Aminopyridines

OVERVIEW

2-Aminopyridine, 3-aminopyridine, and 4-aminopyridine came to the attention of the NCI Division of Cancer Biology as representatives of the monoaminopyridines, a class of chemicals with a significant gap in knowledge as to their chronic toxicity and carcinogenic activity. Information on chemical identification, exposure, and toxicity of each aminopyridine is discussed separately in this review. A structure-activity analysis for the class of chemicals and the references are presented at the end of the document.

Of the three monoaminopyridines, 2-aminopyridine appears to have the highest production, ranging from 10,000 to 500,000 pounds from 1986-2002 except in 1998 when production climbed to 1,000,000 to 10,000,000 pounds. 2-Aminopyridine has various uses, but is primarily a starting material in the production of various drugs. Information in the patent literature suggests many possible uses for 3-aminopyridine, and this chemical is clearly a chemical of commerce. 4-Aminopyridine has various uses, but is most noted as an experimental drug for the treatment of various neurological diseases and as a somewhat controversial bird "frightening" agent that also causes the death of both target and non-target species.

Readily absorbed through the skin and the gastrointestinal tract, monoaminopyridines are widely distributed in the body, including the brain. Studies in animals and humans have shown that the monoaminopyridines are acutely toxic compounds. Part of this toxic response may be due to its ability to block K^+ channels causing, among other effects, convulsions or seizures. The structurally similar compound, pyridine, was positive in a 2-year rodent carcinogenesis assay. Based on structure-activity analysis and actual data, the monoaminopyridines are unlikely to be mutagenic in standard genotoxicity assays.

DATA GAPS IDENTIFIED BY NCI

The following studies were identified as data gaps needed to characterize the toxicity of the

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aminopyridines:

- Complete toxicological characterization, including histopathology, in a 2-year carcinogenesis bioassay of 2-aminopyridine.
- Evaluation of 3- and 4-aminopyridine in short-term mechanistic studies to characterize the similarities and differences among this class of chemicals.
- Conduct comparative neurotoxicity studies, as needed, to characterize similarities and differences among the monoaminopyridines.

INPUT FROM GOVERNMENT AGENCIES/INDUSTRY

In 2001, Dr. John Walker, Executive Director of the TSCA Interagency Testing Committee (ITC), EPA, provided information on the annual production range of 2-pyridinamine (2-aminopyridine).

An earlier draft of the Aminopyridines Summary Sheet was reviewed by the Chemical Selection Working Group (CSWG) in July 2003. At that time, Dr. Walker added that the ITC was investigating halogenated pyridines and the NTP has concluded that some of them are carcinogenic. CSWG members expressed additional concerns about the neurotoxicity of this class of chemicals that can easily pass through the blood-brain barrier. The group noted the high 1998 production volume for 2-aminopyridine and asked that this quantity be confirmed through the ITC's use of a Preliminary Assessment Information Rule (PAIR) under section 8(a) of TSCA.

Based on the request of the CSWG, the pyridinamines, 2-pyridinamine, 3-pyridinamine, and 4-pyridinamine, were added to the Priority Testing List to obtain information on annual production and importation volumes and trends; information on specific uses; and estimates of the number of persons potentially exposed during manufacture and use (53rd Report of the TSCA Interagency Testing Committee) (EPA, 2004). Based on the response received, which did not support an increasing trend in the use of 2-aminopyridine, the ITC removed the three pyridinamines from the Priority Testing List because of low potential for occupational exposure

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(56th Report of the TSCA Interagency Testing Committee) (EPA, 2005a).

It should be noted that under PAIR, producers and importers of a listed chemical are exempted from reporting if production or importation is for the sole purpose of research and development or if production or importation is less than 500 kilograms during the reporting period at a single plant site. Companies whose total annual sales from all sites are below $30 million for the reporting period and who produced or imported less than 45,400 kilograms of the chemical are also exempt.

NOMINATION OFAMINOPYRIDINES TO THE NTP

Based on a review of the literature available as of December 15, 2005, and the recommendations of the Chemical Selection Working Group (CSWG) on that date, NCI nominates this chemical for testing by the National Toxicology Program (NTP) and forwards the following information:

- The attached Summary of Data for Chemical Selection
- Copies of references cited in the Summary of Data for Chemical Selection and Dr. Walker's post-meeting comments
- CSWG recommendations to:

(1) Conduct genotoxicity tests on all three aminopyridines.
(2) Conduct short term mechanistic studies on all three aminopyridines.
(3) Comparative studies of metabolism

The CSWG assigned the priority for testing these chemicals as high.

Comments:
The primary concern regarding these chemicals is the lack of information to permit QSAR analysis for chronic effects for the entire class of monoaminopyridines even though it is clear that they possess biological activity.

The limited number of workers exposed during manufacture, as determined by the PAIR rule, does not take into account downstream uses or exposure potential.

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SUMMARY OF DATA FOR CHEMICAL SELECTION

CHEMICAL IDENTIFICATION

2-Aminopyridine

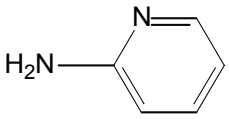
CAS Registry Number: 504-29-0

Chemical Abstracts Service Name: 2-Pyridinamine (9CI)

Synonyms and Trade Name: α-Aminopyridine, 2-pyridinamine, 2-pyridylamine, o-aminopyridine, 2-AP (ChemID, 2003; Trochimowicz *et al*., 1994)

Structural Class: Heterocyclic aromatic tertiary amine derivatives

Structure, Molecular Formula, and Molecular Weight:



$C_5H_6N_2$ Mol. wt.: 94.12

Chemical and Physical Properties:

Description: White leaflets or large colorless crystals (Lewis, 2002; Merck, 2001)

Boiling Point: 210.6 ºC (Lewis, 2002; Merck, 2001)

Melting Point: 57.5 ºC, 58.1 EC (Lewis, 2002; Lide, 2003; Merck, 2001)

Flash Point: 92 ºC (Trochimowicz *et al*., 1994)

Density/Specific Gravity: 1.065 at 20 ºC (Scriven *et al*., 1996)

Log $K_{o/w}$: 0.49 (measured) (Hansch *et al.*, 1995)

Solubility: Soluble in water, alcohol, benzene, ether, hot petrol ether, acetone and organic solvents; slightly soluble in chloroform (Lewis, 2002; Lide, 2003; Merck, 2001)

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Reactivity: Incompatible with acids; highly flammable (Sigma-Aldrich, 2003)

Technical Products and Impurities:

2-Aminopyridine (≥99.0%) is available from the Aldrich division of Sigma-Aldrich (Sigma-Aldrich, 2005).

2-Aminopyridine exists as a mixture of tautomeric amino and imino isomers with the predominance of the amino form over the imino form (1000:1) (Shimizu *et al*., 1993).

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EXPOSURE INFORMATION

Producers:

Chemical Sources International (2003) lists 27 U.S. suppliers for 2-aminopyridine.

According to recent issues of chemical directories, 2-aminopyridine is manufactured or distributed by Aceto Corporation; Alfa Aesar/Johnson Matthey Co.; Austin Chemical Co., Inc.; CBC (America) Corp.; Eastar Chemical Corporation; NetChem & Laboratory, Inc.; Reilly Industries Inc.; Richman Chemical Inc.; Seal Sands Chemicals Ltd./Cambrex Co. (Hunter, 2002; Moynihan, 2002; Tilton, 2002).

Production:

Pyridine is commercially produced by synthesis or by isolation from natural sources such as coal tar. 2-Aminopyridine is manufactured using the reaction of pyridine with sodium amide (Chichibabin amination). It is obtained in high yield after the hydrolysis of the intermediate salt (Merck**,** 2001; Shimizu *et al*., 1993).

