

## A FURTHER CONTRIBUTION TO THE KNOWLEDGE OF CARCINOGENIC EFFECT OF AROMATIC AMINES

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### ABSTRACT

Cause-specific mortality of 906 workers first employed between 1922 and 1970 in a dyestuff factory in Northern Italy was compared in this study to national figures: a marked excess of urinary bladder cancer was observed (36 observed vs. 1.23 expected deaths). The mean latent period was 25 years. The excess was higher among those with longer duration of exposure. Mortality from urinary bladder cancer was much higher among those manufacturing benzidine and naphthylamines than in those exposed to these compounds through use or intermittent contact only. The excess urinary bladder cancer was also very high among fuchsine manufacturers. There was evidence that *o*-toluidine and 4,4' methylene bis (2-methylaniline) should be implicated in such excess mortality. Authors therefore suggest caution in handling these compounds and stress the need for further studies to confirm the findings.

This survey has been undertaken on workers engaged in a dyestuff factory in Northern Italy in order to assess whether evidence could be obtained of the carcinogenicity of some aromatic amines not already recognized as being carcinogenic to man.

### SUBJECTS AND METHODS

It has been possible to follow up 868 workers out of 906 first employed between 1922 and 1970 and to compare mortality during the period from 1946 through 1976 to national figures, according to the usual method of man-years<sup>2</sup>.

Detailed information was available from factory archives on chemical compounds manufactured and used as well as on processes carried out in each department of the plant. The date of the start and end of each operation was reported. The type of exposure for each worker was therefore defined by linking individual information and knowledge of chemical processes corresponding to the jobs mentioned in personal records.

### RESULTS

In the period from 1946 through 1976 there were 36 deaths from urinary bladder cancer, whereas only 1.23 were expected to occur on national basis.

TABLE 1  
Urinary bladder cancer. Observed and expected deaths by duration of exposure and latent period.

Duration of exposure (years)	Observed	Expected	$\frac{\text{Observed}}{\text{Expected}}$	Latent period (years)	Observed	Expected	$\frac{\text{Observed}}{\text{Expected}}$
Up to 10	8	0.64	12.50	Up to 10	0	0.10	—
10-20	13	0.38	34.21	10-20	8	0.37	21.62
21 and over	15	0.21	71.43	21 and over	28	0.76	36.84
Total	36	1.23	29.27	Total	36	1.23	29.27

Table 1 shows the distribution of these deaths and the expected number according to the duration of exposure and the elapsed time since the first exposure or latent period. It can be seen that there is an increasing risk with increasing duration of exposure, while no deaths were observed until at least ten years since the first exposure; the risk thereafter increases with time since first exposure. The mean latent period was 25 years.

TABLE 2  
Urinary bladder cancer. Observed and expected deaths by category of exposure.

Exposure	N <sup>o</sup> in study	Observed	Expected	$\frac{\text{Observed}}{\text{Expected}}$
Benzidine and naphthylamines manufacture	155	23	0.19	121.05
Benzidine and naphthylamines use and intermittent contacts	455	8	0.68	11.76
Fuchsine and safranine T manufacture	53	5	0.08	62.50
Finished product workers	79	0	0.10	—
Other exposures	126	0	0.18	—
All exposures	868	36	1.23	29.27

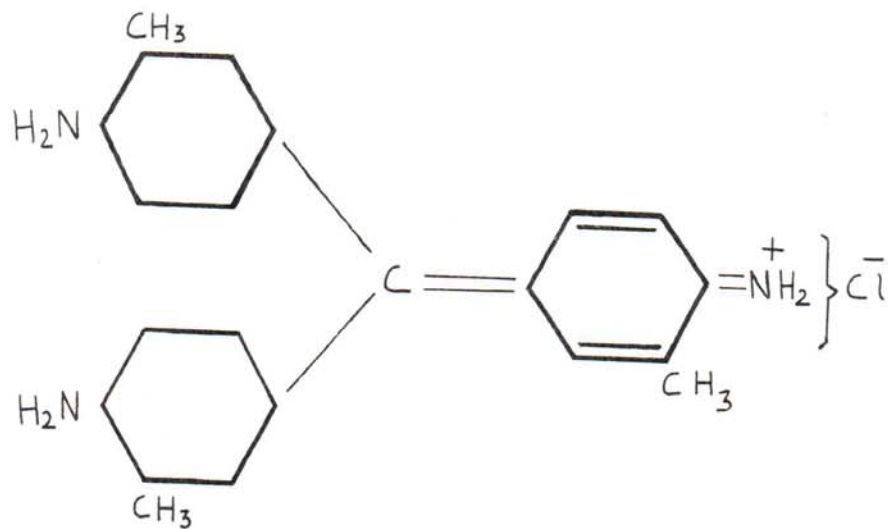
Detailed information on individual jobs and on technical processes at the plant allowed the risk of dying from urinary bladder cancer to be related to specific exposure. This is shown in Table 2, where workers were grouped in five categories of exposure.

