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GMS PUBLISSO ZB MED-Publikationsportal Lebenswissenschaften

4-Nitrobenzoic acid

MAK Value Documentation – Translation of the German version from 2017

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Keywords

4-nitrobenzoic acid; olfactory epithelium; methaemoglobin; clitoral gland; carcinogenicity; reproductive toxicity; genotoxicity; irritation; MAK value; peak limitation

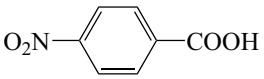
Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has evaluated 4-nitrobenzoic acid [62-23-7]. Available study reports and publications are described in detail. 4-Nitrobenzoic acid caused a statistically significantly increased incidence of adenomas and of the sum of adenomas and carcinomas of the clitoris gland in female F344 rats in an oral, 2-year carcinogenicity study at doses of 60 mg/kg body weight and day and above. The incidence of adenomas and the sum of adenomas and carcinomas was above the range limit of the historical controls of the contract laboratory. As 4-nitrobenzoic acid is a metabolite of 4-nitrotoluene, which also induced tumours of the clitoral gland as well as tumours in other organs, 4-nitrobenzoic acid is classified in analogy to 4-nitrotoluene in Carcinogen Category 3B. 4-Nitrobenzoic acid has a weak mutagenic potency in vitro. It did not induce micronuclei in vivo or in vitro. There are no suitable data in humans to derive a MAK value. In rats, a 15-month study and a 2-year study resulted in a NOAEL of 60 mg/kg body weight and day for delayed body weight gain in the females. Toxicokinetic scaling results in a concentration of 147 mg/m^3 in workplace air. The LOAEC for local effects on the olfactory epithelium of rats in a 14-day study is 150 mg/m^3, the NOAEC 20 mg/m^3. Since 2014, the Commission uses an empirical approach to set MAK values for substances with critical effects on the upper respiratory tract or the eyes. Based on this approach, a MAK value of 1 mg/m^3 for the inhalable fraction was derived. The dose of 60 mg/kg body weight and day which resulted in increased incidences of clitoral gland tumours corresponds to a concentration which is about 150 times higher than the MAK value. In view of the evaluation of carcinogenicity, this margin is considered to be sufficiently large. As local effects are critical, the substance is assigned to Peak Limitation Category I. The difference between NOAEC and LOAEC is large, thus an excursion factor of 2 has been established. 4-Nitrobenzoic acid is assigned to Pregnancy Risk Group D as the available data for prenatal toxicity are insufficient. Skin absorption does not contribute significantly to systemic toxicity and 4-nitrobenzoic acid is not expected to lead to contact sensitization.

Citation Note:
Hartwig A, MAK Commission. 4-Nitrobenzoic acid. MAK Value Documentation – Translation of the German version from 2017. MAK Collect Occup Health Saf. 2022 Mar;7(1):Doc010. https://doi.org/10.34865/mb6223e7_1or

Manuscript completed:
24 Feb 2016

Publication date:
31 Mar 2022

MAK value (2016)	**1 mg/m³ I (inhalable fraction)**
Peak limitation (2016)	**Category I, excursion factor 2**
Absorption through the skin	–
Sensitization	–
Carcinogenicity (2016)	**Category 3 B**
Prenatal toxicity (2016)	**Pregnancy Risk Group D**
Germ cell mutagenicity	–
BAT value	–
Synonyms	1-carboxy-4-nitrobenzene *p*-nitrobenzoic acid *p*-nitrobenzenecarboxylic acid
Chemical name	4-nitrobenzoic acid
CAS number	62-23-7
Structural formula	
Molecular formula	$C_7H_5NO_4$
Molar mass	167.12 g/mol
Melting point	238–240 °C (ECHA 2015, 2016) 242 °C (NLM 2014)
Vapour pressure	3.3×10^{-6} hPa (calc.; NCBI 2014)
log K_{OW}	1.89 (ECHA 2015; NCBI 2014)
pKa	3.44 (NCBI 2014)
pH at 20 °C	3.1 (ECHA 2016)
Solubility	200 mg/l (NCBI 2014), 379 mg/l water, pH 3.3 (ECHA 2015), 420 mg/l (ECHA 2016)
Stability	no data
Production	oxidation of 4-nitrotoluene with pure oxygen or oxidation with 15% nitric acid at 175 °C (NCBI 2014); nitration and subsequent oxidation of polystyrene (NCBI 2014)
Purity	no data
Impurities	no data
Uses	organic synthesis; manufacture of intermediates, reagent for alkaloids, manufacture of pesticides, paints, explosives, industrial solvents (NTP 1994)

1 Toxic Effects and Mode of Action

4-Nitrobenzoic acid does not cause irritation of the skin in rabbits, but does cause severe irritation of the eyes.

In a 2-week inhalation study, rats showed minimal to slight necrosis in the dorsal olfactory epithelium at 4-nitrobenzoic acid concentrations of 150 mg/m^3 and above.

In rats, 4-nitrobenzoic acid was completely absorbed after ingestion, and up to 63% of the administered dose was excreted as free 4-nitrobenzoic acid mainly with the urine. In marmosets, the half-life in the blood was about 1 hour after oral administration. During metabolism, the nitro group is reduced to form 4-hydroxyaminobenzoic acid and 4-aminobenzoic acid and its conjugates. After ingestion, the substance is broken down mainly by intestinal bacteria.

In a 15-month study and a 2-year study, the body weight gains in female rats were decreased after oral administration of 4-nitrobenzoic acid at 125 mg/kg body weight and day. In a 13-week study, congestion and haemosiderin deposits were observed in the spleens of rats after 4-nitrobenzoic acid doses of 160 mg/kg body weight and day and above. In a 15-month study, changes in haematological parameters, such as an increase in the number of reticulocytes, a decrease in the erythrocyte count and decreases in the haemoglobin and haematocrit levels, were found after 4-nitrobenzoic acid doses of 210 mg/kg body weight and day and above.

4-Nitrobenzoic acid was mutagenic in bacteria and clastogenic only in CHO cells (a cell line derived from Chinese hamster ovary). 4-Nitrobenzoic acid was not clastogenic in the bone marrow cells of Chinese hamsters, and the incidences of micronuclei were not increased in the bone marrow cells or peripheral erythrocytes of mice.

In an oral 2-year carcinogenicity study, 4-nitrobenzoic acid significantly increased the incidences of adenomas in the clitoral gland of female F344 rats at doses of 60 mg/kg body weight and day and above; this effect was found only in this species.

In a 13-week study in rats, testicular lesions were observed at a dose of 660 mg/kg body weight and day. After exposure of mice to 4-nitrobenzoic acid before, during and after continuous mating of the F0 generation and subsequent exposure of the F1 generation, the period until pregnancy was prolonged and the number of offspring per litter or litters per pair was reduced after doses of about 1100 mg/kg body weight and day and above.

No clinical findings or animal studies are available for sensitizing effects of 4-nitrobenzoic acid.

2 Mechanism of Action

Nitro aromatic compounds typically lead to adverse effects on the haematological system. They are characterized by the induction of methaemoglobin formation (Greim 2005). Reduction of the nitro group leads to the oxidation of haemoglobin, thereby forming methaemoglobin. In 9-day and 14-day studies and in 13-week and 15-month studies in rats, methaemoglobin formation was increased after repeated ingestion of 4-nitrobenzoic acid (NTP 1994). In addition, 4-hydroxylaminobenzoic acid, which is the metabolite responsible for this reaction, was identified in liver homogenates from Wistar rats (Kato et al. 1969). Erythrocytes that are highly loaded with methaemoglobin release redox-active iron. As a catalyst of auto-oxidation reactions, redox-active iron induces the formation of oxygen radicals via Fenton and Haber-Weiss reactions. The impairment of the redox equilibrium in erythrocytes may lead to changes in the membranes by lipid peroxidation, disturbance of the reversible interactions between the thiol groups of haemoglobin and glutathione, and the binding of haemoglobin to the erythrocyte membrane. Erythrocytes with reduced elasticity and deformability have difficulties in passing or cannot pass the splenic sinuses of the red pulp. Cell debris accumulates in the splenic vessels resulting in swelling, inflammatory reactions, and hyperplastic and fibrotic changes of the splenic stroma (Hartwig 2015, available in German only). Likewise, the degradation of the erythrocytes caused by 4-nitrobenzoic acid led to changes in the spleen such as congestion and haemosiderin deposits (see Section 5.2.2).

There are marked species differences in NADH-dependent methaemoglobin reductase activity, which is responsible for the regeneration of the functioning haem from methaemoglobin. Reductase levels are higher in mice than in rats.

Therefore, mice are considerably less susceptible than rats to substances that lead to haematological changes and splenic lesions by inducing methaemoglobin formation (NTP 1994; Srivastava et al. 2002).

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

3.1.1 Absorption

When marmosets (Callithrix jacchus) were given oral doses of 4-nitrobenzoic acid labelled with ^{14}C at the acid C atom (0.4 mmol; 67 mg/kg body weight), the highest concentration in the blood was determined after 30 to 40 minutes (Kuzniar and James 1981).

Fluxes of 6.92, 0.66 and 0.63 µg/cm^2 and hour were calculated for a saturated aqueous solution using the models of Fiserova-Bergerova et al. (1990), Guy and Potts (1993) and Wilschut et al. (1995), respectively. Assuming the exposure of a surface area of 2000 cm^2 of skin for 1 hour, this would correspond to absorbed amounts of 14, 1.3 and 1.26 mg, respectively.

3.1.2 Distribution

When Wistar rats were given intraperitoneal injections of ^{14}C-4-nitrobenzoic acid, significant amounts of the substance were detected in the caecum and colon after 4 hours; at this time, the urine contained metabolites (Gardner and Renwick 1978).

3.1.3 Elimination

When single oral or intraperitoneal doses of 25 mg ^{14}C-4-nitrobenzoic acid (100 mg/kg body weight) were given to female Wistar rats weighing 250 g, 94% and 83%, respectively, of the radioactivity was found in the urine and 1% to 8% was found in the faeces within 24 hours (Gardner and Renwick 1978). When marmosets were given an oral ^{14}C-4-nitrobenzoic acid dose of 0.4 mmol/kg body weight (67 mg/kg body weight), the half-life in the blood was about 1 hour. After 48 hours, 69% to 91% of the administered dose was found in the urine and 2% to 6% was found in the faeces. After the intraperitoneal injection of 0.4 mmol/kg body weight, about 91% of the administered dose was detected in the urine, and 1.1% to 4.5% was detected in the faeces within a period of 48 hours (Kuzniar and James 1981).

