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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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3-Amino-4-methoxy-benzanilide

No. 119

CAS No. 120-35-4

BG Chemie
Berufsgenossenschaft der chemischen Industrie

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3-Amino-4-methoxybenzanilide

1 Summary and assessment

The acute toxicity of 3-amino-4-methoxybenzanilide is low (LD_{50} rat oral and dermal > 2000 mg/kg body weight).

The substance is practically devoid of irritation of the skin and eye.

In the Salmonella/microsome test, 3-amino-4-methoxybenzanilide is not mutagenic in the absence of metabolic activation but exhibits concentration-dependent mutagenicity in the majority of strains after metabolic activation. When a micronucleus test was carried out in rats which were treated with two oral doses of 2000 mg/kg body weight at an interval of 24 hours, 3-amino-4-methoxybenzanilide did not induce chromosome mutations in bone marrow cells.

2 Name of substance

2.1	Usual name	3-Amino-4-methoxybenzanilide
2.2	IUPAC name	3-Amino-4-methoxybenzanilide
2.3	CAS No.	120-35-4
2.4	EINECS No.	204-388-4

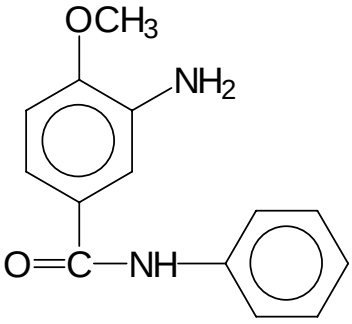
3 Synonyms, common and trade names

3-Amino-p-anisanilid
Aminoanisbase
Aminoanissäureanilid
3-Aminoanissäureanilid
3-Amino-4-methoxybenzanilid
3-Amino-4-methoxybenzoesäureanilid
2-Amino-1-methoxybenzol-4-benzanilid
N-(3-Amino-4-methoxybenzoyl)anilin
3-Amino-4-methoxy-N-phenylbenzamid

3-Amino-4-methoxy-N-phenylbenzamide
Anisbase
4-Methoxy-3-aminobenzanilid

4 Structural and molecular formulae

4.1 Structural formula



4.2 Molecular formula $C_{14}H_{14}O_2N_2$

5 Physical and chemical properties

5.1	Molecular mass, g/mol	242.30
5.2	Melting point, °C	153 (Hoechst, 1985) 154–155 (Hoechst, 1990; Clariant, 1999)
5.3	Boiling point, °C	No information available
5.4	Vapour pressure, hPa	No information available
5.5	Density, g/cm³	No information available
5.6	Solubility in water	1.9 g/l (at 20 °C) (Hoechst, 1990; Clariant, 1999)
5.7	Solubility in organic solvents	Soluble in methanol and acetone (Hoechst, 1991)
5.8	Solubility in fat	No information available
5.9	pH value	Ca. 8 (at 1.9 g/l and 20 °C) (Hoechst, 1990; Clariant, 1999)
5.10	Conversion factor	1 ml/m³ (ppm) ≙ 9.89 mg/m³ 1 mg/m³ ≙ 0.10 ml/m³ (ppm) (at 1013 hPa and 25 °C)

6 Uses

Intermediate for azo pigments (Hoechst, 1991).

7 Experimental results

7.1 Toxicokinetics and metabolism

No information available.

7.2 Acute and subacute toxicity

The acute oral toxicity of 3-amino-4-methoxybenzanilide (99.6% pure) was tested in 5 male and 5 female Wistar rats (mean initial weights 191 and 184 g, respectively) in accordance with OECD guideline No. 401. The dose level was 2000 mg/kg body weight, and the observation period was 14 days. There were no deaths, body weight gain was not depressed and the clinical signs were unspecific and reversible after one day. A necropsy conducted following terminal sacrifice yielded no remarkable macroscopic findings. Thus the LD_{50} was > 2000 mg/kg body weight (Hoechst, 1988 a).

In an exploratory feeding study, 5 deer mice (*Peromyscus maniculatus*, native to North America) were individually offered test substance-treated wheat seeds for a period of 3 days. The animals were placed under observation for 4 days. Compared with the control group, consumption of wheat seeds was reduced by 5%. More than 50% of the mice died within the observation period. The average amount of chemical ingested by the animals was 1188 mg/kg body weight/day (no further details; Schafer and Bowles, 1985).

