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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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4-Nitro-4'-aminodiphenylamine-2-sulfonic acid

No. 120

CAS No. 91-29-2

BG Chemie
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4-Nitro-4’-aminodiphenylamine-2-sulfonic acid

1 Summary and assessment

The acute toxicity of 4-nitro-4’-aminodiphenylamine-2-sulfonic acid is low (LD_{50} rat oral > 5000 mg/kg body weight; LD_{50} rat dermal > 2000 mg/kg body weight). In cats the substance does not lead to methaemoglobin formation when administered in oral doses of 10 and 50 mg/kg body weight.

Subacute oral toxicity of 4-nitro-4’-aminodiphenylamine-2-sulfonic acid is also low. In the rat, a 4-week administration in the feed is tolerated without relevant toxicological findings up to the highest test concentration of 12000 mg/kg feed (equivalent to doses of 1253 mg/kg body weight/day and 1191 mg/kg body weight/day in male rats and female rats, respectively).

When applied to the skin of the rabbit, 4-nitro-4’-aminodiphenylamine-2-sulfonic acid does not produce irritation, and it is not, or only slightly, irritating to the rabbit eye. Following application to the skin of the guinea pig 4-nitro-4’-aminodiphenylamine-2-sulfonic acid shows a sensitising effect.

In summary, the overall data indicate that although the substance reproducibly leads to frame-shift mutations in the Salmonella/microsome assay upon metabolic activation, it does not cause any chromosome aberrations in vitro. The substance does not produce any systemic toxicity following oral administration up to doses of 1200 mg/kg body weight and in particular no changes are seen in the differential blood count, the bone marrow and the spleen. Moreover, the substance does not lead to methaemoglobin formation, which is likely to be attributable to the hydrophilic properties of the sulfo group. As the introduction of a sulfo group leads to a reduction in biological effect in vivo, on the whole there is not much reason to suspect any mutagenic effect in vivo.

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (”MAK-Kommission”) of the Deutsche Forschungsgemeinschaft will investigate the necessity for classification of the chemical’s sensitising potential.

2 Name of substance

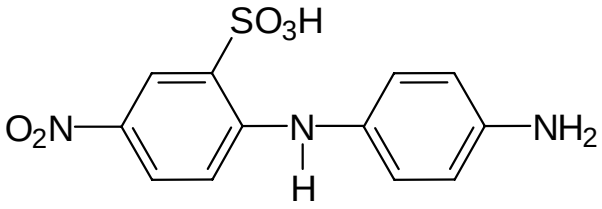
2.1	Usual name	4-Nitro-4’-aminodiphenylamine-2-sulfonic acid
2.2	IUPAC name	4-Nitro-4’-aminodiphenylamine-2-sulfonic acid
2.3	CAS No.	91-29-2
2.4	EINECS No.	202-057-9

3 Synonyms, common and trade names

2-(4-Aminophenylamino)-5-nitrobenzenesulfonic acid
2-(4-Aminophenylamino)-5-nitrobenzolsulfonsäure
4-Nitro-4'-amino-diphenylamine-2-sulfonic acid
4-Nitro-4'-aminodiphenylamin-2-sulfonsäure
4-Nitro-4'-aminodiphenylamin-6-sulfosäure
Nitrophenylparaminsäure
p-Nitrophenylparaminsäure

4 Structural and molecular formulae

4.1 Structural formula



4.2 Molecular formula $C_{12}H_{11}O_5N_3S$

5 Physical and chemical properties

5.1	Molecular mass, g/mol	309.30

5.2	Melting point, °C	No information available	
5.3	Boiling point, °C	Decomposition	(Bayer, 1989)
5.4	Vapour pressure, hPa	No information available	
5.5	Density, g/cm³	No information available	
5.6	Solubility in water	7.8 g/l (at 20 °C)	(Bayer, 1989)
5.7	Solubility in organic solvents	No information available	
5.8	Solubility in fat	No information available	
5.9	pH value	4–5 (at 7.8 g/l water)	(Bayer, 1989)
5.10	Conversion factor	1 ml/m³ (ppm) ≙ 12.62 mg/m³ 1 mg/m³ ≙ 0.08 ml/m³ (ppm) (at 1013 hPa and 25 °C)	

6 Uses

Intermediate in the manufacture of dyestuffs (Hoechst, 1988).