Approximately 80% of workers had been engaged in jobs involving exposure to naphthylamines and benzidine.

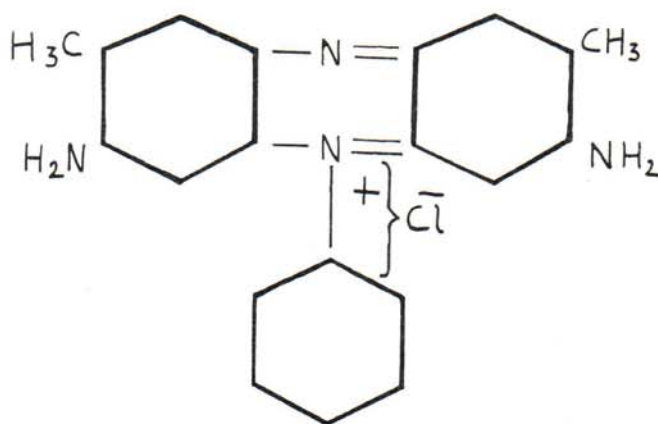
The mortality was exceedingly higher among those employed in benzidine and naphthylamines manufacture than in those only exposed in their use or intermittent contact, but a striking excess of urinary bladder cancer was found

among those only engaged in fuchsine and safranine T manufacture. Looking for causal agents of these tumours requires an examination of chemical processes as well as of location of operations at the plant.

The type of fuchsine manufactured at this plant was the so called New Fuchsine or New Magenta (Basic Violet 2 - Colour Index n<sup>o</sup> 42520)<sup>7</sup>. Safranine T is also called Basic Red 2 (Colour Index n<sup>o</sup> 50240)<sup>8</sup>.



New Fuchsine - Basic Violet 2 (Colour Index n. 42520)



Safranine T - Basic Red 2 (Colour Index n. 50240)

FIG. 1 - Structural formula of New Fuchsine and safranine T.

Figure 1 shows structural formulas of these compounds. The full process to obtain fuchsine and safranine T was carried out in two separate departments located in two different buildings within the factory area. In the first department the synthesis of *o*-toluidine and 4,4'-methylene bis (2-methylaniline) was carried out according to the sequence illustrated in Figure 2. In the second department processes to obtain fuchsine and safranine T were carried out. In this process a mixture of *o*-toluidine, 4,4'-methylene bis (2-methylaniline) and *o*-nitrotoluene was heated to obtain fuchsine. Safranine T was obtained by oxidizing a mixture

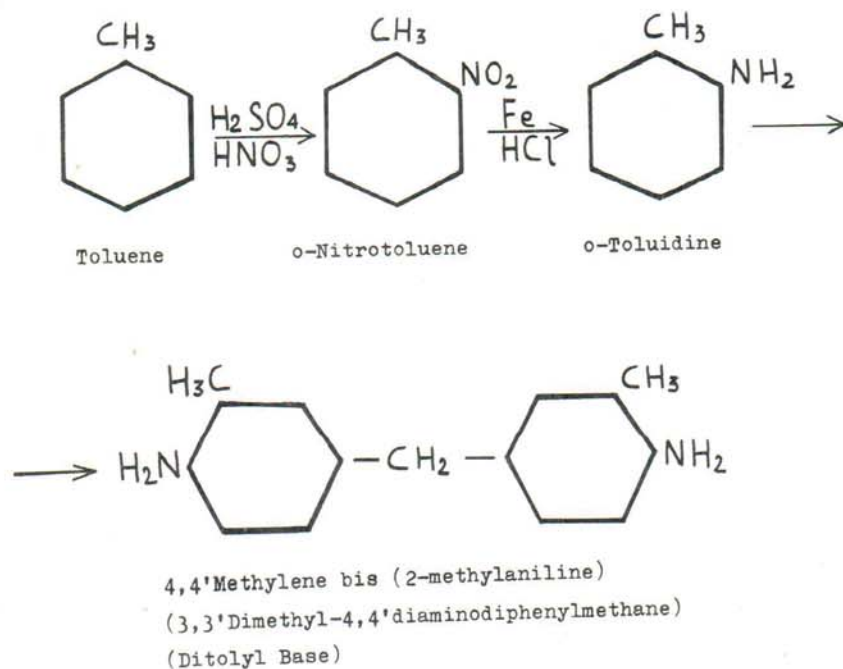


FIG. 2 - Synthesis of *o*-toluidine and 4,4'-methylene bis (2-methylaniline).

