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4-Nitroaniline

MAK Value Documentation, supplement – Translation of the German version from 2020

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Keywords

4-nitroaniline; methaemoglobin; haemangiosarcoma; fertility; developmental toxicity; genotoxicity; carcinogenicity; skin absorption; sensitization; germ cell mutagenicity

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated 4-nitroaniline [100-01-6] considering all toxicological end points. Available publications are described in detail. The critical effect of 4-nitroaniline is methaemoglobin formation in humans and animals. 4-Nitroaniline induces mutations in bacteria and is clastogenic to mammalian cells, although not in vivo. Long-term animal studies did not demonstrate that the germ cells are reached. Compared with other aromatic amino and nitro compounds, 4-nitroaniline has a much lower genotoxic potential in vitro and in vivo. Therefore, an assignment to a Germ Cell Mutagenicity Category is not necessary. The substance is not carcinogenic in Sprague Dawley rats up to doses of 9.9 mg/kg body weight and day, which are toxic to the spleen. In male B6C3F1 mice, however, incidences of haemangiosarcomas in the liver and of haemangiomas or haemangiosarcomas (combined) at all sites were increased. Thus, a carcinogenic potential of 4-nitroaniline is likely. This is also supported by its structural similarity with other carcinogenic aromatic amino and nitro compounds as well as its genotoxicity in vitro. 4-Nitroaniline is therefore assigned to Carcinogen Category 3B. As the substance is genotoxic in vitro, a maximum concentration at the work place (MAK value) cannot be derived. Prenatal toxicity studies found lower foetal body weights in rats at 85 mg/kg body weight and day, but no developmental toxicity in rabbits up to the highest dose tested of 125 mg/kg body weight and day. Clinical data in humans did not describe a discrete contact sensitizing effect for 4-nitroaniline. Also, animal studies performed with low concentrations did not provide explicit evidence of a contact sensitizing potential. In spite of the suspected contact sensitizing effect, the substance is not designated with an "Sh" notation. For lack of data, the "Sa" designation is not applied. Dermal absorption is higher than the systemically tolerable amount calculated after oral administration in rats. Hence, the "H" designation is retained.

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MAK value	–
Peak limitation	–
Absorption through the skin (1958)	**H**
Sensitization	–
Carcinogenicity (2019)	**Category 3 B**
Prenatal toxicity	–
Germ cell mutagenicity	–
BAT value	–
CAS number	100-01-6

4-Nitroaniline was classified in 1999 in category 3 for suspected carcinogens (Hartwig 2014 b). In 2000, the substance was considered a candidate for category 5. However, due to the lack of quantitative and comparative data for the assessment of the carcinogenic risk, a MAK value could not be derived and the substance was classified in category 3 A (Hartwig 2014 c).

This supplement re-evaluates the classification of the carcinogenicity of 4-nitroaniline. Since its germ cell mutagenicity has not been evaluated to date, this is carried out in the present supplement. In addition, the evaluation of the sensitization potential has been updated.

Toxic Effects and Mode of Action

4-Nitroaniline is a strong methaemoglobin former in humans. Methaemoglobin formation is observed also in rats and mice.

In male B6C3F1 mice, 4-nitroaniline led to haemangiosarcomas after gavage doses of 100 mg/kg body weight and day. The increase in the incidence of all haemangiosarcomas was not statistically significant compared with the numbers in the concurrent controls, but it was significant compared with the incidence in historical controls. In Sprague Dawley rats, 4-nitroaniline was not carcinogenic despite dose-dependent effects on the spleen.

Investigations with 4-nitroaniline yielded heterogeneous results in bacterial mutagenicity tests for genotoxicity, as typical of nitroaromatic substances. The addition of flavin mononucleotide, which was added to facilitate nitro reduction, revealed a mutagenic potential with and without a metabolic activation system. In CD1 mice, the substance was not clastogenic after intraperitoneal administration.

In a prenatal developmental toxicity study in Sprague Dawley rats with gavage administration, reduced foetal weights were observed at 85 mg/kg body weight and day and above. At 250 mg/kg body weight and day, pronounced maternal toxicity was accompanied by an increased number of resorptions per dam and malformations of the tail, urogenital system and ribs not specified in greater detail. In New Zealand White rabbits, a prenatal developmental toxicity study with gavage administration did not reveal any teratogenic effects, even at maternally toxic doses of up to 125 mg/kg body weight and day.

Contact allergy induced by 4-nitroaniline in humans has not been reported to date, although the substance may lead to positive reactions in patch tests in persons with existing sensitization to *p*-phenylenediamine. The questionably valid results from experimental studies in animals and the result of an in vitro study do not indicate a pronounced contact sensitizing potential.

Mechanism of Action

Based on an analysis of chemicals that led to an increased incidence of haemangiosarcomas in male B6C3F1 mice in NTP studies, a correlation between Kupffer cell pigmentation and the development of haemangiosarcomas in the liver has been established. Haemosiderosis, which is due to the haemolytic effects of the chemicals, was observed also in female mice without increased tumour incidences. However, the reason for the gender-specific sensitivity to chemical-induced haemangiosarcomas is not known. The increased sensitivity could be due to a hormone-related, reduced antioxidative defense capacity resulting from the modulation of the activity of antioxidative enzymes (Nyska et al. 2004). It is also conceivable that the haemangiosarcomas could develop as a sequel to methaemoglobin formation.

Toxicokinetics and Metabolism

4-Nitroaniline is readily absorbed by inhalation, ingestion and through the skin. Toxicokinetic studies of absorption by inhalation are not available. However, the 4-week inhalation study in rats likewise indicates ready absorption after inhalation exposure (Hartwig 2014 b). The amount absorbed after a single oral dose was found to be 75% to 81% in rats, and 100% after dermal application in monkeys (BUA 1987; Hartwig 2014 b).

Twenty-four hours after the application of 4 µg of 4-nitroaniline in acetone per cm^2 of skin to isolated human skin in vitro or to the shaved abdominal skin of monkeys, 34.5% was absorbed in vitro and 100% in vivo, taking into account the evaporated portion. The maximum absorption took place within the first 2 hours (Hartwig 2014 b).

