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N-Phenyl-2-naphthylamine

MAK Value Documentation, addendum – Translation of the German version from 2021

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Keywords

N-phenyl-2-naphthylamine; human carcinogen; risk assessment; germ cell mutagenicity; skin absorption; toxicokinetics; metabolism; genotoxicity; carcinogenicity; sensitization

Abstract

The German Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) summarized and re-evaluated the data for the carcinogenicity and germ cell mutagenicity of *N*-phenyl-2-naphthylamine [135-88-6]. Relevant studies were identified from a literature search. *N*-Phenyl-2-naphthylamine is used as antioxidant e.g. in rubber and lubricants. *N*-Phenyl-2-naphthylamine is metabolized to 2-naphthylamine, a well-known human bladder carcinogen. 2-Naphthylamine exerts its carcinogenic potential after enzymatic conversion to reactive metabolites able to form adducts with macromolecules such as DNA or haemoglobin. As 2-naphthylamine-haemoglobin adducts were found after oral administration of *N*-phenyl-2-naphthylamine to rats, this shows that *N*-phenyl-2-naphthylamine is activated to carcinogenic metabolites. The study concluded that approximately 1% (absolute) of the 2-naphthylamine that originates from *N*-phenyl-2-naphthylamine can be further metabolized. As occupational exposure to *N*-phenyl-2-naphthylamine may lead to the formation of 2-naphthylamine, and this in turn may induce the development of bladder tumours, *N*-phenyl-2-naphthylamine has been classified in Carcinogen Category 1. Exposure-risk relationships for the induction of bladder tumours by *N*-phenyl-2-naphthylamine and 2-naphthylamine are established. In analogy to 2-naphthylamine, *N*-phenyl-2-naphthylamine is regarded as a germ cell mutagen and has been classified in Category 3 A for germ cell mutagens. *N*-Phenyl-2-naphthylamine is absorbed via the skin in small amounts. As subsequent metabolic activation to the carcinogen 2-naphthylamine is likely, *N*-phenyl-2-naphthylamine has been designated with “H”. The data for skin sensitizing effects confirm the “Sh” designation. There is no data on a potential sensitization of the airways.

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MAK value	–
Peak limitation	–
Absorption through the skin (2020)	**H**
Sensitization (2010)	**Sh**
Carcinogenicity (2020)	**Category 1**
Prenatal toxicity	–
Germ cell mutagenicity (2020)	**Category 3 A**
BAR/BLW/EKA	–
CAS number	135-88-6

The classification of *N*-phenyl-2-naphthylamine in Carcinogen Category 3 B was confirmed in 2011 on the basis that its dephenylation product, the metabolite 2-naphthylamine, is classified as a human carcinogen. In workers exposed by inhalation to *N*-phenyl-2-naphthylamine and in test persons, rats, rabbits and dogs given oral doses of *N*-phenyl-2-naphthylamine, 2-naphthylamine was detected in the urine in concentrations that were too high to be attributed solely to contamination with 2-naphthylamine. In humans, it is estimated that up to 1% of *N*-phenyl-2-naphthylamine is metabolized to 2-naphthylamine. Other findings that support the classification of the substance in Carcinogen Category 3 B are the induction of tumorigenic effects in female mice, the doubtful reliability of the negative findings reported by carcinogenicity studies in dogs resulting from methodological shortcomings and the weak clastogenic effects determined in vitro (Hartwig 2015).

Until recently, it was not known whether the 2-naphthylamine that forms from *N*-phenyl-2-naphthylamine is available for further metabolism and thus leads to the subsequent formation of carcinogenic products such as *N*-hydroxy-2-naphthylamine and the 2-naphthylamine nitrenium ion. A relevant study has now become available which provides indirect evidence of the formation of these products. The study is described in this supplement, followed by an evaluation of whether the carcinogenicity classification of the substance requires amendment and whether the substance should be classified in a category for germ cell mutagens. In addition, two studies investigating skin penetration and new findings relating to skin sensitizing effects in humans have been published.

