HAZARD REVIEW

OF

ALPHA-NAPHTHYLAMINE (1-NA)

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Prepared by

Donald V. Lassiter, Ph.D.
Office of Research and Standards Development

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
U.S. Public Health Service
National Institute for Occupational Safety and Health
Rockville, Maryland

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Occupational exposure to aromatic amines has long been associated with development of bladder cancer. Historically, the population-at-risk has been limited to individuals employed in the "aniline" dye industry. The term "aniline cancer" was used in earlier days to denote cancer of the urinary bladder in workers engaged in the production of "aniline" dyes. Hueper [1] published a paper in 1934 in which he reviewed the history, significance, and epidemiologic evidence of "aniline cancer" in the dye industry. Hueper credited Rehn with reporting the first cases of occupational bladder tumors in 1895 in three workers in a German dye factory. In his review article Hueper mentioned the beginning of what was to become a growing controversy concerning the etiology of occupational bladder cancer. He stated that aniline, benzidine, and naphthylamine were the principal etiologic candidates but emphasized that Hamilton [2] had recognized major epidemiologic "pitfalls" including:

a) Worker exposure to more than one suspect compound; further complicated by shifting of workers between departments

b) Different degrees of exposure hazard between processes

c) Unsuspected impurities in trace amounts possibly more harmful than the parent compound

d) Composition of dyes and production methodology in different factories complicating statistical comparison

To a very great extent these same epidemiologic "pitfalls" are valid to the present day

Alpha-naphthylamine (1-NA) is primarily used in the manufacture of azo dyes and in the rubber industry. Scott [3] stated in 1962 that, under contemporary industrial practices, the beta-naphthylamine (2-NA) content of 1-NA was approximately 4 percent. It is understood that in current industrial practices this level of contamination by 2-NA can be kept below 0.5 percent. [4] In his evaluation of the literature and state-of-the-art concerned with occupational exposure to 1-NA, Scott [3] stated, "It is, therefore, difficult to escape the conclusion that alpha-naphthylamine is carcinogenic to man. . . . The standard of precautionary measures in its manufacture and use should be as high as that recommended for benzidine." It should be noted that, as concerns industrial exposure, Scott believed 1-NA should not

be considered in terms of purity but ". . . as it is found in industry and there can be no doubt epidemiologically and theoretically of the risk attached to it."

In 1937 Berenblum and Bonser [5] considered an additional factor in the recognition of a 10 to 25 year latent period for development of clinical symptoms of bladder tumors in man. These investigators attacked the problem of experimental investigation into occupational bladder cancer by two approaches. First, rabbits were injected (i.p.) with 15 cc of a 1 percent emulsion of 1-NA in water once a week for approximately a year and one-half. Secondly, mice were skin painted with extracts made from the urine of workers engaged in the production of dyes. Neither approach was fruitful in inducing tumor formation, although both approaches (i.e. chronic animal exposure and urinary metabolites extraction) are major research avenues today in the search for etiologic agents in formation of bladder tumors.

Barsotti and Vigliani [6] published the results in 1952 of an epidemiologic survey conducted among workers exposed to aromatic amines. Of the 902 workers surveyed, 30 had been exposed to 1-NA and 23 of these had been examined cystoscopically: 14 were normal, 7 had bladder congestion, and 2 had sessile papillomas. One of the papillomas appeared following four year's exposure and 20 year latency period and the other following 25 year's exposure and one year of

latency. All workers had additional exposure to toluidines, anisidines, xylidines, chloroanilines and phenetidines.