In an in vitro study, 200 µl of an aqueous ^{14}C-nitroaniline solution with a concentration of 0.8 mg/ml (saturation concentration) was applied to human skin (200 to 500 µm; 63.6 mm^2) in Bronaugh diffusion cells for 10 minutes, 1 hour and 24 hours. The concentration in the receptor fluid (Hanks' Balanced Salt Solution with 6% polyethoxylate) was determined several times during the 24-hour exposure and only at the end in the case of the shorter exposures. From these values and the amount of radioactivity in the different skin layers at the end of each exposure, a permeability constant of 0.00881 cm/h was calculated. After application for 24 hours, between 43% and 71% of the applied dose was absorbed. After exposure for 10 minutes and 1 hour, it was about 1% and 2.7% to 5.5%, respectively. The flux rates were 14.4 µg/cm^2 and hour after exposure for 10 minutes, and 9.35 µg/cm^2 and hour after exposure for 1 hour (In Vitro Technologies 2005). From these data, the absorption of 18.7 mg of 4-nitroaniline can be calculated for the exposure of 2000 cm^2 of skin (area of hands and forearms) to a saturated aqueous solution for 1 hour.

4-Nitroaniline is distributed rapidly in all tissues.

After oral or intraperitoneal administration, the metabolism (see Figure 1) of 5 mg ^{14}C-4-nitroaniline per rat (about 20 mg/kg body weight assuming a body weight of 250 g) in male albino rats took place mainly by C-oxidation—probably via an epoxide—to 2-hydroxy-4-nitroaniline (43% of the radioactivity in urine). By reduction of the nitro group 1,4-diaminobenzene (*p*-phenylenediamine) was formed (26% of the radioactivity in the urine). A proportion of 14% of the radioactivity in the urine was not metabolized. Regardless of the route of administration, about 80% of the radioactivity administered was found in the 24-hour urine and only small amounts after 48 and 72 hours. The amount excreted with the faeces remained below 1%. Urine that was not hydrolysed contained about 2% free 4-nitroaniline (Hartwig 2014 b; Maté et al. 1967). Also, the N-oxidation of the amino group to 4-nitrophenylhydroxylamine was detected; this metabolite is responsible mainly for methaemoglobin formation (Hartwig 2014 b). After in vitro incubation, also *N*-acetyl-4-nitroaniline was found (Hartwig 2014 b; Maté et al. 1967).

The metabolites were excreted rapidly and mainly via the kidneys.

Thus, 2 hours after a single oral dose of 0.28 or 13.8 mg ^{14}C-4-nitroaniline/kg body weight in male F344 rats, 75% to 81% of the administered radioactivity was excreted with the urine, and 13% with the faeces. In this study, investigations of biliary excretion in male bile duct-cannulated rats revealed that after intravenous injection of 10 µmol ^{14}C-4-nitroaniline/kg body weight (1.38 mg/kg body weight) about 19% of the administered radioactivity was excreted via the bile within 4 hours after the administration, which suggests enterohepatic circulation. The whole-body half-life of

the radioactivity was 1 hour. From a 2-component exponential decay curve, half-lives of 0.8 and 16.6 hours could be calculated for elimination from the blood (Chopade and Matthews 1984; Hartwig 2014 b).

Quantitative data for metabolites in blood are not available.

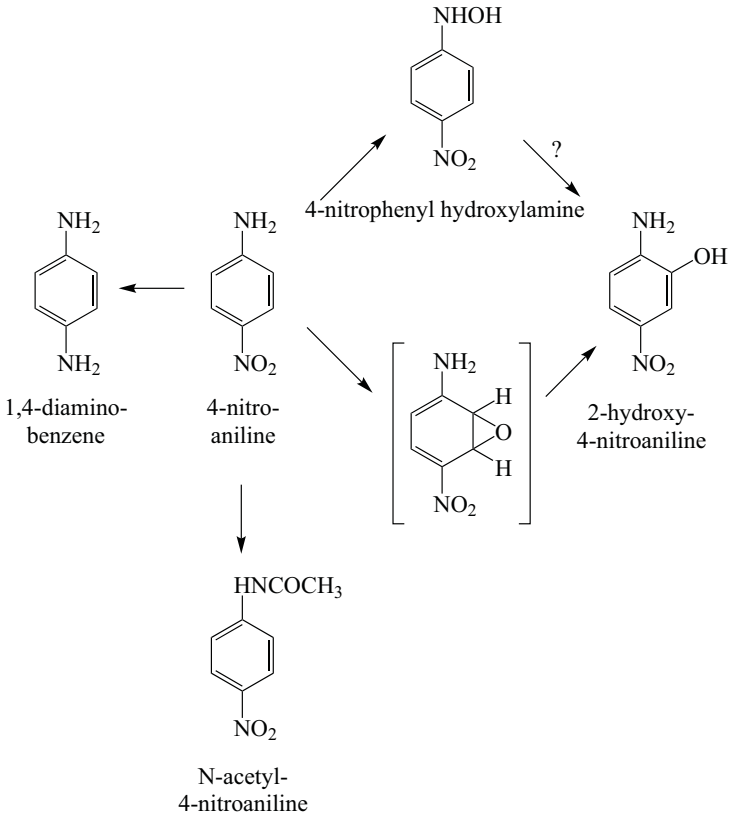


Fig. 1 Metabolism of 4-nitroaniline (according to BUA 1987)

Effects in Humans

The formation of methaemoglobin was described in detail in the 1999 documentation (Hartwig 2014 b).

Since the 1999 documentation (Hartwig 2014 b), no new data for end points other than allergenicity in humans have become available.

Allergenic effects

Only a few new patch test results with 4-nitroaniline have been published since the documentation of 1999 (Hartwig 2014 b) but still no findings of respiratory sensitization.

Ten patients allergic to Disperse Orange 1 or Disperse Yellow 3 in previous tests were patch tested with *p*-phenylenediamine and three *p*-phenylenediamine derivatives, as well as the two products that are formed by reductive cleavage of the azo group of the disperse dyes. All substances were examined in at least three dilutions; readings were taken on days 4 and 7. In 2 of the patients, the initial reactions to the dyes were not reproducible and they produced no reaction to 4-nitroaniline. Of the remaining 8 patients, 5 reacted also to a 0.43% preparation of 4-nitroaniline in petrolatum (one 1+, three 2+ and one 3+). Four of the 5 persons developed marked reactions (mostly 3+) to at least 7 of the around

20 to 40 preparations tested. Two of the 5 persons produced a questionable reaction to 0.043% 4-nitroaniline, and 1 of those tested produced a 1+ reaction (Malinauskiene et al. 2012).