Toxicokinetics and Metabolism

Absorption, distribution, elimination

Dermal exposure

Two studies investigating skin penetration have become available since the publication of the supplement in 2011 (Hartwig 2015). Unlike the findings of a study carried out in 2008, these ex vivo studies found evidence that the substance is able to penetrate the skin of humans (Dennerlein et al. 2017) and pigs (Marek et al. 2017):

Samples of human skin that had been stored in a frozen condition were treated with the aromatic amines *N*-phenyl-2-naphthylamine and 2-naphthylamine, either individually or in combination dissolved in hexane or as a mixture in a technical-conform lubricant. The samples of abdominal skin were taken from 2 female donors. The samples were

exposed for 8 hours in an ex vivo diffusion cell model and the receptor fluid was analysed during exposure (2, 4, 8 hours) and after the end of exposure (16, 24, 48 hours). The test formulations were prepared with 2 g of *N*-phenyl-2-naphthylamine or 4.1 mg of 2-naphthylamine per litre of hexane or with concentrations of 1% and 0.002%, respectively, in lubricant. All scenarios involved the application of either 259 µg *N*-phenyl-2-naphthylamine or 0.52 µg 2-naphthylamine per 0.64 cm^2. When applied in hexane, 5% of the *N*-phenyl-2-naphthylamine and 38% of the 2-naphthylamine were absorbed. During the first 8 hours, 7% to 15% of the total absorbed amount of *N*-phenyl-2-naphthylamine penetrated the skin. The substance continued to penetrate the skin even after the end of exposure. The maximum flow rate was determined to be 0.27 µg/cm^2 and hour. By the end of the test (48 hours after the beginning of application), the substance had not fully penetrated the skin. During the first 8 hours, 82% to 92% of the total absorbed amount of 2-naphthylamine penetrated the skin at a maximum flow rate of 0.06 µg/cm^2 and hour during the first 4 hours. If the two substances were applied together in lubricant, 1.9% of the *N*-phenyl-2-naphthylamine and 2.9% of the 2-naphthylamine were absorbed. The fluxes were calculated to be 0.12 and 0.002 µg/cm^2 and hour, respectively. The cumulative amount of *N*-phenyl-2-naphthylamine that penetrated the skin was 2 µg after 48 hours, which is equivalent to 3.1 µg/cm^2. If only 2-naphthylamine was applied, the first traces of the substance were detected in the receptor fluid after about 38 minutes. In a mixture with *N*-phenyl-2-naphthylamine dissolved in hexane or in the lubricant, the breakthrough time was 3.5 and 2.9 times shorter, respectively. By comparison, *N*-phenyl-2-naphthylamine was first determined in the receptor fluid about 4 hours after application (alone or in a mixture with 2-naphthylamine dissolved in hexane). When applied as a mixture in lubricant, the first traces of the substance were detected more than 7 hours later. The accumulation of the two substances in the various skin layers was likewise dependent on the application conditions (co-exposure and formulation). Co-exposure increased the intradermal absorption of both substances (Dennerlein et al. 2017).

N-Phenyl-2-napththylamine was likewise found to penetrate freshly prepared samples of porcine skin. The experimental conditions of the study resembled the working conditions typical for the printing industry in Germany during the 1960s/1970s, when the use of solutions containing *N*-phenyl-2-naphthylamine in dichloromethane was widespread. The test formulations were prepared with a 1% solution in 0.5 ml dichloromethane (96%) and corn oil (4%), which is equivalent to a concentration of 12 g/l and an *N*-phenyl-2-naphthylamine concentration of 1.91 mg per cm^2 of skin. Under dynamic occlusive conditions and exposure for 1 hour, the flux of *N*-phenyl-2-naphthylamine through the skin was very slow (0.02 ± 0.01 µg/cm^2 and hour) with a cumulative absorbed amount of 0.80 ± 0.26 µg/cm^2 in 48 hours and a lag time of 6.33 ± 2.21 hours. The percutaneous absorption of *N*-phenyl-2-naphthylamine increased 2-fold when the substance was dissolved in dichloromethane in comparison with the level of absorption determined after the substance was dissolved in physiological saline solution with 5% ethanol. Additionally, *N*-phenyl-2-naphthylamine was found to accumulate in the subcutaneous layers of the skin and is continuously released into the organism from this reservoir. After exposure for 1 hour followed by the removal of the solution and the rinsing of the skin samples, 2 µg/cm^2 was found to have been absorbed over a period of 160 hours. These findings indicate that the systemic exposure continues even after exposure of the workers has terminated (Marek et al. 2017).