An average amount of 298 µg 4-aminobenzoic acid per hour was excreted with the urine of female Wistar rats 2 hours after intraperitoneal treatment with 200 mg 4-nitrobenzoic acid. Ethanol increased the 4-nitrobenzoic acid metabolism (Tani and Kourounakis 1993).

In female ferrets given 4-nitrobenzoic acid labelled with ^{14}C at the acid C atom (100 mg/kg body weight) by intraperitoneal injection, about 74% of the administered dose was excreted with the urine after 24 hours (Idle et al. 1978).

3.2 Metabolism

3.2.1 In vitro

In vitro studies with homogenates from rat liver and rat intestinal wall or with the contents of the caecum and colon of rats yielded evidence of reduction at the nitro group of 4-nitrobenzoic acid (Gardner and Renwick 1978; Peters and Fouts 1968) and identified the metabolite 4-hydroxyaminobenzoic acid (Kato et al. 1969). In microsomal preparations from different rabbit tissues, the reduction of the nitro group of 4-nitrobenzoic acid was strongest in the liver, followed by the renal cortex and bladder (Zenser et al. 1981). Among the various rabbit strains, the highest level of activity was

found in New Zealand White rabbits (Cram et al. 1965). In microsomes from the liver of Swiss mice, 4-nitrobenzoic acid was reduced to 4-aminobenzoic acid at a rate of 0.03 to 0.04 µmol/(mg protein × minute) (ECB 2000). Similar studies with microsomes from the liver of Sprague Dawley rats found that reduction proceeded at a rate of 0.151 nmol/(mg protein × minute) (Norred et al. 1975). It was higher in male Sprague Dawley rats than in the females and lowest during gestation (ECB 2000; Kato et al. 1970). A comparative study yielded reductase activities in rats, guinea pigs and mice of 2.72, 2.08 and 2.04 µmol/(g protein × hour), respectively (Adamson et al. 1965).

In the microsomal fractions of the liver, intestines and kidneys of 8 to 26-week-old human foetuses, the enzyme activities involved in 4-nitrobenzoic acid metabolism were detected in the fractions from the liver and intestines, but not in those from the kidneys. The enzymes seem to belong to the same class of multifunctional $NADPH_2$-dependent oxidoreductases found in adult humans and animals (Pelkonen et al. 1971). Even the liver tissue of an 8-week-old foetus was found to have the capacity to metabolize 4-nitrobenzoic acid or to reduce the nitro group; this foetus was the youngest to be examined and weighed 6 to 10 g. The metabolizing activity increased with the size of the foetuses and was consistently about 0.05 to 0.08 µmol/g tissue and hour at a body weight of 100 g and above and from an age of about 13 weeks onwards (Pelkonen 1973).

A nitroreductase activity rate of 255 pmol/(mg protein × minute) was determined for the reduction of 4-nitrobenzoic acid in Salmonella typhimurium TA98, whereas this kind of activity was not detected in CHO cells (ECB 2000).

In a microsomal fraction of the liver of Wistar rats, the metabolites of 4-nitrobenzoic acid were determined to be 4-hydroxyaminobenzoic acid and 4-aminobenzoic acid (Kato et al. 1969).

3.2.2 In vivo

In a study in Wistar rats, 25% of the administered dose of 4-nitrobenzoic acid was metabolized and recovered in the urine as 4-aminobenzoic acid (Wheeler et al. 1975 b).

After a single oral dose or intraperitoneal injection of ^{14}C-labelled 4-nitrobenzoic acid, the examination of the urine of female Wistar rats revealed that 2% and 1%, respectively, was metabolized to free 4-aminobenzoic acid, 20% and 17%, respectively, was metabolized to a conjugated aminobenzoic acid and about 25% was metabolized to other metabolites that were not reported. The fraction of unchanged 4-nitrobenzoic acid in the urine was 55% and 63%, respectively (Gardner and Renwick 1978).

In marmosets, the following percentages of 4-nitrobenzoic acid were recovered in the urine 48 hours after oral administration: 22.6% to 61.2% as 4-nitrohippuric acid, 4.6% to 21.9% as 4-aminobenzoic acid, 6.2% to 24.4% as 4-acetamidobenzoic acid and 27.4% to 51.1% as unchanged substance. When oral administration and intraperitoneal injection of 4-nitrobenzoic acid were preceded by antibiotic treatment, the reductive metabolism was inhibited by about 81% and 73%, respectively. This shows that the substance is reduced by intestinal bacteria after ingestion (Kuzniar and James 1981).

The metabolism of 4-nitrobenzoic acid/kg body weight was examined on the basis of the elimination products excreted in the urine by female ferrets after intraperitoneal injection of 4-nitrobenzoic acid/kg body weight labelled with ^{14}C at the acid C atom. Thin layer chromatography identified 4% of the products as unchanged substance, 38% as glycine conjugates and 29% as glucuronic acid conjugates. 4-Nitrohippuric acid (52%) was the major glycine conjugate and 4-acetamidobenzoic acid (3%) was a minor glycine conjugate. A different pattern of metabolism was found in rats. Rats excreted 43% as unchanged acid. Likewise, glycine conjugates were detected. In addition, 52% of 4-nitrobenzoic acid was reduced to 4-aminobenzoic acid in rats, whereas this fraction was only 3% in ferrets (Idle et al. 1978).

Two hours after intraperitoneal treatment of female Wistar rats with 200 mg 4-nitrobenzoic acid, 298 µg 4-aminobenzoic acid per hour was excreted on average in the urine, 255 µg per hour of this in conjugated form. The fraction in the plasma was 5 µg 4-aminobenzoic acid per hour, 3.7 µg per hour of this as conjugates. Ethanol increased the 4-nitrobenzoic acid metabolism (Tani and Kourounakis 1993).

3.3 Summary

In rats, 4-nitrobenzoic acid is completely absorbed after ingestion and up to 63% of the administered dose is excreted as free 4-nitrobenzoic acid mainly with the urine. The half-life in the blood of marmosets is about 1 hour. During metabolism, the nitro group is reduced to form 4-hydroxyaminobenzoic acid and 4-aminobenzoic acid and its conjugates. After ingestion, the substance is reduced mainly by intestinal bacteria.

4 Effects in Humans

Urine samples from workers of a factory producing pharmaceuticals and explosives were examined for mutagenic activity after a 4-week holiday (control samples) and at the end of the shift after several days of work (exposed samples). The urine samples from 7 workers exposed to 4-nitrobenzoic acid concentrations of up to 5.4 mg/m^3 did not lead to a statistically significant increase in the mutation frequency in Salmonella typhimurium TA98. In contrast, the urine from 12 workers exposed to trinitrotoluene caused a statistically significant increase in mutations in this Salmonella strain (Ahlborg et al. 1985).

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

There are no data available.

5.1.2 Oral administration

Within 24 hours after ingestion, an LD_{50} for 4-nitrobenzoic acid of 1960 mg/kg body weight was determined in rats (Caujolle et al. 1966). In mice, the LD_{50} for 4-nitrobenzoic acid lied between 1470 mg/kg body weight (Caujolle et al. 1966) and 3000 mg/kg body weight (Clariant GmbH 1955). The signs of toxicity were increased irritability, aggressiveness, convulsions, paralysis of the hind limbs in some animals, exhaustion, rapid breathing or bloody purulent lacrimation. 4-Nitrobenzoic acid induced the infiltration of erythrocytes in the liver of some animals and myeloid metaplasia in the red pulp of the spleen (Caujolle et al. 1966).

5.1.3 Dermal application

There are no data available.

5.1.4 Intraperitoneal and intravenous injection

Within 24 hours after intraperitoneal injection, the LD_{50} values determined for 4-nitrobenzoic acid were 1210 mg/kg body weight in rats and 880 mg/kg body weight in mice (Caujolle et al. 1966).

Within 24 hours after intravenous injection, the LD_{50} value for mice was 770 mg/kg body weight (Caujolle et al. 1966).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

Groups of 10 male rats were exposed nose-only to 4-nitrobenzoic acid concentrations of 0, 20 (8–31), 150 (90–260) or 1000 (540–1500) mg/m^3 for 6 hours a day, on 5 days a week, for 2 weeks. The mass median aerodynamic diameter of the particles was about 5 µm at 20 mg/m^3, about 9 µm at 150 mg/m^3 and about 13 µm at 1000 mg/m^3. The aerodynamic diameter was below 10 µm in 75%, 57% and 45% of the particles per concentration. Five animals per group were examined at the end of treatment or after a recovery period of 14 days. At 4-nitrobenzoic acid concentrations of 150 mg/m^3 and above, minimal to slight necrosis was observed in the frontal to middle region of the dorsal olfactory epithelium (no other details) immediately after the end of exposure; this had healed after 14 days. No unusual findings were obtained in the histopathological examinations of the lungs and other organs, blood examinations or urinalysis (DuPont 1989, 2012).

5.2.2 Oral administration

The studies that investigated the toxicity of 4-nitrobenzoic acid after repeated oral administration are shown in Table 1.

In a 13-week study in F344 rats, the methaemoglobin level in the blood increased with the dose mainly in the male animals at 40 mg/kg body weight and above. In every dose group, the methaemoglobin level was highest after 30 days and considerably lower after 90 days (see Table 1). After administration of a 4-nitrobenzoic acid dose of 660 mg/kg body weight and day, the methaemoglobin level reached a maximum of 4.03% (control group: 0.49%) after 30 days and 3.33% after 90 days (NTP 1994). At the highest dose of 210 mg/kg body weight and day, the methaemoglobin level was 0.28% after 15 months (NTP 1994).