In another study, 4-nitroaniline and 4,4′-azodianiline were investigated in patch tests as potential oxidation products of *p*-phenylenediamine in 14 persons who had previously produced an epicutaneous reaction to *p*-phenylenediamine. In 13 persons, the reaction to *p*-phenylenediamine was reproducible. Of these, 1 person produced a 1+ reaction to a 0.013% preparation of 4-nitroaniline, 1 person a 1+ reaction to a 0.0013% preparation and 1 person a 2+ reaction to a 0.13% preparation in acetone (and additionally a 2+ reaction to 0.01% *p*-phenylenediamine). In 2 other persons a 1+ reaction to a 1% preparation of 4-nitroaniline in ethanol/acetone (2:3) was found. None of 15 controls who had previously shown no reaction to *p*-phenylenediamine reacted to the highest concentration tested (Young et al. 2016).

Animal Experiments and in vitro Studies

Subacute, subchronic and chronic toxicity

There are no new data available for 4-nitroaniline, except for those from an oral study with a mixture containing this substance.

Oral administration

A mixture of 4-aminophenol : *p*-nitrophenol : 4-nitroaniline (1 : 3.5 : 6) was administered to groups of 20 male and 20 female Sprague Dawley rats at doses of 0, 5, 25 or 50 mg/kg body weight and day by gavage for 4 weeks. The purity of all substances was of analytical grade. None of the animals died. At 25 and 50 mg/kg body weight and day, the following effects occurred at the end of the experiment: reduced body weight gains, increased methaemoglobin levels, decreased erythrocyte counts and haemoglobin levels, increased leukocyte and reticulocyte counts, and histopathological changes in the liver, kidneys, spleen, cerebellum and haematopoietic system (Wang et al. 2010). The individual substances were not examined.

Allergenic effects

In a maximization test in guinea pigs, 4-nitroaniline did not produce a result that could be considered positive; however, the substance was used only in low concentrations. A 0.5% preparation in propylene glycol was used for intradermal and topical induction treatment, and a 1% and 0.25% preparation of 4-nitroaniline for the challenge treatment. Acetone was probably the vehicle used for the topical treatments, and an open application of 10% sodium lauryl sulfate in dimethylacetamide/acetone/ethanol (4 : 3 : 3) was performed before the topical induction treatment. After challenge treatment, reactions were observed in 5 of the 24 animals pretreated with 4-nitroaniline and in 2 of the 12 control animals (Malinauskiene et al. 2013).

Other information on more recent experimental studies in animals is not available.

An in vitro test using cocultures of human keratinocytes and peripheral blood monocytes as a surrogate for dendritic cells yielded negative results for 4-nitroaniline. The cells were cultured in serum-free medium with the addition of 100 ng interleukin-4, 100 ng GM-CSF (granulocyte-macrophage colony-stimulating factor) and 10 ng TGF-β (transforming growth factor β) per ml. After 48 hours of incubation with up to 200 µmol 4-nitroaniline/l, the expression of CD86 was not increased and no cytotoxicity was observed. *p*-Phenylenediamine caused a half-maximal increase in CD86 expression at 42 µmol/l (Sonnenburg et al. 2012).

Reproductive and developmental toxicity

There are no new data available for this end point.

Fertility

In a 2-generation study in Sprague Dawley rats, gavage doses of 4-nitroaniline of up to 9 mg/kg body weight and day (highest dose tested) did not lead to effects on fertility or the offspring (Hartwig 2014 b; Nair et al. 1990).

Developmental toxicity

In a prenatal developmental toxicity study, similar to OECD Test Guideline 414 (but without a detailed tabular listing of all individual malformations), groups of 24 Sprague Dawley rats were given gavage doses of 4-nitroaniline of 0, 25, 85 or 250 mg/kg body weight and day from gestation days 6 to 19. On gestation day 20, the foetuses were examined after caesarean section. At 85 mg/kg body weight and day and above, signs of maternal toxicity were pale eye colour, dark-yellow urine and anogenital staining. No dam died. At 250 mg/kg body weight and day, 2 dams were observed to have convulsions following dosing. During the treatment period, the body weight gains of the dams were retarded at this dose. At 85 mg/kg body weight and day and above, the foetal body weights were decreased, and at 250 mg/kg body weight and day, the number of resorptions per dam was increased. At 250 mg/kg body weight and day, malformations occurred, mainly of the tail (49/273 foetuses ≙ 17.9%; 10/22 litters ≙ 45.5%), the urogenital system (15/131 foetuses ≙ 11.5%; 5/22 litters ≙ 22.7%) and the ribs (50/142 foetuses ≙ 35.2%; 14/22 litters ≙ 63.6%). The frequency of foetuses with skeletal variations was likewise increased (Hartwig 2014 b; Nair et al. 1985). The malformations of the tail are not described in detail. The description of the malformations of the urogenital system includes not only malformations of the kidney (absence, small or misshapen, of which by today's standards only absent kidneys are considered a malformation) but also malformations of the uterus and the reproductive tract, which are likewise not further described. The malformations of the ribs are described as angulated, wavy or fused ribs (by today's standards, fused ribs are a malformation and wavy ribs are a variation). The individual malformations are not further specified. Thus, the extent of the malformations is unclear.

In a screening study in 50 CD1 mice per dose, which had been given 4-nitroaniline doses of 0 or 1200 mg/kg body weight and day, dissolved in corn oil, from gestation days 7 to 14, pronounced maternal toxicity in the form of convulsions and an increased number of deaths occurred (21 of 50). Of the surviving animals, 16 were pregnant, 6 were not pregnant and in 7, despite fertilization, implantation had not taken place. Eleven of 16 dams delivered live offspring, 4 dams had only dead offspring. In the offspring of one litter, malformations were found on all limbs in the form of ectromelia (reduction or absence of the part of the limbs close to the body). The number of live offspring per litter, the body weights of the offspring and their survival during the first three days were significantly reduced compared with the findings in the control animals. In 1 of the 14 dams that did not produce offspring, resorptions were found in utero (Hardin et al. 1987; Hartwig 2014 b; NIOSH 1983). Due to the high mortality in the dams, the study is not suitable for inclusion in the evaluation of developmental toxicity.

In a prenatal developmental toxicity study, similar to OECD Test Guideline 414 (but without a detailed tabular listing of all individual malformations), 18 New Zealand White rabbits per dose group were given gavage doses of 4-nitroaniline of 0, 15, 75 or 125 mg/kg body weight and day from gestation days 7 to 19. The foetuses were examined on gestation day 30 after caesarean section. At 125 mg/kg body weight and day, 7 dams died (no other details). At 15 mg/kg body weight and day and above, the dams were found to have yellowish staining of the anogenital fur and at 75 mg/kg body weight and day and above, greyish-coloured eyes. The intrauterine development of the foetuses was normal. Teratogenic effects were not observed in any dose group (Hartwig 2014 b; Nair et al. 1985).

Genotoxicity

Apart from a micronucleus test in CD1 mice (Monsanto Company 1989), no new data for genotoxicity have become available since the documentation of 1999 (Hartwig 2014 b).