The in vitro findings were confirmed by the findings in living pigs. A defined amount (no other details) of a 1% solution of *N*-phenyl-2-naphthylamine in dichloromethane/oil (96/4, v/v) was sprayed on 200 cm^2 of skin of 4 German domestic pigs on 5 consecutive days; each day, the substance was applied 4 times within a 60-minute period. After this repeated, non-occlusive application, mean concentrations of *N*-phenyl-2-naphthylamine of up to 2.3 µg/l (range 1.2–4.0 µg/l) were determined in the blood of the animals after completion of the fifth application cycle under the same conditions as used for the in vitro experiments (the working conditions typical for the printing industry) (Koslitz et al. 2016).

Oral or inhalation exposure

Table 1 was previously published in the 2011 supplement and shows the excretion levels of *N*-phenyl-2-naphthylamine and 2-naphthylamine determined in the 24-hour urine of test persons and workers after oral and inhalation exposure to *N*-phenyl-2-naphthylamine, respectively. The amount of 2-naphthylamine detected in the urine of the test persons cannot be explained solely by contamination with 2-naphthylamine, as the excreted amounts were 50 to 400 times

higher than the amount contained in the applied dose. Overall, up to 0.03% of the absorbed *N*-phenyl-2-naphthylamine dose (oral or inhalation) was recovered in the urine as 2-naphthylamine.

Tab. 1 Amount of *N*-phenyl-2-naphthylamine and 2-naphthylamine in the urine within 24 hours after oral or inhalation exposure to *N*-phenyl-2-naphthylamine (Kummer and Tordoir 1975)

Exposure (n persons)	Amount of *N*-phenyl-2-naphthylamine [mg]	2-Naphthylamine (impurity, 0.8 µg/g) [µg]	*N*-Phenyl-2-naphthylamine in the urine [µg]	2-Naphthylamine in the urine [µg]
oral (19 test persons)	10	0.008	0–163	0.4–3
oral (1 test person)	30	0.024	290 (48 hours)	10
inhalation (4 workers)	about 40	0.032	22–213	3–8

The 2-naphthylamine concentrations excreted with the urine after daily uptake of *N*-phenyl-2-naphthylamine by inhalation can be calculated using the data from the workplace study: the estimated total absorbed amount of 40 mg per day is equivalent to an extrapolated concentration of 4 mg/m^3 at a respiratory volume of 10 m^3 per 8-hour working day. Between 3 and 8 µg of 2-naphthylamine (mean: 5.5 µg) were recovered in the 24-hour urine (about 1.5 l) of 4 exposed workers; this is equivalent to a mean 2-naphthylamine concentration of 3.7 µg/l. The study with the 4 workers demonstrates that about 1 µg of 2-naphthylamine would be excreted per litre of urine after inhalation exposure to an *N*-phenyl-2-naphthylamine concentration of 1 mg/m^3.

Metabolism

Figure 1 shows the postulated metabolic pathway from N-phenyl-2-naphthylamine to 2-naphthylamine and the subsequent metabolic steps (Weiss et al. 2013). To determine the quantitative fraction of *N*-phenyl-2-naphthylamine that is first dephenylated to 2-naphthylamine and then further metabolized to carcinogenic metabolites, a study in rats was carried out to detect 2-naphthylamine–haemoglobin adducts. Groups of 5 male and 5 female CD rats were given single gavage doses of *N*-phenyl-2-naphthylamine of 0, 2, 50, 200 or 550 mg/kg body weight or 2-naphthylamine of 0, 0.075, 0.2, 2 or 75 mg/kg body weight. Corn oil was used as the vehicle. The 2-naphthylamine concentration was determined in the pooled urine 0 to 24 or 24 to 48 hours (only in the group given the highest *N*-phenyl-2-naphthylamine dose) after administration of either *N*-phenyl-2-naphthylamine doses of 200 or 550 mg/kg body weight or a 2-naphthylamine dose of 0.075 mg/kg body weight. The haemoglobin adduct levels were determined 24, 48 and 72 hours after treatment with both substances and additional blood samples were taken 96, 120 and 168 hours after the administration of *N*-phenyl-2-naphthylamine. In the control group, the background urinary excretion of 2-naphthylamine was on average below 10 ng/l, which is equivalent to the daily excretion of less than 0.1 ng 2-naphthylamine.

