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REVIEW PAPER

2-NAPHTHYLAMINE TOXICITY

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Abstract

In the past, 2-naphthylamine (2-NA) was used for the production of azo dyes, as an antioxidant in the cable industry and in the rubber industry. Despite the fact that 2-NA is not produced on an industrial scale, it is still used in small quantities as a model bladder carcinogen in laboratories, and also for sewage control, water analysis and oxytocinase assays. In addition, it is detected in the air in coke ovens, where it is formed as one of the pyrolysis products. The main aim of this work is to provide an actual literature review for health risk assessments related to 2-NA which is still used in laboratories. Occupational exposure to 2-NA is important for the respiratory tract, mucous membranes and the skin, and, to a lesser extent, for absorption from the gastrointestinal tract. It is absorbed into the body through the skin and by inhalation, and then undergoes metabolic changes. Most of the absorbed 2-NA dose is excreted in the urine, in the form of metabolites, metabolites conjugated to acids, and even in an unchanged form. Based on literature data, the effects of 2-NA toxicity in sub-chronic and chronic exposure include contact dermatitis, chronic cystitis and bladder cancer. The authors have concluded that it is recommended to determine the occupational exposure limit which will allow preparing the exposure assessment of people at work. Med Pr. 2020;71(2):205–20

Key words: bladder cancer, occupational health, carcinogen, toxicology, health effects, 2-naphthylamine

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BACKGROUND

Basically, 2-naphthylamine (2-NA; CAS 91-59-8) occurs as colorless crystals which turn pink under the influence of light, with a weak, aromatic odor. It is not found in the natural state and is currently not produced on an industrial scale [1]. In the past it was used as an antioxidant in the rubber industry to produce 2-chloronaphthalene, or as an intermediate in dye production and in the cable industry [2]. The European Union banned this procedure in 1998 due to its proven carcinogenic effect. The same prohibition took effect in Italy in 1960, in Great Britain in 1952, and in Switzerland in 1938 [2]. In the USA, 2-NA is considered carcinogenic and is regulated by the Occupational Safety and Health Administration (OSHA); therefore, the associated exposure must be closely monitored [3].

Nowadays, 2-NA is used in analytical laboratories for wastewater control, water analysis and oxytocinase assays [2,4]. Thus, occupational exposure to 2-NA occurs in laboratories in which it is used as a model substance in cancer research, in workers exposed to smoke containing 2-NA generated as a result of pyrolysis (foundry fumes, cigarette smoke, fumes arising during the heating of food oils), and in workers dealing with nitric polycyclic aromatic hydrocarbons that are metabolized to 2-NA [1].

Exposure to 2-NA is possible not only in occupational environment, but also for the general population through exposure to tobacco smoke or other fumes containing 2-NA, as well as in contact with dyes and hair dyes. However, the Working Group of the International Agency for Research on Cancer (IARC) has found no evidence of the presence of 2-NA in consumer products. In addition, exposure to 2-nitronaphthalene, which is obtained through an incomplete combustion of organic substances, and is present in the environment in a mixture with other aromatic nitro compounds and

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PAHs, may be an indirect source of 2-NA [1]. Interesting is the fact that 2-NA has been detected in the fumes of cooking oil in the heating process [5].

According to the data from the Polish Registry on Exposure to Chemicals, Their Mixtures, Factors or Technological Processes on Carcinogenic or Mutagenic Effects, 201 workers were exposed to 2-NA in 2017. In 2015, 54 employees were reported to have been exposed to 2-NA, and in 2016 – 375.

According to Regulation (EC) No. 1272/2008 of the European Parliament and of the Council on classification, labeling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No. 1907/2006, 2-NA has been classified as a substance: carcinogenic category 1A (Carc. 1A), acute toxic category 4 (Acute tox. 4), and hazardous to the aquatic environment category 2 (Aquatic chronic 2) [6]. In addition, 2-NA has been included in Annex XVII of the REACH Regulation [7], containing a list of substances subject to restrictions (which may not be marketed or used, whether as separate substances or in mixtures, in concentrations greater than 0.1% by weight) [7].

METHODS

The literature review was made on the basis of the Internet databases of peer-reviewed scientific journals, including the EBSCO Discovery Service (EDS), PubMed, MedLine and data available on the European Chemicals Agency website. In the preparation of this study, papers published until 2019, mainly in English, were considered and the following key words: "2-naphthylamine application," "2-naphthylamine toxicity," "toxicokinetics," "exposure," and "occupational exposure limits" were used. The presented article includes all of the papers related to the influence of exposure to 2-NA on health.

RESULTS

Acute and sub-acute toxicity

There is limited literature data on the acute and sub-acute human toxicity, due to the fact that the carcinogenicity of 2-NA is a critical effect in occupational exposure conditions.

Acute toxicity of 2-NA in humans results in methemoglobinemia, hypoxia of the brain and other organs, as well as hemorrhagic cystitis – no data on the route of exposure. Symptoms of acute poisoning include weakness, dizziness, blue discoloration of the skin and mucous membranes, euphoria, painful urination and dyspnoea that may occur even several hours after the end of exposure. In addition, 2-NA dust can cause skin and eye irritation [4,8].