Humans react more sensitively to methaemoglobin formers than rats. Therefore, it is important to know at which dose levels 4-nitrobenzoic acid impairs the oxygen transport in humans through the formation of methaemoglobin. In the general population, a mean background level of 0.78% was determined for methaemoglobin in the blood. Methaemoglobin levels of up to 5% are regarded as safe (Leng and Bolt 2008). As no data are available for 4-nitrobenzoic acid, aniline is used as a reference substance. The 6-hour exposure of humans to an aniline concentration of 2 ml/m^3 led to an increase in methaemoglobin levels from 0.7% to 1.2% and thus an increment of 0.5% (Käfferlein et al. 2014). In rats, 6-hour exposure to an aniline concentration of 90 mg/m^3 (24 ml/m^3) led to a methaemoglobin increment of about 1.2% (Pauluhn 2004). By applying linear extrapolation, the increment determined for the methaemoglobin level in humans at a concentration of 24 ml/m^3 would be 6%. Therefore, assuming linear extrapolation, the increase in the methaemoglobin level would be 5 times as high in humans as that in rats after exposure to aniline at the same concentration level. In the 13-week study, the maximum increment in the methaemoglobin level induced by exposure to 4-nitrobenzoic acid at a dose level of 40 mg/kg body weight and day was 0.72% in male rats after 30 days. If the species difference above is applied to 4-nitrobenzoic acid, the increment in humans would be 5 times as high, and thus 3.6%. When the background level of about 0.78% is additionally taken into account, the total haemoglobin level that results is below the critical limit of 5%. The methaemoglobin concentration in male rats given 4-nitrobenzoic acid at a dose level of 160 mg/kg body weight and day was 1.92% after 30 days. This dose is regarded as the LOAEL (lowest observed adverse effect level) because congestion and haemosiderin deposits in the spleen were observed. The corresponding NOAEL (no observed adverse effect level) was 70 mg/kg body weight and day. Haematological changes characteristic of regenerative anaemia were observed after 4-nitrobenzoic acid doses of 660 mg/kg body weight and day and above. The changes were manifest as increased reticulocyte counts and decreased erythrocyte counts, haemoglobin levels and haematocrit values (NTP 1994). Therefore, the NOAEL in the 13-week study was 40 mg/kg body weight and day. In the 2-year study, the methaemoglobin level was determined only at the interim evaluation after 15 months. This was increased only in the males of the high dose group. In the 2-year study, it was not possible to evaluate the changes in the spleen because of the accumulation of mononuclear leukaemia cells. The accumulation of mononuclear leukaemia cells in the spleen was found in all untreated and treated animals (NTP 1994).

Other systemic effects that were noted in rats were decreased body weight gains. These effects were observed in female animals in the 15-month study and in the 2-year study at 4-nitrobenzoic acid levels of 125 mg/kg body weight and day and above. The NOAEL for these effects was 60 mg/kg body weight and day (NTP 1994).

Therefore, the chronic NOAEL in rats was 60 mg/kg body weight and day.

Other effects were induced only after 14-day exposure to very high doses: lesions in the thyroid gland were observed at 1000 mg/kg body weight and day and atrophy in the testes, ovaries, thymus and bone marrow were found at 2000 mg/kg body weight and day (NTP 1994).

In a 13-week study, 4-nitrobenzoic acid caused a decrease in body weight gains in male mice at 170 mg/kg body weight and day and above and in female mice at 670 mg/kg body weight and day and above. However, adverse effects on the body weights of the males were not observed after exposure to up to 300 mg/kg body weight and day for 13 weeks. No haematological changes or abnormalities in the spleen were observed in mice after chronic administration of 4-nitrobenzoic acid doses of up to 905 mg/kg body weight and day. Therefore, the chronic NOAEL in mice for reduced body weight gains was 300 mg/kg body weight and day for males and 365 mg/kg body weight and day for females (NTP 1994).

Further data from these studies are shown in Table 1.

Tab. 1 Studies that investigated the toxicity of 4-nitrobenzoic acid after repeated oral administration

Species, strain, number per group	Exposure	Findings	References
rat, 5, (no other details)	**9 days**, 1000 mg/kg body weight and day, 7 × in 9 days, examination: 2 days after administration	**1000 mg/kg body weight**: blood: 37% decrease in erythrocyte count, 26% decrease in haemoglobin level, MetHb (no data); no unusual gross-pathological or histopathological findings	ECB 2000
rat, F344, 5 ♂, 5 ♀	**14 days**, 0, 2500, 5000, 10 000, 20 000, 40 000 mg/kg diet (about 0, 300, 600, 1200, 2400, 4800 mg/kg body weight and day[a])	**about 300 mg/kg body weight**: blood: ♀: MetHb 0.16% (controls: 0.09%); **about 600 mg/kg body weight**: blood: ♀: MetHb 0.35%, ♂: reticulocyte count ↑; **about 1200 mg/kg body weight and above**: 13–18% decrease in body weights, blood: ♀: MetHb 0.53%, haemoglobin level, haematocrit level and erythrocyte count ↓, reticulocyte count ↑, ♀ and ♂: number of nucleated erythrocytes ↑, spleen: relative and absolute weights ↑, thyroid gland: ♂: congestion, ♀: hypertrophy of the follicular epithelium; **about 2400 mg/kg body weight and above**: 32–40% decrease in body weights, blood: ♂: haemoglobin level, haematocrit level and erythrocyte count ↓, MetHb: ♀: 1.63%, ♂: 3.06% (controls: 0.14%), spleen: ♀: congestion, thyroid gland: ♂: hypertrophy of the follicular epithelium, testes and ovaries: atrophy, thymus: ♂ and ♀: atrophy, bone marrow: ♀: atrophy; **about 4800 mg/kg body weight**: 48%–60% decrease in body weights, blood: MetHb: ♀: 1.27%, ♂: 2.48%, thyroid gland: hyperplasia of the follicular epithelium	NTP 1994

Tab. 1 *(continued)*

Species, strain, number per group	Exposure	Findings	References
rat, F344, 10 ♂, 10 ♀	**13 weeks**, 0, 630, 1250, 2500, 5000, 10 000 mg/kg diet (according to the authors: ♂: 0, 40, 70, 160, 310, 660 mg/kg body weight and day; ♀: 0, 40, 80, 170, 340, 680 mg/kg body weight and day)	**40 mg/kg body weight and above**: blood: MetHb: ♂: controls: 0.49%, maximum of 1.21% after 30 days (after 90 days: 0.66%, controls: 0.79%), kidneys: ♂: mild hyaline droplet accumulation (α2u-globulin) in epithelial cells of the proximal tubules (dose-dependent), **NOAEL**; **160/170 mg/kg body weight and above**: ♂: 5% decrease in body weights, blood: MetHb: ♂: maximum of 1.92% after 30 days (after 90 days: 0.89%), spleen: ♂ and ♀: haemosiderin deposits in the red pulp and macrophages, ♂: congestion, kidneys: tubular epithelial cells with larger nuclei, ♂: haemosiderin deposits; **310/340 mg/kg body weight and above**: 9–11% decrease in body weights, spleen: relative weights ↑, blood: MetHb not determined; **660/680 mg/kg body weight**: 17–26% decrease in body weights, blood: haematological parameters as in the case of regenerative anaemia, maximum MetHb level after 30 days: ♂: 4.03% (control value: 0.49%), after 90 days: 3.33% (control value: 0.79%), ♀: 3.01% (control value: 1.24%), after 90 days: 2.08% (control value: 0.78%), Heinz bodies and reticulocyte count ↑, haemoglobin level, haematocrit value and erythrocyte count ↓, spleen: relative and absolute weights ↑, ♀: pigmentation, testes: congestion in the seminiferous tubules; pigmentation (haemosiderin) is a sign of haemolytic anaemia; with the exception of the values after 30 days, MetHb increased in a dose and time-dependent manner, reaching a maximum of 4%	NTP 1994; Williams et al. 2001
rat, F344, 10 ♂, 10 ♀	**15 months**, 0, 1250, 2500, 5000 mg/kg diet (according to the authors: ♂: 0, 50, 100, 210 mg/kg body and day; ♀: about 0, 60, 125, 250 mg/kg body weight and day)	**60 mg/kg body weight**: **NOAEL**; **125 mg/kg body weight**: ♀: about 6% decrease in body weights; **210/250 mg/kg body weight**: blood: ♀: haemoglobin level, haematocrit value and erythrocyte count ↓ and ♂: MetHb 0.28% (control value: 0.22%), spleen: ♀: weights ↑ and haemosiderin accumulation in macrophages, kidneys: ♂: dose-dependent severity of age-related nephropathy ↓; no unusual findings in the histopathological examinations	NTP 1994; Williams et al. 2001
rat, F344, 50 ♂, 50 ♀	**2 years,** 0, 1250, 2500, 5000 mg/kg diet (according to the authors: ♂: 0, 50, 100, 210 mg/kg body weight and day; ♀: 0, 60, 125, 250 mg/kg body weight and day)	**60 mg/kg body weight**: ♀: **NOAEL**; **100/125 mg/kg body weight**: ♀: about 8% decrease in body weights (not specified whether statistically significant), ♂: **NOAEL**; **210/250 mg/kg body weight**: ♀: 10–16% decrease in body weights, kidneys: ♂: dose-dependent severity of age-related nephropathy ↓, spleen: haemosiderin accumulation in macrophages and extramedullary haematopoiesis difficult to evaluate histopathologically because spleen filled with mononuclear leukaemia cells in all ♂ and many ♀ untreated and treated animals; tumour findings in ♀: see Section 5.7	NTP 1994; Williams et al. 2001
mouse, CD1, 5 ♂, 5 ♀	**14 days**, 0, 2500, 5000, 10 000, 20 000, 30 000 mg/kg diet (according to the authors: ♂: 0, 410, 830, 1500, 3700, 5770 mg/kg body weight and day; ♀: 0, 520, 1130, 2060, 3990, 7060 mg/kg body weight and day)	**1500/2060 mg/kg body weight and above**: body weight gains ↓; range-finding study for 33-week fertility study	NTP 1990

