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A retrospective mortality study of substituted anthraquinone dyestuffs workers

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ABSTRACT Because short-term bacterial tests have previously shown that about one-third of substituted anthraquinones tested are capable of causing reverse mutation, and two-year feeding studies of three such dyestuffs in rats have shown an excess of hepatocellular carcinomas, a retrospective cohort mortality study was carried out on a population of 1975 male workers employed in a dyestuffs manufacturing plant in Scotland. The population was identified as having worked for more than six months within the factory during the decade 1 January 1956–31 December 1965, and their mortality experience was followed up to 30 June 1980. Age-standardised mortality rates did not show any excess in total or cancer-related mortality.

In 1966 Laham[1] and his co-workers reported the induction of benign mammary adenomas "and other benign tumours" in female rats, and one small-cell sarcoma of the intestine and a neurofibrosarcoma in male rats fed with 1-aminoanthraquinone once a week as a corn-oil suspension.

In 1975 Tamaro *et al*[2] observed that 1-amino-4-hydroxyanthraquinone was a bacterial frameshift mutagen without metabolic activation, whereas 1,4-diaminoanthraquinone, 1-methyl-amino-4(2-hydroxyethyl)anthraquinone, and 1,5-diamino-4,8-dihydroxy-3-anthraquinone sulphanilic acid (sodium salt) were not mutagens.

Further evidence was produced a year later when Brown and Brown[3] reported that, of 90 substituted anthraquinones and related anthracene derivatives, 35% proved to be mutagenic in *Salmonella typhimurium* reverse mutation tests. These workers showed that certain anthraquinone compounds with free hydroxyl groups were direct frameshift mutagens; certain anthraquinone compounds with primary amino, and in a few cases secondary amino, groups were frameshift mutagens; and certain anthraquinone compounds with one or more nitro groups were also frameshift mutagens, although these showed the least specificity regarding both strain of bacteria reverted and microsomal activation. The mutagenic effect of the latter two groups of compounds was potentiated by mammalian microsome preparations.

Feeding studies with mice by Murthy *et al* in 1977[4] showed that, at dietary levels of 0·03% and 0·06%, 1-amino-2-methylanthraquinone induced subcutaneous tumours in 97% of the animals tested. Similar studies in rats, reported by the National Cancer Institute in 1979,[5] showed excess hepatocellular tumours in animals fed with 2-amino-anthraquinone, 1-amino-2-methylanthra-quinone, and 2-methyl-1-nitroanthraquinone.

The results of these studies suggest that a carcinogenic hazard for man may be associated with exposure to substituted anthraquinones. No epidemiological evidence was available from reports and it was therefore considered appropriate to test the toxicological findings by setting up a historical cohort study among a group of dyestuffs manufacturing workers. At the same time it was decided to carry out *S typhimurium* reverse mutation (Ames) tests on 13 highly purified substituted anthraquinones and five commercial grade anthraquinone dyestuffs at the central toxicology laboratory of ICI. Five of these selected purified chemicals had already been reported positive in the Ames test.[3] The theoretical possibilities of combinations with DNA were also considered.[6] All except two compounds tested were positive in the Ames test, confirming some published results and increasing our concern that it was important to examine the health records of workers in our dyestuffs factory.

A talk on this subject was given by Dr Gardiner at a meeting of Medichem in September 1980 at Tokyo.

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Manufacture

Substituted anthraquinones have been used in the dyeing industry for over 100 years. Production of these dyes and manufacture of their intermediates began at the Grangemouth factory of ICI in Scotland in the early 1920s and has continued until the present date. During this time they have represented the main class of dyestuffs produced at this factory. Several other products (pharmaceuticals, for example) have also been manufactured during and since the second world war, but these have never amounted to more than 20% of the total output of the factory.

Traditionally, substituted anthraquinone dyestuffs have been produced in small batch multi-product plants grouped in a number of buildings known locally as sheds.

No retrospective hygiene measurements are available for the study group, although the assessment of the works medical officer and process managers was that exposure during the years before the identification of the cohort, in the 1930s, 1940s, and 1950s, would not be acceptable by today's standards, in that workers were subjected to skin contact and dust exposure both from manufactured dyestuffs and from their intermediates.

Proportional mortality studies

Causes of death of those employees who had died in service or as pensioners from 1962 onwards were available for examination in a company computer system. The number of deaths and the proportional mortality did not differ from those expected on the basis of Scottish national statistics. It was, nevertheless, thought important to carry out an epidemiological study in greater detail.

