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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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Benzonitrile No. 260

CAS No. 100-47-0

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Benzonitrile

1 Summary and assessment

On oral administration of benzonitrile to rabbits, approx. 60% of the dose is excreted in the urine within 48 hours as oxidation products (m- and p-hydroxybenzonitrile as well as benzoic acid) and as the sulfate, glucuronide and glutathione conjugates. Approximately between 5 and 6% is recovered as mercapturic acid.

Acute oral administration and acute dermal application of benzonitrile has proved harmful (LD_{50} rat oral 690 to 1500 mg/kg body weight; LD_{50} rat dermal 1200 mg/kg body weight). In inhalation hazard tests lasting up to 8 hours, no animals died after exposure to a benzonitrile-saturated atmosphere.

Following repeated oral administration of benzonitrile for 2 weeks, doses of 1000 mg/kg body weight have been found to cause damage to the forestomach and the kidneys in rats, whereas in mice liver damage has been observed after 600 mg/kg body weight. Repeated, 6-hour daily inhalation of an analytically verified benzonitrile concentration of 97 ppm, equivalent to approx. 410 mg/m³, for a period of 2 weeks is tolerated without symptoms by cats, rabbits, guinea pigs and rats.

Benzonitrile has a slightly irritant effect on rabbit skin and the rabbit eye.

In the 90-day study in rats receiving oral doses of benzonitrile at levels of up to 300 mg/kg body weight, female rats exposed to the top dose showed reduced grip strength and a delayed reaction to thermal stimuli. In the male rats, the relative kidney weights were increased at doses of 75 mg/kg body weight and above. Histopathological examination of the kidneys revealed dose-dependent hyaline droplet degeneration and dilatation of the tubules. The female rats receiving 150 and 300 mg/kg body weight showed kidney changes that were characterised by vacuolation of the tubular cells. The *no effect level* for male and female rats is reported as 37.5 mg/kg body weight and 75 mg/kg body weight, respectively. In an analogous study conducted in mice treated with benzonitrile doses of up to 600 mg/kg body weight, female mice were found to exhibit a statistically significant increase in acoustic startle response latency at the high dose. Starting at a dose level of 75

mg/kg body weight, the absolute and relative liver weights were increased. Histopathological changes of the liver (centrilobular hypertrophy, proliferation of Kupffer cells, mineralisation and cellular necrosis) were found at 300 mg/kg body weight and above, and the kidneys exhibited changes (tubular dilatation) starting at 150 mg/kg body weight. The maximum tolerated dose is reported as 37.5 mg/kg body weight.

Benzonitrile is devoid of mutagenicity, with and without metabolic activation, both in the Salmonella/microsome assay using *Salmonella typhimurium* strains TA 97, TA 98, TA 100, TA 1535, TA 1537, TA 1538, in the mutagenicity assay using *Escherichia coli* WP2uvrA and in the mouse lymphoma assay using cell line L5178YTK+/–, and it does not cause DNA damage in the sister chromatid exchange assay in Chinese hamster CHO cells. Malsegregation of the chromosomes in *Saccharomyces cerevisiae* D61.M can only be induced by means of cold treatment; under standard conditions, benzonitrile failed to cause aneuploidy in these yeast cells. In the in-vitro chromosome aberration test in Chinese hamster CHO cells, benzonitrile induces an increase in the aberration rates at the high concentration of approx. 1500 µg/ml in the presence of metabolic activation. In the absence of metabolic activation, however, there is no evidence from this test system or the in-vivo mouse micronucleus test to demonstrate that benzonitrile has a chromosomal mutagenicity potential following subchronic administration of doses of up to 600 mg/kg body weight.

In humans, skin and mucous membrane contact with benzonitrile have been observed to cause irritation. In the maximisation test and according to other studies and observations, there is no evidence of a sensitising potential in humans.

2 Name of substance

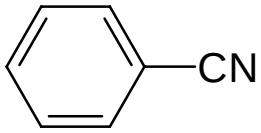
2.1	Usual name	Benzonitrile
2.2	IUPAC Name	Benzonitrile
2.3	CAS No.	100-47-0
2.4	EINECS No.	202-855-7

3 Synonyms, common and trade names

Benzene carbonitrile
Benzene, cyano-
Benzenenitrile
Benzoic acid nitrile
Benzonitril
Cyanobenzene
Phenylcyanid
Phenyl cyanide

4 Structural and molecular formulae

4.1 Structural formula



4.2 Molecular formula C_7H_5N

5 Physical and chemical properties

5.1	Molecular mass, g/mol	103.12	
5.2	Melting point, °C	–12.7	(Lide and Frederikse, 1996)
		–12.75	(Budavari et al., 1989)
		–13	(EC, 1996)
		–13.8	(Maki and Suzuki, 1985)
5.3	Boiling point, °C	28.2 (at 1.333 hPa)	
		69.2 (at 13.33 hPa)	
		123.5 (at 133.3 hPa)	
		190.7 (at 1013 hPa)	(Budavari et al., 1989)
		191.1	(Lide and Frederikse, 1996; Maki and Suzuki, 1985)