Tab. 1 *(continued)*

Species, strain, number per group	Exposure	Findings	References
mouse, B6C3F1, 5 ♂, 5 ♀	**14 days**, 0, 2500, 5000, 10 000, 20 000, 40 000 mg/kg diet (0, 500, 1000, 2000, 4000, 8000 mg/kg body weight and day[b])	**1000 mg/kg body weight and above**: body weights ♀: ↓; **2000 mg/kg body weight and above**: body weights ♂: ↓, relative organ weights ♀: liver ↑ and thymus ↓; **4000 mg/kg body weight and above**: mortality: 3 ♂ and 2 ♀, lethargy and ataxia, body weights ♂: ↓, relative organ weights ♂: liver ↑ and thymus ↓, testes: degeneration, bone marrow: ♀: haemorrhages and atrophy	NTP 1994
mouse, B6C3F1, 10 ♂, 10 ♀	**13 weeks**, 0, 1250, 2500, 5000, 10 000, 20 000 mg/kg diet (according to the authors: ♂: 0, 170, 330, 670, 1900, 4000 mg/kg body weight and day; ♀: 0, 240, 460, 970, 2500, 4900 mg/kg body weight and day)	**170 mg/kg body weight and above**: ♂: 7% decrease in body weights; **460 mg/kg body weight**: ♀: **NOAEL**; **970 mg/kg body weight and above**: ♀: 6% decrease in body weights; no unusual findings in organ weights or in the histopathological examinations	NTP 1994
mouse, B6C3F1, 10 ♂, 10 ♀	**15 months**, 0, 1250, 2500, 5000 mg/kg diet (according to the authors: ♂: 0, 150, 300, 675 mg/kg body weight and day; ♀: 0, 170, 365, 905 mg/kg body weight and day)	**up to 675/905 mg/kg body weight**: no unusual findings in the haematological or histopathological examinations	NTP 1994
mouse, B6C3F1, 50 ♂, 50 ♀	**2 years**, 0, 1250, 2500, 5000 mg/kg diet (according to the authors: ♂: 0, 150, 300, 675 mg/kg body weight and day; ♀: 0, 170, 365, 905 mg/kg body weight and day)	**300/365 mg/kg body weight**: **NOAEL**; **675/905 mg/kg body weight**: ♀: 19% decrease in body weights; ♂: 10% decrease; no unusual findings in the histopathological examinations	NTP 1994

[a] conversion factor: 0.12 according to EFSA (2012)
[b] conversion factor 0.2 according to EFSA (2012)
MetHb: methaemoglobin

Summary: On the basis of the 15-month study and 2-year study with 4-nitrobenzoic acid in rats, a NOAEL of 60 mg/kg body weight and day was derived for reduced body weight gains in the female animals and a NOAEL of 100 mg/kg body weight and day was derived for increased methaemoglobin levels and splenic changes in the male animals. However, 4-nitrobenzoic acid doses of 60 mg/kg body weight and day and above significantly increased the incidences of adenomas in the clitoral gland of female rats (see Section 5.7).

In the 15-month and 2-year studies with mice, the NOAEL for reduced body weight gains induced by 4-nitrobenzoic acid was 365 mg/kg body weight and day.

5.2.3 Dermal application

Aliquots of 0.5 ml of a 10% solution of 4-nitrobenzoic acid in sesame oil were rubbed into the shaved flank skin of 5 rabbits 21 times within 28 days. Blood examinations and urinalysis were carried out at the beginning and at the end of the treatment period. Histopathological examinations of the internal organs were carried out after an observation period of 3 days. No unusual findings were obtained for the skin, the blood count, in the urinalysis or in the examination of the internal organs (Clariant GmbH 1955). However, this study has not been included in the evaluation of the dermal effects because 4-nitrobenzoic acid was applied only as a 10% solution.

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

Aliquots of 0.02 ml of undiluted 4-nitrobenzoic acid or solutions of 4-nitrobenzoic acid diluted up to 1:1000 were injected intracutaneously into the shaved skin of groups of 3 rabbits. Erythema was observed at all concentrations including the highest dilution and persisted for 2 days (Clariant GmbH 1955).

Amounts of 500 mg 4-nitrobenzoic acid (mixed to a paste with 0.35 ml polyethylene glycol 400) were applied semi-occlusively to the shaved skin of 3 New Zealand White rabbits for 4 hours. The skin was examined 24, 48 and 72 hours after removal of the substance. Mean values of 0 for erythema and eschar formation as well as for oedema were calculated for all animals on the basis of the individual findings obtained after 24, 48 and 72 hours (Clariant GmbH 1989 b).

5.3.2 Eyes

In rabbits, the instillation of 0.5 ml of a 10% 4-nitrobenzoic acid solution in sesame oil in the eyes induced mild redness within the following 24 hours (Clariant GmbH 1955).

Amounts of 100 mg 4-nitrobenzoic acid were applied to the conjunctival sac of the left eye of 3 New Zealand White rabbits for 24 hours. Marked hyperaemia up to severe diffuse redness and very mild to marked swelling were observed on the conjunctivae within 1 hour and up to 72 hours afterwards. The iris of 2 animals was reddened up to 24 hours following instillation and in the 3rd animal up to 72 hours after treatment. Diffuse corneal opacity was observed in 1 animal 24 to 72 hours following instillation. The signs of irritation were accompanied by a clear colourless or white mucous discharge. All signs of irritation had disappeared 7 days after treatment. The mean values calculated from the individual findings after 24, 48 and 72 hours were 0.3 for corneal opacity (individual values: 0, 0 and 1), 0.4 for iritis (individual values: 0.3, 0.3 and 0.7), 2.4 for conjunctival redness (individual values: 2.7, 2.3 and 2.3) and 1.4 for conjunctivitis (1.7, 1.0 and 1.7) (Clariant GmbH 1989 a). On the basis of the findings of severe conjunctival redness, 4-nitrobenzoic acid has been classified as severely irritating to the eyes according to the GHS criteria (ECHA 2016).

5.4 Allergenic effects

There are no data available.

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

The results from the available fertility studies are shown in detail in Table 2.

In rats fed 4-nitrobenzoic acid with the diet for 13 weeks, congestion was observed in the seminiferous tubules at a dose of 660 mg/kg body weight and day (NTP 1994).

In a feeding study in CD1 mice with exposure to 4-nitrobenzoic acid for 33 weeks before, during and after continuous mating of the F0 generation and exposure of the F1 generation, the period until pregnancy occurred was prolonged at dose levels of 1100 mg/kg body weight and day and above. The number of offspring per litter or the number of litters per pair was reduced. When female mice treated with a 4-nitrobenzoic acid dose of 1100 mg/kg body weight and day were cross-mated with untreated male mice, the number of live F2 offspring was reduced by 40%. 4-Nitrobenzoic acid prolonged the oestrus cycle and damaged the spermatogonia in the F1 generation (NTP 1990, 1994).

Tab. 2 Effects of 4-nitrobenzoic acid on fertility

Species, strain, number per group	Exposure	Findings	References
rat, F344, 5 ♂, 5 ♀	**14 days**, 0, 2500, 5000, 10 000, 20 000, 40 000 mg/kg diet (about 0, 300, 600, 1200, 2400, 4800 mg/kg body weight and day)	**2400 mg/kg body weight and day and above**: 32–40% decrease in body weights, testes and ovaries: atrophy; for further findings see Table 1	NTP 1994
rat, F344, 10 ♂, 10 ♀	**13 weeks**, 0, 630, 1250, 2500, 5000, 10 000 mg/kg diet (according to the authors: ♂: 0, 40, 70, 160, 310, 660 mg/kg body weight and day; ♀: 0, 40, 80, 170, 340, 680 mg/kg body weight and day)	**70 mg/kg body weight**: **NOAEL**; **160 mg/kg body weight and above**: ♂: body weights ↓; **340 mg/kg body weight and above**: ♀: body weights ↓; **660 mg/kg body weight**: testes: congestion in the seminiferous tubules; for further findings see Table 1	NTP 1994
rat, F344, 10 ♂, 10 ♀	**15 months, 2 years**, 0, 1250, 2500, 5000 mg/kg diet (according to the authors: ♂: 0, 50, 100, 210 mg/kg body weight and day; ♀: 0, 60, 125, 250 mg/kg body weight and day)	**60 mg/kg body weight and above**: ♀: in 2nd year: body weight gains ↓; **210/250 mg/kg body weight**: no unusual findings in the histopathological examinations of the gonads; for further findings see Table 1	NTP 1994
mouse, B6C3F1, 5 ♂, 5 ♀	**14 days**, 0, 2500, 5000, 10 000, 20 000, 40 000 mg/kg diet (0, 500, 1000, 2000, 4000, 8000 mg/kg body weight and day)	**1000 mg/kg body weight and above**: ♀: body weights ↓; **2000 mg/kg body weight and above**: : ♂: body weights ↓; **4000 mg/kg body weight and above**: mortality: 3 ♂ and 2 ♀, testes: degeneration; for further findings see Table 1	NTP 1994
mouse, B6C3F1, 10 ♂, 10 ♀	**13 weeks**, 0, 1250, 2500, 5000, 10 000, 20 000 mg/kg diet (according to the authors: ♂: 0, 170, 330, 670, 1900, 4000 mg/kg body weight and day; ♀: 0, 240, 460, 970, 2500, 4900 mg/kg body weight)	**170/240 mg/kg body weight and above**: ♂: body weight gains ↓; **330/460 mg/kg body weight and above**: ♀: body weight gains ↓; no unusual findings in the weights or histopathological examination of the gonads; for further findings see Table 1	NTP 1994
mouse, CD1, 10 ♂, 10 ♀	**13 weeks**, 0, 1250, 2500, 5000, 10 000, 20 000 mg/kg diet (according to the authors: ♂: 0, 170, 330, 670, 1900, 4000 mg/kg body weight and day ♀: 0, 240, 460, 970, 2500, 4900 mg/kg body weight)	**1900 mg/kg body weight and above**: ♂: body weight gains ↓; **4000 mg/kg body weight**: weights of testis and epididymis ↓, ♀: body weight gains ↓, oestrus cycle prolonged; no unusual findings in the sperm count	Chapin et al. 1987; NTP 1994

