives of VC polymerization workers (study group) were contrasted with data for the wives of PVC fabrication and rubber workers ("controls"), who were known to have had very low or no VC exposure, respectively.

The data in Table VI show the paternal age distribution for fetal deaths according to the husband's exposure. Prior to husband's exposure, the crude fetal death rates for the control and study group were 6.9 and 10.1%, respectively. Subsequent to husband's exposure, the crude rates were 8.8 and 16.5%, respectively.

As can be seen in Table VI, the excess in fetal mortality subsequent to husband's exposure, was associated with younger-aged husbands. For husbands 30 years of age and older, the rates for the control group 17/142 (12.0%) and primary VC exposure group 9/69 (13%) were about the same; whereas, for husbands less than 30 years of age, the rates for the control group 7/131 (5.3%) and primary VC exposure group 14/70 (20.0%) were significantly different, $P < 0.001$, $\chi^2 = 10.52$, with one degree of freedom (df). The excess of fetal mortality among wives of younger-aged husbands may be a reflection of a practice of placing newly hired personnel because of little or no seniority, in jobs where occupational exposures to VC may have been worse. This hypothesis, however, needs further assessment in other working populations. Since parental age was positively correlated with fetal mortality in this population and in a previous study [25], fetal mortality rates for the study group were adjusted to the age-distribution of the control group.

TABLE VI

PATERNAL AGE DISTRIBUTION FOR FETAL DEATHS ACCORDING TO HUSBAND'S VC EXPOSURE

Paternal age group (years)	"Controls"			Primary VC Exposure		
	Pregnancies	Fetal deaths		Pregnancies	Fetal deaths	
	(N)	(N)	(%)	(N)	(N)	(%)
	Prior to husband's exposure					
<20	31	2	6.5	7	0	0.0
20—24	80	4	5.0	44	2	4.5
25—29	38	4	10.5	56	7	12.5
30—34	6	1	16.7	27	5	18.5
≥35	4	0	0.0	14	1	7.1
All ages crude rate	159	11	6.9	148	15	10.1
Mean paternal age at conception (year)	23.0			26.4		
Age-adjusted [a] rate			(6.9)			(6.1)
	Subsequent to husband's exposure					
>20	1	0	0.0	0	0	0.0
20—24	43	4	9.3	22	3	13.6
25—29	87	3	3.4	48	11	22.9
30—34	87	7	8.0	36	3	8.3
≥35	55	10	18.2	33	6	18.2
All ages crude rate	273	24	8.8	139	23	16.5
Mean paternal age at conception (year)	30.4			30.2		
Age- adjusted [a] rate			(8.8)			(15.8)

[a] Fetal mortality rates for primary VC exposure group are direct age-adjusted to the paternal age-distribution of the pregnancies in the control group (shown in parentheses).

TABLE VII

MEAN PATERNAL NUMBER OF PREGNANCIES AND AGE-ADJUSTED FETAL DEATH RATES ACCORDING TO HUSBAND'S VC EXPOSURE

	"Controls" [a]	Primary VC exposure [b]
	Prior to husband's exposure	
Number of families	95	70
Mean paternal age at conception (years)	23.0	26.4
Number of fetal deaths among wives	11	15
Number of pregnancies	159	148
Age-adjusted fetal deaths/100 preg. [c]	6.9	6.1
	Subsequent to husband's exposure	
Number of families	113	62
Mean paternal age at conception (years)	30.4	30.2
Number of fetal deaths among wives	24	23
Number of pregnancies	273	139
Age-adjusted fetal deaths/100 preg. [c]	8.8	15.8 [d]

[a] Rubber and PVC fabrication workers.
[b] VC polymerization workers.
[c] Rates age-adjusted to "control" group paternal age distribution.
[d] Subsequent to husbands' exposure, the frequency of fetal deaths among wives was significantly greater in the primary VC exposure group as compared to the "controls" ($P < 0.05$) or to the frequency in the study group prior to husband's exposure ($P < 0.02$) by age-adjusted Chi-square testing.