Production/Import Level:

The EPA's Inventory Update Rule reports nonconfidential production ranges of chemicals every four years. The production levels of 2-aminopyridine during the years 1986-2002 are listed in Table 1.

Table 1. Production Levels of 2-Aminopyridine

Year	Production Range (lbs.)
1986	10,000 - 500,000
1990	10,000 - 500,000
1994	10,000 - 500,000
1998	> 1,000,000 - 10,000,000
2002	10,000 - 500,000

Source: EPA (2005b)

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2-Aminopyridine is not listed in the EPA High Production Volume (HPV) Challenge Program. 2-Aminopyridine is listed as a LPV chemical in the European Union, meaning that 10 to 1,000 metric tons were produced or imported from 1990-1994. European producers of 2-aminopyridine are EUTICALS S.P.A., Novartis Grimsby UK Ltd. (formerly Ciba-Geigy Grimsby), and RHODIA LTD (European Chemicals Bureau, 2005).

The Port Import/Export Reporting Service (PIERS) reported 2-aminopyridine exports with a cargo weight of 3,516 pounds over the 7-month period from August 2002 to March 2003. For the 7-month period between September 2001 and April 2002, PIERS also reported exports of aminopyridines as a solution, with a cargo weight of 108,762 lbs (Dialog Information Service, 2003). No additional imports or exports of any monoaminopyridines were identified in the period between March 2003 and October 2005.

The US International Trade Commission (USITC) reported 2-aminopyridine as an end-use chemical for the years 1989-1993. No U.S. production data in the ten most recent volumes of *Synthetic Organic Chemicals, US Production and Sales* was shown for the years 1984-1993. This source is no longer published (US International Trade Commission, 1990, 1991, 1993, 1994a,b).

Use Pattern:

The primary use of 2-aminopyridine is as an intermediate in the manufacturing of pharmaceuticals, particularly antihistamines and piroxicam. 2-Aminopyridine may also be an intermediate in the production of ciclopiroxolamine, diphenpyramide, methaqualone, propiram fumarate, pyrilamine, triprolidine, and zomepirac (Carlucci *et al*., 1989; Lewis, 2002; Merck, 2001; Sittig, 1988).

A total of 1,655 patents containing the name "2-aminopyridine" were on file with the U.S. Patent and Trademark Office between 1976 and October 2005. Many of these patents refer to 2-aminopyridine as a reactive chemical used in the synthesis of a host of other chemicals (US Patent and Trademark Office, 2005).

Human Exposure:

Occupational Exposure: Occupational exposure to 2-aminopyridine may occur via dermal contact with this compound at workplaces where it is produced or used (HSDB, 2005).

The National Occupational Exposure Survey (NOES), which was conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983, estimated that 3,159 workers, including 429 females, in 61 facilities representing 3 industries were potentially exposed to 2-aminopyridine (Sigma-Aldrich, 2003). The NOES database does not contain information on the frequency, level, or duration of exposure to workers of any chemical listed therein.

Consumer Exposure: 2-Aminopyridine is both a synthesis precursor and a decomposition product by acidic cleavage of the NSAID piroxicam and was found as an impurity in piroxicam capsules and suppositories at a concentration of 0.1 percent (Carlucci *et al*., 1989). This suggests that patients taking medications derived from 2-aminopyridine may receive exposure to low concentrations of 2-aminopyridine.

Environmental Exposure: 2-Aminopyridine's production and use as an intermediate for antihistamines and other pharmaceuticals may result in its release to the environment through various waste streams (HSDB, 2005).

Environmental Occurrence:

If released to air, 2-aminopyridine will exist solely as a vapor in the ambient atmosphere. Vapor-phase 2-aminopyridine will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 19 hours. If released to soil, 2-aminopyridine is expected to have very high mobility. The pKa of 2-aminopyridine is 6.86, indicating that this compound will partially exist in the protonated form in the environment and cations generally adsorb to organic carbon and clay more strongly than their neutral counterparts. Volatilization from moist soil surfaces is not expected to be an important fate process. 2-Aminopyridine will not

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volatilize from dry soil surfaces based upon its vapor pressure. 2-Aminopyridine degrades slowly in soil. In one study, 2-aminopyridine was found to completely degrade in greater than 96 days under both aerobic and anaerobic conditions. If released into water, 2-aminopyridine is not expected to adsorb to suspended solids and sediment in water. Volatilization from water surfaces is not expected to be an important fate process. Aminopyridines may be susceptible to photochemical degradation. Biodegradation in water may slowly occur based upon biodegradation studies in soil. Measured BCFs of 3.0-7.7 and <5.1-25 at a concentration of 0.1 and 0.01 mg/l, respectively, suggests bioconcentration in aquatic organisms is low (HSDB, 2005).

Limited information on the toxicity of 2-aminopyridine in terrestrial or aquatic species was found. The 5-30 minute EC_{50} for 2-aminopyridine in *Photobacterium phosphoreum* was 284 mg/l. The 60-hr EC_{50} in *Tetrahymena pyriformis* was 390 mg/L (Verscheuren, 2001). The oral LD_{50} of 2-aminopyridine in the quail was reported to be 133 mg/kg (RTECS, 2000).

Regulatory Status:

The Occupational Safety and Health Administration (OSHA) permissible exposure limit for 2-aminopyridine is 0.5 ppm (2 mg/m^3) averaged over an eight-hour work shift. The NIOSH recommended exposure limit (REL) for 2-aminopyridine is 0.5 ppm (2 mg/m^3) averaged over a ten-hour work shift (RTECS, 2000). The American Conference of Governmental Industrial Hygienists (ACGIH) recommended threshold limit value (TLV) for 2-aminopyridine is a time-weighted average (TWA) of 0.5 ppm (1.9 mg/m^3) (ACGIH, 2001; ACGIH, 2005).

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TOXICOLOGICAL INFORMATION

Human Data:

No epidemiological studies or case reports investigating the exposure of 2-aminopyridine and cancer risk in humans were identified in the available literature.

Several case reports have described 2-aminopyridine intoxication in workers. Inhalation of 2-aminopyridine at 5 ppm for 5 hours during milling operations resulted in a severe pounding headache, nausea, flushing of the extremities, and elevated blood pressure, but the operator recovered fully by the next day. Another case report described severe headache followed by convulsions and a stuporous state. More severe human poisoning incidents have been reported, involving convulsions, respiratory failure, and death. For example, a chemical plant worker died after exposure from a spill during distillation. He continued to work in contaminated clothing for 1½ hours after the spill. 2-Aminopyridine is a severe skin and eye irritant and is readily absorbed through intact skin. 2-Aminopyridine is considered toxic by all routes although skin and eye contact and inhalation are the primary routes of exposure (ACGIH, 2001; HSDB, 2005; Reilly Industries, Inc., 2005; Trochimowicz *et al*., 1994).

Animal Data:

Acute Toxicity: 2-Aminopyridine (0.02 M aqueous solution) caused a slight, transient eye injury on the rabbit cornea (Trochimowicz *et al*., 1994). 2-Aminopyridine lacked skin sensitization in the Buehler Topical Closed Patch Skin Sensitization Test; testing per DOT protocol showed 2-aminopyridine to be negative for corrosivity (Reilly Industries, Inc., 2005).

The LD_{50} values for 2-aminopyridine are given in Table 2.