The median lethal doses (LD50) of 2-NA that were obtained in experimental animal studies indicate that 2-NA is a toxic substance when swallowed.

In the available literature, only 2 LD50 values were described for 2 routes of exposure: intragastric and intraperitoneal. The median value of lethal doses for rats after oral administration is 727 mg/kg, and after intraperitoneal exposure for mice – 200 mg/kg [9,10]. Toxic effects have not been described in detail except for LD50 [11]. The lethal dose low (LDL_0) for dogs after *per os* administration is 500 mg/kg. The dose of 200 mg/kg (via gavage) has been found to cause symptoms of methemoglobinemia [11].

Sub-chronic and chronic toxicity in humans

In the sensitization test of 2-NA in humans, chronic contact dermatitis was observed, as well as obstructed and painful urination, and blood appearance in the urine [12]. At the same time, employees exposed to 2-NA and benzidine showed a decrease in the peripheral blood count of CD4 subpopulations of T4 lymphocytes [13].

As a result of chronic, repeated exposure to 2-NA in increasing doses via gavage (0.3–100 mg/rat/day), for 5 days a week for 52 weeks, in the rats which died during the study, disturbances in weight gain, symptoms of gastritis, gastrointestinal hemorrhage, kidney and liver discoloration, hepatomegaly, intestinal bleeding and bladder deposits were observed [14].

Mutagenicity and genotoxicity

According to the current classification of chemical substances in the European Union, 2-NA is not classified as a mutagen; however, it may be included in the group of promutagens, thus agents that display a genotoxic activity after metabolic activation.

Data available in the literature clearly shows that, in the reaction of DNA with *N*-hydroxy-2-naphthylamine, 3 adducts are formed in pH 5.0 [15,16]. The first of these was characterized as a derivative of *N*-(deoxyguanosine-8-yl)-2-naphthylamine, which is formed in the greatest amount, followed by 1-(deoxyguanosine-N2-yl)-2-naphthylamine and 1-(deoxyadenosine-N6-yl)-2-naphthylamine. The same adducts were observed in the tissues of the urinary epithelium and liver in dogs as early as 2 days after oral administration of 2-NA [16].

It has been found that 2-NA exhibits weak mutagenic effects in both *in vitro* tests on bacteria and in selected

mammalian cells. In *in vivo* tests, 2-NA was positive in the *Drosophila melanogaster* study, but some of the micronucleus tests gave inconclusive results [17–20]. Positive results were obtained revealing a statistically significant increase in the frequency of cells with micronuclei in animals [21–24], as well as in humans [25]. Moreover, 2-NA induces DNA fragmentation in the rodent liver, based on *in vivo* studies [26] and DNA damage in *E. coli* PQ37 bacteria [27].

Positive results were also obtained in a spot test in mice [28], as well as in the case of gene mutations and recombination in yeast and plants [29]. In addition, 2-NA induces unplanned DNA synthesis in human cells [29], as well as single DNA strand breaks in rat hepatocytes and pituitary DNA in *in vitro* studies [28,30]. In an *in vivo* indirect host test, in which *E. coli* K-12 uvrB/recA cells were intravenously implanted into mice and 2-NA was given intraperitoneally (doses: 67 mg/kg, 200 mg/kg), a positive result was also obtained [31].

In addition, 2-NA causes an increase in the frequency of chromosomal aberrations and sister chromatide exchange in human and animal cells, in both *in vitro* and *in vivo* studies [29,32], and induces the formation of adducts from DNA in the bladder and liver cells of dogs in *in vivo* studies [33].

The 2-NA metabolite *N*-hydroxy-2-NA also causes single DNA strand breaks in human skin culture and lung fibroblasts [34,35]. Detailed information concerning mutagenicity are presented in Table 1.

Carcinogenicity

Humans

Detailed data regarding cohort studies is presented in Table 2. On the basis of the available data, several conclusions can be drawn. First of all, contact with 2-NA (production, use) causes a significant increase in the number of bladder cancer cases and hence deaths, in relation to the expected number of cases and deaths in a population that was not exposed [59–65]. In addition, employment in the production of 2-NA causes a much higher risk of bladder cancer than its use (e.g., in the production of antioxidants and dyes, in the rubber industry) or during the performance of ancillary activities [61,66,67]. However, it should be clear that the discrepancies associated with the period of bladder cancer are associated with exposure to 2-NA calculated over the years by mean values at a very wide range of extreme values, e.g., 16 (2–45) [59], 24.9 (12–41) [61], 15–20 [63], 17.6 (4–29) [65]. The exposure period to 2-NA is also an important factor influencing the increase in mortality [61] and the incidence of bladder cancer [62]. However, Case et al. [59] in their work claimed that the number of years worked in the conditions of exposure to 2-NA had no effect on the development of bladder cancer in employees. Stern et al. [63] gave an example of 2 deaths from bladder cancer after 10 and 18 years, which occurred due to short term exposure (2 months).