Within 24 hours after treatment with an oral **2-naphthylamine** dose of 0.075 mg/kg body weight, the mean levels of urinary excretion were 107.9 ng 2-naphthylamine (0.7% of the applied dose) in the males and 116.5 ng (0.6%) in the females. The effects observed after treatment with *N*-phenyl-2-naphthylamine differed between the sexes. Within the first 24 hours after treatment with a single *N*-phenyl-2-naphthylamine dose of 200 mg/kg body weight, amounts of 456 ± 319 and 1656 ± 712 ng **2-naphthylamine**, respectively, were determined in the urine of the males and females. The amount excreted by the females within the first 24 hours was thus about 4 times as high as that excreted by the males and, during the second 24-hour interval, twice as high as the amount excreted by the males. The amounts excreted remained about the same in the males during the two test intervals, while the females excreted twice as much in the first 24 hours as in the second interval from 24 to 48 hours.

In this study, the test formulation of *N*-phenyl-2-naphthylamine contained < 0.0024% (24 ppm) 2-naphthylamine as an impurity. Therefore, the animals treated with *N*-phenyl-2-naphthylamine doses of 200 and 550 mg/kg body weight concurrently absorbed 2-naphthylamine doses of < 0.005 and < 0.0132 mg/kg body weight, respectively. Within the first 24 hours after treatment with *N*-phenyl-2-naphthylamine (see above), the amounts of 2-naphthylamine excreted

with the urine were 70 times as high in the males and 225 times as high in the females as the amounts expected on the basis of the impurities contained in the test substance. This shows that the amount of 2-naphthylamine contained in *N*-phenyl-2-naphthylamine makes up a negligible fraction of the total amount of excreted 2-naphthylamine. **By combining these findings with the data from other studies, the authors determined that about 0.75% to 1.5% (as related to the molar amount of substance) or 0.5% to 1% 2-naphthylamine (absolute), respectively, is formed from the applied amount of *N*-phenyl-2-naphthylamine.** In both sexes, the haemoglobin adduct levels in the blood reached their maximum concentration 96 hours after treatment with *N*-phenyl-2-naphthylamine and 48 hours after treatment with 2-naphthylamine. The adduct levels decreased more rapidly after treatment with 2-naphthylamine than after treatment with *N*-phenyl-2-naphthylamine: with 2-naphthylamine, adduct levels had decreased to about 20% (female animals) and 15% (male animals) of the maximum concentration 24 hours after reaching the maximum concentration. After treatment with *N*-phenyl-2-naphthylamine, adduct levels decreased to about 75% in both sexes. More adducts formed in the female rats at the same dose. Blood samples analysed 120 hours (*N*-phenyl-2-naphthylamine) and 72 hours (2-naphthylamine) after treatment demonstrated a proportionality between the 2-naphthylamine–haemoglobin adduct concentration and the applied dose. This study provides indirect evidence that *N*-phenyl-2-naphthylamine given to rats in oral doses is converted to 2-naphthylamine, which in turn forms reactive oxidative metabolites that are able to covalently bind to haemoglobin. To form the same amount of adducts as from a specific dose of 2-naphthylamine, according to the authors, a 100 to 200-fold dose of *N*-phenyl-2-naphthylamine would be required. After comparing the haemoglobin adduct concentrations and the excreted amounts of 2-naphthylamine, they concluded that the entire amount of 2-naphthylamine that is formed by the dephenylation of *N*-phenyl-2-naphthylamine is available for further *N*-oxidation (Weiss et al. 2013).

