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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
CINCINNATI, OHIO 45268

MEMORANDUM

DATE: June 1, 1994

SUBJECT: Review of ERM's Cancer Risk Assessment and Recommendations for Alternative Provisional Qualitative and Quantitative Assessments (Drake Chemical/Lock Haven, PA)

FROM: Joan S. Dollarhide *Joan S Dollarhide*
Director
Superfund Health Risk Technical Support Center
Chemical Mixtures Assessment Branch

TO: Roy Smith
U.S. EPA
Region III

This memorandum responds to your request for a review of ERM's cancer risk assessment and recommendations for alternative provisional qualitative and quantitative assessments for the Drake Chemical site, Lock Haven, PA.

Attached please find the following:

- Risk Assessment Issue Paper for: Provisional Cancer Risk Assessment for 2-Naphthylamine (CASRN 91-59-8)

Please feel free to contact the Superfund Technical Support Center at (513) 569-7300 if you need additional assistance.

Attachment

cc: R. Harris (Region III)
J. Konz (5204G)

**Risk Assessment Issue Paper for:
Provisional Cancer Risk Assessment for 2-Naphthylamine (CASRN 91-59-8)**

INTRODUCTION

2-Naphthylamine (CASRN 91-59-8) was used in the past as an intermediate in the manufacture of dyes and as a rubber antioxidant, but, since January 1975, has not been commercially manufactured or imported for use in the United States (Dressler, 1981; ACGIH, 1991). It is widely recognized as causing bladder cancer in humans by the scientific community and regulatory agencies including IARC (1974, 1987a,b), NIOSH (1992), OSHA (1989, 1993) and ACGIH (1992). However, there are no regulatory guidelines for 2-naphthylamine on U.S. EPA's IRIS (U.S. EPA, 1994a) or HEAST (U.S. EPA, 1993a).

The EPA CRAVE Work Group discussed cancer risk assessment for 2-naphthylamine on 9/04/91 and verified a qualitative weight-of-evidence classification for 2-naphthylamine of Group A - human carcinogen (U.S. EPA, 1991a, 1994b). Approaches to deriving quantitative cancer risk estimates were discussed, but a particular risk estimate was not verified. Discussion centered on the appropriate analysis of bladder tumor incidence data from a study with monkeys given oral doses of 2-naphthylamine (Conzelman et al., 1969). The quantitative estimate was left "Under Review" pending finalization of a Health and Environmental Effects Profile document on 2-naphthylamine. To date, this document has not been released (U.S. EPA, 1991b, 1993b), although a draft version is available (Syracuse Research Corporation, 1986).

This issue paper reviews the human and animal data pertinent to the carcinogenicity of 2-naphthylamine and critically reviews a cancer assessment presented by Environmental Resources Management, Inc. (ERM, 1994). The paper concludes with recommendations for a provisional cancer risk assessment for 2-naphthylamine. Information to prepare this paper was found in several reviews (IARC, 1974, 1987a,b; Syracuse Research Corporation, 1986; Cohen and Johansson, 1992; Beland and Kadlubar, 1985, 1986; Radomski, 1979) and computer searches of the TOXLINE (1984-1994), MEDLINE (1985-1994) and TSCATS (1984-1994) databases.

CARCINOGENICITY OF 2-NAPHTHYLAMINE

HUMAN DATA

An excess of urinary bladder cancers among workers who used aromatic amines in the production of synthetic dyes was first recognized in the late 1800's by a German physician named Rehn (see Stern et al., 1985 for review). With the introduction of synthetic dye technology to other countries, increased numbers of cases of bladder cancer were reported, and by the 1930's bladder cancer was widely recognized as an occupational hazard associated with this industry. In 1934, 3 cases of bladder cancer were reported to have occurred in workers exposed to 2-naphthylamine and/or benzidine at E.I. duPont de Nemours and Company's dye manufacturing facilities in the U.S.; by 1948, 139 workers at the du Pont facility developed bladder cancer (Stern et al., 1985). The du Pont Company ceased production and use of 2-naphthylamine in 1955 (Kaufmann, 1979; Castleman, 1979). Numerous other case reports of bladder cancer in workers exposed to 2-naphthylamine and other aromatic amines have been published and are reviewed elsewhere (Hueper, 1942; IARC, 1974, 1987a). Initially attempts to reproduce this type of cancer in laboratory animals were unsuccessful until Hueper et al. (1938) reported that exposure of dogs to commercial 2-naphthylamine in the diet and by subcutaneous injection for approximately 2 years produced urinary bladder cancer that was similar to that seen in dye workers.

In a retrospective occupational cohort study of bladder cancer and exposure to aromatic amines in British chemical companies, Case et al. (1954) found that among 209 men employed only in the manufacture of 2-naphthylamine for > 6 months between 1920 and 1950 in England and Wales, 55 developed bladder cancer and 27 died from bladder cancer by the time the study was conducted. Only 0.3 deaths from bladder cancer were expected for this cohort, based on the incidence of deaths from bladder cancer in the general male population of England and Wales between 1921 and 1949. Although this study did not estimate exposure levels or adjust for possible confounding factors such as age or smoking history, Boyko et al. (1985) reported that its impact was sufficient to lead to legislation banning 2-naphthylamine in the United Kingdom.

Several other retrospective cohort studies found evidence for elevated risks of bladder cancer in workers exposed to 2-naphthylamine and other aromatic amines, but none obtained exposure level information. Brief reviews of some of these studies follows.

Goldwater et al. (1965) examined medical and employment records for men employed in a coal-tar dye factory between 1912 and 1962. Among 48 men who were exposed only to 2-naphthylamine, 12 had bladder cancer. A control or reference group was not included in this study.

