APPENDIX I

HAZARD REVIEW

OF

4,4'-METHYLENE-BIS(2-CHLOROANILINE)

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4,4'-Methylene-bis(2-chloroaniline)

A preliminary report concerning the carcinogenicity of orally introduced 4,4'-methylene-bis(2-chloroaniline)* in rats was made by Steinhoff and Grundmann [1] in 1969. In 1970 these two investigators published a more extensive paper [2] of their completed findings. In the later paper the toxicity and carcinogenicity of 4,4'-methylene-bis(2-chloroaniline) was compared with that of 4,4'-diaminodiphenylmethane (DDM). Both of these compounds are used as hardeners or curing agents for epoxy resin systems and isocyanate-containing polymers. [2, 4, 7] Although commercial production of 4,4'-methylene-bis(2-chloroaniline) began in 1962, [3] DDM has been in production for over 25 years. [4] The investigators quote previous work to document the strong toxic effect of DDM on both rat and human liver as well as the carcinogenic effect on rat liver. Schoental [4] has also demonstrated the carcinogenicity of DDM on the rat liver. An accidental acute poisoning episode occured in 1965 in Great

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*4,4'-methylene-bis(2-chloroaniline) or 3,3'-dichloro-4,4'-diaminodiphenylmethane has been given the registered trademark, MOCA, by the E. I. du Pont de Nemours & Co., Inc.

Britain in which 84 persons became ill, some seriously, with jaundice following the consumption of bread accidentally contaminated with DDM. [5] In general, Steinhoff and Grundmann [1 and 2] considered 4,4'-methylene-bis(2-chloroaniline) to be less toxic but more carcinogenic than the non-chlorinated compound, DDM.

In their experiments Steinhoff and Grundmann [1 and 2] maintained fifty 100-day-old Wistar rats (25 male; 25 female) on a low protein diet containing 0.1 percent 4,4'-methylene-bis(2-chloroaniline) for 500 days. (Acute toxicity tests had earlier demonstrated the relative nontoxicity of the compound when all ten experimental animals in the study survived either an oral or a subcutaneous administration of a single dose of 5000 mg/Kg.) Control rats used in the chronic feeding experiment were maintained on an identical low protein diet excluding the test compound. At the termination of the 500-day experimental feeding period (total dose of 27 g/Kg body weight) the experimental animals were maintained on the control diet. The average life span for male rats was 565 test days, the average for females was 535 test days. The average life span for controls was 730 test days.

Of the 25 male animals, 23 died with tumors. Twenty-two animals had liver tumors and in 7 of these, primary lung tumors (not metastases) occured also. Two of the animals with liver tumors had lung metastases and one brain metastasy was observed. One animal without liver tumors exhibited "massive tumor permeation" of the lungs

and benign bladder papillomas were observed in one animal. The two tumor-free animals exhibited fatty livers with isolated necrosis and hemorrhages.

Of the 25 female animals, 20 died with tumors. Eighteen animals had liver tumors and in 4 of these animals, three also had primary lung tumors (not metastases) and one had mammary gland tumors. Two animals had lung tumors without liver tumors and 9 had benign mammary gland tumors. The investigators emphasized that lung tumors in rats are relatively rare. Of the 50 control animals only two mammary fibroadenomas were observed in female rats, although the average life span of the controls was longer than that of the experimentals.

In another set of experiments Steinhoff and Grundmann [6] injected a suspension of 94 percent pure, technical grade 4,4'-methylene-bis(2-chloroaniline) into 34 Wistar rats (17 males, 17 females). Subcutaneous injections of 500 or 1000 mg/Kg body weight were administered on the order of once a week, or at longer intervals, to a total dose of 25 g/Kg body weight. Twenty-two of the 34 animals died with a total of 29 malignant tumors. Nine animals had liver cell carcinomas which, in all but one such animal, were discovered in multiple locations. Primary lung carcinomas were formed in 7 animals with a multi-central distribution in 3 animals. In the 50 control animals (25 males, 25 females) a total of 13 malignant tumors at different sites were discovered, including one lung tumor. No liver tumors developed over an average life span of 1040 days compared to an

average life span of 778 days in experimentals. The investigators stated:

> "Thus, 3,3'-dichloro-4,4'-diaminodiphenylmethane exhibits a definite carcinogenic action in the rat, the liver and lungs being the main organs affected, even after subcutaneous administration and sufficient protein nutrition. However, a greater number of liver tumors appear in a shorter time after feeding the compound in a low-protein diet."