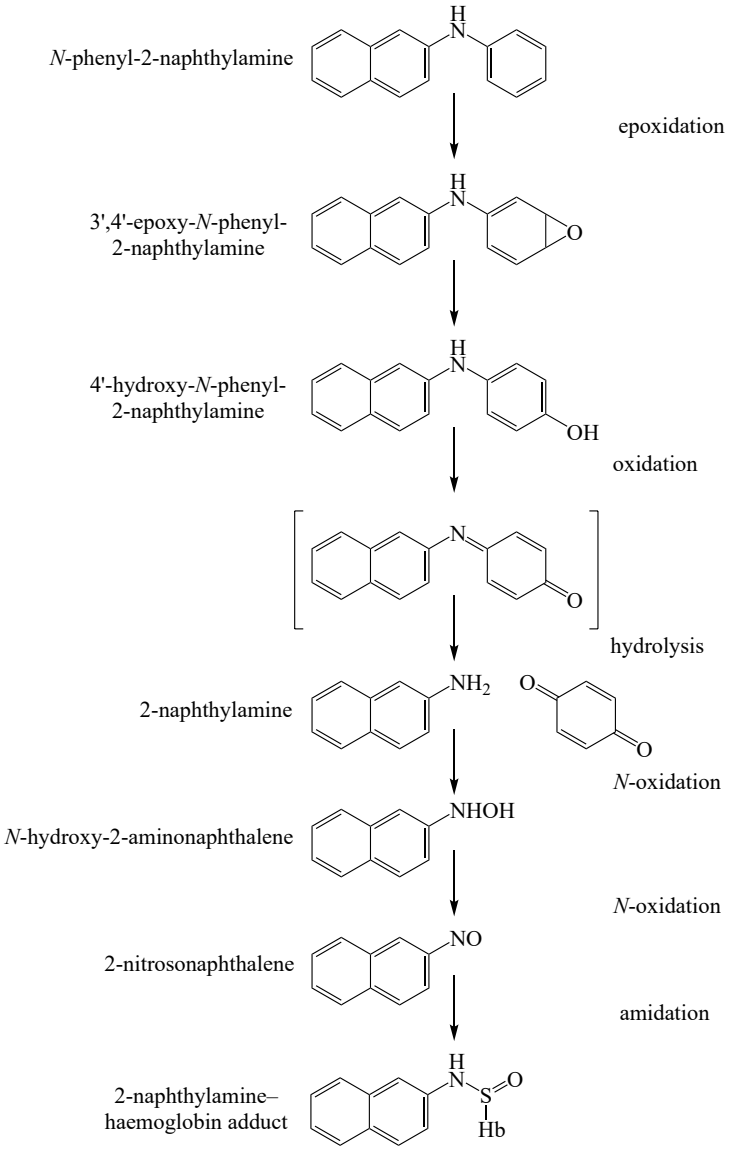

Fig. 1 Postulated metabolic pathway from *N*-phenyl-2-naphthylamine to 2-napthylamine and the subsequent metabolic steps (according to Weiss et al. 2013)

Effects in Humans

Allergenic effects

Of a total of 29 522 patients examined at the centres of the European Surveillance System on Contact Allergies (ESSCA) network, 2870 were tested both with the baseline series and with an additional rubber series. Patch tests with *N*-phenyl-2-naphthylamine were carried out in 559 patients; of these, 1 person (0.18%) produced a strong positive reaction (2+ or 3+ reaction). Questionable or irritant reactions were obtained in 2.82% of the patients. None of the patients produced a 1+ reaction (Uter et al. 2016).

Animal Experiments and in vitro Studies

Allergenic effects

There are no data available.

Genotoxicity

No new studies investigating genotoxicity have become available since the supplement was published in 2011 (Hartwig 2015). The data are summarized below.

The in vitro genotoxicity data suggest a weak clastogenic potential in mammalian cells in the presence of a metabolic activation system. The substance does not cause mutagenic effects in bacteria. The study with positive findings in the $TK^{+/-}$ test did not include information specifying whether the effects were based on chromosomal aberrations or mutations, as the study report did not provide any data about the size of the colonies that formed. There are no valid in vivo studies of genotoxicity (Hartwig 2015).

Analysis of the carcinogenic risk

Below, the risk of developing bladder cancer is calculated for workers exposed to *N*-phenyl-2-naphthylamine. The calculation is based on the data from an animal study investigating carcinogenicity induced by 2-naphthylamine in rhesus monkeys (Conzelman et al. 1969). As the metabolite 2-naphthylamine causes genotoxic effects, the risk was calculated by linear extrapolation. In the carcinogenicity study, gavage doses of 2-naphthylamine of 6.25 to 400 mg/kg body weight and day were given to monkeys on 6 days a week for up to 60 months. A bladder tumour developed in 1 animal of the group of 6 animals that were treated with doses of up to 25 mg/kg body weight and day (time-weighted average: 20.3 mg/kg body weight and day). The risk was therefore 17% or 0.85% per mg/kg body weight and day. Bladder tumours were induced in 2 of 4 animals given a daily dose of 26 to 50 mg/kg body weight (time-weighted average: 48.75 mg/kg body weight and day). Therefore, the risk of developing a bladder tumour was 50% or 1.02% per mg/kg body weight and day for this group. The risk per dose unit was lower in the higher dose groups (not demonstrated). Therefore, an about equally high risk of 1% per mg/kg body weight and day was calculated based on the data for the two low dose groups. As the animals were treated on 6 days a week, the findings were converted to reflect the 5 days of a standard working week, and the dose unit was multiplied by 6/5 (= 1.2 mg/kg body weight and day, 5 days/week). By extrapolating the oral dose from the animal study to a concentration in workplace air (assuming 100% oral absorption, 70 kg body weight, 10 m^3 respiratory volume and the corresponding species-specific correction value for monkeys of 2), this results in a concentration of 4.2 mg/m^3 (1.2 mg/kg body weight / 2 × 70 kg body weight / 10 m^3 = risk 1%).