In vitro

To evaluate the germ cell mutagenicity of 4-nitroaniline, the studies already described in the 1999 documentation (Hartwig 2014 b) are summarized again below.

In several Salmonella mutagenicity tests, 4-nitroaniline was found to be mutagenic in the strains TA98 and TA1538 with and without the addition of a metabolic activation system, but not in the strains TA100, TA1535, TA1537, TA98NR and TA1538NR. In Bacillus subtilis, the substance induced increased DNA repair. In mammalian test systems without the addition of a metabolic activation system, positive results were obtained in the $TK^{+/-}$ test, for chromosomal aberrations in CHO cells (a cell line derived from Chinese hamster ovary) and human lymphocytes and, to a small extent, for sister chromatid exchanges in CHO cells. However, DNA repair was not observed in rat hepatocytes. With the addition of a metabolic activation system, 4-nitroaniline did not induce mutations, but did produce chromosomal aberrations and sister chromatid exchanges (Hartwig 2014 b).

In a modified gene mutation test with preincubation, the addition of flavin mononucleotide, which facilitates nitro reduction, led to an enhancement of the mutagenicity of 4-nitroaniline in the Salmonella typhimurium strains TA98 and TA100. The addition of a metabolic activation system from hamster liver and flavin mononucleotide resulted in mutagenic effects at and above 0.1 µM, with a maximum increase in the number of revertants by a factor of 11; compared with the activation system from rat liver, the effect was stronger (Dellarco and Prival 1989).

The study of the in vitro genotoxicity of 4-nitroaniline (Dellarco and Prival 1989) not included in the 1999 documentation (Hartwig 2014 b) and also not in the BUA report (BUA 1987) and the studies with positive results from the 1999 documentation (Hartwig 2014 b) are shown in Table 1.

Tab. 1 In vitro studies of the genotoxicity of 4-nitroaniline

End point	Test system	Concentration	Cytotoxicity	Results		Remarks	References
				–m.a.	+m.a.		
gene mutation (preincubation)	Salmonella typhimurium TA98	0, 0.1, 0.3, 1.0, 3.0, 10.0 µmol/plate; vehicle: *p*-dioxane; purity: 99%	–	n.d.	+FMN (to facilitate nitro reduction): +m.a. from hamster liver: + at 0.1 µmol/plate and above (max. number of revertants 11-fold); +m.a. from rats: + at 1.0 µmol/plate and above	m.a. from hamsters compared with m.a. from rats: increased mutagenic effect	Dellarco and Prival 1989
			–	n.d.	–FMN: +m.a. from hamster liver: + at 0.1 µmol/plate and above		
	Salmonella typhimurium TA100	see above	no data	n.d.	+ no data		

Tab. 1 *(continued)*

End point	Test system	Concentration	Cytotoxicity	Results		Remarks	References
				–m.a.	+m.a.		
SCE	CHO cells	–m.a.: test 1: 0, 0.5, 1.6, 5, 16, 50, 160 µg/ml; test 2: 0, 50, 100, 200 µg/ml; +m.a.: test 1: 0, 16, 50, 160, 500, 1600, 5000 µg/ml; test 2: 0, 160, 500, 1000 µg/ml; test 3: 0, 250, 500, 750, 1000 µg/ml; vehicle: DMSO; purity: > 99%	–m.a.: at 200 µg/ml; +m.a.: at 1000 µg/ml	+ at 160 µg/ml and above	inconclusive		Hartwig 2014 b; NTP 1993
CA	human lymphocytes	0, 0.005, 0.01, 0.05, 0.10 µmol/ml; vehicle: DMSO; purity: not specified	no data	+ contradictory data: at 0.005 µmol/ml (0.7 µg/ml) and above (Huang et al. 1995); effective dose: 5 µmol/ml (690 µg/ml) (Huang et al. 1996)	n.d.	gaps not included in total number of CA	Hartwig 2014 b; Huang et al. 1995, 1996
CA	CHO cells	0, 173, 345, 690, 1035 µg/ml; vehicle: DMSO; purity: ≥ 99%	TC_{50} 345 ± 18 µg/ml	+ at 173 µg/ml and above (number of aberrant cells: at least 13%, control: 6%)	n.d.	mainly dicentric chromosomes; gaps not included in total number of CA	Chung et al. 1996; Hartwig 2014 b
CA	CHO cells	–m.a.: 0, 50, 160, 500, 1600 µg/ml; +m.a.: 0, 160, 500, 1600, 5000 µg/ml; time: 14 h; vehicle: DMSO; purity: > 99%	–	+ at 1600 µg/ml (percentage of cells with CA: 5-fold increase)	+ at 1600 µg/ml and above (percentage of cells with CA: at least 6.7-fold increase)	gaps not included in total number of CA, mainly simple CA (breaks and terminal deletions)	Hartwig 2014 b; NTP 1993

Tab. 1 *(continued)*

End point	Test system	Concentration	Cytotoxicity	Results		Remarks	References
				–m.a.	+m.a.		
CA	CHO cells	–m.a.: test 1: 0, 16, 50, 160, 500 µg/ml (12 h); test 2: 0, 100, 200, 400, 600, 800, 1200 µg/ml (12.8 h); +m.a.: test 1: 0, 16, 50, 160, 500, 1600 µg/ml (12 h); test 2: 0, 200, 400, 600, 800, 1200 µg/ml (12 h); test 3: 0, 400, 600, 800, 1200 µg/ml (15 h); test 4: 0, 400, 600, 800, 1000 µg/ml (12.5 h); test 5: 0, 400, 600, 800, 1000, 1200, 1600, 2000 µg/ml (22 h); vehicle: DMSO; purity: > 99%	–m.a.: at 1200 µg/ml, +m.a.: at 1200 µg/ml	–	+ at 1000 µg/ml (aberrant cells: 73%, control: 3%)	gaps not included in total number of CA, primarily simple CA (breaks and terminal deletions)	Hartwig 2014 b; NTP 1993
mutation	L5178Y cells, $TK^{+/-}$ test	–m.a.: test 1: 0, 16, 32, 63, 125, 250, 500, 1000 µg/ml; test 2: 0, 16, 32, 63, 125, 250, 500 µg/ml; test 3: 0, 50, 100, 200, 300, 400 µg/ml; +m.a.: 0, 25, 50, 100, 200, 300, 500 µg/ml; vehicle: acetone; purity: > 99%	–m.a.: at 400 µg/ml and above	+ at 250 µg/ml and above (average mutant fraction: 81, control: 23) with simultaneous marked growth inhibition (relative growth: 12%–26%)	–	–m.a.: precipitation at 500 µg/ml and above; +m.a.: precipitation at 300 µg/ml and above; no differentiation between large and small colonies, not possible to decide whether mutagenicity or clastogenicity is the main effect	Hartwig 2014 b; NTP 1993

CA: chromosomal aberrations; DMSO: dimethyl sulfoxide; FMN: flavin mononucleotide; m.a.: metabolic activation system; n.d.: not determined; SCE: sister chromatid exchanges; TC50: toxic for 50% of the cells

The two metabolites of 4-nitroaniline, 1,4-benzene diamine (*p*-phenylenediamine, Henschler 1992) and 2-amino-5-nitrophenol, are known to be mutagenic in bacteria in vitro (NTP 1988).

