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GDCh-Advisory Committee
on Existing Chemicals of
Environmental Relevance (BUA)

Xylidines
(Dimethylanilines)
BUA Report 161
(June 1994)



S. Hirzel

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Committee on Existing Chemicals
of Environmental Relevance

Beratergremium für
Umweltrelevante Altstoffe (BUA)



S. Hirzel

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Foreword

The German Chemicals Act (Chemikaliengesetz - ChemG) of 1980 stipulates that certain existing chemicals must be reported to the competent authority, if they exhibit properties which indicate that they may be hazardous, either alone or in combination with other substances.

In the summer of 1982, an Advisory Committee on Existing Chemicals of Environmental Relevance (BUA) was set up by the German Chemical Society (Gesellschaft Deutscher Chemiker - GDCh). It brings together representatives from the scientific community, the chemical industry and the governmental authorities. This Advisory Committee is responsible for elaborating appropriate solutions for substances of relevance for health and the environment on the basis of voluntary measures. It selects and examines existing chemicals from the aforementioned angles. The testing and evaluation are based on scientific criteria alone.

It was, therefore, necessary to develop priority setting procedures. In a first phase reports were only prepared for priority chemicals. Within the framework of a first priority setting procedure, chemicals were compiled from several priority lists and 135 chemicals were selected for detailed substance reports.

In a second priority setting procedure the survey of the German Chemical Industry Association (VCI) on all substances with a production volume of more than 10 tons per year was used as a starting list. Since this survey covered 4,600 chemicals, BUA decided to process the corresponding list in several stages. The first stage included approx. 1,050 substances with a production volume of more than 1,000 tons per year.

Detailed reports are drawn up on chemicals suspected of having a hazard potential and abridged reports on those presenting only a minor hazard potential, according to the current state of knowledge.

The detailed BUA reports take in both the published literature and data from industry. If data for the evaluation of the chemicals are not available, additional studies are recommended and the results are published as updates to the reports. The reports serve as a basis for the instigation of administrative measures, when there are indications of risks to health or the environment.

Tübingen, May 1993

Ernst Bayer
Chairman of the Advisory Committee
on Existing Chemicals
of Environmental Relevance

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BUA Report on Xylidines

Summary and conclusions

Ecological aspects

Manufacture, usage and distribution among environmental compartments (discharge, occurrence)

No data are available for the manufacture and processing of **2,3-xylidine** in the Federal Republic of Germany. It may be concluded from the Information available that this compound is of no economic importance. There are also no data available on the manufacture of **2,5-xylidine** and **3,4-xylidine** in the Federal Republic of Germany. Demand in Germany is met by Imports. One German manufacturer produced 100 tonnes **3,5-xylidine** in 1990.

In 1990, around 1 000 tonnes of the **2,4-xylidine**, 1 000 to 2 000 tonnes of the **2,6-xylidine** and around 100 tonnes of the **3,5-xylidine** produced in the Federal Republic of Germany underwent further processing there.

One German further processor currently imports < 1 000 t/a **3,4-xylidine** into the Federal Republic of Germany. Another imports from 100 to 500 t/a **2,4-xylidine** and 1 to 10 t/a **2,5-xylidine** for further processing. No other valid data about imports of xylidines into the Federal Republic of Germany are available. In 1990, about 10 tonnes **2,4-xylidine** and > 196 tonnes **2,6-xylidine** were exported from the Federal Republic of Germany.

No information is available about current further processing in the new German federal states (the former German Democratic Republic).

In the Federal Republic of Germany the various isomers serve mainly as starting materials for the following products:

2,4-xylylidine: dyes (primarily azo pigments)

2,6-xylylidine: the herbicide Metazachlor

3,4-xylylidine: riboflavin (vitamin B2)

3,5-xylylidine: paint pigments

Nothing is known about the extent of direct use of the xylylidines in the Federal Republic of Germany as, e. g., fuel additives, absorption agents for sulphur dioxide in flue-gas desulphurization or as anti-oxidants and anti-ozonants for lubricating oil and rubber as well as corrosion inhibitors for metallic materials.