There is a relationship between the age of employees undertaking work in the conditions of 2-NA exposure, the latency period and age at which bladder cancer manifests. The period of latency is the longer, the lower the age of people commencing employment. In addition, there is a tendency to shorten the latency period with the increasing age of people undertaking work (a greater vulnerability of older people to cancer) [59,66].

People exposed to 2-NA are more likely to develop bladder tumors (cancer, papillomas), but also prostate, kidneys, esophagus or lung tumors [60].

On the basis of the above-mentioned data, it can be concluded that the results of the tests performed so far provide sufficient evidence of the carcinogenic effect of 2-NA on the urinary tract. Therefore, the International Agency for Research on Cancer has classified 2-NA as a category 1 carcinogen. However, the carcinogenicity of 2-NA to other organs has not been sufficiently documented in epidemiological studies yet. In addition, there is no data related to the concentration of 2-NA in workplaces.

Animals

Oral administration

All collected data concerning animal exposure to 2-NA is presented in Table 3 [14,82–103] and Table 4 [85, 95–97].

Bonser et al. [92] exposed rats (29 females, 18 males; 43 rats as a control group) to 0.01% 2-NA via diet. The authors observed 4 cases of bladder papillomas. Carcinomas were also observed. In the control group, there were no tumors.

Fisher rats were exposed to 0.5–150 mg of 2-NA/week via gavage per week for 52 weeks. The authors observed many tumors (testicular intersticial cell tumor, adrenal adenoma, fibrosarcoma, mammary adenocarcinoma, mammary fibroadenoma, hepatoma) which were not statistically significant [14].

Hicks and Chowaniec [100] studied the influence of 2-NA given via gavage (in arachis oil) at a dose of 300 mg/kg b.w. per week for 1 year on Wistar rats, with 55 rats (males and females) serving as the control group. In 5 out of 17 rats, bladder tumors were observed.

Table 1. Mutagenicity of 2-naphthylamine

Test type – dose	Results		References
	with metabolic activation	without metabolic activation	
In vitro tests (bacteria, fungus)			
Salmonella typhimurium (TA1538, TA1537, TA1535, TA100, TA98) mutation*	+		36–41
Salmonella typhimurium TA100 mutation			
5–50 μg/plate	+		42
2.510 μg/plate			43
20–80 μg/plate			44
Salmonella typhimurium (TA100, TA102, TA98) mutation*	+		45
Salmonella typhimurium (TA1535) mutation			
10–50 μg/plate	+		46
Salmonella typhimurium (TA98) mutation			
20 μg/plate	+		47
Salmonella typhimurium (TA98, TAMix 7001–7006) mutation	+		48
4–5000 μg/ml			
Salmonella typhimurium (NM2009) mutation	–		49
5 μM			
Salmonella typhimurium mutation	–		50
>100 μM			
Salmonella typhimurium (NM6001, NM6002, NM6000) mutation			
≥1 μM	+ (NM6001, NM6002) – (NM6000)		51
Salmonella typhimurium mutation	+		52
10 μg/plate			
Salmonella typhimurium (NM2009) mutation	+		53
10 μM			
Escherichia coli (uvrB-/rec-; uvrA+/rec+) mutation*	+	+	54
In vitro tests (mammalian cells)			
Chinese hamster ovary cells mutation			
50–100 μg/ml	+	+	55
Chinese hamster ovary cells mutation			
20 μg/ml	–		44
Chinese hamster embryo cells cell transformation test*	+		29, 56
In vivo tests			
Drosophila melanogaster – mitotic recombinations*	+		57, 58

* No dose information available.
"–" – negative result, "+" – positive result.

Wistar rats (20 females) were exposed to 2-NA at a dose of 300 mg/kg b.w. once a week for 57 weeks via gavage (in arachis oil) [101], with 20 rats serving as the control group. One moribund animal was killed. Bladders of 18 rats were histologically tested revealing that 8 out of 18 animals had urothelial hyperplasia, and out of those 8, 4 also developed carcinoma.

Bonser et al. [92] exposed IF mice (12 females, 12 males) to a dose of 400 mg/kg b.w. per week for 90 weeks via gavage (in arachis oil). A group of 11 rats served as the control.