5.4	Vapour pressure, hPa	0.93 (at 25 °C) (MacEwen and Vernot, 1974) < 1 (at 20 °C) (EC, 1996) 13.7 (at 70 °C) 22.7 (at 79 °C) 45.3 (at 94 °C) (Maki and Suzuki, 1985) 1.10 (at 25 °C) 4.96 (at 50 °C) 18.0 (at 75 °C) 54.1 (at 100 °C) 140 (at 125 °C) 322 (at 150 °C) (Lide and Frederikse, 1996)
5.5	Density, g/cm³	1.0052 (at 20 °C) (Maki and Suzuki, 1985) 1.0093 (at 15 °C) (Lide and Frederikse, 1996)
5.6	Solubility in water	Low in cold water (Budavari et al., 1989) Low (Lide and Frederikse, 1996) 4.33 g/l (at 25 °C) (McGowan et al., 1966) 1% (at 100 °C) (Budavari et al., 1989; Maki and Suzuki, 1985)
5.7	Solubility in organic solvents	Dissolves well in acetone, miscible with ethanol and diethyl ether (Lide and Frederikse, 1996) Miscible with the usual organic solvents (Budavari et al., 1989; Maki and Suzuki, 1985)
5.8	Solubility in fat	Partition coefficient n-octanol/water, log P_{ow}: 1.5 (measured) (RCC NOTOX, 1988)
5.9	pH value	–
5.10	Conversion factor	1 ml/m³ (ppm) ≙ 4.21 mg/m³ 1 mg/m³ ≙ 0.24 ml/m³ (ppm) (at 1013 hPa and 25 °C)

6 Uses

For the manufacture of benzylamine, benzoguanamine and benzoguanamine resins; intermediate in the production of pesticides, pharmaceuticals and dyes; used as a solvent (Maki and Suzuki, 1985).

7 Experimental results

7.1 Toxicokinetics and metabolism

Upon single oral administration of 145 mg benzonitrile/kg body weight to 2 rabbits, approx. 60% of the administered dose was excreted in urine within 2 days as sulfate and/or glucuronic acid conjugates of hydroxylated compounds (23% and 27% as sulfate conjugates, 38% and 28% as glucuronic acid conjugates). Another approx. 5% of the administered dose was recovered from urine in the form of mercapturic acids. Moreover, the authors suspected that benzoic acid was a metabolite of benzonitrile and confirmed this in subsequent experiments (see below). In the urine collected between 2 and 4 days following administration, benzonitrile metabolites were still detectable but they were not quantified. In subsequent experiments, the excretion of metabolites was investigated in greater detail. Maximum metabolite excretion was found to be reached 2 to 3 days following oral administration of benzonitrile (no further details). To determine the formation of mercapturic acid more accurately, 3 female rabbits were given a single oral dose of 200 mg benzonitrile/kg body weight. Within 6 days, they excreted 5.2 to 5.7% of the administered dose in the form of mercapturic acids. The authors discussed the possibility that the mercapturic acid in question was the one derived from 4-hydroxybenzonitrile (which they referred to as p-cyanophenylmercapturic acid), but they were not able to confirm the structure. From urine collected for 2 days from another 5 rabbits which together had received a total of 4 g benzonitrile, the authors recovered approx. 10% of the administered dose in the form of benzoic acid, part of which was found to be bound as glycine and glucuronic acid derivatives. Urine collected for 4 days from 6 rabbits which together had received 2.8 g benzonitrile by oral administration was analysed and found to contain 4-hydroxybenzonitrile and 3-hydroxybenzonitrile (referred to by the authors as p- and m-cyanophenol, respectively), with 4-hydroxybenzonitrile accounting for the larger fraction. In addition, there was evidence of dihydroxybenzonitrile (cyanocatechol) formation. The hydroxybenzonitriles war partially recovered in the form of their sulfate conjugates. The authors were not able to detect 2-hydroxybenzonitrile (o-cyanophenol) in the rabbit urine. Metabolic release of thiocyanates, as has been observed to occur with aliphatic nitriles, was precluded by the authors (no further details; Smith and Williams, 1950).

Even Hunt (as early as 1904) considered metabolic release of cyanate from benzonitrile unlikely, as in mouse studies prussic acid antidote had failed to show a protective effect against the acute toxicity of benzonitrile (Hunt, 1904).