Tab. 2 *(continued)*

Species, strain, number per group	Exposure	Findings	References
mouse, CD1, 20 ♂, 20 ♀	**33 weeks**, 0%, 0.35%, 0.75%, 1.5% in the diet (according to the authors: 0, 500, 1100, 2400 mg/kg body weight and day); F0: exposure before and during continuous mating, continuous exposure of all 5 litters per parental animal (duration: 18 weeks); F1 left with dams up to weaning, later same treatment as the corresponding dams, examination of F1 from litters 1–4 on postnatal day 74; cross-mating: treated (only F1 of 0, 1100 mg/kg body weight groups) × untreated F1 from litter 5, exposure before, during and after cross-mating (duration: 15 weeks); examination of F2 on postnatal day 1	**controls**: F0 parental animals: litters/pair = 4.9; **500 mg/kg body weight**: **NOAEL**; **1100 mg/kg body weight and above**: F0 parental animals: ♀ after littering: 7–9% decrease in body weights, litters/pair = 4.5 ⟶ in some cases no litters 4 and 5, period until littering prolonged mainly for litters 3 and 4; F1: duration of development in the uterus ↑, birth: 22% decrease in length, 17% decrease in body weights, 10% decrease in body weight gains; **cross-mating** of treated F1 ♀ × untreated F1 ♂: treated F1 ♀: 9% decrease in body weights, 9% decrease in relative kidney weights, oestrus cycle length: 5.4 days (controls: 4.9 days), substance-induced hepatitis; F2: number of live offspring/litter ↓, 40% decrease in live offspring, 25% decrease in body weights; **cross-mating** of treated F1 ♂ × untreated F1 ♀: no unusual findings, exception: number of spermatogonia with changes ↑; **2400 mg/kg body weight**: F0 parental animals: ♀ after littering: 10–15% decrease in body weights, litters/pair = 3.5, period until littering after litter 1 ↑; F1: number of offspring/litter ↓, birth: 38% decrease in length, 18% decrease in body weights, 30% decrease in body weights	Chapin et al. 1997 a, b; NTP 1990
mouse, B6C3F1, 10 ♂, 10 ♀	**15 months, 2 years**, 0, 1250, 2500, 5000 mg/kg diet (according to the authors: ♂: 0, 150, 300, 675 mg/kg body weight and day; ♀: 0, 170, 365, 905 mg/kg body weight and day)	**675/905 mg/kg body weight**: no unusual findings in weights or histopathological examination; for further findings see Table 1	NTP 1994

Summary: A NOAEL of 310 mg/kg body weight and day was derived from a 13-week study in F344 rats based on the occurrence of testicular lesions. The LOAEL was 660 mg/kg body weight and day (NTP 1994). After exposure of CD1 mice to 4-nitrobenzoic acid before, during and after continuous mating of the F0 generation and exposure of the F1 generation, the period until pregnancy occurred was prolonged and the numbers of offspring per litter or litters per pair were reduced at the dose level of about 1100 mg/kg body weight and day and above. The NOAEL was 500 mg/kg body weight and day (NTP 1990, 1994).

5.5.2 Developmental toxicity

There are no studies available that allow the evaluation of developmental toxicity. In the study in mice described above (see also Table 2) with exposure to 4-nitrobenzoic acid before, during and after continuous mating and the subsequent cross-mating of the F1 animals, delays in the development of the F1 foetuses in the uterus and reduced numbers of offspring per litter in the F1 generation were found at dose levels of about 1100 mg/kg body weight and day and above. In the F1 and F2 offspring, body length and body weights were reduced after birth and body weight gains were later delayed. In the F2 generation, 40% of the offspring died (NTP 1990, 1994).

Summary: A NOAEL of about 500 mg/kg body weight and day was derived from a generation study in mice for the foetotoxicity and maternal toxicity of 4-nitrobenzoic acid. No studies are available for teratogenicity.

5.6 Genotoxicity

5.6.1 In vitro

In the available Salmonella mutagenicity tests (see Table 3), 4-nitrobenzoic acid increased the incidences of frame-shift mutations in a number of studies with Salmonella typhimurium TA98, TA1538 and TA1537 (Chiu et al. 1978; Clariant GmbH 1987; Shimizu and Yano 1986), increased the incidences of base-pair substitutions in Salmonella typhimurium TA100 (Clariant GmbH 1987; Kawai et al. 1987; Shimizu and Yano 1986; Sundvall et al. 1984; Zeiger et al. 1987) and did not increase the mutation frequency in Salmonella typhimurium TA98, TA100, TA1535, TA1537 or TA1538 (Chiu et al. 1978; Dellarco and Prival 1989; Goldman et al. 1977; Kawai et al. 1987; Shimizu and Yano 1986; Sundvall et al. 1984; Suzuki et al. 1983; Wheeler et al. 1975 a). Overall, the mutagenic effects were weak.

Tab. 3 Studies that investigated the genotoxicity of 4-nitrobenzoic acid in vitro

Strain	Concentration [a]	Effective concentration [a]	Cytotoxicity [a]	Result		References
				–m.a.	+m.a.	
frame-shift mutations in Salmonella typhimurium						
TA98	0.1–10 µmol/plate	0.1 µM	1 µM	+ (up to 3.5-fold)	not examined	Chiu et al. 1978
TA98	0.3–10 µmol/plate	–	–	–	–	Dellarco and Prival 1989
TA98	no data	no data	no data	–	–	Sundvall et al. 1984
TA98	100	–	no data	–	–	Suzuki et al. 1983
TA98	250–5000	–	–	–	–	Kawai et al. 1987
TA98	10–5000	100	5000	+ (dose-dependent: 2.7–4.5-fold)	not examined	Shimizu and Yano 1986
TA98	4–5000	no data		+	+	Clariant GmbH 1987
TA1537	10–5000	–	5000	–	not examined	Shimizu and Yano 1986
TA1537	4–5000	no data		+	+	Clariant GmbH 1987
TA1538	10–5000	500	5000	+ (dose-dependent up to 2.9-fold)	not examined	Shimizu and Yano 1986
TA1538	4–5000	no data	500	+	+	Clariant GmbH 1987
TA1538	50	–	no data	–	–	Goldman et al. 1977; Wheeler et al. 1975 a
base-pair substitutions in Salmonella typhimurium						
TA100	0.3–10 µmol/plate	3 µM	–	–	– (standard test) + pre-incubation with FMN	Dellarco and Prival 1989
TA100	100–200	100	no data	+ (1.35 revertants/µg)	–	Sundvall et al. 1984
TA100	0.1–1 µmol/plate	–	–	–	not examined	Chiu et al. 1978
TA100	10–5000	100	–	+ (dose-dependent up to 3-fold)	+ (dose-dependent up to 4-fold)	Zeiger et al. 1987
TA100	100	–	no data	–	–	Suzuki et al. 1983

Tab. 3 *(continued)*

Strain	Concentration [a)]	Effective concentration [a)]	Cytotoxicity [a)]	Result		References
				–m. a.	+m. a.	
TA100	10–5000	500	5000	+ (up to 4.4-fold)	not examined	Shimizu and Yano 1986
TA100	250–5000	500	–	+ (0.87 revertants/µg)	+ (0.59 revertants/µg)	Kawai et al. 1987
TA 100	4–5000	no data	500	+	+	Clariant GmbH 1987
TA1535	10–5000	–	5000	–	not examined	Shimizu and Yano 1986
TA1535	4–5000	no data	500	+	+	Clariant GmbH 1987
TA1535	no data	no data	no data	+ (0.26 revertants/µg)	not examined	Sundvall et al. 1984
mutations in Escherichia coli						
Escherichia coli WP2uvrA	4–5000	no data	500	+	+	Clariant GmbH 1987
SCE in CHO cells						
	58–1750 µg/ml	498 µg/ml	1510 µg/ml	+	–	NTP 1994
DNA repair synthesis (UDS) in rat hepatocytes						
	1 µmol/ml		no data	–	not examined	Probst et al. 1981
CA in CHO cells						
	440–1750 µg/ml	875 µg/ml	–	+	–	NTP 1994
mutations (HPRT) in V79 cells						
	500–2000 µg/ml	–	2000 µg/ml	–	–	Clariant GmbH 1988

[a)] unless otherwise specified, values expressed as µg/plate
FMN: flavin mononucleotide for nitroreduction; m. a.: metabolic activation

In a UDS test with primary rat hepatocytes, DNA repair synthesis was not induced at a 4-nitrobenzoic acid concentration of 1000 nmol/ml (Probst et al. 1981). In CHO cells, 4-nitrobenzoic acid increased the incidences of sister chromatid exchange without metabolic activation at concentrations of 498 µg/ml and above and of chromosomal aberrations at concentrations of 875 µg/ml and above (NTP 1994). In V79 cells, 4-nitrobenzoic acid was not mutagenic up to a concentration of 2000 µg/ml (Clariant GmbH 1988).

5.6.2 In vivo

A study carried out according to OECD Test Guideline 475 with Chinese hamsters did not lead to increased incidences of chromosomal aberrations in bone marrow cells after a single oral dose of 4-nitrobenzoic acid (dissolved in sesame oil) at the level of 5000 mg/kg body weight (Clariant GmbH 1990).

In 1990, a study was carried out in NMRI mice according to OECD Test Guideline 474. In this study, 4-nitrobenzoic acid (dissolved in sesame oil) given as a single dose of 1500 mg/kg body weight by gavage did not induce increased incidences of micronuclei in peripheral immature erythrocytes (ECB 2000).

When female and male B6C3F1 mice were fed a diet containing 4-nitrobenzoic acid for 13 weeks, no micronuclei were detected in the peripheral erythrocytes of the blood at doses up to about 680 mg/kg body weight and day (NTP 1994).

5.6.3 Summary

4-Nitrobenzoic acid caused frame-shift mutations and base-pair substitutions in Salmonella typhimurium, and sister chromatid exchanges and chromosomal aberrations in CHO cells, but did not induce DNA repair synthesis in the UDS test with primary rat hepatocytes or mutations in V79 cells. In vivo, the incidence of chromosomal aberrations was not increased in the bone marrow cells of Chinese hamsters, and the formation of micronuclei in the peripheral erythrocytes of the blood was not increased in mice after single or repeated exposures to 4-nitrobenzoic acid for up to 13 weeks.