From the data available, the following amounts of xylylidines are discharged into the atmosphere and hydrosphere in the Federal Republic of Germany (a and b denote different companies):

Isomer	Discharge during	
	Manufacture (reference year)	Processing (reference year)
<u>Atmosphere</u>		
2,4-Xylylidine	Approx. 7 kg y ⁻¹ (1990)	(a) < 25 kg y ⁻¹ (1990) (b) < 0.1 kg y ⁻¹ (1991/92)
2,5-Xylylidine	Currently not produced in FRG	< 1 kg y ⁻¹ (estimated; 1991/92)
2,6-Xylylidine	(a) Approx. 1 kg kg y ⁻¹ (1990) (b) None (1990)	(b) Approx. 20 kg y ⁻¹ (estimated; 1991/92)
3,4-Xylylidine	Currently not produced in FRG	Unknown
3,5-Xylylidine	< 25 kg y ⁻¹ (1993)	(a) 485 kg y ⁻¹ (1990) (b) 0 – 50 kg y ⁻¹ (estimated; 1991/92)
<u>Hydrosphere</u>		
2,4-Xylylidine	max. 3 kg y ⁻¹ (estimated; 1990)	(a) Approx. 350 kg y ⁻¹ (estimated; 1990) (b) Not detectable in waste- water-treatment plant effluent
2,5-Xylylidine	Currently not produced in FRG	< 1 kg y ⁻¹ in influent to wastewater-treatment plant (estimated; 1991/92)
2,6-Xylylidine	(a) 1 t y ⁻¹ in influent to waste- water-treatment plant (estimated; 1990/91) (b) Approx. 0.5 kg y ⁻¹ (estimated; 1990)	(a) 300 kg y ⁻¹ in influent to to wastewater-treatment plant (estimated; 1990/91)
3,4-Xylylidine	Currently not produced in FRG	< 100 kg y ⁻¹ (1994)
3,5-Xylylidine	< 100 kg y ⁻¹ in influent to wastewater-treatment plant (estimated; 1993)	(a) Unknown (1990) (b) Approx. 500 kg y ⁻¹ in influent to wastewater- treatment plant (1991/92)

It is conceivable that **xylidine** isomers are discharged into the air and water from the use of crude-oil and coal products, via waste air and wastewater generated in their production and via residues and decomposition of dyes, pigments and plant-protection agents. However, in view of the data situation, the levels cannot be quantified for the Federal Republic of Germany. Nor is it possible to quantify discharges into the environment arising from use of the compounds as fuel additives, in flue-gas desulphurization or as anti-oxidants or corrosion inhibitors.

No information is available about discharge levels of xylidines into the geosphere, or biosphere. The compounds may enter the geosphere in the form of residues or through decomposition of dyes, pigments and plant-protection agents derived from xylidines; however, there are insufficient data to quantify these discharge levels for the Federal Republic of Germany.

Work-place concentrations of **2,4-xylidine** in coal-liquefaction pilot plants are quoted as being 0.1 to 0.5 mg/m³. None of the other isomers has been analysed for in the work place or the ambient air.

The mean burden in the longitudinal Rhine profile (from Basle to Lobith) was estimated in 1990 at 13 to 17 kg/d **2,4-** and **2,6-xylidine** (equivalent to 4.7 to 6.2 t/a). In 1991, **2,6-xylidine** was not detected in the Rhine or in its confluences with the Sieg, Erft, Ruhr and Lippe tributaries (lower application limit of the method: < 1 µg/l). The Emscher tributary of the Rhine contained 1.7 µg/l of the compound and the Wupper (subsidiary of the Rhine) contained < 0.1 µg/l (lower application limit of the method).

Nothing is known about the occurrence of the **xylidine** isomers in the geosphere.

All **xylidine** isomers have been detected in mineral-oil and coal products (products from coal liquefaction/coal carbonization, coal tar, coal oils, light oil, diesel oil, fuel oil). For example, distillate from coal-liquefaction products contained between 47 mg/kg for

2,3-xylidine and 370 mg/kg for **3,5-xylidine** determined along with *m*-ethyl aniline, and the water-soluble fractions of fuel oils varied in content from qualitative detection of 2,3- and **2,6-xylidine** to 0.33 mg/l for **3,5-xylidine**.

The **2,3-**, **2,5-**, **2,6-** and **3,4-isomers** of **xylidine** have been detected in tobacco leaves. Blood taken from smokers contained low picogramme quantities of the **2,3-**, **2,4-**, **2,5-** and **2,6-**isomers.

Degradability

Owing to their moderate volatility, xylidines that have been discharged into the hydrosphere will volatilize only in small quantities to the atmosphere where they are photochemically oxidized with a calculated half life $t_{1/2}$ of 1.75 - 2 hours.

Laboratory experiments performed in aerobic conditions with acclimated inoculum show that xylidines discharged into water may undergo elimination or primary degradation in the hydrosphere. The compounds' UV-absorption properties make direct photo-transformation in the upper most water layers unlikely.

One laboratory trial has shown that most of the **2,6-xylidine** discharged into soil is adsorbed on soil particles. At least partial biodegradation is expected in aerobic conditions in soil.