Table 2. Carcinogenicity of 2-NA (2-naphthylamine) – cohort studies

Country	Workers [n]	Minimal employment period	Observation time	Results/Risk assessment/Comments	References
United Kingdom	455	n.d.	n.d.	341 bladder cancer; 26 deaths caused by bladder cancer (SMR = 86.7)	59
Italy	906	1 year	1922–1989	49 cases of bladder cancer (SMR = 30.4), SMR = 142.11 (2-NA production), SMR = 16.7 (2-NA and/or benzidine use), SMR = 150 (2-NA production)	61, 66, 71
USA	1384	n.d.	1940–1992	8 deaths caused by bladder cancer (SMR = 5.6), 7 deaths caused by esophageal cancer (SMR = 2.0), 41 deaths caused by lung cancer (SMR = 1.67), 11 deaths caused by prostate cancer (SMR = 2.1), 21 deaths caused by digestive system cancer (SMR = 1.06), 1 death caused by mouth and throat cancer (SMR = 0.37) (2-NA and other aromatic amines)	62, 63, 76, 77
USA	590	n.d.	1922–2003	56 deaths caused by bladder cancer (SMR = 16.5) (aromatic amines)	64
United Kingdom	2090 men	n.d.	1945–1949	58 cases of bladder cancer (SIR = 1.7)	65, 69
Poland	6978	3 months	1945–1973	299 deaths caused by cancer (SMR = 112.7); 6 deaths caused by bladder cancer (SMR = 2.76)	67
USA	639 men	27 years	1938–1965	14 cases of bladder cancer (SMR = 17.7), 5 cases of prostate cancer, 2 cases of kidney cancer, 6 cases of pancreas cancer, 8 cases of lung cancer (2-NA and benzidine)	68
Japan	3322	n.d.	1950–1978	244 cases of genitourinary cancer, including 11 other cancer cases (liver, bile duct, large intestine, lungs, maxillary sinus cancer) (2-naphthylamine and benzidine)	70
USA	89 men	n.d.	1952–1988	17 deaths caused by cancer, including: 3 cases of bladder cancer (SMR = 12), 2 cases of kidney cancer (SMR = 9.52), 2 cases of nervous system cancer (SMR = 9.1) (benzidine and 2-naphthylamine)	72
Japan	604	n.d.	1945–1971	8 deaths caused by cancer, including: 2 cases of urinary tract cancer (SMR = 11.76), 2 cases of stomach cancer (SMR = 0.78), 2 lung tumors (SMR = 1.73) (benzidine and 2-naphthylamine)	73
Russia	4581 (2409 men, 2172 women)	1 month	1930–1989	8 cases of bladder cancer (SIR = 19.5); in the case of employees hired before attaining the age of 20 SIR = 49.4 (4 cases) (benzidine and 2-naphthylamine)	74
Japan	442 (437 men, 5 women)	n.d.	1935–1992	3 deaths caused by bladder cancer (SMR = 48.4), 3 deaths caused by urinary tract cancer (SMR = 24.4)	75
USA	400 (374 men, 26 women)	n.d.	1960–1998	28 deaths caused by cancer, including 4 deaths due to bladder cancer (SMR = 16.83), 12 deaths due to respiratory tract cancer (SMR = 3.9)	78
Poland	17 747 (11 660 men, 6087 women)	n.d.	1950–1995	increased incidence of cancer and tumors of the lip, tongue, esophagus, stomach, gall bladder, pancreas, peritoneum, cartilage, connective tissue, skin, testis, prostate, bladder, kidney, brain, Hodgkin's lymphoma, myeloma, leukemia (e.g., 2-naphthylamine, 4-aminodiphenol, benzidine, benzene, vinyl chloride, asbestos, styrene, acrylonitrile, N-phenyl-2-naphthylamine, formaldehyde, carbon tetrachloride)	79
Japan	224 men	n.d.	1953–2011	81 cancer cases (SIR = 1.58), 18 cases of lung tumors (SIR = 2.58), 7 cases of bladder cancer (SIR = 4.70), (2-NA and/or benzidine)	80
United Kingdom	n.d.	n.d.	1967–2015	stomach cancer (SMR = 1.26), lung cancer (SMR = 1.25), bladder cancer (SMR = 1.16)	81

n.d. – no data, SIR – standardized incidence ratio, SMR – standardized mortality ratio.

Table 3. Location and incidence of tumors in experimental animals, caused by 2-NA after oral exposure

Species	Dose/exposure period	Location and incidence of cancer	References
Mice			
BALB/c	2000 ppm/40 weeks	adenoma: 10 mice, hepatoma: 3 mice	93
A/J	600 mg/kg b.w./8 weeks	lung tumor: 8/14 males, 4/13 females	99
CBA mice	a) 160 mg/kg b.w./week/90weeks	a) hepatoma 24/57 males, 25/54 females, malignant hepatoma: 16 mic	92
	b) 240 mg/kg b.w./week/90 weeks	b) hepatoma: 6/9 males, 7/14 females	
IF mice	400 mg/kg b.w./week/90 weeks	liver cholangioma: 5/13 males, 5/12 females	92
Rats			
rats (no additional data)	whole life	bladder papilloma: 4/50	92
Fisher	a) 3 mg/rat/52 weeks	a) testicular interstitial cell tumor: 4/12, adrenal adenoma: 1/12	14
	b) 10 mg/rat/52 weeks	b) testicular interstitial cell tumor: 4/12, fibrosarcoma: 1/15, mammary adenocarcinoma: 1/14, mammary fibroadenomas: 2/14	
	c) 30 mg/rat/52 weeks	c) testicular interstitial cell tumor: 3/3, hepatoma: 1/3	
Wistar	a) 300 mg/kg b.w./week/1 year	a) bladder tumor: 5/17	100
	b) 300 mg/kg b.w./once a week/57 weeks	b) urothelial, hyperplasia: 8/18, including bladder carcinoma: 4/8	101
Hamsters			
Syrian golden hamsters	600 mg/week/whole life	bladder carcinoma: 10/23 males, 8/16 females, hepatoma: 1 male, 1 female	82
Rabbits	200 mg/twice a week/over 5 years	transitional-cell bladder papilloma, bladder epithelial hyperplasia	92
Dogs			
dogs (no additional data)	a) 5–30 mg/kg b.w./7.5 months	a) bladder carcinoma: 7/8	103
	b) 30 mg/kg b.w./8.5 months	b) bladder carcinoma: 7/8	
	c) 500600 mg/day/20–26 months	c) bladder carcinoma: 15/15	88
beagle	a) 5 mg/kg b.w./30 days	a) bladder carcinoma: 0/8	89**
	b) 25 mg/kg b.w./26 weeks	b) bladder epithelial carcinoma: 2/4	90
	c) 6.25, 12.5, 25 and 50 mg/kg b.w./2–26 months	c) bladder transitional cell carcinoma: 5/5	91
	d) 400 mg/34 months	d) bladder tumor: 24/34	87
mongrel*	a) 4 mg/day (dogs weighting ≤12 kg) and 5 mg/day (big dogs >12 kg)/14 weeks; for the following 12 weeks the doses were doubled, and tripled for the subsequent 63 weeks; starting oral dose: 100 mg/day, ending oral dose: 300 mg/day	a) bladder papillomas and carcinomas: 13/16	102
	b) 200 mg/day/6 months then 600 mg/day/2 years	b) bladder tumor: 2/4, bladder carcinoma: 1/2	85
	c) 400 mg/day/2 years	c) bladder tumor: 4/4	86
mongrel Airedale	100–700 mg/day	bladder papilloma: 3/3, anaplastic carcinoma: 1/3	84
Monkeys			
rhesus	6.25, 12.5, 25, 50, 100, 200, 400 mg/kg b.w./day/33–60 months	bladder carcinoma: 9/24	83