Additional risk-based values are derived using the following calculations and assumptions:

1. The monkeys were observed for a period of 5 years and have a life expectancy of about 30 years (5/30 = 1/6)
2. The duration of exposure in the monkey study was 5 years at a life expectancy of 30 years. Workers are exposed at the workplace for a period equivalent to about half of their lives (75 years / 40 years of exposure × 52 weeks / 48 weeks minus days of leave)
3. The extrapolation of the duration of exposure in the monkey study to workplace conditions: 5 years of exposure / 30 years life expectancy monkey × 75 years / 40 years exposure of worker × 52 / 48 weeks (= 1/3)
4. 4.2 mg/m^3 × 1/6 × 1/3 = 2-naphthylamine concentration of 0.23 mg/m^3 or 0.039 ml/m^3 (= 1% risk or 1 : 100)

On this basis, a risk of 4 in 1000 exposed persons is calculated for exposure for 40 years to a 2-naphthylamine concentration of 0.017 ml/m^3 (0.1 mg/m^3). At a risk of 4 in 10 000, exposure is reduced to 0.0017 ml/m^3 (0.01 mg/m^3) and a risk of 4 in 100 000 is determined for a 2-naphthylamine concentration of 0.00017 ml/m^3 (0.001 mg/m^3). In comparison with

vinyl chloride, **2-naphthylamine** is 2350 times as carcinogenic (vinyl chloride: 40 ml/m^3 = 4 : 1000; 4 ml/m^3 = 4 : 10 000; 0.4 ml/m^3 = 4 : 100 000) (Table 2) (Hartwig and MAK Commission 2023).

Assuming that 1% 2-naphthylamine forms as a metabolic product of *N*-phenyl-2-napthylamine in both humans and rats, the following risk-based values are derived: a risk of 4 cases of bladder cancer per 1000 exposed persons is calculated for occupational exposure to an ***N*-phenyl-2-napthylamine** concentration of 10 mg/m^3. A risk of 4 : 10 000 is calculated for an *N*-phenyl-2-naphthylamine concentration of 1 mg/m^3, and a risk of 4 : 100 000 for an *N*-phenyl-2-naphthylamine concentration of 0.1 mg/m^3 (Table 2).

Manifesto (MAK value/classification)

The critical effect of *N*-phenyl-2-naphthylamine is the carcinogenicity expected after long-term contact with sufficient amounts of the substance. This is the critical effect also in humans.

Carcinogenicity. As previously discussed in the introductory section, 2-naphthylamine forms as a metabolite during the dephenylation of *N*-phenyl-2-naphthylamine. This metabolite induces carcinogenic effects in humans. In workers exposed by inhalation to *N*-phenyl-2-naphthylamine and in test persons, rats, rabbits and dogs given oral doses of *N*-phenyl-2-naphthylamine, 2-naphthylamine was determined in the urine in concentrations that were too high to be attributed solely to contamination with 2-naphthylamine. In humans, it was estimated that up to 1% of the absorbed amount of *N*-phenyl-2-naphthylamine is metabolized to 2-naphthylamine. In addition, there is evidence of the development of tumorigenic effects in female mice. The negative findings reported by carcinogenicity studies in dogs are of questionable validity. *N*-phenyl-2-naphthylamine induces weak clastogenic effects in vitro (Hartwig 2015).

A recent study found 2-naphthylamine–haemoglobin adducts in rats given a single oral dose of *N*-phenyl-2-naphthylamine. This is indirect evidence that 2-naphthylamine undergoes further metabolism to form the metabolites *N*-hydroxy-2-naphthylamine and 2-nitrosonaphthalene because these are necessary for the formation of haemoglobin adducts. The 2-naphthylamine nitrenium ion may form from *N*-hydroxy-2-naphthylamine; this ion is regarded as responsible for the formation of the relevant DNA adducts. However, to date, no evidence of these adducts has been found. A study found that, overall, up to 1% (absolute) of the absorbed amount of *N*-phenyl-2-naphthylamine is available for further metabolism following its conversion to 2-naphthylamine (Weiss et al. 2013). 2-Naphthylamine was determined also in the urine of humans exposed to *N*-phenyl-2-naphthylamine. It can therefore be assumed that humans are likewise exposed to 2-naphthylamine, a known and potent human carcinogen. As a result, bladder tumours may form after occupational exposure to *N*-phenyl-2-naphthylamine.

