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NDR20599

1 OF 7

NDA 20-599

Rhone-Poulenc Rorer Pharmaceuticals
Attention: Thomas E. Donnelly, Jr., Ph.D.
Group Director
Worldwide Regulatory Affairs
500 Arcola Road
Collegeville, PA 19426

DEC 12 1995

Dear Dr. Donnelly:

Please refer to your June 29, 1995 new drug application and your resubmission dated December 1995 submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Rilutek® (riluzole) Tablets.

We also acknowledge receipt of your amendment and correspondence dated December 4, 1995 and December 8, 1995.

This new drug application provides for the treatment of patients with amyotrophic lateral sclerosis (ALS).

We have completed the review of this application including the submitted draft labeling and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the enclosed draft labeling. Accordingly, the application is approved effective on the date of this letter.

The final printed labeling (FPL) must be identical to the enclosed draft labeling. Marketing the product with FPL that is not identical to this draft labeling may render the product misbranded and an unapproved new drug.

Please submit fifteen copies of the FPL as soon as it is available, in no case more than 30 days after it is printed. Please individually mount ten of the copies on heavy weight paper or similar material. For administrative purposes this submission should be designated "FINAL PRINTED LABELING" for approved NDA 20-599. Approval of this labeling by FDA is not required before it is used.

Should additional information relating to the safety and effectiveness of the drug become available, revision of that labeling may be required.

We remind you of your Phase 4 commitments as specified under the "Clinical" subsection in your submission dated December 4, 1995. As agreed, these commitments will be completed by December 1996. However, we acknowledge that you cannot commit at the present time to the measurement of free riluzole in samples from previous studies. Protocols, data, and final reports should be submitted to your IND for this product and a copy of the cover letter sent to this NDA. Should an IND not be required to meet your Phase 4 commitments, please submit protocol, data, and final reports to

this NDA as correspondences. For administrative purposes, all submissions, including labeling supplements, relating to these Phase 4 commitments must be clearly designated "Phase 4 Commitments."

In addition, please submit three copies of the introductory promotional material that you propose to use for this product. All proposed materials should be submitted in draft or mock-up form, not final print. Please send one copy to the Division of Neuropharmacologic Drug Products and two copies of both the promotional material and the package insert directly to:

Food and Drug Administration
Division of Drug Marketing, Advertising and
Communications, HFD-40
5600 Fishers Lane
Rockville, Maryland 20857

Validation of the regulatory methods has not been completed. At the present time, it is the policy of the Center not to withhold approval because the methods are being validated. Nevertheless, we expect your continued cooperation to resolve any deficiencies that may occur.

Please submit one market package of the drug when it is available.

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

If you have any questions, please contact:

Katurah Higgins, R.Ph.
Project Manager
(301) 594-5529

Sincerely yours,

 12/11/55

Robert Temple, M.D.
Director
Office of Drug Evaluation I
Center for Drug Evaluation and Research

ENCLOSURE

RILUTEK[®] (riluzole)

Package Insert

NDA #20-599

WordPerfect 6.1 Document

11-Dec-95

RILUTEK®
(riluzole)

DESCRIPTION

RILUTEK® (riluzole) is a member of the benzothiazole class. Chemically, riluzole is 2-amino-6-(trifluoromethoxy)benzothiazole. Its molecular formula is $C_8H_5F_3N_2OS$ and its molecular weight is 234.2. Its structural formula is as follows:

Insert Chemical Structure Here

Riluzole is a white to slightly yellow powder that is very soluble in dimethylformamide, dimethylsulfoxide and methanol, freely soluble in dichloromethane, sparingly soluble in 0.1 N HCl and very slightly soluble in water and in 0.1 N NaOH. RILUTEK® is available as a capsule-shaped, white, film-coated tablet for oral administration containing 50 mg of riluzole. Each tablet is engraved with "RPR 202" on one side.

Inactive Ingredients: **Core:** anhydrous dibasic calcium phosphate, USP; microcrystalline cellulose, NF; anhydrous colloidal silica, NF; magnesium stearate, NF; croscarmellose sodium, NF. **Film coating:** hydroxypropylmethyl cellulose, USP; polyethylene glycol 6000; titanium dioxide, USP.

CLINICAL PHARMACOLOGY

Mechanism of Action:

The etiology and pathogenesis of amyotrophic lateral sclerosis (ALS) are not known, although a number of hypotheses have been advanced. One hypothesis is that motor neurons, made vulnerable through either genetic predisposition or environmental factors, are injured by glutamate. In some cases of familial ALS the

enzyme superoxide dismutase has been found to be defective.

The mode of action of RILUTEK® is unknown. Its pharmacological properties include the following, some of which may be related to its effect: 1) an inhibitory effect on glutamate release, 2) inactivation of voltage-dependent sodium channels, and 3) ability to interfere with intracellular events that follow transmitter binding at excitatory amino acid receptors.

Riluzole has also been shown, in a single study, to delay median time to death in a transgenic mouse model of ALS. These mice express human superoxide dismutase bearing one of the mutations found in one of the familial forms of human ALS.

It is also neuroprotective in various *in vivo* experimental models of neuronal injury involving excitotoxic mechanisms. In *in vitro* tests, riluzole protected cultured rat motor neurons from the excitotoxic effects of glutamic acid and prevented the death of cortical neurons induced by anoxia.

Due to its blockade of glutamatergic neurotransmission, riluzole also exhibits myorelaxant and sedative properties in animal models at doses of 30 mg/kg (about 20 times the recommended human daily dose) and anticonvulsant properties at a dose of 2.5 mg/kg (about 2 times the recommended human daily dose).

Pharmacokinetics

Riluzole is well-absorbed (approximately 90%), with average absolute oral bioavailability of about 60% (CV=30%). Pharmacokinetics are linear over a dose range of 25-100 mg given every 12 hours. A high fat meal decreases absorption, reducing AUC by about 20% and peak blood levels by about 45%. The mean

elimination half-life of riluzole is 12 hours (CV=35%) after repeated doses. With multiple dose administration, riluzole accumulates in plasma by about 2 fold and steady-state is reached in less than 5 days. Riluzole is 96% bound to plasma proteins, mainly to albumin and lipoproteins over the clinical concentration range.

The 50 mg market tablet was equivalent, with respect to AUC, to the tablet used in the dose ranging clinical trials, while the C_{max} was approximately 30% higher. Both tablets have been used in clinical trials. However if doses greater than those recommended are given, it is likely that higher plasma levels will be achieved, the safety of which has not been established (See DOSAGE AND ADMINISTRATION).

Metabolism and Elimination

Riluzole is extensively metabolized to six major and a number of minor metabolites, not all of which have been identified. Some metabolites appear pharmacologically active in *in vitro* assays. The metabolism of riluzole is mostly hepatic and consists of cytochrome P450-dependent hydroxylation and glucuronidation.

There is marked inter-individual variability in the clearance of riluzole, probably attributable to variability of CYP 1A2 activity, the principal isozyme involved in N-hydroxylation.

In vitro studies using liver microsomes show that hydroxylation of the primary amine group producing N-hydroxyriluzole is the main metabolic pathway in human, monkey, dog and rabbit. In humans, cytochrome P450 1A2 is the principal isozyme involved in N-hydroxylation. *In vitro* studies predict that CYP 2D6, CYP 2C19, CYP 3A4 and CYP 2E1 are unlikely to contribute significantly to riluzole metabolism in humans. Whereas direct glucuroconjugation of riluzole (involving the glucurotransferase

isoform UGT-HP4) is very slow in human liver microsomes, N-hydroxyriluzole is readily conjugated at the hydroxylamine group resulting in the formation of O- (>90%) and N-glucuronides.

Following a single 150 mg dose of ¹⁴C-riluzole to 6 healthy males, 90% and 5% of the radioactivity was recovered in the urine and feces respectively over a period of 7 days. Glucuronides accounted for more than 85% of the metabolites in urine. Only 2% of a riluzole dose was recovered in the urine as unchanged drug.

Special Populations

The pharmacokinetics of riluzole have not been studied in renally and hepatically impaired subjects, nor is there information about the effects of smoking, age and gender on the pharmacokinetics of riluzole but certain differences in population subsets should be anticipated (see PRECAUTIONS).

Hepatic and Renal Disease: Since riluzole is extensively metabolized and subsequently excreted in the urine, it is likely that functional hepatic and renal impairment will reduce the clearance of riluzole and its metabolites and give higher plasma levels. (see PRECAUTIONS and WARNINGS).

Age: Age-related decreased renal function would be expected to give higher plasma levels of riluzole and metabolites. However, in controlled clinical trials, in which approximately 30% of patients were over 65, there were no differences in adverse events between younger and older patients (see PRECAUTIONS).

Gender: CYP 1A2 activity has been reported to be lower in women than in men. Therefore, a gender effect on riluzole kinetics may be expected in women, resulting in higher blood concentrations of riluzole and its metabolites (see PRECAUTIONS). No

gender effect on favorable or adverse effects of Riluzole was seen in controlled trials, however.

Smoking: Cigarette smoking is known to induce CYP 1A2. Patients who smoke cigarettes would be expected to eliminate riluzole faster. There is no information, however, on the effect of, or need for, dosage adjustment in these patients.

Race: Clearance of riluzole in Japanese subjects native to Japan was found to be 50% lower as compared to Caucasians after normalizing for body weight. Although it is not clear if this difference is due to genetic or environmental factors (e.g. smoking, alcohol, coffee, and dietary preferences), it is possible that Japanese subjects may possess a lower capacity (oxidative and/or conjugative) for metabolizing riluzole. There are no studies, however, of lower doses in Japanese subjects (see PRECAUTIONS).

Clinical Trials

The efficacy of RILUTEK® as a treatment of ALS was established in two adequate and well-controlled trials in which the time to tracheostomy or death was longer for patients randomized to RILUTEK® than for those randomized to placebo.

These studies admitted patients with either familial or sporadic ALS, a disease duration of less than 5 years, and a baseline forced vital capacity greater than or equal to 60%.

In one study, performed in France and Belgium, 155 ALS patients were followed for

at least 13 months (maximum duration 18 months) after being randomized to either 100 mg/day (given 50 mg BID) of RILUTEK® or placebo.

Figure 1, which follows, displays the survival curves for time to death or tracheostomy. The vertical axis represents the proportion of individuals alive without tracheostomy at various times following treatment initiation (horizontal axis). Although these survival curves were not statistically significantly different when evaluated by the analysis specified in the study protocol (Logrank test $p=0.12$), the difference was found to be significant by another appropriate analysis (Wilcoxon test $p=0.05$). As seen, the study showed an early increase in survival in patients given riluzole. Among the patients in whom treatment failed during the study (tracheostomy or death) there was a difference between the treatment groups in median survival of approximately 90 days. There was no statistically significant difference in mortality at the end of the study.

Insert Figure 1 Here.

In the second study, performed in both Europe and North America, 959 ALS patients were followed for at least 1 year (North American centers) and up to 18 months (European centers) after being randomized to either 50, 100, 200 mg/day of RILUTEK® or placebo.

Figure 2, which follows, displays the survival curves for time to death or tracheostomy for patients randomized to either 100 mg/day of RILUTEK® or placebo. Although these survival curves were not statistically significantly different when evaluated by

the analysis specified in the study protocol (Logrank test $p = 0.076$), the difference was found to be significant by another appropriate analysis (Wilcoxon test $p = 0.05$). Not displayed in Figure 2 are the results of 50 mg/day of RILUTEK® which could not be statistically distinguished from placebo and the results of 200 mg/day which are essentially identical to 100 mg/day. As seen, the study showed an early increase in survival in patients given riluzole. Among the patients in whom treatment failed during the study (tracheostomy or death) there was a difference between the treatment groups in median survival of approximately 60 days. There was no statistically significant difference in mortality at the end of the study.

Insert Figure 2 Here

Although riluzole improved early survival in both studies, measures of muscle strength and neurological function did not show a benefit.

INDICATIONS AND USAGE

RILUTEK® is indicated for the treatment of patients with amyotrophic lateral sclerosis (ALS). Riluzole extends survival and/or time to tracheostomy.

CONTRAINDICATIONS

RILUTEK® is contraindicated in patients who have a history of severe hypersensitivity reactions to riluzole or any of the tablet components.

WARNINGS

Liver Injury / Monitoring Liver Chemistries

RILUTEK® should be prescribed with care in patients with current evidence or history of abnormal liver function indicated by significant abnormalities in serum transaminase (ALT/SGPT; AST/SGOT), bilirubin, and/or gamma-glutamyl

transferase (GGT) levels (see PRECAUTIONS and DOSAGE ADMINISTRATION SECTIONS). Baseline elevations of several LFTs (especially elevated bilirubin) should preclude the use of RILUTEK®.

RILUTEK®, even in patients without a prior history of liver disease, causes serum aminotransferase elevations. Experience in almost 800 ALS patients indicates that about 50% of Riluzole-treated patients will experience at least one ALT/SGPT level above the upper limit of normal, about 8% will have elevations > 3 X ULN, and about 2% of patients will have elevations > 5 X ULN. A single non-ALS patient with epilepsy treated with concomitant carbamazepine and phenobarbital experienced marked, rapid elevations of liver enzymes with jaundice (ALT 26 X ULN, AST 17 X ULN, and bilirubin 11 X ULN) four months after starting RILUTEK®; these returned to normal 7 weeks after treatment discontinuation.

Maximum increases in serum ALT usually occurred within 3 months after the start of riluzole therapy and were usually transient when < 5 times ULN. In trials, if ALT levels were < 5 times ULN, treatment continued and ALT levels usually returned to below 2 times ULN within 2 to 6 months. Treatment in studies was discontinued, however, if ALT levels exceeded 5 X ULN, so that there is no experience with continued treatment of ALS patients once ALT values exceed 5 times ULN. (see "PRECAUTIONS, Laboratory Tests"). There were rare instances of jaundice.

Liver chemistries should be monitored (See PRECAUTIONS).

Neutropenia

Among approximately 4000 patients given riluzole for ALS, there were three cases of marked neutropenia (absolute neutrophil count less than 500/mm³), all seen within the first 2 months of riluzole treatment. In one case, neutrophil counts rose on

continued treatment. In a second case, counts rose after therapy was stopped. A third case was more complex, with marked anemia as well as neutropenia and the etiology of both is uncertain. Patients should be warned to report any febrile illness to their physicians. The report of a febrile illness should prompt treating physicians to check white blood cell counts.

PRECAUTIONS

Use in Patients with Concomitant Disease

RILUTEK® should be used with caution in patients with concomitant liver and/or renal insufficiency (see WARNINGS, Clinical Pharmacology). In particular, in cases of RILUTEK®-induced hepatic injury manifested by elevated liver enzymes, the effect of the hepatic injury on RILUTEK® metabolism is unknown.

Special Populations

Riluzole should be used with caution in elderly patients whose hepatic or renal functions may be compromised due to age. Also, females and Japanese patients may possess a lower metabolic capacity to eliminate riluzole compared to males and Caucasian subjects, respectively (see CLINICAL PHARMACOLOGY: Clinical Pharmacokinetics).

Information for the Patient:

Patients should be advised to report any febrile illness to their physicians (see WARNINGS: Neutropenia).

Patients and caregivers should be advised that RILUTEK® should be taken on a regular basis and at the same time of the day (e.g. in the morning and evening) each day. If a dose is missed, take the next tablet as originally planned (see DOSAGE AND ADMINISTRATION).

Patients should be warned about the potential for dizziness, vertigo, or somnolence and advised not to drive or operate machinery until they have gained sufficient experience on RILUTEK® to gauge whether or not it affects their mental and/or motor performance adversely.

Whether alcohol increases the risk of serious hepatotoxicity with RILUTEK® is unknown; therefore, patients being treated with RILUTEK® should be discouraged from drinking excessive amounts of alcohol.

Patients should also be made aware that RILUTEK® should be stored at temperatures between 20° - 25° C (68° - 77° F) and protected from bright light.

RILUTEK® must be kept out of the reach of children.

Laboratory Tests:

It is recommended that serum aminotransferases including ALT levels be measured before and during riluzole therapy. Serum ALT levels should be evaluated every month during the first 3 months of treatment, every 3 months during the remainder of the first year, and periodically thereafter. Serum ALT levels should be evaluated more frequently in patients who develop elevations (see WARNINGS).

As noted in the WARNINGS Section, there is no experience with continued treatment of patients once ALT exceeds 5 X ULN. If a decision is made to continue to treat these patients, frequent monitoring (at least weekly) of complete liver function is recommended. Treatment should be discontinued if ALT exceeds 10 X ULN or if clinical jaundice develops. Because there is no experience with rechallenge of patients who have had RILUTEK® discontinued for ALT > 5 X ULN, no

recommendations about restarting RILUTEK® can be made.

In the two controlled trials in patients with ALS, the frequency with which values for hemoglobin, hematocrit, and erythrocyte counts fell below the lower limit of normal was greater in RILUTEK®-treated patients than in placebo-treated patients, however these changes were mild and transient. The proportions of patients observed with abnormally low values for these parameters showed a dose-response relationship. Only one patient was discontinued from treatment because of severe anemia. The significance of this finding is unknown.

Drug Interactions:

There have been no clinical studies designed to evaluate the interaction of riluzole with other drugs.

As with all drugs, the potential for interaction by a variety of mechanisms is a possibility.

Hepatotoxic Drugs: The clinical trials in ALS excluded patients on concomitant medications which were potentially hepatotoxic, (e.g. allopurinol, methyldopa, sulfasalazine). Accordingly there is no information about the safety of administering RILUTEK® in conjunction with such medications. If the practitioner chooses to prescribe such a combination, caution should be exercised.

Drugs Highly Bound To Plasma Proteins: Riluzole is highly bound (96%) to plasma proteins, binding mainly to serum albumin and to lipoproteins. The effect of riluzole (up to 5 mcg/mL) on warfarin (5 mcg/mL) binding did not show any displacement of warfarin. Conversely, riluzole binding was unaffected by the addition of warfarin,

digoxin, imipramine and quinine at high therapeutic concentrations.

Effect of Other Drugs On Riluzole Metabolism: *In vitro* studies using human liver microsomal preparations suggest that CYP 1A2 is the principal isozyme involved in the initial oxidative metabolism of riluzole, and therefore, potential interactions may occur when riluzole is given concurrently with agents that affect CYP 1A2 activity. Potential inhibitors of CYP 1A2 (e.g. caffeine, phenacetin, theophylline, amitriptyline, and quinolones) could decrease the rate of riluzole elimination, while inducers of CYP 1A2 (e.g. cigarette smoke, charcoal-broiled food, rifampicin, and omeprazole) could increase the rate of riluzole elimination.

Effect of Riluzole On the Metabolism of Other Drugs: CYP 1A2 is the principle isoenzyme involved in the initial oxidative metabolism of riluzole; potential interactions may occur when riluzole is given concurrently with other agents which are also metabolized primarily by CYP 1A2 (e.g. theophylline, caffeine and tacrine). Currently, it is not known whether riluzole has any potential for enzyme induction in humans.

Drug Laboratory Test Interactions: None known

Carcinogenicity - Mutagenesis - Impairment of Fertility

Long-term studies to determine the carcinogenic potential of riluzole have not yet been completed.

The genotoxic potential of riluzole was evaluated in the bacterial mutagenicity (Ames) test, the mouse lymphoma mutation assay in L5178Y cells, the ~~*in vitro* chromosomal aberration assay in human lymphocytes and the *in vivo* rat cytogenetic assay and *in vivo*~~ mouse micronucleus assay in bone marrow. There was no evidence of

mutagenic or clastogenic potential in the Ames test, the mouse lymphoma assay, or the *in vivo* assays in the mouse and rat. There was an equivocal clastogenic response in the *in vitro* lymphocyte chromosomal aberration assay.

Riluzole impaired fertility when administered to male and female rats prior to and during mating at an oral dose of 15 mg/kg or 1.5 times the maximum daily dose on a mg/m² basis. (See PRECAUTIONS, Pregnancy for effects on fertility.)

Pregnancy:

Pregnancy category C:

Oral administration of riluzole to pregnant animals during the period of organogenesis caused embryotoxicity in rats and rabbits at doses of 27 mg/kg and 60 mg/kg, respectively, or 2.6 and 11.5 times, respectively, the recommended maximum human daily dose on a mg/m² basis. Evidence of maternal toxicity was also observed at these doses.

When administered to rats prior to and during mating (males and females) and throughout gestation and lactation (females), riluzole produced adverse effects on pregnancy (decreased implantations, increased intrauterine death) and offspring viability and growth at an oral dose of 15 mg/kg or 1.5 times the maximum daily dose on a mg/m² basis.

There are no adequate and well-controlled studies in pregnant women. Riluzole should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Women:

In rat studies, ¹⁴C-riluzole was detected in maternal milk. It is not known whether riluzole is excreted in human breast milk. Because many drugs are excreted in human milk, and because of the potential for serious adverse reactions in nursing infants from RILUTEK® is unknown, women should be advised not to breast-feed during treatment with RILUTEK®.

Use in the Elderly:

Age related compromised renal and hepatic function may cause a decrease in clearance of riluzole (see CLINICAL PHARMACOLOGY: Clinical Pharmacokinetics). In controlled clinical trials, about 30% of patients were over 65. There were no differences in adverse effects between younger and older patients.

Pediatric Use:

The safety and the effectiveness of RILUTEK® in pediatric patients have not been established.

ADVERSE REACTIONS

The most commonly observed AEs associated with the use of RILUTEK® more frequently than placebo treated patients, were: asthenia, nausea, dizziness, decreased lung function, diarrhea, abdominal pain, pneumonia, vomiting, vertigo, circumoral paresthesia, anorexia, and somnolence. Asthenia, nausea, dizziness, diarrhea, anorexia, vertigo, somnolence, and circumoral paresthesia were dose related.

Approximately 14% (n = 141) of the 982 individuals with ALS who received RILUTEK® in pre-marketing clinical trials discontinued treatment because of an adverse experience. Of those patients who discontinued due to adverse events, the most commonly reported were: nausea, abdominal pain, constipation, and ALT

elevations. In a dose response study in ALS patients, the rates of discontinuation of RILUTEK® for asthenia, nausea, abdominal pain, and ALT elevation were dose related.

Incidence in Controlled ALS Clinical Studies:

Table 1 lists treatment-emergent signs and symptoms that occurred in at least 2% of patients with ALS treated with RILUTEK® (n=794) participating in placebo-controlled trials and were numerically greater in the patients treated with RILUTEK® 100 mg/day than with placebo or for which a dose response relationship is suggested.

The prescriber should be aware that these figures cannot be used to predict the frequency of adverse experiences in the course of usual medical practice where patient characteristics and other factors may differ from those prevailing during clinical studies. Inspection of these frequencies, however, does provide the prescriber with one basis to estimate the relative contribution of drug and non-drug factors to the AE incidences in the population studied.

Table 1				
Adverse Events Occurring in Placebo-Controlled Clinical Trials				
Percentage of patients reporting events*				
Body System / Adverse Event*	Riluzole 50 mg/day (N=237)	Riluzole 100 mg/day (N=313)	Riluzole 200 mg/day (N=244)	Placebo (N=320)
Body as a Whole				
Asthenia	14.8	19.2	20.1	12.2
Headache	8.0	7.3	7.0	6.6
Abdominal pain	6.8	5.1	7.8	3.8
Back pain	1.7	3.2	4.1	2.5
Aggravation reaction	0.4	1.3	2.0	0.9
Malaise	0.4	0.6	1.2	0.0

Digestive				
Nausea	12.2	16.3	20.5	10.6
Vomiting	4.2	4.2	4.5	1.6
Dyspepsia	2.5	3.8	6.1	5.0
Anorexia	3.8	3.2	8.6	3.8
Diarrhea	5.5	2.9	9.0	3.1
Flatulence	2.5	2.6	2.0	1.9
Stomatitis	0.8	1.0	1.2	0.0
Tooth disorder	0.0	1.0	1.2	0.3
Oral Moniliasis	0.4	0.6	1.2	0.3
Nervous				
Hypertonia	5.9	6.1	5.3	5.9
Depression	4.2	4.5	6.1	5.0
Dizziness	5.1	3.8	12.7	2.5
Dry mouth	3.0	3.5	2.0	3.4
Insomnia	2.1	3.5	2.9	3.4
Somnolence	0.8	1.9	4.1	1.3
Vertigo	2.5	1.9	4.5	0.9
Circumoral paresthesia	1.3	1.6	3.3	0.0
Skin and Appendages				
Pruritus	3.8	3.8	2.5	3.1
Eczema	0.8	1.6	1.6	0.6
Alopecia	0.0	1.0	1.2	0.6
Exfoliative dermatitis	0.0	0.6	1.2	0.0
Respiratory				
Decreased lung function	13.1	10.2	16.0	9.4
Rhinitis	8.9	6.4	7.8	6.3
Increased cough	2.1	2.6	3.7	1.6
Sinusitis	0.4	1.0	1.6	0.9
Cardiovascular				
Hypertension	6.8	5.1	3.3	4.1
Tachycardia	1.3	2.6	2.0	1.3
Phlebitis	0.4	1.0	0.8	0.3
Palpitation	0.4	0.6	1.2	0.9
Postural hypotension	0.8	0.0	1.6	0.6
Metabolic and Nutritional Disorders				
Weight loss	4.6	4.8	3.7	4.7
Peripheral edema	4.2	2.9	3.3	2.2
Musculoskeletal System				
Arthralgia	5.1	3.5	1.6	3.4

Urogenital System				
Urinary tract infection	2.5	2.6	4.5	2.2
Dysuria	0.0	1.0	1.2	0.3

Other Adverse Events Observed

Other events which occurred in more than 2% of patients treated with RILUTEK 100 mg/day but equally or more frequently in the placebo group included: accidental injury, apnea, bronchitis, constipation, death, dysphagia, dyspnea, flu syndrome, heart arrest, increased sputum, pneumonia, and respiratory disorder.

The overall adverse event profile for RILUTEK® was similar between females and males, and was independent of age. Because the largest non-white racial subgroup was only 2% of patients exposed to RILUTEK® (18/794) in placebo-controlled trials, there are insufficient data to support a statement regarding the distribution of adverse experience reports by race. In ALS studies, dizziness did occur more commonly in females (11%) than in males (4%). There was not a difference between females and males in the rates of discontinuation of RILUTEK® for individual adverse experiences.

Other Adverse Events Observed During All Clinical Trials:

RILUTEK® has been administered to 1713 individuals during all clinical trials, some of which were placebo-controlled. During these trials, all adverse events were recorded by the clinical investigators using terminology of their own choosing. To provide a meaningful estimate of the proportion of individuals having adverse events, similar types of events were grouped into a smaller number of standardized categories using modified COSTART dictionary terminology. The frequencies presented represent the proportion of the 1713 individuals exposed to RILUTEK® who experienced an event of the type cited on at least one occasion while receiving

RILUTEK®. All reported events are included except those already listed in the previous table, those too general to be informative, and those not reasonably associated with the use of the drug.

Events are further classified within body system categories and enumerated in order of decreasing frequency using the following definitions: *frequent* adverse events are defined as those occurring in at least 1/100 patients; *infrequent* adverse events are those occurring in 1/100 to 1/1000 patients; *rare* adverse events are those occurring in fewer than 1/1000 patients.

* = or AE frequency $\leq$ to placebo

Body as a Whole: *Frequent:* Hostility*. *Infrequent:* Abscess*, sepsis*, photosensitivity reaction*, cellulitis, face edema*, hernia, peritonitis, attempted suicide, injection site reaction, chills*, flu syndrome, intentional injury, enlarged abdomen, neoplasm. *Rare:* Acrodynia, hypothermia, moniliasis*, rheumatoid arthritis.

Digestive System: *Infrequent:* Increased appetite, intestinal obstruction*, fecal impaction, gastrointestinal hemorrhage, gastrointestinal ulceration, gastritis*, fecal incontinence, jaundice, hepatitis, glossitis, gum hemorrhage*, pancreatitis, tenesmus, esophageal stenosis. *Rare:* Cheilitis*, cholecystitis, hematemesis, melena*, biliary pain, proctitis, pseudomembranous enterocolitis, enlarged salivary gland, tongue discoloration, tooth caries.

Nervous System: *Frequent:* Agitation*, tremor. *Infrequent:* Hallucinations, personality disorder*, abnormal thinking*, coma, paranoid reaction*, manic reaction, ataxia, extrapyramidal syndrome, hypokinesia, urinary retention, emotional lability, delusions, apathy, hypesthesia, incoordination, confusion*, convulsion, leg cramps, amnesia, dysarthria, increased libido, stupor, subdural hematoma, abnormal gait, delirium, depersonalization, facial paralysis, hemiplegia, decreased libido, myoclonus. *Rare:* Abnormal dreams, acute brain syndrome, CNS depression, dementia, cerebral embolism, euphoria*, hypotonia, ileus*, peripheral neuritis, psychosis*, psychotic depression, schizophrenic reaction, trismus, wrist drop.

Skin and Appendages: *Infrequent:* Skin ulceration, urticaria, psoriasis, seborrhea*, skin disorder, fungal dermatitis*. *Rare:* Angioedema, contact dermatitis, erythema multiforme, furunculosis*, skin moniliasis, skin granuloma, skin nodule.

Respiratory System: *Infrequent:* Hiccup, pleural disorder*, asthma, epistaxis, hemoptysis, yawn, hyperventilation*, lung edema *, hypoventilation*, lung carcinoma, hypoxia, laryngitis, pleural effusion, pneumothorax*, respiratory moniliasis, stridor.

Cardiovascular System: *Infrequent:* Syncope*, hypotension, heart failure, migraine, peripheral vascular disease, angina pectoris*, myocardial infarction*, ventricular extrasystoles, cerebral hemorrhage, atrial fibrillation*, bundle branch block, congestive heart failure, pericarditis, lower extremity embolus, myocardial ischemia*, shock*. *Rare:* Bradycardia, cerebral ischemia, hemorrhage, mesenteric artery occlusion, subarachnoid hemorrhage, supraventricular tachycardia*, thrombosis, ventricular fibrillation, ventricular tachycardia.

Metabolic and Nutritional Disorders: *Infrequent:* Gout*, respiratory acidosis, edema, thirst*, hypokalemia, hyponatremia, weight gain*. *Rare:* Generalized edema, hypercalcemia, hypercholesteremia.

Endocrine System: *Infrequent:* Diabetes mellitus, thyroid neoplasia. *Rare:* Diabetes insipidus, parathyroid disorder.

Hemic and Lymphatic System: *Infrequent:* Anemia*, leukocytosis, leukopenia, ecchymosis. *Rare:* Neutropenia, aplastic anemia, cyanosis, hypochromic anemia, iron deficiency anemia, lymphadenopathy, petechiae*, purpura.

Musculoskeletal System: *Infrequent:* Arthrosis, myasthenia*, bone neoplasm. *Rare:* Bone necrosis, osteoporosis, tetany.

Special Senses: *Infrequent:* Amblyopia, ophthalmitis. *Rare:* Blepharitis, cataract, deafness, diplopia*, ear pain, glaucoma, hyperacusis, photophobia, taste loss, vestibular disorder.

Urogenital System: *Infrequent:* Urinary urgency, urine abnormality, urinary incontinence, kidney calculus, hematuria, impotence, prostate carcinoma, kidney pain, metrorrhagia, priapism. *Rare:* Amenorrhea, breast abscess, breast pain, nephritis*, nocturia, pyelonephritis, enlarged uterine fibroids, uterine hemorrhage, vaginal moniliasis.

Laboratory Tests: *Infrequent:* Increased gamma glutamyl transferase, abnormal liver function/tests, increased alkaline phosphatase, positive direct Coombs test, increased gamma globulins. *Rare:* increased lactic dehydrogenase.

OVERDOSE

There have been no reports of overdose with RILUTEK®. No specific antidote or information on treatment of overdosage with RILUTEK® is available. In the event of overdose, RILUTEK® therapy should be discontinued immediately. Treatment should be supportive and directed toward alleviating symptoms.

The estimated oral median lethal dose is 94 mg/kg and 39 mg/kg for male mice and rats, respectively.

DOSAGE AND ADMINISTRATION

The recommended dose for RILUTEK® is 50 mg every 12 hours. No increased benefit can be expected from higher daily doses, but adverse events are increased.

RILUTEK® tablets should be taken at least an hour before, or two hours after, a meal to avoid a food related decrease in bioavailability.

Special Populations:

Patients with Impaired Renal or Hepatic Function: Studies have not yet been completed in these populations. (See WARNINGS, PRECAUTIONS, CLINICAL PHARMACOLOGY).

HOW SUPPLIED

RILUTEK® 50 mg tablets are white, film-coated, capsule-shaped and engraved with "RPR 202" on one side. RILUTEK® is supplied in bottles of 60 tablets, NDC 0075-7700-60. These bottles are designed with a special dispensing flip cap to aid dispensing with minimum effort.

NDA 20-599 RILUTEK® Agreed Package Insert
(rilutek7.wp6)
07-Dec-95

Page 21

**STORE AT CONTROLLED ROOM TEMPERATURE 20° - 25° C (68° - 77° F) AND
PROTECT FROM BRIGHT LIGHT.**

KEEP OUT OF THE REACH OF CHILDREN.

Caution: Federal law prohibits dispensing without prescription.

Manufactured in Ireland

RHÔNE-POULENC RORER PHARMACEUTICALS INC.

Collegeville, PA 19426

IN-####X

Rev. X/95

BAR CODE

RHÔNE-POULENC RORER PHARMACEUTICALS INC.

500 ARCOLA ROAD
P.O. BOX 1200
COLLEGEVILLE, PA 19426-0107

THOMAS E. DONNELLY, JR., Ph.D.
GROUP DIRECTOR
WORLDWIDE REGULATORY AFFAIRS
TEL: 610-454-3023
FAX: 610-454-5299
VM# 610-454-8666, BOX 3023

June 29, 1995

Paul D. Leber, MD., Director
Division of Neuropharmacological Drug Products
(HFD-120)
Center for Drug Evaluation and Research
Office of Drug Evaluation I
Document Control Room 4008
Food and Drug Administration
1451 Rockville Pike
Rockville, MD 20852

NDA 20-599
Rilutek® (riluzole)

**ORIGINAL NEW DRUG
APPLICATION**

Dear Dr. Leber:

In accordance with 21 CFR 314.50 of the Federal Food, Drug and Cosmetic Act, Rhone-Poulenc Rorer Pharmaceuticals Inc. is submitting an Original New Drug Application for Rilutek® (riluzole) which demonstrates the efficacy and safety of the product in the treatment of patients with amyotrophic lateral sclerosis (ALS). This compound has Orphan Drug status which was granted under 21 CFR 316.24 on March 16, 1993.

Although the etiology of ALS has not been completely elucidated, it is suggested that glutamic acid plays a major role in this disease. In ALS, it is believed that compromised motor neurons (through genetic predisposition or environmental factors) exposed to glutamate at the synaptic cleft may be more sensitive to its toxic effects; this may result in damage or death of central neurons.

Riluzole (2-amino-6-trifluoromethoxybenzothiazole) is indicated for the treatment of patients with amyotrophic lateral sclerosis (ALS). Riluzole appears to be an antagonist

Dr. Paul D. Leber
Page 2 of 3
June 30, 1995
NDA 20-599

of glutamatergic transmission. In in-vivo animal models, riluzole exhibits potent neuroprotective effects against cerebral ischemia and excitotoxicity. Riluzole also protects experimental animals against the effects of a wide range of convulsant agents, especially excitatory amino acids. High doses of riluzole exert a depressant effect on the central nervous system, decreasing neuronal activity in a number of brain structures and modifying concomitantly the electrocorticogram and brain glucose consumption. In vitro, riluzole protects motoneurons against the toxic effects of glutamic acid. Riluzole does not bind to any known receptors, but it is known to exert a marked inhibitory effect on the spontaneous release of glutamate and on dopamine release induced by glutamate. The antagonism of glutamatergic transmission might be via interaction with voltage-dependent sodium channels or with G-proteins. However, the precise molecular target remains under investigation.

The efficacy and safety of riluzole in the treatment of ALS has been demonstrated in two adequate and well-controlled clinical trials. The full study report for study 216 was submitted to the IND on February 15, 1994, serial no. 036. A preliminary report on study 01 together with full datasets on diskette were submitted to the IND on March 23, 1995, serial no. 081. Additionally, the Nonclinical Pharmacology and Toxicology section and the Human Pharmacokinetics and Bioavailability section were previously submitted to the IND.

In accordance with 21CFR 314.50, this application contains the following technical sections: 1) Chemistry, Manufacturing and Controls, 2) Nonclinical Pharmacology and Toxicology, 3) Human Pharmacokinetics and Bioavailability, 4) Clinical Data, 5) Statistics, and the following additional items 6) Labeling, 7) Case Report Forms (patient profiles per agreement), 8) Patent information, 9) Publications regarding riluzole.

Case report form tabulations for the individual medical reports are included in the appendices of each report when available and are located in the Clinical Data and Statistical sections of the application. An index to these tabulations is provided in Item 11: Case Report Form tabulations.

The format and content of the various sections of the NDA were discussed and agreed to at several pre-NDA meetings, the minutes of which we have previously submitted to the IND. A list of these meetings is provided together with submission information.

Draft labeling is provided on diskette in WordPerfect 5.1 format for ease of review and revision; a hard copy of the file is also enclosed.

Dr. Paul D. Leber
Page 3 of 3
June 30, 1995
NDA 20-599

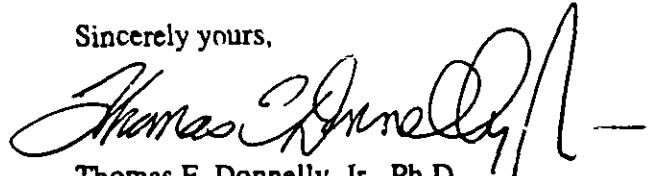
In accordance with the Prescription Drug User Fee Act of 1992, a check (Check No. 727453), in the amount of _____ was sent to the Food and Drug Administration, Philadelphia, Pennsylvania on June 26, 1995. The application was assigned the User Fee Identification Number 2828.

As required by Section 306(k)(1) of the Generic Drug Enforcement Act [21 U.S.C. 335a (k)(1)], we hereby certify that, in connection with this application, Rhone-Poulenc Rorer did not and will not use in any capacity the services of any person debarred under subsections 3-6(a) or (b) of the act.

Rhone-Poulenc Rorer Pharmaceuticals Inc. considers the information in this application to be confidential and proprietary and we request that no portions thereof be disclosed to third parties, under FOI or otherwise, without first obtaining written consent from Rhone-Poulenc Rorer.

If you have any questions or require any additional information during review of this application, please contact me at (610) 454-3023.

Sincerely yours,



Thomas E. Donnelly, Jr., Ph.D.
Group Director
Worldwide Regulatory Affairs

TED/bnh
Attachment



NDA 20-599

14/05/95

Rhone-Poulenc Rorer Pharmaceuticals, Inc.
Attn: Thomas E. Donnelly, Jr., Ph.D.
500 Arcola Road
Collegville, PA 19426

JUL 13 1995

Dear Dr. Donnelly:

We have received your new drug application submitted under section 505(b) of the Federal Food, Drug and Cosmetic Act for the following:

Name of Drug Product: Rilutek® (riluzole) 50mg Tablets
Therapeutic Classification: P
Date of Application: June 29, 1995
Date of Receipt: June 29, 1995
Our Reference Number: NDA 20-599

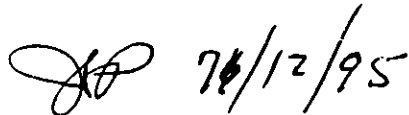
Unless we notify you within 60 days of the receipt date that the application is not sufficiently complete to permit a substantive review, this application will be filed under section 505(b) of the Act on August 28, 1995 in accordance with 21 CFR 314.101(b).

Under 21 CFR 314.102(c) of the new drug regulations and in accordance with the policy described in the Center for Drug Evaluation and Research Staff Manual Guide CDER 4820.6, you may request an informal conference with this Division (to be held approximately 90 days from the above receipt date) for a brief report on the status of the review but not on the application's ultimate approvability. Please request the meeting at least 15 days in advance. Alternatively, you may choose to receive such a report by telephone. Should you wish a conference, a telephone report, or if you have any questions concerning this NDA, please contact:

Katurah H. Higgins
Consumer Safety Officer
(301) 594-2777

Please cite the NDA number listed on the previous page at the top of the first page of any communications concerning this application.

Sincerely yours,

Handwritten signature of John S. Purvis and the date 7/12/95.

John S. Purvis
Chief, Project Management Staff
Division of Neuropharmacological Drug Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

RILUTEK[®]
(Riluzole) Tablets

NDA 20-599

**Administrative Issues
&
Labeling**

Volume 1 of 1

RHÔNE-POULENC RORER PHARMACEUTICALS INC.