Material and methods

IDENTIFICATION OF STUDY POPULATION

A cohort of employees who had worked at the factory at any time from 1 January 1956 to 31 December 1965 was identified from personnel records. These dates were chosen because it was thought that any excess deaths attributable to malignant disease would be observed by the late 1970s. Additionally, records of employees were of a less satisfactory nature before 1956. The following information was recorded when available: name; last known address; ICI Pension Fund number; date of birth; date of first engagement; date of last leaving the factory; reason for leaving; job location (department or shed); and type of job. Those who had worked for under six months were excluded; in all 3162 employees were listed.

Employees not engaged in chemicals manufacture were then classified as clerical, accountant, personnel, and others. They totalled 923, leaving 2239 employees who had real or potential exposure to raw materials, intermediates, and final products of the factory, and these are referred to as the "exposure group." This division is to some extent arbitrary, because no accurate information on the exposure history of any individual was available.

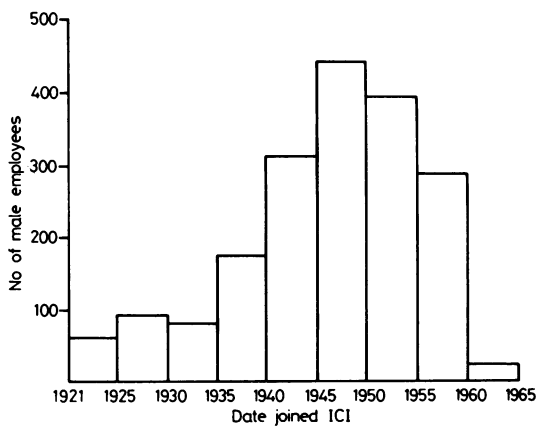
This exposure group contained both men and women: for the purposes of further analysis it was decided that the women should be excluded since it became apparent that a large proportion of them were lost to follow-up. This left 1975 men who were exposed in their working environment to substituted anthraquinones.

The figure shows the distribution for this group of the dates of starting employment. It shows that, although the cohort was defined by employment in 1956–65, it contains many men whose experience at the factory began in previous decades.

FOLLOW-UP OF DIRECT EXPOSURE GROUP

From details listed in personnel records, individuals who were still employed and those who had retired on pension could be followed up using the ICI Pension Fund lists. If they were dead details of the death certificate could also be obtained within the company. People who had died in service could be similarly traced. In this way a large proportion of the group could be traced using company resources.

Of the 1975 individuals in the exposure group, 45 had emigrated, while 676 had left the company voluntarily and therefore had to be traced using the National Records Offices at Edinburgh and Newcas-



Distribution of study group by date of starting work at the factory.

tle upon Tyne. These institutions were able to account for all but 11 of the 676 so that, at the time of writing, 56 people were lost to follow-up, representing 2.8% of the study population. The total number of deaths recorded at 30 June 1980 was 470.

METHOD OF ANALYSIS

The control population for the direct exposure group was taken from Scotland as a whole, as published in the *Annual Report, 1975, of the General Register, Scotland,—Part 1, Mortality Statistics.* This document contains the annual death rates from 1965 to 75 by sex and age, subdivided by region. For this analysis the 1975 data were used, although the effect on the results of using 1966, 1968, 1970, and 1972 data were checked; little difference was noted.

Causes of death were coded according to the World Health Organisation's *International Classification of Diseases, Vol 1, 1977.* Underlying (primary) cause of death only was noted, although if a malignant neoplasm was registered as a contributory (secondary) cause it was included in the analysis of the data (this occurred only once).

The numerical analysis of the data was facilitated by using a computer program designed specifically for epidemiological calculations. This program, MYCL (man-years computer language),[7] enables the expected number of deaths from any cause within an exposure group to be calculated and expressed within specific age ranges. This expected number was compared with the observed number, the data treated as a Poisson variate, and the standardised mortality ratio (SMR) was calculated.

The date of entry of an individual into the study was either 1 January 1956 or the date of engagement if before 31 December 1965, whichever was later. The date of exit from the study was the date of death, if appropriate, or 30 June 1980, the date of analysis.

Results

The calculated SMRs for deaths from all causes, and for the most frequent causes (cardiovascular disease, cerebrovascular disease, and malignant neoplasms) are shown in tables 1–4. In all cases the overall SMRs are lower than would have been expected in Scotland as a whole. The mortality ratio for all cancers falls as age increases: this may be attributable to a survivor effect and to a healthy worker effect.

