UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

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SUBJECT: **Human Studies Review Board:** Weight of Evidence Discussion for 4-Aminopyridine
DP Barcode D340000
PC Code: 069201 Reregistration Case #: 0015

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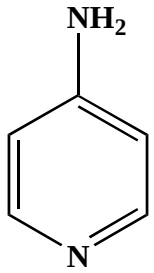
This document describes the scientific support for deriving a point of departure for 4-aminopyridine (4-AP) derived from oral human studies (MRID no. **47093601, 47093602, 47093603**). This point of departure is applicable for use in acute (1-day), subchronic (30 days to 180 days risk assessments for all potential exposure scenarios to 4-AP.

1. Background and Introduction:

4-Aminopyridine is a bird repellent registered under the name Avitrol. It is also being developed by Acorda therapeutics under the name Fampridine for the treatment of multiple sclerosis and spinal cord injury. The US FDA has recently (December 20, 2006) granted orphan drug status for an immediate release, orally administered version of 4-aminopyridine (*Ampydin*, made by Neurorecovery, Inc.) in the treatment of chronic functional motor and sensory deficits resulting from Guillain-Barré syndrome. 4-Aminopyridine has been used in humans to reverse neuromuscular blockade resulting from nondepolarizing neuromuscular blocking agents and certain antibiotics, and as an experimental treatment for Botulinus intoxication, myoneural disorders and Alzheimer's disease (EPA, 1989)



Chemical Structure of 4-aminopyridine

There are no reliable animal toxicity studies to derive an appropriate point of departure for assessing human health risk. However, there are numerous published studies evaluating the efficacy and safety of 4-aminopyridine following its oral administration to human volunteers with spinal cord injury. In accordance with the human studies rule, the Agency is asking the Human Studies Review Board to review the ethical and scientific conduct and design of these studies and their potential utility in assessing human health risk.

2. Hazard Characterization and Database Summary

According to a review by Technical Resources International prepared for NCI to support chemical nomination for testing, 4-aminopyridine is highly toxic to mammals (Toxicity Category I) and birds, with oral LD_{50} ranging from 3.7 mg/kg in dogs to 20 mg/kg in rats. Acute toxicity produced in animals is manifested by hyperexcitability, salivation, tremors, muscular incoordination, clonic and tonic convulsions, cardiac or respiratory arrest, and death. Humans poisoned with an accidental ingestion of 60 mg of 4-AP developed rapid symptoms of weakness, dizziness, intense diaphoresis, dyspnea and profound thirst, and became agitated and combative (Spyker *et al*, 1980).

4-AP is a central nervous system (CNS) toxicant. It acts on the nervous system by releasing acetylcholine (Giovannini *et al*, 2002, cited in the NCI 2006 report), thus enhancing transmission at the neuromuscular junction and other synapses (Spyker *et al*, 1980). This property led to its use in the clinical treatment of prolonged paralysis caused

by antibiotics and muscle relaxants (Spyker *et al*, 1980). The aminopyridines also block potassium channels in excitable membranes and facilitate chemical synaptic transmission at central, autonomic and neuromuscular synapses (Van Diemen *et al*, 1993). Several studies have demonstrated the beneficial effects of 4-AP in disorders of neuromuscular transmission and in multiple sclerosis.

A. Animal Data

The data base exploring the toxicity of 4-AP in animals is very limited. Two studies regarding the subchronic oral toxicity of 4- aminopyridine were summarized in the EPA Health and environmental Effects Document for 4-Aminopyridine (1989). In one study by Cervenka and Vega (1968, cited in EPA 1989), rats (10/sex/dose)) were given dietary concentrations of 4-aminopyridine hydrochloride at 0, 3, 30 or 300 ppm for 90 days. At 300 ppm three rats died during the study and all surviving rats at this dose were hyperirritable to noise and touch. Brain weights of female rats and liver weights of males rats at 300 ppm were significantly elevated ($p<0.05$). Hyperirritability also occurred at 30 ppm and a 3 ppm (0.2 mg/kg body weight/day) was considered a NOEL. In the other study by Cervenka and Vega (1968, cited by EPA 1989) beagle dogs (4/sex/dose) were fed diets containing 4-aminopyridine hydrochloride at 0.1, 1.0, or 2.0-3.25 mg/kg/day for 90 days. At >2.0 mg/kg/day, the dogs exhibited salivation and muscular weakness and the brain weights were slightly decreased.