 500 ARCOLA ROAD
 P.O. BOX 1200
 COLLEGEVILLE, PA 19426-0107
 TEL. 610-454-8000

New Drug Application: #20-599
Form FDA 356h
Item
Rilutek® (riluzole)

Items 13 and 14: Patent Information and Patent Certification

Product Generic Name	riluzole
RPR Brand Name	Rilutek®
Key Compound No	54274
RPR Product Profile	Riluzole is a new benzothiazole with neuroprotective properties, for the treatment of Amyotrophic Lateral Sclerosis (ALS), also known as "maladie de Charcot" (FR), motor neuron disease (GB), or Lou Gehrig's disease (US), a rapidly fatal neurodegenerative disease for which there is no effective treatment. It is the first product to slow the motor function.

United States Patent Information

Patent Number	Filing Date	Patent Grant Date	Expiration Date Extension Date	Claim Types
5,240,948	Jan 29, 1991	5,240,948	Aug. 31, 2010	Composition, Method of use, Formulation
		Aug. 31, 1993	N/A	
5,008,280	Dec. 13, 1989	5,008,280	Apr. 16, 2008	Compound, Process, Method of use
		Apr. 16, 1991	Dec. 13, 2009	
1,866,694	Oct. 26, 1993	1,866,694	Dec. 13, 2004	Trademark
		Dec. 13, 1994	N/A	

EXCLUSIVITY SUMMARY for NDA # 20-599 SUPPL # _____

Trade Name Rilutek Generic Name Riluzole

Applicant Name Rhone-Poulenc Rorer HFD- 120

Approval Date December 12, 1995

PART I IS AN EXCLUSIVITY DETERMINATION NEEDED?

1. An exclusivity determination will be made for all original applications, but only for certain supplements. Complete Parts II and III of this Exclusivity Summary only if you answer "yes" to one or more of the following questions about the submission.

a) Is it an original NDA? YES / / NO / /

b) Is it an effectiveness supplement? YES / / NO / /

If yes, what type? (SE1, SE2, etc.) _____

c) Did it require the review of clinical data other than to support a safety claim or change in labeling related to safety? (If it required review only of bioavailability or bioequivalence data, answer "no.") YES / / NO / /

If your answer is "no" because you believe the study is a bioavailability study and, therefore, not eligible for exclusivity, EXPLAIN why it is a bioavailability study, including your reasons for disagreeing with any arguments made by the applicant that the study was not simply a bioavailability study.

If it is a supplement requiring the review of clinical data but it is not an effectiveness supplement, describe the change or claim that is supported by the clinical data:

PART II FIVE-YEAR EXCLUSIVITY FOR NEW CHEMICAL ENTITIES

(Answer either #1 or #2, as appropriate)

1. Single active ingredient product.

Has FDA previously approved under section 505 of the Act any drug product containing the same active moiety as the drug under consideration? Answer "yes" if the active moiety (including other esterified forms, salts, complexes, chelates or clathrates) has been previously approved, but this particular form of the active moiety, e.g., this particular ester or salt (including salts with hydrogen or coordination bonding) or other non-covalent derivative (such as a complex, chelate, or clathrate) has not been approved. Answer "no" if the compound requires metabolic conversion (other than deesterification of an esterified form of the drug) to produce an already approved active moiety.

YES / ___ / NO / /

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA # _____

NDA # _____

NDA # _____

2. Combination product.

If the product contains more than one active moiety (as defined in Part II, #1), has FDA previously approved an application under section 505 containing any one of the active moieties in the drug product? If, for example, the combination contains one never-before-approved active moiety and one previously approved active moiety, answer "yes." (An active moiety that is marketed under an OTC monograph, but that was never approved under an NDA, is considered not previously approved.)

YES / ___ / NO / ___ /

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA # _____

NDA # _____

NDA # _____

IF THE ANSWER TO QUESTION 1 OR 2 UNDER PART II IS "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8. IF "YES," GO TO PART III.

PART III THREE-YEAR EXCLUSIVITY FOR NDA'S AND SUPPLEMENTS

To qualify for three years of exclusivity, an application or supplement must contain "reports of new clinical investigations (other than bioavailability studies) essential to the approval of the application and conducted or sponsored by the applicant." This section should be completed only if the answer to PART II, Question 1 or 2, was "yes."

1. Does the application contain reports of clinical investigations? (The Agency interprets "clinical investigations" to mean investigations conducted on humans other than bioavailability studies.) If the application contains clinical investigations only by virtue of a right of reference to clinical investigations in another application, answer "yes," then skip to question 3(a). If the answer to 3(a) is "yes" for any investigation referred to in another application, do not complete remainder of summary for that investigation.

YES /___/ NO /___/

IF "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. A clinical investigation is "essential to the approval" if the Agency could not have approved the application or supplement without relying on that investigation. Thus, the investigation is not essential to the approval if 1) no clinical investigation is necessary to support the supplement or application in light of previously approved applications (i.e., information other than clinical trials, such as bioavailability data, would be sufficient to provide a basis for approval as an ANDA or 505(b)(2) application because of what is already known about a previously approved product), or 2) there are published reports of studies (other than those conducted or sponsored by the applicant) or other publicly available data that independently would have been sufficient to support approval of the application, without reference to the clinical investigation submitted in the application.

For the purposes of this section, studies comparing two products with the same ingredient(s) are considered to be bioavailability studies.

- (a) In light of previously approved applications, is a clinical investigation (either conducted by the applicant or available from some other source, including the published literature) necessary to support approval of the application or supplement?

YES /___/ NO /___/

If "no," state the basis for your conclusion that a clinical trial is not necessary for approval AND GO DIRECTLY TO SIGNATURE BLOCK ON PAGE 8:

- (b) Did the applicant submit a list of published studies relevant to the safety and effectiveness of this drug product and a statement that the publicly available data would not independently support approval of the application?

YES /___/ NO /___/

- (1) If the answer to 2(b) is "yes," do you personally know of any reason to disagree with the applicant's conclusion? If not applicable, answer NO.

YES /___/ NO /___/

If yes, explain: _____

- (2) If the answer to 2(b) is "no," are you aware of published studies not conducted or sponsored by the applicant or other publicly available data that could independently demonstrate the safety and effectiveness of this drug product?

YES /___/ NO /___/

If yes, explain: _____

- (c) If the answers to (b)(1) and (b)(2) were both "no," identify the clinical investigations submitted in the application that are essential to the approval:

Investigation #1, Study # _____

Investigation #2, Study # _____

Investigation #3, Study # _____

3. In addition to being essential, investigations must be "new" to support exclusivity. The agency interprets "new clinical investigation" to mean an investigation that 1) has not been relied on by the agency to demonstrate the effectiveness of a previously approved drug for any indication and 2) does not duplicate the results of another investigation that was relied on by the agency to demonstrate the effectiveness of a previously approved drug product, i.e., does not redemonstrate something the agency considers to have been demonstrated in an already approved application.

a) For each investigation identified as "essential to the approval," has the investigation been relied on by the agency to demonstrate the effectiveness of a previously approved drug product? (If the investigation was relied on only to support the safety of a previously approved drug, answer "no.")

Investigation #1	YES /___/	NO /___/
Investigation #2	YES /___/	NO /___/
Investigation #3	YES /___/	NO /___/

If you have answered "yes" for one or more investigations, identify each such investigation and the NDA in which each was relied upon:

NDA # _____	Study # _____
NDA # _____	Study # _____
NDA # _____	Study # _____

b) For each investigation identified as "essential to the approval," does the investigation duplicate the results of another investigation that was relied on by the agency to support the effectiveness of a previously approved drug product?

Investigation #1	YES /___/	NO /___/
Investigation #2	YES /___/	NO /___/
Investigation #3	YES /___/	NO /___/

If you have answered "yes" for one or more investigations, identify the NDA in which a similar investigation was relied on:

NDA # _____	Study # _____
NDA # _____	Study # _____
NDA # _____	Study # _____

- c) If the answers to 3(a) and 3(b) are no, identify each "new" investigation in the application or supplement that is essential to the approval (i.e., the investigations listed in #2(c), less any that are not "new"):

Investigation #__, Study # _____

Investigation #__, Study # _____

Investigation #__, Study # _____

4. To be eligible for exclusivity, a new investigation that is essential to approval must also have been conducted or sponsored by the applicant. An investigation was "conducted or sponsored by" the applicant if, before or during the conduct of the investigation, 1) the applicant was the sponsor of the IND named in the form FDA 1571 filed with the Agency, or 2) the applicant (or its predecessor in interest) provided substantial support for the study. Ordinarily, substantial support will mean providing 50 percent or more of the cost of the study.

- a) For each investigation identified in response to question 3(c): if the investigation was carried out under an IND, was the applicant identified on the FDA 1571 as the sponsor?

Investigation #1 !
 IND # _____ YES /___/ ! NO /___/ Explain: _____
 !

Investigation #2 !
 IND # _____ YES /___/ ! NO /___/ Explain: _____
 !

- (b) For each investigation not carried out under an IND or for which the applicant was not identified as the sponsor, did the applicant certify that it or the applicant's predecessor in interest provided substantial support for the study?

Investigation #1 !
 YES /___/ Explain _____ ! NO /___/ Explain _____
 !
 _____ !
 _____ !
 _____ !

Investigation #2

YES / ___ / Explain _____

NO / ___ / Explain _____

(c) Notwithstanding an answer of "yes" to (a) or (b), are there other reasons to believe that the applicant should not be credited with having "conducted or sponsored" the study? (Purchased studies may not be used as the basis for exclusivity. However, if all rights to the drug are purchased (not just studies on the drug), the applicant may be considered to have sponsored or conducted the studies sponsored or conducted by its predecessor in interest.)

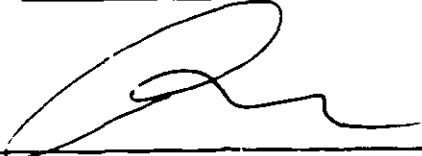
YES / ___ /

NO / ___ /

If yes, explain: _____

Katurah N. Higgins
Signature
Title: CSO

10/25/95
Date


Signature of Division Director

11/5/95
Date

cc: Original NDA

Division File

HFD-85 Mary Ann Holovac

DRUG STUDIES IN PEDIATRIC PATIENTS
(To be completed for all NME's recommended for approval)

NDA # 20-599 Trade (generic) names Rilutek (riluzole) Tablets

Check any of the following that apply and explain, as necessary, on the next page:

1. A proposed claim in the draft labeling is directed toward a specific pediatric illness. The application contains adequate and well-controlled studies in pediatric patients to support that claim.
2. The draft labeling includes pediatric dosing information that is not based on adequate and well-controlled studies in children. The application contains a request under 21 CFR 210.58 or 314.126(c) for waiver of the requirement at 21 CFR 201.57(f) for A&WC studies in children.
- a. The application contains data showing that the course of the disease and the effects of the drug are sufficiently similar in adults and children to permit extrapolation of the data from adults to children. The waiver request should be granted and a statement to that effect is included in the action letter.
- b. The information included in the application does not adequately support the waiver request. The request should not be granted and a statement to that effect is included in the action letter. (Complete #3 or #4 below as appropriate.)
3. Pediatric studies (e.g., dose-finding, pharmacokinetic, adverse reaction, adequate and well-controlled for safety and efficacy) should be done after approval. The drug product has some potential for use in children, but there is no reason to expect early widespread pediatric use (because, for example, alternative drugs are available or the condition is uncommon in children).
- a. The applicant has committed to doing such studies as will be required.
- (1) Studies are ongoing.
- (2) Protocols have been submitted and approved.
- (3) Protocols have been submitted and are under review.
- (4) If no protocol has been submitted, on the next page explain the status of discussions.
- b. If the sponsor is not willing to do pediatric studies, attach copies of FDA's written request that such studies be done and of the sponsor's written response to that request.
4. Pediatric studies do not need to be encouraged because the drug product has little potential for use in children.

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MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: August 8, 1995
FROM: Paul Leber, M.D., Director, *Leber 8/8/95*
Division of Neuropharmacological Drug Products, HFD-120
SUBJECT: Request for Assessment of a Trademark for a Proposed Drug Product
TO: Dan Boring, Chair
Labeling and Nomenclature Committee
HFD-530/NLRC Room 224

Proposed Trademark: Rilutek[®] NDA # 20-599

Established name, including dosage form: Riluzole Tablets *50 mg*

Other trademarks by the same firm for companion products: None

Indications for Use (may be a summary if proposed statement is lengthy):

Rilutek is indicated for the treatment of patients with amyotrophic lateral sclerosis (ALS).

Initial comments from the submitter: (concerns, observations, etc.) None

cc:
ORIG NDA
HFD-120
HFD-120/KHiggins *ket*

Rilutek
1-11-95

MEMORANDUM

Consult #475 (HFD-120)

RILUTEK Riluzole Tablets

A review revealed two names which sound like or look like the proposed name: Rheumatrex and Prilosec. Due to differences in product strengths, the Committee does not believe there is a significant potential for confusion involving the two names and the proposed name. However, the Committee does have a concern that if the strength is not included in a written or verbal order for Rilutek or Rheumatrex, the possibility for a medication error with serious consequences could exist. Division reviewers are urged to carefully consider the Committee's concerns when making a final decision.

Other than the concern expressed above, the Committee has no reason to find the proposed name unacceptable.

CDER Labeling and Nomenclature Committee

D. Borun, Chair

cc: Orig NDA
HFD-120
HFD-120/Leber/Katz/Fcney/Higgins

Rilutek[®]
(Riluzole) Tablets

NDA 20-599

Clinical/Statistical

Volume ___ of ___

CLINICAL / STATISTICAL

NDA 20-599 Rilutek® (riluzole) Tablets Classification: 1P

<u>Date</u>	<u>Document</u>	<u>Reviewer</u>	<u>Tab</u>
	DIVISION DIRECTOR'S MEMO	Paul Leber, M.D.	A
10-17-95	GROUP LEADER'S	Russell Katz, M.D.	B
08-18-95	CLINICAL REVIEW	John Feeney, M.D.	C
09-11-95	GI/CoagulationConsult Review	H. Gallo-Torres, M.D.	D
08-14-95	STATISTICAL REVIEW	David Hoberman, Ph.D.	E
	ORIGINAL NDA: Integrated Efficacy Summary		F
	ORIGINAL NDA: Integrated Safety Summary		G

Memorandum **Department of Health and Human Services**
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

DATE: November 15, 1995

FROM: Paul Leber, M.D.
 Director,
 Division of Neuropharmacological Drug Products
 HFD-120

SUBJECT: Approvable Action Recommendation
 NDA 20-599 Rilutek™ (riluzole) for Amyotrophic Lateral Sclerosis

TO: File NDA 20-599

This memorandum conveys and explicates the basis for the Division's recommendation that Rhone-Poulenc Rorer's NDA 20-599 for Rilutek™ (submitted on 6/29/95) be declared approvable. This recommendation is made with an understanding that Rilutek™ will be marketed under professional product labeling that is identical, minor stylistic and grammatical revisions excepted, to that developed by the Division.¹

Explication:

Efficacy:

The sponsor has submitted an NDA that provides results of two clinical investigations that by current regulatory standards of trial design and conduct can reasonably be viewed as adequate and well controlled clinical investigations of riluzole's effects in patients with Amyotrophic Lateral Sclerosis [ALS].

The evidence adduced in these two investigations, Study 216² and Study 301³,

¹ The division's proposed labeling is attached to the approvable action letter being forwarded to the ODE 1 in the company of this memorandum.

² Study 216, originally reported by Bensimon et al. NEJM 330:585-91 (1994), is a multiclinic parallel, balanced, randomized comparison of riluzole, 100 mg/d (50 mg tablets given bid) and placebo in 155 outpatients with probable or definite ALS who were within 5 years of their disease onset. The study was conducted at 7 sites in France and Belgium and was completed in

has proved sufficient to allow at least some authoritative experts, among them members ⁴ of our Peripheral and Central Nervous System Advisory Committee (PCNS AC), to conclude that riluzole provides a clinical benefit for some patients with ALS. Specifically, in both studies, the distribution of the times to death and/or tracheostomy were, in at least some analyses, more favorable overall for the groups of patients randomized to riluzole than for those randomized to placebo. It bears note, however, that the evidence is far from strong, a point openly acknowledged by virtually all who have reviewed it.

March of 1992. Treatment assignment in the study was stratified by clinic and site of onset of the disease (i.e., bulbar vs. limb) By design, subjects were to be followed on treatment for up to 18 months, but the primary analysis was intended to take place when the last patient enrolled in the study had been on treatment for at least a year.

³ Study 301, is a multiclinic (31 centers-only 8 of which were in North America), randomized, parallel comparison of placebo and 3 doses of riluzole (50, 100 and 200 mg/d) in 959 patients (protocol called for 600) for a time sufficient to allow the last entered patient to be on drug at least 18 months. However, because recruitment in the 8 North American sites did not begin until 6 months after the trial had begun in Europe, there is a systematic difference in the possible duration of observation time between continents. The study was designed to assess the effect of riluzole in mild to moderately symptomatic ambulatory ALS patients. The primary outcome measure specified in the protocol was time to either death, tracheostomy, or intubation undertaken for the purposes of assisting respiration. The primary treatment contrast involves riluzole 100 mg/d (the same dose as used in Study 216) vs. pbo evaluated as per protocol on a log rank statistic (Mantel-Cox) stratified by onset (bulbar vs. limb-girdle). The protocol specified a one tail test at an alpha of 0.05.

⁴ At the PCNS AC meeting on Rilutek (September 18, 1995), 5 of the 9 members present voted affirmatively in regard to the sufficiency of the evidence adduced in regard to the product's effectiveness in use. One of the 5 members voting affirmatively was the consumer nominated member, Ms. Phillips, who, in the course of discussion made clear that her affirmative vote did not reflect an informed opinion about the probative weight of the scientific evidence adduced in the controlled trials but her conviction that an affirmative vote was critical to "keep hope alive..." [see her comments and ensuing discussion at pages 154 and 166 to 1689 of the transcript.]

Subgroup analyses, for example, reveal inconsistencies in the evidence adduced in both studies. In study 216, the overall favorable between treatment difference arises largely from the strata of patient's who had a 'bulbar onset' of their disease. In Study 301, a post hoc subgroup analysis reveals that the evidence which accounts for the trial's positive results derives virtually entirely from European and not from North America centers. In fact, in North America, the outcome among patients on placebo is as good as that among patients randomized to riluzole in Europe.

Although these findings, on face, may be argued to undermine to an affirmative conclusion about Rilutek's value in ALS, they are not conclusive. First, even if riluzole's effectiveness were limited to patients with a bulbar onset, (and the results of Study 301 do not support this conclusion), it would not make Rilutek ineligible for marketing, although it would clearly affect product labeling. As to the possible inconsistencies identified in the post hoc, subgroup analyses of Study 301's results, they can only generate hypotheses. Moreover, the results produced are not all that troubling: the process of 're-partitioning' and/or sub-setting of almost any data set, especially in the face of an overall numerically small treatment effect such as that which exists in Study 301, can be expected to yield results that fail to conform to the trial's overall result.

The interpretation of the experimental results of both studies is also confounded by concerns about the validity of the 'p' values reported in association with the statistical analyses applied.

Study 216 provides results that are analysis dependent. While this may be a consequence of the time-dependency of riluzole's beneficial effects ⁵, the

⁵ Typically in regulatory work, when the statistical significance of a study's results turns on the type of statistical analysis applied, it is taken as a sign that the study, at most, provides evidence of limited probative value. However, when comparisons involve differences in the distribution of failure times among groups of individuals, the dependence of statistical significance on the analysis applied may indicate something else. Time failure analyses are invariably made with incomplete information. This occurs typically because 1) not all those being followed have suffered the failure of interest by the time point chosen for the analysis (censored at trial's end) or 2) because the failure status of some of those who were being followed is unavailable (censored through loss to follow-up). Consequently, data sets used for longitudinal survivorship comparisons typically have greater

finding is, nonetheless, disconcerting to some because the analysis designated by protocol⁶ as the basis for the comparison of the survival distributions among Rilutek and placebo assigned patients, fails at both the protocol specified endpoint (November 1, 1991) and, although by a lesser margin, at the March 1992 endpoint (the one cited in the NEJM report), to attain traditional levels of statistical significance, as the following statistical results taken from Dr. Feeney's review illustrates.

Abstract of Table on page 35 of Dr. Feeney's Efficacy review	Stratified Logrank	Stratified Wilcoxon
November 1991	0.124	0.050
March 1992	0.052	0.034
One year data	0.016	0.010

However, this failure to achieve significance on the protocol designated analysis is not as damaging as it might seem at first viewing. The data do document that riluzole has an effect on survival probability over the first few months of the study. This is evident from an inspection of the plots of the Kaplan Meier product limit curves for both the November 1991 and the March 1992 data sets (See page 21 of Dr. Feeney's effectiveness review). The conclusion is also supported by the findings of the Stratified Wilcoxon, which, as noted in an earlier footnote, is an analysis which gives greater

numbers of subjects at risk at earlier as compared to later time points over their course, and, consequently also have (if the instantaneous risk of suffering the event of interest is constant over time), more events earlier as well. Because analytical methods employed to assess longitudinal data employ different methods for weighting survival information, they not infrequently provide different p values for comparisons made on the same data set. For example, the logrank test (Mantel-Cox/Mantel-Haenszel) gives equal weight to all intervals of observation while the Breslow-Gehan-Wilcoxon gives more weight to those with more individuals at risk. Accordingly, differences among the p values produced by these methods are not an indication of some fundamental problem with a study, but are a result of the different weighting rules employed by these methods.

⁶ stratified logrank analysis

weight to earlier observation periods.

Accordingly, although it is clearly not an easy call, I am persuaded that Study 216 can be considered a source of valid evidence showing that riluzole has a transient beneficial effect upon the probability of survival of patients with ALS.

In Study 301, a comparison of the survival distributions is favorable to Riluzole, and, the protocol designated analysis, the logrank test, technically meets the protocol specified alpha of ≤ 0.05 , one tail. However, the two tail test p value is, 0.076 , and, therefore, the study fails to meet FDA's usual standard for declaring statistical significance. Again, I find this a technical rather than a substantive problem because the evidence collected in Study 301 clearly shows that Rilutek treatment is associated with an improvement in an ALS patient's chance for survival, at least during the first few months of treatment. As in Study 216, the application of stratified Wilcoxon test, an analysis which gives greater weight to earlier time points, attains statistical significance, ($p=0.05$).

Not unexpectedly, some critics of riluzole have expressed concerns about this fact, arguing that a transient beneficial effect should not be viewed as clinically meaningful. This, however, represents a position that turns entirely on personal sentiment; accordingly, it is irrelevant vis a vis a regulatory determination of effectiveness so long as the effects that riluzole has been shown to exert in clinical trials can be judged, fairly and responsibly by experts, to be clinically meaningful effects.

Some who find the survival distribution data in the two clinical trials inadequate as a source of evidence of effectiveness are influenced by the fact that riluzole's effect on survival is not accompanied by a beneficial effect that would explain why survival is enhanced (e.g., there is no drug associated improvement in muscle strength, neurological signs, or respiratory function.) Although the lack of collateral changes to 'explain' riluzole's effects proves nothing in and of itself, it is not inconsistent with the possibility that the improved survival associated with the use of riluzole might be explained by something other than a biological/pharmacological effect, for example, penetration of the treatment blind. This possibility, I must acknowledge, cannot be dismissed out of hand even in a study where

outcome is measured by a clearly defined endpoint⁷; on the other hand, there is no evidence to support a concern that the treatment blind was broken. Accordingly, I find no basis to discount the evidence adduced in these studies.

Concerns have also been voiced by some about the whether or not Study 301 constitutes a truly independent confirmation of Study 216, especially in light of the finding that the evidence of effectiveness in Study 301 derives in large measure from centers that participated in Study 216. Although it would certainly have been preferable for the two studies to have been conducted by entirely independent sets of investigators, the two studies enrolled different patients, were conducted at different times, and, therefore, reasonably allow a conclusion that improved survival is a replicable result of treatment with riluzole. To be clear, replication is never as good a source of confirmation of experimental findings as is evidence derived from truly independent studies because replication provides no protection against results that arise from systematic bias and/or fraud. There is, importantly, no evidence of systematic bias or fraud and I, accordingly, do not find the lack of fully independent corroboration a sufficient basis to reject the two trials as independent sources of substantial evidence of effectiveness.

In sum, although the evidence adduced is modest and doubt clearly exists in the minds of some experts about its probative value, I am able to conclude that it is sufficient to meet the substantial standard of effectiveness imposed by the Act.

Safety

No pharmacologically active drug is free from risk. Accordingly, in most regulatory situations, a conclusion that a drug is safe for use actually means that the evidence, taken as a whole, supports a judgment that the risks associated with the use of the drug are reasonably outweighed by the benefits that are likely to accrue from its use. This process that leads to this determination is often a difficult to describe because the benefits and risks of a drug are typically assessed in different clinical outcome domains on scales of measurement that cannot be compared in any quantitatively

⁷ Although death and tracheostomy are 'hard' endpoints with unambiguously attributable dates of occurrence, patient and caregiver behavior may have an important influence upon the time at which either takes place.

understandable way. (A typical quantitative risk benefit assessment is many respects akin to asking what is bigger: 3 yards or 4 pounds).

The one clear exception to the circumstances just described is when both the risks and benefits of the drug involve death. Here, so long as one subscribes to the view that life is invariably a better state than death, the comparison of risk and benefit is straight forward. If more patients survive longer on riluzole than on placebo, the drug has a favorable risk benefit ratio.

Indeed, even if riluzole caused considerable pain and morbidity, and it does not, it could be deemed safe for use because of the simple fact that the survival distribution of ALS patients given riluzole is superior to that of ALS treated with placebo. Of course, from an ethical and moral perspective, this need not be true in every clinical setting or circumstance (e.g., a drug prolonging survival in a vegetative state), but, in the current circumstances such exceptions do not seem relevant (although it is possible that riluzole might extend life by prolonging the time taken to passage a clinically undesirable stage --relatively speaking--in the disease; unfortunately, the evidence needed to assess such a possibility is not currently available⁶).

Information on the risks associated with the use of riluzole are also important to collect and systematize for another reason: to allow for informative labeling that will provide potential users with information about the drug's expected untoward effects. For this purpose, the experience gained in clinical trials with more than 1900 individuals is more than sufficient.

Labeling:

Beyond meeting regulatory standards for safety and effectiveness, a drug product must, by law, be marketed under labeling that is neither false nor misleading in any particular. This is not always so easily accomplished because it is not merely the words of labeling, but the overall context in which they are presented that determines the truth value of the information being communicated.

⁶ A treatment that prolongs life by extending the time to passage an early clinical stage/state where impairment is mild is clearly superior to one that extends survival by the same absolute amount of time but does so by extending the time taken to passage a very advanced stage.

Accurately describing the modest effects of a drug intended for use in the management of ALS, a devastating relentlessly progressive degenerative disease that lacks any alternative treatment, is especially difficult, because the beneficial effects of riluzole are not only small and transient, and clinically undetectable (neither the physician or patient will know if the drug is actually working), but are measured on an outcome (a shift in probability of survival conditioned on time) that is so arcane that even able clinicians may find it hard to understand, let alone explain to their patients.

Recognizing this, the division has developed labeling that it believes accurately and fairly presents what is and what is not known about riluzole's expected risks and benefits in clinically understandable terms. If riluzole is marketed under this labeling, we believe it will be safe for use, effective in use and accurately labeled.

Recommendation:

Issue the approvable action letter.

A handwritten signature in black ink, appearing to read 'Paul Leber', is written over a horizontal dashed line.

Paul Leber, M.D.
November 15, 1995

MEMORANDUM

DATE: October 17, 1995

FROM: Deputy Director
Division of Neuropharmacological Drug Products/HFD-120

TO: File, NDA 20-599

SUBJECT: Supervisory Review of NDA 20-599 for Riluzole in the Treatment of Patients with Amyotrophic Lateral Sclerosis (ALS)

NDA 20-599 for Riluzole as a treatment for patients with ALS was submitted by Rhone Poulenc Rorer on June 29, 1995. The NDA contains complete reports of 2 randomized controlled trials that the sponsor asserts establish the effectiveness of the treatment, as well as, according to the sponsor, sufficient safety data to permit the conclusion that the drug is safe for use in this population. A detailed review of the safety and effectiveness data has been performed by Dr. John Feeney, Medical Officer in this Division, and detailed reviews of the effectiveness data have been performed by Dr. David Hoberman, Mathematical Statistician of the Division of Biometrics.

The purpose of this memo is to briefly review the safety and effectiveness data, as well as to provide a brief chronology of the relevant aspects of the development of this treatment.

BACKGROUND

IND for the use of riluzole in patients with ALS, was submitted by Rhone-Poulenc Rorer in May, 1993. At the time of submission of the IND, a controlled trial (Study 216) had been performed in France and Belgium which ostensibly demonstrated increased survival in patients receiving riluzole compared to those receiving placebo. A report of this trial was published in the New England Journal of Medicine in 1994.

In the May, 1993 original IND submission, the sponsor submitted a protocol for a larger, multi-national, placebo controlled trial (Study 301) designed to replicate the findings of Study 216. At the time of this submission, European centers had already begun enrollment in Study 301, and the IND submission contained the protocol for the domestic centers. This protocol was essentially identical to the European protocol according to the sponsor, (although we had not seen the European version), and it was clear from the initial IND submission that the data from the US study was to be combined with the data from the European centers. Indeed, Study 301 was performed in several countries in Europe, Canada, and the United States.

When Study 301 was completed, and reportedly confirmed the findings of Study 216, it was felt that sufficient data existed to support the establishment of a Treatment IND. This was discussed with the sponsor, who submitted a Treatment Protocol request on May 19, 1995. This request was granted on June 20, 1995.

Since the Treatment IND was granted, a third controlled trial in ALS patients has been completed. Although a complete report of this trial has not been submitted, we have enough information about it to be able to come to some preliminary conclusions. Also, a fourth controlled trial is on-going in Japan, about which we know very little at this time.

At this point, I will briefly review the major findings of the 2 controlled trials presented as providing substantial evidence of effectiveness of riluzole in patients with ALS.

STUDY 216

This was a randomized, double blind, parallel group study comparing riluzole 50 mg BID to placebo that was performed at 7 centers (6 in France, 1 in Belgium), and enrolled 155 patients with ALS of less than 5 years duration. The primary outcome was a survival analysis, to be performed when the last patient entered had completed one year of treatment (the protocol actually stated a calendar date-November 30, 1991-that would serve as the time at which the primary analysis was to be performed, and, somewhat remarkably, this turned out to be the date on

which the last patient completed a year in study). At the time of the last patient's completion of one year in study, however, a number of patients had completed considerably more time in study, up to a maximum of 18 months (enrollment occurred over a 6 month period). It is worth emphasizing that the primary analysis was to be performed utilizing all the data that had accrued up until November 30, 1991, and was not to be limited to include only the first year of data for all patients. This is important to point out because the analysis that was presented in the NEJM (and which was ostensibly positive by usual standards) was based on only the one year data for all patients, not the protocol stated analysis. The NEJM article did not describe the analysis presented as a post hoc analysis performed after the data were examined, although that is what it was. In addition, another analysis was performed using data accrued up until March, 1992. This latter analysis was performed because the study was continued in a blinded fashion until this date, this being the time that the results of the November 1991 analysis became available. This March, 1992 analysis, then, is based on the longest duration of treatment of all the analyses. Because the protocol specified analysis was the one performed on the basis of all data accrued as of November 30, 1991, I will focus on the results of this analysis.

In this study, death or tracheostomy were the endpoints considered in the survival analyses. In addition, a number of scales of neurologic function were rated. In fact, the randomization was stratified on the location of the onset of the disease (not the patient's condition at enrollment) as either "bulbar" or "limb" onset in order to facilitate the interpretation of these functional scales, not because it was thought that location of onset of disease would affect the primary outcome.

RESULTS

A total of 77 patients were randomized to riluzole, 78 to placebo. According to the sponsor, 24/77 (31%) of the riluzole patients left the trial because they reached an endpoint of death or tracheostomy, compared to 36/78 (46%) of the placebo patients. However, an additional 26 riluzole patients left the trial for other reasons, and 15 of these were reported to have gone on to reach an endpoint. This compares to an additional 15 placebo patients who left the trial for other reasons, 13 of

whom were reported to have ultimately gone on to reach an endpoint. The remaining patients who discontinued (11 riluzole, 2 placebo) were considered to have been alive for the remainder of the trial. However, at the time of the report in the NEJM, as Dr. Feeney discovered, the sponsor had no affirmative evidence that these patients actually were alive without tracheostomy at study's end, although they were reported as such, and were considered as such in the survival analysis (as opposed to having been considered censored at the time of their discontinuation). When we brought this fact to their attention, the sponsor initiated attempts to discern the actual status of these patients at the appropriate time point. Currently, we believe that the sponsor has accounted for all of these 13 patients except 2. What we cannot be sure of at this time, however, is how many (if any) of the 15 riluzole and 13 placebo patients who discontinued and then went on to die received a tracheostomy prior to death, information that could theoretically alter the results as reported.

In any event, if the data are accepted as given, the results of the primary analysis (based on 11/30/91 data, stratified logrank test as called for in the protocol) are presented on page 35 of Dr. Feeney's review, and are reproduced here (the p-values for the results that follow are based on an 8/1/95 update on the data):

	Median Survival (Days)	Logrank	Cox Model
Riluzole	502	p=0.124	p=0.082
Placebo	469		

(P values are 2 sided). The Cox Model was an analysis stated in the protocol, but was not to be primary (based on a February, 1992 amendment).

For interest, the following results are presented for the March, 1992 cut-off, and then for the one year data (these are the analyses presented in the NEJM, although the p-values are slightly different, based on the sponsor's 8/1/95 submission):

March, 1992 Results

	Median Survival	Logrank	Cox Model
Riluzole	531	p=0.052	p=0.06
Placebo	456		

One Year Results

	Cumulative Survival	Logrank	Cox Model
Riluzole	74%	p=0.016	p=0.004
Placebo	58%		

The sponsor contends that this latter analysis is the most appropriate analysis, because when they performed the protocol specified (11/30/91) analysis, they noted that the survival curves were not parallel. As can be seen in Dr. Feeney's review, page 20, the curves appear to be coming together at the end of the trial, and this is due to the fact that the hazards (the likelihood of surviving for a short interval of time, given survival up until that interval, and which are the underlying mathematical quantities utilized for the construction of the survival curves) are not proportional between the treatments-in fact, as can be seen from the diagram on page 22 of Dr. Feeney's review, the hazards for intervals after approximately 300 days on treatment actually cross. That is, after this time point, the hazards are greater for patients on riluzole than on placebo. According to the sponsor, the logrank test relies upon an assumption of proportional hazards. According to Dr. Hoberman, however, this is not correct, although the logrank test does lose power when the hazards are not proportional. Therefore, the logrank test is not an invalid test in this setting. Dr. Hoberman does suggest, however, that there are other tests that are more sensitive in the setting of non-proportional hazards (e.g., Kolmogorov-Smirnov test; see Dr. Hoberman's 6/2/95 review of Study 301, page 3), because these analyses compare the survival curves directly, not the underlying hazards, as do the logrank and Cox tests. In essence, this test examines the maximum difference between the 2

survival curves. However, when he performed this test, he found no statistically significant difference between the treatments.

For the most part, the other measures of functional changes, muscle strength, pulmonary function, and global outcomes yielded no significant differences. However, the between group comparison on Muscle Testing yielded a p value of 0.026, based on an analysis of the mean slopes of decline calculated on the basis of the individual slopes of decline of each patient (an analysis not specified in the protocol but performed to correct for a pattern of informative censoring of data; that is, a situation in which, over time, patients who leave a study are fundamentally different from those who continue in the trial). Drs. Feeney and Hoberman devote considerable effort to the differential effects of the drug on bulbar and limb onset patients, but these analyses, while interesting, do not contribute significantly to the overall assessment of these results.

Dr. Feeney does discuss the potential for the blind to have been penetrated in this study, due to the fact that the investigators had access to lab data during the conduct of the trial. Riluzole is known to elevate LFTs, and by Dr. Feeney's count, 42 patients had at least one elevation of ALT. Thirty one (31) of these were receiving riluzole, 11 placebo. A survival analysis (see Dr. Feeney's review, page 33) comparing patients with and without LFT elevations shows differences numerically (though not statistically) in favor of those with LFT elevations. This, of course, does not prove that the blind has been broken, although it does raise the possibility. It should be noted that Dr. Feeney points out that plasma levels of riluzole were drawn during the trial. Were these known to the investigators, the blind would have been penetrated without question. We have repeated assurances from the sponsor, however, that these samples were not analyzed until the trial had been completed. This issue of possible penetration of the blind will be further discussed below, with respect to Study 301.

STUDY 301

Study 301 was a multi-national, randomized, parallel group trial

comparing 3 doses of riluzole (25, 50, and 100 mg BID) to placebo. The primary outcome was survival, with the endpoint, as in Study 216, death or tracheostomy. In most other important aspects, the study was similar in design to Study 216. The primary comparison of interest, as stated in the protocol, was the outcome of the 50 mg BID group compared to placebo. The trial was to enroll 600 patients (150 per treatment group), and the primary analysis was to be a stratified logrank test utilizing each patient's 18 month data. However, an amendment to the protocol changed the final analysis to include all data up to a cut-off date of December 31, 1994 (enrollment had begun in Europe in December, 1992, and in the US in June, 1993). This change was instituted because enrollment in the study was more rapid than originally planned, and December 1994 corresponded to the time at which approximately the first 600 patients (the original planned total enrollment) had completed 18 months in study. As a result of this amendment, an unknown number (but undoubtedly most) of the patients in the US did not receive a full 18 months of treatment at the time of the final analysis.

RESULTS

This trial was performed in 5 European countries (France, Belgium, UK, Germany, and Spain), and in Canada and the US. A total of 959 patients (25 BID-237; 50 BID-236; 100 BID-244; Placebo-242) were enrolled at 31 centers.

Focusing on the 50 mg BID and placebo groups, 69/236 (29%) of the riluzole, and 84/242 (35%) of placebo patients left the trial because they reached an endpoint. An additional 54 riluzole patients left the trial early for other reasons, and 33 of these reached an endpoint (total of 102/236 or 43%), compared to an additional 50 placebo patients who left the trial early for other reasons, 36 of whom went on to reach an endpoint (a total of 120/242, or 50%). Unlike Study 216, the sponsor assures us that they prospectively and reliably determined the status of patients every 2 months throughout the trial, so that we can be assured of reasonably accurate information regarding time to endpoints, status at end of trial,

whether an endpoint was reached by death or tracheostomy, etc, except for a total of 8 patients who were lost to follow-up (2 riluzole, 1 placebo).

The results of the protocol specified analyses are described below:

	25% Quartile Survival (Days)	Logrank	Cox Model
Riluzole	351	p=0.076	p=0.002
Placebo	264		

25% Quartile Survival replaces Median Survival because the survival curves did not fall below the 50th percentile. The results of the 25 BID vs placebo comparison were numerically in favor of riluzole (p=0.25), and the results of the 100 BID vs placebo analyses were essentially identical numerically and statistically to the 50 BID vs placebo (primary) comparison. Survival curves for each individual dose compared to placebo, as well as for all doses combined can be found as Figure 1 in Dr. Hoberman's June 2, 1995 review of Study 301.

As in Study 216, there were no significant findings on other measures (functional, muscle strength, pulmonary function, globals).

A finding of interest, however, concerns the pattern of responses seen in specific geographic areas. Specifically, Dr. Hoberman has constructed survival curves and hazard diagrams not only for the study as a whole, but also for French centers alone, and non-French centers. This has been done because the French centers in Study 301 are the same centers that participated in Study 216. It was felt important to examine the effects of excluding these centers from the analysis, because their inclusion might be considered to violate the spirit of independent replication/corroboration of the results of Study 216. Also, 400 patients were enrolled in the French centers.

When these analyses were conducted, some interesting results emerged.

As Figures 3 and 4 in Dr. Hoberman's June 2, 1995 review of Study 301

depict, the survival curves in the French centers continue to separate at the end of the study, but the curves in the non-French centers converge. Of great interest also are the survival curves for Riluzole and Placebo (see Dr. Feeney's review, page 59) which are essentially superimposable. Analogous to what was seen in Study 216, as Figures 6 and 7 of his review suggest, the hazards cross in the non-French centers, but, for the most part, do not in the French centers (interestingly, mortality in general is considerably higher in France compared to outside France). Again, Dr. Hoberman suggests that the more sensitive Smirnov test might be more appropriate in this setting, although when it was performed on the entire data set (all European and North American centers), it failed to detect statistically significant differences. *for US centers*

Regarding the question of the potential for knowledge of LFT elevations to allow investigators to penetrate the blind, Dr. Feeney has performed an analysis which compares the results of patients with LFT elevations to those of patients without elevations (see page 60 of his review). No substantive differences between this analysis and the analysis that compares all drug treated and all placebo treated patients were found.