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Table 2. Acute Toxicity Values for 2-Aminopyridine

Species	Route of administration	LD_{50} (mg/kg)	Reference
Mouse	oral	50	ACGIH, 2001
		145	RTECS, 2000
Rat	oral	200	ACGIH, 2001; RTECS, 2000
Rabbit	dermal	>1,000	Reilly Industries, 2005
Guinea pig	dermal	500	RTECS, 2000

In the LD_{50} dermal study in guinea pigs, deaths were associated with convulsions following exposure to 500 mg/kg (Trochimowicz *et al*., 1994).

Subchronic and Chronic Toxicity: No information on the subchronic or chronic toxicity of 2-aminopyridine was found in the available literature.

Short-Term Tests:

Several investigators have examined the mutagenic potential of 2-aminopyridine.

- 2-Aminopyridine was not mutagenic in *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 at concentrations up to 10,000 μg/plate. Mutagenicity was not enhanced by Aroclor-induced rat or hamster S-9 (Zeiger *et al*., 1987).

- 2-Aminopyridine was not mutagenic in *Salmonella typhimurium* TA98 in the presence or absence of S-9. Mutagenicity was not enhanced in the presence of norharman at 200 μg/plate with or without activation (Sugimura *et al*., 1982).

- 2-Aminopyridine was not mutagenic in *Escherichia coli* strain Sd-4-73 using a paper-disk method (Szybalsky, 1958).

Metabolism:

2-Aminopyridine is readily absorbed through the skin (Trochimowicz *et al*., 1994).

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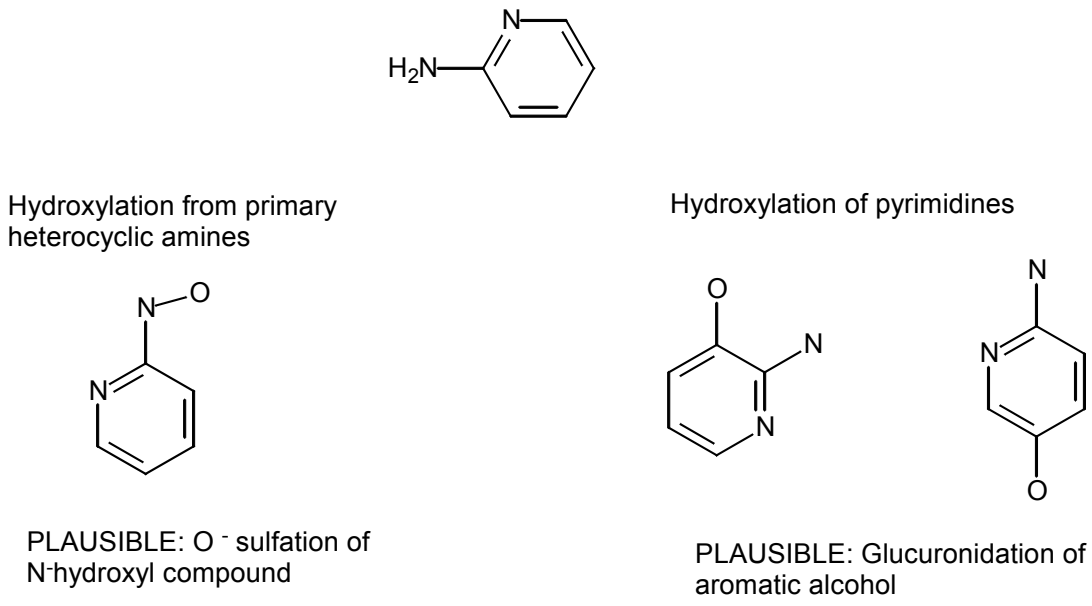
The predictive program, METEOR described the formation of hydroxylamines from primary heterocyclic amines and the hydroxylation of pyrimidines as plausible metabolic pathways for 2-aminopyridine (LHASA, Ltd, 2004). These reactions are shown in Figure 1.

Figure 1: Plausible metabolites of 2-aminopyridine



2-Aminopyridine was identified as one of the major urinary metabolites of the carcinogen *2-N*-nitroso-*N*-methylaminopyridine (2-NMPY) (Heydt-Zapf *et al*., 1983). 2-Amino-pyridine is also a major metabolite of 2-isopropylaminopyrimidine, a drug that was implicated in several cases of hepatitis (Martinat *et al.*, 1992). 2-Aminopyridine was also detected in the urine of male Sprague-Dawley rats treated with methapyrilene, a compound that induces liver tumors in rats (Kammerer *et al.*, 1988).

Other Biological Effects:

Blockage of K^+ Channels: 2-Aminopyridine selectively blocked the K^+ channels of the squid axon internal or external membranes. The steady-state block of K^+ channels was

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more complete for low depolarization, and was gradually relieved at higher depolarizations (Yeh *et al.*, 1976).

Neurological Effects: Injection (intraperitoneal [ip]) of 2-aminopyridine precipitated convulsions in mice (HSDB, 2005).

Aminopyridines are capable of increasing acetylcholine release and spontaneous transmitter release in response to nerve stimulation (Bowman *et al*., 1977).

Other Effects on the Nervous System: Injection (intravenous [iv]) of 2-aminopyridine induced an increase in the blood pressure and respiratory rate in cats (HSDB, 2005).

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CHEMICAL IDENTIFICATION

3-Aminopyridine

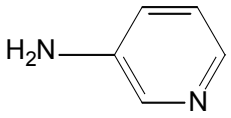
CAS Registry Number: 462-08-8

Chemical Abstracts Service Name: 3-Pyridinamine (9CI)

Synonyms and Trade Name: 3-Aminopyridine, 3-pyridinamine, 3-pyridylamine, m-aminopyridine, amino-3 pyridine (ChemID, 2003)

Structural Class: Heterocyclic aromatic tertiary amine derivatives

Structure, Molecular Formula, and Molecular Weight:



$C_5H_6N_2$ Mol. wt.: 94.12

Chemical and Physical Properties:

Description: Brown crystals (ChemicalLAND21.com, 2003)

Boiling Point: 252 ºC (Lide, 2003)

Melting Point: 64.5 ºC (Lide, 2003)

Flash Point: 124 ºC (ChemicalLand21.com, 2003)

Density/Specific Gravity: 1.24 at 20 EC (Scriven *et al*., 1996)

Solubility: Soluble in water, alcohol, benzene, ether; insoluble in petrol ether (Lide, 2003)

Log $K_{o/w}$: 0.11 (Hansch *et al.*, 1995)

Reactivity: Incompatible with strong oxidizing agents, strong acids, acid chlorides, and acid anhydrides (Sigma-Aldrich, 2003)

Technical Products and Impurities: 3-Aminopyridine, 99% pure, is available from the Aldrich Division of Sigma-Aldrich (Sigma-Aldrich, 2005).

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EXPOSURE INFORMATION

Producers:

Chemical Sources International (2003) lists 20 U.S. suppliers for 3-aminopyridine. 3-Aminopyridine is manufactured or distributed by Alfa Aesar/Johnson Matthey Co.; CBC (America) Corp.; NetChem & Laboratory, Inc.; Seal Sands Chemicals Ltd./Cambrex Co. (Hunter, 2002; Moynihan, 2002; Tilton, 2002).

Production:

3-Aminopyridine is produced from the corresponding pyridinecarboxamides and sodium hypochlorite in alkaline solution (Hofmann reaction). Pyridinecarboxamides are prepared by hydrolysis of pyridinecarbonitriles (Shimizu *et al*., 1993).

Production/Import Level:

3-Aminopyridine is listed in the EPA TSCA Inventory (ChemID, 2003) but is not contained in the EPA IUR for 1986-2002.