The data in Table VII show fetal death rates per 100 pregnancies for the wives of male workers in the control and study groups. Prior to their husband's exposures, the rates were 6.9% and 6.1% for the control and study groups, respectively. Subsequent to husband's exposure, however, the rates were 8.8% for the control group versus 15.8% for the study group. This difference was significant at the $P < 0.05$ level by Mantel-Haenszel Chi-square testing [17] ($\chi^2 = 4.84$, df = 1). Further, the before and after exposure comparisons indicated changes in rates from 6.9 to 8.8% for the control group as compared to a change from 6.1 to 15.8% for the study group. The rates for before and after husband's exposure in the study group were significantly different ($P < 0.025$, $\chi^2 = 5.78$, df = 1). For the study group, it may be noted that before exposure age-adjustment reduced the crude rate from 10.1 to 6.1%; whereas, after exposure the crude rate was reduced from 16.5 to 15.8%. The greater reduction in rates for the before exposure age-adjustment resulted from a difference in the age distribution of the husbands in the two groups. As can be seen in Table VII, prior to husband's exposure, the mean paternal age in the study group was 26.4 years as compared to only 23.0 years for the control group, whereas, subsequent to husband's exposure, the mean ages of the two groups were virtually the same, i.e., 30.4 years versus 30.2 years. In both situations mean age was a good measure of central tendency.

To determine whether women who had chronically experienced abortions might have weighted the results in favor of a higher fetal mortality rate in the primary VC exposure group, data for the pregnancies of women who had two, three or four or more spontaneous abortions were eliminated. The data were then recalculated to determine whether or not the trend of a greater miscarriage rate could be maintained. As shown in Table VIII, the trend was maintained for each analysis.

TABLE VIII

NUMBER OF PREGNANCIES AND AGE-ADJUSTED FETAL DEATH RATES ACCORDING TO HUSBAND'S VC EXPOSURE EXCLUDING PREGNANCIES IN WOMEN WITH ≥2, 3 OR 4 FETAL DEATHS

Rates for the primary VC exposure group are age-adjusted to the Control group

	Controls		Primary VC exposure	
	Number of pregnancies	Fetal death rate	Number of pregnancies	Fetal death rate
	≥2 Fetal deaths excluded			
Before husband's exposure	155	5.8%	126	1.7%
After husband's exposure	255	4.7%	111	6.2%
	≥3 Fetal deaths excluded			
Before husband's exposure	159	6.9%	141	3.1%
After husband's exposure	265	6.8%	120	10.8%
	≥4 Fetal deaths excluded			
Before husband's exposure	159	6.9%	142	5.8%
After husband's exposure	265	6.8%	127	11.8%

As reported previously [7a], additional analyses suggested that the significant excess in fetal mortality after the husband's exposure would not seem to be the result of bias from interviewers nor from respondents. Because of the highly volatile nature of vinyl chloride [27], carry-home exposure to the wife would seem unlikely. Therefore, the leading possibility for the mechanism involved would seem to be germ-cell damage in the male through direct VC exposure.