STUDY 302

This was a multi-center, double blind, placebo controlled parallel group trial in patients with ALS who could not satisfy the Inclusion Criteria for Study 301. Specifically, this trial enrolled older (over 75 years) and sicker (disease duration > 5 years or Vital Capacity < 60%) patients. In this study, patients were randomized to Placebo or riluzole, 100 mg/day.

Results

This study was conducted at 10 centers (9 in France, 1 in Belgium) and enrolled 168 patients. While a formal effectiveness analysis has not been performed or submitted by the sponsor, we do know that the proportion of patients alive at the end of the study was essentially the same in both groups (27-28%). Of the 82 patients randomized to riluzole, 63 left the

trial; 41 died and 22 left for other reasons. Of these 22, 19 subsequently died. Of the 86 Placebo patients, 66 left the trial; 53 died and 13 left for other reasons. Of these 13, 9 subsequently died. However, since we do not have detailed data for this study, we can make no comment about the shape of the survival curves.

SAFETY

Exposure

In 40 clinical studies, 1908 people have received riluzole. Of these, there are 1014 exposures to doses equal to or greater than 100 mg/day, the dose purported to be effective (this number does not represent the number of individual patients who have received this dose; a particular patient may have received several doses. In these cases, that patient would have been counted more than once in the dose/duration displays). Of these exposures, 731 have been at this dose or greater for 3 months or longer, with 574 of these being in patients with ALS. Of the 731, there have been 527 exposures to the drug for 6 months or greater, with 507 being in patients with ALS.

Deaths

As noted earlier, there were many deaths in the ALS studies. According to the sponsor, however, in Study 216 all but 4 were considered to be unrelated to the treatment by the investigators. Of the 4, 2 were in placebo treated patients (liver disease and pulmonary embolus), and 2 were in the riluzole treated patients (respiratory or cardiac cause). In Study 301, 52 patients died who were not considered to have died from respiratory or direct ALS-related causes. Most of these deaths occurred in the placebo group (20), with 12, 11, and 9 deaths each in the 50, 100, and 200 mg/day groups, respectively. An inspection of the causes of these deaths does not point to any specific drug related causation.

In the total data base, there were 3 additional deaths. One 20 year old male died in a motorcycle accident 3 weeks after receiving a single 100

mg dose of riluzole in a Phase 1 study. Two other patients who received 2 intravenous injections of 10 mgs of riluzole (30-40 minutes apart) to treat status epilepticus died at least 6 days after the injections.

Discontinuations

In the combined 216/301 database, 112 (14% of) Riluzole treated patients were discontinued secondary to adverse events (17% and 15% in the 100 and 200 mg/day groups, respectively) compared to 11% of placebo patients. The most common AEs resulting in discontinuation included asthenia, ALT elevations, nausea, and respiratory disorder.

In the non-ALS patients enrolled in clinical trials, the only AEs resulting in discontinuation at a rate of greater than 1% were anxiety and AST elevation (each occurring in 1/28 patients at a daily dose of 200 mg/day), and exfoliative dermatitis and headache (each occurring in 1/93 patients receiving 100 mg/day). Additionally, in the 250-500 mg/day group, there is one report each of CVA, hemiplegia, and facial paralysis (N=17), although it is possible that these 3 event terms all apply to a single patient.

Adverse events

Approximately 90% of patients in the combined 216/310 populations reported one or more adverse event during the trials. The most common AEs reported included the following:

	50 mg/d N=237	100 mg/d N=313	200 mg/d N=244	Placebo N=320
Event				
Asthenia	15%	19%	20%	12%
Nausea	12%	16%	20%	11%
Decreased Lung Fxn	13%	10%	16%	10%

Rhinitis	9%	7%	8%	6%
Dizziness	5%	4%	13%	3%
Abd Pain	7%	5%	8%	4%
Diarrhea	6%	3%	9%	3%
Pneumonia	6%	4%	7%	4%
Anorexia	4%	3%	9%	4%
Vomiting	4%	4%	5%	2%
Vertigo	3%	2%	5%	1%
Somnolence	1%	2%	4%	1%
Circumoral paresthesia	1%	2%	3%	0%

Many other AEs have been reported in the ALS trials, (see Dr. Feeney's Safety Review, pp.13-17), but appear to not be drug/dose related, or occurred in fewer than 1% of patients.

In Phase 1 and 2 studies in healthy volunteers and non-ALS patients, doses of up to 500 mg/day were given. At these higher doses in normal volunteers, there was a marked increased incidence/first appearance of vertigo (42%), thinking abnormal (10%), paresthesia (19%), and circumoral paresthesia (19%).

Laboratory Examinations

No consistent abnormalities were seen on routine clinical chemistries (excluding liver function tests) or CBCs, nor were there significant changes in blood pressure. Although there were sporadic reports of various EKG changes, there were no consistent findings that could be classified as drug/dose related.

Rash

Three patients discontinued treatment in the ALS studies due to rash or related dermatologic AEs (1 rash, 1 angioedema, 1 pruritis).

Liver Function Tests

A considerable number of patients experienced elevations above the upper limit of normal (ULN) of at least 1 LFT during the ALS trials. For example, close to 50% of patients in the 200 mg/day group in study 301 had at least one such elevation during the trial, and the number of such patients in the 100 mg/day group is not yet clear, but may be on the same order (the sponsor has not presented the data in a way that allows us to know these numbers accurately-this applies to the placebo rate as well). The only other consistently apparently drug related increases were seen in bilirubin, for which 15% of the 100 mg/day patients and 25% of the 200 mg/day groups had at least one increase, compared to 11% of the placebo groups.

Although the number of patients with any elevation of LFTs above the ULN is not known with great accuracy, the number of patients with LFT elevations greater than 3 times the ULN (the level of clinical interest), is. In Studies 216 and 301 combined, 5%, 7%, 12%, and 2% of patients in the 50, 100, 200 mg/day, and placebo groups respectively, had at least one ALT elevation between 3 and 5 times the ULN. No more than 3% of patients in the drug treated groups had ALT elevations greater than 5 times the ULN, compared to 2% in the placebo group (the protocol of Study 301 required that patients be discontinued if such an elevation occurred). The number of patients with AST elevations between 3 and 5 times the ULN was considerably smaller, with 1%, 4%, 1%, and 1% in the 50, 100, 200 mg/day, and placebo groups, respectively.

These elevations were usually seen within the first 2 months, with shorter latencies associated with higher doses. Most patients with ALT levels less than 5 times the ULN continued on treatment, with subsequent fall of the levels to less than 2 times the ULN over 2-6 months. In those patients discontinued for elevations greater than 5 times the ULN, the levels typically returned to less than 2 times the ULN over 2 months, although latencies of up to 6 months were seen.

As noted above, there were a number of discontinuations secondary to ALT elevations. However, there were several other discontinuations secondary to liver abnormalities.

One patient receiving 200 mg/day in Study 301 was discontinued after a

GGT elevation of greater than 6 times the ULN and an Alk Phos of greater than 2 times the ULN. This patient died one week later secondary to respiratory insufficiency.

Another patient was discontinued secondary to an ALT of 7 times the ULN after 6 weeks on 200 mg/day. The ALT dropped to under 3 times the ULN at week 7, but rose to 20 times the ULN at week 8. The ALT normalized by week 10, but rose to 3 times the ULN at week 11. After a period of normalization, the ALT rose to 5 times the ULN at month 6, after which it normalized again.

Three patients receiving 200 mg/day in Study 301 developed jaundice, with none having ALTs greater than 3 times the ULN. One patient was discontinued. Another patient was hospitalized with "subicterus" (ALT 2.5 times the ULN) after 75 days on 200 mg/day. Biopsy revealed massive microvascular steatosis, mild lobular hepatitis, and granulocytes. The ALT normalized after treatment discontinuation.

In the non-ALS studies, similar patterns of LFT elevations were seen. One case was particularly severe. A 20 year old male with epilepsy, who was also receiving phenobarbital and carbamazepine, had been treated with riluzole for 3 months, at which time his bilirubin rose to 3 times the ULN (he was receiving 250 mg/day of riluzole at that time). The dose was reduced to 100 mg/day, but at month 4 he was diagnosed with jaundice and neutropenia. His ALT was 26 times the ULN, AST was 17 times the ULN, and bilirubin was 11 times the ULN. The riluzole was discontinued, and by 7 weeks post-treatment, the chemistries had normalized.

ADVISORY COMMITTEE MEETING

On September 18, 1995, the Peripheral and Central Nervous Systems Drugs Advisory Committee met to discuss the NDA for Riluzole. There was considerable discussion about the lack of demonstrated treatment effect in North America, as well as of the convergence of the survival curves at the end of each study, and the lack of traditional statistical significance by the protocol specified analyses. Nonetheless, the final vote was 5-4 in favor of recommending approval.

OVERALL COMMENTS

The sponsor has submitted detailed results of 2 controlled trials in patients with ALS comparing riluzole with placebo, and preliminary data from a third controlled trial in patients who were considerably sicker at baseline than those in the first 2 trials.

As noted above, there are a number of elements of the data set presented that give rise to concern.

First, although the 2 main studies are adequate by design to demonstrate the effectiveness of riluzole, neither, when judged by the results of the protocol specified analyses, meet the usual standard for the demonstration of effectiveness, although the p-value for the primary comparison in Study 301 is reasonably close to the usual standard ($p=0.076$). However, inspection of the curves comparing survival between patients randomized to Riluzole 100 mg/day to that of patients receiving placebo in both studies makes clear that, for the majority of the time in study, these curves describe a bona fide difference between treatments. Further, alternate appropriate analyses do detect a statistically significant difference between treatments in each study. While it is difficult to describe the differences seen (e.g., there is no one number that accurately quantitates the effect of the drug on mortality), and the meaning and interpretation of the "crossing hazards" after one year are not entirely clear, nonetheless consistent differences are obtained.

Of additional concern is the apparent discrepancy between the results in the French centers and those in the non-French centers. While in the French centers, the results appear clearly positive (the survival curves continue to separate at trial's end), the curves clearly converge in the other centers, and, importantly, the curves for the North American centers are essentially superimposable. Although Dr. Hoberman has performed some preliminary work on these data (including examining the differential effects of some covariates on these "geographic specific" analyses), it is not immediately obvious why these discrepancies exist. It is interesting to note, for example, that the estimate of mortality of the placebo

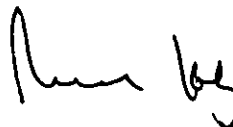
patients in North America is better than the best estimate of mortality on drug in any of the European centers, a finding consistent with many explanations (e.g., fundamental differences in disease, population, standard care, etc.). However, it should be pointed out that the trial was designed to be analyzed as a single trial, and was not to be primarily analyzed by geographic region. Given this view, the differences seen by geographic region can be considered an interesting finding, perhaps one deserving of additional study and/or analysis, but not necessarily of primary consideration in determining the effectiveness of the treatment.

In addition, the essentially complete absence of treatment effects on other measures of neurologic function raises questions. In particular, it is difficult to understand how the drug may be decreasing mortality if it cannot be detected to be having an effect on the functions we would ordinarily expect to contribute to this outcome (e.g., respiratory function, muscle strength). One explanation, of course, is that these other measures are simply insensitive, individually, to the effect of the treatment on these functions, but that the sum total of the treatment's effects results in an effect on mortality. Whatever the explanation, however, for the absence of an effect on these outcomes, the similar findings on mortality in both studies remain.

Despite the panoply of difficulties noted in the interpretation of the data, in my view the sponsor has presented the results of 2 adequate and well controlled trials that demonstrate the effectiveness of Rilutek as a treatment for patients with ALS. Further, the safety data base adequately supports approval of the NDA.

RECOMMENDATION

The sponsor should be issued an Approvable letter with draft labelling.



Russell Katz, M.D.

CLINICAL REVIEW AND EVALUATION

NDA 20-599

Rilutek (riluzole)

Reviewer:	John Feeney, M.D.
Date:	August 18, 1995
Sponsor:	Rhone-Poulenc Rorer
Indication:	Amyotrophic Lateral Sclerosis (ALS)
NDA Submission Date:	June 29, 1995
Treatment IND Date:	June 20, 1995

RILUZOLE EFFICACY FINDINGS

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Introduction

Riluzole has been studied for various indications in man since 1984. In the United States, the IND for investigation of riluzole in amyotrophic lateral sclerosis (ALS) was opened in May 1993. In support of the IND, the sponsor, Rhone-Poulenc Rorer (RPR), submitted protocol NNY301, a multinational, placebo-controlled study of three different doses of riluzole. NNY301 had already been initiated at European sites by May 1993 with 420 patients recruited out of a total planned enrollment of 600 worldwide.

By May 1993, Study **AG216**, a 155-patient placebo-controlled study had already been completed in France and Belgium. That preliminary study had suggested a beneficial effect of riluzole on survival. Those results were subsequently published in the New England Journal of Medicine in 1994.

Riluzole does not bind to any known receptors, but it is known to exert an inhibitory effect on the spontaneous release of glutamate and on dopamine release induced by glutamate. In all likelihood, riluzole inhibits voltage-dependent sodium channels in much the same way that phenytoin, carbamazepine, and lamotrigine inhibit sodium channels; such inhibition results in decreased glutamate release from presynaptic neurons. Evidence exists that supports a role of glutamate in the pathogenesis of motor neuron loss in ALS.

ALS is estimated to occur in 100,000 people worldwide. Average survival is 3-5 years. Death usually results from respiratory compromise and weakness of bulbar musculature. In theory, survival might depend on three factors: 1) the original site of weakness (face, arm, or leg), 2) the rate at which new regions of the body become involved, and 3) the rate at which weakness progresses in the regions already involved.

Other Studies in ALS Patients

AG216LT: The long term extension of study AG216 began March 1992 and continued through December 1993. Patients have continued receiving riluzole beyond that date also.

NNY301LT: The long term extension of study 301 began June 1994 and continues at present.

Study 302: A double-blind, placebo-controlled trial designed to capture patients who did not meet inclusion/exclusion criteria for Study 301 in France and Belgium.

302LT: The long term extension of Study 302.

Study 304: An ongoing double-blind, placebo-controlled trial of riluzole in Japanese patients.

Study AG216 (France/Belgium)

Investigators

Center	1	Meininger	n=68
	2	Bouche	n=27
	3	Hugon/Couratier	n=12
	4	Blin	n=11
	5	Peyro-Saint-Paul/David	n=14
	6	Viader	n=9
	7	Maloteaux/Delwaide (Belgium)	<u>n=14</u>
	Total		n=155

A. Study Design

This was a double-blind, placebo-controlled, parallel study of the effectiveness of Riluzole 50 mg bid vs. Placebo in patients diagnosed with ALS. ALS was defined as the combination of a lesion of the anterior horn along with a pyramidal syndrome. Both clinical and EMG criteria were used. A monoclonal gammopathy was cause for exclusion.

Inclusion/exclusion criteria dictated that patients should have the diagnosis for no greater than 5 years at entry and be between the ages of 20 and 75 years. Pts with tracheotomies were excluded as were pts expected to require tracheotomy within the next 2 months. Pts with a restrictive pulmonary function > 40% were also excluded.

Pts on concomitant medications which could induce or inhibit hepatic metabolism were excluded. Women on oral contraceptives were excluded. Abnormal liver function as manifested by ALT or AST greater than twice the upper limit of normal were excluded.

If patients met the inclusion/exclusion criteria, they were randomized to either drug or placebo. The randomization was stratified based on limb onset vs. bulbar onset. If a patient had both limb and bulbar onset, that pt was classified as bulbar onset. The reason for this stratification was stated in the final study report, but not the protocol: to better evaluate

NEUROLOGICAL EXAMINATION

Date of assessment:

m	d	y			

REGION	SIGNS OF CENTRAL IMPAIRMENT (UMN)	SIGNS OF PERIPHERAL IMPAIRMENT (LMN)			
	CLINICAL SIGNS	CLINICAL SIGNS		EMG SIGNS	
	NO (i.e. Absent)	YES (i.e. Present)	NO (i.e. Absent)		YES (i.e. Present)
Bulbar	e.g. Brisk Jaw Jerk (Masseter)	☐	☐		☐
If NO, present before study entry?		☐	☐		☐
Upper Limbs	Brisk Tendon Reflexes and/or Hoffmann	☐	☐	☐	☐
If NO, present before study entry?		☐	☐		☐
Lower Limbs	Brisk Tendon Reflexes and/or Babinski	☐	☐	☐	☐
If NO, present before study entry?		☐	☐		☐
ALS Diagnosis ☐ Definite: UMN + LMN Signs in three regions each ☐ Probable: UMN + LMN Signs in 2 different regions each with UMN signs rostral to the LMN signs					

Operational Definition of ALS from Study 3

the functional scale employed in the study. That scale, the Norris Scale, is divided into a bulbar scale and a limb scale. (The onset form of the disease was a historical variable collected at baseline by the investigator, presumably by patient recall or record review.) "A differential effect of riluzole on survival, depending on site of disease onset, was not initially anticipated."(study report, p139)

A dose of 50 mg bid was chosen, corresponding to 0.5 mg/kg to 1.0 mg/kg or doses that antagonized the effects of glutamate in animal models. 50 mg is about one-fourth the highest dose of riluzole (200mg) that was well tolerated in previous non-ALS clinical trials. Administration during the trial was before meals. Gastrostomized pts were allowed to take crushed tablets.

Clinical observations included:

1. Time of death
2. Time of tracheostomy
3. Muscle strength recorded with the MRC 0-5 scale, added over preselected muscles, and collected at 2 month intervals (see next pages)
4. Norris Bulbar Score, a functional bulbar scale, collected at 2 month intervals (see next pages)
5. Norris Limb Score, a functional limb scale, collected at 2 month intervals (see next pages)
6. Pulmonary function studies collected at 6 month intervals e.g. FEV1, VC, and FEV1/VC
7. Analogue scales performed by the patient for selected clinical symptoms e.g. fasciculations, cramps, stiffness, and fatigability.

The protocol specified primary outcome was **time to death or onset of respiratory insufficiency necessitating respiratory assistance.** The protocol specified study cutoff time was November, 1991, a time when the last patient recruited had been in the study for at least 12 months. (The protocol did state that "between the one year date (11/30/91) and result availability (3/31/92), blind follow-up will be pursued.") The protocol specified analysis was a survival analysis using the Cox model. Some confusion was added by the addition of a **statistical analysis plan** dated Feb 7, 1992 (after the Nov 30, 1991

	Normal	Impaired	Moderate	None
1. Blow _____	☐ 3	☐ 2	☐ 1	☐ 0
2. Whistle _____	☐ 3	☐ 2	☐ 1	☐ 0
3. Blowing out cheeks _____	☐ 3	☐ 2	☐ 1	☐ 0
4. Jaw movement _____	☐ 3	☐ 2	☐ 1	☐ 0
5. Clicking tongue _____	☐ 3	☐ 2	☐ 1	☐ 0
6. Tongue protrusion _____	☐ 3	☐ 2	☐ 1	☐ 0
7. Tongue against the cheek _____	☐ 3	☐ 2	☐ 1	☐ 0
8. Tongue against the palate _____	☐ 3	☐ 2	☐ 1	☐ 0
9. Cough _____	☐ 3	☐ 2	☐ 1	☐ 0
	Absent	Moderate	Present	Severe
10. Hypersialorrhea _____	☐ 3	☐ 2	☐ 1	☐ 0
11. Nasalisation _____	☐ 3	☐ 2	☐ 1	☐ 0
12. Speech: mumbling _____	☐ 3	☐ 2	☐ 1	☐ 0
	Normal	Tender	Minced	1/2 liquid
13. Swallowing: food _____	☐ 3	☐ 2	☐ 1	☐ 0
ATTENTION: If food is given through nasogastric tube, swallowing must be rated 0.				

Bulbar Norris Scale

	Normal	Impaired	Moderate	None
1. Hold up head _____	☐ 3	☐ 2	☐ 1	☐ 0
2. Turn in bed _____	☐ 3	☐ 2	☐ 1	☐ 0
3. Sit up in bed _____	☐ 3	☐ 2	☐ 1	☐ 0
4. Writing ability _____	☐ 3	☐ 2	☐ 1	☐ 0
5. Buttoning, zipping _____	☐ 3	☐ 2	☐ 1	☐ 0
6. Dress oneself with a shirt, a blouse _____	☐ 3	☐ 2	☐ 1	☐ 0
7. Dress oneself with pants, a skirt _____	☐ 3	☐ 2	☐ 1	☐ 0
8. Cutting meat _____	☐ 3	☐ 2	☐ 1	☐ 0
9. Holding a fork _____	☐ 3	☐ 2	☐ 1	☐ 0
10. Filling up a glass and drinking from it _____	☐ 3	☐ 2	☐ 1	☐ 0
11. Standing up and shaking hands _____	☐ 3	☐ 2	☐ 1	☐ 0
12. Combing one's hair _____	☐ 3	☐ 2	☐ 1	☐ 0
13. Brushing one's teeth _____	☐ 3	☐ 2	☐ 1	☐ 0
14. Lift book or tray _____	☐ 3	☐ 2	☐ 1	☐ 0
15. Lift fork or pencil _____	☐ 3	☐ 2	☐ 1	☐ 0
16. Change arm position _____	☐ 3	☐ 2	☐ 1	☐ 0
17. Climb stairs _____	☐ 3	☐ 2	☐ 1	☐ 0
18. Walk around a block _____	☐ 3	☐ 2	☐ 1	☐ 0
19. Walk alone _____	☐ 3	☐ 2	☐ 1	☐ 0
20. Walk with assistance _____	☐ 3	☐ 2	☐ 1	☐ 0
21. Stand up _____	☐ 3	☐ 2	☐ 1	☐ 0

Limb Norris Scale

DATE OF ASSESSMENT:

m	d	y		

		No contraction	Flicker or trace of contraction	Active movement with gravity eliminated	Active movement against gravity but not against resistance	Active movement against gravity and resistance	Normal power	First Test
I UPPER LIMB POWER								
1. Thumb opposition _____	Left	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
	Right	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
2. Wrist flexion _____	Left	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
	Right	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
3. Wrist extension _____	Left	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
	Right	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
4. Elbow flexion _____	Left	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
	Right	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
5. Elbow extension _____	Left	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
	Right	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
6. Shoulder abduction _____	Left	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
	Right	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
II LOWER LIMB POWER								
7. Ankle dorsiflexion _____	Left	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
	Right	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
8. Knee flexion _____	Left	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
	Right	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
9. Knee extension _____	Left	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
	Right	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
10. Hip flexion _____	Left	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
	Right	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
III NECK								
11. Neck flexion _____		☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	
12. Neck extension _____		☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	

Muscle Strength Scale

date) which altered the analysis plan in the protocol somewhat. For instance, the **logrank test** was substituted for the primary analysis with the Cox model used for the "final analysis."

The secondary outcome was stated to be **Norris Bulbar and Limb Scores**, studied over time. The **Norris Bulbar Scale** assesses 13 functional tests involving brainstem motor neurons (e.g. swallowing, whistling, blowing) and grades them each from 0 (none) to 3 (normal). It has a range from 0 (total incapacity) to 39 (normal bulbar function). The **Norris Limb Scale** assesses the ability of the patient to use their limbs to perform 21 daily activities (e.g. walking, writing, sitting) and grades each from 0 to 3. It has a range from 0 to 63 (normal). The protocol specified analysis of the Norris scales and muscle scores (see below) was an analysis of **slopes** by the estimator maximum likelihood method.

Muscle testing tested 6 movements of each upper limb and 4 movements of each lower limb and 2 neck movements. Altogether 22 movements were each graded from 0 (no movement) to 5 (normal), resulting in a total score from 0 (total incapacity) to 110 (normal).

With a projected enrollment of 110 pts and a dropout rate of 10%, the study was powered to detect a difference of 30% in survival i.e. 85% survival in the drug group vs. 55% survival in the placebo group.

The study was also powered to detect a difference in the slopes of the overall **Norris Score** over time between treatment groups of 50% i.e. -1.2 points/month in the drug group vs. -2.4 points/month in the placebo group.

Standard safety assessments were performed at regular intervals to include liver enzymes. A schedule of time and events is attached on the next page. Lab work was performed every 2 weeks for the first 4 months, and every 2 months thereafter. Plasma levels of riluzole were also collected and recorded in the CRFs. The protocol does not discuss how the blind was maintained in the face of these two points.

	Inclusion visit	D14	M1	W6	M2	W10	M3	W14	M4	M6	M8	M10	M12	M14	M16	[M18]	[M20]	[M22]	Control visit
Demographics	○																		
Failed information	○																		
Informed consent	○																		
Diagnosis	○																		
Inclusion criteria	○																		
Exclusion criteria	○																		
Eligibility	○																		
Previous treatment	○																		
Study treatment	○																		
Concomitant treatment	○																		
Survival evaluation	○																		
Norris scale	○																		
Muscular testing	○																		
RFT	○																		
CGI	○																		
VAS	○																		
DBP, SBP and HR determination	○																		
ECO	○																		
Standard lab work-up	○																		
Abridged lab work-up	○																		
Plasma samples	○																		
Adverse event collection	○																		
Comments	○																		
Conclusion	○																		

Study 216 Time and Events Schedule

B. Subject Disposition and Baseline Comparison

The study was conducted between June 1990 and November 1991. Recruitment occurred over a 5-month period, so that, for the **November 1991** analysis, pts were in the study for between 12-18 months. As per protocol, pts remained on double-blind treatment after November 1991, while data was being collated and analyzed. This double-blind extension lasted until March 1992, so that, for the **March 1992** analysis, pts were in the study for between 15-21 months. Altogether 155 pts with ALS were enrolled: 123 with limb onset form and 32 with bulbar onset form.

Baseline demographic data is presented in the table below. No significant differences between groups were detected. Note that no systematic data on gastrostomy was reported although this would have been collected in the case report form as part of the general physical exam. Only five pts had a family history of ALS, 4 in the riluzole group and 1 in the placebo group.

	Riluzole	Placebo
	N=77	N=78
Male/Female	45/32	46/32
Age (years)	57	58
Weight (kgs)	66	65
Duration of Disease (years)	2.2	2.3
Vital Capacity Ratio (VCR=pt's VC divided by lower limit normal for VC)	0.92	0.86
Norris Limb Score (normal=63)	42	41
Norris Bulbar Score (normal=39)	31	30
Muscle Testing (normal=110)	83	79

Protocol deviations at Inclusion occurred. 5 riluzole pts vs. 6 placebo pts had VCR reductions of 40% or greater. 3 riluzole pts vs 6 placebo pts had disease duration greater than 5 years. 0 riluzole pts vs 2 placebo pts had ages outside the inclusion/exclusion criteria (a 19 yr-old and a 76 yr-old).

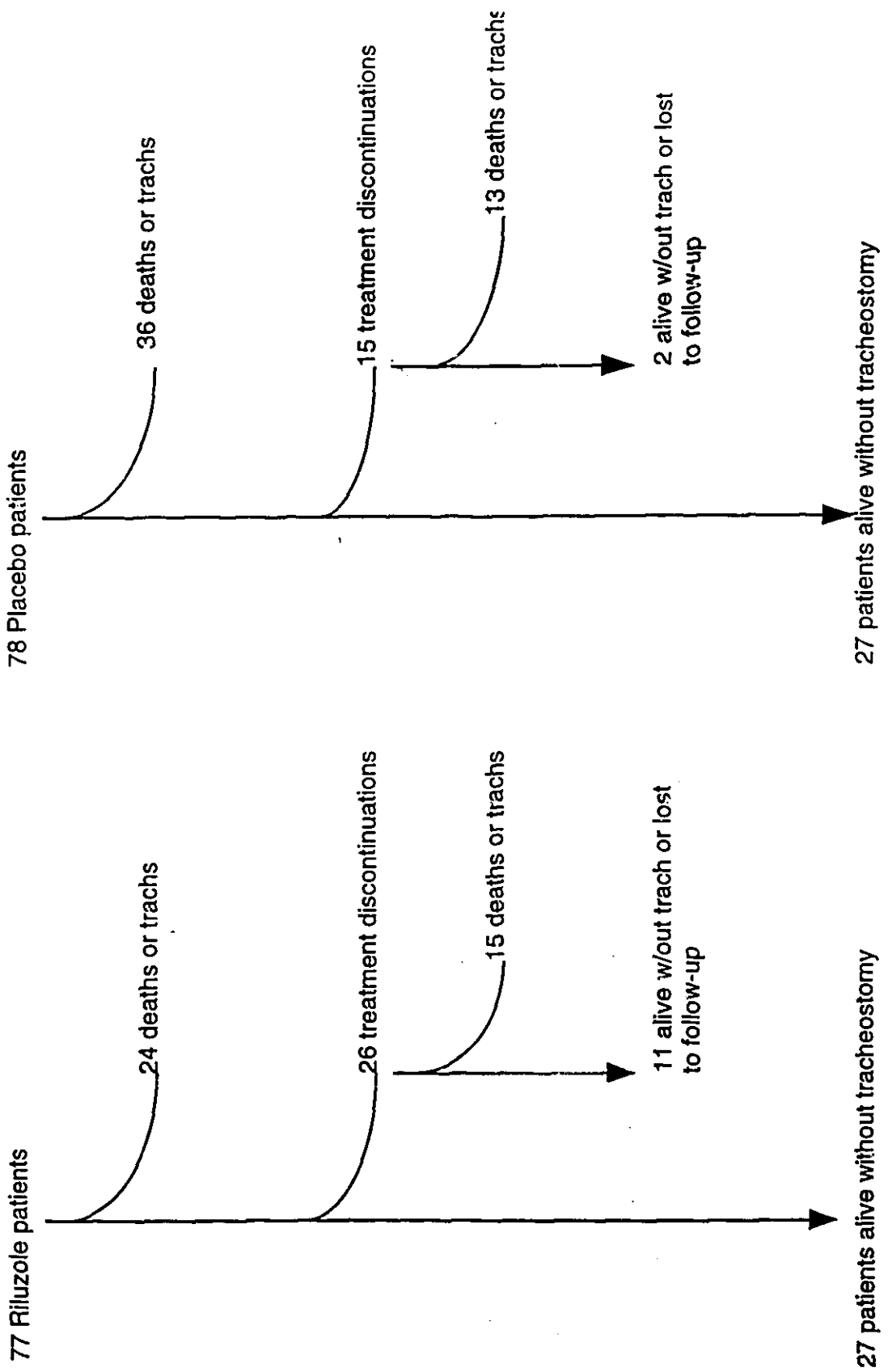
There were more pts in the riluzole group than in the placebo group who received disallowed concomitant medications: 22(29%) vs 14(18%) respectively. In particular, 17(22%) riluzole pts vs 10(13%) placebo pts received medications that at various times have been proposed as slowing disease progression in ALS (calcium channel blockers, dextromethorphan, etc). Within the bulbar onset stratum, however, the latter numbers were balanced.

Discontinuations from drug or placebo occurred as outlined in the following Table. Note that this table lists the **earliest** reason for discontinuing treatment. Patients who discontinued treatment for an adverse event could have died at a later date. Those patients are counted only under "adverse experience" and not "death" in this table.

	Riluzole		Placebo	
	N	%	N	%
Adverse Experience	19	38	9	18
Lack of Efficacy	2	4	3	6
Lost to Follow-Up	3	6	2	4
Tracheostomy	1	2	2	4
Death	23	46	34	67
Other	2	4	1	2
All	50	100	51	100

Thus, 101 pts discontinued treatment before March 12, 1992. 60 of the 101 treatment discontinuations were due to death or tracheostomy, 24 in the riluzole group and 36 in the placebo group. 41 of the 101 treatment discontinuations were due to reasons other than death or tracheostomy; 28 of these 41 went on to tracheostomy or death prior to March 1992. 54 pts continued treatment until March 1992.

The NEJM article states, "In the analysis of survival, there was no loss to follow-up." The flow diagram on the following page demonstrates patient disposition during the conduct of the study.



From the flow diagram, there were 41 treatment discontinuations during the conduct of the study, 26 in the riluzole group and 15 in the placebo group. This differential discontinuation rate created a potential for bias in the study that needed to be explored. In particular, the difference in total number of deaths or trachs per treatment group seems to come from the 11 riluzole patients who were treatment discontinuations, but who were called alive without trach at study end.

Therefore, we asked the sponsor to clarify how the primary outcome was documented for patients who discontinued treatment (and would have potentially been lost to follow-up). We received different answers at different times:

April 19, 1994 submission: "The lost to follow-up patients did not return to the investigator for assessment. Their final status was determined by checking local court houses for records of death. Of the 5, three deaths were determined, one additional patient was tracheotomized, and one could not be found. This patient is listed as censored, with no data available after the date of the last visit."

April 18, 1995 submission: "There were 13 patients whose life status was unconfirmed as of the 12 March 92 cutoff... The remaining 11 patients were counted as alive in the intent-to-treat analysis." This same submission also implied that more patients might have been counted as deaths based on the finding of death certificates in local city halls, but for whom information on tracheostomy was lacking.

In the survival analyses that the sponsor performed, the mentioned patients were not only "presumed alive"; they were also not censored when they were lost to follow-up. For instance, if a patient was lost to follow-up after 2 months, that patient was given credit as being alive without tracheostomy for the full 15-21 months of study. The appropriate way to handle this would have been to censor the patient alive at 2 months.

My initial concern was for the 13 treatment discontinuations (11 riluzole, 2 placebo) who were counted alive without trach at study end. These are patients:

Placebo: 302,403

Riluzole: 114,137,138,220,244,303,406,506,602,614,702

The sponsor has addressed "13 patients who never entered the long term extension of Study 216 and who could be considered lost to follow-up at that time." This is a different working definition which does not include pts 244,302,303,and 403. It includes 2 pts who were already known to have been trached during the study and who were therefore not lost-to-follow-up. The sponsor's 13 patients are:

Placebo: 119,505,612

Riluzole: 114,133,137,138,220,406,506,602,614,702

Of the sponsor's 13, outcome has been clarified in the past few months for all but 2, pts 406 and 614. For purposes of analysis, these patients are now being counted as alive with allocated times of 34 days and 234 days.

Still a concern that the sponsor has not addressed is the ascertainment of outcome for the 28 patients who discontinued treatment but are known to have met endpoint. If the endpoint was death, and death was ascertained by locating a death certificate at a city hall, how can we know whether or not the patient was trached prior to death ?

Given the low number of trachs overall, as well as the balance between treatment groups for this subset of outcomes (15 riluzole vs 13 placebo), I do not expect the final analysis to change considerably as more information is collected on these patients.

Alternatively, for other outcome assessments, including muscle testing, Norris Scores, and pulmonary function tests, there was often missing data due to missed visits. If data is missing because of death, informative censoring has taken place. Since more deaths occurred in the placebo group, this informative censoring could create a biased analysis. This was addressed by utilizing unweighted slopes of decline on these scales.

The protocol specified cutoff date was November 30, 1991. During the

subsequent 3 months, while the sponsor organized the database and began to analyze the data, investigators continued to collect information on patients. Thus, data was accrued through March, 1992. This second batch of data was incorporated into an analysis of this second study population.

C. Efficacy Evaluation

Primary Outcome: The protocol specified endpoint analysis included data through November 1991. The results are presented below:

	Median Survival in days	Stratified Logrank Test 2-sided P value	Cox Model 2-sided P value
Riluzole	502	0.140	0.088
Placebo	469		

While the Cox Model p value approached statistical significance, the effect size was small i.e. an increase in median survival of one month. Again, the February 1992 statistical analysis plan stated the primary analysis would be the stratified logrank while the protocol specified analysis was the Cox model.

A point to note in the above analysis is that tracheostomy is incorporated into the endpoint variable along with death. Because time of tracheostomy is as much determined by patient and clinician discretion as by the clinical state of the patient, time of tracheostomy has to be considered a possible source of bias. For Study AG216, however, the number of tracheostomies was so small that this was not considered an important contributor to the final result. If more tracheostomies had been performed, a separate analysis using death alone as the endpoint would have been warranted. In AG216, 11 pts underwent tracheostomy prior to March 1992, 6 placebo pts and 5 riluzole pts.

For reasons that are never fully explained in the final study report, the sponsor chooses to primarily rely on the data collected through March, 1992. The results of this analysis are presented below:

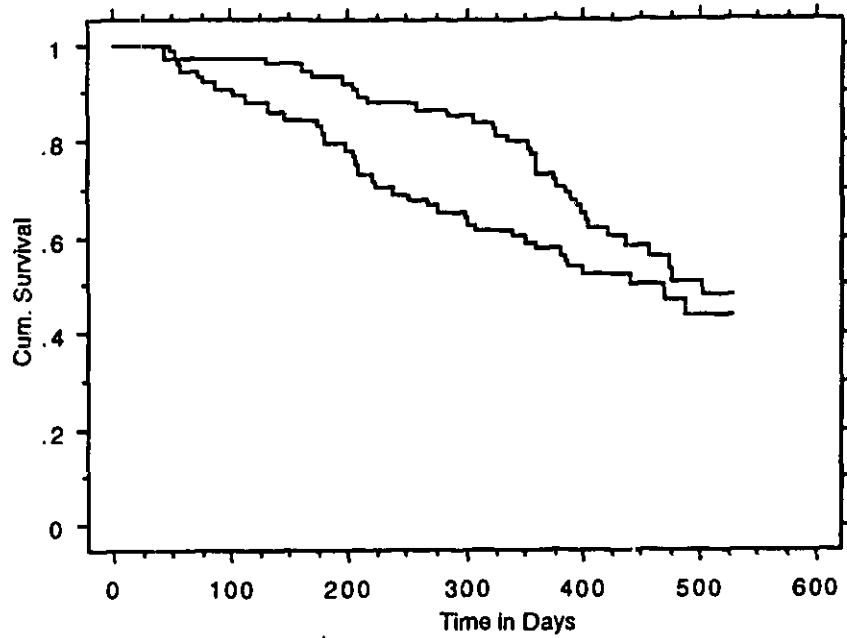
	Median Survival in days	Stratified Logrank Test 2-sided P value	Cox Model 2-sided P value
Riluzole	531	0.061	0.066
Placebo	456		

The p values are lower for this analysis than for the November 91 analysis. Likewise the clinical effect appears larger than in the November 91 analysis, 75 days vs. 30 days. Curiously, part of this improvement arises from a worsening in median survival for the placebo group, 469 days to 456 days.

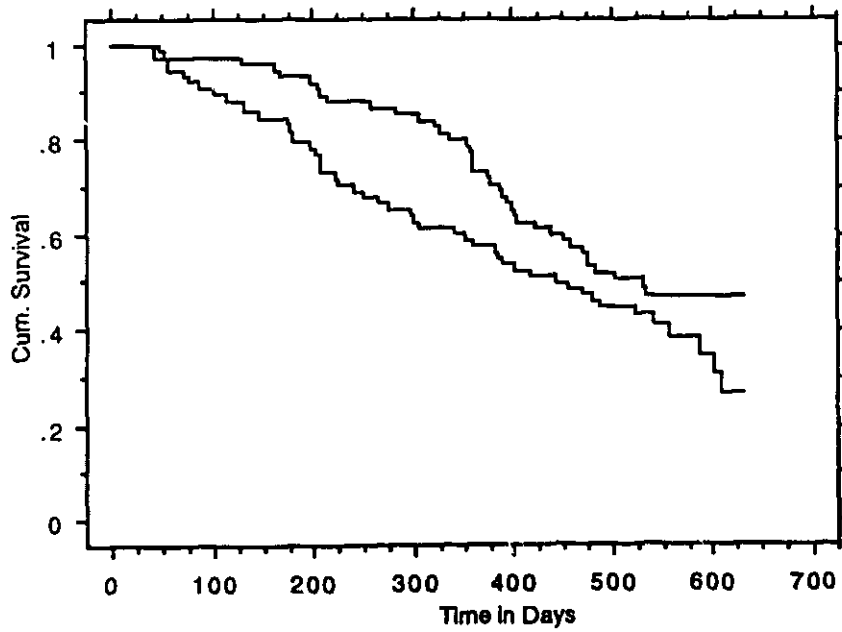
The sponsor also presents an analysis of a third patient population, using only the first 12 months of data for each patient. This analysis was not prospectively outlined and ignores a substantial part of the data collected. In the final study report, the sponsor states, "When the results of the Nov 1991 analysis were displayed, it appeared that the survival curves for the two treatments were not parallel and that the assumption of proportional hazards was questionable. Consequently, the data cutoff of one year of treatment was applied, yielding another analysis of survival data." As will be seen later, the one year cutoff is coincidentally the time when the hazard for the riluzole group increases over the hazard of placebo, raising the possibility that this is an analysis chosen retrospectively to portray riluzole in its most favorable light. Suffice it to say that it might be interesting to know the results of an endpoint analysis when all patients had contributed only 1 year of data toward the analysis. The results are summarized below:

	Cumulative Survival at One Year	Stratified Logrank Test 2-sided P value	Cox Model 2-sided P value
Riluzole	74%	0.018	0.006
Placebo	58%		

Shape of the Kaplan-Meier Plots: The Kaplan-Meier plots are shown below for both the November cutoff and the March cutoff. The most striking feature about the curves is the early separation with a later tendency to come together.