In a major in-depth epidemiologic study published by Case and co-workers [7] in 1954 it was stated that those investigators who considered 1-NA to possess tumorigenic risk also believed that the small beta-naphthylamine (2-NA) contamination of 1-NA was the etiologic agent. The investigators concluded from their epidemiologic survey that occupational exposure to benzidine, 1-NA, and 2-NA resulted in increased risk of bladder tumors over the number expected. The expected number of tumor cases was 0.7 but 28 cases were discovered in a population of 3,198 workers (p 0.005). The average induction period for tumors was 16 years for 2-NA as contrasted and with 22 years for 1-NA. An interesting conclusion was reached by the investigators:

> The average induction time is not appreciably influenced by the severity or duration of the exposure. It therefore appears to be a characteristic of the causal agent. This suggests that it is possible that the beta-naphthylamine content of alpha-naphthylamine is not the sole causative agent in the latter substance unless it is assumed that alpha-naphthylamine could retard the production

of beta-naphthylamine tumors.

No malignant tumors were noted in a study by Bonser and co-workers [8] published in 1956 in mice injected (s.c.) twice weekly for 52 weeks with 1-NA (free from 2-NA) in arachis oil, but some benign intestinal polyps were discovered. Based on their assessment of the literature in 1958, however, these investigators [9] concluded that there could be no doubt that occupational exposure to 2-NA, and benzidine was responsible for a large proportion of industrial tumors.

The principal direction of research efforts to determine causation of bladder tumors induced by the naphthylamines has been the identification of those metabolites which possess this potentiality.

The principal route of metabolism of the naphthylamines is via the liver, where they are conjugated with either glucuronic or sulfuric acid or hydroxylated, and excreted via the urinary bladder. It is, in fact, the diversity and multiplicity of metabolites which has obscured the identification of the respective proximate carcinogenic metabolite (or metabolites) of the naphthylamines. It is generally accepted that both 1-NA and 2-NA require metabolic modification to elicit their carcinogenic response.

In 1963, Clayson & Ashton [10] published the results of their investigation with mice fed 1-NA (100 mg/liter), as the hydrochloride,

in drinking water. The number of hepatomas observed and the latency period was not significantly different from control animals. These investigators also discovered that the urinary excretion products of the 1-NA used in this study, which was free of 2-NA, were similar, although not identical, in the dog, rat, mouse, cavy, ferret, hamster and rabbit. A greater abundance of 1-amino-4-napthol derivatives were excreted compared to 1-amino-2-naphthol derivatives. The investigators considered the excess of 1-amino-4-naphthol derivatives to suppress the liberation of free 1-amino-2-naphthol from conjugates. Such ortho-hydroxylated aromatic amines were considered to possess a greater carcinogenic potential than para- or meta-hydroxylated amines.

Boyland et al [11] tested for the carcinogenicity of the N-hydroxylated derivative of 1-NA and discovered it to produce bladder adenoma or papilloma in 3/26 mice and bladder carcinoma in 5/26 mice. The method of exposure involved surgical implantation of a stearic acid pellet impregnated with 1-naphthylhydroxylamine. Thirty-six (36) percent of the 26 animals exhibited tumors compared to 14 percent of the vehicle controls. Although the technique of bladder implantation has been critized, Clayson [12] concluded that this technique was a valid test for carcinogenicity.

Much interest in recent years has centered on the carcinogenicity of the N-hydroxylated derivatives of the naphthylamines. Belman et al [13], in 1966, found the N-hydroxylated derivative of 1-NA, 1-

naphthylhydroxylamine (N-OH-1-NA), to possess greater tumorigenic potential than its 2-NA counterpart, 2-naphthylhydroxylamine (N-OH-2-NA). In this study, and as updated by a later report [14], the investigators injected (i.p.) Wister rats with 50 mg N-OH-1-NA or N-OH-2-NA/kg body weight twice a week for three months. Peritoneal sarcomas were observed in 11/14 rats injected with N-OH-1-NA in 9 months but in only 1/15 rats injected with N-OH-2-NA after 10 months observation.