* Oral route of exposure + subcutaneous injection, ** no data on the route of exposure.

Table 4. Location and incidence of tumors in experimental animals, caused by 2-NA after a subcutaneous or intraperitoneal injection

Species	Injection	Dose/exposure period	Location and incidence of cancer	References
Mice				
BALB/c	subcutaneous	a) 50 µg/mouse/single injection	a) lung tumors: 15/71, hepatoma: 1/71	96
		b) 100 µg/5 days	d) lung adenoma: 9/41	97
Swiss	subcutaneous	a) 30 µl of 3% solution in gelatin	a) bronchial adenoma: 6/63	95
		b) 30 µg/1, 3, 5 day of life	b) hepatoma: 2/26, lymphosarcoma: 1/21	
Swiss albino	subcutaneous	0.1 ml of a 3% solution of 2-NA/50 weeks	sarcoma: 0/13; 10/16, hepatoma: 2/4, 4/5	85
mice (no additional data)	subcutaneous	312 mg/mouse/52 weeks	sarcoma: 12 mice, hepatoma: 7 mice, intestinal tumor: 1 malignant, 1 non-malignant	94
CBA mice	subcutaneous	0.1 ml of a 3% solution of 2-NA-HCl/10 months	sarcoma 0/11, hepatoma 4/11	85
Rats				
Chester Beatty	intraperitoneal	50 mg/kg b.w./twice a week/3 months	sarcoma: 2/14, salivary gland tumor: 1/14	98

The authors observed only liver cholangiomas. There were no tumor incidences. In another study conducted by Bonser et al. [92] on 23 CBA mice (14 females, 9 males), where the animals were receiving 2-NA at a dose of 240 mg/kg b.w. per week for 90 weeks via gavage (in arachis oil), the authors observed only hepatoma incidences. There were no tumors even in the control group (14 mice).

Groups of CBA mice (12–15 females, 14–15 males) were exposed to 2-NA via diet at a dose of 160 mg/kg b.w. per week for 90 weeks. There was no data concerning the control group. The authors observed that 42% of males and 46% of females had developed hepatomas. Malignant hepatomas were observed in 16 mice [92].

BALB/c mice (20 females) were exposed to 2000 ppm of 2-NA via diet for 40 weeks. In 6 out of 16 animals, hyperplasia of the bladder epithelium was observed at week 55. In the livers of the surviving animals, 3 hepatomas, 10 adenomas and hyperplastic nodules were seen [93].

Stoner et al. [99] administered 2-NA in tricaprylin via gavage 3 times a week, resulting in a total dose of 600 mg/kg b.w. for 8 weeks. After treatment, 57% of males (8/14) and 31% of females (4/13) developed lung tumors although this was not a significant increase in comparison to the control group.

Golden Siberian hamsters (30 males, 30 females) were exposed to 2-NA via diet at a total dose of 600 mg/kg b.w. per week throughout their life. First bladder carcinomas appeared after 45 weeks in 43% of males (10/23) and in 50% of females (8/16). In addition, 2 hamsters developed hepatomas. There were no bladder carcinomas in the control group. In the second experiment where hamsters were exposed to a lower dose of 2-NA via gavage (10 mg for 40 weeks), no tumors were observed [82].

Bonser et al. [92] exposed orally 6 rabbits to 2-NA at a dose of 200 mg per animal twice a week for over 5 years. One animal developed epithelial hyperplasia with a downgrowth in the bladder and 1 had a bladder papilloma.