Mancuso and El-Attar (1967) established a cohort of workers employed in 1938-1939 by a company manufacturing 2-naphthylamine and benzidine. By 1965, 79 deaths had occurred among 216 workers exposed to 2-naphthylamine and/or benzidine compared with 36

deaths among 134 non-exposed workers. Fourteen of the deaths in the exposed group were associated with bladder or urinary tract cancer, while no bladder or urinary tract cancer cases were reported for the deaths in the non-exposed group.

Within another cohort of 1385 workers employed between 1940 and 1972 in a U.S. dye plant that manufactured and used 2-naphthylamine, benzidine and other aromatic amines (Stern et al., 1985; Schulte et al., 1985, 1986), 5 cases of bladder cancer were found by 1983 among 118 exposed workers. Exposed workers were defined as those with > "1 year of employment or any employment in two high-exposure departments in the plant". In contrast, 8 cases of bladder cancer occurred among 1267 non-exposed workers (Schulte et al., 1986). An odds ratio of 6.96 (with 95% confidence interval limits of 3.9 and 12.4) was calculated indicating an approximate 7-fold increased risk of bladder cancer in the exposed group.

Bladder cancer accounted for 49 of 271 deaths that occurred by 1989 among a group of 664 workers employed for at least 1 year between 1922 and 1970 in an Italian dye manufacturing plant that used aromatic amines including 2-naphthylamine (DeCarli et al., 1985; Piolatto et al., 1991). Based on national mortality rates, only 1.6 deaths from bladder cancer were expected. Using a statistical analysis of the data described by Brown and Chu (1983), DeCarli et al. (1985) noted no marked effect of age at first exposure on absolute excess risk of bladder cancer and a marked negative effect of age at first exposure on relative risk. Absolute excess risk was found to increase sharply during exposure, and to continue to rise, although less sharply, after exposure had ceased. Relative risk decreased after cessation of exposure. These findings were interpreted by the authors, in the context of the multistage theory of carcinogenesis, to be consistent with 2-naphthylamine having both early stage (i.e., tumor initiating) and late stage (i.e., tumor promoting) effects.

Clinical experiences with a cytostatic drug, chlornaphazine [N,N-bis(2-chloroethyl)-2-naphthylamine], provide some indirect support for the suggestion that exposure to fairly low doses of 2-naphthylamine can cause bladder tumors in humans. Chlornaphazine was used in the 1950's in Denmark and Italy for the treatment of polycythemia vera and Hodgkin's disease (Thiede et al., 1964; Thiede and Christensen, 1969; Schmähl et al., 1977). Prescribed daily dosages ranged from approximately 100-400 mg (1.4-5.7 mg/kg/day) (Thiede et al., 1964; Thiede and Christensen, 1969; Schmähl et al., 1977). Use of the drug was discontinued by 1963 in Denmark after cases of bladder cancer were reported among treated patients. Hydrolysis of the two chloroethyl groups from chlornaphazine is expected to occur *in vivo* leading to the formation of 2-naphthylamine (Schmähl, 1977).

Schmähl et al. (1977) compiled 15 examples of bladder cancer in patients treated with chlornaphazine. The cases included seven men and eight women (average age = 57 years) who received daily doses of chlornaphazine ranging from 100-400 mg/day for periods ranging from 9 months to 10 years (average duration of treatment was 6 years). Estimated latency periods for bladder tumor development ranged from 2.5 to 10 years (average = 6 years). Thiede and Christensen (1969) reported that among 61 polycythemic patients treated

with chlornaphazine for various periods of time between 1951 and 1962, 10 developed bladder cancer and another five displayed atypical cells in their urine. It is possible that confounding factors may have contributed to the observed carcinogenic effects in these patients (e.g., smoking habits, disease predisposition, other treatments administered to the patients and differential pharmacokinetic behavior between chlornaphazine and 2-naphthylamine). However, the relatively short exposure durations and latent periods for development of cancer suggest that chlornaphazine is an unusually potent carcinogenic agent.

Interpretation of the relationship between chlornaphazine administration and 2-naphthylamine-induced bladder cancer is complicated by the fact that chlornaphazine itself is one of a class of compounds known as nitrogen mustards. These chemicals, which contain the N,N-bis(2-chloroethyl)- moiety, can alkylate DNA and produce DNA cross-links. Bladder cancer has been observed in patients treated with at least one other nitrogen mustard (cyclophosphamide) which does not contain the 2-naphthylamine substituent (Schmähl et al., 1977). This suggests that, for cyclophosphamide, sufficient alkylating agent reaches the bladder to induce cancer. No data are available from humans to estimate the extent to which chlornaphazine is excreted unchanged in the urine (i.e., reaches the bladder tissue), or conversely, is dealkylated to yield 2-naphthylamine. Dealkylated metabolites have been identified as excretion products in rats injected with chlornaphazine (Boyland and Manson, 1963a) and would be predicted to occur after oral administration in humans. However, lacking more specific data, the relative contributions to the induction of human bladder cancer by chlornaphazine itself, or its putative metabolite 2-naphthylamine, cannot be determined. Therefore, the human data for chlornaphazine cannot be used to estimate risk from exposure to 2-naphthylamine.

In summary, case reports and retrospective cohort studies, in conjunction with data from animal studies, have causally linked exposure to 2-naphthylamine with the occurrence of bladder cancer in workers exposed while manufacturing or using the compound (IARC, 1974, 1987a). The available studies of occupational exposure, however, do not provide sufficient information concerning levels of exposure to 2-naphthylamine that can be used to estimate cancer risk as a function of exposure level. Clinical experiences with chlornaphazine, a cytostatic drug expected to be biotransformed to 2-naphthylamine, likewise do not provide sufficient data to support a quantitative estimate of risk.