Table 5 shows an analysis of deaths due to malignant neoplasms, classified by anatomical site. Deaths from cancer of the respiratory tract (ICD 162–162·9: trachea, bronchus, and lung) make up, as expected, the largest proportion but, for the

Table 1 *Deaths from all causes*

No of deaths			
Age groups	*Expected*	*Observed*	*SMR*
15–24	1·5	—	—
25–34	7·1	7	98·6
35–44	28·5	24	84·2
45–54	94·3	79	83·8
55–64	186·6	155	83·1
65–74	201·8	136	67·4
75–84	85·9	66	76·8
≥85	5·8	3	51·7
			Overall SMR
Total	611·5	470	76·9

SMR = Standardised mortality ratio.

Table 2 *Deaths from cerebrovascular disease*

No of deaths			
Age groups	*Expected*	*Observed*	*SMR*
15–24	0	0	—
25–34	0·4	1	—
35–44	1·7	0	—
45–54	5·9	4	66·7
55–64	16·8	6	35·3
65–74	26·7	18	66·7
75–84	13·8	7	50·4
≥85	1·2	0	—
			Overall SMR
Total	66·5	36	54·1

SMR = Standardised mortality ratio.

Table 3 *Deaths from cardiovascular disease*

No of deaths			
Age groups	*Expected*	*Observed*	*SMR*
15–24	0	0	—
25–34	0·6	0	—
35–44	9·0	10	111·1
45–54	40·5	37	91·4
55–64	77·4	62	80·1
65–74	75·8	51	67·3
75–84	30·4	27	88·8
≥85	1·9	1	—
			Overall SMR
Total	235·6	188	79·7

SMR = Standardised mortality ratio.

Table 4 *Deaths from all malignant neoplasms*

No of deaths			
Age groups	*Expected*	*Observed*	*SMR*
15–24	0·1	0	—
25–34	1·0	2	200
35–44	5·2	5	96·2
45–54	23·2	23	99·1
55–64	51·1	49	95·9
65–74	52·0	33	63·5
75–84	16·5	17	103·0
≥85	0·6	0	—
			Overall SMR
Total	149·7	129	86·2

SMR = Standardised mortality ratio.

cohort as a whole, no anatomical site showed a significant increase in fatal neoplasms.

Table 6 shows the distribution of deaths from cancer, classified according to shed or department. In such an analysis significant excesses are almost sure to be found, even if no causative hazard exists: it is therefore necessary to seek a causative chemical before assuming medical as well as statistical significance. Table 6 shows that an excess of deaths from cancer are found at several sites—in the engineering and labour departments, and in sheds 1, 2, 3, 5, 11, and 13. All six patients with oesophageal cancer found in the cohort were men in the engineering department. These cases have been discussed separately because, within this department only, they form a significant excess.

Three process sheds show significant statistical differences between observed and expected deaths at the 5% level, although the numbers concerned are so small that this is unlikely to have any medical significance.

A prostatic cancer that was a contributory cause of death has been included for shed 3, which brings the total number of identified prostatic cancers in this shed to three, compared with an expected 0·09. This is a highly statistically significant result.

Table 4 has been recalculated, setting the date of entry to the study at 1 January 1966. Individuals in the cohort who had died before this date were excluded from the calculations. The results are shown in table 7, with little effect noted in the overall SMRs.

Discussion

The calculations outlined in tables 1–4 indicate that for any of the main causes of death there is no overall excess mortality at the factory producing substituted anthraquinone dyestuffs. There is a pronounced lowering of the SMRs for the older age-groups, which is probably due to a survivor effect.[8]

Excess mortality has been found in individual departments, particularly engineering and shed 3 of the process department. In the engineering department an excess of oesophageal cancer was found: indeed, this disease appeared nowhere else within the factory. No common employment experience could be traced on examination of the factory records of the individuals concerned, and though it is known that they spent most of their time in and around the manufacturing sheds, there is nothing to connect them with any particular exposure that was not experienced by a large proportion of the process operators. Engineering department contributed 45% of the total group. It would, therefore, be reasonable to assume that if there is a causative factor for this disease then it should be evenly distributed throughout the whole of the study population and, therefore, the data indicated that the excess of oesophageal cancer is unlikely to be associated with chemical exposure.