A group of mongrel Airedale dogs (3 males, 1 female) were exposed to 150 mg of 2-NA once a day in milk for 3 weeks. Then, the dose was reduced to 100 mg per day (no data concerning the period) and gradually increased to 700 mg. One animal died after 1 year of treatment. Multiple bladder papillomas developed between 3.7–5 years, and an anaplastic carcinoma developed in 1 animal [84].

Bonser et al. [85] exposed a group of 4 female mongrel dogs to 2-NA encapsulated in gelatin at a dose of

200 mg per animal for 6 months (6 days a week). After that time, the dose was raised to 600 mg per animal and the treatment was continued for 2 years. Bladder tumors were observed in 2 animals but only 1 had an invasive bladder carcinoma.

A group of 4 mongrel dogs (all 4 female) were exposed to 2-NA (400 mg per animal) once a day for 2 years. All the animals developed bladder tumors within 9–18 months from the beginning of the exposure. In addition, all the animals received 5-fluorouracyl intravenously and intracavitarily to observe transient tumor regression. The observation time was 23–55 months or until death/sacrifice. The authors observed metastases of the bladder carcinoma in the lungs of 2 animals and in the kidney of 1 animal [86].

Conzelman and Moulton [87] exposed beagle dogs (34 dogs; sex not specified) orally to 2-NA (doses: 6.25, 12.5, 25, 50 mg/kg b.w.), mixed with lactose encapsulated in gelatin, 6 days a week for 2–26 months. The control group included 2 males and 2 females. The authors observed the development of bladder tumors in 24 out of 34 animals in all dose groups. An invasive transitional-cell carcinoma was diagnosed in 6 dogs (in 2 animals in the groups receiving 6.25, 12.5 and 50 mg/kg b.w., and in 5 animals in the group receiving 25 mg/kg b.w.). Invasive squamous-cell carcinomas developed in 1, 2, 3 and 2 dogs, respectively, in the increasing dose groups. In addition, papillary carcinomas were noticed in 1, 3 and 4 dogs in the highest dose groups. No tumors were observed in the control group.

Romanenko and Martynenko [103] treated 8 dogs (females) with 2-NA given orally at doses of 5–30 mg/kg b.w. for 7.5 months (4–6 times a week) and 30 mg/kg b.w. for 8.5 months (4–6 times a week). Bladder carcinomas developed in 7 out of 8 dogs (55 months after the first exposure).

A group of 15 dogs (females) was exposed orally to a capsule containing 500–600 mg of 2-NA daily for 20–26 months. All the animals developed hematuria and bladder carcinomas [88].

Radomski et al. [90], in the first experiment, exposed beagle dogs (5 males) to 2-NA (25 mg/kg b.w.) in corn oil, given orally in the form of capsules, for 5 days a week for 26 weeks. In the second experiment, the authors exposed 4 beagle dogs (2 males, 2 females) to the same dose of the examined substance for 1, 6 or 36 weeks and, after that time, the animals were sacrificed. In the third experiment, 8 beagle dogs (4 males, 4 females) were treated with the same doses of 2-NA for 26 weeks, following which 4 animals were killed and the remaining 4 lived for 3 years without any treatment. In some of the dogs, a loss of the bladder luminal membrane, lymphocyte infiltration of the submucosa and hyperplasia of the bladder epithelium were observed after 1 and 6 weeks of treatment. In addition, 2 out of 4 dogs developed bladder carcinomas.

Purchase et al. [91] exposed 5 beagle dogs (3 males, 2 females) to 400 mg of 2-NA encapsulated in gelatin for 5 days a week for 34 months. A group of 8 dogs (4 males, 4 females) served as the control. All the treated animals developed transitional cell carcinomas of the bladder. No neoplasia signs were found to occur in the control group.

In a study conducted by Conzelman et al. [83], rhesus monkeys were exposed to 2-NA, mixed with lactose given in gelatin capsules, for 6 days a week for 33–60 months, at doses of 6.25, 12.5, 25, 50, 100, 200 and 400 mg/kg b.w. per day. In 9 out of 24 treated animals, transitional cell bladder carcinomas, bladder papillary carcinomas or bladder carcinomas were observed.

Subcutaneous injections

Bonser et al. [85] exposed Swiss albino mice to 0.1 ml of a 3% solution of 2-NA in arachis oil twice a week for 50 weeks. The control group included 17 mice. There were no hepatomas in 13 mice, but in 2 out of 4 animals which had survived for more than 77 weeks hepatomas were observed. In a similar experiment, the prepared solution of 2-NA was left to stand for 4 weeks. The details were the same: 0.1 ml of a 3% solution of 2-NA in a subcutaneous injection twice a week for 50 weeks, which resulted in subcutaneous injection-site sarcomas in 10 out of 16 mice which had survived for more than 20 weeks. Four out of 5 animals which had survived for more than 80 weeks had hepatomas. No carcinomas were noted in the control group.