No acute, subchronic, or chronic inhalation toxicity studies of 4- aminopyridine were available. There are no oral chronic toxicity studies, published or unpublished, available either.

Limited information is known about 4-AP developmental and reproductive toxicity. In a 1972 study by Mistov and Uzunov summarized by EPA (1989) and NCI (2006), 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, parenchymatous degeneration and hepatic fatty degeneration were observed. No malformations were noted in the offspring born to treated rats.

No human or animal data for assessing the carcinogenic potential of 4-AP were available. Based on the lack of this data and the weight of evidence, IRIS (1993) classified the carcinogenic potential of 4-aminopyridine as D category: not classifiable as to human carcinogenicity (http://www.epa.gov/iris/subst/0440.htm).

4-Aminopyridine was negative in reverse mutation assays in *Salmonella typhimurium* (Ogawa *et al*., 1986; Wakabayashi *et a*l., 1982 and Sugimura *et al*, 1982 as summarized in EPA IRIS, 2003 and NCI 2006). Ogawa et al. (1986) tested the mutagenicity of 4-aminopyridine in *S. typhimurium* strains TA98, TA100, TA1537 and TA2637; 4-aminopyridine was not mutagenic both alone and in the presence of cobalt (II) chloride (study data not reported). [Cobalt (II) chloride was found to enhance the mutagenicity of

other heteroaromatic compounds (i.e., 9-aminoacridine, 4-aminoquinoline and harman) in *Salmonella*]. In the Wakabayashi *et al*. (1982) and Sugimura *et al*, 1982 studies, 4-aminopyridine at concentrations of up to 2 mg/plate in the presence or the absence of S9 hepatic homogenates or in the presence of norharman, a tryptophan pyrolysate (200 ug/plate), was not mutagenic for *S. typhimurium* strains TA98 and TA100.

B. Human Data

1. MRID 47093602. Safety and efficacy of 4-AP in humans with cord injury (SCI): a long-term, controlled trial. Segal *et al*, 1999 study:

In a study approved by the U.S. Food and Drug Administration investigating the safety and efficacy of 4-AP in treating spinal cord injury (SCI) in humans, 21 healthy men and women outpatients suffering from traumatic SCI for at least 2 years were administered 4-AP (as an immediate release formulation) at 30 mg/day (16 subjects) or 6 mg/day (5 subjects: low dose group) for 3 months (Segal *et al*, 1999). The low dose group served as an active-treatment control. All patients provided institution-approved written informed consent. Eleven patients who did not have prior exposure to 4-AP were randomly assigned to receive high- or low-dose 4-AP. Ten patients who had participated previously in a short-term (24 hour) study of the effects of a single 10-mg dose and/or 2 weeks of 35 mg/day orally more than 1 year before the present study were unblinded and assigned to receive high-dose 4-AP. Study subjects did not differ significantly in age, height, weight, or injury duration. Dosages of 4-AP were titrated to tolerance in increments of 2, 5, or 10 mg over 2 weeks using an immediate-release formulation of encapsulated crystalline 4-AP in admixture (w/w) with pharmaceutical-grade microcrystalline cellulose. The encapsulated 4-AP was stable for at least one year.

Improvement in and recovery of sensory and motor function, enhanced pulmonary function and diminished spasticity were seen in these patients after one month and persisted during the 3-month study duration. No clinically significant adverse effects or measurable toxicity was noted. Nervousness, giddiness or dizziness, and gastrointestinal upset manifesting as mild abdominal cramping or nausea were the most frequent side effects. All side effects were transient, self-limited, or disappeared with changes in dosage or the timing of drug ingestion to coincide with meals or snacks. Patients routinely reported mood elevation and an unabated enhanced sense of well-being. Seizure or seizure-like activity was not observed nor was it reported by patients or caregivers at any time or at any dosage. Serially acquired EEG, ECG, biochemical and hematologic profiles, and urinalyses remained within normal range. The results of this study confirmed earlier work by these investigators that oral administration of 40 mg of 4-AP/day was tolerated by patients and produced the desired therapeutic effects. They attributed this record of safety to careful patient selection and implementing individualized dosing regimens based on population-specific pharmacokinetic behavior. They also warned that 4-Aminopyridine is a potentially toxic drug with a narrow therapeutic index.