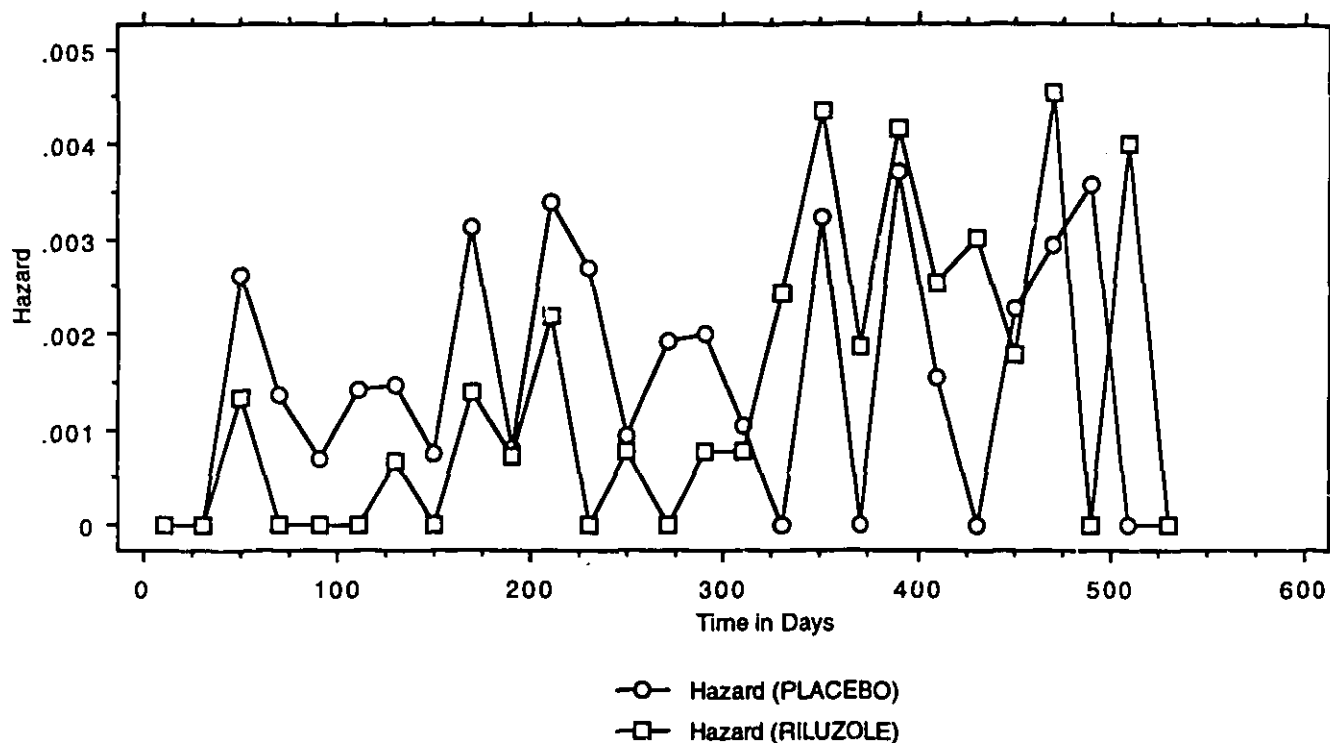


November 1991 Cutoff Date, riluzole vs placebo



March 1992 Cutoff Date, riluzole vs placebo

This tendency for the curves to come together implies that, after about 1 year of treatment, the **hazard** for the riluzole group actually rises above that for the placebo group. That is, for patients still alive at a certain time interval beyond 350 days, the risk of that patient dying or undergoing tracheostomy in the immediate future is higher for the riluzole treated patient. This can be shown graphically below by plotting the hazard for increments of 20 days over the length of the study. Note that the hazards for placebo tend to be above those for riluzole for the first half of the study and then, for the second half of the study, the riluzole hazards tend to rise higher than placebo hazards.



Of course, the unanswered question is whether the Kaplan-Meier curves eventually meet and follow a common path thereafter. Potentially the curves could cross with cumulative survival being worse on drug after 2-3 years.

The comparison of Kaplan-Meier curves as discussed up to this point has essentially been a comparison of their respective hazards over time. The hazards can all be treated equally as is done in a logrank analysis or the hazards can be weighted so that earlier hazards are given more emphasis

as is done in Wilcoxon analyses (either Peto-Peto-Wilcoxon or Breslow-Gehan-Wilcoxon). Obviously in designing a clinical trial in ALS, the hope would be that the two curves in question (drug vs placebo) would separate and remain separate. For this reason, RPR must have chosen the stratified logrank statistic for the primary analysis. Because the curves come together in this riluzole trial, the Wilcoxon statistics will make the results appear more favorable than the logrank. Given the wide array of p-values discussed, the following table was prepared by RPR to consolidate all of them in one place.

	Stratified Logrank	Stratified Wilcoxon	Cox Model
November 1991	0.14	0.058	0.088
March 1992	0.062	0.04	0.066
One year data	0.018	0.012	0.006

Primary Outcome by Stratum (Bulbar vs Limb Onset): To reiterate, the stated reason for this stratification was to better evaluate the functional scale employed in the study. That scale, the Norris Scale, is divided into a bulbar scale and a limb scale. The onset form of the disease was a historical variable collected at baseline by the investigator, presumably by patient recall or record review.

Interestingly, in Study AG216, the overall effect on "survival" arose from the bulbar onset stratum. Regardless of the study population (November or March cutoff), the median survival for the limb onset groups was essentially the same for the drug and placebo groups (502 vs 487 days in Nov or 531 vs 523 days in March). For the **bulbar-onset group**, the following results emerged:

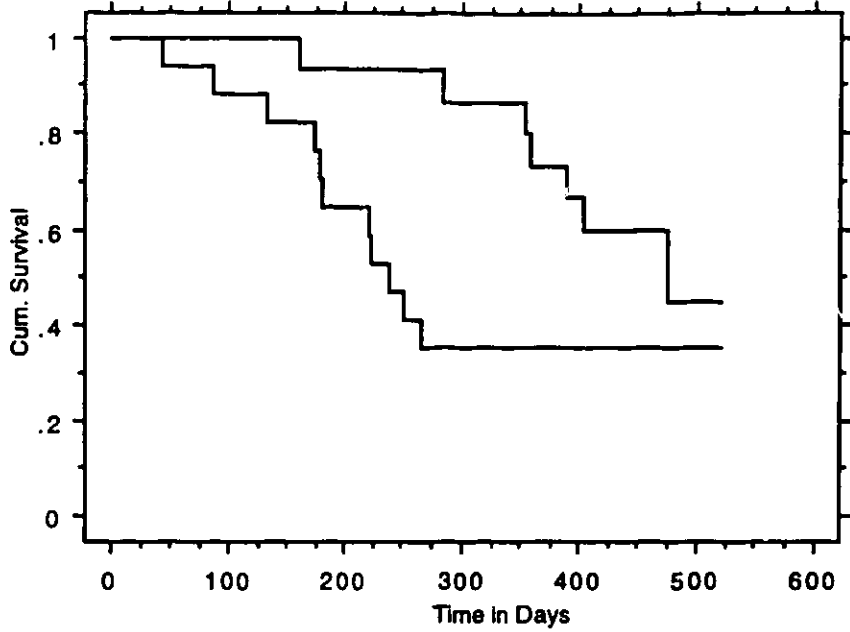
November 1991 Cutoff
Bulbar-onset patients

	Median Survival in days	Logrank Test 2-sided P value	Cox Model 2-sided P value
Riluzole	476	0.078	0.060
Placebo	239		

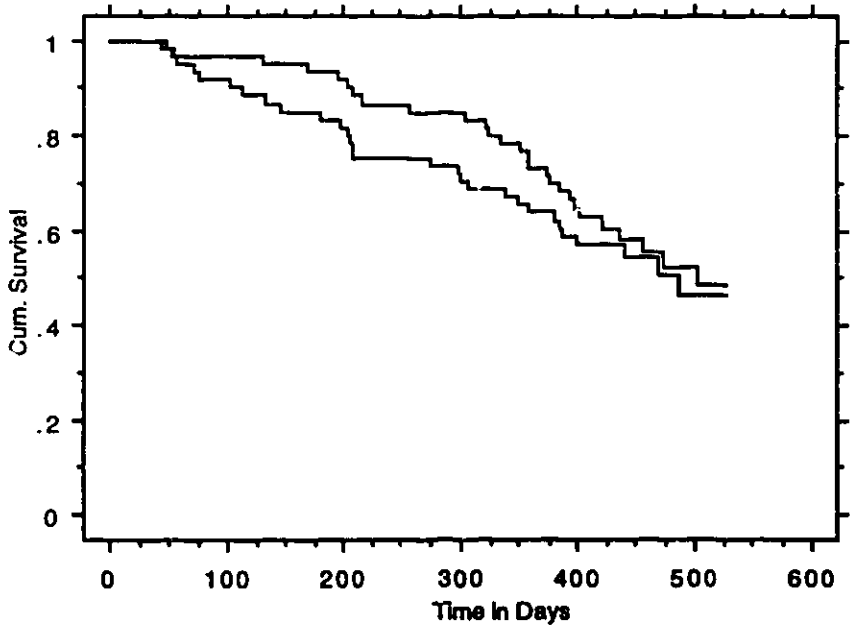
March 1992 Cutoff
Bulbar-onset patients

	Median Survival in days	Logrank Test 2-sided P value	Cox Model 2-sided P value
Riluzole	>476	0.014	0.010
Placebo	239		

No obvious explanation for the discrepancy between the results in the bulbar onset group and the limb onset group exists. The Kaplan-Meier curves for the two strata using the November cutoff data are shown on the following page.



Bulbar-Onset Stratum, riluzole vs placebo



Limb-Onset Stratum, riluzole vs placebo

Cox Model: The stratified logrank test was the specified analysis in the February 1992 analysis plan, using onset form of the disease for stratification. In the final study report, the sponsor also reports the Cox model, used to adjust for prognostic variables at baseline. In this study, results do not differ considerably between the stratified logrank analysis and the Cox model. In Study 301, the Cox model p-values differ by an order of magnitude from the logrank p-values. Dr. Hoberman has discussed this at some length in his statistical review, where he comments on the possible misinterpretations that may arise using the Cox model.

The candidate prognostic variables here were:

- o sex
- o age
- o duration of disease
- o baseline VCR
- o baseline Norris Bulbar Score
- o baseline Norris Limb Score
- o baseline muscle testing score
- o baseline CGI severity
- o baseline VAS scores for fasciculation, cramp, stiffness, and tiredness

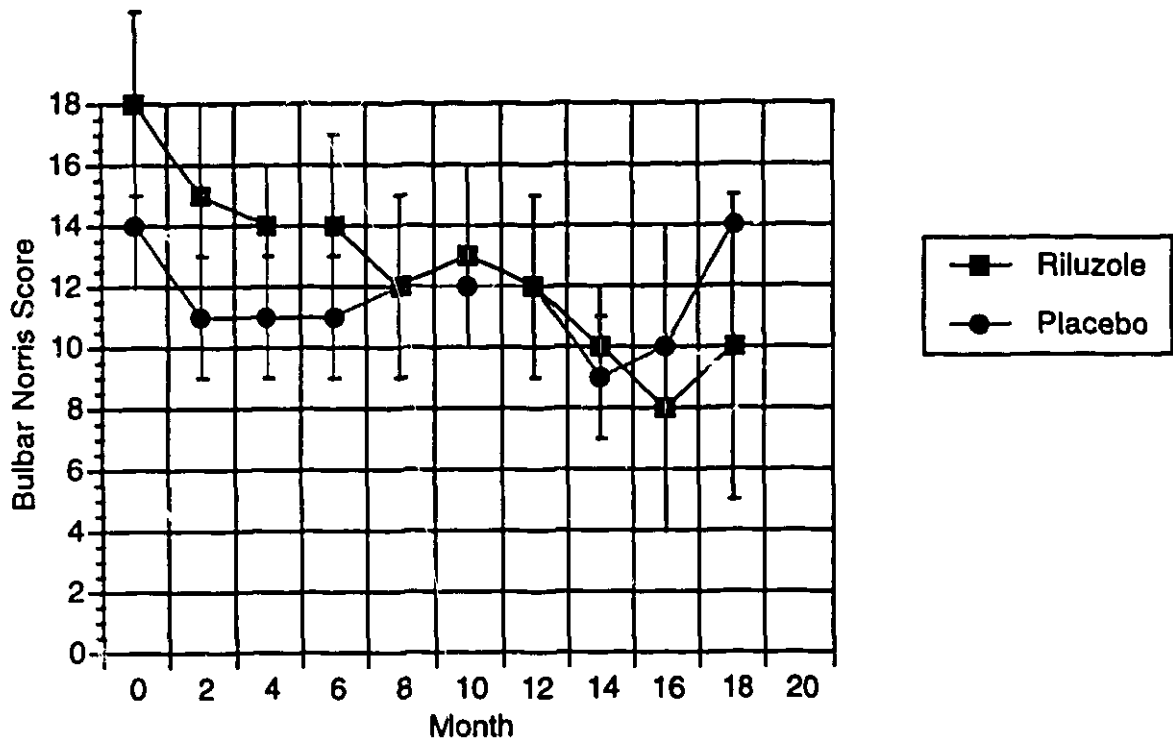
Using a stepwise procedure, the following prognostic variables selected in the 3 procedures (Nov 91, Mar 92, and one year) were retained for the final model:

- o age
- o duration of disease
- o baseline VCR
- o baseline Norris Bulbar Score
- o baseline VAS stiffness
- o baseline VAS tiredness

A center effect was also evaluated, but because of the small number per center and stratum, the 7 centers were pooled into two groups: the 2 centers in Paris versus the 5 remaining centers. The center effect was initially included in the Cox model, but was found not to be a relevant prognostic factor.

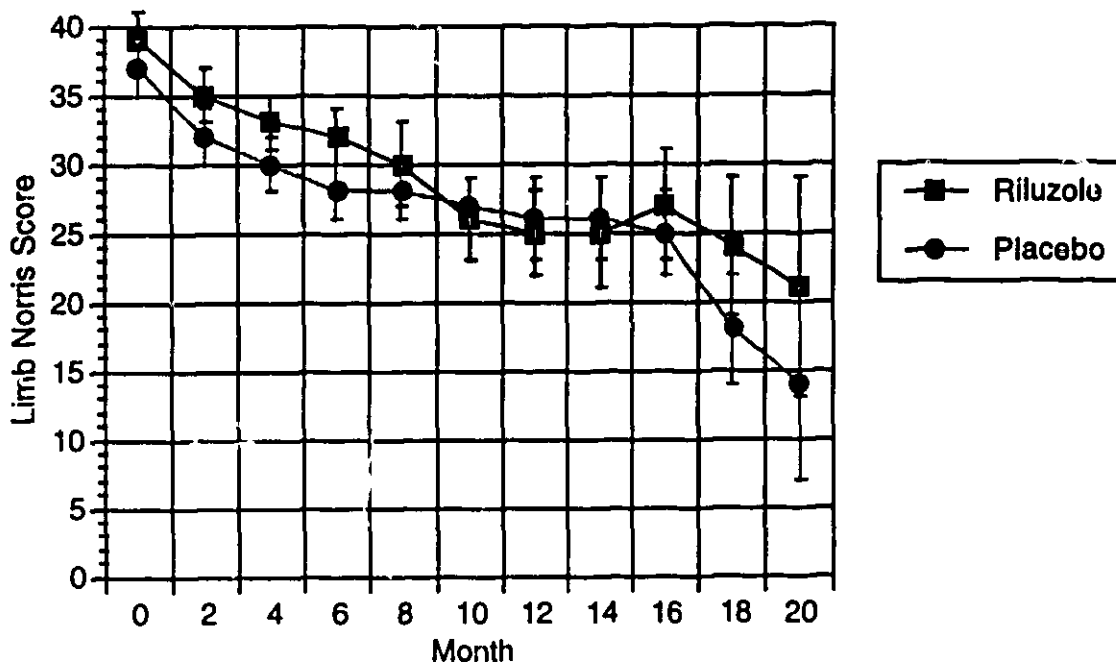
Secondary Outcomes (Norris Scales): The crude data for the bulbar Norris scale is plotted below for patients with bulbar-onset disease.

Graph: Average bulbar Norris scores with S.E. bars for each treatment group and visit. Only pts with bulbar-onset disease are included.



Likewise, the crude data for the limb Norris scale is plotted below for patients with limb-onset disease.

Graph: Average limb Norris score by treatment group and month with S.L. bars. Only pts with limb-onset disease are shown.



However, applicable to both of these graphs, informative censoring was probably occurring over time in the different treatment groups. That is, patients with the worst slopes of decline on the Norris scales were most likely to die and thus, those patients with the worst scores did not contribute to later visits. Moreover, since patients appeared to die sooner on placebo than on riluzole, a potential bias is introduced.

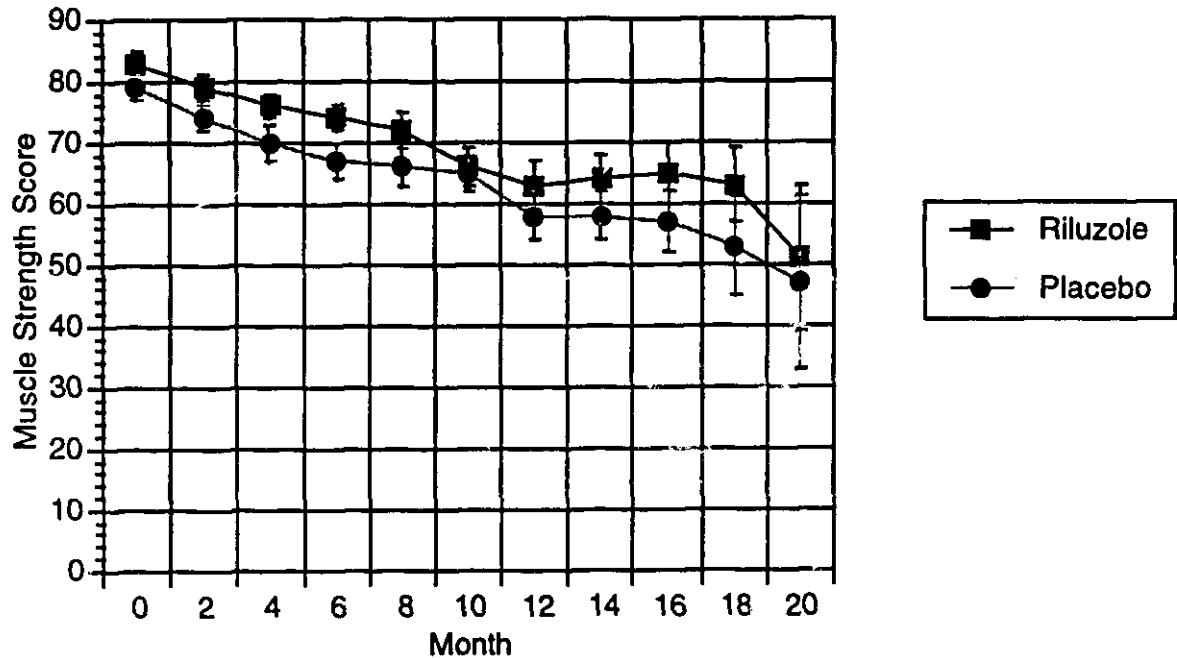
Therefore, the sponsor computed the slopes of decline on the Norris Bulbar Scale and Norris Limb Scale for each patient and then compared mean slopes of decline between treatment groups. The sponsor did this 1) incorporating data for each patient for up to one year of follow-up and 2) incorporating data for each patient up through March 1992. No statistically significant differences in slopes of decline were found for either the bulbar or limb scale in the sponsor's analyses. The sponsor does not present analyses of the effect of riluzole on rate of deterioration in Norris scales for the different strata.

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Secondary Outcomes (Muscle Testing): The crude data for the muscle testing scale is plotted below for all patients.

Graph: Average muscle scores with standard error bars at each visit for the different treatment groups

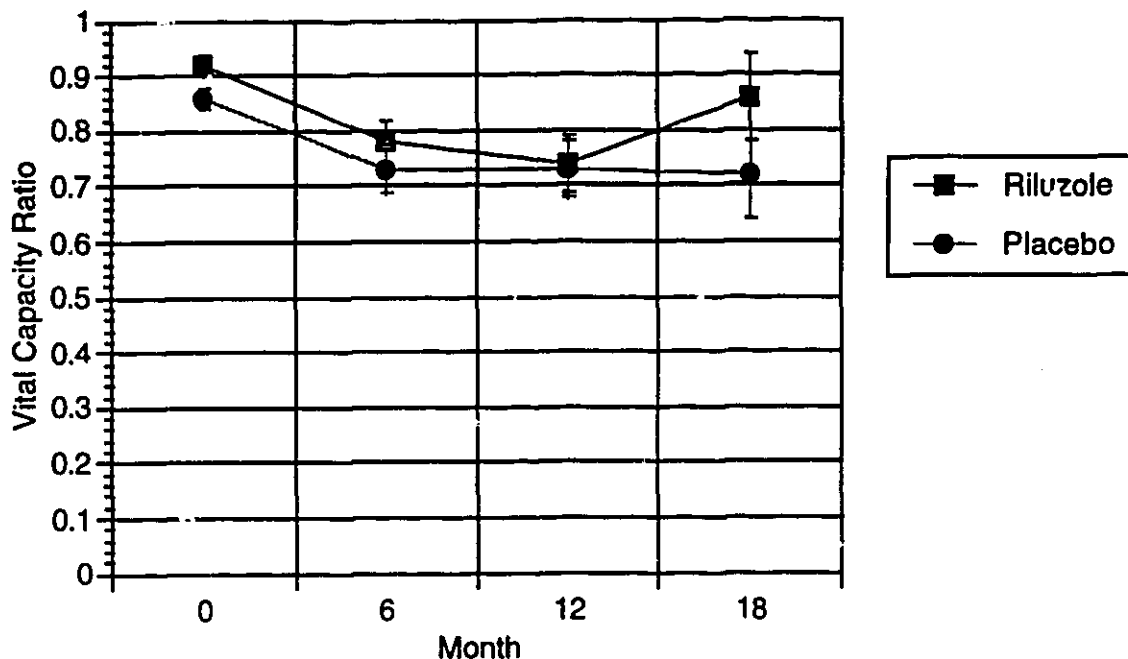


Again, given the possibility of informative censoring over time, and the introduction of bias, the sponsor chose to compute slopes of decline in total muscle score over time for each patient and then compare mean slopes for the two treatment groups.

A difference of 11 units/year was found, favoring drug ($p=0.026$). According to the sponsor (study report, p150), riluzole slowed the rate of deterioration in muscle function to about the same extent in both strata, bulbar onset and limb onset.

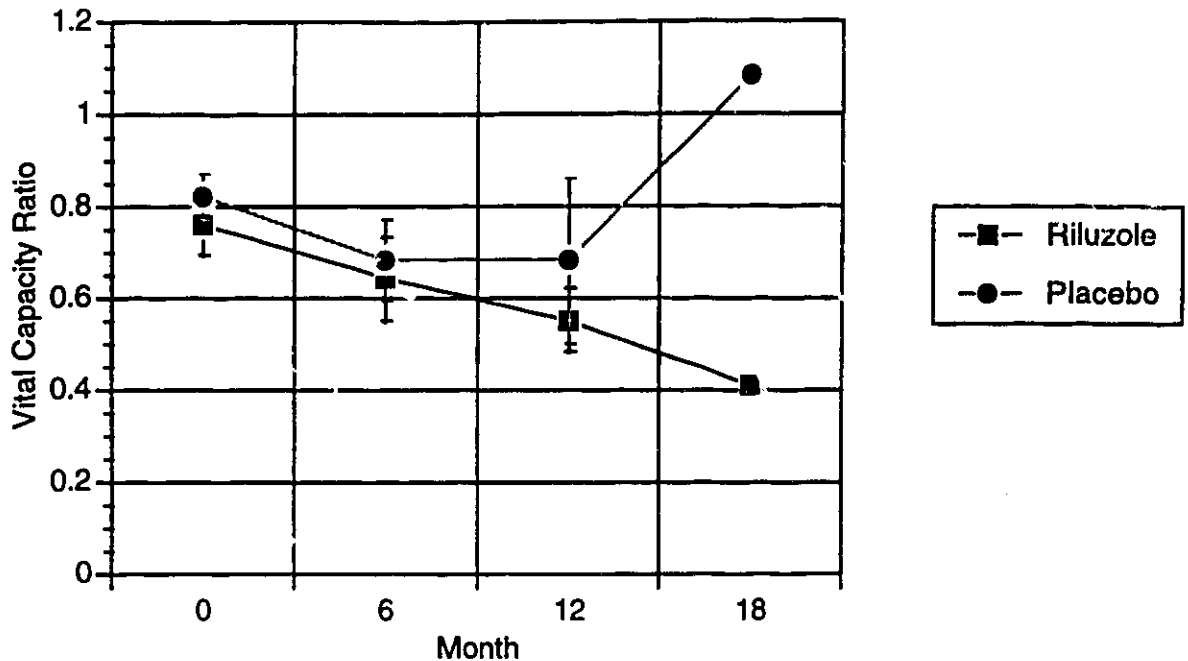
Secondary Outcomes (Pulmonary Function): The crude data for vital capacity ratios is plotted below for all patients.

Graph: All patients; average vital capacity ratios with SE bars.



The same crude data is plotted below only for patients with bulbar-onset disease.

Graph: Bulbar onset pts; vital capacity ratio and SE bars.



The sponsor made no statistical comparisons between groups for these variables. For VCR, fully 25% of data points are missing for both the riluzole and the placebo groups. The sponsor reports no apparent differences between treatment groups.

Secondary Outcomes (Analogue Scales of Symptoms): The sponsor presents no statistical tests of significance for the four analogue scales.

Secondary Outcomes (Global Impressions): The sponsor reports no apparent differences between treatment groups.

Gastrostomies: The sponsor was asked to provide data on gastrostomy from this study. In a Feb, 94 submission, the sponsor identified 6 patients with gastrostomy:

<u>Pt</u>	<u>Time gastro. noted</u>	<u>Group</u>	<u>Strata</u>	<u>Outcome</u>
135	M8 visit	Placebo	bulbar	Died
136	M16 visit	Placebo	bulbar	Surviving
143	M4 visit	Riluzole	bulbar	Died
604	M14 visit	Placebo	limb	Died
605	M16 visit	Placebo	limb	Surviving
624	M14 visit	Placebo	bulbar	Died

Plasma Concentrations: Plasma concentrations of riluzole were determined at monthly intervals for 4 months, and then every other month for the study duration. There was large inter-individual variability in plasma concentrations with coefficients of variation between 49 and 117%. The sponsor attributes some of this variability to not dosing on a mg/kg basis and variable length of time since last dose.

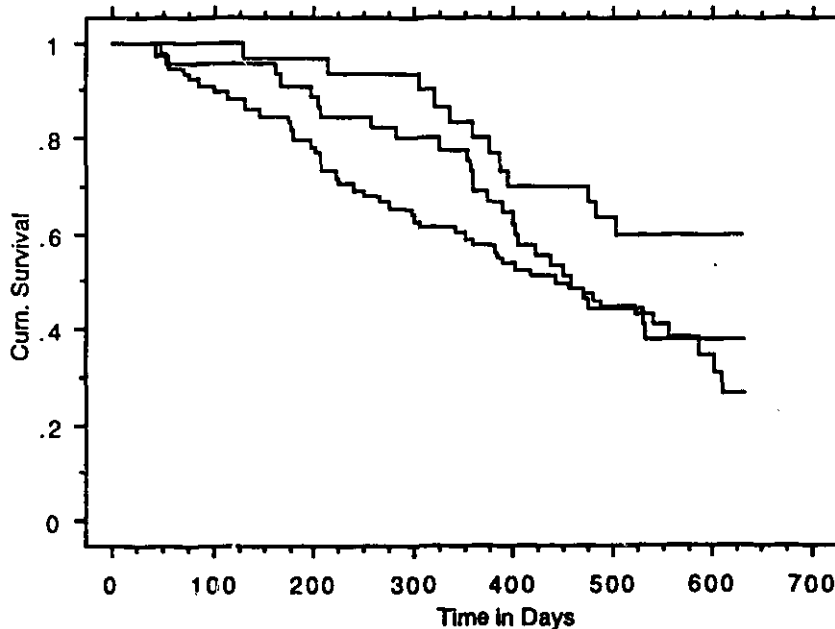
Unblinding: Knowing the potential of riluzole to cause SGPT(ALT) elevations and using a list of SGPT(ALT) values over time for each patient, I generated the following list of patients that I would have guessed were on riluzole:

111	206	404
114	207	503
122	212	506
123	219	601
127	220	602
128	233	607
129	237	611
130	240	614
131	246	702
133	305	706
145	306	708
203	307	719
204	309	720
205	401	724

My guesses were correct 31 times and false 11 times. The following table can be generated.

	True	False
Riluzole	31	11
Placebo	67	46

If the 3 larger groups created from this table are placed in the Kaplan-Meier plot below, we see that the 31 riluzole patients that I guessed were on drug did better than the 46 riluzole patients that I guessed were not on drug ($p=0.089$; logrank). Both groups did better than the 67 placebo patients that I guessed were not on drug. Unfortunately, there are only 11 placebo patients that I guessed were on drug; that curve (not shown) most follows the 67 placebo patients.



D. Conclusions

The protocol-specified primary analysis was the stratified logrank test including data through November 1991. The 2-sided p value for this analysis was 0.14, which does not meet the usual standard for approval of a new drug product.

However, the protocol called for continuation of double-blind treatment beyond November 1991 until March 1992, without ever specifying an analysis plan for this additional data. The nominal p value (stratified logrank; 2-sided) using this March 92 cutoff was 0.061. The stratified Wilcoxon, 2-sided p value for this same March 92 cutoff was 0.04.

Concerns were initially raised about the integrity of the data for riluzole patients who discontinued treatment and had variable follow-up. The sponsor has addressed most of these concerns (see Addendum below).

One area of deficiency that may never be fully resolved in Study 216 is the ascertainment of tracheostomy (and therefore time-to-trach) in patients lost-to-follow-up and later ascertained to have died. As mentioned above, this is unlikely to affect the overall results of Study 216 significantly.

The issue of unblinding due to serum ALT elevations is discussed above. However, when only patients without ALT elevations are considered, a separation in Kaplan-Meier curves persists between the riluzole and placebo groups.

A difference in the average slope of decline on the Muscle Strength Scale was found between the riluzole and placebo groups. The difference, 11 units/year, favored drug with a p value of 0.026.

Addendum: On August 1, 1995, the sponsor submitted updated information on patient 406. Previous to this, the last known status of this patient was November 91. "It was recently determined that patient 406 died June 18, 1992 without tracheostomy." Based on this information, the sponsor has revised the p values as shown below. Small differences exist between these p values and those reflected in the above review.

	Stratified Logrank	Stratified Wilcoxon	Cox Model
November 1991	0.124	0.050	0.082
March 1992	0.052	0.034	0.06
One year data	0.016	0.010	0.004

Study NNY301 (International)

<u>Country</u>	<u>Number of Centers</u>
France	9
Belgium	1
United Kingdom	8
Germany	4
Spain	1
United States	5
<u>Canada</u>	<u>3</u>
Total	31

Regions	N	%
France/Belgium	444	46
Other European Countries	327	34
North America	188	20

Country	N	%
Belgium	22	2
France	422	44
Germany	95	10
Spain	24	3
United Kingdom	208	22
United States	120	13
Canada	68	7
Total	959	100

Investigators in France and Belgium:

Investigator	N
Meininger	222
Bouche	24
Couratier	24
Blin	24
Camu	45
Lagueny	24
Clanet	24
Desnuelle	19
Viader	16
Maloteaux/Delwaide	22

Investigators in United Kingdom, Spain, and Germany:

Investigator	N
Leigh	24
Shaw	45
Swash	24
Lane	24
Pall	23
Jefferson	24
Mitchell	23
Schady	21
Mora	24
Dengler	24
Przuntek	24
Ludolph	23
Schimrigk	24

Investigators in USA and Canada:

Investigator	N
Miller	24
Munsat	24
Sufit	24
Harati	24
Rothstein	24
Duquette	23
Eisen	22
Bouchard	23

A. Study Design

This was a double-blind, placebo-controlled study of 3 different doses of riluzole. Except for the additional two dose groups, the study was almost identical in design to AG216. The mid-dose group in 301 was the same dose used in 216, 50 mg bid. The low-dose group was 25 mg bid and the high-dose group was 100 mg bid.

Because enrollment began in Europe prior to North American enrollment, there is a European protocol (October 92) and a North American protocol (April 93). The first 2 amendments to the European protocol were incorporated into the North American protocol. Subsequent amendments to the study are therefore numbered differently for the European and North American protocols.

Inclusion/exclusion criteria were almost identical to study 216. Protocol 216 really provided no operational definition of ALS, but protocol 301 specified that patients meet the definition of definite or probable ALS by the World Federation of Neurology criteria. Amendment #1 to the European protocol allowed the inclusion of patients who presented only with lower motor neuron signs, provided that in the course of the disease, upper motor neuron signs were documented. Also, the trunk evaluation

was deleted from the classification of definite or probable ALS due to uncertainty in distinguishing central vs peripheral signs at that level.

Inclusion criteria were:

- o age between 18-75 years
- o definite or probable ALS by the World Federation of Neurology criteria
- o the duration of ALS (defined as starting from the first symptoms of the disease) must be less than five years
- o patients must have an FVC $\geq$ 60% predicted within 2 months before study entry
- o women of childbearing potential must use acceptable birth control methods
- o patients could be included who had gastrostomies (In 216, this issue was not addressed in the protocol and it is not clear if patients with gastrostomy were included.)

Exclusion criteria were:

- o concomitant use of drugs known to be hepatotoxic, enzyme-inducing, or enzyme-inhibiting (Note that the list of allowed concomitant medications was modified by amendment #2.)
- o serious concomitant disease
- o tracheostomy, or tracheostomy expected within next 2 months
- o baseline ALT or AST values $>$ 2 times normal
- o monoclonal gammopathy
- o conduction block on nerve conduction studies

The protocol for study 301 states "a patient who simultaneously presents with bulbar and peripheral signs at onset of ALS disease should be stratified to the bulbar onset group. The neurologic progression of such patients matches that of the bulbar onset ALS patients." This rule is not clearly stated in the study 216 protocol.

Randomization was stratified by site of onset, bulbar onset vs limb onset, and balanced by study site and country.

The protocol-specified outcome measures were essentially identical to study 216. The 301 protocol was more precise than the 216 protocol in

the definition of endpoint, clearly stating death, tracheostomy, and intubation followed by tracheostomy as the outcome.

Note that for patients who underwent tracheostomy, the investigator would use clinical judgment to decide whether the patient could remain on double-blind treatment. This had obvious ramifications for the secondary outcome measures i.e. whether muscle or functional scores recorded in tracheotomized patients would be included in analyses. Amendment #2 to the European protocol clarified that only safety data would be collected once tracheostomy was performed.

As in 216, the investigators had access to blood test results during the conduct of the study. Knowing the potential of riluzole to cause ALT elevations, this had the potential to compromise the blind.

By protocol, if the ALT value rose above 3x the upper limit of normal, treatment could continue, but the investigator had to report that patient to the medical monitor, check viral hepatitis serology, and implement more frequent monitoring of ALT thereafter. If the value rose above 5x the upper limit of normal, the patient had to be discontinued.

Statistical Analysis: The protocol states both: 1) "The primary analysis for this study will be the one-sided, stratified log rank test comparing the survival curves between riluzole treatment (50 mg bid) and placebo, for all randomized patients during the 18 month period following their randomization;" and 2) "The detailed formal plan for statistical analysis will be written and filed in the study Central File before unblinding of the study. This plan will provide the framework for the statistical inferences to be drawn from this protocol and will specify in detail both primary and secondary outcome measures."

For the functional evaluations, "a model of analysis of variance will be carried out using the estimates of the individual slopes of the total score versus the time curve. From this model, we will compare slopes between treatment groups. Due to the assumption of linearity of the slopes of the functional scale versus time, we will carry out an analysis taking into account only evaluations under treatment. An intent to treat analysis will also be performed."

The sample size was calculated assuming a 35% survival at 18 months in

the placebo group. With 150 patients per group, the study has a power of 97 to detect a difference of 20% between the placebo and the 50 mg bid group using a one-sided test with $\alpha=5\%$

The original protocol had an expected sample size of 600 patients, but total enrollment was much larger than this without any amendment to the protocol addressing this fact.

Protocol 6 to the European protocol changed the time of final analysis from April 95 to December 94. The protocol states, "The number of patients included in the study was substantially higher than the number planned originally. Therefore it has been decided to advance the time of final analysis. The end of December 1994 corresponds approximately to a follow-up period of 18 months for the first 600 included patients."

An Independent Advisory Board was set up, consisting of neurologists, hepatologists and statisticians, none of whom participated in the conduct of the study. The composition of this board and the rules to be followed by the board were outlined in amendment #3 to the European protocol (amendment #1 to the North American protocol). The board met in August 1993, October 1993, and May 1994 to evaluate unblinded data.

B. Subject Disposition and Baseline Comparison

Study 301 began recruiting patients in Europe in December 1992. Enrollment in the United States began in June 1993. The study was originally designed so that all patient would be entered for at least 18 months. Using this rule, the study would have reached completion in April 1995. However, amendment #6 assigned a cutoff date for the final analysis as December 31, 1994 for the primary efficacy variable and November 15, 1994 for the secondary efficacy variables. Enrollment of 600 patients was planned, but 959 patients at 31 centers were enrolled.

Group	N
Placebo	242
50mg	237
100mg	236
200mg	244
All	959

	Placebo	R 50mg	R 100mg	R 200mg
Male/Female	152/90	144/93	143/93	136 /108
Age (years)	56	57	57	57
Weight (kgs)	68	68	68	67
Duration of Disease (years)	1.8	1.9	1.7	1.8
Vital Capacity Ratio (VCR=pt's VC divided by lower limit normal for VC)	88	89	88	88
Norris Limb Score (normal=63)	44	44	45	46
Norris Bulbar Score (normal=39)	30	32	31	31
Muscle Testing (normal=110)	88	89	89	89

Forty patients enrolled with a positive family history of ALS. These were evenly distributed across treatment groups: 11 placebo, 11 50mg/day, 10 100mg/day, and 8 200mg/day.

The following table lists discontinuations (other than for trach or death) as well as the primary reason for discontinuation in the investigator's judgment.