The acute dermal toxicity of 3-amino-4-methoxybenzanilide TTR (99.9% pure) was tested in 5 male and 5 female Wistar rats (mean initial weights 202 and 199 g, respectively) in accordance with OECD guideline No. 402. The dose level was 2000 mg/kg body weight and the observation period was 14 days. Dermal exposure of the animals was preceded by mechanical depilation of approx. 30 cm² of their dorsal skin. The substance was mixed into a paste with 0.9-percent NaCl solution on a piece of aluminium foil (sized 6 cm x 8 cm), applied to the intact shorn dorsal skin, covered with a

piece of aluminium foil (sized 6 cm x 8 cm) and subsequently sealed with adhesive plaster. After an exposure period of 24 hours, the dressing was removed and the treated area of skin washed off with lukewarm water. Throughout the 24-hour exposure and the 14-day observation period, no signs of intoxication, depression of body weight gain or deaths occurred. The rats which were sacrificed at the end of the 14-day observation period showed no macroscopically visible changes. The acute dermal toxicity of 3-amino-4-methoxybenzanilide was therefore > 2000 mg/kg body weight in male and female Wistar rats (Hoechst, 1991).

The intravenous LD_{50} for the mouse was given as 320 mg/kg body weight (no further details; U.S. Army, year not given)).

7.3 Skin and mucous membrane effects

The primary skin irritancy of 3-amino-4-methoxybenzanilide (99.6% pure) was tested in 3 New Zealand white rabbits (weighing 1.9 to 2.5 kg) in accordance with OECD guideline No. 404. The animals underwent 4 hours' exposure to 500 mg of the chemical (mixed into a paste with 0.3 ml polyethylene glycol 400) which was applied semi-occlusively to an area of about 25 cm^2 of skin on the dorsal trunk which had been shaved with electric clippers 24 hours in advance. Following 4 hours' exposure, any remaining chemical was removed from the skin with lukewarm tap water. The effects were assessed after 30 to 60 minutes and after 24, 48 and 72 hours. The mean irritation indices were 0.1 for erythema and eschar formation and 0.0 for oedema formation. From 48 hours after patch removal onwards, all animals were free of signs of irritation. Thus, 3-amino-4-methoxybenzanilide proved to be practically devoid of irritation in the rabbit skin (Hoechst, 1988 b).

The eye irritancy of 3-amino-4-methoxybenzanilide (99.6% pure) was tested in 3 New Zealand white rabbits (weighing 2.0 to 2.5 kg) in accordance with OECD guideline No. 405. The animals were given a single instillation of 100 mg into the conjunctival sac of one eye, and the results were recorded after 1, 24, 48 and 72 hours. The mean irritation indices for reddening of the conjunctiva, conjunctival swelling, iritis and clouding of the cornea were 0.3, 0.0, 0.0 and 0.0, respectively. Thus, 3-amino-4-methoxybenzanilide proved to be practically devoid of irritation of the eye (Hoechst, 1988 b).

7.4 Sensitisation

No information available.

7.5 Subchronic and chronic toxicity

No information available.

7.6 Genotoxicity

7.6.1 In vitro

3-Amino-4-methoxybenzanilide (purity not specified) was tested in the Salmonella/microsome assay using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 as well as *Escherichia coli* WP2 uvrA at concentration levels of 0, 0.16, 0.8, 4, 20, 100 and 500 µg/plate in the absence and presence of metabolic activation (S-9 mix prepared from Aroclor 1254-induced rat liver). Positive controls which were treated with the chemicals 9-aminoacridine and 2-aminoanthracene, a methylhydrazone derivative or streptocotocin were included in the study. No bacteriotoxic range was observed. In the absence of S-9 mix, none of the bacterial strains were found to have increased revertant counts relative to the corresponding controls. In the presence of the metabolising system, 3-amino-4-methoxybenzanilide produced concentration-dependent increases in the numbers of revertant colonies in all *Salmonella typhimurium* tester strains other than TA 1535 (i.e. in TA 98, TA 100, TA 1537 and TA 1538) at levels of and above 4 µg/plate and in the *Escherichia coli* strain WP2 uvrA at levels of and above 100 µg/plate. The chemical thus exhibited mutagenic activity (Hoechst, 1982).

A further study retested 3-amino-4-methoxybenzanilide (99.9% pure) for mutagenic activity in the Salmonella/microsome plate assay using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 and *Escherichia coli* WP2 uvrA in the absence and presence of metabolic activation (S-9 mix from Aroclor 1254-induced rat liver). The main study was preceded by a range-finding study in all strains. Both studies employed DMSO to aid solubilisation. The chemicals used for positive controls in both studies without metabolic activation were 2-nitrofluorene for strains