In 1968, Daly et al. confirmed in in-vivo studies conducted in male rats and in in-vitro studies in rabbit liver microsomes that benzonitrile undergoes biotransformation to hydroxybenzonitrile. The rats were treated with intraperitoneal doses of 100 mg ^{2}H-benzonitrile/kg body weight and their urine was subsequently collected over a period of 48 hours. Without further quantification, the urine was stated to contain 4-hydroxybenzonitrile and smaller amounts of 3-hydroxybenzonitrile and 2-hydroxybenzonitrile. Upon incubation of the liver microsomes (one hour at 37 °C and pH 8) with 50 µmol ^{2}H-benzonitrile, 4-hydroxybenzonitrile and 2-hydroxybenzonitrile were detectable in traces (Daly et al., 1968).

The results obtained by Kutsenko and Shilov (1991) are in good agreement with the formation of mercapturic acids, as in their studies intraperitoneal administration of benzonitrile to rats was found to result in decreased hepatic glutathione levels.

Male rats received single intraperitoneal doses of 6.8 mg benzonitrile/kg body weight. At 0.25, 3, 8 and 24 hours after administration, the liver and brain glutathione levels and the brain cytochrome oxidase activities were determined in groups of 6 rats per necropsy time point. Hepatic glutathione levels were significantly lowered at 3 and 8 hours after administration but were back to control levels after 24 hours. In the brain, the glutathione levels and cytochrome oxidase activity levels remained within the control range at all necropsy time points (no further details; Kutsenko and Shilov, 1991)

7.2 Acute and subacute toxicity

Acute toxicity

Following single oral, dermal, subcutaneous, intraperitoneal and intravenous administration of benzonitrile, the following LD_{50} values were determined (cf. Table 1). Based on the data available, benzonitrile is to be evaluated as harmful upon acute oral administration or dermal application.

Table 1. Acute toxicity (LD_{50}) of benzonitrile				
Species	Sex	Route of administration	LD_{50} value and findings (mg/kg body weight)	Reference
Rat	male female	oral	690	TNO, 1978 a
Rat	male	oral	720	Carpenter, 1974
Rat	no information	oral	800, approximate LD_{50} value	Zeller et al., 1969
Rat	no information	oral	840	Moreno, 1977
Rat	no information	oral	1000	Calo, 1974
Rat	male female	oral	1131–1501	Cheav and Foussard-Blanpin, 1990
Rat	male	oral	1500 ± 200	Agaev, 1975; Agaev et al., 1977
Mouse	no information	oral	971	Tanii and Hashimoto, 1984
Mouse	no information	oral	1240	Cheav et al., 1983
Mouse	male female	oral	1081–1242	Cheav and Foussard-Blanpin, 1990
Mouse	no information	oral	1400	Agaev, 1975
Rabbit	no information	oral	800 lethal, 400 tolerated	Zeller et al., 1969
Cat	no information	oral	800 lethal, 400 tolerated	Zeller et al., 1969
Rat	no information	dermal (no further details)	1200	Calo, 1974
Rat	no information	dermal (1 hour) dermal (15 minutes)	ca. 2000 lethal ca. 2000 tolerated	Zeller et al., 1969
Rabbit	no information	dermal (no further details)	1250	Moreno, 1977
Rabbit	no information	dermal (occlusive, 24 hours)	ca. 1500 lethal, 200 tolerated	Zeller et al., 1969
Rabbit	no information	subcutaneous	200 lethal	Verbrugge, 1899
Rat	male female	intraperitoneal	740–781	Cheav and Foussard-Blanpin, 1990
Mouse	no information	intraperitoneal	400, approximate LD_{50} value	Zeller et al., 1969
Mouse	male female	intraperitoneal	861–901	Cheav and Foussard-Blanpin, 1990
Rabbit	no information	intravenous	430 lethal	Cheav and Foussard-Blanpin, 1990

Mice, rats, and rabbits have been observed to show signs of toxicity including irregular and laboured breathing, somnolence, tremor, convulsions,

paresis, paralysis, atonia, failure of reflexes (e. g. the pain and righting reflexes), ptosis and prostration. Macroscopic examination revealed no substance-related changes. Histopathological examination revealed congestion of the lungs with oedema formation and intra-alveolar haemorrhages as well as dose-independent degeneration of the centrilobular hepatocytes (Cheav and Foussard-Blanpin, 1990; TNO, 1978 a; Verbrugge, 1899; Zeller et al., 1969).

The acute inhalation toxicity of benzonitrile is summarised below in Table 2. Based on these findings, benzonitrile is to be evaluated as a low-toxicity chemical.