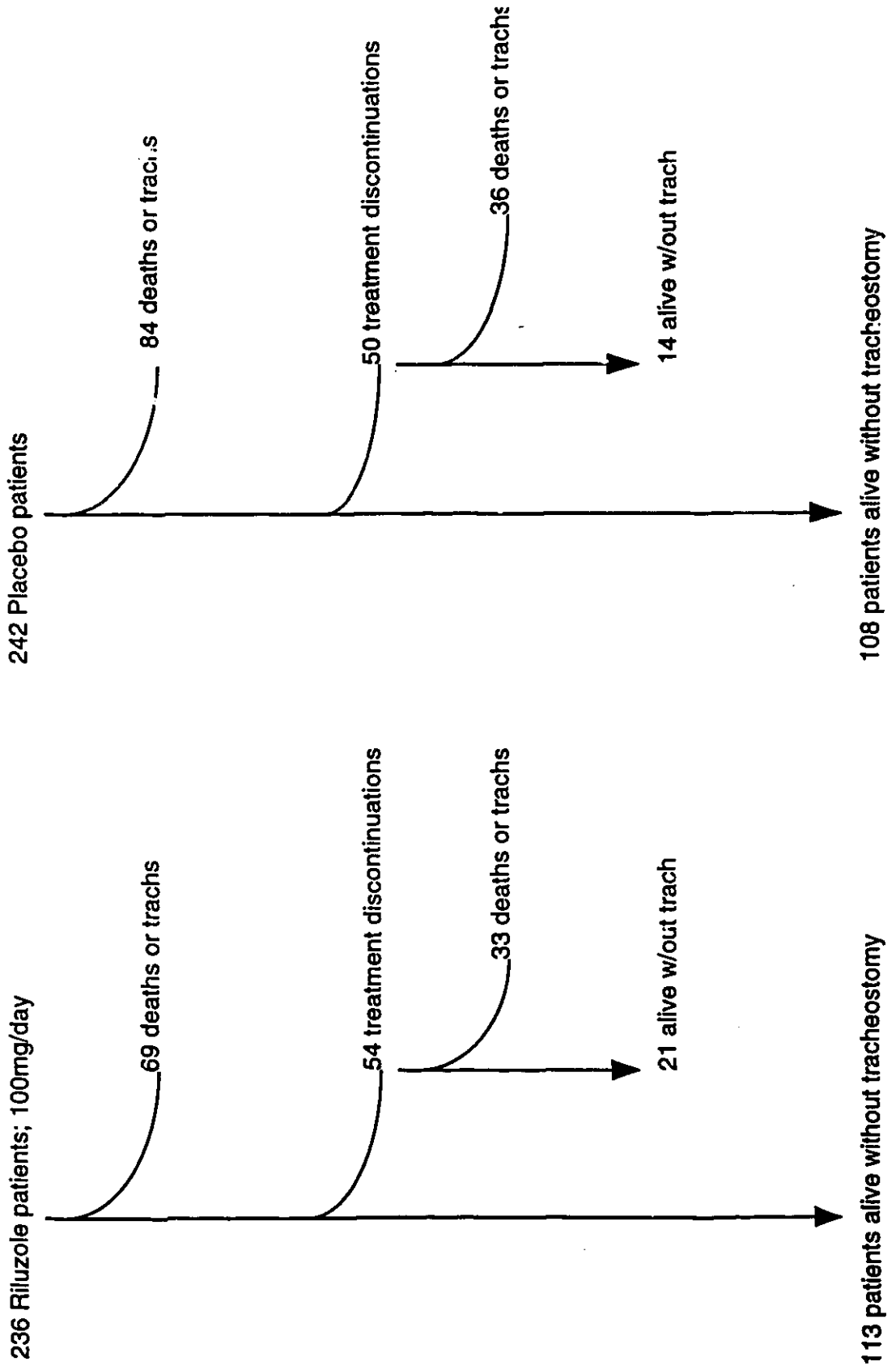
Premature Discontinuations from Treatment

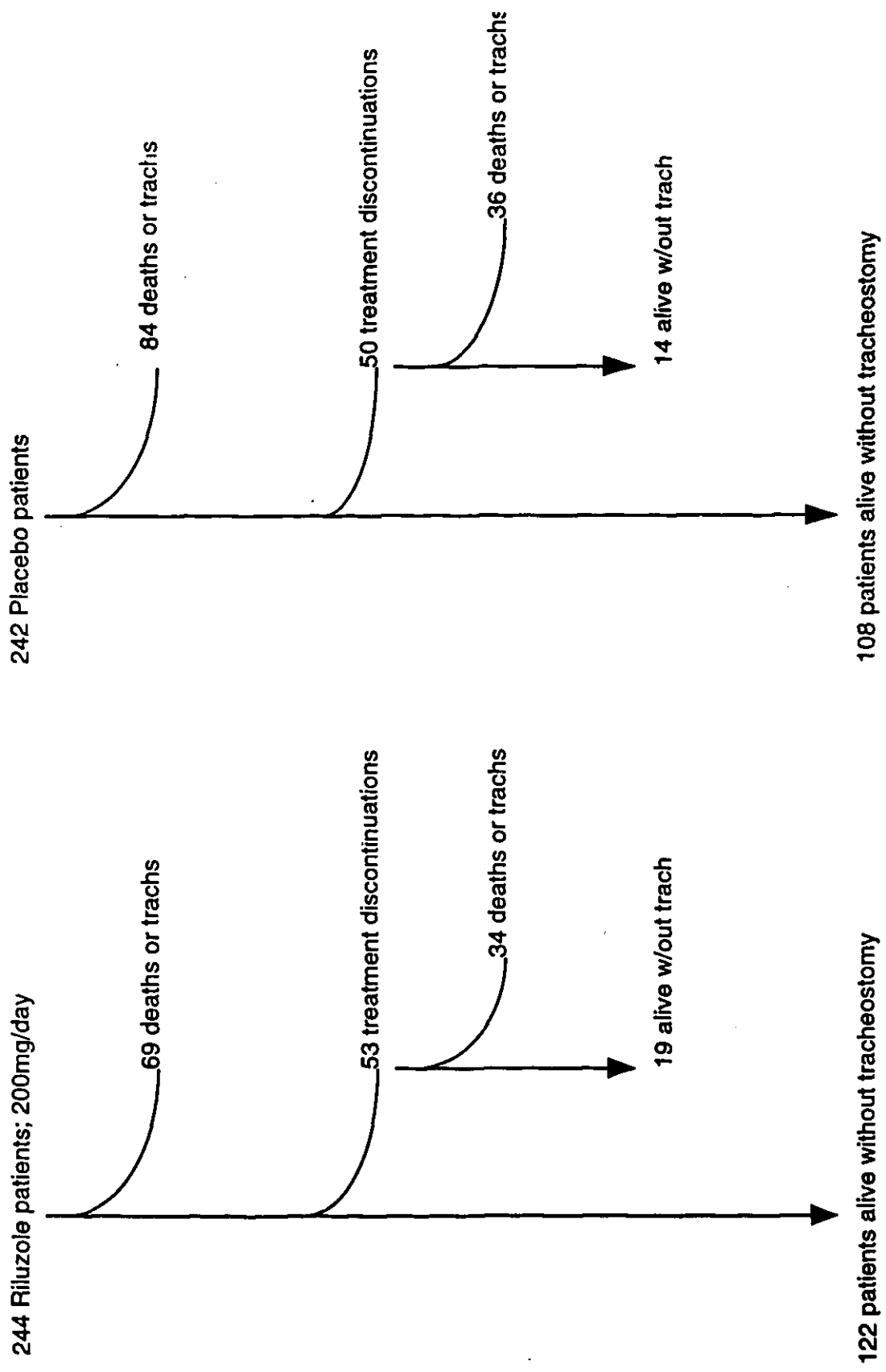
	Placebo N (%)	R 50mg N (%)	R 100mg N (%)	R 200mg N (%)
Adverse Clinical Experience	19 (8)	20 (8)	25 (11)	27 (11)
Adverse Laboratory Experience	8 (3)	3 (1)	9 (4)	9 (4)
Lack of Efficacy	13 (5)	11 (5)	14 (6)	8 (3)
Consent Withdrawn	10 (4)	10 (4)	3 (1)	6 (3)
Other	0	4 (2)	3 (1)	3 (1)

Altogether, only 8 patients were lost to follow-up; their survival status at the end of 18 months, or at the cutoff date December 31, 94, was unknown. They were distributed evenly across treatment groups. Note that one intubated patient was extubated later and, therefore, was not taken into account as a failure in the survival analysis.

A ninth patient was censored for the analyses because he was randomized twice in the study, once at each of two independent centers. Censoring was done for one treatment, for which follow-up lasted less than 1 month.

Flow diagrams for the 100 mg/day group vs placebo and for the 200 mg/day group vs placebo are illustrated on the next two pages. Contrary to the conduct of Study 216, the sponsor has repeatedly assured us that, except for the 8 patients mentioned above, patients who discontinued treatment were routinely followed over time for ascertainment of tracheostomy or death.





C. Efficacy Evaluation

Primary Outcome: The protocol specified endpoint was time to death or tracheostomy, analyzed (according to the statistical analysis plan, amendment #3 to the European protocol and amendment #1 to the N.American protocol) using a stratified logrank test. The comparison 50 mg bid vs placebo was identified in the protocol as the primary outcome comparison. The results for this comparison are presented below. Because the survival curves did not both drop below the 50th percentile, 25% quantile for survival has replaced "median survival in days."

	25% quantile for survival	Logrank Test 2-sided P value	Cox Model 2-sided P value
Riluzole 50 mg bid	351	0.076	0.002
Placebo	264		

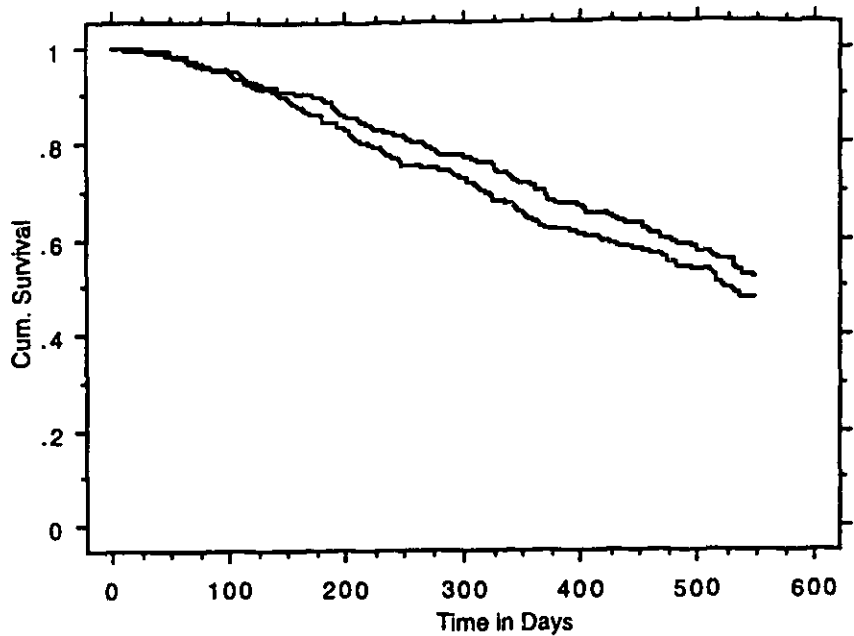
The p value for the Cox model meets the usual standard for statistical significance. The logrank p value approaches statistical significance. A third p value generated by a Wilcoxon test is 0.050, again statistically significant. The effect size was an increase in survival of 3 months at the 25% quantile for survival.

A point to note in the above analysis is that tracheostomy is incorporated into the endpoint variable along with death. Because time of tracheostomy is as much determined by patient and clinician discretion as by the clinical state of the patient, time of tracheostomy has to be considered a possible source of bias. For Study 301, however, a separate analysis using death alone as the endpoint yielded essentially the same results.

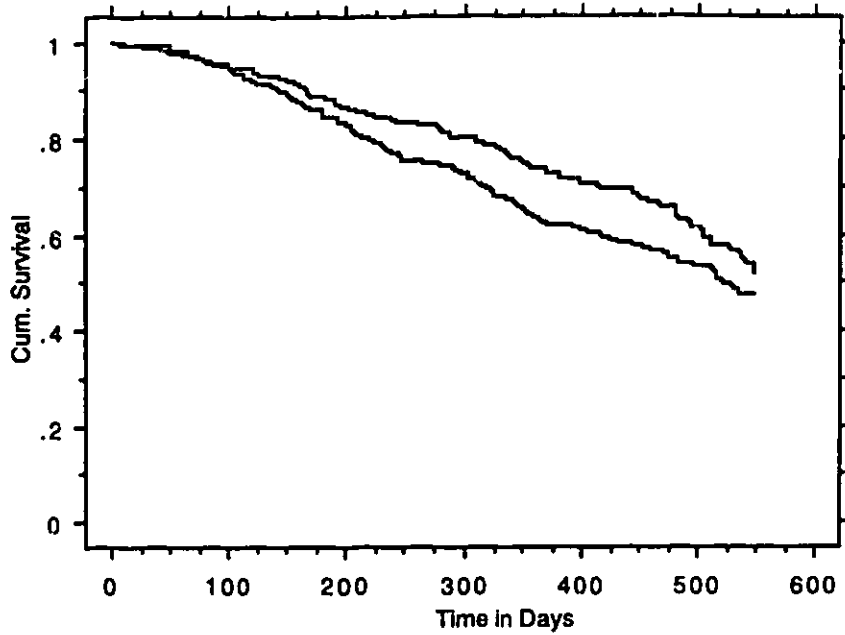
Failures are summarized in the table below. In this table, patients who died after having been trached are counted only in the tracheo/intubation category:

	Placebo	50mg	100mg	200mg
Tracheo/intubation	10	5	12	11
Death	110	101	90	92
Combined Failure	120	106	102	103

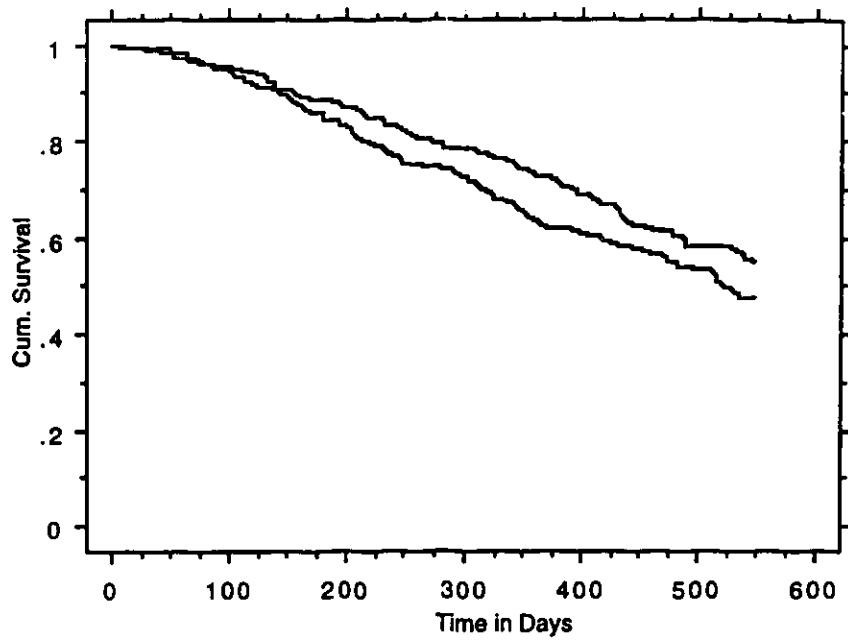
Kaplan-Meier survival curves for the three different dose comparisons are illustrated on the following pages.



For the 50mg/day vs placebo analysis, the 25th percentile survival was 326 days for riluzole vs 264 days for placebo, a difference of 2 months. However, the p value was only 0.25 (stratified logrank).



For the 100mg/day vs placebo analysis, the 25th percentile survival was 351 days for riluzole vs 264 days for placebo, a difference of 3 months. The p value was 0.075 (stratified logrank).



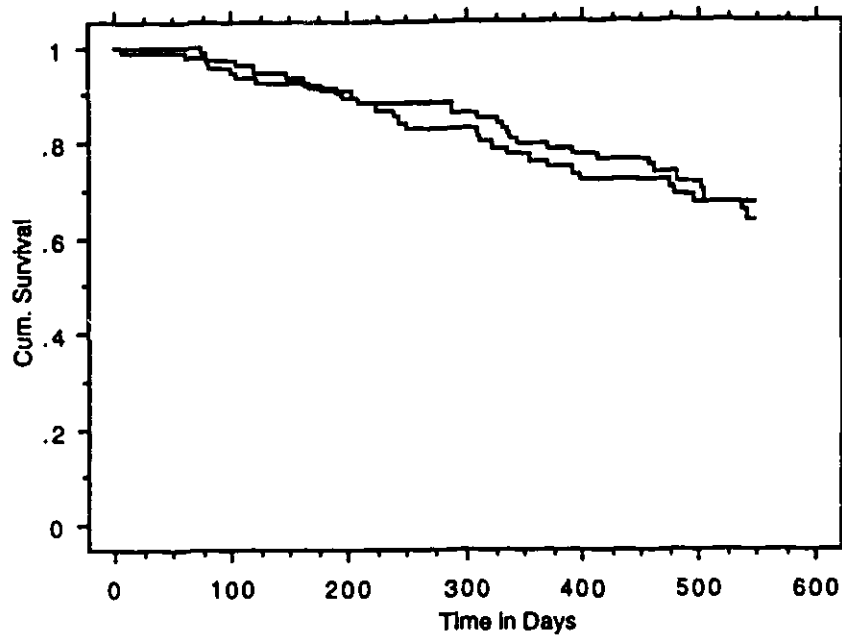
For the 200mg/day vs placebo analysis, the 25th percentile survival was 344 days for riluzole vs 264 days for placebo, a difference of slightly less than 3 months. The p value was 0.075 (stratified logrank).

Interim Analysis: A single interim analysis for efficacy was performed in October 1994 with a cutoff date of August 31, 1994. Only patients who had been on study medication for one year were included and it was based on the binary outcome "failure-free survival at 12 months (yes/no)." A stringent p-value of 0.001 was set for this analysis in order to stop the trial only in case of an overwhelming effect of the drug.

In the riluzole 100mg/day group, 26% of patients failed (50/192) versus 38% in the placebo group (74/195). The p-value was equal to 0.012 (chi-square).

Primary Outcome by Stratum (Bulbar vs Limb Onset): The sponsor provided analyses showing that the effect of riluzole on survival was not different for the two strata, as it was in Study 216.

Primary Outcome by Bulbar vs Limb-Only Involvement at Baseline: Bulbar involvement at baseline was defined by a simple yes-no question in the CRF answered by the investigator. This essentially defined a group of patients with limb-onset disease who, by the time of randomization, still had no bulbar involvement. While the sponsor showed that patients with limb-onset disease contributed to the favorable results in Study 301, we were interested in testing whether patients with limb-only involvement at baseline also contributed to the favorable effect. For the 100mg/day group vs placebo, the results are shown on the next page; no effect is demonstrated in this subgroup.



Limb-Only Involvement at Baseline, 100 mg/day vs placebo.

Secondary Outcomes (Norris Scales): No differences in slope of decline on either the Norris Bulbar Scale or the Norris Limb Scale were seen between treatment groups when all patients, both bulbar-onset and limb-onset, were considered.

No differences in slope of decline on the Norris Bulbar Scale were seen between treatment groups when only bulbar onset patients were considered.

No differences in slope of decline on the Norris Limb Scale were seen between treatment groups when only limb onset patients were considered.

Secondary Outcomes (Muscle Testing): No differences in slope of decline on the Total Muscle Strength Score were seen between treatment groups.

Secondary Outcomes (Pulmonary Function): For VCR, fully 25% of data points are missing for both the riluzole and the placebo groups. The sponsor reports no apparent differences between treatment groups.

Secondary Outcomes (Analogue Scales of Symptoms): The sponsor reports no differences between treatment groups.

Secondary Outcomes (Global Impressions): The sponsor reports no apparent differences between treatment groups.

Gastrostomies: 158 patients had gastrostomies performed during the study. These were distributed equally across treatment groups: 46 placebo, 36 50mg/day, 40 100mg/day, and 36 200mg/day. An additional 8 patients already had gastrostomies upon entering the study. This is summarized below:

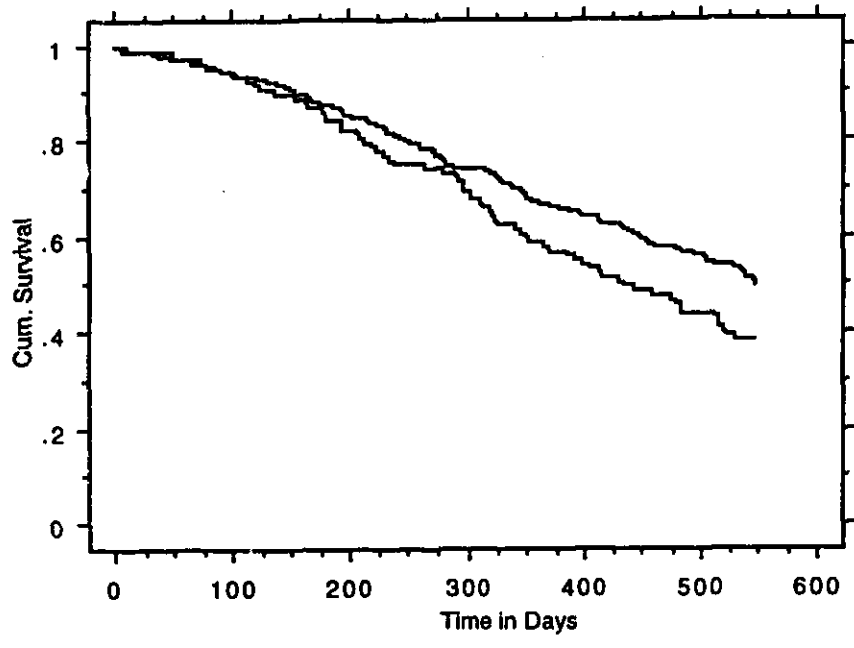
Gastrostomy	Placebo	50 mg	100 mg	200 mg	All
Before study	2	2	1	3	8
During study	46	36	40	36	158 (16%)
Mean time to gastrostomy	251 days	238 days	277 days	226 days	249 days

An analysis was performed by the sponsor which combined gastrostomy with death and tracheostomy into the common survival endpoint. The results of this analysis are essentially the same as when only death and tracheostomy are combined into a common survival endpoint.

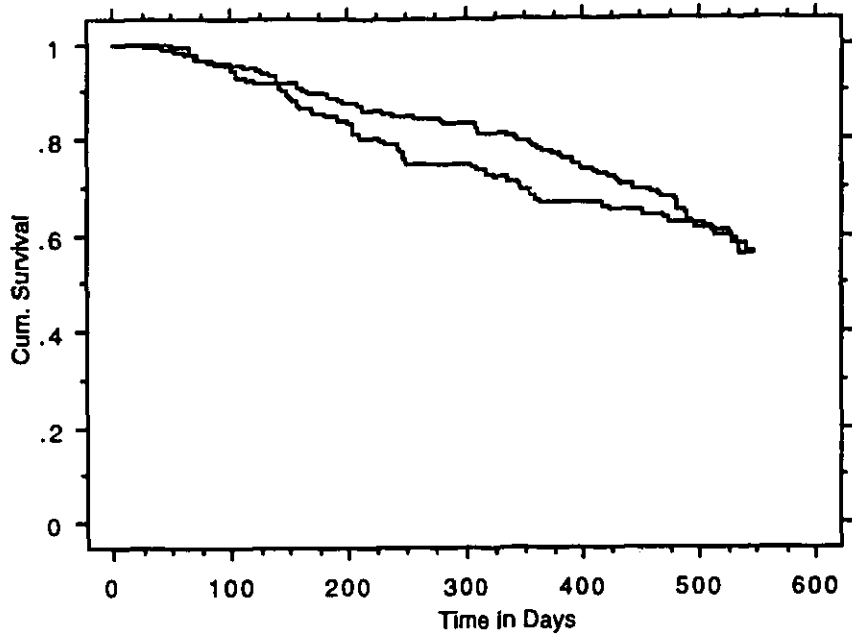
Plasma Concentrations: Amendment #4 to the European protocol (amendment #2 to the North American protocol) outlined a population PK analysis to be added to the study. The minimum number of patients needed for this kinetic analysis was considered to be 300, among them 225 (75%) were expected to be taking riluzole. Four plasma samples per patient were to be taken, 2 on one visit day and 2 on another visit day. The results of this population PK study are not available.

Geographical Interaction: For this exploratory analysis, the 100 mg/day and 200 mg/day groups were combined and then compared to placebo. Study 216, the initial study of the efficacy of riluzole, was primarily a French study. France contributed the bulk of patients to Study 301 (400 out of 960). The results for French centers alone are shown on the next page. Results for all centers outside of France and for North American centers alone are shown on the following pages.

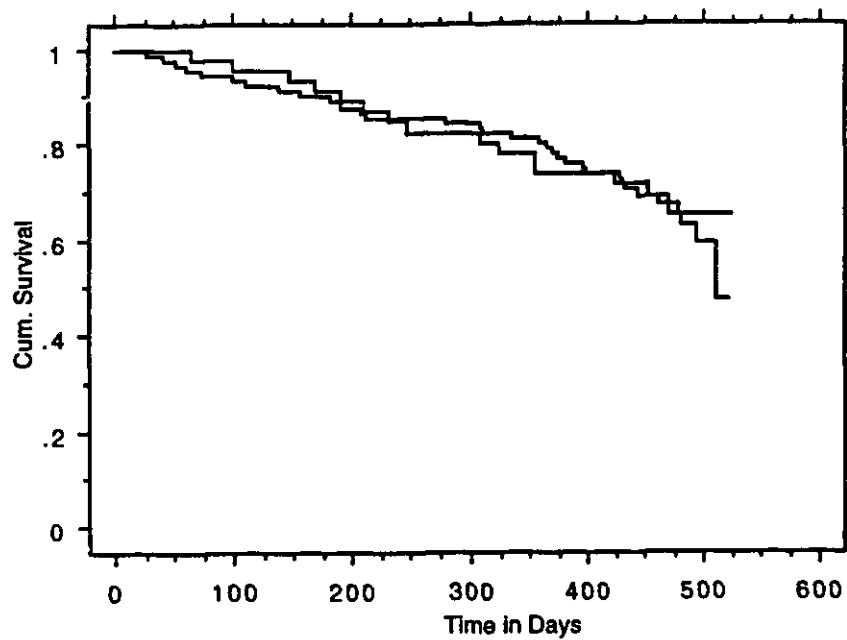
French Centers



All non-French centers



North American centers



Unblinding: Given the potential for riluzole to cause elevations in ALT (SGPT) in a large percentage of patients, the issue of unblinding was explored in Study 216. In that study, when patients without ALT elevations were considered alone, the riluzole-treated group continued to perform better than the placebo-treated group.

In Study 301, investigators again had access to laboratory data during the conduct of the study. Roughly half of all patients in the 100mg and 200mg treatment groups had ALT elevations $> 1.3 \times \text{ULN}$ between days 10-150 (during the first 5 months of the study and excluding baseline). Only about 15% of placebo patients had similar ALT values between days 10-150.

To explore the possible effect of unblinding on the study outcome, I generated Kaplan-Meier curves for the placebo patients without ALT elevations by 5 months and the combined 100- and 200-mg/day group without ALT elevations by 5 months. The curves continued to demonstrate the favorable separation between placebo- and riluzole-treated patients.

Post Hoc Analysis (Regional Spread Within the Body): Given that rate of deterioration in muscle strength and function was not slowed on these scales, Dr. Hoberman and I explored another possible explanation for riluzole's effect. We examined whether riluzole slowed the regional spread of motor neuron involvement. To do this, we identified patients with limb-only involvement at baseline and followed them for time-to-bulbar-involvement. For this purpose, bulbar involvement was defined as a Norris Bulbar Score ≤ 36 . For patients with limb-only involvement at baseline, no difference was seen between treatment groups in time of progression to bulbar involvement.

D. Conclusions

The protocol-specified primary analysis was the stratified logrank test comparing the mid-dose group to placebo. The 2-sided p value for this analysis was 0.076, which does not meet the usual standard for approval of a new drug product.

However, the stratified Wilcoxon 2-sided p value was 0.05. It is also important to note that the Kaplan-Meier curve for each of the 3 dose groups in Study 301 fell above the curve for the placebo group.

The selective effect of riluzole seen in bulbar-onset patients in Study 216 was not seen in Study 301. However, patients with limb-onset disease in Study 301, who had not progressed to bulbar involvement by the time of randomization, showed no effect. Still, in at least one of the studies, either 216 or 301, the effect on survival has been seen in the bulbar-onset subgroup, the limb-onset subgroup, pure bulbar patients at baseline, and patients with limb-only involvement at baseline. That a particular subgroup effect may not have been reproduced in both studies may not be important at this point.

Study 302 (France/Belgium)

Investigators

Center	1	Meininger	n=71
	2	Camu	n=19
	3	Bouche	n=12
	4	Couratier	n=11
	5	Desnuelle	n=11
	6	Viader	n=11
	7	Clanet	n=10
	8	Blin	n=7
	9	Lagueny	n=7
	10	Maloteaux/Delwaide (Belgium)	<u>n=9</u>
	Total		n=168

A. Study Design

This was a multicenter, double-blind, randomized, placebo-controlled, parallel group study in patients who did not satisfy the inclusion criteria for the 301 study. In particular, patients either older than 75 years of age, with a disease duration > 5 years, or with a vital capacity ratio < 60% were enrolled in Study 302. Also eligible for inclusion were patients whose vital capacity could not be measured, patients with stomach tubes, and patients with monoclonal gammopathies of undetermined significance.

The study essentially ran concurrently with Study 301 and had a similar study design as 301. Study 302 LT was the longterm extension of Study 302; it began enrolling patients in July 1994.

Patients were randomized to either placebo or 100mg/day riluzole.

Study 302 was designed as both a safety and efficacy study. However, the preliminary report included in the NDA presents only the serious adverse events from Study 302 and the SAEs that occurred in the 302 LT study prior to January 1995.

B. Subject Disposition and Baseline Comparison

168 patients were enrolled from 9 centers in France and 1 center in Belgium.

The data currently provided do not make it clear what clinical or laboratory parameter made each individual patient ineligible for enrollment in Study 301.

Sponsor's Tables 5, 6, and 7 are included on the following page. Obviously, patients included in Study 302 were, on average, much more advanced in their disease than patients included in Studies 216 and 301. This is reflected in the mean scores for vital capacity ratio, muscle testing scale, and the Norris scales. The overall mortality during the conduct of the study was also much higher than in Studies 216 and 301.

Table 5: Patient characteristics at baseline: Demographics

	Placebo	P. 100 mg	All
ALL PATIENTS COMBINED			
N	86	82	168
SEX M/F	48/38	34/48	82/86
AGE (years)*	62.8 ± 1.4	57.8 ± 1.4	60.4 ± 1.0
RACE (%)			
- Caucasian	98.8	97.6	98.2
- Black	1.2	0	0.6
- Oriental	0	2.4	1.2
- Other	0	0	0
WEIGHT (kg)*	61.8 ± 1.4 (N = 74)	59.7 ± 1.4 (N = 70)	60.8 ± 1.0 (N = 144)

Table 6: Disease features at baseline

	Placebo	R 100 mg	All
ALL PATIENTS COMBINED			
N	86	82	168
ALS diagnosis:			
definite (%)	51 (59.3)	51 (62.2)	102 (60.7)
probable (%)	35 (40.7)	31 (37.8)	66 (39.3)
Form of ALS at onset			
bulbar (%)	26 (30.2)	29 (35.4)	55 (32.7)
limb (%)	60 (69.8)	53 (64.6)	113 (67.3)
Duration of disease (years)*	3.9 ± 0.4	3.4 ± 0.2	3.6 ± 0.2
Familial (%)	6 (7.0)	3 (3.7)	9 (5.4)
Sporadic (%)	80 (93.0)	79 (96.3)	159 (94.6)
Vital capacity ratio*	55.1 ± 2.6 (N = 74)	51.9 ± 3.1 (N = 59)	53.7 ± 2.0 (N = 133)

Table 7: Patient functional characteristics at baseline: Disease severity

	Placebo	R 100 mg	All
ALL PATIENTS COMBINED			
MUSCLE TESTING SCALE* (max. score = 110)	70.3 ± 2.8 (N = 86)	63.8 ± 3.2 (N = 82)	67.1 ± 2.1 (N = 168)
BULBAR NORRIS SCALE* (max. score = 39)	20.5 ± 1.3 (N = 86)	18.9 ± 1.4 (N = 80)	19.7 ± 1.0 (N = 166)
LIMB NORRIS SCALE* (max. score = 63)	28.8 ± 2.2 (N = 86)	26.5 ± 2.1 (N = 82)	27.7 ± 1.5 (N = 168)

The flow diagram on the next page was created by me, utilizing information from the following sources:

1. Sponsor's Table 3: Premature discontinuations from treatment
2. Sponsor's Table 9: Incidence of serious AEs with outcome death
3. Sponsor's Table 10: Deaths on treatment
4. Sponsor's Appendix II, page 71: Deaths after treatment discontinuation

If the flow diagram is correct, the total number of deaths would be 60/82 (73%) in the riluzole group and 62/86 (72%) in the placebo group. Intubation and tracheostomy are not accounted for in the flow diagram at this point in time.

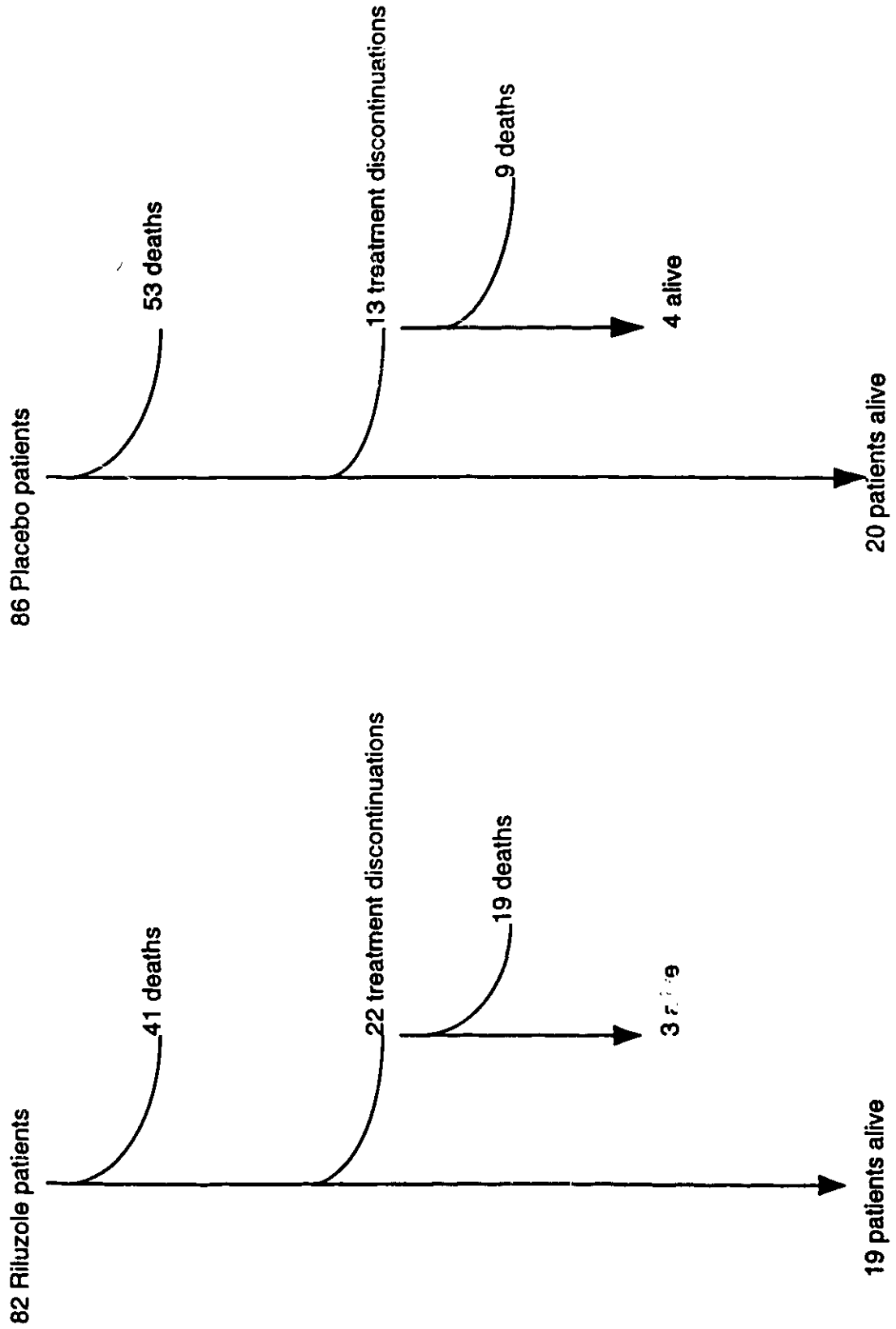
We are told that a total of 20 patients from the riluzole arm of the trial went on to participate in 302LT while 21 placebo patients went on to 302LT.

C. Efficacy Evaluation

The preliminary report of Study 302, submitted in the NDA, does not include an efficacy analysis. From the patient flow diagram above, however, we can see that the proportion of patients alive at study end was the same in both treatment groups, 27-28%. Of course, the distribution of deaths (and trachs) over time may have differed for the two treatment groups, again presenting the situation of survival curves that separate and then come together (crossing hazards). The efficacy data has been requested from the sponsor.

No information on secondary outcome scales is presented in the submitted report.

The serious AEs were comparable in the riluzole and placebo groups during the double-blind phase of the study. In addition, no serious AEs of special concern were reported either during 302 or 302 LT (through January 95).



Study 304 (Japan)

A double-blind, placebo-controlled study of riluzole 50 mg bid vs placebo in ALS patients for 18 months. A total of 200 patients at 42 centers are currently enrolled. Enrollment began in October 1993.

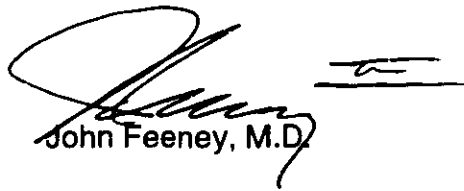
Inclusion/Exclusion criteria are similar to Studies 216 and 301. The primary endpoint was planned to be time to failure where failure was defined as inability to walk alone, loss of upper limb function, trach, intubation, artificial nutrition, or death. To be comparable to studies 216 and 301, this has been changed to time to intubation, trach, or death.

The final analysis is planned for February 1996. Interim analyses were performed in October 1994 and March 1995.

The current submission summarizes only serious AEs reported prior to January 1995. A review of that listing reveals no serious AEs of special concern.

Overall Conclusions

The body of evidence, taken as a whole, supports an effect of riluzole on time-to-tracheostomy or death in patients with ALS. This, in conjunction with a favorable safety profile, warrants the approval of riluzole for marketing in the United States.



John Feeney, M.D.

CC: Orig NDA # 20-599
HFD-120/Div. File
HFD-120/Leber/Katz/Feeney/Higgins

RILUZOLE SAFETY FINDINGS

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Preclinical Safety Profile

In all non-rodent species, emesis was observed.

No consistent changes were induced in hematology parameters in repeated dose toxicity studies in mice, rats, and non-human primates.

Nevertheless, hemolytic anemia was observed in dogs given riluzole up to 30mg/kg/day for 2 weeks. The mechanism of hemolytic anemia in dogs is unknown. Osmotic fragility tests, intra-erythrocytic G-6-PD activity and intra-erythrocytic electrolyte levels were unaltered by treatment.

Inconsistent elevations in liver enzymes and liver weights were observed in repeated dose toxicity studies. However, no clear evidence of hepatotoxicity was observed.

1.0 Exposure

Riluzole has been studied in various neurologic and psychiatric conditions in man since 1984. Forty clinical trials have been conducted in 2423 subjects and patients, of whom 1908 have received riluzole. These studies include 24 Phase 1 trials in healthy subjects, 12 Phase 2 trials in non-ALS patients, and 4 Phase 2/3 trials in patients with ALS. Three of the latter trials have long-term, open-label extensions.

For purposes of the NDA submission, all adverse events are tabulated for ALS trials 216 and 301. All AEs from ALS trials 302 and 304 have not been tabulated at this point in time, although serious AEs are listed for those individual studies.

Only one longterm extension study, Study 216LT, has been fully tabulated for the NDA. The sponsor has chosen to present the safety data from 216LT separate from other studies.

Therefore, 4 populations have been created by the sponsor for presentation of the safety data:

1. Phase 1 trials in healthy subjects; maximum dosing duration 14 days
2. Phase 2 trials in non-ALS patients
3. Studies 216 and 301 in ALS patients, placebo-controlled
4. Study 216LT in ALS patients, open-label

Table :: Study Identifiers used in the

Phase	Text Reference	Protocol No.	Study Design (1)	Dosing regimen	Phase	Text Reference	Protocol No.	Study Design (1)	Dosing regimen
I	Study 01 (2)	PK26124/01	Dose escalation	Single dose	I	Study 406	AG406	Cross over	Single dose
I	Study 02 (2)	PK26124/02	Single treatment	Single dose	I	Study 407	AG407	Cross over	Multiple dose
I	Study 04 (2)	PK26124/04	Single treatment	Single dose	II	Study 001	AG001	Variable dose	Multiple dose
I	Study 05 (2)	PK26124/05	Parallel group	Multiple dose	II	Study 002	AG002	Variable dose	Multiple dose
I	Study 14 (2)	PK26124/14	Dose escalation	Single dose	II	Study 003	AG003	Variable dose	Multiple dose
I	Study 010	AG010	Parallel group	Multiple dose	II	Study 211	AG211	Cross over	Multiple dose
I	Study 011	AG011	Cross over	Single dose	II	Study 213	AG213	Single treatment	Multiple dose
I	Study 015	AG015	Dose escalation	Single dose	II	Study 215	AG215	Single treatment	Multiple dose
I	Study 101 (2)	RFS4274V-101	Parallel group	Single dose	II	Study 401 (2)	AG401	Variable dose	Multiple dose
I	Study 106	AG106	Cross over	Multiple dose	II	Study 402	AG402	Variable dose	Multiple dose
I	Study 114	AG114	Single treatment	Single dose	II	Study 403	AG403	Variable dose	Multiple dose
I	Study 152	NNY152	Single treatment	Single dose	II	Study 404	AG404	Parallel group	Multiple dose
I	Study 156 (2)	NNY156	Cross over	Single dose	II	Study 408 (2)	AG408	Parallel group	Multiple dose
I	Study 157 (2)	NNY157	Parallel group	Multiple dose	II	Study 441 (2)	AG441	Parallel group	Multiple dose
I	Study 158	NNY158	Parallel group	Multiple dose	II/III	Study 216	AG216	Parallel group	Multiple dose
I	Study 159 (2)	RFS4274X-159	Single treatment	Single dose	III	Study 216L(T2)	AG216 LT	Single treatment	Multiple dose
I	Study 160	NNY160	Single treatment	Single dose	III	Study 301	NNY301	Parallel group	Multiple dose
I	Study 161 (2)	RFS4274X-161	Cross over	Single dose	III	Study 301L(T2)	NNY301 LT	Single treatment	Multiple dose
I	Study 201	AG201	Cross over	Multiple dose	III	Study 302(2)	NNY302	Parallel group	Multiple dose
I	Study 202	AG202	Cross over	Single dose	III	Study 302L(T2)	NNY302 LT	Single treatment	Multiple dose
I	Study 203	AG203	Cross over	Multiple dose	III	Study 304(2)	NNY304	Parallel group	Multiple dose
I	Study 405	AG405	Cross over	Multiple dose					

(1) Study design for purposes of the safety discussion. For example, a cross over pharmacokinetic study of the same dose given in two different formulations (Study 152) is a single treatment study for purposes of the safety discussion, since the same dose was given in both arms of the cross over.