ANIMAL DATA

Observed carcinogenic responses to orally administered 2-naphthylamine in animals include bladder tumors in dogs (Hueper et al., 1938; Bonser et al., 1956; Deichmann et al., 1965; Conzelman and Moulton, 1972; Radomski et al., 1978; Purchase et al., 1981), bladder tumors in rhesus monkeys (Conzelman et al., 1969), liver tumors in mice (Bonser et al., 1952; Yoshida et al., 1972; Osanai, 1976), bladder tumors in rats (Hicks and Chowaniec, 1977; Hicks et al., 1982), and bladder tumors in hamsters (Saffioti et al., 1967). Parenteral

administration of 2-naphthylamine produced pulmonary adenomas (Theiss et al., 1981; Walters et al., 1967) and liver tumors (Bonser et al., 1956) in mice. Studies examining carcinogenic responses in animals following inhalation or dermal exposure to 2-naphthylamine were not located in the databases or literature examined for this issue paper.

Animal studies providing sufficient and appropriate dose-response information for the extrapolative estimation of lifetime oral cancer risk from 2-naphthylamine in humans as a function of exposure level are restricted to a dog study by Conzelman and Moulton (1972), a monkey study by Conzelman (1969) and a hamster study by Saffioti et al. (1967). These studies, described in further detail below, were judged to be appropriate because they all included control groups and groups treated with multiple oral doses. In addition, the carcinogenic response in dogs, monkeys and hamsters occurs in the same target organ as in humans exposed to 2-naphthylamine (i.e., bladder cancer). Although reporting of the details of the Saffioti et al. study (1967) was sparse, this study was selected because the animals were exposed for their lifetime. The dog and monkey studies did not provide lifetime exposure but were otherwise adequately designed, conducted and reported. Mice appear to develop liver tumors, not bladder tumors, in response to oral 2-naphthylamine making them a less desirable model species for quantitation of cancer risk to humans exposed to 2-naphthylamine. The rat studies were not selected, because rats appear to be less sensitive than dogs and monkeys to the carcinogenicity of 2-naphthylamine due to differences in urine pH, frequency of micturition and resorption (Young and Kadlubar, 1982).

Conzelman et al. (1969) gave gelatin capsules containing 2-naphthylamine at various doses ranging from 6.25-400 mg/kg, 6 days/week for up to 60 months to 16 female and 8 male rhesus monkeys. The test material was purified by gradient sublimation before incorporation into gelatin capsules, but percentage purity was not reported. Three female monkeys served as controls. Complete necropsy was performed after 60 months or when the monkeys appeared moribund. The urogenital tracts were examined histologically. Table 1 describes the dosing schedule and findings concerning bladder tumors for the individual animals in this study. Seven treated monkeys appeared moribund before the end of the 60-month dosing period; 6/7 of these monkeys were found to have bladder tumors. Moribundity associated with the occurrence of bladder cancer was observed as early as 33 months after commencement of treatment. No bladder tumors were found in the controls. Bladder carcinomas (described as either transitional cell carcinomas, carcinoma in situ or papillary carcinomas) were identified in 9/24 treated monkeys. Additionally, two treated monkeys displayed bladder adenomas and 1 treated monkey displayed a benign bladder papilloma (see Table 1).

Conzelman and Moulton (1972) administered 2-naphthylamine in gelatin capsules at doses of 0, 6.25, 12.5, 25.0 or 50 mg/kg, 6 days/week for 2-26 months to groups of 4-10 dogs. The test material was purified by gradient sublimation before incorporation into gelatin capsules, but the purity was not reported. Necropsies were performed immediately after treatment or at various periods (1-5 months) after treatment for a number of dogs (see Table 2). Urinary tracts were grossly and histologically examined for tumors. Early

mortalities did not occur in control or treated dogs. Table 2 describes the dosing schedules and the histological findings for the individual dogs. Bladder tumors described as late squamous metaplasia with invasion into the mucosa or submucosa, invasive transitional carcinoma, invasive squamous carcinoma or papillary carcinoma were found at incidences of 0/4, 4/9, 4/10, 8/10 and 4/5 for the control through high-dose groups, sequentially (Table 2). Benign bladder papillomas without invasive metaplasia or carcinomas were observed in an additional 3 dogs in the 12.5-mg/kg group and one dog in the 50-mg/kg group (Table 2).

Saffioti et al. (1967) provided groups of 30 male and 30 female Syrian golden hamsters with diets containing 0, 0.1 or 1.0% (w/w%) 2-naphthylamine (purity not specified) starting at 8 weeks of age and continuing until spontaneous death occurred. The authors estimated that animals received 0, 60 or 600 mg 2-naphthylamine per week, based on average historical food consumption data for their hamster colony. Body weight and survival data were recorded, but were not given in the only available report of this study. All tumors and bladders, and "most" livers, kidneys and adrenals, were histologically examined post-mortem. No bladder tumors were found in the control group or in "several hundred" historical control hamsters from the authors' laboratory. No "significant changes were observed" in the group fed the 0.1% diet with the exception of the finding of one female at 97 weeks with "papillomatous hyperplasia" of the bladder. Bladder tumors were observed in the 1.0% group. "Ten out of 23 effective males and 8 out of 16 effective females had bladder tumors, almost all typical transitional cell carcinomas." The first tumors were found in a male that died at 45 weeks and in a female that died at 49 weeks. Further details concerning the findings of this study were not provided. The authors stated that "more detailed description of the experimental details and detailed pathological findings" were to be reported elsewhere, but a more detailed account was not located in the databases or literature examined for this issue paper.