TA 98 and TA 1538, sodium azide for TA 100 and TA 1535, 9-aminoacridine for TA 1537 and N-methyl-N'-nitro-N-nitrosoguanidine for *Escherichia coli* WP2 uvrA, while the studies with metabolic activation included 3,4-benzopyrene and 2-aminoanthracene. The range-finding study was carried out using 2 plates/concentration (0, 4, 20, 100, 500, 2500 and 10000 µg/plate). At concentration levels of 2500 and 10000 µg/plate precipitation of the test substance and inhibition of bacterial growth occurred in all 6 strains. In the absence of S-9 mix none of the bacterial strains were found to have increased revertant counts relative to the concurrent controls. In the presence of S-9 mix, 3-amino-4-methoxybenzanilide levels of and above 4 µg/plate generated mostly concentration-dependent increases in revertant counts in all tester strains other than strain TA 1535. The mutagenic effect on *Escherichia coli* WP2 uvrA was weak. The main study used 3 plates per concentration. In the absence of S-9 mix 3-amino-4-methoxybenzanilide was tested in all 6 bacterial strains at concentration levels of 0, 4, 20, 100, 500, 2500 and 5000 µg/plate. The same concentrations were also used when *Escherichia coli* WP2 uvrA was tested with metabolic activation. Tests in the presence of S-9 mix were carried out on the 5 strains of *Salmonella typhimurium* at concentration levels of 0, 0.16, 0.8, 4, 20 and 100 µg/plate; TA 100, TA 98 and TA 1538 were additionally tested at 0.032 µg/plate. In the absence of S-9 mix, revertant counts in the main study as well were not increased in any of the bacterial strains relative to the corresponding controls. In the presence of the metabolising system, 3-amino-4-methoxybenzanilide produced concentration-dependent increases in the numbers of revertant colonies in the *Salmonella typhimurium* tester strains other than TA 1535 (i.e. in TA 98 and TA 100) at levels of and above 0.8 µg/plate and in strains TA 1537 and TA 1538 at levels of and above 4 µg/plate. In the assay performed with *Escherichia coli* WP2 uvrA there were slight but not concentration-dependent increases in revertant counts at test concentrations of and above 20 µg/plate. In the presence of the metabolising system, 3-amino-4-methoxybenzanilide thus exhibited concentration-dependent mutagenicity in the majority of the bacterial strains (Hoechst, 1985).

7.6.2 In vivo

3-Amino-4-methoxybenzanilide (98.6% pure) was tested in accordance with OECD guideline No. 474, the U.S. EPA:OPPTS 870.5395 test guideli-

ne and EEC Directive 92/69 by means of a micronucleus test which was carried out in approx. 6-week-old male and female Hsd:Sprague-Dawley rats with respective mean initial weights of 181.5 and 138.1 g. In both the dose-range finding study and the main study, 3-amino-4-methoxybenzanilide was suspended at a concentration of 20% in a 0.5-percent Tylose H4000 G4 PHA solution and administered to groups of 5 males and 5 females by oral gavage (the volume administered was 10 ml/kg body weight). In the dose-range finding study, a single 2000 mg/kg body weight dose of 3-amino-4-methoxybenzanilide was tolerated without toxic effects over an observation period of 7 days. Therefore, the 10 rats in the main study received two doses of 2000 mg/kg body weight by oral gavage at an interval of 24 hours. The group of vehicle controls (5 male and 5 female rats) were treated with Tylose H4000 G4 PHA in the same manner. The 5 males and 5 females in the positive control group were treated with a single oral dose of 40 mg/kg body weight cyclophosphamide in distilled water. Twenty-four hours after the single dose or the second dose, all 30 rats in the study were sacrificed for examination of bone marrow cells. For each animal, 2000 polychromatic erythrocytes were counted and the number of cells with micronuclei was recorded. The number of individual micronuclei was not ascertained. In addition, the ratio of polychromatic erythrocytes to 200 normochromatic erythrocytes was determined. All rats survived after treatment without exhibiting any signs of toxicity. Dissection of the animals revealed no substance-related macroscopic findings. The incidence of micronucleated polychromatic erythrocytes observed in the group treated with 3-amino-4-methoxybenzanilide was 0.1 ± 0.06% and thus within the range of the Tylose control group (0.2 ± 0.05%). The ratio of polychromatic erythrocytes to total erythrocytes remained unaffected by the test compound (mean values: test group 0.51 ± 0.05, Tylose control group 0.56 ± 0.08). Cyclophosphamide induced a marked increase in the number of polychromatic erythrocytes with micronuclei (1.8 ± 0.38%) and a small but statistically significant reduction of polychromatic erythrocytes relative to total erythrocytes (mean value 0.45 ± 0.06). Under the conditions of the study, 3-amino-4-methoxybenzanilide did not induce chromosome mutations in the rat micronucleus test (Aventis, 2001).

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

No information available.

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