(2) Study not included in the (1) Study had a long-term (LT) extension phase which was not treated as a separate protocol.

The proposed labeling recommends a dosing regimen of 50mg bid. From Studies 216 and 301, there are 313 ALS patients who were treated with this dose. From the same studies there are 320 ALS patients who were treated with placebo.

The combined databases represent 14 Phase 1 studies with 238 riluzole-treated subjects, 11 Phase 2 non-ALS studies with 438 riluzole-treated patients.

Dose and duration data for the Phase 1 studies is provided in Sponsor's Tables 5 and 6 from the Integrated Summary of Safety.

Dose and duration data for the Phase 2 studies is provided in Sponsor's Tables 8 and 9 from the ISS.

Sponsor's Table 12 summarizes dose and duration data in ALS patients in Studies 216, 301, and 216LT.

Single doses of riluzole as high as 500mg were tested in Phase 1 studies. In Phase 2 studies, doses as high as 375mg/day were taken.

Riluzole exposure in Phase 1 was for 10 days or less and generally at doses < 200mg. In Phase 2, riluzole exposure was primarily for < 3 months and at doses of 75 and 150mg/day.

The Phase 2/3 ALS safety database represents extensive safety information for approximately 200 to 300 patients at each of the 3 dose levels tested. There are safety data for 15 to 18 months of treatment for about half the riluzole- and placebo- treated populations.

Total Daily Dose	No. of Subjects	% of Total
Single Dose Studies		
TOTAL (1)	121	
Riluzole, 25 - 75 mg	80	66%
Riluzole, 100 mg	68	56%
Riluzole, 150 mg	18	15%
Riluzole, 200 mg	45	37%
Riluzole, 250-500 mg	31	26%
Placebo	56	46%
Multiple Dose Studies		
TOTAL (1)	133	
Riluzole, 25 - 75 mg/day	72	54%
Riluzole, 100 mg/day	34	26%
Riluzole, 150 mg/day	42	32%
Riluzole, 200 mg/day	12	9%
Placebo	58	44%

(1) Subjects may have received both riluzole and placebo in the same crossover study and therefore, may be counted more than once.

Source: Appendices A.1.1.2 and A.1.1.3.

Duration of treatment	Riluzole Total Daily Dose				Total (1)
	25 - 75 mg	100 mg	150- 200 mg	250 - 500 mg	
TOTAL (1)	152	102	117	31	254
Riluzole, single dose	80	68	63	31	120
Riluzole, ≤ 5 days.	30	25	30	0	43
Riluzole, 7 - 10 days.	42	9	24	0	75
Placebo					114

(1) Subjects may have received single and multiple doses, as well as placebo in the same study and therefore, may be counted more than once.

Source: Appendix A.1.1.4.

Total Daily Dose	No. of Patients	% of Total
TOTAL ⁽¹⁾	520	
Riluzole, 25 - 75 mg/day	347	67%
Riluzole, 100 mg/day	93	18%
Riluzole, 150 mg/day	310	60%
Riluzole, 200 mg/day	28	5%
Riluzole, 225 - 375 mg/day	17	3%
Placebo	92	18%

⁽¹⁾ Patients may have received more than one dose level of riluzole, or riluzole and placebo in the same study and therefore, may be counted more than once.

Source: Appendix A.2.2.

Duration of treatment	Riluzole Total Daily Dose				Total ⁽¹⁾
	25 - 75 mg	100 mg	150- 200 mg	225 - 375 mg	
TOTAL ⁽¹⁾	347	93	318	17	520
Riluzole, $\leq$ 1 mo.	304	89	177	12	356
Riluzole, 3 mo.	38	3	129	5	175
Riluzole, 6 mo.	2	1	14	0	17
Riluzole, 9 mo.	3	0	5	0	8
Placebo					92

⁽¹⁾ Patients treated at different dose levels for different durations may be counted more than once.

Source: Appendix A.2.3.

Table 12: Duration of dosing for dose levels administered in Phase II/III ALS studies Studies 216, 216LT, 301 ⁽¹⁾ Number of Patients					
Duration of Treatment	Riluzole Total Daily Dose			ALL Riluzole ⁽²⁾	Placebo
	50 mg/day	100 mg/day	200 mg/day		
TOTAL	237	342	244	823	320
≤ 1 mo.	9	5	7	21	3
3 mo.	23	43	24	90	37
6 mo.	15	28	16	59	44
9 mo.	20	30	18	68	32
12 mo.	17	27	17	61	19
15 - 18 mo. ⁽³⁾	149	168	157	474	171
21 mo.	4	9	4	17	13
24 mo.	0	8	1	9	0
27 mo.	0	4	0	4	0
30 mo.	0	0	0	0	1
> 30 mo.	0	20	0	20	0

⁽¹⁾ Cutoff date for Study 216LT: 31 Dec 1993.

⁽²⁾ Includes 29 patients who received placebo in Study 216 and who then received riluzole, 100 mg/day, in Study 216LT; these patients are counted in both treatment groups.

⁽³⁾ This range covers the duration of dosing around the 31 Dec 1994 cutoff for Study 301.

Source: Appendix A.3.1.1

2.0 Deaths

ALS is a uniformly fatal disease. Death was a common outcome for patients in the Phase 2/3 ALS studies. In Study 216, all but 4 deaths were unrelated to test drug according to the investigators. For 2 patients in the riluzole group and for 2 patients in the placebo group, the cause of death was thought to be test-drug-related: respiratory or cardiac causes in the riluzole group and increased AST/ALT or pulmonary embolus in the placebo group.

In Study 301, 12 deaths were deemed "remotely" related to study treatment by the investigator: 2 placebo pts, 3-50mg/day pts, 2-100mg/day pts, and 5-200mg/day pts. In no cases were deaths deemed "possibly" or "probably" related to study treatment by the investigator.

Additionally, for Study 301, a list of cases where death was not attributed to pulmonary or ALS-related causes is provided by the sponsor. It includes 20 placebo patients, 12-50mg/day pts, 11-100mg/day pts, and 9-200mg/day pts. The diagnoses include accidental death, suicides, sudden deaths, cardiac arrest, cerebral hemorrhage, and pulmonary embolism.

Sponsor's Table 41 which follows tabulates all deaths not clearly related to ALS from combined Studies 216 and 301.

Table 41: Cause of death in Phase II/III ALS studies - Deaths not clearly related to ALS Studies 216 and 301		
Treatment	Patient No. (1)	Cause of death
Riluzole 50 mg/day		
Riluzole 100 mg/day		
Riluzole 200 mg/day		
Placebo		

(1) Patient numbers beginning with "00" are from Study 216. Five digit patient number beginning with a "1" or a "2" are from Study 301.

(2) This patient discontinued treatment because of increased transaminases and died one month later. Transaminases did not normalize during that interval and so the AE outcome was recorded as death. No other cause of death was indicated.

Source: CMRs for Studies 216, 301.

3.0 Assessment of Dropouts

One subject discontinued from Phase 1 studies because of ECG abnormality. The pt developed PVCs and left posterior fascicular block noted on the ECG performed 20 minutes after riluzole administration. The ECG abnormalities, which were judged to be of unknown relationship to study drug by the investigator, persisted at evaluations performed 10 hours and 2 days after study drug administration.

In 11 Phase 2 non-ALS studies, a total of 39 pts had AEs leading to treatment discontinuation. Riluzole was associated with AEs leading to treatment discontinuation more frequently than was placebo, although there was no single AE leading to treatment discontinuation that was reported with frequency $\geq 1\%$

Sponsor's Table 44 presents the treatment discontinuations for AEs from Studies 216 and 301. In the combined 216/301 database, the breakdown of the 107 (13%) of AEs that led to treatment discontinuation for riluzole pts favored the categories body as a whole, digestive, respiratory and nervous.

Table 44: Treatment discontinuation for Adverse Experiences in Phase II/III ALS studies Studies 216 and 301 Number of patients discontinued (% of Total)					
	Riluzole Total Daily Dose			ALL Riluzole	Placebo
	50 mg/day	100 mg/day	200 mg/day		
Total Patients	237	313	244	794	320
No. discontinued for AEs (%)	23 (10%)	53 (17%)	36 (15%)	112 (14%)	36 (11%)
STUDY					
216		19		19	9
301	23	34	36	93	27

Source: CMRs for Studies 216 and 301.

4.0 ADR Incidence Tables

4.1 Incidence in Controlled Clinical Trials in ALS Patients

Sponsor's Table 1 includes ALS patients from Studies 216/301 who received 100mg/day. All AEs are listed where the frequency is greater than or equal to the frequency in the placebo treatment group. AEs are listed in decreasing order of frequency within each body system.

Table I: Adverse Experiences for which the frequency in the 100 mg/day treatment group is greater than or equal to the frequency in the placebo treatment group.

Phase II/III ALS studies

Studies 216 and 301

% of patients reporting

COSTART term	Riluzole 100 mg/d	Pla- cebo	COSTART term	Riluzole 100 mg/d	Pla- cebo	COSTART term	Riluzole 100 mg/d	Pla- cebo
	% of pa- tients	% of pa- tients		% of pa- tients	% of pa- tients		% of pa- tients	% of pa- tients
Body as a Whole			Cardiovascular			Digestive System		
Asthenia	19.2	12.2	Hypertension	5.1	4.1	Nausea	16.3	10.6
Headache	7.3	6.6	Tachycardia	2.6	1.3	Vomiting	4.2	1.6
Abdominal pain	5.1	3.8	Pulmonary embolus	1.6	1.3	Flatulence	2.6	1.9
Pain	5.1	2.2	Phlebitis	1.0	0.3	Esophagitis	1.6	0.9
Back pain	3.5	2.5	Atrial fibrillation	0.3	0.3	Colitis	1.3	0.3
Infection	1.6	1.6	Heart failure	0.3	0.3	Tooth disorder	1.0	0.3
Aggravation reaction	1.3	0.9	Migraine	0.3	0.3	Stomatitis	1.0	0
Chest pain	1.3	1.3	Peripheral vasc. disease	0.3	0.3	Fecal impaction	0.6	0.6
Neck pain	1.3	1.3	Thrombophlebitis	0.3	0.3	G.I. disorder	0.6	0.6
Neck rigidity	1.0	0.3	Embolus lower extrem.	0.3	0	Increased appetite	0.6	0.3
Face edema	0.6	0				Oral moniliasis	0.6	0.3
Hernia	0.6	0				Gastroenteritis	0.3	0.3
Malaise	0.6	0				Melena	0.3	0.3
Pelvic pain	0.6	0				Mouth ulceration	0.3	0.3
Body odor	0.3	0.3				Rectal disorder	0.3	0.3
Chills	0.3	0.3				Esophageal stenosis	0.3	0
Photosensitivity rxn	0.3	0.3				Fecal incontinence	0.3	0
Abscess	0.3	0				Gastritis	0.3	0
Acrodynia	0.3	0				Glossitis	0.3	0
Allergic reaction	0.3	0				Hematemesis	0.3	0
Injection site reaction	0.3	0				Pancreatitis	0.3	0
Overdose (lithium)	0.3	0				Peptic ulcer	0.3	0
						Salivary gland enlarged	0.3	0
						Tongue discoloration	0.3	0

Source: Appendices A.3.1.4 and A.3.1.15.

Table is continued...

Table 1: Adverse Experiences for which the frequency in the 100 mg/day treatment group is greater than or equal to the frequency in the placebo treatment group.
Phase II/III ALS studies
Studies 216 and 301
% of patients reporting

COSTART term	Rilu- zole 100 mg/d	Pla- cebo	COSTART term	Rilu- zole 100 mg/d	Pla- cebo	COSTART term	Rilu- zole 100 mg/d	Pla- cebo
	% of pa- tients	% of pa- tients		% of pa- tients	% of pa- tients		% of pa- tients	% of pa- tients
Endocrine System			Nervous System			Respiratory System		
Thyroid neoplasia	0.3	0	Hypertonia	6.1	5.9	Lung function decrease	10.2	9.7
			Dizziness	3.8	2.5	Rhinitis	6.7	6.3
Hemic & Lymphatic			Dry mouth	3.5	3.4	Cough increased	2.6	1.6
Echymosis	0.6	0	Somnolence	1.9	1.3	Laryngismus	1.0	0.9
Dir. Coombs Test (+)	0.3	0	Vertigo	1.9	0.9	Sinusitis	1.0	0.9
Leukopenia	0.3	0	Nervousness	1.6	1.6	Epistaxis	0.6	0.6
Lymphadenopathy	0.3	0	Circumoral paresthesia	1.6	0	Hiccups	0.6	0.3
			Neuralgia	1.0	0.9	Aspiration pneumonia	0.3	0.3
Metabolic/Nutritional			Twitching	1.0	0.3	Hemoptysis	0.3	0
Weight loss	4.8	4.7	Vasodilatation	0.6	0.3	Hypoxia	0.3	0
Peripheral edema	3.2	2.2	Ataxia	0.3	0.3	Laryngitis	0.3	0
ALT increased	1.9	0.9	Convulsion	0.3	0.3	Pleural effusion	0.3	0
AST increased	1.6	0.3	Tremor	0.3	0.3			
Edema	0.3	0	Abnormal gait	0.3	0	Skin & Appendages		
γ-globulin increased	0.3	0	Amnesia	0.3	0	Pruritus	3.8	3.4
LDH increased	0.3	0	Coma	0.3	0	Eczema	1.6	0.6
Thirst	0.3	0	Emotional lability	0.3	0	Alopecia	1.0	0.6
			Facial paralysis	0.3	0	Nail disorder	1.0	0
Musculoskeletal			Myoclonus	0.3	0	Skin ulcer	0.6	0.6
Arthralgia	3.5	3.4				Exfoliative dermatitis	0.6	0
Bursitis	1.0	0.3				Herpes zoster	0.6	0
Arthritis	0.6	0.3				Psoriasis	0.6	0
Bone pain	0.3	0.3				Dry skin	0.3	0.3
Generalized spasm	0.3	0				Urticaria	0.3	0.3
Tetany	0.3	0				Acne	0.3	0
						Angioedema	0.3	0
						Erythema multiforme	0.3	0

Source: Appendices A.3.1.4 and A.3.1.15.

Table is continued ...

Table I: Adverse Experiences for which the frequency in the 100 mg/day treatment group is greater than or equal to the frequency in the placebo treatment group.
Phase II/III ALS studies
Studies 216 and 301
% of patients reporting

COSTART term	Rilu- zole 100 mg/d	Pla- cebo	COSTART term	Rilu- zole 100 mg/d	Pla- cebo
	% of pa- tients	% of pa- tients		% of pa- tients	% of pa- tients
Special Senses			Urogenital System		
Otitis media	1.0	0.6	Urinary tract infection	3.2	2.2
Taste perversion	1.0	0.6	Urinary frequency	1.3	0.9
Amblyopia	0.3	0	Dysuria	1.0	0.3
Cataract	0.3	0	Kidney calculus	0.6	0.3
Deafness	0.3	0	Hematuria	0.3	0.3
Ear pain	0.3	0	Amenorrhea	0.3	0
Lacrimation disorder	0.3	0	Breast pain	0.3	0
Ophthalmitis	0.3	0	Impotence	0.3	0
			Kidney pain	0.3	0
			Urination impaired	0.3	0

Source: Appendices A.3.1.4 and A.3.1.15.

4.2 Adverse Events Commonly Observed in Controlled Clinical Trials in ALS Patients

The most frequent AEs seen in the combined 216/301 database are shown in Sponsor's Table 35. AEs are shown wherever the frequency of occurrence was $\geq 5\%$ at any dose.

Table 35: Adverse Experiences: Phase II/III ALS studies Studies 216 and 301 Number of patients reporting (% of Total) with Frequency $\geq 5\%$ at any dose					
	Riluzole Total Daily Dose			ALL Riluzole	Placebo
	50 mg/day	100 mg/day	200 mg/day		
Total Patients	237	313	244	794	320
No. with AEs (%)	207 (87%)	287 (92%)	223 (91%)	717 (90%)	288 (90%)
COSTART term					
Death	77 (32%)	87 (28%)	72 (30%)	236 (31%)	110 (34%)
Asthenia	35 (15%)	60 (19%)	49 (20%)	144 (18%)	39 (12%)
Dysphagia	41 (17%)	49 (16%)	41 (17%)	131 (17%)	54 (17%)
Nausea	29 (12%)	51 (16%)	50 (20%)	130 (16%)	34 (11%)
Bronchitis	39 (16%)	43 (14%)	34 (14%)	116 (15%)	47 (15%)
Lung function decrease ⁽¹⁾	31 (13%)	32 (10%)	39 (16%)	102 (13%)	31 (10%)
Accidental Injury	37 (16%)	32 (10%)	28 (11%)	97 (12%)	36 (11%)
Constipation	26 (11%)	30 (10%)	21 (9%)	77 (10%)	34 (11%)
Apnea	22 (9%)	25 (8%)	19 (8%)	66 (8%)	30 (9%)
Respiratory disorder	14 (6%)	38 (12%)	10 (4%)	62 (8%)	44 (14%)
Rhinitis	21 (9%)	21 (7%)	19 (8%)	61 (8%)	20 (6%)
Headache	19 (8%)	23 (7%)	17 (7%)	59 (8%)	21 (7%)
Dizziness	12 (5%)	12 (4%)	31 (13%)	55 (7%)	8 (3%)
Abdominal Pain	16 (7%)	16 (5%)	19 (8%)	51 (6%)	12 (4%)
Hypertonia	14 (6%)	19 (6%)	13 (5%)	46 (6%)	19 (6%)
Diarrhea	14 (6%)	9 (3%)	22 (9%)	45 (6%)	11 (3%)
Dyspnea	14 (6%)	14 (4%)	17 (7%)	45 (6%)	22 (7%)
Pneumonia	15 (6%)	11 (4%)	18 (7%)	44 (6%)	13 (4%)
Anorexia	9 (4%)	10 (3%)	21 (9%)	40 (5%)	12 (4%)
Hypertension	16 (7%)	16 (5%)	8 (3%)	40 (5%)	13 (4%)
Depression	10 (4%)	14 (4%)	15 (6%)	39 (5%)	16 (5%)
Dyspepsia	6 (3%)	12 (4%)	15 (6%)	33 (4%)	16 (5%)
Pain	8 (3%)	16 (5%)	6 (2%)	30 (4%)	7 (2%)
Arthralgia	12 (5%)	11 (4%)	4 (2%)	27 (3%)	11 (3%)

⁽¹⁾ Includes the more commonly used terms: respiratory decompensation / distress / insufficiency (with or without intubation) and tracheostomy.

Source: Appendix A.3.1.4.

Sponsor's Table 38 shows AEs wherever the frequency in any riluzole treatment group was more than 3 percentage points greater than in the placebo treatment group. Those that appeared to demonstrate a dose-response relationship are: asthenia, nausea, vomiting, vertigo, somnolence, and circumoral paresthesia.

Table 38: Adverse Experiences for which the frequency in any Riluzole-treatment group is ≥ 3 percentage points greater than in the Placebo-treatment group: Phase II/III ALS studies Studies 216 and 301 Number of patients reporting (% of Total)					
	Riluzole Total Daily Dose			ALL	Placebo
	50 mg/day	100 mg/day	200 mg/day	Riluzole	
Total Patients	237	313	244	794	320
No. with AEs (%)	207 (87%)	287 (92%)	223 (91%)	717 (90%)	288 (90%)
COSTART term					
Asthenia ⁽¹⁾	35 (15%)	60 (19%)	49 (20%)	144 (18%)	39 (12%)
Nausea ⁽¹⁾	29 (12%)	51 (16%)	50 (20%)	130 (16%)	34 (11%)
Lung function decrease ⁽²⁾	31 (13%)	32 (10%)	39 (16%)	102 (13%)	31 (10%)
Accidental injury ⁽³⁾	37 (16%)	32 (10%)	28 (11%)	97 (12%)	36 (11%)
Dizziness	12 (5%)	12 (4%)	31 (13%)	55 (7%)	8 (3%)
Abdominal pain	16 (7%)	16 (5%)	19 (8%)	51 (6%)	12 (4%)
Hypertension	16 (7%)	16 (5%)	8 (3%)	46 (5%)	13 (4%)
Diarrhea	14 (6%)	9 (3%)	22 (9%)	45 (6%)	11 (3%)
Pneumonia	15 (6%)	11 (4%)	18 (7%)	44 (6%)	13 (4%)
Anorexia	9 (4%)	10 (3%)	21 (9%)	40 (5%)	12 (4%)
Vomiting ⁽¹⁾	10 (4%)	13 (4%)	11 (5%)	34 (4%)	5 (2%)
Vertigo ⁽¹⁾	8 (3%)	6 (2%)	11 (5%)	23 (3%)	3 (1%)
Somnolence ⁽¹⁾	2 (1%)	6 (2%)	10 (4%)	18 (2%)	4 (1%)
Circumoral paresthesia ⁽¹⁾	3 (1%)	5 (2%)	8 (3%)	16 (2%)	0 (0%)

⁽¹⁾ Frequency shows a dose-response relationship.

⁽²⁾ Includes the more commonly used terms: respiratory decompensation / distress / insufficiency (with or without intubation) and tracheostomy.

⁽³⁾ Includes fractures and bruises secondary to falling down.

Source: Appendix A.3.1.4.

4.3 Other Events Observed During the Pre-Marketing Evaluation of Riluzole

The most frequent Phase 1 AEs are shown in Sponsor's Table 31 that follows. The dose-response relationship of dizziness and vertigo is obvious. Also note the conspicuous frequency of paresthesia and circumoral paresthesia at doses > 200mg/day.

Table 31: Adverse Experiences: Phase I studies. AEs with Frequency $\geq$ 5% at any dose Number of subjects reporting (% of Total)							
	Riluzole Total Daily Dose (mg)					ALL	Placebo
	25 - 75	100	150	200	250 - 500	Riluzole	
Total Subjects (1)	152	102	60	57	31	238	114
No. with AEs(%)	55 (36%)	45 (44%)	26 (43%)	33 (58%)	22 (71%)	124 (52%)	34 (30%)
COSTART term							
Headache	13 (9%)	12 (12%)	9 (15%)	4 (7%)	5 (16%)	38 (16%)	7 (6%)
Asthenia	12 (8%)	6 (6%)	4 (7%)	11 (19%)	9 (29%)	36 (15%)	3 (3%)
Somnolence	13 (9%)	11 (11%)	2 (3%)	13 (23%)	2 (6%)	31 (13%)	9 (8%)
Dizziness	5 (3%)	7 (7%)	6 (10%)	12 (21%)	6 (19%)	27 (11%)	6 (5%)
Vertigo	0	0	0	0	13 (42%)	13 (5%)	0
Thinking abnormal	3 (2%)	2 (2%)	1 (2%)	2 (4%)	3 (10%)	10 (4%)	4 (4%)
Nausea	2 (1%)	0	3 (5%)	2 (4%)	2 (6%)	9 (4%)	1 (1%)
Paresthesia	3 (2%)	1 (1%)	2 (3%)	0	3 (10%)	8 (3%)	0
Circumoral paresthesia	2 (1%)	0	0	0	6 (19%)	7 (3%)	0
Euphoria	0	0	1 (2%)	0	2 (6%)	3 (1%)	0
Reflexes increased	0	0	0	0	3 (10%)	3 (1%)	0
Hypotonia	0	0	0	0	2 (6%)	2 (1%)	0

(1) Subjects in crossover studies are counted in all dose levels at which they were treated.

Source: Appendices A.1.1.9 and A.1.1.10.

The most frequent Phase 2, non-ALS AEs are shown in Sponsor's Table 33 that follows. Dizziness and vertigo do not appear on this list, while paresthesia were seen in only one patient (representing 6% of patients dosed at > 200mg/day). Anxiety and somnolence were more likely to be seen among riluzole-treated pts in this primarily psychiatric population. Dose-response relationship cannot be reliably assessed due to the small numbers of patients.

Table 33: Adverse Experiences: Phase II non-ALS studies. AEs with Frequency $\geq$ 5% for any treatment Number of patients reporting (% of Total)							
	Riluzole Total Daily Dose (mg/day)					ALL	
	25 - 75	100	150	200	250 - 500	Riluzole	Placebo
Total Patients ⁽¹⁾	347	93	310	28	17	438	92
No. with AEs (%)	85 (24%)	14 (15%)	141 (45%)	8 (29%)	4 (24%)	207 (47%)	52 (57%)
COSTART term							
Insomnia	23 (7%)	0	38 (12%)	0	0	51 (12%)	24 (26%)
Anxiety	14 (4%)	0	27 (9%)	0	0	35 (8%)	3 (3%)
Agitation	5 (1%)	0	16 (5%)	0	0	19 (4%)	7 (8%)
Somnolence	6 (2%)	4 (4%)	9 (3%)	0	1 (6%)	18 (4%)	1 (1%)
Hostility	3 (1%)	0	13 (4%)	0	0	16 (4%)	11 (12%)
Asthenia	3 (1%)	0	10 (3%)	0	1 (6%)	13 (3%)	2 (2%)
Abdominal pain	3 (1%)	0	6 (2%)	0	1 (6%)	10 (2%)	0
Personality disorder	2 (1%)	0	8 (3%)	0	0	10 (2%)	8 (9%)
Hypotension	2 (1%)	0	4 (1%)	2 (7%)	0	7 (2%)	0
Arrhythmia	1 (.3%)	0	1 (.3%)	2 (7%)	0	4 (1%)	0
GGT increased	0	0	2 (1%)	2 (7%)	0	4 (1%)	0
Depression	1 (.3%)	0	2 (1%)	0	1 (6%)	4 (1%)	0
Incoordination	2 (1%)	1 (1%)	1 (.3%)	0	1 (6%)	4 (1%)	0
Paresthesia	2 (1%)	0	0	0	1 (6%)	3 (1%)	0
Eye disorder	1 (.3%)	0	0	0	1 (6%)	2 (.5%)	0

⁽¹⁾ Patients in crossover studies are counted in all dose levels at which they were treated.

Source: Appendices A.2.7 and A.2.8.

5.0 Other Safety Findings

5.1 Laboratory Findings

The information that follows is taken from the combined ALS studies. On page 99 of the ISS, the sponsor makes the case for not analyzing means for laboratory parameters in the Phase 2/3 ALS studies because of the large number of normal ranges applied to lab evaluations in these multicenter trials. Instead, the sponsor has chosen to present the percentages of pts with lab values above and below the "limit of normal." Presumably, pts' lab values were compared to the normal ranges at their own centers.

Hematology

Sponsor's Table 54 summarizes the % of pts with hematology values below the LLN at any time during the study, regardless of baseline value.

For platelet counts, no differences between riluzole and placebo emerged. For leukocyte counts, no differences between riluzole and placebo emerged.

However, the frequency with which Hgb, Hct, and erythrocyte counts fell below the LLN was greater in riluzole treated pts, compared to placebo pts. Further, the frequency with which low values were observed for these 3 parameters showed a dose-response relationship.

During the trials, investigators had the opportunity to classify abnormal lab values as laboratory adverse events. No strict rules were in place to guide this decision. Sponsor's Table 56 summarizes the frequencies of laboratory AEs as coded by the investigators.

For platelet counts, no differences between riluzole and placebo emerged.

For leukocyte counts, no differences between the combined riluzole groups and placebo emerged, but the high-dose riluzole group showed 7% vs the placebo 3%

For Hgb, Hct, and erythrocyte counts, no differences between the combined riluzole groups and placebo emerged, but the high-dose riluzole group showed 5-7% vs the placebo 2-3%

Table 54: Frequency of Abnormal Laboratory Values in Phase II/III ALS studies: Hematology
Number of patients (% of Total) with any result
Studies 216 and 301
BELOW the Lower Limit of the Normal Range

	Riluzole Total Daily Dose			ALL Riluzole	Placebo
	50 mg/day	100 mg/day	200 mg/day		
TOTAL	237 (%)	313 (%)	244 (%)	794 (%)	320 (%)
HEMATOLOGY					
Hemoglobin	80 (34%)	103 (33%)	136 (56%)	319 (40%)	67 (21%)
Hematocrit	46 (19%)	70 (22%)	77 (32%)	193 (24%)	42 (13%)
Erythrocytes	96 (41%)	146 (47%)	147 (60%)	380 (49%)	94 (29%)
Leukocytes	20 (8%)	40 (13%)	26 (11%)	86 (11%)	42 (13%)
Neutrophils	4 (2%)	11 (4%)	8 (3%)	23 (3%)	9 (3%)
Lymphocytes	7 (3%)	22 (7%)	10 (4%)	39 (5%)	33 (10%)
Platelets	11 (5%)	17 (5%)	13 (5%)	41 (5%)	14 (4%)

Source: CMRs for Studies 216, 301.

Table 56: Frequency of Adverse Laboratory Experiences in Phase II/III ALS studies
Hematology
Studies 216 and 301
Number of patients (% of Total)

	Riluzole Total Daily Dose			ALL Riluzole	Placebo
	50 mg/day	100 mg/day	200 mg/day		
Total Patients	237	313	244	794	320
No. with AEs (%)	69 (29%)	128 (41%)	109 (45%)	306 (39%)	69 (22%)
HEMATOLOGY					
Hemoglobin	6 (3%)	11 (4%)	18 (7%)	35 (4%)	9 (3%)
Hematocrit	1 (.4%)	7 (.2%)	13 (5%)	21 (3%)	5 (2%)
Erythrocytes	7 (3%)	15 (5%)	16 (7%)	38 (5%)	9 (3%)
Leukocytes	8 (3%)	9 (3%)	17 (7%)	34 (4%)	8 (3%)
Neutrophils	5 (2%)	6 (2%)	7 (3%)	17 (2%)	4 (1%)
Lymphocytes	9 (4%)	7 (2%)	5 (2%)	21 (3%)	4 (1%)
Monocytes	4 (2%)	1 (.3%)	2 (1%)	7 (1%)	1 (.3%)
Eosinophils	3 (1%)	2 (1%)	2 (1%)	7 (1%)	3 (1%)
Basophils	1 (.4%)	2 (1%)	2 (1%)	5 (1%)	2 (1%)
Platelets	5 (2%)	2 (1%)	4 (2%)	11 (1%)	3 (1%)

Source: CMRs for Studies 216, 301.

Nevertheless, there were only 2 reports of anemia in the clinical AE database. Pt 16082 on 100mg/day had a positive direct Coombs test, but no other lab findings of a hemolytic process. Hgb fell as low as 8g/dL at month 10, but rebounded to 10.4 shortly thereafter. IgG gammopathy was also detected.

Pt 12275 on 200mg/day had a transient low Hgb of 10.2g/dL and continued on riluzole.

Clinical Chemistry (excluding liver-related parameters)

Sponsor's Table 57 summarizes the % of pts with chemistry values above the ULN at any time during the study, regardless of baseline value. No clinically relevant differences were seen between riluzole and placebo.

The sponsor did not initially present a similar analysis for chemistry values below the LLN. Since this would be of interest, especially for glucose, sodium, and potassium, the sponsor was asked to prepare such an analysis. It was received on August 15 and is labeled "Companion to Table 57 of the ISS." No clinically relevant differences were seen between riluzole and placebo.

The sponsor does summarize the frequencies of chemistry laboratory AEs as coded by the investigators.

The only difference noted was for serum glucose, 1% of the combined riluzole group vs 0.3% of placebo. A suggestion of a dose-response relationship was also seen.

Pt 12294 was discontinued for hyponatremia; this pt was on placebo.

Liver-Related Chemistries

Sponsor's Table 59 is supposed to represent the number of patients who had values for liver-related chemistries that were above the ULN at any time during the studies. However, using a search strategy designed to capture all pts who had ALT values $\geq 1.3 \times$ ULN in the first 5 months of treatment, I located approximately 115 pts in the 200mg/day riluzole group (47%), in contrast to the 62 pts(25%) located by the sponsor for the entire study duration.

The sponsor was asked to resolve this discrepancy, perhaps by clarifying the definition of "ULN." The response (never submitted in writing) was

Table 57: Frequency of Abnormal Laboratory Values in Phase II/III ALS studies: Biochemistry					
Number of patients (% of Total) with any result					
Studies 216 and 301					
ABOVE the Upper Limit of the Normal Range					
	Riluzole Total Daily Dose			ALL Riluzole	Placebo
	50 mg/day	100 mg/day	200 mg/day		
TOTAL	237 (%)	313 (%)	244 (%)	794 (%)	320 (%)
BIOCHEMISTRY					
Creatinine	0	1 (.3%)	1 (.4%)	2 (.3%)	2 (1%)
Glucose	19 (8%)	42 (13%)	30 (12%)	91 (11%)	37 (12%)
Uric acid	2 (1%)	9 (3%)	6 (2%)	17 (2%)	9 (3%)
Protein	2 (1%)	12 (4%)	4 (2%)	18 (2%)	12 (4%)
Sodium	2 (1%)	22 (6%)	4 (2%)	28 (4%)	31 (10%)
Potassium	3 (1%)	43 (13%)	4 (2%)	50 (6%)	28 (9%)
Chloride	26 (11%)	24 (8%)	22 (9%)	72 (9%)	20 (6%)
Bicarbonate	37 (16%)	65 (21%)	38 (16%)	140 (18%)	78 (24%)

Source: CMRs for Studies 216, 301.

Companion to Table 57 of the ISS:

Frequency of Abnormal Laboratory Values in Phase II/III ALS studies: Biochemistry					
Studies 216 and 301					
Number of patients (% of Total) with any result					
BELOW the Lower Limit of the Normal Range					
	Riluzole Total Daily Dose			ALL Riluzole	Placebo
	50 mg/day	100 mg/day	200 mg/day		
TOTAL	237 (%)	313 (%)	244 (%)	794 (%)	320 (%)
BIOCHEMISTRY					
Creatinine	56 (24%)	129 (41%)	64 (26%)	249 (31%)	122 (38%)
Glucose	2 (1%)	35 (11%)	4 (5%)	41 (5%)	30 (9%)
Uric acid	2 (1%)	14 (4%)	6 (2%)	22 (3%)	11 (3%)
Protein	16 (7%)	34 (11%)	22 (9%)	72 (9%)	23 (7%)
Sodium	4 (2%)	38 (12%)	26 (11%)	68 (9%)	31 (10%)
Potassium	4 (2%)	33 (11%)	5 (2%)	42 (5%)	49 (15%)
Chloride	8 (3%)	68 (22%)	10 (4%)	86 (11%)	78 (24%)
Bicarbonate	11 (5%)	27 (9%)	8 (3%)	46 (6%)	27 (8%)

Companion to Table 59 of the ISS:

Frequency of Abnormal Laboratory Values in Phase II/III ALS studies: Liver-related					
Studies 216 and 301					
Number of patients (% of Total) with any result Greater Than 1.3 times the Upper Limit of the Normal Range					
	Riluzole Total Daily Dose			ALL	Placebo
	50 mg/day	100 mg/day	200 mg/day	Riluzole	
TOTAL	237 (%)	313 (%)	244 (%)	794 (%)	320 (%)
LIVER-RELATED					
ALT	73 (31%)	152 (49%)	128 (52%)	353 (44%)	90 (28%)
AST	28 (12%)	113 (36%)	67 (27%)	208 (26%)	81 (25%)
GGT	29 (12%)	67 (21%)	54 (22%)	150 (19%)	44 (14%)
Bilirubin, total	6 (3%)	18 (6%)	29 (12%)	53 (7%)	13 (4%)
Alk. phosphatase	7 (3%)	13 (4%)	12 (5%)	32 (4%)	10 (3%)
CPK	53 (22%)	75 (24%)	50 (20%)	178 (22%)	76 (24%)

Table 59: Frequency of Abnormal Laboratory Values in Phase II/III ALS studies: Liver-related					
Number of patients (% of Total) with any result					
Studies 216 and 301					
ABOVE the Upper Limit of the Normal Range (1)					
	Riluzole Total Daily Dose			ALL	Placebo
	50 mg/day	100 mg/day	200 mg/day	Riluzole	
TOTAL	237 (%)	313 (%)	244 (%)	794 (%)	320 (%)
LIVER-RELATED					
ALT	30 (13%)	95 (30%)	62 (25%)	187 (24%)	47 (15%)
AST	6 (3%)	85 (27%)	25 (10%)	116 (15%)	72 (23%)
GGT	12 (5%)	53 (17%)	27 (11%)	92 (12%)	41 (13%)
Bilirubin, total	17 (7%)	46 (15%)	61 (25%)	124 (16%)	34 (11%)
Alk. phosphatase	1 (0.4%)	9 (3%)	2 (1%)	12 (2%)	16 (5%)
CPK	24 (10%)	48 (15%)	27 (11%)	99 (12%)	45 (14%)

Source: CMRs for Studies 216, 301.

that a dual cutoff was used: all values from 216 above the ULN are included while only values $> 2 \times \text{ULN}$ from 301 are included. Since 216 and 301 both have placebo and 100mg/day groups, the placebo and 100mg/day groups represent a strange mix of pts with either $> 1 \times \text{ULN}$ or $> 2 \times \text{ULN}$. The 50mg/day and 200mg/day groups are only represented by Study 301, so these groups represent only pts with $> 2 \times \text{ULN}$ values.

The sponsor agreed to modify this table to include % of pts with lab values $> 1.3 \times \text{ULN}$. This was submitted on August 15 and is labeled "Companion to Table 59 of the ISS." This shows a dose-response relationship for ALT, GGT, and total bilirubin. Fully, half of the 100 and 200mg/day groups had ALT values $> 1.3 \times \text{ULN}$, twice as many as the placebo group.

From Table 59, the sponsor believes that there is an increased frequency of high ALT (but not AST) and of bilirubin concentration in riluzole patients vs placebo. The findings showed an apparent dose-response relationship.

The sponsor summarizes the frequencies of liver-related chemistry laboratory AEs as coded by the investigators in Table 60. Lab AEs of ALT, AST, GGT, and bilirubin were all more frequent in riluzole vs placebo pts. In addition, these lab AEs showed a dose-response relationship.

By protocol, pts whose ALT rose above $5 \times \text{ULN}$ were discontinued from treatment in Study 301; pts whose ALT rose above $3 \times \text{ULN}$ were usually discontinued from treatment in Study 216 although no written rules for this existed in the 216 protocol. Patients whose ALT rose above $3 \times \text{ULN}$ had increased monitoring. Sponsor's Table 61 presents the breakdown for ALT and AST according to these categories.

Of note is that increases in serum ALT were not consistently associated with increases in AST. Between 11-45% of pts with ALT $> 3 \times \text{ULN}$ also had AST $> 3 \times \text{ULN}$.

Aside from ALT and AST, there was one treatment discontinuation for a liver-related chemistry. Pt 17006 discontinued from 200mg/day when GGT rose suddenly to $6.4 \times \text{ULN}$ with an associated increase in alk phos to $2.3 \times \text{ULN}$. The pt died one week later due to respiratory insufficiency.