7 Experimental results

7.1 Toxicokinetics and metabolism

No information available.

7.2 Acute and subacute toxicity

An acute toxicity study of 4-nitro-4'-aminodiphenylamine-2-sulfonic acid was carried out in 5 male (195 to 205 g) and 5 female (185 to 188 g) Wistar rats. The substance, formulated in starch gruel, was administered by gavage at a dose of 5000 mg/kg body weight. The observation period was 14 days. During this period no animals died. Thus, the LD_{50} was more than 5000 mg/kg body weight. The clinical signs of toxicity observed on the day of administration included motor hyperactivity, ruffled fur, crouch position, retracted flanks and widening of the palpebral fissure. In all animals the urine showed an orange colour up to 3 days after administration of the sub-

stance. Autopsy at the end of the observation period did not yield any pathological findings (Hoechst, 1983 a).

Each of 5 male and 5 female rats (SPF-Wistar, approx. 150 g and approx. 9 to 14 weeks old) received by gavage a single dose of 5000 mg of the substance in polyethylene glycol 400 per kg body weight (20 ml/kg body weight). The observation period was 14 days. All rats tolerated the treatment without signs of toxicity, and no influence was seen on the development of body weight. This study thus also yielded an LD_{50} of more than 500 mg/kg body weight (Bayer, 1981).

An acute dermal toxicity study of 4-nitro-4'-aminodiphenylamine-2-sulfonic acid was carried out according to OECD guideline No. 402. Each of 5 male and 5 female rats (Tif:RAIf, SPF; initial weight 184 to 224 g) received a single semi-occlusive application to the shaved dorsal skin of 2000 mg of 4-nitro-4'-aminodiphenylamine-2-sulfonic acid (70.3-percent) per kg body weight in a 50-percent formulation with distilled water. The observation period was 14 days. All animals survived. The symptoms observed included dyspnoea, ruffled fur, curved and ventral body position as well as transient sedation and diarrhoea. There were no local reactions. Autopsy revealed no unusual findings. The dermal LD_{50} was > 2000 mg/kg body weight (Ciba-Geigy, 1984).

4-Nitro-4'-aminodiphenylamine-2-sulfonic acid was administered by gavage to two cats (weighing 3.9 and 4 kg) as single doses of 10 and 50 mg/kg body weight, respectively (solutions in polyethylene glycol 400, containing 10 mg/ml and 50 mg/ml). The methaemoglobin content of the animals' blood was determined prior to treatment as well as 3, 7, 24 and 30 hours afterwards (by means of the photometric cyanmethaemoglobin method) as were the numbers of Heinz bodies (nile blue stain, 1000 erythrocytes per smear). The methaemoglobin content measured following administration of 10 mg/kg body weight was 2% at all sampling times and no more than 3% following administration of 50 mg/kg body weight. Moreover, the maximum of 2% for the number of Heinz bodies was not elevated (the reference values in untreated cats are methaemoglobin content: 1.7% (n = 176); content of Heinz bodies: 5% (n = 163)). Following administration of 10 mg/kg body weight impaired general condition and lack of appetite were observed up to 8 hours after the treatment, and lack of appetite up to 8 hours, but no

other impairment of general condition, was also observed following administration of 50 mg/kg body weight (Bayer, 1984).

In a preliminary experiment to a subacute study in rats no signs of toxicity occurred following administration of 2000 or 10000 mg of 4-nitro-4'-aminodiphenylamine-2-sulfonic acid per kg feed during a period of 14 days. Body weight gain and feed consumption were not affected by administration of the test substance. Haematology results revealed no unusual findings. At autopsy the animals exhibited no macroscopic changes (no further data; Hoechst, 1990).

In a subsequent subacute toxicity study 4-nitro-4'-aminodiphenylamine-2-sulfonic acid (purity: 58.9%, 40.2% water) was tested in male and female Wistar rats (approx. 6 weeks old at the beginning of the study) in accordance with the OECD guideline for testing, No. 407. Each of 5 male and 5 female animals received 0 (controls), 480, 2400 or 12000 mg/kg feed for 28 days (corresponding to an average daily substance intake of 50, 243 and 1253 mg/kg body weight by the male rats and 47, 234 and 1191 mg/kg body weight by the female rats). All animals tolerated the treatment without any symptoms even at the highest dosage. All haematological and clinical chemistry parameters under investigation remained unchanged. The urine of the animals in the highest dose group showed brown discoloration. However, urinary status and sediments were not unusual. Post-mortems showed that 2 male rats of the 2400 mg/kg group and all male animals of the 12000 mg/kg group exhibited ocherous kidneys. Histology revealed no cases of pathological findings. The *no observed effect level* was thus found to be 12000 mg/kg feed, which corresponded to an average substance intake of 1253 mg/kg body weight/day and 1191 mg/kg body weight/day by the male rats and the female rats, respectively (Hoechst, 1990).