In a later report on this work [14] the total number of rats with peritoneal sarcomas (injected with N-OH-2-NA) after 2 years observation was 4/14. In another study with these same two metabolites, 5 peritoneal sarcomas were observed in a group of 12 rats injected with N-OH-1-AN and one bladder carcinosarcoma and one peritoneal sarcoma in a group of 12 rats injected with N-OH-2-NA. The investigators compared the results of their experiments to those of Boyland [14], who discovered the N-hydroxylated metabolites of 1-AN and 2-NA were carcinogenic for the mouse bladder. In a corollary study, mice were fed a single dose (600 mg) of 2-NA and 4 others a single dose (250 mg) of 1-NA. No major differences were observed between the groups in the amount of N-hydroxylated metabolites formed over a 24 hour period.

The N-hydroxylated derivative of 2-NA has been reported to be a metabolite in both man and the dog [15,16,17]. In 1967 Brill and

Radomski [18] published a study in which they demonstrated that N-OH-1-NA was a urinary metabolite of 1-NA in the dog. The investigators said of their findings:

> These results demonstrated that unconjugated N-OH-1-AN is a urinary metabolite of 1-AN in the dog. This finding plus the observation of Troll and co-workers that N-OH-1-AN is a stronger carcinogen that [sic] N-OH-2-AN in the rat suggests that the reported non-carcinogenicity of 1-AN in the dog needs to be re-examined. That the dog could metabolize 1-AN to produce a carcinogenic metabolite and remain refractory to its effect poses an interesting problem.

Radomski et al [19] confirmed the work of Belman et al [13&15] in a study published in 1971 in which they compared the carcinogenicity of the N-hydroxylated and 1- and 2-nitroso compounds of 1-NA and 2-NA. Rats were injected (i.p.) with 50 mg of N-OH-1-NA or N-OH-2-NA/kg body weight twice weekly for 12 weeks. Of the 27 animals injected with N-OH-1-NA, 12 developed tumors including: fibromas (3), granulomas (3), fibrosarcomas (4), lymphosarcoma (1), and hepatoma (1). None of the 27 animals injected with N-OH-2-NA developed tumors. Although the acute toxicity of the 1- and 2-nitroso compounds precluded a direct comparison with the N-hydroxylated derivatives, 4/27 rats injected

with 1-nitrosonaphthalene (1-NO) developed tumors while none of the animals injected with 2-nitrosonaphthalene (2-NO) developed tumors.

Radomski and Brill [20] considered that although 1-NA is N-hydroxylated to N-OH-1-NA, which is carcinogenic, only negligible amounts are found in the urine of dogs and, it is assumed, in the urine of humans exposed to 1-NA.

The relationship between carcinogenicity and mutagenicity has been evaluated by several investigators [21-23] who demonstrated that N-OH-1-NA was mutagenic both in bacteria [22,23] and in Neurospora. Ong and de Serres [21] concluded from their investigation with Neurospora that a positive correlation was demonstrated between the carinogenicity and mutagenicity of N-OH-1-NA.

Finally it should be mentioned that 1-NA has been discovered [24-26] in cigarette smoke. However, the amount of 1-NA per cigarette was very minute, on the order of 2.7×10^{-8} grams.

The contamination of 1-NA by 2-NA in the industrial environment has long precluded a direct evaluation concluding that 1-NA per se is carcinogenic, given the well-established carcinogenic potential of 2-NA. Both 1-NA and 2-NA are readily metabolized to various derivatives, several of which have a demonstrated carcinogenic

potential (proximate carcinogens). As yet, however, the ultimate carcinogenic metabolite(s) has not been clearly identified. Although the final resolution of this situation may be only academic to the occupational environment, the fact that an ultimate, active hazardous substance has not been identified for even 2-NA, precludes the dismissal of 1-NA as noncarcinogenic until it is clearly demonstrated that its metabolites are not carcinogenic for man. The demonstration that one such metabolite, N-OH-1-NA, was carcinogenic for rats and mice and was found to possess a greater carcinogenic potential than its 2-NA counterpart [13] underscores this particular point. In addition, the extensive epidemiologic study in the dyestuffs industry by Case [1] failed to eliminate an active role for 1-NA as a bladder carcinogen.

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