In 1972, Sherman and Zapp [7] presented investigations in which rats fed a normal diet, but containing 1000 ppm of 4,4'-methylene-bis(2-chloroaniline), for 18 months subsequently developed lung tumors with some spreading to the pleural cavity. The investigators also observed an increased incidence of liver tumors. When animals were maintained on a low protein diet containing the compound, the incidence and malignancy of both liver tumors (males) and mammary tumors (females) was found to increase.

A contemporary paper by the National Cancer Institute reports on the work of the Weisburgers [8] concerning the carcinogenicity of 4,4'-methylene-bis(2-chloroaniline) in mice and rats. Preliminary studies established the maximally tolerated dose of this compound in the diet was 1000 mg/Kg body weight in rats and 2000 mg/Kg body weight in mice. Control animals were maintained on Purina laboratory chow

during the chronic feeding investigations while equal numbers of experimental animals (25 male mice; 25 female mice; 25 male rats) were dosed at the above levels and other groups at half these levels. Tumors observed in experimental animals and absent in controls included: hepatomas in rats (4/19 effective rats at the high dose and 1/22 effective at the low dose); 1 glioma; 2 adenocarcinomas of the lung; 2 gastrointestinal adenocarcinomas; 1 ear duct tumor; 2 tumors of the urinary bladder; and 7 adenomata of the lung.

In female mice, hepatomas were observed in 50 percent of the animals at the high dose and 43 percent at the low dose. No hepatomas were observed in female control mice. In male mice there was no significant difference between experimentals and controls concerning the incidence of hepatomas. Although no vascular tumors (hemangiomas and hemangiosarcomas) were found in control mice, such tumors were observed in 40 percent of the males and 43 percent of the females receiving the high dose. At the low dose 23 percent of the males and none of the females were observed to develop vascular tumors. Malignant lymphomas which were common in control mice were not as common in the experimental animals.

It is interesting that three independent studies [6 through 8] have reported the development of lung tumors in rats exposed to 4,4'-methylene-bis(2-chloroaniline). As emphasized by the investigators of two of these studies, [6 and 8] the rat is not highly susceptible to lung tumor formation. The influence of diet is known to alter the

carcinogenic potential of various substances and diet apparently affects the carcinogenic potential of 4,4'-methylene-bis(2-chloroaniline), but the results of two studies [7 and 8] in which the experimental animals were maintained on a normal diet, to which the test substance was added, clearly demonstrate that the effect of diet, alone, is not sufficient to account for the oncogenic activity of 4-4'-methylene-bis(2-chloroaniline).

A single plant cohort study involving a group of 31 employees and an equal number of controls was published by Linch et al [3] in 1971. The length of exposure of the control group was not specified. When compared to the control group no significant findings were observed utilizing the Pap technique as a screening tool for the early identification of bladder cancer.

Medical records for 178 employees were reviewed for evidence of acute illnesses, specific systemic illnesses, chronic disease, and malignancy. With the exception of 4 individuals all individuals in this group had not been exposed to 4,4'-methylene-bis(2-chloroaniline) for the last 10 years. In this group the elapsed time since first exposure was:

a)	less than 10 years	-	no employees
b)	from 10 to 15 years	-	158 employees
c)	more than 15 years	-	20 employees

If the assumption is made that, of the group of 158 employees, 15 years had elapsed since the first exposure, and that no exposure had

occured for 10 years, then their total exposure was 5 years. Likewise, the total exposure of the group of 20 employees in which more than 15 years had elapsed since first exposure would be a maximum of approximately 2 years. Because of the short exposure durations of both groups, it should not be considered unusual that negative findings were reported since the known average latency period for development of occupational bladder cancer is approximately 20 years.

The fact that the rate of cancer deaths in the plant population was better than national cancer statistics is not surprising when consideration is given to the differences between the total U.S. population and the able working population of the plant.

These investigators considered the principal route of absorption to be other than respiratory and recommended biologic rather than air monitoring as the procedure of choice for exposure control.

Another industrial study involved the finding by Mastromatteo [9] in 1965 that two of six employees, both in their thirties, who had a mixed exposure to 4,4'-methylene-bis(2-chloroaniline), TDI and several isocyanate-containing resins developed urinary frequency with hematuria in addition to eye irritation, respiratory irritation with cough and tightness in the chest. The hematuria can best be related to the 4,4'-methylene-bis(2-chloroaniline) than to the other substances. The author considered the conditions to be mild but also

considered that exposure to this substance, primarily by dust inhalation, was the cause of the observed cystitis.