Table 1. Dosing schedule and bladder tumor occurrence in rhesus monkeys orally exposed to 2-naphthylamine (Conzelman et al., 1969)

Animal Number	Sex	Dose and Duration (mg/kg, 6 days/week)	Microscopic findings in bladder
1129*, 1422#, 1683*	F	0 mg/kg for 60 months	no tumors
1425#	F	6.25 mg/kg for 6 months, 12.5 mg/kg for 54 months	papillary carcinoma
1426#	F	12.5 mg/kg for 60 months	no tumors
620*	M	12.5 mg/kg for 6 months, 25.0 mg/kg for 54 months	no tumors
1136*	F	12.5 mg/kg for 6 months, 25.0 mg/kg for 54 months	no tumors
1418#, 1289*	F	25.0 mg/kg for 60 months	no tumors
671*, 1421#	F	25.0 mg/kg for 6 months, 50.0 mg/kg for 54 months	no tumors
1183*, 1279+	F	50.0 mg/kg for 60 months	benign papilloma (1183); papillary carcinoma (1279)
1264*+	F	100 mg/kg for 55 months	no tumors
2345	M	200 mg/kg for 33 months	transitional cell carcinoma
1772*	M	200 mg/kg for 18 months, 100 mg/kg for 42 months	no tumors
2338	F	200 mg/kg for 42 months, 100 mg/kg for 18 months	papillary adenoma
2333	M	200 mg/kg for 42 months, 100 mg/kg for 4 months	transitional cell carcinoma, invasive
1798*, 1803*	M	200 mg/kg for 40 months, 100 mg/kg for 20 months	no tumors (1798); carcinoma in situ (1803)
1770*	F	400 mg/kg for 4 months, 200 mg/kg for 32 months, 100 mg/kg for 24 months	no tumors
1792*	F	200 mg/kg for 34 months, 100 mg/kg for 12 months	papillary adenoma
2377*	F	200 mg/kg for 33 months	carcinoma in situ
2616*	F	200 mg/kg for 32 months, 100 mg/kg for 10 months	transitional cell carcinoma, invasive
1877*	M	200 mg/kg for 24 months, 100 mg/kg for 12 months	carcinoma in situ
1894*+	M	400 mg/kg for 12 months, 200 mg/kg for 24 months, 100 mg/kg for 24 months	no tumors
2321*	F	200 mg/kg for 33 months, 100 mg/kg for 27 months	transitional cell carcinoma, invasive

* - These monkeys were infected with Plasmodium cynomolgi and treated with chloroquine at least 12 weeks before exposure to 2-naphthylamine.
+ - These monkeys were splenectomized during the 12-week period before 2-naphthylamine exposure.
- These monkeys were given single doses of N-(3-bromopropionamidomethyl)acrylamide before assignment to this study.

Table 2. Dosing schedule and bladder tumor occurrence in beagle dogs orally treated with 2-naphthylamine in gelatin capsules (Conzelman and Moulton, 1972).

Animal Number and Sex	Dose and Duration (mg/kg, 6 days/week) ¹	Microscopic findings in the bladder
1362(M), 1501(F), 1335(M), 1594(F)	0 mg/kg for 6 (1362), 9 (1501), 12(1335) or 24 (1594) months	No preneoplastic lesions or tumors found in controls.
1306(F), 1547(M), 1162(F), 1577(F), 1565(M), 1573(M), 1566(M), 1635(M), 1623(M)	6.25 mg/kg for 6 (1306), 9 (1547), 12 (1162), 15 (1577), 18 (1565), 21 (1573), 24 (1566, 1635**), or 25 (1623**) months	No lesions: 1573 Preneoplastic lesions but no tumors: 1306, 1547, 1162, 1577. Late squamous metaplasia with invasion into mucosa and submucosa: 1565, 1635. Invasive transitional carcinoma: 1566, 1623.
1550(M), 1519(F), 1381(M), 1567(M), 1574(M), 1563(F), 1655(F), 1658(M), 1608(F), 1621(F)	12.5 mg/kg for 6(1550), 9 (1519), 12 (1381), 15(1567), 18(1574), 21(1563), 22(1655), 24(1658, 1608*), or 26(1621**) months.	Preneoplastic lesions but no tumors: 1550, 1519, 1381. Early papilloma, benign: 1574, 1563, 1655. Late squamous metaplasia with invasion into mucosa and submucosa: 1567, 1658. Invasive transitional carcinoma: 1608, 1622.
1502(F), 1548(M), 1417(F), 1569(M), 1578(F), 1571(M), 1671(M), 1575(M), 1636(M), 1624(M)	25 mg/kg for 2(1502), 9(1548), 12(1417), 15(1569), 18(1578), 21(1571), 22(1671**), 24(1575, 1636**), or 25(1624*) months.	No lesions: 1502. Preneoplastic lesions: 1548. Late squamous metaplasia with invasion into mucosa and submucosa: 1578. Papillary carcinoma with invasion into mucosa or submucosa: 1575, 1624. Invasive transitional carcinoma: 1636, 1571, 1671, 1569, 1417.
1677(M), 1698(F), 1697(F), 1689(M), 1609(F)	50 mg/kg for 9(1677), 18(1698*, 1697*, 1689**), or 21(1609*) months.	Early papilloma, benign: 1677. Invasive transitional carcinoma: 1698, 1697, 1689, 1609.

¹ All dogs were necropsied immediately following the dosing period with the following exceptions:

* - These dogs were necropsied 1-2 months after termination of the dosing period.

** - These dogs were necropsied 3-5 months after termination of the dosing period.

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SUPPORTING DATA

2-Naphthylamine has a wide range of genotoxic activities. 2-Naphthylamine induced reverse mutations in *Salmonella typhimurium* in the presence of mammalian metabolic systems (Mayer, 1982; Connor et al., 1983; Langenbach et al., 1983), mutations in growing, but not resting, cells of *Saccharomyces cerevisiae* and *Neurospora crassa* (Ong and deSerres, 1972; Callen and Philpot, 1977; Mayer, 1971, 1973) and unscheduled DNA synthesis in rat and hamster hepatocytes (Althaus, 1982; Kornbrust and Barfknecht, 1984). 2-Naphthylamine induced sister chromatid exchanges, chromosomal aberrations and morphological transformations in mammalian cell cultures; it caused mutations in a mouse lymphoma cell assay, but not in a chinese hamster cell assay (deSerres and Ashby, 1981). Intraperitoneal administration of 2-naphthylamine to pregnant mice produced coat-color mutations in their offspring (Chauhan et al., 1983).