Table 2. Acute inhalation toxicity (LD_{50}) of benzonitrile

Species	Sex	Findings	Reference
Rat	no information	800 mg/m³, 4 hours; not lethal (no further details)	Calo, 1974
Rat	male	ca. 3790 mg/m³, 4 hours (nominal concentration); mortality: 0/6 animals; depressed body weight gain in 5 of 6 animals; observation period was 14 days	MacEwen and Vernot, 1974; Vernot et al., 1977
Rat	no information	4000 mg/m³, 4 hours (saturated atmosphere); not lethal (no further details)	Carpenter, 1974
Rat	no information	4000 mg/m³, 8 hours (saturated atmosphere); not lethal (no further details)	Carpenter, 1974
Rat	no information	8000 mg/m³, 4 hours; 3 of 10 animals died (no further details)	Calo, 1974
Rat	no information	LC_{50} = 38600 mg/m³ (no details as to duration of exposure)	Agaev, 1975
Rat	no information	in the inhalation risk test, none of the animals died following a more than 4-hour exposure to a stream of air saturated with the fraction of benzonitrile which is volatile at 20 °C; observation period 7 days	Zeller et al., 1969
Mouse	male	2950 mg/m³, 4 hours (analytical concentration); mortality 10/10 animals; observation period 14 days	MacEwen and Vernot, 1974
Mouse	male	3750 mg/m³, 2 hours (analytical concentration); mortality 1/7 animals; observation period 14 days	MacEwen and Vernot, 1974
Mouse	no information	LC_{50} = 6000 mg/m³ (no details as to duration of exposure)	Agaev, 1975

Upon inhalation, rats and mice showed signs of toxicity, including laboured breathing, ataxia, prostration, sedation and narcosis (MacEwen and Vernot, 1974; Zeller et al., 1969). Macroscopic examination revealed no substance-induced changes in the rat and the mouse. Histopathologically, the mice showed congestion of the lungs (with accompanying emphysema) and the liver (with sinusoidal dilation), and the rat lungs displayed multifocal areas of lymphoid hyperplasia with foamy macrophage accumulations (MacEwen and Vernot, 1974).

Subacute toxicity

Groups of 5 male and 5 female Fischer 344 rats received daily doses of 0 (controls), 11, 33, 100, 300 and 1000 mg benzonitrile/kg body weight by oral gavage as solutions in maize germ oil (corn oil) for a period of 2 weeks. Following 1000 mg/kg body weight, all animals exhibited signs of toxicity, including ataxia, loss of fine motor control as well as salivation, and they died within 5 days. Lower doses (≤ 300 mg/kg body weight) were survived. The 300 mg/kg body weight dose resulted in salivation, lacrimation and nasal discharge. In the female rats, some of the symptoms were seen down to the lowest test dose of 11 mg/kg body weight. Macroscopic examination of the lethally poisoned rats of the highest dose group primarily revealed lesions in the forestomach which were histopathologically diagnosed as acute inflammatory changes with acanthosis, hyperkeratosis, mucosal erosions and ulcers, and in 9 of the 10 rats vacuolar degeneration of the proximal convoluted tubule occurred. The low doses did not cause any kidney damage (NTP, 1994).

In 5 male and 5 female Wistar rats (180 ± 10 g) receiving daily doses of benzonitrile (in olive oil) by oral gavage at as low a level as 40 mg/kg body weight for 4 weeks, no substance-related changes (behaviour, body weight gain, macroscopic examination) occurred. Groups of 5 untreated animals per sex and 5 animals per sex receiving olive oil served as controls (no further details; Cheav and Foussard-Blanpin, 1990).

Groups of 5 male and 5 female B6C3F1 mice received doses of 0 (controls), 22, 66, 200, 600 and 2000 mg benzonitrile/kg body weight by oral gavage as solutions in maize germ oil (corn oil) for a period of 2 weeks. The highest dose level was lethal to all animals within 2 days, the mice ex-

hibiting tremor, hypoactivity, prostration and ataxia before dying. At the lower dose levels there were no deaths, whereas 600 mg also resulted in hypoactivity and ataxia. Histopathological examination of the 600 mg/kg group revealed karyomegaly and necrosis of the hepatocytes in animals of both sexes. No findings were reported for the dose levels ≤ 200 mg/kg body weight (NTP, 1994).

Groups of 2 cats, 2 rabbits and 2 guinea pigs as well as groups of 2 male and 2 female rats and 5 male and 5 female mice inhaled a nominal benzonitrile concentration of 460 ppm, equivalent to 1937 mg/m³, for 6 hours per day on 3 consecutive days (dynamic study design). The observation period was 14 to 21 days. The cats showed no signs of toxicity during and after the first inhalation, but in the course of the subsequent exposures developed disturbances of equilibrium and were easily startled. Moreover, both animals exhibited pupil dilation, lack of appetite and weight loss. One cat died 7 days after exposure (purulent pleuropneumonia, fibrinous pericarditis). The survivor was free of symptoms after 5 days. The rabbits and the guinea pigs showed no signs of toxicity. One of the 4 rats died after the third exposure (throat abscess). The other rats remained without symptoms. The mice showed transient apathy, disturbances of equilibrium, lying on the side (one animal) and narcosis. Six mice and 2 mice died after the second and the third exposure, respectively. Necropsy revealed that in 5 cases the animals had atelectasis in the lungs and definitive marks on the lobes of the liver. In another 2 animals, considerable adipose infiltration of the liver was observed (BASF, 1961).