Anaerobic degradation in the hydrosphere or soil has not been studied.

Bioaccumulation

Results of laboratory tests on the bioaccumulation of **2,5-**, **2,6-** and **3,4-xylidine**, together with measured and calculated log P_{OW} values,

suggest that the **xylidine** isomers have a weak potential for bioaccumulation.

Ecotoxicological effects

Cell-Proliferation-Inhibition tests revealed a 16-hour toxic limiting concentration (TLC) of 8 mg/l **2,4-xylidine** for *Pseudomonas putida* and an 8-day TLC of 0.43 mg/l for the cyanobacterium *Microcystis aeruginosa*. The same type of test on **2,4-xylidine** yielded a 24-h IC₅₀ value of 351 mg/l (minimum inhibiting concentration, MIC: 994 mg/l) for *Escherichia coli* and a 96-h MIC value of 87 mg/l for *Mycobacterium smegmatis*.

The Cell-Proliferation-Inhibition test involving beer yeast (*Saccharomyces cerevisiae*) yielded an IC₅₀ value of 775.5 mg/l for proliferation in the exponential growth phase and a 24-h IC₅₀ value of 824.0 mg/l for the cell density.

The Oxygen-Consumption-Inhibition test involving activated sludge returned an EC₂₀ value of 254.7 mg/l and an EC₅₀ value > 1 000 mg/l (nominal values) for **2,6-xylidine**.

The Static Cell-Proliferation-Inhibition test produced 48-h EC₅₀ values ranging from 235 mg/l (**3,4-xylidine**) to 329 mg/l (**2,6-xylidine**) for the effect of **2,3-**, **2,4-**, **2,5-**, **2,6-**, **3,4-** and **3,5-xylidine** on ciliates (*Tetrahymena pyriformis*).

The Cell-Proliferation-Inhibition test for the effect of **2,4-xylidine** on protozoa yielded a 72-h TLC concentration of 9.8 mg/l for *Entosiphon sulcatum*, a 20-h TLC of 12 mg/l for *Uronema parduczi* and a 48-h TLC of > 40 mg/l for *Chilomonas paramecium*.

2,6-xylidine applied to silty clay at a rate of 100 mg/kg caused significant inhibition of denitrification (anaerobic incubation; 36 h).

Nitrite and dinitrogen monoxide accumulated temporarily in the test system.

Cell-Proliferation-Inhibition tests performed on the unicellular green alga *Scenedesmus subspicatus* for 48 and 168 hours yielded the following results:

		IC ₁₀	IC ₅₀
		(mg/l)	
2,3-xylydine	48 h	19	44
2,4-xylydine	168 h	0.75	11.6
3,4-xylydine	48 h	5.5	24

A 30-minute IC₁₀ value of 0.21 mg/l and an IC₅₀ value of 9.34 mg/l were measured as the effect of **2,4-xylydine** on the fluorescence of *Scenedesmus subspicatus* in a flow-through cuvette following a pulse of light.

Photosynthesis-Inhibition tests on *Selenastrum capricornutum* in the exponential growth phase yielded a 4-h EC₅₀ value of 50 mg/l **2,3-xylydine**, as measured by CO₂ fixation. On addition of fulvic acid to act as humic material, the effective concentration fell markedly to an EC₅₀ of 1.8 mg/l.

In studies of the effect of **2,4-xylydine** on photosynthesis in protoplasts (cells with no walls) of the broad bean (*Vicia faba*), the EC₂₀ value for inhibition of light-dependent oxygen evolution after 15 - 20 minutes' exposure was 6.1 mg/l (EC₁₀: 0.008 mg/l); the EC₂₀ value for inhibition of the CO₂ enzyme ribulose-1,5-biphosphate carboxylase was 157.5 mg/l (EC₁₀: 36.3; EC₅₀: 957.3 mg/l) after 36 hours.

The following concentrations for the acute effect of xylydines on the swimming ability of the water flea *Daphnia magna* were obtained experimentally:

		EC ₀ (mg/l)	EC ₅₀ (mg/l)
2,3-xylydine	24 h	1.6	10
2,4-xylydine	24 h	0.4 - 3.1	16 - 650
	48 h	3.1	9.9
3,4-xylydine	24 h	0.2	2.9

The extended Daphnia test yielded an NOEC value of 0.16 mg/l for re production for 21 days' exposure to **2,3-xylydine**; the NOEC for **3,4-xylydine** was 0.016 mg/l (nominal values).