In vivo

Somatic cells

In the publication already cited in the 1999 documentation (Hartwig 2014 b), the induction of DNA repair in hepatocytes of F344 rats was not observed (Mirsalis et al. 1983). However, the study is only a summary and provides no information on dose levels.

In a micronucleus test in male and female CD1 mice (5 animals per group and sex, highest dose: 6 animals) given two intraperitoneal injections of 4-nitroaniline at dose levels of 0, 80, 400 or 800 mg/kg body weight (purity: 99%, vehicle: corn oil), no significant increase in micronuclei frequency in comparison to solvent controls was found in polychromatic erythrocytes up to the highest dose tested. In the males, the ratio between polychromatic and normochromatic erythrocytes was reduced after 48 hours at the highest dose tested of 800 mg/kg body weight; one animal died at this dose. The ratio of polychromatic to normochromatic erythrocytes was not decreased in the female animals at 800 mg/kg body weight after 24 and 48 hours, but toxicity in the form of unresponsiveness and listlessness occurred even at

the lowest dose. The results with the positive control cyclophosphamide confirmed the sensitivity of the test system (Monsanto Company 1989).

Germ cells

Three tests for sex-linked recessive lethal mutations (SLRL) in Drosophila yielded negative results (BUA 1987; NTP 1993; US EPA 2009).

In a sperm head abnormality test in BALB/c mice, 4-nitroaniline doses of up to 500 mg/kg body weight and day, administered intraperitoneally for 5 days, did not result in an increase in the number of sperm head abnormalities (BUA 1987). Changes in sperm morphology are not reliable indicators of mutations, the relevance of the effects with regard to germ cell mutagenicity is questionable (ICPEMC 1983; Salamone 1988; Wild 1984), and the results can be interpreted only as cytotoxic effects.

Carcinogenicity

There are no new data available.

In a carcinogenicity study of the NTP, groups of 70 male and 70 female B6C3F1 mice were given gavage doses of 4-nitroaniline of 0, 3, 30 or 100 mg/kg body weight and day in corn oil on 5 days per week for up to 103 weeks. At 3 mg/kg body weight and day and above, increased incidences of hypercellularity (hyperplasia) in the bone marrow of the males and of haemosiderosis of the spleen in the females occurred. At 30 mg/kg body weight and day and above, methaemoglobin formation and histological changes in the spleen (congestion, haematopoiesis, haemosiderosis), liver (Kupffer cell pigmentation) and bone marrow (hyperplasia) occurred in addition if not already existing. In the males, the incidences of haemangiosarcomas of the liver were 0 of 50, 1 of 50 (2%), 2 of 50 (4%) and 4 of 50 (8%) at 0, 3, 30, 100 mg/kg body weight and day, respectively, and those of haemangiosarcomas and haemangiomas (combined) at all sites were 5 of 50 (10%), 3 of 50 (6%), 4 of 50 (8%) and 10 of 50 (20%) at 0, 3, 30, 100 mg/kg body weight and day, respectively. In the high dose group, they were thus slightly, but not significantly increased, based on a subsequent calculation using Fisher's exact test ($p = 0.06$). However, they were higher than the historical control data given in this NTP report (haemangiosarcomas in the liver: 15/699; mean ± standard deviation: 2.1 ± 2.1%; range: 0–6%; haemangiosarcomas and haemangiomas (combined) at all sites: 46/700; 6.6 ± 3.6%; 0–12%). No increased tumour incidences were observed in the female animals. NTP evaluated the result in the male mice in this experiment as equivocal (Hartwig 2014 b; NTP 1993).

In a 2-year carcinogenicity study in Sprague Dawley rats, groups of 60 males and 60 females were given gavage doses of 4-nitroaniline of 0, 0.25, 1.5 or 9.9 mg/kg body weight and day in corn oil, on 7 days a week. At 0.25 mg/kg body weight and day and above, pigmentation of the spleen was found and, at 1.5 mg/kg body weight and day and above, increased methaemoglobin levels and spleen weights. The tumour incidence in the treated animals was not increased compared with that in the controls (Hartwig 2014 b; Nair et al. 1990).

Manifesto (MAK value/classification)

Critical effects of 4-nitroaniline are the formation of methaemoglobin in humans and animals, the suspected carcinogenicity based on haemangiosarcomas induced in male B6C3F1 mice and the genotoxicity in vitro.

Carcinogenicity. In Sprague Dawley rats, 4-nitroaniline was not carcinogenic despite dose-dependent effects on the spleen at gavage doses of 0.25 mg/kg body weight and day and up to 9.9 mg/kg body weight and day (Hartwig 2014 b; Nair et al. 1990). However, in male B6C3F1 mice, an increased incidence of haemangiosarcomas was found after gavage administration of 100 mg/kg body weight and day. The increase in the incidence was not statistically significant compared with the incidence in the controls, but was higher than that in historical controls (Hartwig 2014 b; NTP 1993). Thus, also in view of the genotoxicity in vitro, a carcinogenic effect is suspected.

A comparison of the monocyclic aromatic amino and nitro compounds shows that a common basic pattern is recognizable in the organotropy of the developing tumours. In rats and mice, blood vessel tumours are the most prevalent. Haemangiosarcomas may be limited to the spleen, but also occur elsewhere (Greim 2005). Thus, also due to the structure of 4-nitroaniline, carcinogenic effects are suspected.