An additional inhalation study was conducted with an analytically monitored benzonitrile concentration of 97 ppm, equivalent to 408 mg/m³. Groups of 2 cats, 2 rabbits and 2 guinea pigs, and groups of 2 male and 2 female rats and 5 male and 5 female mice inhaled this concentration for 6 hours per day on 5 days per week over a period of 2 weeks. The observation period was 14 to 21 days. With the exception of 2 mice which died after the second and the ninth exposure without any externally visible findings, all animals survived without symptoms. A post-mortem performed on the deceased mice and the rats and mice sacrificed at the end of the observation period yielded no significant findings (BASF, 1961).

On repeated intraperitoneal administration of 30 mg benzonitrile/kg body weight to groups of 5 male and 5 female Wistar rats (180 ± 10 g) over a pe-

riod of 4 weeks, 3 rats died between study days 19 and 22. Macroscopic examination of all rats at the end of the study revealed that they had congested spleens, necrotic pancreatitis, bilateral haemorrhagic necrosis of the renal cortex and a pulmonary abscess. Groups 5 animals receiving the vehicle, olive oil, served as controls (no further details; Cheav and Foussard-Blanpin, 1990).

7.3 Skin and mucous membrane effects

The primary skin irritancy of benzonitrile was tested in the intact and scarified skin of groups of 6 New Zealand white rabbits by means of the occlusive patch test (Draize method). The volume applied was 0.5 ml and the duration of exposure was 24 hours. The skin reaction was assessed immediately on removal of the patch as well as after another 48 hours. In the intact skin, benzonitrile caused very mild erythema in 2 of the 6 rabbits (primary irritation indices of 0.3 and 0.2 from a maximum irritation index of 8); whereas the scarified skin showed very slight erythema in 5 of the 6 rabbits and very slight oedema in one of the 6 rabbits. The primary irritation indices for the scarified skin were 1.3 and 1.0 from a maximum irritation index of 8. Based on these findings, benzonitrile was judged to be a very slight primary skin irritant (TNO, 1978 b).

The acute skin irritancy of benzonitrile was tested with the patch test on the dorsal skin of white rabbits, the test substance being applied once to the intact skin for 15 minutes or for 20 hours by means of a small textile patch (approx. 2.5 cm x 2.5 cm). Following the 15-minute application, the exposed skin area was washed first with undiluted polyethylene glycol 400 and subsequently with a 50-percent aqueous polyethylene glycol solution. Following the 20-hour exposure, the skin was not washed. The skin reactions were assessed on removal of the patches as well as after 1, 3 and 8 days. Benzonitrile caused mild skin irritation both after 15 minutes and after 20 hours of exposure (Zeller et al., 1969).

In an occlusive patch test (24-hour exposure), undiluted benzonitrile had an irritant effect on the intact and the scarified rabbit skin (no further details; Moreno, 1977).

To test the acute eye irritancy of benzonitrile, 6 New Zealand white rabbits each had 0.1 ml of the chemical instilled into the conjunctival sac (FDA me-

thod). The untreated eye served as the control. The findings were assessed 24, 48 and 72 hours as well as 7 days after instillation. After 24, 48 and 72 hours, reddening of the conjunctivae was observed in 6, 4 and 4 of the 6 animals, respectively (irritation index 1 from a maximum score of 3). Chemosis occurred in 2 of the 6 animals 24 hours after instillation (irritation index 1 from a maximum score of 4). The cornea and iris were without findings. The slight conjunctival irritation had cleared up completely after 7 days. Based on these findings and the FDA criteria, benzonitrile was evaluated as not irritating (TNO, 1978 b).

Instillation of benzonitrile into the conjunctival sac of the rabbit eye (number of animals not given) resulted in slightly injected vessels. The applied volume was 50 µl. The findings were assessed 10 minutes, 1 hour and 24 hours as well as 3 and 8 days after instillation (no information on reversibility; Zeller et al., 1969). Benzonitrile thus proved to be mildly irritating in this study.

7.4 Sensitisation

No information available.