The sponsor makes the case that normal baseline ALT predicts a low

Table 60: Frequency of Adverse Laboratory Experiences in Phase II/III ALS studies Liver-Related Parameters Studies 216 and 301 Number of patients (% of Total) (1)					
	Riluzole Total Daily Dose			ALL	
	50 mg/day	100 mg/day	200 mg/day	Riluzole	Placebo
Total Patients	237	313	244	794	320
No. with AEs (%)	69 (29%)	128 (41%)	109 (45%)	306 (39%)	69 (22%)
LIVER-RELATED					
ALT	38 (16%)	84 (27%)	76 (31%)	198 (25%)	36 (11%)
AST	18 (8%)	48 (15%)	51 (21%)	117 (15%)	22 (7%)
GGT	13 (5%)	33 (11%)	35 (14%)	81 (10%)	14 (4%)
Bilirubin, total	3 (1%)	5 (2%)	18 (7%)	26 (3%)	3 (1%)
Alk. phosphatase	3 (1%)	7 (2%)	8 (3%)	18 (2%)	3 (1%)
CPK, total	10 (4%)	13 (4%)	5 (2%)	28 (4%)	12 (4%)

Source: CMRs for Studies 216, 301.

Table 61: Patients with Hepatic-Associated Laboratory Values ≥ 3 times the Upper Limit of the Normal Range (ULN): Phase II/III ALS Studies Studies 216 and 301						
Laboratory Parameter	Maximum value	Riluzole Total Daily Dose			ALL	
		50 mg/day N = 237	100 mg/day N = 313	200 mg/day N = 244	Riluzole N = 794	Placebo N = 320
ALT	$\geq 3N$, but $< 5N$	11 (5%)	22 (7%)	29 (12%)	62 (8%)	7 (2%)
	$\geq 5N$	3 (1%)	9 (3%)	8 (3%)	20 (2%)	5 (2%)
AST	$\geq 3N$, but $< 5N$	2 (1%)	13 (4%)	3 (1%)	18 (2%)	2 (1%)
	$\geq 5N$	0	1 (3%)	2 (1%)	3 (4%)	2 (1%)

Source: CMRs for Studies 216, 301.

likelihood for later ALT elevations. The sponsor compares ALT elevations at 6 weeks between pts with normal baseline values (6%) and baseline values above normal (20%).

Across all dose groups, most ALT elevations occurred within the first 2 months of treatment. At higher doses, the elevations occur earlier. In Study 301, the median time to ALT elevation $> 3 \times \text{ULN}$ was between 40 and 60 days. 16 of 20 riluzole pts with ALT elevations requiring discontinuation ($> 5 \times \text{ULN}$) occurred within the first 3 months of treatment, regardless of dose.

The sponsor states that, in Study 301, among pts with ALT $> 3 \times \text{ULN}$, the ALT usually fell to $< 2 \times \text{ULN}$ within 2 to 6 months, despite continued riluzole treatment. The exception was Pt 12091, whose ALT remained $3 \times \text{ULN}$ throughout the study.

For pts discontinued from drug for ALT $> 5 \times \text{ULN}$, ALT fell to $< 3 \times \text{ULN}$ and usually $< 2 \times \text{ULN}$ within 2 to 8 weeks after stopping drug. In some cases, the return to normal took as long as 6 months.

Rechallenge Data

Only a handful of pts were rechallenged with medication after initially being discontinued because of elevated ALT. In Study 301, rechallenge was prohibited by protocol.

Pt 245 in Study 408 (phase 2, non-ALS) was discontinued after ALT rose to $5 \times \text{ULN}$ at week 6. Two weeks later, after ALT had normalized, riluzole was restarted. ALT rose again, but remained $< 2 \times \text{ULN}$ for the remaining 10 months of the study. Dose was 75 mg/day.

In Study 216, two pts were rechallenged after AST (not ALT) elevations $> 3 \times \text{ULN}$. After rechallenge, the AST remained $< 2 \times \text{ULN}$ or $< 4 \times \text{ULN}$ for the remainder of the study.

Liver Biopsy

When autopsies were performed, liver histopathology was not examined according to the sponsor. However, 2 pts had transjugular biopsies performed in Study 301.

Pt 12298 had an ALT $2-3 \times \text{ULN}$ along with "subicterus." Biopsy showed

massive microvascular steatosis, mild lobular hepatitis, and the presence of granulocytes but no cytolytic lesions. ALT normalized after treatment (200mg/day) discontinuation.

Pt 12293 had a normal liver biopsy, although ALT was $> 3 \times \text{ULN}$.

Recommended Monitoring

The sponsor believes that ALT $> 5 \times \text{ULN}$ should prompt discontinuation of treatment.

The baseline ALT should be measured because of the suggestion that baseline ALT $>$ normal but $< 3 \times \text{ULN}$ is associated with a greater likelihood of later ALT elevations. The sponsor maintains that a baseline value $> 3 \times \text{ULN}$ should preclude use of riluzole.

The sponsor proposes monthly ALT monitoring for the first 3 months of therapy, then every third month thereafter.

If treatment is discontinued because of ALT $> 5 \times \text{ULN}$, the sponsor proposes monitoring every 2 weeks until ALT $< 2 \times \text{ULN}$, then every 2 months until 2 consecutive ALT results are $< 2 \times \text{ULN}$.

For pts with ALT $> 5 \times \text{ULN}$, there is no data regarding the effect of lowering of dose, nor on the results of rechallenge after treatment is discontinued.

Urinalysis

The sponsor does not present an analysis of the effect of riluzole on routine parameters in the urinalysis.

Other Non-ALS Studies

Phase 1 studies revealed a pattern of lab changes similar to that seen in the ALS studies above. Of note is that one pt developed a marked drop in glucose and potassium 1-2 hours after an I.V. infusion of 100mg riluzole. An increase in insulin was noted. ECG abnormalities were then noted and attributed to the low glucose.

Phase 2, non-ALS studies revealed a similar pattern of lab changes as was seen in the ALS studies. One case deserves special comment:

Pt 3-12-1 in Study 003 developed hepatitis and jaundice after doses of riluzole were escalated to 250mg/day. This 20-yr-old male had epilepsy treated with carbamazepine and phenobarb. Alk phos and GGT were mildly elevated at baseline. After 3 months on riluzole (? duration at dose of 250/day), bilirubin rose to 3 X ULN. The dose was reduced to 100/day for several weeks, but at month 4 the pt had jaundice and neutropenia. ALT was 26 X ULN, AST was 17 X ULN and bilirubin was 11 X ULN. Riluzole was stopped at month 4 and the hepatitis began to improve immediately thereafter. 7 weeks after riluzole was stopped, liver-related chemistries had normalized.

5.2 Vital Signs and Weight

The sponsor does not present a systematic combined analysis of the effects of riluzole on pulse during the clinical trials, although the statement is made that no apparent adverse effects of riluzole treatment on heart rate were observed.

No grouped data on weight change is presented.

Studies with individual subject/pt listings for BP were reviewed in order to identify cases of notably high BP, that is SBP > 180 or DBP > 110. The sponsor did this focused search because of the concern raised by Study 216 where the frequency of the AE hypertension was 5% in the riluzole group and 0% in the placebo group.

In Phase 1 studies in healthy subjects, none of 330 subjects whose BPs were reviewed had a systolic BP > 180. A total of 5 subjects were found who had a DBP > 110. This was recorded in only one instance for each subject. Only one subjects had an AE recorded as hypertension: this subject's sitting BP rose from a baseline of 140/80 to 170/110 after 1 week at 50mg/day. BP returned to normal despite an increased dose of riluzole to 150mg/day.

In Phase 2, non-ALS studies, the frequency of newly emergent BP > 180/110 was 31/410, or 8%. This compares to 4/82, or 5%, in placebo pts. No pts discontinued due to hypertension. The sponsor notes that high BPs

returned to baseline despite continued riluzole dosing, and even dose escalation in some cases.

In the ALS studies, average SBP and DBP for riluzole treatment groups showed small decreases during the conduct of the studies; that is, no trends were observed toward BP elevation with riluzole treatment. The proportion of pts with SBP > 180 or DBP > 110 was nearly comparable between treatment groups. While 6% riluzole pts had this increase in DBP vs only 3% in the placebo group, there was no clear dose-response relationship for increased DBP for riluzole. No discontinuations for hypertension occurred in the ALS studies.

5.3 Electrocardiograms

In Phase 1 studies, 2 pts had ECG changes considered clinically significant. The first pt was Pt 5007 from Study 101. This pt is the one previously described with hyperinsulinemia, hypoglycemia, and hypokalemia after 100mg IV riluzole. Alterations in intra-atrial conduction and repolarization and increased U-waves were all seen, along with a 28% increase in QTc. The sponsor believes these ECG changes all occurred secondary to the metabolic changes; the hyperinsulinemia is believed to be exogenous by the sponsor because C-peptide was not concurrently increased. A rechallenge 10 months later was uneventful.

Pt 7 in Study 406 developed left posterior fascicular block and frequent PVCs 20 minutes following 100mg riluzole. The ECG abnormalities were judged of unknown relationship to study drug and persisted 2 days after study drug administration.

In Phase 2 studies, the following ECG abnormalities were noted.

One pt had flattened ST segment noted on Day 30 after receiving 25 mg riluzole. Another pt with a history of coronary artery disease and no baseline ECG was found to have a right bundle branch block on the final study ECG.

In Study 215, Pt 01 had diffuse trouble with repolarization, QT prolongation, and left anterior hemi-block noted at the final visit. No baseline ECG was available.

In Study 408, 8 riluzole 25 mg/day pts presented with ECG abnormalities at some point during the study, including ventricular extrasystoles, bradycardia, tachycardia, and "hypokalemic T wave flattening."

In the ALS studies, sporadic ECG abnormalities were reported in both studies. In Study 216, ECG abnormalities were noted in 13 riluzole pts and 7 placebo pts. Newly occurring ECG findings in riluzole pts included extrasystoles, conduction defects, sinus tach, and sinus bradycardia. None of these were considered to be adverse events by the investigator.

In Study 301, the proportion of pts in each of the 4 treatment groups was the same for each of the following categories: 1) pts with at least one abnormal ECG during study, 2) pts with newly occurring abnormal ECG findings, and 3) pts with abnormal ECG findings at baseline.

The sponsor reports that no particular pattern of ECG abnormalities was noted across treatment groups; no dose-related changes were noted.

The sponsor has not presented mean ECG data for the different treatment arms. Since ECGs were performed at regular intervals throughout the ALS studies, such data is available and would lend itself to an analysis of change from baseline for heart rate, PR interval, QRS interval, and QT interval.

5.4 Special Studies

Long-term Adverse Effects as Seen in 216 LT

Study 216LT began in March 1992. The cutoff for the NDA database is December 1993. Only the serious AEs from Jan 94 to Dec 94 are tabulated for the NDA. The full AE profile for 216LT through Dec 94 will be submitted with the 90-day Safety Update.

58 pts enrolled in 216LT, 29 were switching from placebo to 100mg/day riluzole while 29 pts were continuing on riluzole 100mg/day. As of December 93 (the cutoff for the NDA full AE profile), the median duration of exposure to riluzole was 13 months for the "new" group (range 1 day-21 months) while the median was 38 months for the continuation group (range 19 months-42 months). As of July 1995, the longest possible exposure in 216LT would be 60 months or 5 full years.

As of Dec 93, 21/58 pts had died and 1 pt had discontinued treatment due to elevated LFTs. Sponsor's Appendix A.3.2.4 which tabulates the serious AEs for 1994 lists an additional 8 deaths; discontinuations cannot be discerned from the list. Thus, by the beginning of 1995, at least 50% of pts who entered the long-term extension study were dead.

A review of the AEs reported as of Dec 93 raises no new concerns about the safety of riluzole.

Pt 237 discontinued after 22 months of treatment because of an ALT > 7 X ULN and AST > 6 X ULN, both of which were reversible upon discontinuation.

One pt developed a bone neoplasm and one pt developed prostate cancer.

The sponsor did note an upward trend in mean supine and standing BP and pulse. However, the number of pts with recordings was small (9-11) so that interpretation of this finding is difficult. Also, any high blood pressure measurements were sporadic and interspersed with normotensive recordings despite continued riluzole.

There were 3 AE reports of hypertension. One report of serious AE hypertension was received; however, this pt had a prior history of labile hypertension without any concurrent antihypertensive medication.

Pt 509 experienced a drop in platelet count to 28,000 at month 4 of the open-label extension study, recovering to normal by month 7. Another pt, 504, experienced a platelet count of 47,000 after 28 months of treatment. Platelet count remained low but improved (75% LLN) until the last measured value at month 39.

5.5 Withdrawal Phenomena/Abuse Potential

While classical physiological or psychological dependence on riluzole has not been reported, I do not think this has been studied in any systematic way.

The sponsor's formal analysis of AEs occurring after treatment discontinuation is still in progress and will be included in the 90-day Safety Update. The AE "withdrawal" was not reported.

5.6 Human Reproduction Data

There have been no clinical trials which included pregnant women. No riluzole-treated pts are known to have become pregnant.

6.0 Overdose Experience

None known.

7.0 Summary of Important Events Considered Drug Related

7.1 Rash

One discontinuation for rash, one for angioedema, and one for pruritus occurred in the ALS studies. Pt 13063 was hospitalized for itching erythema associated with swelling of face and hands. Symptoms began 5 weeks after starting riluzole and subsided 2 days after drug was stopped. The ingestion of herring may have played a role in this reaction. The other 2 discontinuations were considered non-serious events.

Pt 12071 in Study 301 developed face erythema with desquamation while Pt 12210 in the same study developed squamous lesions of the face and the palms. Treatment was not stopped for either patient. 3 other patients experienced some degree of peeling skin. All 5 of these "exfoliative" events were considered mild, non-serious, and did not result in treatment discontinuation.

3 additional pts in 301 developed cutaneous allergy, coded non-serious.

7.2 Allergic Reaction

A life-threatening anaphylactoid reaction was reported for Pt 17009. 17 days after starting 100mg/day, gum swelling, fever blisters, myalgia, and shortness of breath occurred. A type II hypersensitivity reaction was diagnosed that was similar to a previous penicillin reaction. The pt recovered within 1 week after discontinuation of riluzole.

7.3 Hematologic

Across all studies, there was a small drop in values for Hgb, Hct, and

erythrocyte counts. Because of the finding of hemolysis in dogs, a focused search for hemolysis was undertaken, but revealed no evidence for hemolysis in humans. One discontinuation did occur for a positive Coombs test as described above.

7.4 Pancreatitis

Two cases of pancreatitis were reported in Study 301. Pt 12056 had an elevated amylase secondary to gastrostomy. Amylase returned to normal without discontinuing riluzole.

Pt 22100 had an elevated amylase to 3000 at 18 months. Treatment was stopped and amylase decreased. The pt died from respiratory failure.

A third pt (Pt 28010) developed pancreatic cancer in Study 301.

7.5 Hepatic Adverse Events

The most dramatic case of what appears to be drug-induced hepatitis with riluzole was described earlier, but the case is repeated here. Notable about this case is the dose of 250mg/day as well as the concomitant use of carbamazepine and phenobarb. Pt 3-12-1 in Study 003 developed hepatitis and jaundice after dose of riluzole was escalated to 250mg/day. This 20-yr-old male had epilepsy treated with carbamazepine and phenobarb. Alk phos and GGT were mildly elevated at baseline. After 3 months on riluzole (? duration at dose of 250/day), bilirubin rose to 3 X ULN. The dose was reduced to 100/day for several weeks, but at month 4 the pt had jaundice and neutropenia. ALT was 26 X ULN, AST was 17 X ULN and bilirubin was 11 X ULN. Riluzole was stopped at month 4 and the hepatitis began to improve immediately thereafter. 7 weeks after riluzole was stopped, liver-related chemistries had normalized.

The occurrence of elevations in ALT and AST are summarized earlier in the laboratory section of this safety review. Elevations were common, resulting in treatment discontinuation as per protocol in some pts.

Pt 22028 developed a saw-tooth pattern of ALT elevations which was unique in that it continued even after treatment discontinuation. After 6 weeks on 200mg/day, the ALT rose to 7 X ULN resulting in treatment discontinuation; the ALT fell to <3 X ULN at week 7; rose to 20 X ULN at

week 8. ALT normalized by week 10 but increased again one week later to 3 X ULN. ALT again normalized, rose to 5 X ULN at month 6, then normalized again. An AE of cytolytic hepatitis was described.

Jaundice was reported as an AE in 5 pts in Study 301: 1 placebo, 1-50mg/day, and 3-200mg/day. None of the pts had an ALT > 3 X ULN. Only one treatment discontinuation resulted, for a 200mg/day pt:

Pt 12298 in Study 301 was hospitalized with weakness and "subicterus" after 75 days on 200mg/day. ALT reached 2.5X ULN. A transjugular biopsy showed massive microvascular steatosis, mild lobular hepatitis and granulocytes, but no cytolytic lesions. ALT normalized after treatment was stopped.

7.6 Hypertension

In Study 216, hypertension was reported for 4 riluzole treated pts. In these 4 pts, the diastolic BP rose 10 to 30 mmHg above baseline. The systolic BP rose 15 to 50 mmHg above baseline. The actual BPs were 145 to 190 mmHg SBP and 80 to 90 mmHg DBP at the time hypertension was recorded. In 3/4 pts, BP returned to normal later in the study. One pt's BP remained high (160/90) despite 3 months treatment with nifedipine. None of the 4 pts were dropped from treatment.

In fact, the label hypertension seems to have been inconsistently applied. A total of 26 pts (14 riluzole; 12 placebo) had SBPs of 180 or greater and/or DBPs of 100 or greater in Study 216, but only 2 of these 26 pts had an AE of hypertension noted. This group of 26 pts had blood pressures as high as 160 to 200 SBP in combination with DBPs of 100 to 110 that were not considered hypertensive.

Across both ALS studies, there were no discontinuations for hypertension. No clear trend for increases in BP were demonstrated.

7.8 Gastrointestinal

Aside from the hepatic effects of riluzole and the case of pancreatitis above, GI AEs of nausea, vomiting, and diarrhea were noted. Administration of riluzole with food may lessen these side effects.

7.9 Neuro-Psychiatric

Somnolence as an adverse event seemed to demonstrate a dose-response effect, with an excess of cases compared to placebo when the dose was 100mg/day or greater.

Isolated cases of neuro-psychiatric AEs leading to treatment discontinuation occurred in the ALS studies. These included insomnia, hallucinations, anxiety, and amnesia (one case each). An excess of anxiety in Phase 2 studies was attributed by the sponsor to the psychiatric patient population being studied.

Rare cases of suicide were equally distributed between placebo and riluzole treatment groups.

8.0 Important Events Considered Not Drug Related

8.1 Deaths

Deaths are discussed earlier in this safety review. Where cause of death was not clearly related to ALS, no clear differences emerged between riluzole- and placebo- treated groups to suggest that riluzole contributed to mortality. None of the deaths can be reasonably attributed to riluzole.

8.2 Myocardial Infarction and Cardiovascular

There were no discontinuations due to myocardial infarction or angina pectoris in the ALS studies. Only one discontinuation occurred in the cardiovascular body system, postural hypotension in a pt on 50mg/day riluzole.

Myocardial infarct was listed as a serious AE in the ALS studies, but with a greater number (3) in the placebo group than in any one riluzole dose group.

Likewise, no particular pattern of ECG abnormalities was noted by the sponsor across treatment groups in the ALS studies.

Two pts developed palpitations considered possibly drug-related. Both pts had ECG abnormalities noted at the prestudy exam; neither pt had an ECG that accompanied the AE of palpitations. Both pts continued on treatment.

8.3 Cancer

Preclinical carcinogenicity testing of riluzole continues. Several isolated cases of cancer (one case of prostate cancer, one case of bone neoplasm, and one case of pancreatic cancer) did emerge during the development process. Based on review of these cases, no clear relationship to riluzole is seen.

8.4 Deafness

Pt 506 in Study 216 developed transient unilateral deafness after 4 months of treatment. Deafness was an acute recurrence of a pre-existing condition and was not felt to be drug-related.

9.0 Summary of Drug Interactions

9.1 Drug-Demographic Interactions

Of note, here, is that blood samples were obtained from pts in selected centers in Study 301. The population PK analysis is not currently available. The sponsor states that it will be submitted as part of the 90-day Safety Update to the NDA.

Differences between males and females in AE profile were not notable in Phase 2, non-ALS studies and in Phase 2/3 ALS studies. Only a small percent of subjects in Phase 1 testing were female. Notably, in non-ALS studies, somnolence showed a gender difference of > 5%, being more frequent in males (6%) than in females (1%). In ALS studies, accidental injury and dizziness were more frequent in females than in males, with > 5% differences. Injury was reported in 16% of females and 9% of males. Dizziness occurred in 11% of females and 4% of males.

In the ALS studies, 30% of pts were > 65 years old. The % of these older pts experiencing AEs was the same as in the < 65 year-old age group, 90%. Most of the AEs for which there was a frequency difference between older and younger pts were actually more common in the younger pts. Among AEs reported more frequently by older pts, only 4 were more frequent in riluzole pts as opposed to placebo pts in the same age group (> 65 years): diarrhea (riluzole 8%; placebo 5%), pneumonia (riluzole 7%; placebo 5%), arthralgia (riluzole 5%; placebo 3%), and pulmonary embolus (riluzole 3%; placebo 2%). The differences between riluzole and placebo groups were not large for these AEs.

Racial differences in AE frequency are not discussed by the sponsor. In fact, with rare exceptions, the study population in all phases of study was Caucasian.

9.2 Drug-Disease Interactions

226 pts with medical history listings available for review were identified in Phase 2, non-ALS studies. 13/226 had a history of hypertension. The sponsor's review of BP in these pts did not suggest an exacerbation of this underlying condition.

2 pts from those same studies were identified with a history of diabetes. Riluzole did not result in an end-of-study serum glucose that was different from baseline.

No special studies were performed in pts with underlying liver disease or renal impairment.

9.3 Drug-Drug Interactions

Specific studies addressing the interaction of riluzole with other drugs have not been performed in humans. The sponsor 1) draws some conclusions using in vitro serum binding and metabolism study results, and 2) reviewed concomitant medications taken by pts in Phase 2/3 ALS trials who had elevations of ALT.

The sponsor identified potentially hepatotoxic concomitant meds in Study 301 using the HEPATOX database. In 301, the frequency of ALT elevations > 3 X ULN was not greater among those who took potentially hepatotoxic medications than it was among those who did not. Of 82 riluzole pts in Study 301 whose ALT > 3 X ULN, only 8 were identified who took no other meds that were potentially hepatotoxic.

The major inhibitors of riluzole metabolism in human liver microsomes in vitro were the known inhibitors of CYP1A, including caffeine and naphthoflavone. The drugs amitriptyline, clomipramine, imipramine, diclofenac, and diazepam were potential inhibitors of metabolism, but all showed low potency in this regard. In 301, 25 pts had an ALT > 5 X ULN, 20 of whom received riluzole. Only 6/20 had been taking potential riluzole metabolism inhibitors.

Riluzole is 96% protein bound. In vitro studies showed no effect of warfarin, digoxin, imipramine, or quinine on riluzole binding. Likewise, riluzole had no effect on warfarin serum binding. There was little clinical evidence of drug interactions related to protein binding. For example, there were no reports of hemorrhagic complications in ALS pts receiving riluzole and warfarin.

DIVISION OF GASTROINTESTINAL AND COAGULATION DRUG PRODUCTS

MEDICAL OFFICER'S CONSULT REVIEW

NDA: 20-599
Date Sent to HFD-180: 08/24/95
Date Assigned to HFD-180 Consultant: 08/31/95
Sponsor: Rhone-Poulenc Rorer
Drug: RILUTEK[®] (riluzole=RIL)
Route of Administration: Oral
Pharmacological Category: Neuroprotective
(Antagonist of glutamatergic transmission)
Material Reviewed: Information from ISS re: liver toxicity with Rilutek.
Data from Clinical trials in NDA 20-599.
Response to specific questions posed by John Feeney, M.D. (HFD-120).

08/31/95

SEP 1 1995

I. Questions the Consultant is Asked to Address

Riluzole (RIL; 2-amino-6-trifluoromethoxybenzothiazole), is a novel psychotropic agent which possesses anticonvulsant and neuroprotective activity in animal models, with a spectrum different from that of benzodiazepines.

In an attached note, Dr. J. Feeney (HFD-120) states that (RIL) has been shown to have an effect on time to tracheostomy or death in amyotrophic lateral sclerosis (ALS), a life-threatening disease. [According to the CSO RIL is going to an Advisory Committee in September 1995 and will most likely result in an approvable letter to the sponsor soon after.] Dr. Feeney explains that during the two pivotal trials (#216 and #301), patients were not allowed to take potentially hepatotoxic drugs and those with liver function tests (LFTs) >2x ULN were excluded. If during the trial liver function tests (LFTs) rose >5x ULN, patients were discontinued and not allowed rechallenge.

NOTE: It is not clarified whether the excluded potentially hepatotoxic drugs included ethanol, acetaminophen and smooth endoplasmic reticulum inducers. It is also not clear what is meant for LFTs (one=ALT or all=ALT, AP, GGT, LDE, bilirubin (BIL)). Note that if the ULN for bilirubin is 1.2 mg/dl, 5x ULN is 6 mg/dl. Since this constitutes pronounced hyperbilirubinemia, it is a good justification not to include patients in the trial.

Dr. Feeney's questions are:

If the drug is approved, how restrictive should labeling be with regard to LFT elevations?

1. Should baseline LFT elevations preclude use of the drug?
2. Should elevations of LFTs on treatment >5x ULN mandate treatment discontinuation?

3. Can concomitant medications like Dilantin and phenobarbital be allowed without unnecessarily raising the risk involved?

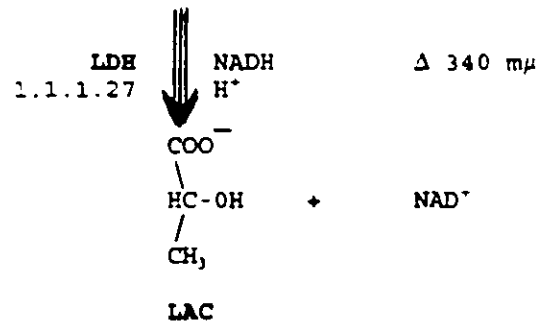
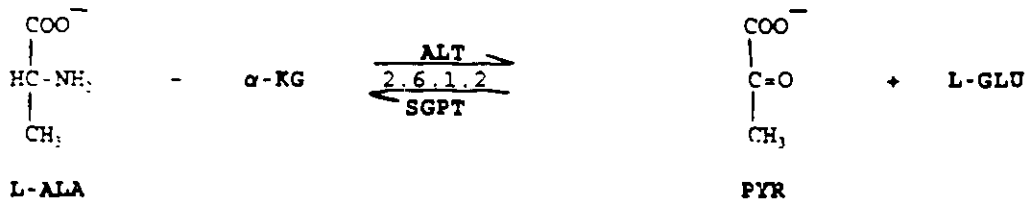
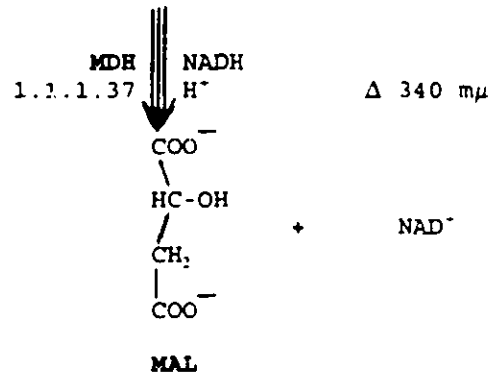
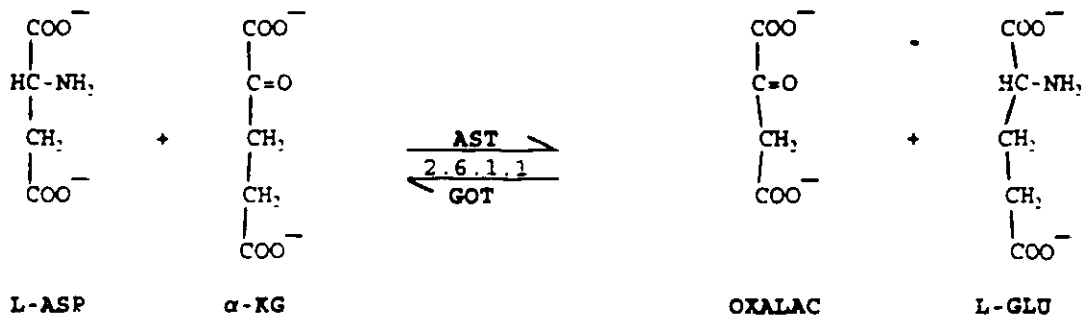
During the review of the existing evidence on liver toxicity with RIL the consultant has used information from Dr. Feeney's Safety Review as well as from sponsor's vol. 1.179 from the NDA which contains the ISS.

II. Background; Scope of Consult Review

ALS is a type of motor neuron disease (MND) with onset of clinical symptoms at a mean age of 57y. The disease affects motor cranial tracts in the lower brain stem from cranial nerve nuclei or corticobulbar tract regeneration. This results in progressive weakness affecting distal more than proximal structures, especially the small muscles of the hand. The disease may develop quite asymmetrically. There is prominent and early muscle atrophy, fasciculations in the entire upper extremities and spasticity, particularly in the lower extremities. The patient may have speech and swallowing difficulties and emotional lability. Intellectual functions deteriorate in ca. 5%. The majority of patients experience a significant linear deterioration. Mean survival for ALS and all forms of MNDs is 4.1y from onset of symptoms. Death in ALS is usually attributed to cardiac or pulmonary failure, resulting from complications of breathing muscle involvement. Upper respiratory infection from pooling of secretions or aspiration are common events. It is important to note that abnormality of liver function is not a characteristic of ALS [J.T. Carosco et al., *Neurol. Clin.* 5:1-8 (1987); K. Kondo and I. Hemmi, *Neuroepidemiol.* 3:129-148 (1984)] but no information is available on the histopathologic profile of the liver of the untreated ALS patient.

The etiopathogenesis of ALS remains unknown but a systemic defect in glutamate metabolism, resulting in plasma concentration of glutamate significantly higher than in normals, has been shown [A. Plaitakis and J.T. Carosco, *Ann. Neurol.* 22:575-579 (1987)]. Of interest, in this study, oral glutamate loading resulted in significantly greater elevations in the plasma glutamate and aspartate levels in the ALS patients than in the controls. The high plasma levels of glutamate are presumably due to abnormal clearance of this compound. But the abnormal glutamate clearance in those patients is not due to a defect in the transamination pathway. According to the aforementioned authors these high levels of glutamate are rather due to neuroexcitotoxic mechanisms (resulting in less utilization of glutamate?).

Because the transamination reactions are important in the context of the present review, they are presented schematically below. The transaminases represent a major group of pyridoxal phosphate-catalyzed enzymes. The α -amino group of amino acids such as aspartic acid (L-ASP), alanine (L-ALA), arginine, asparagine, cysteine, isoleucine, lysine, phenylalanine, tryptophan, tyrosine and valine is removed by transamination. Usually α -ketoglutarate (α -KG) serves as the final acceptor of the amino groups for channeling into reactions whereby the nitrogenous end products such as urea are formed. Following deamination, the carbon skeletons of the amino acids undergo oxidative degradation to compounds that are metabolized in the TCA cycle. Pyridoxal phosphate functions in transaminases in a Schiff base (ketimine) mechanism. Also represented below are the [irreversible] enzyme systems, [malic dehydrogenase=MDH and lactic dehydrogenase=LDH] used in the diagnostic test. Not all cofactors are shown.



It is very important to mention that the enzymes vary in their intracellular distribution. AST exists primarily in the mitochondria. ALT is more ubiquitous and is found in the liver cytosol. For this reason, ALT is more readily leaked out in cases of liver injury. But such leakage is not an indication of liver function.

Some information on liver function in ALS has been obtained in patients treated with placebo in clinical trials.

The evidence assessed in the present consult review includes pre-clinical information and safety clinical data related to the liver (deaths, withdrawals, serious/severe AEs, biochemical alterations and histopathological information, when available). As already pointed out, the information was taken from both sources, the Medical Officer's Safety Findings and vol. 179 of 211 (NDA 20-599). A brief summary of findings is usually followed by a consultant's comment.

A. Pre-clinical Safety Data

Species (Length of Treatment)	MAIN FINDINGS		
	Clinical/Biochemical (Specified Doses/Kg/day)	Macroscopic	Histopathological
1. Mice (3-mo.)	None [10-30]	NR	NR
2. Rats (3-mo.)	Slight ↑ in AP [45 x 1 mo. 25 x 3 mo.]	Mild ↑ in absolute and/or relative liver weights	No. Tx-related changes
F rats (15 mg x one month)	Mild ↑ in ALT	NR	NR
3. Rats (6-mo.)	Mild ↑ in ALT [20; F at W4; both sexes at W12] W26: No changes	NR	Minimal enlargement of centrilobular hepatocytes in the liver of both sexes [20]. No EM changes attributable to RIL.
4. Baboons (3-mo.)	Marked ↑ in serum transaminases in a F euthanized on Day 16 because of heavy sedation, hypothermia and increased HR	NR	Multiple changes secondary to poor clinical condition and/or stress, but no specific changes in the liver
5. Cynomolgus Monkeys (1-mo., I.V.) oral (6-mo., gavage)	None [10] None [20]	None Mild, dose-related ↑ in liver weights at 5 and 10 mg	None Foci of hepatocytes with fat droplets in 2/5 F treated with 10 mg/Kg/day

NR = Not reported

COMMENT

The consultant agrees that these findings in animals are inconsistent and that, based on the variables evaluated, there is no clear evidence of

hepatotoxicity. However, the 6-month observations in rats and cynomolgus monkeys, both of which exhibited some histopathological changes in the liver, are relatively short in relation to the entire life of the animal. Larger animal exposure is needed if one considers that, if approved, RIL is to be administered to the ALS patient for several years. Long-term (L-T) toxicity studies in rats and monkeys, both for >2 years may be needed to arrive at firmer conclusions regarding liver toxicity of RIL in animals.

In addition, no information appears to be available on the effect of RIL on the isolated hepatocyte or liver perfusion. The former gives important *in vitro* data whereas the latter is a useful *in vivo* system. Both systems test for liver toxicity. The information gathered from these tests is important to expand the human data.

B. Clinical Safety Data

The recommended dosing regimen in the proposed labeling is 100 mg per day, in two divided doses (50 mg b.i.d.) but studies with higher daily doses have been carried out. For purpose of presentation of the safety data, four populations listed below and addressed separately in their submission, have been created by the sponsor. [Note populations 3. and 4., referring to ALS patients, are melted into one.]

1. Phase I trials in healthy subjects (n=254)
Data come from 14 studies, testing single daily doses as high as 500 mg, with a maximum dosing duration of 14 days, generally for 10 days or less at doses <200 mg.
2. Phase II trials in non-ALS patients (n=520)
Data come from 11 studies, testing b.i.d. doses as high as 375 mg/day, with a maximum dosing duration of usually <3 months (75 or 150 to 200 mg/day). Few patients (any dose) were treated for 6 to 9 mo.
3. Phase II/III ALS (n=1114)
These data come from two sources. One is the two randomized, double-blind, placebo-comparison pivotal studies, No. 216 [RIL=77; PL=78], and No. 301 [RIL=717; PL=242]. The other is study No. 216LT, where patients who completed study No. 216 were newly treated with RIL in a long-term extension (RIL=29; PL=0).

The bulk of the safety information come from these Phase II/III ALS studies, from patients given three dose levels of RIL: 50 mg/day (n=237), 100 mg/day (n=236) and 200 mg/day (n=244) or PL (n=242). The total number of RIL-treated patients in the ISS, half of them treated ca. for 15 to 18 months, is RIL, n=823, PL, n=320.

In the Section that follows, the safety data are evaluated on the basis of liver-related AEs.

a. Deaths

Comment

The overall incidence of deaths in the groups of patients given RIL was not different from that given PL. From the data displayed in sponsor's Table 41, none of the deaths among RIL-treated patients appears to be directly or overtly related to the liver.¹

¹No cause of death is described as liver insufficiency, liver necrosis, jaundice, encephalopathy, bleeding from esophageal varices or ascites.

The cause of death in one PL-treated patient (#00146) was indicated as increased ALT/AST. This patient, who D/C treatment because of increased transaminases, died one month later. Transaminases (levels not given) did not normalize during that interval and the AE outcome was recorded as death. No other cause of death was indicated.

b. Serious AEs

Comment

None of the tables presented by the sponsor list liver-related serious AEs. Of note, the increases in ALT and AST listed in the Withdrawals Sections, are not listed as serious.

c. Withdrawals

[Sponsor's Tables 44 through 46]

In 11 Phase II non-ALS studies, in which a total of 39 pts. had AEs leading to treatment D/C, RIL was associated with AEs leading to treatment D/C more frequently than was PL. However, there was no single AE leading to treatment D/C that was reported with frequency $\geq 1\%$. In the combined Studies 216/301 database, the breakdown of the 107 (13%) of AEs that led to treatment D/C for RIL patients favored the categories body as a whole, digestive, respiratory and nervous. The number (% of total) of patients reporting liver-related AEs with a frequency $\geq 1\%$ at any dose is summarized below.

Liver-related AEs Leading to Treatment D/C in
Study Nos. 216 and 301

	RIL Total Daily Dose (mg/day)			All RIL	PL
	50	100	200		
Total n=	237	313	244	794	320
no./(% D/C	21 (9%)	57 (18%)	29 (12%)	107 (13%)	42 (13%)
COSTART TERM:					
ALT ↑ ^a	3 (1%)	9 (3%)	8 (3%)	20 (3%)	5 (2%)
AST ↑	0	5 (2%)	0	5 (1%)	0
Asthenia ^b	1 (0.4%)	12 (4%)	4 (2%)	17 (2%)	4 (1%)
Nausea ^c	1 (0.4%)	9 (3%)	5 (2%)	15 (2%)	1 (0.3%)

a) Frequency taken from the laboratory database

b, c) Non-specific for liver

Comment

In the Table above, the consultant has included asthenia and nausea but these are very non-specific AEs (not specifically related to the liver). Data on ALT and AST allow the conclusion that liver-related withdrawals in studies 216/301 did not occur at an incidence higher than those observed among PL-treated patients.

d. Incidence of Liver-related AEs

Aside of the aforementioned asthenia, nausea and vomiting, the COSTART terms listed in sponsor's Table II (Pts. given 100 mg/day) do not include terms related to the liver (jaundice, ascites, encephalopathy, bleeding esophageal varices, etc.). Under the subtitle metabolic-nutritional, the following is listed re: liver function tests (modified by consultant for ease of presentation). This shows a slightly higher numerical incidence of enzyme increases among RIL-treated patients in comparison with those given PL.

	RIL 100 mg/day	PL
	<u>% of Pts.</u>	
ALT †	6/316 (1.9%)	0.9
AST †	1/316 (0.3%) ^a	0
LDH †	1/316 (0.3%) ^b	0

a,b) The 0.3% represents one patient

NOTE: The information summarized in the Table above is very incomplete. It is not clear if the same patient experienced † ALT, † AST and † LDH. It is also important to know the magnitude of the elevation and when did the increase occur. It is important to know the extent and time course of ALT (elevation) and whether it subsided despite continuing drug. Also of interest is whether the ALT elevation was associated with adding another drug such as ethanol, acetaminophen, etc.

Similarly, liver-related AEs (clinical or otherwise) are not listed in sponsor's Table 35, depicting the most frequent AEs seen in the combined Studies 216/301 database (occurring at ≥5% at any dose) and in sponsor's Table 38, listing AEs occurring in any RIL Tx group at >3% points greater than in the PL group. In this Table, asthenia, nausea, vomiting, vertigo, somnolence and circumoral paresthesia appeared to demonstrate a dose-response relationship. But liver-related AEs are not listed in this Table or in Table 31 (AEs with frequency ≥5% at any dose in Phase I trials).

The most frequent Phase II, non-ALS liver-related AEs occurring with a frequency ≥5% for any Tx included the following on GGT:

	RIL Total Daily Dose (mg/day)					All RIL	PL
	25-75	100	150	200	250-500		
Total n=	347	93	310	28	17	438	92
no./(% with AEs)	85 (24%)	14 (15%)	141 (45%)	8 (29%)	4 (24%)	207 (47%)	52 (57%)
GGT †	0	0	2 (1%)	2 (7%)	0	4 (1%)	0

Comment

The data displayed in this section show minor numerical differences between RIL and PL in the incidence of liver enzymes, namely ALT, AST, LDH and GGT. But these differences are very minor, not denoting dose-response relationship and most likely the consequences of the small number of patients in some cells, in comparison to others.

e. Liver-related Changes in Laboratory Parameters

Laboratory parameters commonly accepted as denoting liver function include: serum total bilirubin (BIL), transaminases (ALT and AST), alkaline phosphatase (AP), gamma-glutamyl transpeptidase (GGT), lactic dehydrogenase (LDH), albumin and prothrombin time. A discussion on the sensitivity/specificity of these tests to diagnose liver function is beyond the scope of the present consult. Emphasis is put on the available clinical information, which appears to be rather incomplete.

The material that follows was taken primarily from Dr. Feeney's Safety Findings Section.