The genotoxic and carcinogenic activities of 2-naphthylamine and other aromatic amines that cause bladder cancer are generally thought to be initiated by the oxidative metabolic formation of reactive intermediates that covalently modify DNA. Identified metabolites of 2-naphthylamine include acetyl, glucuronosyl and sulfamyl conjugates at the amino group and C-(ring) and N-hydroxylation products and their conjugates (Boyland and Manson, 1963b; Kadlubar et al., 1981a). N-hydroxylation is viewed as a critical metabolic step in the activation of 2-naphthylamine, whereas N-acetylation, if it precedes N-hydroxylation, has been proposed as a detoxification step (Beland and Kadlubar, 1986). A popular mechanistic theory that has substantial empirical support proposes that 2-naphthylamine undergoes N-hydroxylation in the liver (via the cytochrome P-450 system), followed by N-glucuronidation, transport to the bladder and acid-catalyzed hydrolysis, to yield the free N-hydroxylamine derivative in the target organ (Radomski, 1979; Miller and Miller, 1981; Kadlubar et al., 1977; Beland and Kadlubar, 1985, 1986). A reactive electrophile, a nitrenium ion, is presumably formed by acid-hydrolysis of the N-hydroxy compound; the electrophile is envisioned to covalently bind to DNA, initiating genetic and carcinogenic effects.

Critical evidence in support of this hypothesis includes the demonstration that, when DNA and N-hydroxy-2-naphthylamine were incubated under in vitro acidic conditions (pH 5), 3 major DNA adducts were formed that were also found in liver and bladder DNA extracted from a 9-kg dog given an oral dose of 72 mg [^3H]2-naphthylamine (Kadlubar et al., 1981b; Kadlubar et al., 1981c; Beland et al., 1983). Levels of DNA adducts in bladder DNA were about 4-fold greater than levels in liver DNA (Kadlubar et al., 1981c; Beland et al., 1983). Evidence is also available that an alternative oxidation system found in the bladder (prostaglandin H-synthase) can produce reactive free radical derivatives of 2-naphthylamine and other arylamines, (presumably ring-oxidation products) that also are capable of covalently modifying DNA (Yamazoe et al., 1985; Boyd and Eling, 1987; Flammang et al., 1989).

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Comparisons of the carcinogenic potency and metabolism of 1-naphthylamine with that of 2-naphthylamine provide supporting evidence. Whereas 2-naphthylamine causes bladder cancer in several animal species, 1-naphthylamine has failed to produce tumors in experimental animals (Radomski et al., 1980; Poupko et al., 1983). The lack of a carcinogenic response to 1-naphthylamine has been correlated with decreased N-hydroxylation (Brill and Radomski, 1971; Orzechowski et al., 1992) and increased N-glucuronidation (Orzechowski et al., 1992) compared with 2-naphthylamine.

REVIEW OF CANCER ASSESSMENT BY ERM, INC.

In the absence of cancer risk estimates for 2-naphthylamine on IRIS or the HEAST, Environmental Resources Management, Inc. (ERM, 1994) derived a soil cleanup level of 3,500 mg/kg soil for the Drake Superfund site. A key thesis to ERM's approach was the judgement that 2-naphthylamine exerts its carcinogenicity predominantly "through promotion, rather than initiation", and thus that a threshold approach to cancer risk assessment for this compound is appropriate. The derivation involved the interpretation that the Conzelman and Moulton (1972) dog study identified a "cancer threshold dose" between 5-10 mg/kg body weight for 2-naphthylamine. A "human carcinogenic threshold" dose of 0.005 mg/kg/day was derived by dividing the dog dose of 5 mg/kg/day by a "safety factor" of 1000. The proposed cleanup level of 3,500 mg/kg soil was derived by applying standard reference values for body weight (70 kg) and daily ingestion of soil (10^{-4} kg soil/day) to the human threshold dose. ERM defined a cancer threshold dose as a dose at which the compound "poses no risk of cancer". ERM's assessment has two major problems which make it an inappropriate assessment of the carcinogenicity of 2-naphthylamine. Discussion of these problems follows.

The first major problem is ERM's thesis that the mechanism governing 2-naphthylamine's carcinogenicity predominately involves tumor promotion, and thus that a threshold approach to quantitative risk assessment is appropriate. ERM's arguments against using a non-threshold cancer modeling approach for 2-naphthylamine are not consistent with predominant ideas concerning the mechanism of its carcinogenicity.

ERM argued that the use of a non-threshold cancer model such as the linearized multistage model is not appropriate for a compound that is not a tumor initiator. However, as discussed in the previous section of this issue paper, the weight of evidence regarding the mechanism by which 2-naphthylamine and other aromatic primary amines causes bladder cancer supports a mechanism in which metabolites of 2-naphthylamine (especially, but not exclusively, N-hydroxy derivatives) initiate tumor production. Consistent with tumor initiation capabilities are the findings that 2-naphthylamine and its N-hydroxy derivative display genotoxic activities in a wide range of assays, and the same DNA adducts, formed when N-hydroxy-2-naphthylamine and DNA are reacted *in vitro*, are also found in the

bladder of dogs treated with 2-naphthylamine (Beland et al., 1983; Beland and Kadlubar, 1985).