7.5 Subchronic and chronic toxicity

To determine the subchronic toxicity of benzonitrile, groups of 10 male and 10 female Fischer 344 rats received daily doses of 0 (controls), 19, 37.5, 75, 150 and 300 mg benzonitrile/kg body weight by oral gavage as solutions in maize germ oil (corn oil) for a period of 13 weeks. There were no deaths. In rats of both sexes, hyperactivity as well as aggressive behaviour were observed following 150 and 300 mg/kg body weight. The females of the 300 mg/kg dose group showed reduced hindlimb grip strength and a delayed reaction to thermal stimuli after 7 and 13 weeks. In the males and females of this dose group, body weight gain was reduced by 10.6 and 6.7% after 13 weeks, relative to controls. At the end of the study, the male rats were found to have significantly increased absolute and relative kidney weights at the 75 mg/kg body weight dose level. In the male rats, histopathological examination of the kidneys revealed dose-dependent hyaline droplet degeneration and dilation of the tubules at and above 75 mg/kg body weight, which according to the authors resembled “hydrocarbon nephropathy”. In the females of the two top dose groups, the kidney changes cha-

racteristically consisted in vacuolar degeneration of the cortical tubules with anisokaryotic cells. The *no effect levels* for nephropathy were 37.5 mg/kg body weight and 75 mg/kg body weight for male and female rats, respectively (no further details; NTP, 1994). The possibility of α_{2u}-globulin nephropathy in the male rats was not discussed.

In addition, the subchronic toxicity of benzonitrile was tested in groups of 10 male and 10 female B6C3F1 mice. They received daily doses of 0 (controls), 37.5, 75, 150, 300 and 600 mg benzonitrile/kg body weight by oral gavage as solutions in maize germ oil (corn oil) for a period of 13 weeks. There were no substance-related deaths. In the 300 and 600 mg/kg dose groups, both the males and the females displayed hypoactivity and ataxia. In addition, the females of the 600 mg/kg group showed a significant increase in acoustic startle response latency. Body weight gain in the mice of the 600 mg/kg dose was increased by 21.5% and 14.6% (males and females, respectively) relative to controls. At the end of the study, both sexes were found to have significantly increased absolute and relative liver weights at dose levels of and above 75 mg/kg body weight. In the female mice of the 37.5 mg/kg dose group, there was only an increase in absolute liver weight. Histopathological examination of the male mice of the 300 and 600 mg/kg dose groups as well as the females of the 600 mg/kg dose group revealed centrilobular hypertrophy of the liver cells, proliferation of the Kupffer cells, mineralisation and cellular necrosis. The kidneys of the male mice of the three highest dose groups and the females of the two top dose groups showed dose-dependent dilation of the tubules of the inner renal cortex. The maximum tolerated doses reported for male and female mice was 37.5 mg/kg body weight (no further details; NTP, 1994).

Inhalation exposure of male rats (weighing 160 to 180 g) to 70 ± 10 mg benzonitrile/m³ air for 4 hours/day on 5 days per week for a period of 4.5 months resulted in significant reduction in red blood cell counts and haemoglobin content at 3 and 4 months, whereas leukocyte counts, hippuric acid excretion and cytochrome oxidase activities in the liver, kidney and heart were significantly increased relative to controls. From these results, the authors concluded that the maximum allowable concentration in the air of work areas was 1 mg/m³ (Agaev et al., 1977). Insufficient documentation of the conduct and the results of the study render it unsuitable for the assessment of the systemic toxicity of benzonitrile following repeated inhalation administration.

7.6 Genotoxicity

7.6.1 In vitro

Tests for mutagenic activity

There were no indications of a mutagenic potential of benzonitrile (98.56% pure) in the Salmonella/microsome assay (standard-plate incorporation test) using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 with and without metabolic activation (S-9 mix from Aroclor 1254-induced rat liver). Concentrations ranging from 8 to 5000 µg/plate were used. The test was repeated in an independent experiment. Even at the highest test concentration, there was no bacteriotoxicity (Microtest, 1988).

In another Salmonella/microsome assay, which was also conducted as a standard-plate incorporation test, benzonitrile concentrations ranging from 33 to 3333 µg/plate were again found to be devoid of mutagenicity in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 with and without metabolic activation (S9 mix from Aroclor 1254-induced rat or hamster liver) (no further details; NCI, 1984).

In the Salmonella/microsome preincubation assay using *Salmonella typhimurium* strains TA 97, TA 98, TA 100 and TA 1535TA 1538 with and without metabolic activation (S-9 mix from Aroclor 1254-induced rat or hamster liver), there were also no indications of a mutagenic potential of benzonitrile (99% pure) in the same concentration range (Zeiger et al., 1988) .

A further preincubation test with *Salmonella typhimurium* strain TA 1535 revealed no mutagenic effect of 500 to 7500 µg benzonitrile/plate. The assay was conducted only in the absence of metabolic activation (no further details; NCI, 1984).

In addition, benzonitrile (99% pure) was studied for mutagenicity in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 as well as in *Escherichia coli* WP2uvrA. The tests were carried out using the preincubation method in the presence and absence of metabolic activation (S-9 mix from phenobarbital-induced and 5,6-benzoflavone-induced rat liver). The test concentrations (in DMSO) were in the range from 20 to 5000 µg/plate. Starting at a concentration of 625 µg/plate bacteriotoxicity was

observed. Thus, this study also failed to produce any evidence of mutagenicity from benzonitrile (JETOC, 1996).