References

1 Bartsch, H., C. Malaveille and R. Montesano, Human, rat and mouse liver-mediated mutagenicity of vinyl chloride in *S. typhimurium* strains, Int. J. Cancer, 15 (1975) 429—437.
2 Creech, J.L., Jr. and M.N. Johnson, Angiosarcoma of liver in the manufacture of polyvinyl chloride, J. Occup. Med., 16 (1974) 150—151.
3 Ducatman, A., K. Hirschhorn and I.J. Selikoff, Vinyl chloride exposure and human chromosome aberrations, Mutation Res., 31 (1975) 163—168.
4 Funes-Cravioto, F., B. Lambert and J. Lindsten, Chromosome aberrations in workers exposed to vinyl chloride, Lancet 1 (1975) 459.
5 Gedrigk, P., R. Muller and H. Bechtelsheimer, Morphology of liver damage among polyvinyl chloride workers, Ann. NY Acad. Sci., 246 (1975) 278—285.
6 Huberman, E., H. Bartsch and L. Sachs, Mutation induction in Chinese hamster V79 cells by two vinyl chloride metabolites, chloroethylene oxide and 2-chloroacetaldehyde, Int. J. Cancer, 16 (1975) 639—644.
7a Infante, P.F., J.K. Wagoner and A.J. McMichael, Genetic risks of vinyl chloride, Lancet, 1 (1976) 734—735.
7b Infante, P.F., J.K. Wagoner, A.J. McMichael, R.J. Waxweiler and H. Falk, Genetic risks of vinyl chloride, Lancet, 1 (1976) 1289—1290.
8 Infante, P.F., Oncogenic and mutagenic risks in communities with polyvinyl chloride production facilities, Ann. NY Acad. Sci., 271 (1976) 49—57.
9 Kramer, C.G. and J.E. Mutchler, The correlation of clinical and environmental measurements for workers exposed to vinyl chloride, J. Amer. Ind. Hyg. Assoc., 33 (1972) 19—30.
10 Lange, C.E., S. Juhe, G. Stein and G. Veltman, Further results in polyvinyl chloride production workers, Ann. NY Acad. Sci., 246 (1975) 16—21.
11 Lester, D., L.A. Greenberg and W.R. Adams, Effects of single and repeated exposures of humans and rats to vinyl chloride, J. Amer. Ind. Hyg. Assoc., 24 (1963) 265—275.
12 Lilis, R., H. Anderson, W.J. Nicholson, S. Daum, A.S. Fischbein and I.J. Selikoff, Prevalence of disease among vinyl chloride and polyvinyl chloride workers, Ann. NY Acad. Sci., 246 (1975) 22—41.
13 Loken, E. and E. Thils-Evensen, Preliminary report on the medical examination of 288 employees at the PVC plant, Norsk Hydro a.s., Porsgrunn Fabrikker, Porsgrunn, Norway, unpublished.
14 Loprieno, N., R. Barale and S. Baroncelli, Evaluation of the genetic effects induced by vinyl chloride monomer (VCM) under mammalian metabolic activation: studies in vitro and in vivo, Mutation Res., 40 (1976) 85—95.
15 Maltoni, C., Preliminary report on the carcinogenicity bioassays of vinyl chloride, U.S. Dept. Labor Informal Fact-Finding Hearing on Possible Hazards of Vinyl Chloride Manufacture and Use, Washington, D.C., February 1974.
16 Maltoni, C. and G. Lefemine, Carcinogenicity bioassays of vinyl chloride, Current results, Ann. NY Acad. Sci., 246 (1975) 195—218.
17 Mantel, N. and W. Haenszel, Statistical aspects of the analysis of data from retrospective studies of disease, J. Natl. Cancer. Inst., 22 (1959) 719—748.
18 Marsteller, H.J., W.K. Leibach, R. Muller and P. Gedigk, Unusual splenomegalic liver disease as evidenced by peritoneoscopy and guided liver biopsy among polyvinyl chloride production workers, Ann. NY Acad. Sci., 246 (1975) 95—134.
19 McMichael, A.J., S.G. Haynes and H.A. Tyroler, Observations on the evaluation of occupational mortality data, J. Occup. Med., 17 (1975) 128—131.
20 Miller, A., A.S. Teirstein, M. Chuang, I.J. Selikoff and R. Warshaw, Changes in pulmonary function in workers exposed to vinyl chloride and polyvinyl chloride, Ann. NY Acad. Sci., 246 (1975) 42—52.
21 Nicholson, W.J., E.C. Hammond, H. Seidman and I.J. Selikoff, Mortality experience of a cohort of vinyl chloride-polyvinyl chloride workers, Ann. NY Acad. Sci., 246 (1975) 225—230.
22 Patty, F.A., W.P. Yant and C.P. Waite, Acute response of guinea pigs to vapors of some new commercial organic compounds, Public Health Reports, 45 (1930) 1963—1971.
23 Purchase, I.F.H., C.R. Richardson and D. Anderson, Chromosomal and dominant lethal effects of vinyl chloride, Lancet, II (1975) 410—411.

24 Rannug, U., A. Johansson, C. Ramel and C.A. Wachtmeister, The mutagenicity of vinyl chloride after metabolic activation, Ambio, 3 (1974) 194—197.
25 Shapiro, S., E.W. Jones and P.M. Densen, A life table of pregnancy terminations and correlates of fetal loss, Millbank Quarterly, 40 (1962) 7—45.
26 Tabershaw, I.R. and W.R. Gaffey, Mortality study of workers in the manufacture of vinyl chloride and its polymers, J. Occup. Med. 16 (1974) 509—518.
27 United States Environmental Protection Agency, Sampling and analysis of select toxic substances. Task III, vinyl chloride, Contract No. 68—01—2646, Jan 20, 1976.
28 Veltman, G., C.E. Lange, S. Juhe, G. Stein and U. Baehner, Clinical manifestations and course of vinyl chloride disease, Ann. NY Acad. Sci., 246 (1975) 6—17.
29 Viola, P.L., A. Bigotti and A. Caputo, Oncogenic response of rat skin, lungs and bones to vinyl chloride, Cancer Res. 31 (1971) 516—519.
30 Waxweiler, R.J., W. Stringer, J.K. Wagoner, J. Jones, H. Falk and C. Carter, Neoplastic risk among workers exposed to vinyl chloride, Ann. NY Acad. Sci., 271 (1976) 39—48.