ERM argued (page 8, section 3.1.3) that if the liver metabolite, N-hydroxy-2-naphthylamine, "were the ultimate carcinogenic species related to exposure to β NA, it would be expected that cancers would be initiated at many body organ sites". Because this is not observed, ERM argued that the operating mechanism "would be through promotion, rather than initiation". The basis for ERM's expectation and resultant argument is not entirely clear, but they do not appear to consider a current working hypothesis that a detoxification step, glucuronidation, occurs in the liver before transport to other organs and that the acid conditions of the urine (of certain species) enhance the hydrolysis and formation of a proposed reactive electrophilic intermediate from the glucuronide of N-hydroxy-2-naphthylamine (Kadlubar et al., 1981b; Kadlubar et al., 1981c; Beland et al., 1983). In support of this working hypothesis, Young and Kadlubar (1982) used a pharmacokinetic model, with parameters of urine pH, voiding interval and extent of resorption, to accurately predict species differences in bladder tumor susceptibility.

ERM also cites (page 8, section 3.1.3) observations that 1-naphthylamine is not a bladder carcinogen and that its N-hydroxy derivative undergoes "stronger DNA adduction" (presumably than N-hydroxy-2-naphthylamine) as reasons for rejecting the tumor initiation role and accepting the promoter role for 2-naphthylamine. The rationale for this conclusion is not clear. Current observations comparing the carcinogenicity and metabolism of 1-naphthylamine and 2-naphthylamine are consistent with the hypothesis that N-hydroxy derivatives of aromatic primary amines are ultimate tumor-initiating carcinogens, and that the *in vivo* formation of N-hydroxy-1-naphthylamine occurs only to a limited extent. In contrast to 2-naphthylamine, pure 1-naphthylamine has not produced tumors in oral bioassays with rats, mice or dogs (Radomski, 1979). N-hydroxy-1-naphthylamine, however, produced carcinogenic responses at sites of parenteral administration in rats and newborn mice (Radomski, 1979). *In vitro* reaction of N-hydroxy-1-naphthylamine with DNA under acid conditions (pH 5) yields at least 3 DNA adducts, but when [3 H]-1-naphthylamine was administered to a dog, DNA adducts were not found in the urothelial DNA (Kadlubar et al., 1981c; Beland et al., 1983). N-hydroxy derivatives of 1-naphthylamine could not be detected in experiments with liver microsomal preparations dogs, rats and humans (Hammons et al., 1985) or with isolated rat hepatocytes (Orzechowski et al., 1992). N-Hydroxy-2-naphthylamine was detected when these systems were incubated with 2-naphthylamine (Hammons et al., 1985; Orzechowski et al., 1992).

The second major problem with ERM's assessment involves their analysis of the key study, the dog study by Conzelman and Moulton (1972). ERM (page 7, section 3.1.2.) stated the "the best index of dose-response" in this study was the observation of "diffuse masses of tumors filling the bladder", but provided no further explanation for their choice of this index. ERM stated that the frequency of occurrence for this index was 0%, 0%, 20%,

40% and 60% in the control, 6.25, 12.5, 25 and 50 mg/kg groups respectively and that these data indicated that the "potential bladder carcinogenesis threshold" (i.e., a no cancer effect level) was between 5 and 10 mg/kg (see page 9, section 3.14 and Figure 1).

Examination of the original report shows that ERM's choice of cancer index misrepresents the findings of the Conzelman and Moulton study. As shown in Table 2 of this issue paper, 4 of the 9 dogs exposed to the 6.25 mg/kg dosage level were diagnosed with histologically confirmed, malignant bladder lesions described either as late squamous metaplasia with invasion into mucosa and submucosa or as invasive transitional carcinoma. Although these lesions had not progressed to the stage of filling the bladder, their diagnosis by the investigators as malignant lesions suggests they would have done so given the time for development. Thus, ERM's determination of a cancer threshold for 2-naphthylamine-induced cancer is based on an experimental dose that, rather than producing no cancer, produced cancer in 4/9 dogs within only a portion of (no more than approximately 2/15) of the normal lifespan of the experimental species, beagle dogs.

In summary, ERM's cancer risk assessment for 2-naphthylamine is not recommended for 2 major reasons. The assessment is based on a mechanistic assumption (i.e., that 2-naphthylamine is predominately a tumor promoter) that is not consistent with current predominant mechanistic ideas on 2-naphthylamine carcinogenicity, and it misrepresents the findings of the study on which its risk number is based.

PROVISIONAL CANCER RISK ASSESSMENT FOR 2-NAPHTHYLAMINE

A weight-of-evidence classification of A, human carcinogen, is appropriate for 2-naphthylamine based on sufficient evidence from case reports and cohort mortality and morbidity studies of bladder cancer in occupationally exposed humans, as reviewed earlier in this paper. There also is sufficient evidence for bladder cancer in orally exposed monkeys, dogs, rats and hamsters, and for liver and lung tumors in orally or subcutaneously exposed mice. When metabolically activated, 2-naphthylamine displays genotoxic effects in a wide range of assays. The U.S. EPA CRAVE Work Group verified an A classification for 2-naphthylamine in September, 1991 (U.S. EPA, 1991a, 1994b).

As discussed earlier in this paper, studies regarding cancer in humans exposed to 2-naphthylamine do not provide sufficient information for quantitative dose-response modeling. Thus, dose-response modeling of 3 animal studies was carried out in order to provide a provisional quantitative risk estimate for lifetime oral exposure of humans to 2-naphthylamine: a monkey study by Conzelman et al. (1969); a dog study by Conzelman and Moulton (1972) and a hamster study by Saffioti et al. (1969). The rationale for selecting these studies was discussed earlier in this paper.

The strengths of the monkey study include the appropriateness of monkeys as a general model species for humans and the inclusion of several dosage levels; weaknesses of the study include the small number of animals and the short duration of the study (60 months, which is approximately 1/7 of the lifespan of the rhesus monkey). The dog study, likewise, used several dosage levels and an appropriate animal species, but had small numbers of animals and was designed to study the sequential development of bladder tumors after less-than-lifetime exposures. Within each dose group, 1-2 dogs were scheduled for sacrifice at 2-4 month intervals up to a total of 26 months (see Table 2). Use of the dog and monkey data requires extrapolation to lifetime exposure.