Benzonitrile was tested for mutagenicity in the mouse lymphoma assay using cell line L5178YTK+/–. The tests were carried out in the presence and absence of metabolic activation (S-9 mix from Aroclor 1254-induced rat liver). In the absence and presence of metabolic activation, the test concentrations (in DMSO) were in the ranges from 0.5 to 1.07 µl/ml and 0.39 to 0.80 µl/ml, respectively. The survival rates were in the 71 to 11% and the 104 to 39% ranges in the absence and presence of activation, respectively. Benzonitrile caused no significant increase in mutation rates in this study (Microbiological Associates, 1982; NCI, 1984).

Tests for DNA-damaging activity

Benzonitrile (as a solution in DMSO) caused no increase in sister chromatid exchange rate in Chinese hamster ovary (CHO) cells with or without metabolic activation (S-9 mix from Aroclor 1254-induced rat liver). The concentrations were in the range from 5 to 166.7 µg/plate. A concentration of 166.7 µg/ml was cytotoxic (NTP, 1988 a, 1995).

Tests for chromosome-damaging activity

In addition, benzonitrile was studied in CHO cells of the Chinese hamster for chromosome-damaging activity. The concentrations employed were in the ranges from 200 to 1500 µg/ml and 200 to 3000 µg/ml in the absence and presence of metabolic activation (S-9 mix from Aroclor 1254-induced rat liver), respectively. Without metabolic activation, there was no increase in the rate of chromosome aberrations. With metabolic activation, 1500 µg/ml resulted in chromosome aberrations (total percentage of cells with aberrations 72%, simple aberrations 64%, complex aberrations 8%; DMSO controls 3, 1.5 and 1.5%, respectively). In the absence and presence of activation, 1500 µg/ml and 3000 µg/ml, respectively, were cytotoxic. A repeat experiment with metabolic activation and concentrations in the 1005 to 1993 µg/ml range was not able to confirm a similar order of magnitude for the strongly positive result obtained in the initial experiment at a concentration level of 1495 µg/ml. There was a total of 11% of cells with aberrations, of which 3% were simple and 9% were complex aberrations (DMSO con-

trols had 3, 1 and 2%, respectively). A concentration of 1993 µg/ml was cytotoxic (NTP, 1988 a, 1995).

Studies on chromosome malsegregation

In a study of chromosome loss in *Saccharomyces cerevisiae* D61.M, benzonitrile (99% pure) concentrations of up to 2190 µg/ml did not lead to chromosome loss under standard conditions (16-hour incubation at 30 °C). Modification of experimental conditions (interruption of incubation at 30 °C by a 16-hour cold treatment at 0 °C in order to optimise induction of aneuploidy) resulted in significantly higher levels of chromosome loss. The authors suspected that benzonitrile induces aneuploidy by interacting with the spindle apparatus (Whittaker et al., 1990).

7.6.2 In vivo

A micronucleus test was conducted at study termination in groups of 9 or 10 male and 7 to 10 female B6C3F1 mice/dose from the 13-week subchronic exploratory study (cf. Section 7.5). Even at the highest dose (600 mg/kg body weight/day), no significant increase was observed in the number of micronucleated polychromatic erythrocytes (no further details; NTP, 1995). The result of the micronucleus test carried out at the end of a 90-day inhalation study must be considered relevant in accordance with OECD guideline No. 474 (1995) as in this study systemic toxicity was observed at the lowest test concentration of 37.5 mg/m³ in the female mice and at concentration levels of and above 75 mg/m³ in the male mice, and also because the mice received benzonitrile treatment up to the time of sacrifice (when blood smears were obtained).

7.7 Carcinogenicity

No information available.

7.8 Reproductive toxicity

Benzonitrile had no adverse effect on sperm morphology and vaginal cytology (no further details; NTP, 1988 b).

7.9 Effects on the immune system

No information available.

7.10 Neurotoxicity

Male Swiss mice (10 animals/dose group) received single doses of 240, 480 and 720 mg benzonitrile/kg body weight (in olive oil) by oral gavage. In the high-dose group spontaneous mortality was significantly reduced. Levels of motor and exploratory activity were lower in all 3 dose groups. Neuromotor co-ordination (rotating rod test) was dose-dependently impaired at concentrations of 240 mg/kg body weight and higher. In the high-dose group, body temperature was significantly reduced. Thirty minutes following administration, catalepsy occurred in 30, 40 and 70% of the animals in the low, mid and top dose groups, respectively. At 30 and 60 minutes, hyperaesthesia was seen in the low and mid dose groups, while in the top dose group analgesia was observed. Based on these results and other findings, the authors considered it possible that benzonitrile has an effect on the action of the catecholaminergic neurotransmitters (Cheav et al., 1990).