For the 7-month period from September 2001 and April 2002, PIERS reported exports of aminopyridines as a solution, with a cargo weight of 108,762 lbs (Dialog Information Service, 2003).

USITC reported 3-aminopyridine as an end-use chemical for the years 1991-1993. No US production data in the ten most recent volumes of *Synthetic Organic Chemicals, US Production and Sales* was shown for the years 1984-1993. This source is no longer published. No quantitative information on annual production was found in any other available literature (US International Trade Commission, 1990, 1991, 1993, 1994a,b).

Use Pattern:

3-Aminopyridine is an intermediate in the production of pharmaceuticals, agrochemicals; and dyes (ChemicalLand21.com, 2003; Merck, 2001; Shimizu *et al*., 1993). This chemical is also listed as a plant growth regulator (Cotner, 2005).

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A total of 832 patents containing the name "3-aminopyridine" were on file with the U.S. Patent and Trademark Office since 1976 as of October 2005 (US Patent and Trademark Office, 2005).

Human Exposure:

No information on human exposure was found in the available literature. Use patterns suggest that there is a potential for worker exposure in the manufacturing of some pharmaceuticals, agrochemicals, and dyes.

Environmental Occurrence:

Use as a plant growth regulator could result in the release of 3-aminopyridine into the environment.

Only limited information on the toxicity of 3-aminopyridine in terrestrial or aquatic species was found in the available literature. The oral LD_{50} of 3-aminopyridine in the quail was reported to be 178 mg/kg (Sigma-Aldrich, 2003).

Regulatory Status:

No regulatory information for 3-aminopyridine was identified in the available literature. No standards or guidelines have been set by NIOSH or OSHA for occupational exposure to or workplace allowable levels to this chemical. 3-Aminopyridine was not on the American Conference of Governmental Industrial Hygienists (ACGIH) list of compounds for which recommendations for a Threshold Limit Value (TLV) or Biological Exposure Index (BEI) are made.

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TOXICOLOGICAL INFORMATION

Human Data:

No epidemiological studies or case reports investigating the exposure of aminopyridines and cancer risk in humans were identified in the available literature.

Animal Data:

Acute Toxicity: The intraperitoneal (i.p.) LD_{50} of 3-aminopyridine in the mouse was reported to be 28 mg/kg (Sigma-Aldrich, 2003). No acute toxicity values for the oral, dermal, or inhalation routes were found in the available literature.

Subchronic and Chronic Toxicity: No information on the subchronic or chronic toxicity of 3-aminopyridine was identified in the available literature.

Short-Term Tests:

3-Aminopyridine was not mutagenic in *Escherichia coli* strain WP2 *uvrA* (Pai *et al*., 1978). However, it was mutagenic in *Salmonella typhimurium* TA98 in the presence of S-9 and norharman at 200 μg/plate (Sugimura *et al*., 1982; Wakabayashi *et al*., 1982).

Metabolism:

No information was found in the available literature regarding the metabolism of 3-aminopyridine.

The program, METEOR, predicts that hydroxylamines from primary heteroaromatic amines and 3-hydroxylation of pyridines would be plausible metabolic pathways (, Ltd., 2004).

Other Biological Effects:

Blockade of K^+ Channels: 3-Aminopyridine selectively blocked the K^+ channels of the squid axon internal or external membranes. The steady-state block of K^+ channels was more complete for low depolarization, and was gradually relieved at higher depolarizations (Yeh *et al*., 1976).

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Neurological Effects: Injection (i.p.) of 3-aminopyridine precipitated convulsions in mice (HSDB, 2005).

Aminopyridines are capable of increasing acetylcholine release and spontaneous transmitter release in response to nerve stimulation (Bowman *et al*., 1977).

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CHEMICAL IDENTIFICATION

4-Aminopyridine

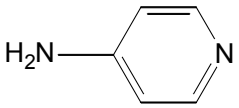
CAS Registry Number: 504-24-5

Chemical Abstracts Service Name: 4-Pyridinamine (9CI)

Synonyms and Trade Name: 4-Aminopyridine; 4-pyridinamine; 4-pyridylamine, *p*-aminopyridine; Avitrol®; Fampridine® (ChemID, 2003)

Structural Class: Heterocyclic aromatic tertiary amine derivatives

Structure, Molecular Formula, and Molecular Weight:



$C_5H_6N_2$ Mol. wt.: 94.12

Chemical and Physical Properties:

Description: Crystals from chloroform (Lewis, 2002; Merck, 2001)

Boiling Point: 273 ºC (Lide, 2003)

Melting Point: 158.5 ºC (Lide, 2003)

Density/Specific Gravity: 1.25 at 20 ºC (Scriven *et al*., 1996)

Log $K_{o/w}$: 0.26 (Hansch *et al*., 1995)

Solubility: Soluble in water, alcohol, benzene, and ether (Lewis, 2002; Lide, 2003; Merck, 2001)

Reactivity: Incompatible with strong oxidizing agents, strong acids, acid chlorides, and acid anhydrides (Sigma-Aldrich, 2003)

Technical Products and Impurities:

4-Aminopyridine, at ≥99% purity, is available from the Aldrich division of Sigma-Aldrich (Sigma-Aldrich, 2005).

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EXPOSURE INFORMATION

Producers:

*C*hemical Sources International (2003) lists 29 U.S. suppliers for 4-aminopyridine.

4-Aminopyridine is manufactured or distributed by Akor Corporation; Alfa Aesar/Johnson Matthey; CBC (America) Corp.; Davos Chemical Corp.; Eastar Chemical Corporation; Elpar International Inc.; National Biochemicals; NetChem & Laboratory, Inc.; Regis Technologies, Inc.; Richman Chemical Inc.; SATTVA Chemical Div.of Pechiney World Trade (USA) Inc; Siddharth International; Spectrum Chemical Mfg. Corp. (Hunter, 2002; Moynihan, 2002; Tilton, 2002).

The pesticide, Avitrol®, which contains 4-aminopyridine as the active ingredient, is manufactured by Avitrol Corporation (Avitrol, 2005). The experimental drug, Fampridine®, is produced by Acorda Therapeutics (National Spinal Cord Injury Association, 2005).

Production:

4-Aminopyridine is produced by heating pyridine with sodium amide in *N*,*N*-dimethylaniline at 180ºC. It is derived as well from 2-aminopyridine and synthesized through peroxidation/nitration/nitro reduction of pyridine (HSDB, 2003; Lewis, 2002).

4-Aminopyridine is also produced from the corresponding pyridinecarboxamides and sodium hypochlorite in alkaline solution (Hofmann reaction). Pyridinecarboxamides are prepared by hydrolysis of pyridinecarbonitriles (Shimizu *et al.*, 1993).

Production/Import Level:

4-Aminopyridine is listed in the EPA TSCA Inventory (ChemID, 2003) but is not listed in the EPA Inventory Update Rule for 1986-2002. 4-Aminopyridine is listed as a LPV chemical in the European Union, meaning that 10 to 1,000 metric tons were produced or

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imported from 1990-1994. The European producer of 4-aminopyridine is INTEROR PRODUCTION (European Chemicals Bureau, 2005).

In a 1980 registration standard report, the annual production of Avitrol® was 330-350 pounds with 104 pounds used in agricultural crops and 240 pounds applied in agricultural premises and urban areas (EPA, 1980). Preliminary pesticide use data from the state of California reported that 29.49 pounds of 4-aminopyridine were used in agricultural applications in the year 2000 (California Environmental Protection Agency, 2001).