The hamster study included more desirable numbers of animals per dose group (60) and was designed as a lifetime exposure bioassay. However, details of the experiment were minimally reported in the only available report. In addition, the tumor dose-response observed in this study indicates that hamsters may be considerably less sensitive to the bladder carcinogenicity of 2-naphthylamine than dogs or monkeys. No tumors were observed in chronically exposed hamsters at a dosage level (0.1% in the diet, approximately 64 mg/kg/day; see Table 3) considerably higher than the lowest dosage levels that produced tumors in dogs (5.4 mg/kg/day, see Table 4) or monkeys (10.2 mg/kg/day, see Table 5) exposed for periods that represented less than 20% of their projected lifespans.

The linearized multistage procedure was judged to be appropriate for the modeling. Although it is possible that high doses of 2-naphthylamine may influence the risk for bladder cancer by inducing increased cell proliferation, 2-naphthylamine clearly behaves as a classic genotoxic carcinogen and thus is assumed to present a finite risk for development of cancer even at low doses of exposures (i.e., a no-threshold model is appropriate). As discussed by Cohen and Ellwein (1993), genotoxic bladder carcinogens such as 2-acetylaminofluorene and 2-naphthylamine operate through a different mechanism (i.e., they have both genotoxic and cell proliferative properties) than certain other bladder carcinogens such as sodium saccharin which apparently cause cancer in sensitive species only through a cell proliferative mechanism that has an apparent threshold.

Dose-response modelings of the three data sets are described in Tables 3-5. The three data sets provide slope factors for lifetime oral exposure that span several orders of magnitude. The hamster estimate, $2.9\text{E}-3$ per (mg/kg)/day, is approximately 44,000-fold lower than the estimate based on the dog data, $1.3\text{E}+2$ per (mg/kg)/day. The estimate based on the monkey data is of an intermediate value, $7.5\text{E}+0$ per (mg/kg)/day.

The oral slope factor derived from the dog data, $1.3\text{E}+2$ per (mg/kg)/day, is recommended to serve as the quantitative risk estimate for human oral exposures to 2-naphthylamine for two major reasons. First, this estimate is the most protective among the three derived herein from animal studies and is thus the most conservative choice. Among the several animal species that have been tested (dog, rhesus monkey, hamster, rats and

mice), dogs appear to be the most sensitive (Radomski, 1979; also see Tables 3, 4 and 5). Secondly, indirect evidence suggests that humans may be more or equally sensitive to the bladder carcinogenicity of 2-naphthylamine compared to the dog; thus the choice of the estimate based on the dog data does not appear to be overly conservative. Radomski (1979) estimated that occupationally exposed workers, who were at a definite risk to develop bladder cancer, may have been exposed to 2-naphthylamine doses on the order of 10 mg/kg/day, a dosage level that is comparable to those which produced bladder tumors in dogs. This estimate was based on the observations that 1-naphthylamine workers excreted 10-40 mg free amine/day in their urine and that, after 1-naphthylamine or 2-naphthylamine exposure, about 5% of the total amine derived substances present in urine is free amine $[(40 \text{ mg free amine in urine/day}) \times [1/70 \text{ kg body weight}] \times [1 \text{ mg 2-naphthylamine administered}/0.05 \text{ mg free amine in urine}] = 11.4 \approx 10 \text{ mg 2-naphthylamine/kg/day}]$. Young and Kadlubar (1982) argued that humans may be more sensitive than dogs and other animal species based on theoretical pharmacokinetic considerations. These investigators modeled bladder exposure to N-hydroxy-2-naphthylamine, a proposed ultimate carcinogen for 2-naphthylamine, in humans, dogs, monkeys and rats, using a model that included species-specific values for urine pH and urinary voiding intervals. The predicted order of species susceptibility, indicated as total bladder exposure to N-hydroxy-2-naphthylamine, was human $\geq$ dog $>$ monkey $>$ rat.

In conclusion, an oral slope factor of $1.3\text{E}+2$ per (mg/kg)/day is recommended for 2-naphthylamine. This risk estimate is based on bladder tumor incidence data for dogs exposed orally to 2-naphthylamine in gelatin capsules on a 6-days/week basis for periods up to 26 months (Conzelman and Moulton, 1972). Dogs appear to be the most appropriate animal model for estimating bladder cancer risk in humans. The principal study was well conducted and adequately reported, but had a small number of animals and was not optimally designed to estimate risk from lifetime exposure. The slope factor was derived using the linearized multistage low-dose extrapolation procedure and a procedure that extrapolated to lifetime exposure. Confidence in the risk estimate would be improved with the availability of a lifetime oral exposure bioassay with dogs.

Derivation of an inhalation unit risk for 2-naphthylamine is not recommended. Although occupationally exposed workers who developed bladder cancer are expected to have been exposed by both the dermal and inhalation routes, no quantitative information regarding exposure levels was located in the available studies. No studies were located that looked for the occurrence of cancer in animals following inhalation exposure to 2-naphthylamine. An extrapolation from oral data to the inhalation exposure scenario is not recommended, because no pharmacokinetic data were located that would support an estimation of air concentrations that would deliver doses (to the whole body or to the target organ) similar to those from oral exposure.

Table 3. Dose-response modeling of tumor incidence data for hamsters orally exposed for life to 2-naphthylamine in their diet. Source: Saffioti et al., 1967.