On this basis, *N*-phenyl-2-naphthylamine has been classified in Carcinogen Category 1.

Using the findings of the inhalation study with monkeys and the risk of developing bladder tumours calculated from the study data (Conzelman et al. 1969; see the Section „Analysis of the carcinogenic risk“), the exposure–risk relationships shown in Table 2 have been derived for 2-naphthylamine and *N*-phenyl-2-naphthylamine. For purposes of comparison, the table includes the exposure–risk relationship for the induction of angiosarcomas in the liver by vinyl chloride.

A comparison of the calculated values demonstrates that 2-naphthylamine is 2350 times and *N*-phenyl-2-naphthylamine is 40 times as carcinogenic on a ml/m^3 basis than equal amounts of the human carcinogen vinyl chloride. The risk may have been overestimated for *N*-phenyl-2-naphthylamine because the derivation of risk from data from animal studies requires many extrapolation steps and assumptions, whereas the risk calculation for vinyl chloride was derived directly from human data. The risk values for vinyl chloride are also markedly higher when these are calculated using data from animal studies.

Tab. 2 Exposure–risk relationships for bladder tumours induced by 2-naphthylamine and *N*-phenyl-2-naphthylamine and, for purposes of comparison, for the induction of liver angiosarcomas by vinyl chloride (Hartwig and MAK Commission 2023)

Risk	Concentration [ml/m³] (related to molar mass)	Concentration [mg/m³]
2-naphthylamine (Carcinogen Category 1)		
4 : 1000	0.017	0.1
4 : 10 000	0.0017	0.01
4 : 100 000	0.00017	0.001
N-phenyl-2-naphthylamine		
4 : 1000	1.1	10
4 : 10 000	0.11	1
4 : 100 000	0.011	0.1
vinyl chloride (Carcinogen Category 1)		
4 : 1000	40	100
4 : 10 000	4	10
4 : 100 000	0.4	1

Germ cell mutagenicity. *N*-Phenyl-2-naphthylamine is weakly clastogenic in vitro. No mutations were induced in the Salmonella mutagenicity test. Whether the positive findings obtained in the $TK^{+/-}$ test were caused by chromosomal aberrations or mutations is unclear, as the study report did not provide any data about the size of the colonies that formed. Valid genotoxicity data in vivo, particularly data for germ cells, are not available. The metabolite 2-naphthylamine has been classified in Category 3 A for germ cell mutagens. Decisive for this classification were the mutagenic effects determined in vitro and in vivo, the evidence showing that the substance crosses the placental barrier and, in analogy to structurally related aromatic amines, its assumed ability to cross the blood-testis barrier, which is evidence that the substance is able to reach the germ cells. As *N*-phenyl-2-naphthylamine is bioavailable, it is assumed that both the parent substance as well as the critical metabolite reach the germ cells. Therefore, in analogy to 2-naphthylamine, *N*-phenyl-2-naphthylamine has been classified in Category 3 A for germ cell mutagens.

Absorption through the skin. On the basis of the findings of the studies carried out with porcine skin (Marek et al. 2017) and with human skin (Dennerlein et al. 2017), the cumulative absorption was determined to be in the lower µg/cm² range depending on the concentration applied, the period of time the substance was left on the skin and the application medium. Therefore, *N*-phenyl-2-naphthylamine has been demonstrated to penetrate the skin and this leads to the formation of the carcinogenic and genotoxic 2-naphthylamine. *N*-Phenyl-2-naphthylamine has therefore been designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Allergenic effects. The supplement from 2011 (Hartwig 2015) reviewed an extensive number of positive clinical findings relevant for the evaluation of the skin sensitizing effects induced by *N*-phenyl-2-naphthylamine. Since then, only a few new findings in humans have become available, but no animal studies. Likewise, there are no data available for the sensitizing effects induced by *N*-phenyl-2-naphthylamine on the airways. For this reason, *N*-phenyl-2-naphthylamine remains designated with "Sh" (for substances which cause sensitization of the skin), but not with "Sa" (for substances which cause sensitization of the airways).

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

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