The Animal Damage Control (ADC) program under the Animal and Plant Health Inspection (APHIS) of the U.S. Department of Agriculture provides assistance when wild animals cause damage to property, threaten public health and safety, feed on valuable crops, kill livestock and pets, or harm endangered species. The ADC also has responsibilities for such damage on public lands. The ADC program has used and supervised the use of Avitrol® on pigeons, sparrows, gulls, crows, blackbirds, and starlings. In FY 2000, ADC oversaw the application of 1,529 ounces of Avitrol® on private and public lands. In FY 2001, these figures were 295 ounces plus an additional 24 grams used on gulls (ADC, 2005).

For the 7-month period from September 2001 and April 2002, PIERS reported exports of aminopyridines as a solution, with a cargo weight of 108,762 lbs (Dialog Information Service, 2003).

The USITC reported 4-aminopyridine as an end-use chemical for the years 1991-1993; 4-aminopyridine was also listed for the year 1989. No US production data in the ten most recent volumes of *Synthetic Organic Chemicals, US Production and Sales* was shown for the years 1984-1993. This source is no longer published. No quantitative information on annual production was found in any other available literature (US International Trade Commission, 1990, 1991, 1993, 1994a,b).

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Use Pattern:

Pesticide: As an EPA registered pesticide (PC Code 069201), Avitrol® is used against red-winged blackbirds, grackles, and blackbirds in agricultural fields; pigeons and sparrows in public buildings; and various birds around livestock feeding pens. Avitrol® repels birds by poisoning individual members of a flock of birds, causing them to utter distress calls which signal other birds to leave the site. Avitrol® has five active registered products, Avitrol concentrate, Corn Chops, Double Strength Corn Chops, Mixed Grains, and Whole Corn (Cornell University, 1985, 1996; EPA, 1989; Schafer *et al.*, 1975; Wisconsin Department of Agriculture, Trade & Consumer Protection, 2005).

An example of the use of Avitrol® is its application on sunflower crops. This involves the repeated application of Corn Chops, containing 0.03% 4-aminopyridine diluted 1:99 and broadcast by air or ground equipment at a treatment rate of 3 pounds of bait per field acre. Although approximately 2.5 million acres are planted with sunflowers, Avitrol® is not applied uniformly to all of these acres. Damage is also not evenly spread, with 20 percent of the acreage suffering serious damage. Other methods, e.g., controlling cattails to reduce suitable habitat, are also used (Jacobs, 2005; Kleingartner, 2005).

Intermediate: 4-Aminopyridine is an intermediate in the production of pharmaceuticals and agrochemicals (Shimizu *et al*., 1993).

Experimental Drug: 4-Aminopyridine is a potassium channel blocker that may enhance the release of acetylcholine from nerve terminals. It has been examined for the treatment of patients with Lambert-Eaton syndrome, myasthenia gravis, human botulism, Huntington's disease, Alzheimer's disease, and spinal cord injuries. It has also been used to reverse the effects of non-depolarizing muscle relaxants. The sustained release formula of 4-aminopyridine, oral Fampridine-SR®, is presently in Phase III clinical trials for the treatment of multiple sclerosis (MS) (Köller *et al*., 1997; Murray & Newsom-Davis, 1981; National Multiple Sclerosis Society, 2005a; National Spinal Cord Injury Association, 2005;

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Potter *et al*., 1998; Rossini *et al*., 2001; Segal *et al.*, 1999; Stork & Hoffman, 1994; Van Diemen *et al.*, 1993; Wesseling *et al*., 1984).

In Bulgaria, 4-aminopyridine has been used for many years as an antagonist to nondepolarizing neuromuscular blocking agents (De Roos, 2002).

Veterinary Medicine: 4-Aminopyridine has been used off-label, alone, or in combination with yohimbine hydrochloride as an antagonist of xylazine sedation in dogs and cattle, and xylazine-ketamine anesthesia in horses (Hendricks *et al*., 1984).

Other: A total of 1,091 patents citing the term "4-aminopyridine" were on file with the U.S. Patent and Trademark Office since 1976 as of October 2005 (U.S. Patent and Trademark Office, 2005).

Human Exposure:

Occupational Exposure: Occupational exposure to 4-aminopyridine may occur via dermal contact or inhalation in workplaces where 4-aminopyridine is produced or used (HSDB, 2003).

The National Occupational Exposure Survey (NOES), which was conducted by NIOSH between 1981 and 1983, estimated that 4,618 workers in 452 facilities representing 3 industries were potentially exposed to 4-aminopyridine (Sigma-Aldrich, 2003). The NOES database does not contain information on the frequency, level, or duration of exposure to workers of any chemical listed therein.

4-Aminopyridine bait preparation involves manual mixing of the active ingredient with bait material, therefore, workers who handle dust formulations may be exposed to 4-aminopyridine through dermal contact and inhalation (EPA, 1980).

Consumer Exposure: Exposure to 4-aminopyridine may occur when handling Avitrol® products or from handling of dead birds killed with Avitrol®. Formulations of this avicide are commercially available as grain baits with 0.03 to 1% 4-aminopyridine, or powder concentrates consisting of 25 or 50% 4-aminopyridine (Avitrol Corporation, 2005; California Environmental Protection Agency, 2003).

The general population may be exposed to 4-aminopyridine through ingestion of contaminated grain and seed products (HSDB, 2003).

Environmental Exposure: 4-Aminopyridine's production and use as a chemical intermediate and in agriculture as a bird repellent and avicide will result in its release to the environment through various waste streams (HSDB, 2003). However, no detectable levels of 4-aminopyridine in field corn and sunflower seeds were reported after multiple applications of baits containing 4-aminopyridine (EPA, 1980).

Considerable attention has been given recently to the issue of water pollution from consumer use of drugs either disposed of in the water or excreted and disposed of through sewer systems. Should Fampridine-SR® receive approval for the treatment of MS, a potential patient population of 400,000 would become available to receive 4-aminopyridine (National Multiple Sclerosis Society, 2005a,b). The narrow therapeutic window and severe side effects associated with 4-aminopyridine would probably greatly restrict this number.

Environmental Occurrence:

Atmospheric: If released to air, 4-aminopyridine will exist solely as a vapor in the ambient atmosphere. Vapor-phase 4-aminopyridine will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 19 hours (HSDB, 2003).

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Biodegradation and Soil Accumulation: Aromatic amines undergo rapid and reversible covalent binding with humic materials in aqueous solution which may explain why leaching of 4-aminopyridine was negligible in three alkaline soils and four acidic soils (EPA, 1989).

Although 4-aminopyridine volatilization from soil was reported to be very low, this chemical is readily adsorbed to soil particles and is highly persistent. Even though it remains near the soil surface, microbial degradation is not likely to occur. Microorganisms isolated from soil (*Pseudomonas fluorescens*, *Enterobacter aerogenes*, *Aspergillus niger*, *Streptomyces griseus*, and *Agrobacterium tumefaciens*) during 120 hrs of incubation were unable to metabolize 4-aminopyridine. Under aerobic conditions, 4-aminopyridine showed half-lives ranging between 3 to 32 months in different types of soil. Under flooded conditions half-lives ranged between 8 months in clay soil to 10 months in sandy loam soil. Metabolism to carbon dioxide was also negligible under anaerobic conditions (Betts *et al.*, 1976; Cornell University, 1996; EPA, 1980, 1989).

Aquatic: If released into water, 4-aminopyridine is not expected to adsorb to suspended solids and sediment. Volatilization from water surfaces is not expected to be an important fate process. Biodegradation in water may slowly occur based upon biodegradation studies in soil. A measured BCF of <0.2-0.6 and <1.8-7.2 at a concentration of 50 and 5 mg/l, respectively, suggests bioconcentration in aquatic organisms is low (HSDB, 2003).