5.7 Carcinogenicity

In 2-year carcinogenicity studies, groups of 50 male and 50 female F344 rats were given 4-nitrobenzoic doses of 0, 1250, 2500 or 5000 mg/kg diet (according to the authors: ♂: 0, 50, 100 or 210 mg/kg body weight and day; ♀: 0, 60, 125 or 250 mg/kg body weight and day) and groups of 50 male and 50 female B6C3F1 mice were given 4-nitrobenzoic acid doses of 0, 1250, 2500 or 5000 mg/kg diet (according to the authors: ♂: 0, 150, 300 or 675 mg/kg body weight and day; ♀: 0, 170, 365 or 905 mg/kg body weight and day). A significant increase in the incidence of clitoral gland adenomas was observed only in female F344 rats at 4-nitrobenzoic acid doses of 60 mg/kg body weight and day and above (see Table 4). When the incidences of adenomas and carcinomas were combined, the increases in all treatment groups were statistically significant, but not dose-dependent. The incidences were above those of the mean values and upper range of the historical controls of the contract laboratory. The incidences of hyperplasia, which is considered to be a tumour precursor, were not increased and the mean time to the diagnosis of the tumours was not reduced. Therefore, the result was interpreted merely as "some evidence of carcinogenicity" (NTP 1994).

Particularly in male F344 rats the spleens were packed with mononuclear leukaemia cells. The incidences were 29/50 (control group), 35/50 (60 mg/kg body weight), 26/50 (125 mg/kg body weight) and 2/50 (250 mg/kg body weight), and in the females 17/50 (control group), 11/50 (60 mg/kg body weight), 3/50 (125 mg/kg body weight) and 0/50 (250 mg/kg body weight) (NTP 1994).

Tab. 4 Study of the carcinogenicity of 4-nitrobenzoic acid in F344 rats

Author:		NTP 1994			
Substance:		**4-nitrobenzoic acid** (purity: > 99%)			
Species:		**rat**, F344/N, 50 ♂ and 50 ♀			
Administration route:		diet			
Dose:		0, 1250, 2500, 5000 mg 4-nitrobenzoic acid/kg diet (♂: 0, 50, 100, 210 mg/kg body weight and day, ♀: 0, 60, 125, 250 mg /kg body weight and day)			
Duration:		104 weeks			
Toxicity:		**125 mg/kg body weight and above**: body weight gains ♀: 8% ↓;			
		mg/kg body weight and day			
		0	60	125	250
surviving animals	♀	27/50 (54%)	23/50 (46%)	21/50 (42%)	21/50 (42%)
tumours and pre-neoplasms clitoral gland:					
hyperplasias	♀	10/50	6/49	6/49	7/50
adenomas	♀	4/50 (8%)	12/49 (24%)*	10/49 (20%)	12/50 (24%)*
adjusted for surviving animals		11.9%	42.5%	33.7%	42.1%
life table test		p = 0.034	p = 0.013	p = 0.030	p = 0.013
logistic regression test		p = 0.046	p = 0.013	p = 0.050	p = 0.023

Tab. 4 *(continued)*

		mg/kg body weight and day			
		0	60	125	250
carcinomas	♀	1/50 (2%)	2/49 (4%)	5/49 (10%)	4/50 (8%)
adjusted		3.7%	6.0%	19.3%	11.7%
life table test		p = 0.085	p = 0.460	p = 0.056	p = 0.139
logistic regression test		p = 0.117	p = 0.459	p = 0.084	p = 0.224
adenomas or carcinomas (combined)	♀	4/50 (8%)	14/49 (29%)**	15/49 (31%)**	15/50 (30%)**
adjusted		11.9%	45.9%	48.9%	47.7%
life table test		p = 0.008	p = 0.005	p = 0.001	p = 0.002
logistic regression test		p = 0.011	p = 0.004	p = 0.003	p = 0.004
mean time to diagnosis [days]		699	712	672	683
historical controls of the contract laboratory up to 20.08.1992 (NTP 1994**)**					
adenomas	♀	27/367 (7.4%), 0–19%			
carcinomas	♀	21/367 (5.7%), 0–15%			
adenomas or carcinomas (combined)	♀	47/367 (12.8%), 2–21%			

*p ≤ 0.05; **p ≤ 0.01

The clitoral and preputial glands are located in the genital areas of female and male rats and mice and produce pheromones.

Spontaneous neoplasms of the clitoral gland occur mainly in F344 rats. They are relatively rare in Sprague Dawley or Wistar rats and in B6C3F1 or CD1 mice (Gad 2008).

An NTP study of 4-nitrotoluene in F344 rats included a table that showed the incidences of clitoral gland neoplasms in control animals (NTP 2002). The data are listed in Table 5. The animals of the carcinogenicity study with 4-nitrobenzoic acid were fed NIH-07 diet. The study was carried out at the Southern Research Institute.

Tab. 5 Incidences of spontaneous neoplasms in the clitoral gland of female F344 control rats in NTP studies after administration of NIH-07 diet (NTP 2002)

	Adenomas	Carcinomas	Adenomas or carcinomas
data of the Southern Research Institute (up to 21.12.1999)			
benzyl acetate (started in 1988)	0/50	1/50	1/50
2,2-bis(bromomethyl)-1,3-propanediol	4/48	1/48	5/48
butyl benzyl phthalate	3/50	4/50	7/50
D&C Yellow No 11 (started in 1990)	11/49	6/49	17/49
emodin	10/49	2/49	12/49
2-nitroanisole	3/45	4/45	7/45
4-nitrobenzoic acid (started in 1988)	4/50	1/50	4/50

Tab. 5 *(continued)*

	Adenomas	Carcinomas	Adenomas or carcinomas
overall incidence in all feeding studies given NIH-07 diet			
total (%)	89/968 (9.2%)	30/968 (3.1%)	118/968 (12.2%)
mean ± standard deviation	9.2±6.0%	3.1±3.2%	12.2±7.7%
range	0–22%	0–12%	2–35%

The historical control data of the contract laboratory for the sum of adenomas and carcinomas of the clitoral gland covered an incidence range of 2% to 35%. The lowest spontaneous incidence (2%) was observed in a study with benzyl acetate, which began in the same year (1988) as the study with 4-nitrobenzoic acid. The highest spontaneous incidence was obtained in a study with D&C Yellow No 11, which was started 2 years later (1990) than the study with 4-nitrobenzoic acid. Thus, within a period of only 2 years, the spontaneous incidences determined by the same laboratory varied between 2% and 35%. The mean values of the spontaneous clitoral gland tumours in all feeding studies carried out by the NTP up to 1999 (Table 5) approximately correspond to the mean values of the historical control incidences of the contract laboratory up to 1994 (Table 4).

However, a number of publications demonstrated that clitoral gland tumours are of relevance (McConnell et al. 1986; Reznik and Ward 1981). Spontaneous clitoral gland adenomas are rare except in F344 rats. Like adenocarcinomas, adenomas originate from acinar cells (Rudmann et al. 2012). Some authors consider them to be precursors of carcinomas (Reznik and Ward 1981). Substances that induce these tumours also cause tumours in other tissues, for example on the skin and skin appendages, such as the sebaceous glands (Reznik and Ward 1981).

Summary: Significant, but not dose-dependent increases in the incidences of clitoral gland adenomas were observed only in female F344 rats at 4-nitrobenzoic acid doses of 60 mg/kg body weight and day and above. In all treatment groups the combined incidences of adenomas and carcinomas were higher than the mean value of the historical controls of the contract laboratory. The incidences of the clitoral gland tumours that spontaneously occur in F344 rats are highly variable and range from 2% to 35%. Clitoral gland tumours are rare in other rat strains.

6 Manifesto (MAK value/classification)

The critical effects of 4-nitrobenzoic acid are the local effects on the olfactory epithelium. Systemic effects are methaemoglobin formation, effects on the haematopoietic system, splenic changes and reduced body weight gains. In addition, significant increases in the incidences of clitoral gland adenomas were observed in female F344 rats.

Carcinogenicity. 4-Nitrobenzoic acid increased the incidence of clitoral gland adenomas in female F344 rats, reaching statistical significance at dose levels of 60 mg/kg body weight and day and above. However, this effect was not dose-dependent, and the increases in incidences of carcinomas were not statistically significant. When the incidences of adenomas and carcinomas were combined, the increase was statistically significant in all treatment groups and was higher than the incidences in the historical controls of the contract laboratory. However, the tumours were considered to be only "some evidence of carcinogenic activity" because the increases in tumour incidences were not dose-dependent, the mean latency period up to diagnosis was not reduced and the incidences of hyperplasia were not increased. Similar findings were reported by an NTP study with 4-nitrotoluene, a substance that is classified in Carcinogen Category 3B. 4-Nitrotoluene induced this type of tumour in female F344 rats given the same concentrations with the diet (statistically significant increase in the incidences of adenomas; no increase in the incidences of carcinomas). However, this substance additionally caused bronchoalveolar lung tumours (statistically significant increase in the incidences of adenomas; no increase in the incidences of carcinomas) in male mice and subcutaneous tumours (statistically significant increase in the incidences of fibromas; no increase in the incidences of fibrosarcomas) in male rats. In F344 rats, 68% of 4-nitrotoluene is metabolized to 4-nitrobenzoic acid and its metabolites (see Hartwig and MAK Commission 2016). 4-Nitrotoluene may induce additional types of tumours because the substance first has to be metabolized to excretable products and therefore remains in the body longer than 4-nitrobenzoic acid, which

is eliminated mainly unmetabolized and thus rapidly. However, 4-nitrotoluene likewise induced only those types of tumours that have a high incidence of spontaneous tumours in the respective species.

As regards the clitoral gland neoplasms induced by 4-nitrobenzoic acid, the incidences of adenomas or the sum of adenomas and carcinomas were above the range of the respective historical controls of the contract laboratory. 4-Nitrobenzoic acid remains a suspected carcinogen because 4-nitrotoluene likewise caused clitoral gland tumours in addition to other types of spontaneous tumours, the activity of 4-nitrobenzoic acid is similar although weaker compared with that of 4-nitrotoluene, and mutagenicity was detected in Salmonella typhimurium. In analogy to 4-nitrotoluene, 4-nitrobenzoic acid has been classified in Carcinogen Category 3B.