7.3 Skin and mucous membrane effects

In an acute skin irritancy study of 4-nitro-4'-aminodiphenylamine-2-sulfonic acid (59.8 percent, the rest being water) which was carried out in accordance with OECD guideline No. 404, 3 rabbits (New Zealand albino rabbits) were exposed to 500 mg of the substance under semi-occlusive cover for 4 hours following application to the mechanically depilated dorsal skin. Inspection of the sites took place 30 to 60 minutes and 24, 48 and 72 hours

after the end of exposure. The test substance was not irritating to the skin (Hoechst, 1983 b).

In a another study, 4-nitro-4'-aminodiphenylamine-2-sulfonic acid (purity: 58.1%, the rest being water) was tested in accordance with OECD guideline No. 404 for primary skin irritancy in 3 adult female albino rabbits (strain: HC:NZW, weight: 3.2 to 3.3 kg). The animals were subjected to a single 4-hour exposure to 500 mg of the test substance, mixed into a paste with water and applied semi-occlusively to the mechanically depilated skin of the flanks. The findings were scored after 1, 24, 48 and 72 hours as well as 7 days in accordance with the Draize method. At none of the time points were any signs of irritation observed (average irritation scores for erythema and oedema were 0). 4-Nitro-4'-aminodiphenylamine-2-sulfonic acid thus also proved to be not irritating to the rabbit skin in this experiment (Bayer, 1986).

Each of 3 rabbits (albino New Zealand rabbits) had 100 mg of the test substance (59.8 percent, the rest being water) instilled into the conjunctival sac of the left eye (OECD guideline No. 405). The eyes were scored 1, 24, 48 and 72 hours after administration of the substance. Signs of irritation (clouding of the cornea, diffuse redness and marked swelling of the conjunctiva) were seen in 1 of the 3 treated animals, but the effects were reversible within 3 days (Hoechst, 1983 c). The test substance thus exhibited a mildly irritating effect in this study.

A further eye irritation study of 4-nitro-4'-aminodiphenylamine-2-sulfonic acid (purity: 58.1%, the rest being water) was carried out in accordance with OECD guideline No. 405 in 3 adult female albino rabbits (strain: HC:NZW, weight: 2.9 to 3.6 kg). They were given a single instillation of 100 µl (approx. 50 mg) into the conjunctival sac of one eye, and the results were recorded after 1, 24, 48 and 72 hours as well as after 7 days. Evaluation was carried out in accordance with the Draize method. At none of the time points were any signs of irritation observed (average irritation scores for cornea, iris and conjunctivae were 0). 4-Nitro-4'-aminodiphenylamine-2-sulfonic acid thus proved to be not irritating to the rabbit eye in this study (Bayer, 1986).

7.4 Sensitisation

In order to assess the skin sensitisation potential of 4-nitro-4'-aminodiphenylamine-2-sulfonic acid (60.6% test compound; 3.4% dicondensation pro-

duct, 0.8% p-diaminobenzene, 0.3% chloronitrous acid, 33.2% water), 20 female Dunkin-Hartley guinea pigs (average initial weight: 385 g) were subjected to the Magnusson and Kligman maximisation test in accordance with OECD guideline No. 406. Another 20 animals served as controls. Based on the results of preliminary investigations, intradermal induction and topical induction were carried with a 2.5-percent aqueous solution of the test compound, and the topical challenge was performed using 25-percent and 12.5-percent aqueous solutions. All of the 20 guinea pigs that underwent induction showed positive reactions to both the 25-percent and the 12.5-percent solutions, while the 20 controls did not exhibit skin reactions. 4-Nitro-4'-aminodiphenylamine-2-sulfonic acid thus clearly proved to be sensitising to the guinea pig skin in this study (HRC, 1993).