4-Aminopyridine is not expected to be present in surface water as a result of land application of formulated products (Cornell University, 1996).

Environmental Toxicology: As an avicide, the toxicity of 4-aminopyridine to the environment has been studied extensively. 4-Aminopyridine induces mortality in many non-target avian species. 4-Aminopyridine, or its hydrochloride salt, were highly toxic to 39 species of birds, including doves, parakeets, magpies, grackles, ducks, jays, robins, finches, sparrows, pheasants, quails, starlings, blackbirds, bobwhites, queleas, chicken,

pigeons, and mannikins. The oral LD_{50} ranged from 2.4 to 20 mg/kg (EPA, 1989; Schafer *et al*., 1973).

EPA has characterized 4-aminopyridine as moderately toxic to warm water fish (EPA, 1980). Based on log $K_{o/w}$ and water solubility, EPA projected that 4-aminopyridine will not concentrate in aquatic organisms (EPA, 1989).

Several acute toxicity values for 4-aminopyridine in aquatic and terrestrial species are summarized in Table 3.

Table 3. Selected Ecotoxicity Values for 4-Aminopyridine

Organism	Study Time	Toxicity Endpoint	Toxic Dose (mg/l)	Reference
Photobacterium phosphoreum	5-30 min	EC_{50}	284	Verscheuren, 2001
Tetrahymena pyriformis	60 hr	EC_{50}	260	Verscheuren, 2001
Daphnia magna	24 hr	LC_{50}	17	EPA, 1989
	96 hr	LC_{50}	3.2	
River horn snail (*Elimia catenaria*)	24 hr	LC_{50} (static)	100	EPA, 1989; Orme & Kegley, 2002
	96 hr	LC_{50} (static)	62	
Frog larvæ (*Rana sphenocephala*)	24 hr	LC_{50}	7.2	EPA, 1989
	96 hr	LC_{50}	2.4	
Bluegill fish (*Lepomis macrochirus*)	96 hr	LC_{50} (static)	2.82-7.56	EPA, 1980; Verscheuren, 2001
Channel catfish (*Ictalurus punctatus*)	96 hr	LC_{50} (static)	2.43-5.80	EPA, 1980; Verscheuren, 2001

In studies where flesh-eating birds and mammals were fed blackbirds killed with 4-aminopyridine, no indication of secondary hazard toxicity was observed even though the equivalent of the LD_{50} dose for 4-aminopyridines was exceeded (EPA, 1989; Schafer *et al.*,

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1974). Cumulative toxicity in non-target avian species has also not been observed (Schafer & Marking, 1975).

Two field studies conducted in the Midwest in sunflower fields treated with 4-amino-pyridine showed that in two small areas (36.65 and 51.5 acres), 258 target and 42 non-target birds were found dead. In an 18,000 area field of which only 128 acres were searched, 29 target and 3 non-target birds were found (EPA, 1980).

Regulatory Status:

No standards or guidelines have been set by NIOSH or OSHA for occupational exposure to or workplace allowable levels of 4-aminopyridine. 4-Aminopyridine was not on the American Conference of Governmental Industrial Hygienists (ACGIH) list of compounds for which recommendations for a Threshold Limit Value (TLV) or Biological Exposure Index (BEI) are made.

Based on potential hazards to fish and non-target birds, 4-aminopyridine formulations are classified by the EPA as Restricted Use Pesticides (RUP) and are on the EPA's list of "Acutely Hazardous" commercial pesticides (Cornell University, 1996).

As indicated in Section 180.312 of 40 CFR, a tolerance of 0.1 ppm is established for negligible residues of 4-aminopyridine in or on the raw agricultural commodities corn fodder and forage, corn grain (including popcorn grain), fresh corn (including sweet corn kernels plus cob with husks removed), and sunflower seeds (Jacobs, 2005).

4-Aminopyridine is regulated under the Emergency Planning and Community Right-to-Know Act (EPCRA) as an Extremely Hazardous Substance (EHS). Under section 302 of EPCRA, reporting requirements are triggered when 4-aminopyridine is stored in amounts in excess of 500/10,000 lbs. Under section 304 of EPCRA, releases > 1,000 lbs must be reported. 4-Aminopyridine is listed in the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and the Resource Conservation and Recovery

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Act (RCRA) as a hazardous waste. The reportable quantity (RQ) is 1,000 lbs and it must be managed according to federal and/or state hazardous waste regulations as indicated in 40 CFR 261.33 (EPA, 2001; HSDB, 2003).

The Modernization Act of 1997 directs the Food and Drug Administration (FDA) to develop a list of bulk substances for use in pharmacy compounding. Although 4-aminopyridine was nominated for inclusion, FDA was concerned about reports of seizures associated with the use of 4-aminopyridine and did not include this substance on the initial list of bulk substances (FDA, 1999a).

TOXICOLOGICAL INFORMATION

Human Data:

No epidemiological studies or case reports investigating the exposure of aminopyridines and cancer risk in humans were identified in the available literature. The EPA's Integrated Risk Information System (IRIS) assigned a classification of D (not classifiable) for the carcinogenicity for lifetime exposure to 4-aminopyridine. The basis was no human data and no animal data available (EPA, 1993).

Acute toxicity, including tonic-clonic seizures, perioral parasthesis, unsteadiness during walking, restlessness, and slight increase in systolic blood pressure have been observed from the accidental ingestion of 4-aminopyridine, in patients receiving 4-aminopyridine, and in clinical trial subjects (EPA, 1980, 1989; FDA, 1999b).

- Two men (100 kg) who ingested ~60 mg of 4-aminopyridine were hospitalized and treated for nausea, weakness, dizziness, profuse perspiration, altered mental status, and hypertension. One had three seizures. A third person who also ingested 4-aminopyridine induced vomiting within 10 minutes of ingestion and denied any adverse effects (Spyker *et al.*, 1980).

- A 28-yr old female MS patient who had been treated at 2 mg/d for 2 years, discontinued her medication and took a catch up dose of 6 mg that produced two seizures. On admission to the emergency room, she had a serum 4-aminopyridine level of 136.3 ng/ml (Stork & Hoffman, 1994).

- A 54-year old quadriplegic woman with MS had a grand-mal seizure after 3 days of dosing with 4-aminopyridine (Stork & Hoffman, 1994).

- A 52-year old woman with MS fell into a prolonged coma after ingesting 4-aminopyridine, but she recovered fully after 5 days (Stork & Hoffman, 1994).

- A previously healthy 8-month old boy presented to an emergency room 40 minutes after accidental ingestion of up to 20 mg of 4-aminopyridine. He was jittery, tachycardic, and tachypneic and later developed dramatic opisthotonic posturing and vermiform tongue fasciculations. His symptoms responded to the administered treatment and he was asymptomatic 20 hours after admission (Velez *et al.*, 2003).

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A number of clinical trials involving oral administration of 4-aminopyridine to patients with MS or spinal cord injury have been conducted. Some studies reported significant side effects of 4-aminopyridine, such as a confusional state and epileptic fits. Restlessness, confusion, and generalized tonic-clonic seizures have been reported at doses higher than 0.8 mg/kg bw (Potter *et al*., 1998; Rossini *et al*., 2001; van der Bruggen *et al*., 2001).

Eight MS patients received three treatments on three separate days with placebo or 4-aminopyridine to a target peak serum concentration of 30 to 59 ng/ml or 60-100 ng/ml. All subjects experienced side effects at the high serum concentration; a grand mal seizure occurred at a serum level of 104 ng/ml and an acute confusional episode occurred at 114 ng/ml (Bever *et al*., 1994).