4-Nitroaniline is mutagenic in bacteria and causes chromosomal aberrations in mammalian cells in vitro. The in vivo micronucleus test in mice that yielded negative results (Monsanto Company 1989) does not completely invalidate the in vitro genotoxicity results, since this test examined only clastogenicity. Mutagenicity tests in vivo were not carried out. A metabolism study revealed the formation of at least nine metabolites (BUA 1987; Chopade and Matthews 1984), two of which, 1,4-benzenediamine (*p*-phenylenediamine, Carcinogen Category 3 B, Henschler 1992) and 2-amino-5-nitrophenol, are likewise genotoxic in vitro. In an investigation of the 24-hour urine of rats given single oral or intraperitoneal doses of about 20 mg/kg body weight and day, 26% of the urinary radioactivity was found in the form of *p*-phenylenediamine and 43% in the form of 2-amino-5-nitrophenol subsequent to acid hydrolysis (Hartwig 2014 b; Maté et al. 1967). Thus, at least two genotoxic metabolites could be systemically available.

All in all, 4-nitroaniline is still suspected to be carcinogenic based on the haemangiosarcomas in male B6C3F1 mice, its structure and its genotoxicity in vitro. The substance is therefore classified in Carcinogen Category 3 B.

MAK value and peak limitation. Due to the suspected carcinogenic effect and the genotoxicity in vitro, no MAK value can be derived. Peak limitation is therefore not applicable.

Prenatal toxicity. Since no MAK value can be derived, assignment to a pregnancy risk group is not applicable.

In a prenatal developmental toxicity study with gavage administration to Sprague Dawley rats, reduced foetal weights were observed at 85 mg/kg body weight and day and above. At 250 mg/kg body weight and day, a dose level with pronounced maternal toxicity (delayed body weight gains, convulsions), the number of resorptions per dam was increased. In addition, malformations of the tail, the urogenital system and the ribs occurred at this dose; the individual data were, however, not reported (Hartwig 2014 b; Nair et al. 1985). In some cases, today they are no longer regarded as malformations but as variations or as neither malformations nor variations.

A prenatal developmental toxicity study in New Zealand White rabbits with gavage administration did not reveal any teratogenic effects even at maternally toxic doses of up to 125 mg/kg body weight and day (Hartwig 2014 b; Nair et al. 1985).

The effects in rats are possibly the result of anaemia.

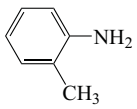
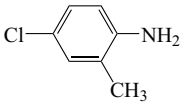
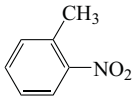
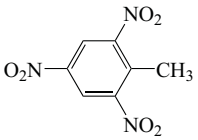
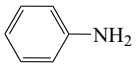
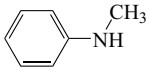
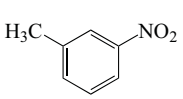
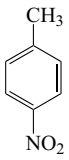
Germ cell mutagenicity. 4-Nitroaniline is genotoxic in vitro, but the results in bacterial mutagenicity tests are heterogeneous. Thus, the substance behaves like a typical nitroaromatic compound. Bacterial mutagenicity tests in vitro are not suitable for the adequate determination of the genotoxic potential of monocyclic aromatic amino and nitro compounds (Greim 2005). After the addition of flavin mononucleotide, 4-nitroaniline was found to have a mutagenic potential with and without the addition of a metabolic activation system (Dellarco and Prival 1989).

In vitro, the substance is clastogenic in mammalian cells and mutagenic in bacteria (Hartwig 2014 b). Therefore, the negative result in an in vivo micronucleus test in mice (Monsanto Company 1989) does not completely invalidate the genotoxicity observed in vitro. Mutagenicity tests have not been performed in animal experiments. Long-term studies in rats (Hartwig 2014 b; Nair et al. 1990) and mice (Hartwig 2014 b; NTP 1993) have not provided evidence that 4-nitroaniline is able to affect the reproductive organs, that is to reach the germ cells.

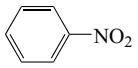
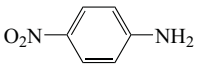
An evaluation of the monocyclic aromatic amino and nitro compounds in the List of MAK and BAT Values as regards their germ cell mutagenicity (see Table 2) shows that four compounds are classified in a category for germ cell mutagens. *o*-Toluidine and 4-chloro-*o*-toluidine are both classified in category 3 A for germ cell mutagens and in category 1 for carcinogens, while 2-nitrotoluene and 2,4,6-trinitrotoluene are classified in category 3 B for germ cell mutagens and in category 2 for carcinogens. For these four compounds, marked carcinogenic effects in humans or in animal experiments, marked genotoxic effects in vivo and the ability of the substance to reach the germ cells have been proven or there are strong indications that this is the case.

With 4-nitroaniline, however, the carcinogenicity in animal experiments and the genotoxicity in vivo are less pronounced. From long-term studies in rats and mice and from the sperm morphology test, no effects on the reproductive organs are suspected. Thus, there is no evidence that the substance reaches the germ cells. On the basis of these data, 4-nitroaniline is not classified in one of the categories for germ cell mutagens.

Tab. 2 Evaluation of the germ cell mutagenic effects of the amino and nitro aromatics included in the List of MAK and BAT Values

Substance	GCMut Cat	Carc Cat	Structural formula	In vivo genotoxicity	Ability to reach the germ cells	References
classifed as a germ cell mutagen						
o-toluidine	3A	1		micronuclei: peripheral erythrocytes, rat; covalent DNA binding: liver cells, rat; sister chromatid exchange: bone marrow, mice	degeneration of seminiferous tubules, rat	Hartwig 2014 d
4-chloro-*o*-toluidine	3A	1		mammalian spot test (somatic cells), mouse	passes placental barrier, therefore presumably reaches the germ cells	Greim 2003, available in German only
2-nitrotoluene	3B	2		micronuclei: peripheral erythrocytes, mouse	damage to testis and epididymis, rat	Greim 2002, available in German only
2,4,6-trinitrotoluene	3B	2		clastogenicity, humans	effects in testes, rat, possibly secondary effects	Hartwig 2014 a
not classified as a germ cell mutagen						
aniline	–	4		dominant lethal test, rat, negative; micronuclei: bone marrow, rat, 2 tests positive, 1 test negative results; chromosomal aberrations, bone marrow, rat, 1 test positive, 1 test negative results	no effects on testes in long-term studies, rats	Hartwig 2010
N-methylaniline	–	3B		no examinations; analogous to aniline	no data	Hartwig and MAK Commission 2019
3-nitrotoluene	–	3B		covalent binding macromolecules: liver, rat; UDS: hepatocytes, rats, negative analogous to 4-nitrotoluene	testicular degeneration, rat	Hartwig and MAK Commission 2016 a
4-nitrotoluene	–	3B		covalent binding macromolecules: liver, rat; UDS: hepatocytes, rats, negative; micronuclei: rat, mouse, negative	testicular atrophy and testicular degeneration, rat	Hartwig and MAK Commission 2016 b