In the same study, Bonser et al. [85] administered 2-NA (a fresh solution of 2-NA-HCl) subcutaneously to CBA mice twice a week for 6 months, and then once a week for another 4 months. No sarcomas were observed, but 4 out of 11 mice developed hepatomas. In a similar experiment where 2-NA-HCl was purified by gradient sublimation, the authors observed the same results: no sarcomas and 4 of 11 mice developed hepatomas. In the control group consisting of 15 mice, 1 hepatoma had developed.

In another study, Bonser et al. [94] exposed a group of mice (45 males and 45 females) to a subcutaneous injection of 0.1 ml 2-NA in arachis oil (both freshly prepared and a 4-week-old suspension) twice a week for 52 weeks. The total dose was 312 mg per mouse. The

authors observed that 12 mice developed sarcomas, 7 mice hepatomas and 2 mice intestinal tumors (1 malignant, 1 benign). There is no data concerning the control group.

Roe et al. [96] exposed 91 newborn BALB/c mice to a single subcutaneous injection with 50 μg of 2-NA in 1% aqueous gelatin. The authors observed that 15 out of 71 mice which had not been sacrificed developed lung tumors which were not significant, and 1 had hepatoma. In the control group, lung tumors were observed in 2 of 21 mice.

In another study, 12 groups of 50–60 newborn BALB/c mice were exposed to a single subcutaneous injection of 2-NA (100 μg in 20 μL arachis oil) within 24 hours after birth, or once a day for the first 5 days of their life. The authors observed that incidences of pulmonary adenomas were not statistically different between the treatment and control groups. In the second experiment, newborn BALB/c mice were given 100 μg of 2-NA in 3% aqueous gelatin in subcutaneous injections for the first 5 days of their life. In 9 out of 41 surviving mice, and in 4 of 48 animals belonging to the control group, lung adenomas were observed, this number not being significant. The authors concluded that the metabolic system of newborn mice was immature and no tumors were observed [97].

Newborn Swiss mice (68 animals, sex unknown) were exposed to a single subcutaneous injection of 30 μL 2-NA as a 3% suspension in gelatin. After 10 months of exposure, bronchial adenomas were observed in 6 out of 63 treated mice. In the control group (57 mice), no tumors were noted [95].

In another study, Radomski et al. [95] administered 30 μg of 2-NA suspended in 3% gelatin (100 μg/dose) in a subcutaneous injection on the first, third and fifth day of life to newborn Swiss mice (28 males, 23 females). After 12 months, 2 out of 26 surviving mice developed hepatoma and 1 female had a lymphosarcoma. In the control group, (20 males, 30 females) 1 case of lymphosarcoma was observed.

Mongrel dogs (16 females) were exposed to subcutaneous injections of 2-NA at doses of 4 mg/day for dogs weighting ≤12 kg, and 5 mg/day for larger dogs, for 14 weeks. These doses were doubled in the following 12 weeks and tripled in the following 63 weeks. For the subsequent 63 weeks, the animals also received a daily dose of 2-NA in capsules, directly to their food (the starting dose: 100 mg/day). Until the subcutaneous injections were terminated, a daily oral dose of 2-NA was 300 mg. The control group included 4 dogs. After 20–26 months of the completed treatment, bladder papillomas and carcinomas were observed in 13 out of 16 dogs [104].

Intraperitoneal injections

Chester Beatty rats (16 males) were exposed to an intraperitoneal injection of 2-NA (a dose of 50 mg/kg b.w.), suspended in arachis oil, twice a week for 3 months. Two out of 14 animals developed sarcomas, and in 1 animal a salivary gland tumor was observed. There is no data concerning the control group [98].

The carcinogenic effects of 2-NA have been repeatedly tested on various animal species, via various routes of exposure, but there is no data regarding inhalation exposure. Detailed data regarding the incidence and location of tumors is presented in Table 3. However, malignant and benign tumors were most often found in the bladder and liver. Urinary bladder cancers (adenomas and carcinomas from transient cells) were observed in hamsters, dogs and monkeys after oral administration of 2-NA [82–91].

A small number of bladder tumors were observed in rats receiving 2-NA for 57 weeks via gavage at a dose of 30 mg/rat [14]. Chronic oral exposure to 2-NA at doses of 0.1–30 mg/rat for 52 weeks resulted in male adenocarcinomas and mammary fibroadenomas [14].

One of 6 rabbits exposed to 2-NA for 5 years developed bladder papilloma [92].

It was found that 2-NA administered via gavage to the stomach or via diet resulted in hepatocellular carcinomas of the liver [92,93]. Following subcutaneous administration in both mice and rats, sarcomas were observed at the injection site [94,95,98], and pulmonary carcinomas were observed following intraperitoneal administration in mice [96].

Intravesical administration of 2-NA (10 mg) in dogs did not cause bladder tumors or other cancers within 45 months [90].

In addition, 2-NA metabolites have been tested for carcinogenicity, with N-hydroxy-2-naphthylamine being shown to be much more carcinogenic than 2-NA. Direct evidence was obtained from a study conducted on neonatal mice in which lung adenomas had developed after a subcutaneous injection of N-hydroxy-2-naphthylamine [97]. The incidence of adenoma after administration of N-hydroxy-2-naphthylamine was higher than after administration of 2-NA. Studies conducted on mice, rats and dogs, intravesically doped with N-hydroxy-2-naphthylamine, confirmed the carcinogenic effects of this 2-NA metabolite [104,105].