The results of the experimental animal studies involving rats and mice, as reported by three independent groups of investigators, [1,2,6,8] clearly demonstrate an active oncogenic role for 4,4'-methylene-bis(2-chloroaniline).

The absence of definitive industrial experience with only 2 reported studies, [3 and 9] and the positive findings in two animal studies by 3 independent investigators, preclude the elimination of 4,4'-methylene-bis(2-chloroaniline) as a human carcinogen.

References for 4,4'-methylene-bis(2-chloroaniline)

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2. Grundmann E, Steinhoff D: Z Krebsforsch 74(1):28-39, 1970
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7. Stula FF et al: Tox and Appl Pharmacol 19:380, 1971
8. Russfield AB, Homburger F, Roger F, Weisburger EK, Weisburger JH: submitted to Tox Appl Pharmcol
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APPENDIX II

METHOD FOR SAMPLING AND ANALYSIS OF 4,4'-METHYLENEBIS (2-CHLOROANILINE) IN AIR

The NIOSH recommended methods for sampling and analysis of 4,4'-Methylenebis(2-chloroaniline) in air may be found in the NIOSH Manual of Analytical Methods (1), and the NIOSH Manual of Sampling Data Sheets (2).

The recommended methodology utilizes a two stage sampler consisting of a high efficiency glass-fiber filter, followed by a bed of silica gel sorbent to collect 4,4'-Methylenebis(2-chloroaniline) aerosol and vapor, and a high performance liquid chromatograph (HPLC) equipped with a UV detector for analysis. This method has been evaluated at levels as low as 0.15 μg per sample collected (50 l of air at 3.0 μg/m3) and found suitable (3).

While the recommended method is thought by NIOSH to be the best available at this time, it is still subject to further review and refinement. If research by NIOSH results in the development of improved methods for sampling and analysis of 4,4'-Methylenebis(2-chloroaniline) in air from the occupational environment, the information will be forwarded to the Department of Labor.

Monitoring for work surface contamination and for workers' urinary 4,4'-Methylenebis(2-chloroaniline) content should be routinely undertaken to supplement monitoring of workplace air. Industrial

experience has indicated that build-up of 4,4'-Methylenebis(2-chloroaniline) on workplace surfaces, even from low airborne levels may account for an increase of workers' urinary 4,4'-Methylenebis(2-chloroaniline) content above that which would normally be expected (15). Baseline data on individual workers' urinary 4,4'-Methylenebis(2-chloroaniline) content and for work surface contamination should be gathered under ideal working conditions, i.e., workers are provided with adequate protective clothing and equipment, work surfaces have been washed down (see Section 6 (f)), and airborne 4,4'-Methylenebis(2-chloroaniline) concentrations are at or below the recommended permissible exposure level. Assuming that the recommended airborne level for 4,4'-Methylenebis(2-chloroaniline) is being met, detection of the chemical at levels above the baseline in either urinary monitoring or spot tests would indicate a need for improving work practices and for washdown of work surfaces. Analysis for urinary 4,4'-Methylenebis(2-chloroaniline) content should be undertaken weekly at the end of the workshift. Spot tests should also be done on a frequent basis for the detection of work surface contamination and as confirmatory tests for unusual findings in urinary monitoring and following accidents, spills, leaks or any changes in the industrial process which might produce an increase in the accumulation of 4,4'-Methylenebis(2-chloroaniline) on workplace surfaces. Methodology for both spot tests and monitoring of urinary 4,4'-Methylenebis(2-chloroaniline) content have been developed and used successfully by LASL (17) and the DuPont Company (Appendix III and IV).

References

1. Manual of Analytical Methods. National Institute for Occupational Safety and Health, DHEW/PHS. Cincinnati, DHEW (NIOSH) Publication No. 77-157-A, April 1977.

2. Manual of Sampling Data Sheets. National Institute for Occupational Safety and Health, DHEW/PHS. Cincinnati, DHEW (NIOSH) Publication No. 77-159, March 1977.

3. Rappaport, S.M., Morales, R., Weeks, R.W., Campbell, E.E., Ettinger, H.J., "Development of Sampling and Analytical Methods for Carcinogens," July 1 - December 31, 1975, LA-6387-PR, Los Alamos Scientific Laboratory. Los Alamos, N.M. 1976.