In tests of the acute toxicity of **2,4-**, **2,6-** and **3,5-xylydine** towards different freshwater fish, the lowest LC₅₀ value (for **3,5-xylydine**) after 48 hours' exposure was 17.0 mg/l. Otherwise the 24-h to 48-h LC₅₀ values lay in the range 35 - 196 mg/l.

The 18-h LD₅₀ for **3,4-xylydine** (administered as a single oral dose) was 10 mg/kg in the starling (*Sturnus vulgaris*) and 5.6 mg/kg in the red-shouldered blackbird (*Agelaius phoeniceus*).

Toxicological aspects^{*)}

2,4-, 2,5-, 2,6- and **3,4-xylidine** are absorbed well from the gastro intestinal tract and are excreted either unchanged or metabolized along with the urine. The main metabolite of **2,4-xylidine** in the urine of rats is N-acetyl-4-amino-3-methylbenzoic acid and in the urine of dogs, 6-hydroxy-2,4-xylidine. **2,6-xylidine** is primarily metabolized to 4-hydroxy-2,6-xylidine by rats and dogs; the urine of rats also contained 4-hydroxy-2,5-xylidine as main metabolite after application of **2,5-xylidine**. The metabolites of **3,4-xylidine** in rats were 4-acet-amido-2-methylbenzoic acid, 2-amino-4,5-dimethylphenyl sulphate and 4-amino-2-methylbenzoic acid. No information is available about absorption and metabolism of **2,3-** or **3,5-xylidine** but absorption is expected after oral application on account of toxic effects.

The effect of the xylidine isomers at different end points is shown schematically in the table (p. XIV).

Most xylidines are harmful to health after single, oral application. **2,4-xylidine** is poisonous by inhalation (tested as aerosol).

Methaemoglobin formation induced in adult cats by acute application of xylidine isomers was pronounced for **2,5-** and **3,5-xylidine**, moderate for **2,3-** and **3,4-xylidine** and weak for **2,4-** and **2,6-xylidine**. **2,4-xylidine** is an eye irritant and a weak skin irritant. **2,6-xylidine** is a weak eye irritant and a skin irritant. **3,5-xylidine** is a weak eye irritant but not a skin irritant.

No data on the sensitization effect of xylidines are available.

Repeated oral application of **2,4-xylidine** results in extensive dose dependent liver and kidney damage.

*) Toxicological data on 2,4-Xylidine were taken from (with permission): "Toxikologische Bewertung Nr. 64 2,4-Xylidin (8/93)", Berufsgenossenschaft der chemischen Industrie. Heidelberg, Germany.

Toxicological data for xylidine isomers

Isomer	Acute toxicity Rats		Irritation (I) Sensitization(S)	Repeated dose toxicity	Mutagenicity <i>in vitro</i>		Geno- toxicity <i>in vivo</i>	Carci- noge- nicity	Repro- duction toxicity
	LD ₅₀ oral in mg kg ⁻¹	inhalation LC ₅₀ (4 h)			Bacteria	Mam- malian cells			
2,3- Xylidine	930	N.I.	I skin: N.I. I eyes: N.I. S: N.I.	N.I.	+ ^a	N.I.	N.I.	N.I.	N.I.
2,4- Xylidine	470- 600	1.53 mg/l	I skin: weak irritation I eyes: irritant S: N.I.	Rat (inhalation. 28 d): Hepatotoxic, thrombo-cythaemia LOAEL 0.03 mg/l (6 h/d) Rat: (feed, 6 m): hepatotoxic and nephrotoxic ^b , target cell anaemia, NOEL < 375 ppm	+	+	N.I.	+ ?	N.I.
2,5- Xylidine	1 300	N.I.	I skin: N.I. I eyes: N.I. S: N.I.	Rat (p.o.. 4w): Hepatotoxic, lowered haematocrit values+ haemoglobin concentrations Dog (p.o.. 4 w): hepatotoxic, NOEL 2 mg/kg/d	+ ^a	+ ^a	N.I.	+ ?	N.I.
2,6- Xylidine	840- 1 300	N.I.	I skin: irritant I eyes: weak irritation S: N.I.	Rat/mouse (p.o.. 12 d): Rat/mouse polychromasia; rat leucocytosis, anisocytosis, poikilocythaemia, NOEL both species 160 mg/kg/d Rat (p.o., 13 w): Lowered haematocrit value and haemoglobin concentrations, reduced lympho- and leucocyte count, LOEL 20 mg/kg/d Rat (feed, 26 w): nephrotoxic ^c , weakly hepatotoxic	- ^d	+	+/- ^e	+	N.I.
3,4- Xylidine	810	N.I.	I skin: N.I. I eyes: N.I. S: N.I.	N.I.	+	N.I.	N.I.	N.I.	N.I.
3,5- Xylidine	710	N.I.	I skin: not irritant I eyes: weak irritation S: N.I.	N.I.	- ^d	N.I.	N.I.	N.I.	N.I.

a Approximately as many positive as negative results

b After subchronic or chronic application of high doses

c After chronic application of high doses

d Weak or no mutagenic effect

e Negative: Micro test, SLRL test, UDS test; positive: DNA binding

N.I. No information

+ Positive

- Negative

+ ? Positive, but doubtful
validity

XX

Six months' administration of **2,4-xylidine** hydrochloride in rat feed yielded a no-effect level < 375 ppm (< approx. 19 mg/kg/d body weight).