Tab. 2 *(continued)*

Substance	GCMut Cat	Carc Cat	Structural formula	In vivo genotoxicity	Ability to reach the germ cells	References
nitrobenzene	–	4		clastogenicity (micronuclei, comet assay), liver, kidneys, thyroid gland, rat, at toxic doses; chromosomal aberrations, bone marrow, rat, negative; SCE, bone marrow, rat, negative; UDS, liver, rat, negative	testicular atrophy, rat	Hartwig and MAK Commission 2018
4-nitroaniline	–	3B		micronuclei: mouse, negative; SLRL, Drosophila, negative	no effects on testes in long-term studies, rats and mice, sperm morphology test results negative	

SCE: sister chromatid exchanges; SLRL: sex-linked recessive lethal; UDS: DNA repair synthesis

Absorption through the skin. For humans, the maximum dermal absorption of 18.7 mg after exposure to a saturated aqueous solution under standard conditions (2000 cm^2 skin surface, 1-hour exposure) can be estimated from an in vitro study. The LOAEL (lowest observed adverse effect level) in a 2-year study was 0.25 mg/kg body weight and day after oral administration in rats. The following toxicokinetic data are taken into consideration for the extrapolation of this dose (systemic LOAEL) to humans: the species-specific correction value for the rat (1 : 4), the experimental oral absorption of 100% (Maté et al. 1967), the daily exposure of the animals in comparison with the 5 days per week exposure at the workplace (7 : 5), the body weight (70 kg) of the person, and the extrapolation of the data from experimental studies with animals to humans (1 : 2). The amount calculated from this is 3 mg, for which effects are still to be expected. The systemically tolerable amount is therefore less than 3 mg. The amount absorbed through the skin is thus higher than the systemically tolerable amount, and designation with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts) has been retained.

Sensitization. Two new studies show that reactions to 4-nitroaniline can occur in patients with existing sensitization to (disubstituted) aromatic amino compounds. Since there are no data for previous exposure available, a contact sensitization potential of 4-nitroaniline itself in humans has not been demonstrated beyond doubt. Experimental studies in animals, carried out at relatively low concentrations, did not provide explicit evidence of a contact sensitization potential and the result of an in vitro study was negative. 4-Nitroaniline has therefore not been designated with "Sh" (for substances which cause sensitization of the skin) in spite of the suspected contact sensitization potential. Studies of respiratory sensitization are not available to date, so that 4-nitroaniline has not been designated 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.

References

BUA (GDCh-Advisory Committee on Existing Chemicals), editor (1987) p-Nitroaniline (4-nitrobenzeneamine). BUA report No. 19. Stuttgart: Hirzel

Chopade HM, Matthews HB (1984) Disposition and metabolism of p-nitroaniline in the male F-344 rat. Fundam Appl Toxicol 4(3 Pt 1): 485–493. https://doi.org/10.1016/0272-0590(84)90207-0

Chung KT, Murdock CA, Zhou Y, Stevens SE, Li YS, Wei CI, Fernando SY, Chou MW (1996) Effects of the nitro-group on the mutagenicity and toxicity of some benzamines. Environ Mol Mutagen 27(1): 67–74. https://doi.org/10.1002/(SICI)1098-2280(1996)27:1<67::AID-EM9>3.0.CO;2-B

Dellarco VL, Prival MJ (1989) Mutagenicity of nitro compounds in Salmonella typhimurium in the presence of flavin mononucleotide in a preincubation assay. Environ Mol Mutagen 13(2): 116–127. https://doi.org/10.1002/em.2850130206

Greim H, editor (2002) 2-Nitrotoluol. In: Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten. 34th issue. Weinheim: Wiley-VCH. Also available from https://doi.org/10.1002/3527600418.mb8872d0034

Greim H, editor (2003) 4-Chlor-o-toluidin. In: Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten. 36th issue. Weinheim: Wiley-VCH. Also available from https://doi.org/10.1002/3527600418.mb9569d0036

Greim H, editor (2005) Monocyclic aromatic amino and nitro compounds. MAK Value Documentation 2003. In: The MAK-Collection for Occupational Health and Safety. Part I: MAK Value Documentations. Volume 21. Weinheim: Wiley-VCH. p. 3–45. Also available from https://doi.org/10.1002/3527600418.mb0maryvere0021

Hardin BD, Schuler RL, Burg JR, Booth GM, Hazelden KP, MacKenzie KM, Piccirillo VJ, Smith KN (1987) Evaluation of 60 chemicals in a preliminary developmental toxicity test. Teratog Carcinog Mutagen 7(1): 29–48. https://doi.org/10.1002/tcm.1770070106

Hartwig A, editor (2010) Aniline. MAK Value Documentation, 2007. In: The MAK-Collection for Occupational Health and Safety. Part I: MAK Value Documentations. Volume 26. Weinheim: Wiley-VCH. p. 57–106. Also available from https://doi.org/10.1002/3527600418.mb6253e0026b

Hartwig A, editor (2014 a) 2,4,6-Trinitrotoluene (and isomers in technical mixtures). MAK Value Documentation, 2008. In: The MAK-Collection for Occupational Health and Safety. Part I: MAK Value Documentations. Weinheim: Wiley-VCH. https://doi.org/10.1002/3527600418.mb11896e4514

Hartwig A, editor (2014 b) 4-Nitroaniline. MAK Value Documentation, 1999. In: The MAK-Collection for Occupational Health and Safety. Part I: MAK Value Documentations. Weinheim: Wiley-VCH. https://doi.org/10.1002/3527600418.mb10001e2814

Hartwig A, editor (2014 c) 4-Nitroaniline. MAK Value Documentation, 2000. In: The MAK-Collection for Occupational Health and Safety. Part I: MAK Value Documentations. Weinheim: Wiley-VCH. https://doi.org/10.1002/3527600418.mb10001e3014

Hartwig A, editor (2014 d) o-Toluidine. MAK Value Documentation, 2007. In: The MAK-Collection for Occupational Health and Safety. Part I: MAK Value Documentations. Weinheim: Wiley-VCH. https://doi.org/10.1002/3527600418.mb9553e4314

Hartwig A, MAK Commission (2016 a) 3-Nitrotoluene. MAK Value Documentation, 2007. MAK Collect Occup Health Saf. https://doi.org/10.1002/3527600418.mb9908e4216

Hartwig A, MAK Commission (2016 b) 4-Nitrotoluene. MAK Value Documentation, 2007. MAK Collect Occup Health Saf. https://doi.org/10.1002/3527600418.mb9999e4216

Hartwig A, MAK Commission (2018) Nitrobenzene. MAK Value Documentation, 2017. MAK Collect Occup Health Saf 3(4): 1932–1982. https://doi.org/10.1002/3527600418.mb9895e6318