In a Phase II trial, involving 69 patients with MS treated with a mean dosage of 31.2 mg/day for 12 weeks (maximum dose of 0.5 mg/kg/day), 14 patients needed dose reduction and three patients withdrew from the study because of the side effects. Even so, the side effects from oral administration were less serious when compared with the effects observed after intravenous administration (Van Diemen *et al.*, 1993).

In a Phase II trial of Fampridine-SR® involving 206 MS patients, side effects of drug administration at 10, 15, or 20 mg for 12 weeks included dizziness, insomnia, and nausea. Two persons in the 20-mg group had seizures, one from an accidental overdose (National Multiple Sclerosis Society, 2004).

Animal Data:

Acute Toxicity: 4-Aminopyridine is highly toxic in mammals as well as birds, producing hyperexcitability, salivation, tremors, muscular incoordination, clonic and tonic convulsions, cardiac or respiratory arrest, and death. Among mammals, dogs are most sensitive and rats the least sensitive. Birds were approximately as sensitive to 4-aminopyridine intoxication as mammals and showed no obvious differences in sensitivity between species or genera. Birds showed the same signs of 4-aminopyridine intoxication as mammals (Schafer *et al*., 1973).

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The LD_{50} values for 4-aminopyridine in mammals are given in Table 4.

Table 4. Acute Toxicity Values for 4-Aminopyridine

Species	Route of administration	LD_{50} (mg/kg)	Reference
Mouse	oral	19	Sigma-Aldrich, 2003
		42	Trochimowicz *et al*., 1994
Dog	oral	3.7, 4.0 11.9 (HCl salt)	Schafer *et al*., 1973
Rat	oral	20 28-32.5 (HCl salt)	Schafer *et al*., 1973
Rabbit	dermal	327 (HCl salt)	EPA, 1980
Hog	oral	17.8 (HCl salt)	Schafer *et al*., 1973
Guinea pig	oral	6	Sigma-Aldrich, 2003
		7.5	HSDB, 2003

4-Aminopyridine hydrochloride (50 mg) applied to abraded and intact skin of rabbits did not produce irritation at 24 or 72 hours (EPA, 1980).

A clinical trial of 4-aminopyridine was carried out in 39 dogs with traumatic paraplegia or paraparesis. 4-Aminopyridine was administered in total doses between 0.5 and 1 mg/kg bw, and transient improvements of neurological status were observed in 64 percent of the dogs. Significant side effects were seen in 6 dogs, with elevation of body temperature and apparent anxiety, leading to mild seizures in 3 dogs (Blight *et al.*, 1991).

In horses, cumulative iv doses of 4-aminopyridine of 300 to 500 μg/kg caused muscle tremors, stilted gait, and signs of excitement. Once signs of excitement occurred, additional

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administration of 4-aminopyridine resulted in severe muscle tremors and exaggerated responses to external stimuli. Increases in respiratory rates were noted in all horses after such doses, but significant alterations in heart rate occurred only in horses manifesting obvious signs of CNS excitement (Klein & Hopkins, 1981).

Subchronic and Chronic Toxicity: No 2-year carcinogenicity studies of aminopyridines in animals were identified in the available literature.

4-Aminopyridine hydrochloride was given in the diet to rats at concentrations of 3, 30, or 300 ppm for 90 days. At 300 ppm, the animals were hyperirritable to noise and touch and the brain weights of female rats and liver weights of male rats were significantly increased. No changes were observed in blood, urinalyses, or in gross and histopathological examinations (EPA, 1980, 1989).

4-Aminopyridine hydrochloride was given in the diet to dogs at concentrations of 0.1, 1.0, or 2.0-3.25 mg/kg/day for 90 days. At > 2.0 mg/kg/day, the animals showed salivation and muscular weakness and brain weights were slightly decreased. No changes were observed in blood, urinalyses, or in histopathological examinations (EPA, 1980, 1989).

Short-Term Tests:

Two studies related to the mutagenic potential of 4-aminopyridine were found in the available literature.

- 4-Aminopyridine was not mutagenic in *Salmonella typhimurium* strains TA98 and TA100 at concentrations up to 2 mg/plate in the presence or absence of S-9. Mutagenicity was not enhanced in the presence of norharman at 200 :g/plate with or without activation (Sugimura *et al*., 1982; Wakabayashi *et al*., 1982).
- 4-Aminopyridine was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1537, and TA2637 in the presence or absence of cobalt(II) chloride (Ogawa *et al*., 1986).

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Metabolism:

4-Aminopyridine is readily absorbed through the skin and gastrointestinal tracts of animals (Cornell University, 1996). 4-Aminopyridine administered to nine healthy volunteers as enteric coated 20 mg tablets was also rapidly absorbed (EPA, 1989; Uges *et al.*, 1982).

A study in which mongrel dogs were injected with 1 mg/kg of 4-aminopyridine showed a volume of distribution of 8.6 times the volume of serum, suggesting extensive distribution to the tissues. In this report, only 0.01 ± 0.01% of the administered dose was recovered in the bile while 60 ± 9% was recovered from the urine 10 hr after dosing. Renal excretion of 4-aminopyridine involved tubular excretion with an elimination half-life of 125 min (EPA, 1989).

The program, METEOR, predicts that hydroxylamines from primary heteroaromatic amines and 3-hydroxylation of pyridines would be plausible metabolic pathways (LHASA, Ltd., 2004).

4-Aminopyridine was detected in the urine of rats exposed to the non-carcinogen, 4-*N*-nitroso-*N*-methylaminopyridine (4-NMPY) (Heydt-Zapf *et al*., 1983).

Other Biological Effects:

Developmental and Reproductive Effects: 4-Aminopyridine injected ip to white rats (10 animals/sex) for 1 month at 1 or 5 mg/kg/day or for 6 months at 1 or 4 mg/kg/day, did not induce changes in body weight, hemoglobin concentration, or red and white blood cell counts. In the 1-month study, cerebral edema and proliferation of capillaries in the myocardial interstitium were noted. In the 6-month study, hepatic parenchymatous degeneration and hepatic fatty degeneration were observed. No malformations were noted in the offspring born to treated rats (EPA, 1989).

A single subacute oral dose of 4-aminopyridine (5.62 mg/kg) was given to coturnix quail (*Coturnix coturnix*). When these animals were paired with untreated mates, no effect on

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reproductive performance of males was observed. Egg production of females was reduced the third week after treatment but the hatchability of eggs was not affected. When mated pairs were fed 1,000 ppm 4-aminopyridine, the birds did not produce live chicks. The F_1 progeny from quails fed 31.6, 100, and 316 ppm of 4-aminopyridine showed no reproductive abnormalities (EPA, 1980, 1989; Schafer *et al*., 1975).

Blockade of K^+ Channels: 4-Aminopyridine selectively blocked the K^+ channels of the squid axon internal or external membranes. The steady-state block of K^+ channels was more complete for low depolarization, and was gradually relieved at higher depolarizations (Yeh *et al*., 1976).

The beneficial effects of 4-aminopyridine observed in MS patients may be due to the restoration of the conduction of demyelinating fibers through the delayed repolarization that occurs as a consequence of the blockade of the fast inactivating K^+ current (EPA, 1980; Fujihara and Miyoshi, 1998; Köller *et al*., 1997; Targ and Kocsis, 1985). However, 4-aminopyridine may also increase synaptic activity and/or exert an antagonism to factors present in the cerebrospinal fluid of MS patients that promote Na^+ channel inactivation (Köller *et al*., 1997; Mei *et al*., 2000).