2. MRID 47093601. Efficacy and Safety of 4-AP in patients with long-term spinal cord injury (SCI): a randomized, double-blind, placebo-controlled trial. Grijalva *et al*, 2003 study:

This study was conducted from September 1999-June 2000 at the Spinal Cord Clinic (a referral center of institutional practice mainly of ambulatory care) of the Research Medical Unit for Neurological Diseases at the Specialties Hospital, Centro Médico Nacional Siglo XXI, of the Instituto Mexicano del Seguro Social (IMSS) in Mexico City. The study was approved by both the local research committee of the hospital and the National Research Council of the IMSS. All patients were fully informed about the trial, all received a written description of the trial, and their questions and concerns were answered orally. All patients signed an informed consent letter. SCI patients with more than 1.5 years duration and aged 18-60 years were selected for this study. The study required that women patients be postmenopausal, surgically sterile or were using an acceptable method of birth control.

In this randomized, double-blind, crossover design placebo-controlled trial, there were two groups: 4-AP-placebo (14 patients) and placebo-4-AP sequence (13 patients). Identical capsules containing 4-AP 5 mg or placebo were prepared. The 4-AP was given in gelatin capsules containing 5 mg 4-AP and microcrystalline cellulose as the excipient (inert carrier). Placebo capsules contained only the excipient. Each patient was administered two capsules every 8 hours (6 capsules/day). Initially, all patients completed a run-in period of 2 weeks with placebo. Patients randomized to the 4-AP-placebo sequence then received one 4-AP capsule and five placebo capsules/day for 1 week (i.e., 4-AP dosage was 5 mg/day). The 4-AP dosage was increased by 5 mg/week by substitution of placebo by 4-AP capsules, such that patients received six capsules/day throughout the study. At 6 weeks, patients in the 4-AP group were receiving 4-AP 30 mg/day. The 30-mg/day dosage was maintained for 7 weeks. These patients then switched to the opposite treatment and received placebo (six capsules/day) for 12 weeks.

Patients in the placebo-4-AP sequence received placebo for 12 weeks after the run-in period. Then they received 4-AP starting with 5 mg/day, increasing by 5 mg/week to a maximum of 30 mg/day, and maintaining the 30-mg/day dosage for 7 weeks, as described above. There was no washout period. The strategy of giving six capsules/day allowed the investigators to increase the 4-AP dosage gradually while maintaining the double-blind study design.

Patients were evaluated every 4 weeks for adverse reactions, vital signs assessments, physical examination and laboratory tests. Blood and urine samples were obtained during each patient visit to determine concentrations for glucose; creatinine; blood urea nitrogen; total cholesterol; triglycerides; total, direct, and indirect bilirubin; alanine aminotransferase (ALT); aspartate aminotransferase (AST); alkaline phosphatase (ALP); creatine kinase (CK); lactic acid dehydrogenase; sodium; potassium; chloride; calcium; and phosphorus. A complete blood cell count with differentials and a routine urinalysis and urine culture were also obtained at each visit.

4-Aminopyridine improved neurologic function in patients with long-term SCI and had a persistent effect 12 weeks after its discontinuation. Although no serious adverse reactions (e.g., epileptic seizures, severe laboratory-determined abnormalities) were encountered during this study, there were several mild adverse, brief and transitory reactions (such as dry mouth, dizziness, nausea, gastritis, and oral and peripheral paresthesia (an abnormal or tingling, pricking, or burning sensation)) and no patient required dosage reduction. Dry mouth, dizziness, and gastritis began with 4-AP at 5 or 10 mg/day; oral and peripheral paresthesia appeared only with 4-AP at 30 mg/day. Adverse reactions that had not been previously reported, such as memory alteration, bitter taste in the mouth, global pinching pain, and cramps were observed. Blood tests did not show any significant effects of 4-AP on renal function and electrolyte balance, but showed some mild effects on hematologic parameters and enzyme levels. Six patients had increased enzyme levels (ALT = alanine aminotransferase, AST = aspartate aminotransferase , ALP = alkaline phosphatase, and CK - creatine kinase), and one had mild thrombocytopenia (platelet count 115-135 x $10^3/mm^3$). Abnormal enzyme levels never were higher than 2 times the upper limit of normal levels and diminished gradually. Both enzyme and platelet alterations resolved during 4-AP treatment or within 8 weeks after drug discontinuation. One patient was excluded with a moderate adverse reaction characterized by posterior tibial artery vasospasm while receiving 4-AP 20 mg/day. This patient was hospitalized and successfully treated with an oral calcium antagonist (nifedipine 30 mg/day) with complete recovery. According to the study results, the drug was safe, with only mild and tolerable adverse reactions, and transitory enzyme and platelet alterations. However, in patients receiving 4-AP 30 mg/day, platelet counts and serum enzyme levels (ALT, AST, ALP, and CK) should be monitored, and patients should be monitored for possible arterial vasospasms and other mild adverse reactions.