Reported Concentration in diet (%w/w)	Reported Dose (mg/week)	Calculated Dose ¹ (mg/kg/day)	Lifetime Human Equivalent Dose ²	Incidence of Bladder Tumors ³
0	0	0	0	0/60
0.1%	60	64.0	7.9	0/60
1%	600	639.7	79.4	18/39

¹ A reference body weight of 0.134 kg (U.S. EPA, 1987) was assumed for this calculation.

² Human Equivalent Dose = Animal Dose x (Animal body weight/ Human body weight)^{1/3} = Animal Dose x 0.12416.

³ Male and females were combined. The "effective" number of animals in the control and low-dose groups are assumed to be equal to the initial numbers of animals in these groups. In the high-dose group, bladder tumors were reported to have occurred in "ten out of 23 effective males and 8 out of 16 effective females".

Quantitative estimate of carcinogenic risk from oral exposure:

Oral slope factor: 2.9E-3 per (mg/kg)/day

Extrapolation method: Linearized multistage procedure, extra risk

Table 4. Dose-response modeling of tumor incidence data for dogs orally exposed to 2-naphthylamine in gelatin capsules for up to 10-26 months. Source: Conzelman and Moulton, 1972.

Reported Dose (mg/kg, 6 days per week)	Adjusted Animal Dose (mg/kg/day)	Human Equivalent Dose ¹ (mg/kg/day)	Malignant Bladder Tumor Incidence ²
0	0	0	0/2
6.25	5.4	2.9	4/7
12.5	10.7	5.7	4/8
25.0	21.4	11.5	8/8
50	42.9	23.4	4/4

¹ Human Equivalent Dose = Adjusted Animal Dose x (Animal body weight / human body weight)^{1/3}
= Adjusted Animal Dose x (10.8/70)^{1/3}, where 10.8 and 70 kg are reference body weights for beagle dogs and humans, respectively (U.S. EPA, 1987).

² The denominator includes only those dogs that lived as long as the dog in which bladder tumors were noticed earliest (10 months, Dog No. 1698 in the 50 mg/kg group, see Table 2).

Quantitative estimate of carcinogenic risk from oral exposure:

Oral slope factor, unadjusted for lifetime exposure (unadjusted q1*):
3.0E-1 per (mg/kg)/day.

Extrapolation method: Linearized multistage procedure, extra risk. The estimate was obtained using data for the controls and the first 3 dose groups. When the highest dose group data were included, the model would not converge.

Oral slope factor, adjusted for lifetime exposure (adjusted q1*):
 $3.0E-1 \text{ per (mg/kg)/day} \times (15 \text{ years} / 2 \text{ years})^3 = 3.0E-1 \times 421.88 = 1.3E+2 \text{ per (mg/kg)/day.}$

The lifetime exposure extrapolation is based on the assumptions that:

1. the age specific rate of cancer will continue to increase with age as a constant function of the background rate (U.S. EPA, 1980),
2. the slope of the dose-response curve, calculated at time, L_e , would increase by at least the 3rd power of L/L_e , where L is the full lifespan (U.S. EPA, 1980),
3. the lifespan for beagle dogs is 15 years (U.S. EPA, 1987).

Table 5. Dose-response modeling of tumor incidence data for monkeys orally exposed to 2-naphthylamine in gelatin capsules for up to 60 months. Source: Conzelman et al., 1969.

Animal Number	Time-Weighted Average Animal Dose ¹ (mg/kg/day)	Human Equivalent Dose ² (mg/kg/day)	Incidence for malignant bladder tumors
1129, 1422, 1683	0	0	0/3
1425	10.2	5.5	1/1
1426	10.7	5.8	0/1
1136, 620	20.4	11.0	0/2
1418, 1289	21.4	11.5	0/2
671, 1421	40.7	21.9	0/2
1183, 1279	42.9	23.1	1/2
1264	85.7	46.1	0/1
1772	111	59.7	0/1
2333	125.7	67.6	1/1
2321	132.9	71.5	1/1
1877, 1798, 1803	142.9	76.9	2/3
2338	145.7	78.4	1/1
1770	148.6	79.9	0/1
1792	149.1	80.2	1/1
2616	151.0	81.2	1/1
1894, 2377, 2345	171.4	92.2	2/3

¹ Sample calculation: Administered dose = 6.25 mg/kg for 6 months and 12.5 mg/kg for 54 months, 6 days/week.
TWA Animal Dose = $\{[(6.25 \text{ mg/kg} \times 6 \text{ mo.}) + (12.5 \text{ mg/kg} \times 54 \text{ mo.})]/60 \text{ mo.}\} \times 6\text{d}/7\text{d} = 10.2 \text{ mg/kg/day}$.

² Human Equivalent Dose = TWA Animal Dose $\times$ (Animal body weight/ human body weight)^{1/3} = TWA Animal Dose $\times$ (0.53799). Calculation assumes reference body weights of 10.9 and 70 kg for monkeys and humans, respectively (U.S. EPA, 1987).

Quantitative estimate of carcinogenic risk from oral exposure:

Oral slope factor, unadjusted for lifetime exposure (unadjusted q1*):
2.2E-2 per (mg/kg)/day.

Extrapolation method: Linearized multistage procedure, extra risk.

Oral slope factor, adjusted for lifetime exposure (adjusted q1*):
 $2.2\text{E-}2 \text{ per (mg/kg)/day} \times (35 \text{ years}/ 5 \text{ years})^3 = 2.2\text{E-}2 \times 343 = 7.5\text{E+}0 \text{ per (mg/kg)/day}$.

The lifetime exposure extrapolation is based on the assumptions that:

1. the age specific rate of cancer will continue to increase with age as a constant function of the background rate (U.S. EPA, 1980),
2. the slope of the dose-response curve, calculated at time, L_e , would increase by at least the 3rd power of L/L_e , where L is the full lifespan (U.S. EPA, 1980),
3. the lifespan for rhesus monkeys is 35 years (U.S. EPA, 1987).

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