of *o*-toluidine and 2,5-diaminotoluene in the presence of aniline. *o*-Aminoazotoluene was the intermediate of the reaction. Workers engaged in the first department were therefore only exposed to compounds reported in Figure 2 sequence, whereas workers manufacturing fuchsine and safranine T were exposed to finished products and their precursors. Three deaths from urinary bladder cancer were reported among those engaged in the first department, that is in the intermediates synthesis, and two deaths among workers engaged in fuchsine and safranine T synthesis. It should be remembered that the total expectation for these deaths was shown to be 0.08. These workers had never changed their section or specific job during employment at the plant.

## DISCUSSION

These findings point to the conclusion that precursors of fuchsine and safranin T [namely *o*-toluidine or 4,4'-methylene bis (2-methylaniline)] should be causal agents of urinary bladder cancer both during manufacture and use. This does not exclude possible carcinogenicity of the intermediate *o*-aminoazotoluene, and of the finished product.

A support to the hypothesis that *o*-toluidine would be aetiological factor of the observed tumours comes from reviewing the available literature. An excess urinary bladder cancer was found among those manufacturing magenta by Case and Pearson in 1954<sup>1</sup>, who were not able to indicate what compounds could have been implicated. According to Colour Index<sup>7</sup> the classical name magenta indicates a dye differing from New Magenta by the number of methyl groups. In commercial production of magenta, *o*-toluidine or *p*-toluidine is used but not 4,4'-methylene bis (2-methylaniline). Based on the hypothesis that Case's study population was engaged in such manufacture, it might be suggested that *o*-toluidine should be the common causal agent.

Results of other studies were accurately evaluated by the IARC Working Group<sup>3</sup>, who stated that at present no human or animal data are available which clearly demonstrated the carcinogenic effect of *o*-toluidine. On the contrary results of two animal experiments<sup>5,6</sup> demonstrated a carcinogenic effect in rats after oral administration of 4,4'-methylene bis (2-methylaniline). No data related to observation in man are available at present.

Whereas no complete information is available on the current production and use of 4,4'-methylene bis (2-methylaniline), IARC Working Group quoted the annual production of *o*-toluidine as high as 1–5 million kg in Western Europe only<sup>3</sup>.

Current legislations in many countries do not include toluidine as a carcinogen in their lists<sup>4</sup>. Since it may be concluded that both *o*-toluidine and 4,4'-methylene bis (2-methylaniline) should be regarded as almost certainly causing cancer of urinary bladder in man, severe rules should be established in handling both these chemicals.

## REFERENCES

1. Case R. A. M., Pearson J. T. Tumours of the Urinary Bladder in Workmen Engaged in the Manufacture and Use of Certain Dyestuff Intermediates in the British Chemical Industry. Part II. Further Consideration of the Role of Aniline and of the Manufacture of Auramine and Magenta (Fuchsine) as Possible Causative Agents. *Br. J. Ind. Med.*, **11** (1954) 213–216.
2. Case R. A. M., Lea A. J. Mustard gas poisoning, chronic bronchitis and lung cancer. *Br. J. Prev. Soc. Med.*, **9** (1955) 62–72.
3. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemical to Man. International Agency for Research on Cancer. Lyon, 1978, Vol. 16, pp. 349–366.
4. Montesano R., Tomatis L. Legislation Concerning Chemical Carcinogens in Several Industrialized Countries. *Cancer Res.*, **37** (1977) 310–316.

5. *Munn A.* Occupational bladder tumours and carcinogens: recent developments in Britain. In: Deichmann W. and Lampe K. F. ed. *Bladder Cancer. A Symposium*, Birmingham, Alabama, Aesculapius, 1967, p. 187.
6. *Stula E. F., Sherman H., Zapp J. A.* Experimental neoplasia in ChR-CD rats with the oral administration of 3,3'-dichlorobenzidine, 4,4'-methylene bis (2-chloroaniline), and 4,4'-methylene bis (2-methyl-aniline). *Toxicol. Appl. Pharmacol.*, **19** (1971) 380-386.
7. *The Society of Dyers and Colourists.* *Colour Index* 3rd ed., **4** (1971) 4389.
8. *The Society of Dyers and Colourists.* *Colour Index* 3rd ed., **4** (1971) 4451.