3. MRID 47093603. 4-Aminopyridine in patients with multiple sclerosis: dosage and serum level related to efficacy and safety. Van Diemen *et al*, 1993 study:

This study was conducted at the Free University Hospital in Amsterdam in the Netherlands. Informed consent was obtained from all patients before admission to the study and the study protocol was approved by the Ethical Committee of the Free University Hospital. In this randomized, double blind, placebo-controlled, cross-over design study, 37 men and 43 women (23-68 years old) with clinically definite or laboratory supported definite MS (2 months to 25 year duration) were treated with 4-AP in two phases (The study report was silent regarding the status of women if they were sterile or using birth control method). In phase I, 4-AP was administered intravenously in increments of 1 mg in 1-2 minutes every 20 minutes during the first hour and increased to 2.5 mg in 1-2 minutes every 20 minutes afterwards until troublesome side effects developed or to a maximum dose of 0.5 mg/kg body weight. The infusion duration ranged from 60 to 260 minutes. This treatment was repeated after one week. The administered dosage of infused 4-AP in this phase ranged from 4.5 to 30.5 mg (0.07 to 0.5 mg/kg body weight). A saline solution (0.9% NaCl) served as a placebo control.

One week after the second iv administration, patients were administered 4-AP orally (phase II). Nonenteric-coated capsules of 4-AP or placebo (avicel) were administered

orally for a period of 12 weeks in a randomized sequence. Initially the 4-AP dosage was 10-25 mg per day in 2-3 doses. It was increased with 5-15 mg at week 2 and 6, or week 14 and 18. The maximum dosage was 0.5 mg/kg body weight/day. Blood was collected prior to phase II and at weeks, 2, 12, 14 and 24 for determination of creatinine, hemoglobin, white cell count, platelets, urea, total protein, sodium, potassium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine kinase (CK). All subjective side effects or concomitant diseases were noted. 4-AP serum levels were determined in blood collected immediately after the termination of iv administration and at the end of the first and second oral treatment periods 1.5-2.5 hours after the last ingestion of 4-AP (Weeks 12 and 24).

In the intravenous phase, the 4-AP serum levels ranged from 24 to 114 ng/mL (mean of 61.8 ng/mL). On average, the 4-AP serum levels increased 3.8 ng/mL per mg of 4-AP. Side effects following the infusion were paresthesias in infusion arm (49 patients), perioral paresthesias (6 patients), dizziness/light headedness (19), dizziness/light headedness and nausea/vomiting (2 patients), dizziness/light headedness and a feeling of restlessness (4 patients) and headache (1 patient). Only seven patients did not have any side effects during the 4-AP infusion. Paresthesias occurred at a minimal dose of 1 mg while the other effects occurred at 9-20 mg dose.

In the oral treatment, the mean oral dose was 31.2 mg/day (range 10-50 mg/day equivalent to 0.17 to 0.55 mg/kg body weight /day) divided in 2-4 doses. Serum levels of 4-AP during the oral treatment ranged from 7-107 ng/mL (mean 53.6 ng/mL). On average, the 4-AP serum levels increased 1.3 ng/mL per mg of 4-AP per day. Side effects during the oral treatment were paresthesias/dysesthesias e.i. : uncomfortable sensations, often described as burning, tingling, or numbness, unpleasant abnormal sensation, impairment of any of the senses, esp. of touch (perioral, acral e.i. affecting peripheral parts, such as limbs, fingers, or ears) (15 patients), dizziness/light headedness (36), gait instability (11), nausea and vomiting (9) restlessness/anxiety (4 patients), abdominal pain (5 patients) and obstipation (severe constipation) (1 patient). These side effects, generally mild, were reported at a daily dosage of 5-50 mg occurring after single doses of 5-20 mg of 4-AP. Fourteen patients needed dose reduction and 3 patients withdrew from the study because of these side effects. These side effects appeared 30-45 minutes after ingesting 4-AP and generally resolved within 2-5 hours. Fifteen patients did not experience side effects. In these 15 patients, the total daily dose of 4-AP ranged from 25-30 mg (0.38-0.55 mg/kg body weight/day) with serum levels ranging from 7 to 106 ng/mL. There were no effects of the 4-AP treatment on any of the blood parameters measured ($p>0.05$). The study investigators concluded that based on lack of abnormalities in the blood tests or ECGs, 4-AP can be used safely in MS patients at doses up to 0.5 mg/kg body weight.