7.5 Subchronic and chronic toxicity

No information available.

7.6 Genotoxicity

7.6.1 In vitro

4-Nitro-4'-aminodiphenylamine-2-sulfonic acid (purity: 58.5%, the rest being water) was tested in the Salmonella/microsome assay (standard-plate incorporation assay) using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 as well as *Escherichia coli* WP2uvrA at concentrations ranging from 4 up to 5000 µg/plate in the absence and presence of a metabolising system (S-9 mix prepared from Aroclor 1254-induced rat liver). In preliminary studies concentrations of more than 1000 µg/plate proved to be toxic to most of the bacterial strains. In the absence of metabolic activation the test substance exhibited only a weak mutagenic effect in TA 1538, whereas in the presence of metabolic activation it caused a significant concentration-dependent increase in the revertant counts in strains TA 1537, TA 1538 and TA 98 (Hoechst, 1984 a).

In a further Salmonella/microsome assay (standard-plate incorporation assay) 4-nitro-4'-aminodiphenylamine-2-sulfonic acid (purity > 99.7%) was investigated in the *Salmonella typhimurium* strains TA 1538 and TA 98 in the absence and presence of a metabolising system (S-9 mix prepared from

Aroclor 1254-induced rat livers). The concentrations of test substance ranged from 4 up to 5000 µg/plate. In the absence of metabolic activation, the test substance exhibited only a weak, but concentration-dependent effect in strain TA 1538; in the presence of the metabolic activation system, the test substance produced a significant concentration-dependent increase in the number of revertants in strains TA 1538 and TA 98 (Hoechst, 1984 b).

The original reports of the two investigations mentioned above indicate that at the concentration levels of 100 and 250 µg/plate the pure substance (> 99.7%) is 1.4 up to 1.9 times more effective than the technical-grade (58.5-percent) substance is in the comparable strains TA 1538 and TA 98. This corresponds approximately to the ”dilution” of technical-grade 4-nitro-4’-aminodiphenylamine-2-sulfonic acid. At higher concentrations, however, there appears to be a tendency towards a reversal of the effect. The results further indicate that the impurities contained in technical-grade 4-nitro-4’-aminodiphenylamine-2-sulfonic acid do not causally contribute to the substance’s mutagenic effect.

4-Nitro-4’-aminodiphenylamine-2-sulfonic acid (purity: 94.9%) was tested in vitro for chromosome-damaging effects in V79 cells of the Chinese hamster in the absence and the presence of metabolic activation (S-9 mix from Aroclor 1254-induced rat liver). The investigations were carried out in accordance with OECD guideline No. 473. DMSO served as the solvent, and cyclophosphamide and ethylmethane sulfonate served as the positive controls. In the preliminary experiment, 4-nitro-4’-aminodiphenylamine-2-sulfonic acid was soluble up to a concentration of 88.9 µg/ml and precipitated at 158 µg/ml. For this reason, concentrations of 0 (DMSO), 50, 88.9 and 158 µg/ml were used in the main study. In the first series of experiments exposure lasted 5 hours and the cells were prepared 18 hours after the start of treatment (with and without metabolic activation). In the second series the same concentrations were used, the exposure periods being 18 and 28 hours (without metabolic activation), and 5 hours followed by preparation after 18 hours (with metabolic activation). The highest concentration and the negative controls were additionally evaluated after 28 hours (in the absence and presence of metabolic activation). For the purposes of evaluation, the two series of experiments were combined. At none of the preparation times, neither in the absence nor in the presence of metabolic activation, was any significant increase seen in the numbers of cells with chromosome aberrations or the numbers of polyploid cells; no changes in the mitotic index were

detected at the highest concentration. 4-Nitro-4'-aminodiphenylamine-2-sulfonic acid thus did not show any clastogenic activity in this study (Merck, 1997).

7.6.2 In vivo

No information available.

7.7 Carcinogenicity

No information available.

7.8 Reproductive toxicity

No information available.

7.9 Effects on the immune system

No information available.

7.10 Neurotoxicity

No information available.

7.11 Other effects

No information available.

8 Experience in humans

No information available.

9 Classifications and threshold limit values

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area ("MAK-Kommission") of the Deutsche Forschungsgemeinschaft will investigate the necessity for classification of the chemical's sensitising potential (DFG, 2000).

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