Germ cell mutagenicity. 4-Nitrobenzoic acid is mutagenic in bacteria. It caused frame-shift mutations and base-pair substitutions in Salmonella typhimurium and sister chromatid exchanges and chromosomal aberrations in CHO cells. DNA repair synthesis was not found to occur in primary rat hepatocytes. In vivo, the incidences of chromosomal aberrations were not increased in the bone marrow cells of mice given a single oral dose of 1500 mg/kg body weight, and the formation of micronuclei was not increased in the peripheral erythrocytes of the blood of Chinese hamsters following the administration of 5000 mg/kg body weight. In mice given an oral dose of 4-nitrobenzoic acid of 680 mg/kg body weight and day for 13 weeks, the formation of micronuclei in the peripheral erythrocytes of the blood was not increased. Therefore, classification in one of the germ cell mutagen categories is not justified on the basis of the available data.

MAK value. There are no data for human exposure available that are suitable for the derivation of a MAK value. On the basis of the findings of a 15-month study and a 2-year study in rats, a NOAEL of 60 mg/kg body weight and day was derived for reduced body weight gains in female animals.

The following toxicokinetic data are used to extrapolate the NOAEL of 60 mg/kg body weight and day to a concentration in workplace air: the corresponding species-specific correction value for the rat determined on the basis of the toxicokinetic data (1:4), the assumed oral absorption (100%), the body weight (70 kg) and the respiratory volume (10 m^3) of the person, the assumed 100% absorption by inhalation, and 5 days per week exposure at the workplace (7:5). A 4-nitrobenzoic acid concentration of 147 mg/m^3 was calculated from this. According to the procedures developed by the Commission, the MAK value derived on the basis of systemic effects, considering the extrapolation of the NOAEL from animal studies to humans (1:2) and the preferred value approach, would be 50 mg/m^3.

In the 14-day study, a NOAEC (no observed adverse effect concentration) of 20 mg/m^3 was determined for local effects on the olfactory epithelium of rats. At this concentration, the aerodynamic diameter of 75% of the particles was < 10 µm. No conclusions can be drawn about an intensification of the effects over time because a long-term inhalation study has yet to be carried out. After taking into consideration a possible intensification of effects after chronic exposure (1:6) and extrapolating the effects on the olfactory epithelium to humans (1:2), a concentration of 1.7 mg/m^3 I (inhalable fraction) was derived for workplace air according to the method of Brüning et al. (2014). By applying the procedures of the Commission, a MAK value of 1 mg/m^3 I is derived from this for the local effects on the olfactory epithelium.

At a MAK value for 4-nitrobenzoic acid of 1 mg/m^3, a 150-fold margin lies between this value and the dose level of 60 mg/kg body weight and day (which corresponds to a 4-nitrobenzoic acid concentration in air of 147 mg/m^3) at which clitoral gland adenomas were observed in female F344 rats. This margin is regarded as sufficiently large in view of the evaluation of the carcinogenicity of the substance.

Peak limitation. 4-Nitrobenzoic acid has been classified in Peak Limitation Category I because the MAK value for this substance was derived on the basis of the local effects on the olfactory epithelium. An excursion factor of 2 has been established for 4-nitrobenzoic acid because there is a large margin between the NOAEC and the LOAEC (lowest observed adverse effect concentration) of 150 mg/m^3.

Prenatal toxicity. A feeding study in mice with exposure of the F0 generation for 33 weeks before, during and after continuous mating in addition to exposure of the F1 generation determined a NOAEL of 500 mg/kg body weight and day for 4-nitrobenzoic acid based on maternal toxicity and foetotoxicity. After toxicokinetic extrapolation (see above) and considering the species-specific correction value for the mouse (1:7), this value corresponds to a concentration of

700 mg/m^3 in air and is far higher than the MAK value. No studies of teratogenicity are available. As there are not sufficient data for a final evaluation, 4-nitrobenzoic acid has been classified in Pregnancy Risk Group D.

Absorption through the skin. Studies of dermal absorption are not available. On the basis of model calculations (Section 3.1) and assuming a surface area of 2000 cm^2 of skin exposed to a saturated aqueous solution for 1 hour, the maximum amount absorbed through the skin is estimated to be 14 mg in humans. The following toxicokinetic data are used to extrapolate the chronic oral NOAEL of 60 mg/kg body weight derived from rats: the corresponding species-specific correction value for the rat determined on the basis of the toxicokinetic data (1:4), the assumed oral absorption (100%), the body weight (70 kg), 5 days per week exposure at the workplace (7:5) and the extrapolation of the NOAEL from animal studies to humans (1:2). This results in a systemically tolerable amount of 4-nitrobenzoic acid of 735 mg. Absorption through the skin accounts for much less than 25% of the systemically tolerable amount, and the substance has not been designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. As clinical findings or results from experimental animal studies are not available for sensitization, 4-nitrobenzoic acid has not been designated with "Sh" or "Sa" (for substances which cause sensitization of the skin or 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

Adamson RH, Dixon RL, Francis FL, Rall DP (1965) Comparative biochemistry of drug metabolism by azo and nitro reductase. Proc Natl Acad Sci U S A 54(5): 1386–1391. https://doi.org/10.1073/pnas.54.5.1386

Ahlborg G, Bergström B, Hogstedt C, Einistö P, Sorsa M (1985) Urinary screening for potentially genotoxic exposures in a chemical industry. Br J Ind Med 42(10): 691–699. https://doi.org/10.1136/oem.42.10.691

Brüning T, Bartsch R, Bolt HM, Desel H, Drexler H, Gundert-Remy U, Hartwig A, Jäckh R, Leibold E, Pallapies D, Rettenmeier AW, Schlüter G, Stropp G, Sucker K, Triebig G, Westphal G, van Thriel C (2014) Sensory irritation as a basis for setting occupational exposure limits. Arch Toxicol 88(10): 1855–1879. https://doi.org/10.1007/s00204-014-1346-z

Caujolle F, Caujolle D, Moisand C (1966) Toxicités comparées des acides nitrobenzoïques pour la souris et le rat [Comparative toxicity of nitrobenzoic acids for mice and rats]. C R Seances Soc Biol Fil 160(5): 1097–1100

Chapin RE, Gulati DK, Russell SL (1987) Para-nitrobenzoic acid: sperm morphology vaginal cytology evaluations in rodents. N01-ES-3–5026. Research Triangle Park, NC: National Toxicology Program

Chapin R, Gulati D, Hope E, Mounce R, Russell S, Poonacha K (1997 a) Reproductive toxicology. p-Nitrobenzoic acid. Environ Health Perspect 105(Suppl 1): 327–328

Chapin RE, Sloane RA, Haseman JK (1997 b) The relationships among reproductive endpoints in Swiss mice, using the reproductive assessment by Continuous Breeding database. Fundam Appl Toxicol 38(2): 129–142. https://doi.org/10.1006/faat.1997.2341

Chiu CW, Lee LH, Wang CY, Bryan GT (1978) Mutagenicity of some commercially available nitro compounds for Salmonella typhimurium. Mutat Res 58(1): 11–22. https://doi.org/10.1016/0165-1218(78)90090-3

Clariant GmbH (1955) p-Nitrobenzoesäure. Report No. 55.0059, 28 Oct 1955, Frankfurt: Gewerbetoxikol. Lab. Hoechst AG, unpublished

Clariant GmbH (1987) p-Nitrobenzoesäure. Study of the mutagenic potential in strains of Salmonella typhimurium (Ames Test) and Escherichia coli. Report No. 87.1620, 07 Dec 1987, Frankfurt: Pharma Forschung Toxikologie und Pathologie, Hoechst Aktiengesellschaft, unpublished

Clariant GmbH (1988) p-Nitrobenzoesäure. Detection of gene mutations in somatic cells in culture, HGPRT-test with V79 cells. Report No. 88.1222, 18 Aug 1988, Frankfurt: Pharma Forschung Toxikologie und Pathologie, Hoechst Aktiengesellschaft, unpublished

Clariant GmbH (1989 a) p-Nitrobenzoesäure. Prüfung auf Augenreizung am Kaninchen. Report No. 89.1329, 19 Sep 1989, Frankfurt: Pharma Forschung Toxikologie und Pathologie, Hoechst Aktiengesellschaft, unpublished

Clariant GmbH (1989 b) p-Nitrobenzoesäure. Prüfung auf Hautreizung am Kaninchen. Report No. 89.1217, 25 Aug 1989, Frankfurt: Pharma Forschung Toxikologie und Pathologie, Hoechst Aktiengesellschaft, unpublished

Clariant GmbH (1990) Evaluation of p-Nitrobenzoesäure in the in vivo cytogenetic test in bone marrow cells of the Chinese hamster-chromosome analysis. Report No. 90.0498, 18 May 1990, Frankfurt: Pharma Research Toxicology and Pathology, Hoechst Aktiengesellschaft, unpublished

Cram RL, Juchau MR, Fouts JR (1965) Differences in hepatic drug metabolism in various rabbit strains before and after pretreatment with phenobarbital. Proc Soc Exp Biol Med 118: 872–875. https://doi.org/10.3181/00379727-118-29994

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

DuPont (1989) Two-week inhalation study with 4-nitrobenzoic acid (PNBA) in rats. Haskell Laboratory for toxicology and industrial medicine. Medical Research No. 8155–001, Haskell Laboratory Report No. 78–89, 06 May 1989, Newark, DE: DuPont Haskell Global Centers for Health and Environmental Sciences, unpublished

DuPont (2012) 4-Nitrobenzoic acid. Document 8EHQ-1218731, control number: 88120000274, 18 Jul 2012, Newark, DE: DuPont Haskell Global Centers for Health and Environmental Sciences, unpublished

ECB (European Chemicals Bureau) (2000) 4-Nitrobenzoic acid. IUCLID dataset, 18.02.2000. Ispra: ECB

ECHA (European Chemicals Agency) (2015) 4-Nitrobenzoic acid (CAS Number 62-23-7). Registration dossier. Individual submission, first publication 27 Feb 2013, last modification 24 Dec 2015. http://echa.europa.eu/web/guest/information-on-chemicals, accessed 09 Jun 2016