Table 5. Hygienic standards for 2-naphthylamine in the world [106]

Country	OEL-TWA [ppm]	OEL-TWA [mg/m^3]	STEL [mg/m^3]
France	0.001	0.005	–
Poland		0	0
Hungary		–	0.005
Italy			0.001

OEL-TWA – occupational exposure limit – time weighted average, STEL – short term exposure limit.

Table 6. Concentration of 2-naphthylamine in the air, corresponding to the subsequent risk levels

Risk level	2-NA concentration [mg/m^3]
10^{-5}	0.000318
10^{-4}	0.00318
10^{-3}	0.0318

In the available literature, there is a lack of data on the carcinogenicity of 2-NA on experimental animals after inhalation and through skin exposure.

Embryotoxicity, teratogenicity and reprotoxicity

The authors of this article have not found any data confirming the embryotoxicity, teratogenicity and reprotoxicity in human studies. However, they have identified 1 study carried out on Wistar rats where histopathological data revealed testicular atrophy in males and uterine polyps in females [14].

Occupational exposure limits in the world

The hygienic standards of 2-NA are presented in Table 5, where the occupational exposure limit values have been divided into 2 standards (occupational exposure limit – time weighted average – OEL-TWA and short term exposure limit – STEL). Many countries have not set their OEL for 2-NA due to its proven carcinogenicity and the fact that bladder cancer is assumed as a critical effect.

In 1974, the International Agency for Research on Cancer assumed 2-NA as a carcinogen to humans (category 1). The Slope Factor coefficient value (the dose-response ratio, the tangent of the dose-response curve in the low-dose range with a positive OX axis direction) estimated for humans, and published by California Environmental Protection Agency [107], is SF = 1.8 (mg/kg-day)$^{-1}$. The lifetime average daily dose (LADD) was calculated based on the risk level of 10^{-5} as 0.4 μg/day [107], based on a study conducted by Conzelman et al. [83]:

$$D = \frac{RSI}{70} = \frac{0.0004}{70} = 5.7\times10^{-6}\ \text{mg/kg b.w./day} \quad (1)$$

where:
D – LADD [mg/kg b.w./day],
RSI – the risk specific intake (0.0004 mg/day on the basis of the risk level of 10^{-5}),
70 – human body weight [kg].

The risk of developing cancer caused by exposure to the tested carcinogen was calculated based on the formula:

$$R = SF\times D = 1.8\times5.7\times10^{-6} = 0.00001 \quad (2)$$

where:
R – risk,
D – LADD [mg/kg b.w./day],
SF – slope factor.

After substituting the formula, the appropriate OEL-TWA value is obtained:

$$OEL = D\times\frac{24}{8}\times\frac{365}{240}\times\frac{70}{40}\times\frac{1}{10}\times70 = 0.000318\ \text{mg/m}^3 \quad (3)$$

where:
D – LADD [5.7×10^{-6} mg/kg b.w./day],
24 – number of hours,
8 – number of working hours,
365 – number of days in a year,
240 – number of working days,
70 – age [years],
40 – seniority [years],
10 – human lung ventilation [m^3],
70 – human body weight [kg].

The concentration values of 2-NA in the air were obtained, corresponding to the subsequent risk levels, and are presented in Table 6.

Assuming an acceptable risk of cancer as 10^{-4}, the authors propose to adopt the OEL value for 2-NA at 0.003 mg/m^3.

CONCLUSIONS

In the past, 2-NA was used for the production of azo dyes, as an antioxidant in the cable industry and in the rubber industry. Currently, it is used mainly in research laboratories, *inter alia*, as a model substance in cancer research, in workers exposed to fumes containing 2-NA generated as a result of pyrolysis, as well as in workers dealing with nitric polycyclic aromatic hydrocarbons that are metabolized to 2-NA. Exposure can also occur on products in which 2-NA is present as an impurity (some rubber components).

In addition, exposure to 2-NA is possible not only in the occupational environment but also in general, through exposure to tobacco smoke or other fumes containing 2-NA (during the heating of food oils).

Occupational exposure to 2-NA is important for the respiratory tract, mucous membranes and the skin, and, to a lesser extent, for absorption from the gastrointestinal tract. It is absorbed into the body through the skin and by inhalation, and then undergoes metabolic changes. Most of the absorbed 2-NA dose is excreted in the urine, in the form of free metabolites, metabolites conjugated to acids, and even in an unchanged form. Based on literature data, the effects of 2-NA toxicity in sub-chronic and chronic exposure include contact dermatitis, chronic cystitis and bladder cancer. In addition, 2-NA may be included in the group of promutagens, thus agents that display a genotoxic activity after metabolic activation. Literature data clearly shows that adducts are formed in the reaction of DNA.

The authors concluded that it is recommended to determine OEL values which will allow preparing an exposure assessment of people at work and propose a value at a level of 0.003 mg/m3. Unfortunately, this value fails to guarantee full protection against cancer risk. It merely implies that 1 cancer case per 10 000 cases is an acceptable risk.

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