Hartwig A, MAK Commission (2019) N-Methylaniline. MAK Value Documentation, 2017. MAK Collect Occup Health Saf 4(3): 1146–1170. https://doi.org/10.1002/3527600418.mb10061e6319

Henschler D, editor (1992) p-Phenylendiamin. In: Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten. 18th issue. Weinheim: VCH. Also available from https://doi.org/10.1002/3527600418.mb10650d0018

Huang Q, Wang L, Han S (1995) The genotoxicity of substituted nitrobenzenes and the quantitative structure-activity relationship studies. Chemosphere 30(5): 915–923. https://doi.org/10.1016/0045-6535(94)00450-9

Huang QG, Kong LR, Liu YB, Wang LS (1996) Relationships between molecular structure and chromosomal aberrations in in vitro human lymphocytes induced by substituted nitrobenzenes. Bull Environ Contam Toxicol 57(3): 349–353. https://doi.org/10.1007/s001289900197

ICPEMC (International Commission for Protection against Environmental Mutagens and Carcinogens) (1983) Screening strategy for chemicals that are potential germ-cell mutagens in mammals. Committee 1 Final Report. Mutat Res 114(2): 117–177

In Vitro Technologies (2005) Human percutaneous absorption and cutaneous disposition of [14C]-nitroaniline in vitro. Baltimore, MD: In Vitro Technologies. https://downloads.regulations.gov/EPA-HQ-OPPT-2003-0006-0320/content.pdf, accessed 21 Dec 2021

Malinauskiene L, Zimerson E, Bruze M, Ryberg K, Isaksson M (2012) Patch testing with the textile dyes Disperse Orange 1 and Disperse Yellow 3 and some of their potential metabolites, and simultaneous reactions to para-amino compounds. Contact Dermatitis 67(3): 130–140. https://doi.org/10.1111/j.1600-0536.2012.02080.x

Malinauskiene L, Zimerson E, Bruze M, Ryberg K, Isaksson M (2013) Sensitizing capacity of Disperse Orange 1 and its potential metabolites from azo reduction and their cross-reactivity pattern. Contact Dermatitis 69(1): 40–48. https://doi.org/10.1111/cod.12078

Maté C, Ryan AJ, Wright SE (1967) Metabolism of some 4-nitroaniline derivatives in the rat. Food Cosmet Toxicol 5(5): 657–663. https://doi.org/10.1016/s0015-6264(67)83217-6

Mirsalis J, Tyson K, Beck J, Loh F, Steinmetz K, Contereras C, Austere L, Martin S, Spalding J (1983) Induction of unscheduled DNA-synthesis (UDS) in hepatocytes following in vitro and in vivo treatment. Environ Mutagen 5: 482

Monsanto Company (1989) Micronucleus assay with p-nitroaniline (final report). NTIS/OTS0532109. Alexandria, VA: NTIS. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0532109.xhtml, accessed 14 Feb 2018

Nair RS, Johannsen FR, Schroeder RE (1985) Evaluation of teratogenic potential of para-nitroaniline and para-nitrochlorobenzene in rats and rabbits. In: Rickert DE, editor. Toxicity of nitroaromatic compounds. New York, NY: Hemisphere Publishing Corporation. p. 61–85

Nair RS, Auletta CS, Schroeder RE, Johannsen FR (1990) Chronic toxicity, oncogenic potential, and reproductive toxicity of p-nitroaniline in rats. Fundam Appl Toxicol 15(3): 607–621. https://doi.org/10.1016/0272-0590(90)90045-L

NIOSH (National Institute for Occupational Safety and Health) (1983) Screening of priority chemicals for reproductive hazards with cover letter. NTIS/OTS04830240. Alexandria, VA: NTIS. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS00002400.xhtml, accessed 06 May 2019

NTP (National Toxicology Program) (1988) Toxicology and carcinogenesis studies of 2-amino-5-nitrophenol (CAS No 121-88-0) in F344/N rats and B6C3F1 mice (gavage studies). NTP TR 334. Research Triangle Park, NC: NTP. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr334.pdf, accessed 06 May 2019

NTP (National Toxicology Program) (1993) Toxicology and carcinogenesis studies of p-nitroaniline (CAS No 100-01-6) in B6C3F1 mice (gavage studies). NTP TR 418. Research Triangle Park, NC: NTP. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr418.pdf, accessed 09 Feb 2018

Nyska A, Haseman JK, Kohen R, Maronpot RR (2004) Association of liver hemangiosarcoma and secondary iron overload in B6C3F1 mice–the National Toxicology Program experience. Toxicol Pathol 32(2): 222–228. https://doi.org/10.1080/01926230403200201

Salamone MF (1988) Summary report on the performance of the sperm assays. In: Ashby J, deSerres FJ, Shelby MD, Margolin BH, Ishidate M Jr, Becking GC, editors. Evaluation of short-term tests for carcinogens: report of the International Programme on Chemical Safety's collaborative study on in vivo assays. Volume 2. Cambridge, MA: Cambridge University Press. p. 2229–2234

Sonnenburg A, Ahuja V, Schreiner M, Platzek T, Stahlmann R (2012) Assessment of the sensitizing potential of textile disperse dyes and some of their metabolites by the loose-fit coculture-based sensitization assay (LCSA). Arch Toxicol 86(5): 733–740. https://doi.org/10.1007/s00204-012-0811-9

US EPA (US Environmental Protection Agency) (2009) Provisional peer reviewed toxicity values for 4-nitroaniline (CASRN 100-01-6). EPA/690/R-09/038F. Cincinnati, OH: US EPA. https://cfpub.epa.gov/ncea/pprtv/documents/Nitroaniline4.pdf, accessed 09 Feb 2018

Wang G, Zhang X, Yao C, Tian M (2010) Four-week oral toxicity study of three metabolites of nitrobenzene in rats. Drug Chem Toxicol 33(3): 238–243. https://doi.org/10.3109/01480540903414156

Wild D (1984) The sperm morphology test, a rapid in vivo test for germinal mutations. In: Baß R, Glocklin V, Grosdanoff P, Henschler D, Kilbey B, Müller D, Neubert D, editors. Critical evaluation of mutagenicity tests. BGA-Schriften, No. 3/84. München: MMV Medizin Verlag. p. 299–306

Young E, Zimerson E, Bruze M, Svedman C (2016) Two sensitizing oxidation products of p-phenylenediamine patch tested in patients allergic to p-phenylenediamine. Contact Dermatitis 74(2): 76–82. https://doi.org/10.1111/cod.12488