ECHA (European Chemicals Agency) (2016) 4-Nitrobenzoic acid (CAS Number 62-23-7). Registration dossier. Joint submission, first publication 27 Feb 2013, last modification 03 Feb 2016. http://echa.europa.eu/web/guest/information-on-chemicals, accessed 09 Jun 2016

EFSA (European Food Safety Authority) (2012) Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA J 10(3): 2579. https://doi.org/10.2903/j.efsa.2012.2579

Fiserova-Bergerova V, Pierce JT, Droz PO (1990) Dermal absorption potential of industrial chemicals: criteria for skin notation. Am J Ind Med 17(5): 617–635. https://doi.org/10.1002/ajim.4700170507

Gad SC (2008) Carcinogenicity studies. In: Gad SC, editor. Preclinical development handbook: Toxicology. Hoboken, NJ: John Wiley & Sons. p. 423–458

Gardner DM, Renwick AG (1978) The reduction of nitrobenzoic acids in the rat. Xenobiotica 8(11): 679–690. https://doi.org/10.3109/00498257809069580

Goldman P, Wheeler LA, Carter JH, Ingelfinger JA, Soderberg FB (1977) Properties of the Ames Salmonella mutants lodged in the gastrointestinal tract of gnotobiotic rats. Am J Clin Nutr 30(11): 1921–1926. https://doi.org/10.1093/ajcn/30.11.1921

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

Guy RH, Potts RO (1993) Penetration of industrial chemicals across the skin: a predictive model. Am J Ind Med 23(5): 711–719. https://doi.org/10.1002/ajim.4700230505

Hartwig A, editor (2015) 3-Nitrobenzoesäure. In: Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten. 58th issue. Weinheim: Wiley-VCH. Also available from https://doi.org/10.1002/3527600418.mb12192d0058

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

Idle JR, Millburn P, Williams RT (1978) Taurine conjugates as metabolites of arylacetic acids in the ferret. Xenobiotica 8(4): 253–264. https://doi.org/10.3109/00498257809056147

Käfferlein HU, Broding HC, Bünger J, Jettkant B, Koslitz S, Lehnert M, Marek EM, Blaszkewicz M, Monsé C, Weiss T, Brüning T (2014) Human exposure to airborne aniline and formation of methemoglobin: a contribution to occupational exposure limits. Arch Toxicol 88(7): 1419–1426. https://doi.org/10.1007/s00204-014-1266-y

Kato R, Oshima T, Takanaka A (1969) Studies on the mechanism of nitro reduction by rat liver. Mol Pharmacol 5(5): 487–498

Kato R, Onoda K, Takanaka A (1970) Strain differences in the metabolism and action of drugs in mice and rats. Jpn J Pharmacol 20(4): 562–571. https://doi.org/10.1254/jjp.20.562

Kawai A, Goto S, Matsumoto Y, Matsushita H (1987) [Mutagenicity of aliphatic and aromatic nitro compounds. Industrial materials and related compounds]. Sangyo Igaku 29(1): 34–54. https://doi.org/10.1539/joh1959.29.34

Kuzniar EJ, James SP (1981) Influence of the gut microflora on the metabolism of 4-nitrobenzoic acid in the marmoset. Xenobiotica 11(10): 675–683. https://doi.org/10.3109/00498258109049087

Leng G, Bolt HM (2008) Methemoglobin-forming substances. BAT Value Documentation, 2008. In: The MAK-Collection for Occupational Health and Safety. Part II: BAT Value Documentations. Weinheim: Wiley-VCH. Also available from https://doi.org/10.1002/3527600418.bb6253e1516

McConnell EE, Solleveld HA, Swenberg JA, Boorman GA (1986) Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. J Natl Cancer Inst 76(2): 283–289

NCBI (National Center for Biotechnology Information) (2014) 4-Nitrobenzoic acid. PubChem annotation record. Source: HSDB. https://pubchem.ncbi.nlm.nih.gov/source/hsdb/2140, accessed 19 Aug 2014

NLM (National Library of Medicine) (2014) p-Nitrobenzoic acid. ChemIDplus Data Bank. https://chem.nlm.nih.gov/chemidplus/rn/62-23-7, accessed 08 Aug 2014

Norred WP, Nishie K, Keyl AC (1975) Effects of short-term administration of nitrosamines on rat hepatic microsomal enzymes. Biochem Pharmacol 24(13–14): 1313–1316. https://doi.org/10.1016/0006-2952(75)90344-5

NTP (National Toxicology Program) (1990) Final report on the reproductive toxicity of para nitrobenzoic acid (CAS No 62-23-7) in Swiss CD-1 mice. National Toxicology Program, report #NTP-90-116. Research Triangle Park, NC: NTP. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB90253766.xhtml, accessed 27 Jan 2015

NTP (National Toxicology Program) (1994) Toxicology and carcinogenesis studies of p-nitrobenzoic acid (CAS No 62-23-7) in F344/N rats and B6C3F1 mice (feed studies). NTP Technical Report Series No. 442. Research Triangle Park, NC: NTP. http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr442.pdf, accessed 19 Aug 2013

NTP (National Toxicology Program) (2002) Toxicology and carcinogenesis studies of p-nitrotoluene (CAS No 99-99-0) in F344/N rats and B6C3F1 mice (feed studies). NTP Technical Report Series No. 498. Research Triangle Park, NC: NTP. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr498.pdf, accessed 08 Jan 2016

Pauluhn J (2004) Subacute inhalation toxicity of aniline in rats: analysis of time-dependence and concentration-dependence of hematotoxic and splenic effects. Toxicol Sci 81(1): 198–215. https://doi.org/10.1093/toxsci/kfh187

Pelkonen O (1973) Drug metabolism in the human fetal liver. Relationship to fetal age. Arch Int Pharmacodyn Ther 202(2): 281–287

Pelkonen O, Vorne M, Jouppila P, Kärki NT (1971) Metabolism of chlorpromazine and p-nitrobenzoic acid in the liver, intestine and kidney of the human foetus. Acta Pharmacol Toxicol (Copenh) 29(23): 284–294. https://doi.org/10.1111/j.1600-0773.1971.tb00613.x

Peters MA, Fouts JR (1968) An evaluation of two procedures used in the study of p-nitrobenzoic acid metabolism by rat liver. Toxicol Appl Pharmacol 12(2): 242–248. https://doi.org/10.1016/0041-008x(68)90036-7

Probst GS, McMahon RE, Hill LE, Thompson CZ, Epp JK, Neal SB (1981) Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: a comparison with bacterial mutagenicity using 218 compounds. Environ Mutagen 3(1): 11–32. https://doi.org/10.1002/em.2860030103

Reznik G, Ward JM (1981) Morphology of hyperplastic and neoplastic lesions in the clitoral and preputial gland of the F344 rat. Vet Pathol 18(2): 228–238. https://doi.org/10.1177/030098588101800211

Rudmann D, Cardiff R, Chouinard L, Goodman D, Küttler K, Marxfeld H, Molinolo A, Treumann S, Yoshizawa K, INHAND Mammary, Zymbal's, Preputial, and Clitoral Gland Organ Working Group (2012) Proliferative and nonproliferative lesions of the rat and mouse mammary, Zymbal's, preputial, and clitoral glands. Toxicol Pathol 40(6 Suppl): 7S-39S. https://doi.org/10.1177/0192623312454242

Shimizu M, Yano E (1986) Mutagenicity of mono-nitrobenzene derivatives in the Ames test and rec assay. Mutat Res 170(1–2): 11–22. https://doi.org/10.1016/0165-1218(86)90077-7

Srivastava S, Alhomida AS, Siddiqi NJ, Puri SK, Pandey VC (2002) Methemoglobin reductase activity and in vitro sensitivity towards oxidant induced methemoglobinemia in Swiss mice and beagle dogs erythrocytes. Mol Cell Biochem 232(1–2): 81–85. https://doi.org/10.1023/a:1014853421871

Sundvall A, Marklund H, Rannug U (1984) The mutagenicity on Salmonella typhimurium of nitrobenzoic acids and other wastewater components generated in the production of nitrobenzoic acids and nitrotoluenes. Mutat Res 137(2–3): 71–78. https://doi.org/10.1016/0165-1218(84)90094-6

Suzuki J, Koyama T, Suzuki S (1983) Mutagenicities of mono-nitrobenzene derivatives in the presence of norharman. Mutat Res 120(2–3): 105–110. https://doi.org/10.1016/0165-7992(83)90150-1

Tani E, Kourounakis PN (1993) Effect of ethanol and its interaction with ascorbic acid or tocopherol-acetate on p-nitrobenzoic acid reduction and lipid peroxidation. Res Commun Subst Abuse 14(1): 7–15

Wheeler LA, Carter JH, Soderberg FB, Goldman P (1975 a) Association of Salmonella mutants with germfree rats: site specific model to detect carcinogens as mutagens. Proc Natl Acad Sci U S A 72(11): 4607–4611. https://doi.org/10.1073/pnas.72.11.4607

Wheeler LA, Soderberg FB, Goldman P (1975 b) The relationship between nitro group reduction and the intestinal microflora. J Pharmacol Exp Ther 194(1): 135–144

Williams KD, Dunnick J, Horton J, Greenwell A, Eldridge SR, Elwell M, Sills RC (2001) p-Nitrobenzoic acid alpha2u nephropathy in 13-week studies is not associated with renal carcinogenesis in 2-year feed studies. Toxicol Pathol 29(5): 507–513. https://doi.org/10.1080/019262301317226302

Wilschut A, ten Berge WF, Robinson PJ, McKone TE (1995) Estimating skin permeation. The validation of five mathematical skin permeation models. Chemosphere 30(7): 1275–1296. https://doi.org/10.1016/0045-6535(95)00023-2

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K, Speck W (1987) Salmonella mutagenicity tests: III. Results from the testing of 255 chemicals. Environ Mutagen 9 Suppl 9: 1–109. https://doi.org/10.1002/em.2860090602

Zenser TV, Mattammal MB, Palmier MO, Davis BB (1981) Microsomal nitroreductase activity of rabbit kidney and bladder: implications in 5-nitrofuran-induced toxicity. J Pharmacol Exp Ther 219(3): 735–740