Table 5 *Distribution of malignant neoplasms by anatomical site*

No of neoplasms			
Site	*Expected*	*Observed*	*SMR*
Oesophagus	4·3	6	139·5
Stomach	18·1	14	77·3
Intestinal tract	11·0	7	63·6
Liver and intrahepatic bile duct	3·3	1	—
Pancreas	6·8	4	58·8
Bladder	5·6	4	71·4
Respiratory	66·2	64	96·7
Leukaemia	3·1	2	64·5
Prostate	8·0	4*	50·0
Rectum	6·8	6	88·2
Kidney	2·9	4	137·9
Brain	3·5	4	114·2
Hodgkin's disease	1·6	2	125·0
Testis	0·6	2	333·3
Jaw	0·2	2	1000
Splenic flexure	—	1	—
No primary site	—	2	—
Total		129	

*Another death (not due to malignant neoplasm) included malignant neoplasm of prostate as a secondary cause.

Table 6 *Distribution of malignant neoplasms by works department*

No of neoplasms				
Department	*Population*	*Expected*	*Observed*	*SMR*
Engineering	898	73·7	50	67·8
Warehouses	60	6·4	5	78·1
Labour	108	12·6	13	103·2
Process:				
Shed 1	36	1·8	5	277·8†
Shed 2	17	0·7	2	285·7†
Shed 3	48	2·3	4*	173·9†
Shed 4	170	10·6	9	84·9
Shed 5	49	2·6	3	115·4
Shed 6	31	1·9	2	105·3
Shed 7	44	4·0	2	50·0
Shed 8	37	2·2	1	—
Shed 9	20	2·0	1	—
Shed 10	28	1·8	0	—
Shed 11	71	4·7	7	149·0
Shed 12	10	0·5	0	—
Shed 13	72	4·6	7	152·2
Shed 14	41	2·4	1	—
Shed 15	42	3·2	2	62·5
Shed 16	56	3·0	2	66·7
Shed 17	19	1·7	0	—
Shed 18	3	0·1	0	—
Shed 19	62	3·9	3	76·9
Others	53		10	
Total	1975		129	

*Another death (not due to malignant neoplasm) included malignant neoplasm of prostate as a secondary cause.
†Significant (p 0·05).

Table 7 *Malignant neoplasms in population at 1 January 1966*

No of neoplasms			
Age groups	*Expected*	*Observed*	*SMR*
15–24	—	0	—
25–34	0·4	0	—
35–44	2·7	2	74·1
45–54	14·2	15	105·6
55–64	34·7	35	100·9
65–74	43·5	26	59·8
75–84	16·7	17	101·8
≥85	0·6	0	—
			Overall SMR
Total	112·8	95	84·2

SMR = Standardised mortality ratio.

In process shed 3 three cases of prostatic cancer, including one listed as a secondary death cause, were found. The total for the whole exposure group was five, including the secondary. This significant result is difficult to interpret. No industrial cause of malignant neoplasm of the prostate has so far been identified, although an association with cadmium has been reported.[9] Cadmium in any of its forms has not been used in any part of the factory, so it seems reasonable to discount this source.

Excess mortality from cancer was also observed in the labour department and in sheds 1, 2, 5, 11, and 13. It is difficult to give any exact interpretation to these results, particularly in the process sheds, because of the small number involved, and great care should be exercised when doing so. Examination of the types of cancer within these areas showed that the bulk of them were respiratory and when these were analysed they did not prove to be significant.

The process of admitting subjects to the study on 1 January 1956 or their date of appointment, whichever was later, made use of all the information available but maximised any bias due to the healthy worker effect.[10] Calculations using 1 January 1966 as the date of entry into the study show that the results reported were not significantly affected (table 7).

It is interesting to speculate why, although many substituted anthraquinone compounds are experimental bacterial mutagens and a few are known, albeit weak animal carcinogens, the study population appears to have no abnormal mortality pattern. One conclusion might be that there is a species difference between rat and man for these compounds. The other might be that, even under the poor working conditions described, neither the exposure nor the absorption were sufficient to cause an observable mortality effect.

Conclusions

Whereas long-term animal (rodent) toxicological tests are of great importance in trying to forecast the possible effects of chemicals intended to be introduced into commercial use, and whereas laboratory short-term (eg Ames) tests are also of use for this purpose, the health effects of chemicals that have been in use over several years or decades are much more realistically assessed by studies of human exposed populations.

One-third of substituted anthraquinones studied are "Ames-positive" and in feeding studies in rats three have now been found to be carcinogenic. In this study work with these compounds has not produced an abnormal pattern of mortality in a population producing dyes from 1 January 1956 to 31 December 1965 and who have been followed up to 30 June 1980.

It is concluded that the extrapolation to man of bacterial mutagenicity data for hitherto untested chemicals should be approached with caution and that where possible the effects of chemicals on health are more realistically assessed by studies of exposed populations.

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