4-Aminopyridine prevented adoptive transfer of experimental allergic encephalomyelitis (EAE), the animal model of MS in rats. 4-Aminopyridine blocked K^+ currents and inhibited proliferative and effector cell functions in activated T cells against the immune responsiveness of guinea pig myelin basic protein-reactive Lewis rat T lymphocytes, enabling them to mediate the transfer of EAE to other animals (Judge *et al*., 1997).

4-Aminopyridine directly increases both skeletal and cardiac muscle contractility. Because blocking voltage-sensitive potassium channels increases calcium influx, 4-aminopyridine also increases neurotransmitter release. The net effect on the cardiovascular system is an increase in myocardial contractility and in peripheral vascular resistance (De Roos, 2002).

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Neurological Effects: Injection (ip) of 4-aminopyridine precipitated convulsions in rats (HSDB, 2003).

Aminopyridines are capable of increasing acetylcholine release and spontaneous transmitter release in response to nerve stimulation (Bowman *et al*., 1977).

The *in vitro* inhibition of the membrane potassium conductance by 4-aminopyridine was described in both nerve and muscle. The mechanism of action of 4-aminopyridine was reported to be calcium-dependent and involved an increase in acetylcholine release at the presynaptic membrane of the neuromuscular junction, increasing the force of muscle contraction (EPA, 1980; HSDB, 2003).

In *in vivo* studies, 4-aminopyridine stimulated the release of norepinephrine but not 5-hydroxytryptamine or dopamine in the rat brain and spinal cord (EPA, 1980). 4-Amino-pyridine caused epileptic seizures in rats probably by increasing glutamatergic synaptic function. Not only did it induce the release of glutamate but also induced small levels of glycine and taurine in the hippocampus and entorhinal cortex. Moreover, in the entorhinal cortex, 4-aminopyridine induced a large increase in extracellular glutamine (Medina-Ceja *et al*., 2000; Peña & Tapia, 2000).

Other Effects on the Nervous System: Administration (iv) of 4-aminopyridine prolonged increases in the blood pressure of cats and antagonized barbiturate hypnosis in mice (EPA, 1980).

Effects on Glucose Homeostasis: Administration (iv) of 4-aminopyridine induced a significant increase in plasma glucose levels in mice, and inhibited glucose-induced insulin secretion. In contrast, in animals deprived of their sympatho-adrenal systems, 4-aminopyridine potentiated the glucose-induced insulin secretion. These findings suggest that 4-aminopyridine may affect glucose homeostasis and insulin secretion via an increase in catecholamine release (Ahrén *et al*., 1981).

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Induction of In Vitro Cell Death: 4-Aminopyridine inhibited proliferation of U87 (wild-type p53) and A172 (mutant p53) astrocytoma cell lines in an *in vitro* assay. It induced apoptosis in U87 astrocytoma cells and blocked the outward rectifier K^+ channel in both U87 and A172 cells (Chin *et al*., 1997).

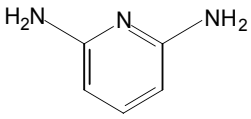
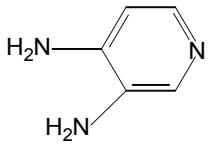
Structure-Activity Relationships for Aminopyridines:

Pyridine (CAS No. 110-86-1) was tested by the National Toxicology Program in F344/N rats, Wistar rats, and B6C3F_1 mice. In groups of 50 male and 50 female F344/N rats exposed in drinking water at 0, 100, 200, or 400 ppm for 104-105 weeks, renal tubule adenomas and carcinomas in high dose males and concentration-related nonneoplastic liver lesions in males and females were observed. Incidences of mononuclear cell leukemia were also increased in the female rats. In groups of 50 male Wistar rats exposed to pyridine in drinking water at concentrations of 0, 100, 200, or 400 ppm, the incidence of testicular interstitial cell adenoma was significantly increased in the high dose animals. Groups of 50 male mice were exposed to pyridine in drinking water at 0, 250, 500, or 1,000 ppm and groups of 50 females were likewise exposed at 0, 125, 250, or 500 ppm. Hepatocellular neoplasms, including hepatoblastomas, in exposed male and female mice were clearly related to pyridine. Many mice had multiple hepatocellular neoplasms; the incidences in exposed males and females exceeded historical control ranges for drinking water studies. Pyridine was not mutagenic in *S. typhimurium* TA98, TA100, TA1535, or TA1537 with or without metabolic activation. Pyridine was also negative in the L5178Y mouse lymphoma assay and did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells. Pyridine did not induce micronuclei in the bone marrow cells of male mice administered i.p. injections of the test material (NTP, 2000).

Two additional compounds structurally related to monoaminopyridines were selected for review. These chemicals were 3,4 diaminopyridine and 2,6-diaminopyridine. No information on carcinogenic activity was found for either compound in a search of the National Library of Medicine TOXNET databases, including Toxline. No information on any of these chemicals was located in the *Survey of Compounds Which Have Been Tested*

for Carcinogenic Activity (CancerChem). Genotoxicity information found in the available literature is presented below in Table 5.

Table 5. Data on Mutagenicity of Compounds Structurally Related to Aminopyridines

Name/Cas No.	Structure	Information on Mutagenicity
2,6-Diaminopyridine 141-86-6		Not mutagenic in *S. typhimurium* TA98 or TA100 with & without PCB-induced S-9 and/or norharman (CCRIS, 2003; Wakabayashi *et al*., 1982) Mutagenic in *S. typhimurium* TA98 with PCB-induced S-9 (CCRIS, 2003)
3,4-Diaminopyidine 54-96-6		Not mutagenic in *S. typhimurium* TA98 or TA100, with & without PCB-induced S-9 and/or norharman (Wakabayashi *et al*., 1982)

Two SAR-based computer software programs were used as tools to assess the toxicity of the three monoaminopyridines. One program, named TOPKAT, uses robust, cross-validated models based on experimental data to calculate a probability value from 0.0-1.0 that a chemical will be positive for a certain endpoint. This program also incorporates a validity diagnostic that indicates if the predicted toxicity values may be accepted with confidence.

Another SAR-based model, DEREK, uses structure alerts to predict the toxicity of a compound. The toxicity predictions made for the monoaminopyridines by TOPKAT and DEREK are shown in Table 6.

Table 6. Toxicity Predictions for 2- , 3-, and 4-Aminopyridine Using SAR-based Programs

Toxicity Endpoint	2-Aminopyridine	3-Aminopyridine	4-Aminopyridine
TOPKAT			
Carcinogenicity (male rat, NTP model)	0.227	0.823*	0.987*
Carcinogenicity (female rat, NTP model)	0.021	0.180	0.185
Carcinogenicity (male mouse, NTP model)	0	No prediction	No prediction
Carcinogenicity (female mouse, NTP model)	0	1.00*	0.999*
Carcinogenicity (male rat, FDA model)	0.098	0.082	0.179
Carcinogenicity (female rat, FDA model)	0.003	0.007	0.087
Carcinogenicity (male mouse, FDA model)	0.621	0.482	0.433
Carcinogenicity (female mouse, FDA model)	0.982*	0.012	0.618
Weight of Evidence Carcinogenicity Call	0.998*	0.998*	0.998*
DEREK			
Carcinogenicity	Plausible for mammalian carcinogenicity	Plausible for mammalian carcinogenicity	Plausible for mammalian carcinogenicity
Skin Sensitization	Plausible as mammalian skin sensitizer	Plausible as mammalian skin sensitizer	Plausible as mammalian skin sensitizer

*Likely to be carcinogenic

Source: Accelrys, Inc., 2004; LHASA Ltd., 2004

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