Benzonitrile was studied together with 5 substituted benzonitrile derivatives to investigate its anticonvulsive action in mice treated with pentetrazol. Following 200 mg/kg body weight (route of administration not stated), convulsions occurred in 4 out of 10 mice, and 2 out of 10 mice died. A 300 mg/kg body weight dose suppressed the convulsions in 7 of 10 mice, and all animals survived (no further details; Cheav et al., 1983).

7.11 Other effects

Groups of 3 or 4 male C57B1/6 mice (weighing 18 to 23 g) received single intraperitoneal doses of 15 and 60 mg benzonitrile/kg body weight in dimethyl sulfoxide. After 24 hours, the olfactory mucosa was examined histologically. In contrast to findings obtained with the herbicide, Dichlobenil (2,6-dichlorobenzonitrile), histological examination revealed no damage to the olfactory epithelium (Eriksson et al., 1992).

Oxidative metabolism in isolated brown adipocytes from hamsters was inhibited by 50 to 59% compared with controls following 5-minute incubation

with 1 mM benzonitrile (equivalent to 103.12 µg/ml phosphate buffer solution) at 37 °C (with oxygen consumption rate serving as the test parameter; Curvall et al., 1984).

A 30-minute incubation of human embryonic lung fibroblasts with 25 mM benzonitrile (equivalent to 2578 µg/ml Tris-buffered saline) at 37 °C resulted in 30 to 39-percent plasma membrane damage compared with controls (using leakage of a cytoplasmic nucleotide marker, [^{3}H]-uridine, as the test parameter; Curvall et al., 1984).

8 Experience in humans

Benzonitrile contamination of the skin and clothing of one worker resulted in unconsciousness despite immediate thorough cleansing of the affected skin areas with water; at that time he had not yet had a change of clothes. On being given a bath, the worker regained consciousness for a short while but suffered from severe dyspnoea. Tonic convulsions of the muscles of the arms and face set in, which subsided upon administration of Luminal and sodium thiosulfate. The unconsciousness persisted for 75 minutes despite oxygen ventilation. The worker was free of symptoms the following day. Several years after the accident, spells of unconsciousness recurred in the worker. Differential diagnosis gave rise to suspicion of pickwickian syndrome (cardiopulmonary syndrome in obesity). Hence it was not possible to establish with certainty that there was a connection with the benzonitrile exposure (Zeller et al., 1969).

In an another 7 cases, skin and mucous membrane contact with benzonitrile was observed to be followed after varying latency periods (frequently several hours) by skin irritations in the form of reddening and subsequent blistering. As the skin changes were in part extensive and/or severe, the average healing time was 10 to 14 days (no further details; Zeller et al., 1969).

One worker was reported to have developed erythema with scaly superficial detachment of the skin on the extensor sides of both lower arms after benzonitrile had run over his protective gloves (no further details; BASF, 1994).

Over an observation period of 13 years and 8 months, employees working in benzonitrile production were not observed to develop skin irritation or

skin damage, altered blood counts, allergies or other disorders likely to be connected with the handling of benzonitrile. The chemical has an unpleasant odour, but this is tolerated after a short time of working with the substance and is then no longer noticed. No irritancy was observed with respect to the nasal mucosa (no further details; SKW, 1994).

In an occlusive patch test in man, no skin irritation was seen following 48-hour skin exposure to a 2-percent formulation of benzonitrile in yellow vaseline (no further details; Epstein, 1977).

From a maximisation test conducted in 35 healthy subjects there were no indications of a sensitising potential for benzonitrile. A further test in 27 subjects also gave no indications of a sensitising potential. Benzonitrile was applied as a 2-percent formulation in yellow vaseline (no further details; Epstein, 1977).

Six patients being treated at a dermatology clinic were allergic to the rubber components of their underwear after it had been bleached with sodium hypochlorite. The hypochlorite had formed reaction products with the accelerator, zinc dibenzyldithiocarbamate, contained in the elastic. Amongst others, benzonitrile was identified by gas chromatography as one of the reaction products. Twenty-five volunteers received 10 consecutive 48-hour treatments with 0.1 ml of the reaction mixture as applications to the skin of the upper arm (induction phase) and an additional single 48-hour treatment (challenge) following a 2-week rest period. Of the 25 subjects, 14 showed a response to the reaction mixture, the same individuals also showing positive reactions to one component, N,N-Dibenzylcarbamoyl chloride (8.2 % of the mixture). Seven subjects underwent testing with the component, benzonitrile (0.5% of the mixture), but showed no allergic reaction. Application was performed with 0.01-percent solutions in ether (Jordan and Bourlas, 1975).

9 Classifications and threshold limit values

No information available.

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