C. Point of Departure and Uncertainty Factor(s)

The three efficacy and safety studies with 4-AP in human subjects described above provide the most useful information for establishing a PoD for the risk assessment from

exposure to 4-AP. In the Van Diemen *et al*, 1993 study, where 4-AP was administered in nonenteric capsules for 12 weeks at mean daily doses of 31.2 mg (range 10-50 mg/day), mean serum concentration of 4-AP was 53.6 ng/mL (range 7-107 ng/mL). The 4-AP serum levels were proportional to the administered dose given orally or by iv. Continuous dosing at a constant level of 4-AP did not result in increased serum levels suggesting that 4-AP residence in the body is short and is quickly eliminated. In the clinical studies discussed in this report, the therapeutic benefit of 4-AP was associated with its continued administration, suggesting again its short residency in the body and rapid elimination. One can conclude that a single PoD value is sufficient for risk assessments of 4-AP for different potential exposure scenarios (short-, intermediate- or long-term exposures). As cited by the NCI report (NCI, 2006), 4-AP is readily absorbed through the skin and gastrointestinal tracts of animals. Therefore, for the purposes of this risk assessment, a dermal absorption factor of 100% is considered for 4-AP.

In the studies reviewed above, 4-AP was tolerated in SCI or MS patients up to 40 mg daily dosages given mostly in 2-6 divided doses in a strictly supervised environment. However, mild transient side effects accompanied these treatments. In very few instances, patients required medical attention or were dropped from continuing in the trials. The medical literature documents cases of severe poisoning following accidental single ingestions of 60 mg 4-AP in human adults. It appears that 4-AP has a very steep dose response of toxicity.

The minimal daily oral dose of 4-AP producing side effects ranges from 5-30 mg. The Van Diemen *et al* study provides detailed information on these side effects as the following table reproduced from this article shows.

Side effects	Number of Patients	Minimal Daily Dose (mg)
Paresthesias/dysethesthesias	15/69	5
Dizziness/light-headedness	36/69	5
Gait instability	11/69	5
Nausea/vomiting	9/69	5
Restlessness/anxiety	4/69	5
Abdominal pain	5/69	10
Obstipation	1/69	25
Needed dose reduction	14/69	-
Withdrawal due to side effects	3/69	-
No side effects	15/69	-

Mild adverse effects were also reported in the Segal *et al*, 1999 study. Nervousness, giddiness or dizziness, and gastrointestinal upset manifesting as mild abdominal cramping or nausea were the most frequent side effects following treatment with doses of an immediate release formulation of 4-AP given in increments of 2, 5, or 10 mg over two weeks to a daily dose of 30 mg/human subject . All side effects were transient, self-limited, or disappeared with changes in dosage or the timing of drug ingestion to coincide with meals or snacks. The Grijalva *et al*, 2003 study concluded that the 4-AP drug is safe at the doses given (5 mg/day gradually increased to a maximum of 30 mg/day/subject)

with mild adverse reactions of such as dry mouth, dizziness, nausea, gastritis, and oral and peripheral paresthesia (tingling, pricking, or burning sensation)) and no patient required dosage reduction. Dry mouth, dizziness, and gastritis began with 4-AP at 5 or 10 mg/day; oral and peripheral paresthesia appeared only with 4-AP at 30 mg/day. Adverse reactions that had not been previously reported, such as memory alteration, bitter taste in the mouth, global pinching pain, and cramps were observed. In this study one patient exhibited arterial vasospasm at 20 mg 4-AP daily dose. The authors cautioned that patients must be carefully monitored for the possible occurrence of peripheral vasospasm.

For the purpose of this risk assessment, a 5 mg daily dose (0.08 mg/kg) is suggested as a minimal LOAEL for a PoD. Because a human study is being used for the risk assessment to 4-AP for different potential exposure scenarios, an interspecies extrapolation factor is not necessary. An uncertainty factor of 10X will be applied to account for individual variability (intraspecies) in the human population. Additional uncertainty factors may be applied to account for the use of a PoD based on a minimal LOAEL.

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