



NTP
National Toxicology Program

Research Concept: Aminopyridines

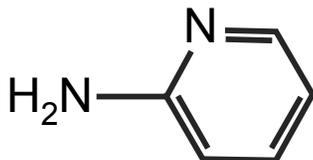
Project Leader: Dr. June K. Dunnick

NTP Board of Scientific Counselors
December 6, 2007

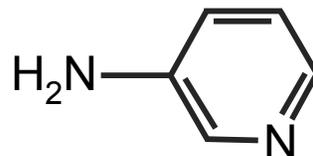




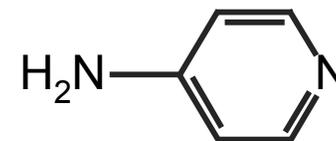
2-Aminopyridine
504-29-0



3-Aminopyridine
462-08-8



4-Aminopyridine
504-24-5



C₅H₆N₂
MW 94.12



Aminopyridine Nomination

- **Nominated by National Cancer Institute because:**
 - Lack of information suitable to predict chronic toxicity for this class of chemicals
 - Interest in conducting structure/activity studies
- **Studies requested:**
 - Toxicological characterization of the 2-, 3-, and 4-aminopyridine including a 2-year cancer study of 2-aminopyridine
 - Short-term mechanistic studies of 2-, 3-, and 4-aminopyridine
 - Neurotoxicity evaluation of 2-, 3-, and 4-aminopyridine



2-Aminopyridine

504-29-0

- 10,000 - 500,000 pounds - 1986-2002 (except for 1998 when production was (1,000,000 - 10,000,000 pounds))
- starting material in drug production
- Oral LD50
 - Mouse - 50 - 145 mg/kg
 - Rat - 200 mg/kg
- No standard toxicity studies reported in literature



3-aminopyridine

462-08-8

- No U. S. production data
- Intermediate in production of pharmaceuticals and dyes
- IP LD50 in mice 28 mg/kg
- No standard toxicity studies reported in literature



4-aminopyridine

504-24-5

- No updated U.S. production data
- Avitrol, pesticide containing 4-aminopyridine; intermediate in production of pharmaceuticals and agrochemicals
- Experimental drug for Huntington's, Alzheimer's, MS
 - Acordia Therapeutics have clinical trials for 4-AP in multiple sclerosis
- Mouse - oral LD50 19-42 mg/kg
- Rat - oral LD50 20 mg/kg
- No standard toxicity studies reported in literature



Salmonella Results

- 2-Aminopyridine negative in Salmonella TA98, 100, 1535, and 1537 (Zeiger *et al* 1987)
- 2-Aminopyridine negative in Salmonella TA98 (Sugimura *et al* 1982)
- 3-Aminopyridine - positive in TA98 with S9 (Sugimura *et al* 1982)
- 4-Aminopyridine negative in Salmonella TA 98, 100, 1537, 2637 (Sugimura *et al* 1982, Ogawa *et al*, 1986, Wakabyashi *et al* 1982))



Aminopyridines (AP) block K⁺ Channels

- 2-AP, 3-AP, 4-AP block K⁺ channels (nerves and myocytes)
- APs interact with K⁺ channels by binding to a site within the channel
- Channels in which APs occupy binding site are occluded and thus cannot conduct K⁺ ions
- Aminopyridines reduce both inward and outward K⁺ currents
- When K⁺ channels are blocked, pre-synaptic action potential is maintained, and nerve signal is increased

Mulgo et al, Eur J Med Chem 20: 149-153, 1985

Caballero et al, Biophys Chem 124: 155-160, 2006



Aminopyridines (AP) block K⁺ Channels

- Both pyridine ring and amino substituent are necessary for activity
- Protonated AP is the active species responsible for blocking K⁺ channels
- Order of in vitro K⁺ blocking activity at physiologic pH:
4-AP > 3-AP > 2-AP
- Structure of K⁺ channels conserved across species
- Blockage of K⁺ channels inhibits proliferation of lymphocytes

Hypothesis: APs will cause neurotoxicity, cardiac toxicity, and/or immunotoxicity at exposure levels that block K⁺ channels

Munoz-Caro & Nino, Biophysical Chemistry 96:1-14, 2002

Doyle et al, Science 280:69-77, 1998

MacKinnon et al, Science 280:106-109, 1998

Judge et al J Biomed Sci 4:169-179, 1997



Liver is a target organ after pyridine

- Pyridine-induced liver toxicity in rats and mice and liver carcinogenesis in mice (NTP TR 470)
- Similarities in the metabolism of the aminopyridines and pyridine suggest that there may be similarities in toxicity

Hypothesis: APs will cause liver toxicity at exposure levels comparable to pyridine exposures that cause liver toxicity



Rationale for Study

- Provide hazard identification information for 2-, 3-, and 4-aminopyridine
- Provide comparative toxicity information for the aminopyridine class



Key Issues to Address

- Use of toxicity tests to detect heart, liver, neurologic, or immunologic toxicity
- Relationship between rodent toxicity endpoints and aminopyridine blood levels reported to block K⁺ channels



Hypotheses to Evaluate

- Aminopyridines will cause neurotoxicity, cardiac toxicity and/or immunotoxicity at exposure levels that block K⁺ channels
- Aminopyridines will cause liver toxicity at exposure levels comparable to pyridine exposures that cause liver toxicity



Aminopyridine Proposed Research Program

- Toxicity studies to identify cardiotoxicity, neurotoxicity, liver toxicity or immunotoxicity
- Genotoxicity studies
- High throughput toxicity screens