The no observed adverse effect level for rats repeatedly inhaling **2,4-xylidine** as an aerosol/vapour mixture is 0.03 mg/l (6 ppm); this concentration caused only marginal effects (closed eyelids, minimum retardation of body weight gain in males).

Repeated oral application of **2,5-xylidine** and **2,6-xylidine** also lead to hepatotoxic effects. Long-term application revealed **2,6-xylidine** also to be nephrotoxic. In rats, **2,4-xylidine** is more toxic than **2,5-** or **2,6-xylidine** whereas in dogs, **2,6-xylidine** is the most toxic.

As for mutagenicity of the xylidines, positive results for bacterial test systems have been obtained only after metabolic activation. In the Ames test, **2,3-**, **2,6-** and **3,5-xylidine** yielded approximately equal numbers of positive and negative results while most tests on **2,4-**, **2,5** and **3,4-xylidine** were positive.

Mutagenicity tests on eucaryotic cells were predomiinantly positive for **2,4-** and **2,6-xylidine** and a positive and a negative result were obtained for **2,5-xylidine**.

Data obtained *in vivo* revealed no evidence of a mutagenic effect exerted by **2,6-xylidine**.

Carcinogenicity tests on **2,4-** and **2,5-xylidine** suggest a carcinogenic potential. However, a definitive statement in this regard is not possible owing to inadequate experimental procedure and documentation. Preliminary evidence of a carcinogenic effect of **2,6-xylidine** has been obtained.

No useful results concerning the reproduction toxicity of the xylidines are available.

Recommendations

Ecological aspects

Not enough data on the effects of the isomers **2,5-**, **2,6-** and **3,5-xylylidine** have been obtained. Only acute fish toxicity has been studied. For final clarification of the potential ecotoxic effects of these isomers, studies of acute alga and daphnia toxicity are recommended.

On the basis of the available studies of the biodegradation of **2,5-xylylidine**, this isomer may only be classified as not readily degradable. Tests of inherent degradability are recommended should relevant discharge into the hydrosphere and atmosphere occur.

If **2,3-xylylidine** were to become economically important in the Federal Republic of Germany in the future (e.g., if it were to be manufactured, or imported or processed in large amounts, the following must be clarified in tests:

- Exposure date
- Inherent degradability
- Acute fish toxicity

Toxicological aspects

No Information concerning skin and eye Irritation is available for **2,3-**, **2,5-** or **3,4-xylylidine**. Testing of **3,4-xylylidine** is recommended. Since exposure to **2,3-** and **2,5-xylylidine** does not take place, such a test is not a priority for these compounds.

None of the isomers has been tested for sensitization potential. Putative carcinogens such as **2,4-**, **2,5-** and **2,6-xylylidine** do not need priority testing; nor does **2,3-xylylidine** since exposure to it does not occur. Testing of **3,4-** and **3,5-xylylidine** is recommended.

In vivo mutagenicity of **3,4-xylidine** should be assessed by a chromosome-aberration test. There is no urgent need to test **3,5-xylidine** *in vivo* since the *in vitro* genotoxicity is very weak and since testicular DNA synthesis was not inhibited after intraperitoneal administration. Rather, the genotoxicity should first be clarified further *in vitro* by an Ames test with and without norharman.

Repeated-dose toxicity should be tested first on **3,4-xylidine** as it is the product produced in by far the greatest amount. Should its toxicity profile closely resemble that of the other studied xylidine isomers, it would be possible to estimate the subacute toxicity of **3,5-xylidine**, especially in view of the comparable acute toxicity of **3,4-** and **3,5-xylidine** and structure-effect relationships. Testing of **2,3-xylidine** is not a priority since it is not manufactured.

Test of reproduction toxicity are not urgent for the following: **2,4-**, **2,5-** and **2,6-xylidine** (suspected carcinogens), **2,3-xylidine** (not manufactured and therefore no exposure) and **3,4-** and **3,5-xylidine** (intermediates).