Sponsor's Table 59, reproduced below, supposedly represented the number of patients who had values for liver-related chemistries that were above the ULN at any time during the studies. However, using a search strategy designed to capture all pts. who had ALT values $\geq 1.3x$ ULN, Dr. Feeney located ca. 115 pts. in the 200 mg/day RIL group (47%), in contrast to the 62 pts. (25%) located by the sponsor. The sponsor was asked to resolve this discrepancy, perhaps by clarifying the definition of "ULN". The response (to be submitted in writing) was that in Table 59 a dual cutoff was used: all values from Study No. 216 above the ULN were included while only values $>2x$ ULN from Study No. 301 were included. Since both studies had PL arms and 100 mg/day groups, the PL and 100 mg/day groups represented a mix of pts. with either $>1x$ ULN or $>2x$ ULN. The 50 mg/day and 200 mg/day groups are only represented by Study 301, so

	RIL Total Daily Dose (mg/day)			All RIL	PL	Δ (RIL > PL)
	50	100	200			
TOTAL n=	237	313	244	794	320	
LIVER-RELATED						
ALT	30 (13%)	95 (30%)	62 ^a (25%)	187 ^b (24%)	47 (15%)	9% ^c
AST	6 (3%)	85 (27%)	25 (10%)	116 (15%)	72 (23%)	-8%
GGT	12 (5%)	53 (17%)	27 (11%)	92 (12%)	41 (13%)	-1%
BIL, Total	17 (7%)	46 (15%)	61 (25%)	124 (16%)	34 (11%)	5%
AP	1 (0.4%)	9 (3%)	2 (1%)	12 (2%)	16 (5%)	-3%

NOTE: It is not specified if the tests were all done in the same (central?) laboratory and whether the same range of normal applied to the various centers.)

a) This becomes 115 (47%) if one includes ALT values $\geq 1.3x$ ULN (Dr. Feeney's calculation).

b) 240 (30.2%) (Dr. Feeney's calculation).

c) 15.2% (According to Dr. Feeney's calculations)

these groups represent only pts. with >2x ULN values. The sponsor agreed to modify their Table 59 to include % of pts. with lab values >1.3x ULN.

From Table 59, the sponsor believes that there is an increased frequency of high ALT (but not AST) and of serum bilirubin concentration in RIL patients vs PL. The findings showed an apparent dose-response relationship. But, at this point, information on time course was not given.

The sponsor summarized the frequencies of liver-related chemistry laboratory AEs as coded by the investigators in Table 60. This Table is reproduced below. Lab AEs of ALT, AST, GGT and BIL were all more frequent in RIL vs PL pts. In addition, these lab AEs showed a dose-response relationship. Again, information on time course is needed.

	RIL Total Daily Dose (mg/day)			All RIL	PL	Δ (RIL > PL)
	50	100	200			
TOTAL n=	237	313	244	794	320	
No. with AEs/(%)	69 (29%)	128 (41%)	109 (45%)	306 (39%)	69 (22%)	
LIVER-RELATED						
ALT	38 (16%)	84 (27%)	76 (31%)	198 (25%)	36 (11%)	14%
AST	18 (8%)	48 (15%)	51 (21%)	117 (15%)	22 (7%)	8%
GGT	13 (5%)	33 (11%)	35 (14%)	81 (10%)	14 (4%)	6%
BIL, Total	3 (1%)	5 (2%)	18 (7%)	26 (3%)	3 (1%)	2%
AP	3 (1%)	7 (2%)	8 (3%)	18 (2%)	3 (1%)	1%

Dr. Feeney noted that across all dose groups, most ALT elevations occurred within the first 2 months of treatment. At higher doses, the elevations occurred earlier. In Study 301, the median time to ALT elevation >3x ULN was between 40 and 60 days. 16 of 20 RIL-healed pts. with ALT elevations requiring D/C (>5x ULN) occurred within the first 3 months of treatment, regardless of dose.

The sponsor stated that, in Study 301, among pts. with ALT >3x ULN, the ALT usually fell to <2x ULN within 2 to 6 months, despite continued RIL treatment. The exception was Pt. 12091, whose ALT remained 3x ULN throughout the study. For pts. withdrawn from drug for ALT >5x ULN, ALT fell to <3x ULN and usually <2x ULN within 2 to 8 weeks after stopping drug. In some cases, the return to normal took as long as 6 months.

Also on page 27 of his review, Dr. Feeney comments on the following case.

Pt. 3-12-1 in Study 003 (multiple, variable dose) developed hepatitis and jaundice after doses of RIL were escalated to 250 mg/day. This 20-y-old M had epilepsy previously and continuously treated with carbamazepine and phenobarbital. AP and GGT were mildly elevated at baseline. After 3 months on RIL (? duration at dose of 250/day), BIL rose to 3x ULN. The dose was reduced to 100/day for several weeks, but at month 4 the pt. had jaundice and neutropenia. ALT was 26x ULN, AST was 17x ULN and bilirubin was 11x ULN. RIL was stopped at month 4 and the hepatitis began to improve immediately thereafter. Seven weeks after RIL was stopped, liver-related chemistries had normalized.

Comment

The data depicted in sponsor's Table 59 demonstrate a dose-related relationship for ALT and especially serum BIL concentration. Although with 100 to 200 mg of compound, the fractions of RIL-treated patients experiencing elevations of ALT and BIL were ca. 28% and 20%, respectively, and there was a 9% (15% if "corrected") and 5% difference between these and PL (Table 59), in reality, the percentage of patients experiencing these laboratory parameters of liver function do not seem high.

For both parameters, the values with 50 mg/day are not dissimilar from those seen with PL. But the percentages seen with 100 and 200 mg/day are certainly numerically higher than those seen with PL. The laboratory parameters listed in Table 60 (Investigator-coded) show a dose-response relationship for both transaminases in addition to GGT, BIL and even AP where the number of observations was rather small. [The column labeled Δ shows all percentages favoring RIL over PL.] This consistency of results suggest but do not prove that something is happening across the liver (not just a mere ALT elevation).

One important point of clarification - assuming that these laboratory changes are drug-related - is how many patients experienced elevations of serum concentration of BIL in addition to concomitant increases in transaminases or other variables. The sponsor should be asked to provide these data. This information is important because an isolated elevation of ALT or AST is less meaningful than a concomitant elevation of BIL and the other parameters of liver function. Also a transient rise is of less concern than a sustained or progressive rise.

Assuming that the abnormal biochemistries in Pt. #3-12-1 were RIL-related, these findings suggest that the manifestation of this hepatocellular-cholestatic injury is reversible upon discontinuation of the drug. It is important to reiterate that, as pointed out by Dr. Feeney, a) regardless of the dose, most ALT occurred within the first 2 months of Tx and that, at higher doses, the elevations occurred earlier. Irrespective of the dose, >5x ULN ALT elevations requiring withdrawal (16 of 20 pts.) occurred within the first 3 mo. of Tx; b) in spite of continued Tx, in Study 301, among patients with ALT >3x ULN this parameter fell to <2x ULN within 2 to 6 months and c) although in some patients the return to normal took as long as 6 months, for most pts. withdrawal from drug because of ALT >5x ULN, ALT fell to <3x ULN and usually <2x ULN within 2 to 8 weeks after stopping of the drug.

The above-described dechallenge information suggests that the alterations in LFTs, especially ALT, were drug-related.

NOTE: For isolated, individual cases of BIL + enzyme elevations, it would be very important to rule out viral hepatitis, of the A, B and especially non-A, non-B (C), in addition to ethanol and concomitant potential hepatotoxic drugs as causative factors to explain biochemical alterations.

f. Rechallenge Data

Little information on rechallenge after transaminase elevations exists. In Study 301, rechallenge was not allowed per protocol. A total of three patients, 2 in Study 216 and one in Study 408 (Phase II, non-ALS) were rechallenged. Information on these three pts. is briefly summarized below.

- In Study 216, two pts. were rechallenged after AST (not ALT) elevations >3x ULN. After rechallenge, the AST remained <2x ULN or <4x ULN for the remainder of the study.
- In Study 408 pt. 245 was withdrawn after ALT rose to 5x ULN at week 6. Two weeks later, after ALT had normalized, RIL was restarted. ALT rose again, but remained <2x ULN for the remaining 10 months of the study. Dose was 75 mg/day (lower than the recommended dose).

Comment

All in all, information on rechallenge is too scanty. These data do not allow meaningful conclusions.

g. Liver Biopsy (Bx) Data

- According to the sponsor, when autopsies were performed, liver histopathology was not examined.
- The following 2 patients, in Study 301, underwent transjugular Bxs.
 - Pt. #12298 (200 mg/day) had an ALT 2-3x ULN along with "subicterus". Bx showed massive microvascular steatosis, mild lobular hepatitis, and the presence of granulocytes but no cytolytic lesions. ALT normalized after Tx discontinuation.
 - Pt. #12293 had a normal Bx. In this patient, ALT was >3x ULN.

Comment

The data on liver Bx involve too few observations and cannot be used to draw meaningful conclusions. It is important to ask the sponsor to clarify if liver histopathology was not examined [this is hard to believe when autopsy had been carried out] even though samples of liver tissue were taken and processed. If no samples were taken, the sponsor has lost a golden opportunity to characterize liver histopathology in PL-treated patients and in those treated with the drug. It is not too late to do this (see Recommendations for Regulatory Action).

In Pt. #12298, the presence of granulocytes in the liver Bx suggests a bacterial infection or alcoholic hepatitis.

III. Sponsor's Recommended Monitoring

This section is reproduced verbatim from page 27 of Dr. Feeney's review.

The sponsor believes that ALT >5x ULN should prompt discontinuation of treatment.

The baseline ALT should be measured because of the suggestion that baseline ALT > normal but <3x ULN is associated with a greater likelihood of later ALT elevations. The sponsor maintains that a baseline value >3x ULN should preclude use of riluzole.

The sponsor proposes monthly ALT monitoring for the first 3 months of therapy, then every third month thereafter.

If treatment is discontinued because of ALT >5x ULN, the sponsor proposes monitoring every 2 weeks until ALT <2x ULN, then every 2 months until 2 consecutive ALT results are <2x ULN.

For pts. with ALT >5x ULN, there are no data regarding the effect of lowering of dose, nor on the results of rechallenge after treatment is discontinued.

Comment

Although, theoretically, certain people may be more sensitive to RIL, it appears that the proposed monitoring resulting in prompt discontinuation of the drug is too restrictive with regards to ALT elevations and not based on the available information. The suggestions that baseline ALT > normal but <3x ULN is associated with greater likelihood of later ALT elevations appears to be an speculation without solid foundation. On the other hand, the sponsor's proposal of monthly ALT monitoring for the first 3 months of therapy, then every third month thereafter appears reasonable. Also reasonable seems to be the monitoring proposed upon treatment discontinuation. Obviously, potential benefits of the drug must play a role in deciding whether to discontinue treatment or not, but neither deaths nor serious liver-related AEs that needed withdrawal of the patient from the assessed trial results seem to be occurring at an alarming frequency. In addition to the obvious monitoring of ALT, the sponsor should consider monitoring serum concentration of BIL (see Recommendations for Regulatory Action).

IV. Recommendations for Regulatory Action

In this section, the specific questions raised by Dr. Feeney are addressed first. This is followed by consultant recommendations for Regulatory Action.

If riluzole is approved to treat ALS, a life-threatening disease, the labeling need not be very restrictive with regard to LFT elevations. Note that there is a very important distinction to be made. The reviewer questions make reference to LFTs. This is plural; LFTs go beyond ALT. On the other hand, the sponsor recommends discontinuation of treatment on the basis of ALT (alone). LFT and ALT are not synonymous. Indeed, technically speaking, ALT is not a measure of function but an indicator of possible hepatocellular injury. But BIL is a liver function test. But since the bulk of the experience reviewed here refers to ALT (and this is what the sponsor proposes), the consultant is addressing primarily ALT.

1. Should baseline elevations of ALT preclude use of the drug?

- This question needs to be expanded [how much elevation?; how often; how long?].

- There is no information in support of the proposal that baseline elevations of ALT should preclude use of the drug (more experience is needed).
 - As a sound clinical approach, baseline elevations of several LFTs especially high increases in serum concentrations of BIL plus concomitantly high increases of transaminases, AP and GGT, as seen for example in full-fledged hepatitis, should definitely preclude the use of any potentially hepatotoxic drug.
 - Based on the information at hand, it appears that, under certain conditions of dose and dose duration, etc., RIL may be hepatotoxic. But it is not yet clear whether the changes induced by this compound are serious and irreversible or non-serious and reversible.
 - An important conclusion from these observations is that more information is needed.
2. Should elevations of ALT on treatment >5x ULN mandate treatment discontinuation.
- Based on information at hand, elevations of ALT on treatment >5x ULN [but <10x ULN] if this abnormality occurs in isolation, should not necessarily mandate treatment discontinuation. This becomes a judgement call. This finding should trigger the assessment of additional LFTs [serum BIL et al.] and a very close monitoring of the patient, including assessment of LFTs every 72h.
 - Elevation of ALT alone on treatment $\geq 10x$ ULN should mandate treatment discontinuation simply because this is too high an elevation.
 - The development of jaundice together with marked elevations of LFTs ($\geq 5x$ ULN), BIL [+ transaminases] should most certainly trigger discontinuation of the treatment.
3. Can committant medications like Dilantin and phenobarbital be allowed without unnecessarily raising the risk involved?
- The available information does not suggest that these anticonvulsants, used concomitantly, raise the risk involved. But there is no information to maintain the contrary. More experience is needed on this matter and this can be gained during post-marketing surveillance.
 - It is important to note that other conditions (alcoholism, viral hepatitis) may confound interpretation of liver-related data and that many drugs, in addition to the two mentioned above, have been shown to be hepatotoxic. This long list include isoniazid, isoniazid-like compounds, cyclosporin A, methotrexate, sulfasalazine, acetaminophen, large doses of aspirin, tacrine, etc., etc.

The EFD-180 Consultant recommends:

- a. Better characterization of LFT abnormalities induced by RIL, beyond the mere determination of ALT proposed by the sponsor [we are not there yet].

These biochemical evaluations of liver function include serum direct BIL concentration and measurement of ALT, AST, GGTP and AP activities. These observations should arise from studies where the compound is being used long-term (example 12, 18, 24 months) at the recommended dose.

- b. Better characterization of the histopathological picture associated with liver biochemical abnormalities apparently induced by the drug.

This recommendation includes examination of liver histopathology in those patients that die. If relatives do not allow the patient to be autopsied, a post mortem Bx of the liver through needle aspiration should be requested. Granted, there are no pre-drug data to compare with but since patients are (were) being treated with the drug, a differentiation could be made between patients with completely normal LFTs and those with abnormal LFTs at the time of dismissal. This approach also offers the possibility of comparing results from these liver histopathological examinations in ALS patients with those of adequate concurrent controls, that is, non-ALS patients with neither biochemical alterations of the liver nor undergoing treatment with RIL.

However, eventually, the confirmatory diagnosis of potential liver injury induced by the drug, should be based on needle aspiration liver Bx with light and electron microscopic and histochemical evaluations. This recommendation should be considered in selected cases to be determined by the hepatologist in consortium with the other specialists. Whatever the injury, one needs to know whether, with continuous treatment, it gets worse, better or stays the same over time.

- c. Full characterization of the effects of the drug on liver function.

No one input can or should be given disproportionate weight in the evaluatory mechanism but owing to the too scarce available information, the sponsor's proposed measurement of serum ALT alone seems inadequate. In addition to the biochemical parameters listed under a. above, the following tests of global liver function are suggested before drug administration and after (varying) periods of dose exposure (2, 16 weeks; 6 and 12 months). The purpose of these evaluations is to demonstrate mild to moderate degrees of liver injury that, although would not be of clinical concern and would not necessarily necessitate discontinuation of the drug, could be missed with biochemical parameters, especially if the ALT is used alone. The results of biochemical parameters say "go ahead, everything is normal". Those of global liver function say "be cautious".

- a) Serum bile acid concentrations

Serum bile acids, especially conjugates of cholic acid, represent a very sensitive index of mild hepatic dysfunction.

- b) Determination of monoethylglycine-xylidide (MEGX)

MEGX is a lidocaine metabolite. This approach is presently used to measure liver function following drug treatment in patients with chronic liver disease and in evaluations around liver transplantation.

- d. Gathering of information to ascertain whether liver dysfunction (moderate vs severe by Child-Pugh classification) has an influence on the PK/PD on the compound.

Although these short-term exposures cannot be used to assess the potential of this drug to cause chronic liver disease, they are important re: the question of whether adjustment of the dose is needed in those patients with liver dysfunction.

- e. Additional studies to determine the potential of the drug to induce liver injury.

The purpose of these assessments is to expand the human data. These evaluations include:

- i) studies in isolated perfused rat liver to evaluate effects of grading amounts of compound on function, such as bile flow and histological changes;

and

- ii) studies employing isolated hepatocytes, among other things to determine if permeability of plasma membranes to ions is altered (by the compound) and to assess effects of the drug on bile acid uptake and release.

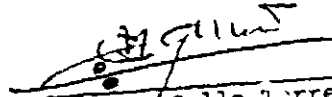
- f. Gathering data that can be used to provide an improved basis for estimation of human risk.

Included should be ADME studies, [which have presumably been already carried out?]. These data should provide answers to the following pertinent questions.

- Is there an entero-hepatic circulation of drug/metabolites?
- Is there accumulation of the drug in the liver?
- What effects do renal and hepatic function (diseases; see above) have in the handling of the drug by the body?
- Do gender and/or age have an influence on the disposition of the drug?

It is possible that answers to these important questions have already been provided in the NDA, but this information was not specifically included in sponsor's vol. 179 of the NDA (ISS).

September 11, 1995


Hugo E. Gallo-Torres, M.D., Ph.D.

CC:
NDA 20-599
HFD-180/SFredd
HFD-180/HGallo-Torres
HFD-181/CSO Consult File
r/g 9/7/95 jgw
e/t 9/11/95 jgw
MED\C\20599509.DRG

9/11/95
Noted. To HFD-120.
PR

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

Date: August 14, 1995
From: David Hoberman, Ph.D., HFD-713
Thru: Satya Dubey, Ph.D.
Chief, Statistical Evaluation and Research Branch (HFD(713))
Subject: Riluzole (NDA# 20-599)
To: File (NDA# 20-599)

The purpose of this memo is to indicate that:

- 1) The 3 attached documents comprise this reviewer's reviews for the Rilutek NDA:
 - a) A review of Study 216, dated May 20, 1994,
 - b) A review of Study 301 which includes overall conclusions based upon both trials, dated June 2, 1995, and
 - c) An addendum to the Review of Study 216 regarding issues in that study arising subsequent to the review of May 20, 1994.

- 2) At the bottom of page 3 in the Study 301 review, there is reference to the use of the Kolmogorov-Smirnov test for the purpose of comparing treatment groups' survival curves. Contrary to the speculation of the reviewer, subsequent ability to perform the test does not provide evidence of a difference between the groups.



David Hoberman, PH.D.

concur: Dr. Nevius *DH for EN*
Dr. Dubey *SD for SD*

cc: Arch NDA# 20-599
HFD-120
HFD-120/PLeber, RKatz, JFeeney, KHiggins
HFD-713/SDubey [File: DRU 1.3.2]
HFD-713/Group2 file

REVISION

Statistical Review and Evaluation

IND#

JUN 2 1995

Applicant: Rhone-Poulenc Rorer

Name of Drug: Rilutek (riluzole)

Document Reviewed: Volume with cover letter dated March 23, 1995

Medical officer: John Feeney, M.D., HFD-120

Background

The sponsor has submitted the results of two randomized, double-blind, placebo controlled trials (Studies 216 and 301) for the purpose of supporting the efficacy and safety of riluzole in patients with ALS (Amyotrophic Lateral Sclerosis). A previous review dated May 20, 1994 and addendum dated June 2, 1995 describe the design and results of Study 216. The current document reviews Study 301 and presents overall conclusions.

Study 301

This trial randomized a total of 959 patients among 31 centers in 7 countries: 9 in France, 1 in Belgium, 8 in the U.K., 4 in Germany, 1 in Spain, 5 in the U.S.A. and 3 in Canada. There were four (4) treatment arms: placebo, 50 mg/day, 100 mg/day, and 200 mg/day. Table 1 displays the distribution of patients by country. France was most represented with 44% of the sample. The French investigators in this trial were substantially the same as those in Study 216, thus raising doubts about the independence of the two trials. Patients in the U.S. and Canada were the last to be enrolled. The study includes patients randomized between 12/17/92 and 11/9/93.

Table 2 displays premature discontinuations from treatment. Approximately 15 % of all patients discontinued due to their lack of efficacy or adverse experiences.

Table 3 displays treatment group sample sizes and baseline demographics. There were approximately 250 patients per group.

Table 4 displays the distribution of disease features at baseline and Table 5 displays functional characteristics (disease severity) at baseline.

Patients were nearly all white, 60% were male, and the average age was almost 60 years. Virtually all had a diagnosis of definite or probable ALS. Two-thirds of the patients were first diagnosed with limb (as opposed to bulbar) onset between 0 and 5 years (average 2 years) before entering the trial. The average vital capacity ratio at baseline was nearly 90%. With respect to categorical functional scales, the average muscle testing score was nearly 90 out of a maximum of 110 (22 muscles), the average bulbar Norris score was 30 out of a maximum of 39, and the

average limb Norris score was nearly 45 out of a maximum of 63. There were no clear imbalances with respect to baseline variables between treatment groups at baseline.

The primary endpoint was patient death or tracheostomy or intubation with artificial ventilation, while the primary treatment comparison was 100 mg vs placebo. All patients were followed for approximately 18 months with a calendar data cutoff date of December 31, 1994. The protocol called for 150 patients per group in order to detect a 15% difference between the placebo and 100 mg riluzole group using a one-sided logrank test at a level of 5% and with a power of 85%. This assumed 65% failure in the placebo group at the end of the 18 month study period.

Supplementary assessments included the Norris Bulbar Scale for 13 tests on a 0(none)-3 scale(normal) scale, the Norris Limb Scale for tests on 21 daily activities (0-3), and segmental muscle strength testing of 22 muscles, each on a 0-5 scale. Other assessments included respiratory function tests (vital capacity and FEV1 ratios), a Clinical Global assessment and various Visual Analogue Scales.

The primary analysis was the logrank test stratified on limb or bulbar onset, as opposed to symptoms at baseline. In addition, the sponsor has conducted Cox model analyses for 1) a dose response trend test, 2) a test pooling the three riluzole groups, and 3) a somewhat different version of a stepwise variable selection procedure specified in the protocol. It incorporated all treatment groups with appropriate dummy variable. The final list of covariates was age, disease duration, VC ratio, muscle testing scale, VAS fatigability, weight, geographical region, VAS stiffness, disease form at baseline and CGI severity .

It is important to note that for both original onset and baseline determinations of disease form, a 'bulbar' patient is one who had any bulbar involvement as assessed by an investigator, whereas a 'limb' patient had no bulbar involvement. These determinations were not made using the Norris scores. Consequently, of the 349 patients listed as being 'limb' patients at baseline, there are 150 with scores under the maximum (best) bulbar score of 39. All but 40 have scores of at least 37. There were 35 patients excluded for the purpose of defining an 'efficacy evaluable' population. Only results of the intent-to-treat analyses will be discussed in this review.

Results

Table 6 displays the results of time-to-event endpoints for events which occurred within 18 months of randomization and before the data cutoff of December 31, 1994. Eight (8) patients' statuses (1 PBO, 3 R50, 2 R100, 2 R200) were unknown as of the data cutoff and were censored as non-failures as of the data cutoff, not as of the date of the last known status. Table 7 displays the p-values for the stratified logrank and generalized Wilcoxon tests.

Figure 1 displays the Kaplan-Meier curves comparing 'failure' distributions in the placebo vs the 3 separate dose groups and the groups combined. The survival comparisons shown which reached nominal statistical significance resulted from the Wilcoxon statistic with only the

100 mg dose group and when all doses were pooled.

Table 8 separates survival rates by disease form at onset. Survival is greater in those who were diagnosed with little or no bulbar symptoms. It also appears that the 'survival' (i.e., non-failure) differences between 100 mg and placebo in the two strata are similar (Bulbar: 45.1% vs 36.5% and Limb: 61.8% vs 56.5%).

Discussion of Overall Survival Analysis

Figure 2 displays the Kaplan-Meier plots for failure in the placebo, 100 mg, and 200 mg arms.

Figures 3 and 4 display the K-M plots for France and outside France, respectively. This geographical distinction is of interest since the original protocol designated France and then centers from other countries were added. Note that the curves stay separated in France but come together after 18 months outside France. Since the profiles of the active groups appear similar, those groups have been pooled in the following. Mortality outside France was similar in the active and placebo groups (36% and 39%, respectively). To the extent that there is a 'treatment effect', it appears in France where the mortalities were 61% and 52%, respectively. The reason for the different levels of mortality between French and non-French centers is not clear. Centers outside France were enrolled after those in France, so that median follow up is somewhat less outside France. However, the K-M curves still indicate substantially less mortality outside France at 18 months (Figures 4 and 5).

Another way to display the primary failure profiles is by estimating the 'hazard', or instantaneous failure rate given survival up to a particular time point, for each treatment group. For purposes of this analysis, an actuarial window of 15 days has been used. Figure 5 displays the hazard functions for placebo and the two combined riluzole arms. Note the tendency for the hazards to increase with time for both groups. Figures 6 and 7 decompose the total hazard function into French and outside-France components, respectively. In France, the placebo hazard remains greater than riluzole's much of the time. However, outside France, where the survival curves merge at approximately 18 months, the hazards cross, whereby the placebo hazard is greater than riluzole's for the first 250-300 days, but the reverse is predominantly true from then on. Also, instead of the placebo hazard increasing for the last part of the study as in France, the placebo hazard outside France stays quite moderate in comparison. In terms of survival, the placebo group outside France did at least as well as the riluzole groups in France.

To the extent that there is evidence that survival may be similar at 18 months but different until then, the logrank test is weak due to the crossing hazards since it is based upon a test of equality of hazards, not the survival distributions, directly. Under these circumstances, the logrank and Wilcoxon tests are less powerful than, say, a two-sample Kolmogorov-Smirnov test which compares the survival curves directly and is not a function of the relative positions of the two hazard functions. Unfortunately, this reviewer has not been able to find a source which gives the null distribution for this statistic. However, one could be confident that it would be statistically

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3 OF 7

significant since the much less powerful ones are nearly so.

Table 9 displays the results of the Cox regression with selected covariates. Note that the p-values for 100 mg and 200 mg have decreased by more than an order of magnitude.

Table 10 displays the p-values comparing treatments for only death as an endpoint. The 100 mg dose and all doses pooled are statistically significant.

Discussion of the Cox Regression with Covariates

The p-values of .002 and .0004 for the 100 mg and 200 mg groups, respectively, appear to strengthen the evidence of a treatment effect. However, this result relies upon 1) very small numerical differences in baseline values and 2) an adjustment in location and not a reduction in standard error. The table below lists the baseline means of covariates for the 100 mg and placebo groups:

	<u>Placebo</u>	<u>100 mg</u>
age	56.0	56.9
disease duration	1.8 yrs	1.7 yrs
vc ratio	87.6	88.4
muscle testing scale	87.9	89.1
vas fatigability	43.3	44.5
vas stiffness	27.7	26.8
weight	68.1	68.1
form at inclusion	69.4% bulbar	60.6% bulbar
CGI severity	4.0	4.0

Based upon the results of the model, baseline imbalances were 'biased against' the 100 mg group for age, disease duration, vas fatigability and vas stiffness. Baseline imbalances were 'biased against' the placebo group for vital capacity, muscle testing scale and disease form at inclusion.

The table below displays the parameter estimate for treatment effect and its standard error for three models: I) includes all covariates, II) includes only those biased against 100 mg, and III) includes only those biased against placebo.

	<u>Estimate</u>	<u>Standard error</u>	<u>p-value</u>
Model I	-.48	.15	.001
Model II	-.46	.14	.001
Model III	-.21	.14	.120

The point of this exercise is to show that the very low p-value resulting from the covariate analysis depends only upon very small baseline differences in a few covariates while having essentially no effect upon reducing 'noise'. Consequently, the model may be 'oversensitive' in the sense that the p-value resulting from adjustment is not reliable. The logrank or Wilcoxon analysis both rely only upon the randomization principle and not upon the vagaries of covariate distributions and complicated modeling whose misspecification may distort the effect of small baseline differences.

The Effect of Limb vs Bulbar Onset Strata, Status at Entry, and Norris Scores

Investigators retrospectively assessed the onset form for each patient. In addition, they classified a patient at entry as being 'limb' or 'bulbar'. This design induces three separate cohorts: 1) those who were 'limb' at onset and 'limb' at baseline (supposedly or no bulbar symptoms, (L)), 2) those who were 'limb' at onset and who developed bulbar symptoms between onset and entry into the study, (L,B), and 3) those who had bulbar symptoms at onset (B). The table below displays the distributions of disease duration for these subgroups (proportion of patients):

		0	1	2	3	4	5
(N=348)	L	8	40	26	12	10	14
(N=315)	L,B	5	36	29	16	10	4
(N=294)	B	13	53	23	8	3	1

Note that average duration of the 'B' patients is substantially less than those in the other two groups. The table below displays the median slopes of deterioration (points/year) for the Norris Limb and Bulbar Scores, and the Muscle Testing Scores for each year of disease duration.

	0	1	2	3	4	5
Limb	29	21	17	11	11	7
Bulbar	15	10	5	4	2	1
Muscle	35	22	17	12	11	11

Clearly, patients who have had ALS longer tend to have less deterioration than those who have recently acquired the disease. Two influences may be at work: 1) Due to entrance criteria which require a certain level of functioning, those who have had the disease longer cannot be progressing too rapidly in order to qualify for entry, and 2) patients who are still alive after some time cannot be progressing too rapidly in order to still be alive at all.

The table below displays the mean baseline functional scores (Muscle and Norris Limb & Bulbar), and corresponding slopes of deterioration for each of the subgroups:

<u>Scale</u>	<u>Group</u>	<u>Mean baseline</u>	<u>Median Slope over Double-Blind Period</u>
Limb	L	44.6	16.8
	L,B	36.2	16.0
	B	54.0	18.2
Bulbar	L	38.1	1.9
	L,B	32.7	8.2
	B	20.6	11.7
Muscle	L	87.1	17.5
	L,B	81.2	20.1
	B	97.9	15.2

Note that despite the variation in baseline limb scores, the rate of deterioration is the same in each subgroup. Also, there is little if any deterioration in bulbar scores among patients without bulbar symptoms at entry. Deterioration in Muscle testing scores is similar among the subgroups.

The reason for inquiring into these subgroups arises from the observation that there was no survival advantage in patients with **limb onset** in Study 216. Evaluation of disease form at baseline in these studies permits an examination of disease form as a covariate at baseline, presumably a more relevant time to assess the effect of symptoms on survival during the study. In fact, among 'limb' patients at baseline, there was absolutely no difference in failure between the placebo and 100 mg groups (32% vs 33%, respectively) in Study 301. This result is in contrast to that reported by the sponsor in which the subgroup with limb only involvement at onset had similar treatment effects in the limb and bulbar randomization strata: (bulbar: 63.5% vs 54.9%, respectively; limb: 45.5% vs 38.2%, respectively). **The discrepancy lies in the fact that the patients who were originally only 'limb' at onset have progressed to bulbar symptoms and therefore are classified as 'bulbar' at baseline. Thus, formerly 'limb' patients contribute to the treatment effect in 'bulbar' patients.**

One question naturally arises: Is there **any** benefit of the drug to patients who remain with only limb involvement at baseline? For instance, does the drug delay progression to bulbar involvement? For these purposes, we defined a 'limb' patient to be one whose Norris bulbar score was at least 37. All riluzole patients were pooled resulting in 64 placebo patients and 236 riluzole patients. A Mantel-Haenszel (logrank) statistic was computed to test 'time to first score below 37.' The result was not statistically significant (p-value approximately .30).

Alternatively, one could ask: Is there a drug effect on any progression of bulbar scores in the 'limb' subgroup? The median slopes of deterioration were 3.8 in the placebo group and 1.7 in the 100 mg riluzole group. The other riluzole groups had similar median slopes. In addition, there was no evidence of a treatment effect on rate of deterioration of bulbar scores among patients who had bulbar involvement at baseline.

Functional Scores

Table 11 displays the results of the sponsor's analyses of variance for each of the three functional scores. The model included treatment onset form of disease, group of countries, and the first order treatment interactions. Interaction terms were removed if their p-value was greater than .15.

Although the sponsor's results indicate scattered statistical significances with some occurring among '12 month completers' (an unplanned analysis), this reviewer has taken the more straightforward approach of using a model with only treatment, onset form of disease, and their interaction.

With respect to bulbar scores, all pairwise comparisons with placebo had p-values above .50. The interaction p-value was significant partly because among the riluzole groups, the slopes appear to be greater in the bulbar stratum.

With respect to Muscle Testing Scores, there was no evidence whatever of a treatment effect, all p-values being above .70.

With respect to limb scores, the only statistically significant pairwise comparison was 100 mg vs placebo. A significant interaction test reflected the fact that the rate of deterioration in the limb stratum of the 100 mg group was greater than in the bulbar stratum, while the opposite relation occurred in the placebo group.

Pulmonary Function

Vital Capacity and FEV1 ratios were measured at months 0, 6, 12, and 18. Due to the large number of failures, there is too much missing data to make reliable inferences about the drugs effect on the entire population. However, two descriptive analyses may be useful: 1) compare the pulmonary endpoints in 'completers', i.e. those who completed approximately 18 months and were alive at the data cutoff, and 2) compare the last measurement of those alive with at least 1 year of follow up.

With respect to 'completers', the table below displays the mean vital capacity ratio by treatment group and disease form at baseline:

<u>Treatment Group (N)</u>	<u>Mean Vital Capacity Ratio</u>
Placebo (70)	Overall: 65% Limb: 71% Bulbar: 58%
50 mg (69)	Overall: 69% Limb: 79% Bulbar: 59%
100 mg (77)	Overall: 61% Limb: 66% Bulbar: 55%
200 mg (81)	Overall: 72% Limb: 80% Bulbar: 63%

There is no statistical evidence that patients in this cohort benefit from the drug.

The table below displays the treatment means of the last measurement for the expanded cohort of those who were alive at the end of their participation in the study and who had **at least 12 months of follow up**.

<u>Treatment Group(N)</u>	<u>Mean Vital Capacity Ratio</u>
Placebo(168)	68%
50 mg (162)	73%
100 mg(178)	66%
200 mg(185)	74%

Discussion

- 1) The sponsor's primary analysis (stratified logrank test) of the primary endpoint (death, tracheostomy or intubation) comparing 100 mg/day to placebo was not statistically significant at the two-sided 5% level. However, the sponsor has reported two supplementary analyses which did reach statistical significance (pooling riluzole groups and 'dose response' including the placebo group. This reviewer found that when the logrank test is stratified by disease form at **baseline**, the p-value was .16 and when disease form at baseline is treated as a covariate in the Cox model and that analysis is stratified by disease form at **onset**, the p-value is .13. Recall that starting at baseline, the comparison was biased against placebo due to a greater proportion of placebo patients with bulbar symptoms at baseline (69% vs 61%).
- 2) The very low p-value resulting from the Cox regression model is too sensitive to small baseline imbalances to qualify as a definitive result.
- 3) When only death is the endpoint, the stratified logrank test achieves significance ($p=.03$).
- 4) The statistical evidence of effect on functional scores is very scanty despite the fact that the analyses of slopes of deterioration would have incorporated 'informative censoring'. That is, even though there is a suggestion of survival benefit for those taking riluzole, that benefit is not reflected (on average) in any difference in rates of deterioration until failure when all patients are included in the analysis.
- 5) There is no reliable evidence that the drug is effective in improving or delaying the decline of vital capacity in patients alive after at least 1 year of follow up (72% of the randomized patients).
- 6) In Study 216, little evidence of a survival benefit appeared in the stratum of 'limb' patients at onset (56.5% riluzole 100 mg vs 52.5% placebo at 18 months follow up). Approximately the same numerical survival difference occurred in 'limb onset' patients in Study 301 (61.8% riluzole 100 mg vs 56.5% placebo). One interesting finding is that there was no treatment difference in survival among patients with only the limb form of disease at baseline in Study 301 (67% riluzole 100 mg vs 70% placebo). In Study 216, the survival difference among patients who were described as 'limb' at baseline was 70% for riluzole 100 mg and 60% for placebo. See Figure 8.
- 7) Since some 'uniform' treatment effect appears not to occur (such as a hazard ratio would be if the ratio of the hazards were constant over 18 months), one could choose an arbitrary point in time to look at the difference in percent mortality or pick a percentile with its associated difference in survival time. For instance, the use of a hazard ratio would be misleading because 1) nothing is known about survival after 18 months and 2) an apparent lack of proportional hazards in a substantial geographical subgroup. By pooling the similar results of the 100 mg and 200 mg groups in Study 301 with respect to the primary endpoint,

one can estimate the 18 month survival of patients on at least 100 mg riluzole to be approximately 6% greater than that on placebo (54% vs 48%). The 95% confidence interval for the true difference is (-4%, 16%). Alternatively, there appears to be a difference of approximately 60 days at the 75th percentile of survival. Thus, when 25% of the patients have failed, those on the drug lived approximately two months longer.

Overall Conclusions

Taking both Study 216 and Study 301 into account, we may conclude that:

- 1) Over 18 months, survival is greater on riluzole than on placebo for much of the time; however, there is a tendency for probabilities of survival to be similar near the end of follow up. In both trials, tests of mortality differences at approximately 18 months do not reject the null hypothesis of no difference. Due to crossing hazard functions it is not possible to state a **uniform** treatment benefit over the courses of the trials. **Ultimately, therefore, any controversy over the results of logrank tests vs Wilcoxon tests is misplaced because the data suggests relative survival patterns which render both of these statistics inappropriate for the question of whether the survival curves are identical. The real issue is how to describe the manifest differences in a clinically meaningful way.**
- 2) Although any effect on survival appears to predominate in patients with whose onset showed evidence of bulbar symptoms, the benefit relative to those with only limb symptoms at onset has not been determined.
- 3) There are no statistically significant results with respect to functional scales or pulmonary function with the exception of slopes of deterioration of muscle strength in Study 216. However, the endpoint analysis was not significant at 3/12/92.



David Hoberman, Ph.D.
Mathematical Statistician

Concur: Dr. Nevius *SEN 6-2-95*

fn Dr. Dubey *SEN 6-2-95*

orig: IND#
HFD-120
HFD-120/Dr. Feeney
HFD-120/Dr. Katz
HFD-120/Dr. Leber

Table 1: Distribution of patients by country

COUNTRY	Placebo	R 50 mg	R 100mg	R 200mg	All
	N (%)	N (%)	N (%)	N (%)	N (%)
FRANCE	109 (25.8)	104 (24.6)	103 (24.4)	106 (25.1)	422 (44.0)
BELGIUM	6 (27.3)	6 (27.3)	4 (18.2)	6 (27.3)	22 (2.3)
UK	51 (24.5)	51 (24.5)	52 (25.0)	54 (26.0)	208 (21.7)
GERMANY	24 (25.3)	24 (25.3)	23 (24.2)	24 (25.3)	95 (9.9)
SPAIN	6 (25.0)	6 (25.0)	6 (25.0)	6 (25.0)	24 (2.5)
USA	30 (25.0)	30 (25.0)	30 (25.0)	30 (25.0)	120 (12.5)
CANADA	16 (23.5)	16 (23.5)	18 (25.6)	18 (26.5)	68 (7.1)
ALL	242 (25.2)	237 (24.7)	236 (24.6)	244 (25.4)	959 (100)

Table 2: Premature discontinuations from treatment

REASON FOR TREATMENT DISCONTINUATION	Placebo	R 50 mg	R 100 mg	R 200 mg	All
	N (%)	N (%)	N (%)	N (%)	N (%)
ADVERSE EXPERIENCES					
- clinical	20 (8.3)	20 (8.4)	25 (10.6)	27 (11.1)	92 (9.6)
- laboratory	7 (2.9)	3 (1.3)	9 (3.8)	9 (3.7)	28 (2.9)
LACK OF EFFICACY	13 (5.4)	11 (4.6)	14 (5.9)	8 (3.3)	46 (4.8)
CONSENT WITHDRAWN	10 (4.1)	10 (4.2)	3 (1.3)	6 (2.5)	29 (3.0)
OTHER	0	4 (1.7)	3 (1.3)	3 (1.2)	10 (1.0)
ALL	50 (20.7)	48 (20.3)	54 (22.9)	53 (21.7)	205 (21.4)

Table 3: Patient characteristics at baseline: Demographics

	Placebo	R 50 mg	R 100 mg	R 200 mg	All
ALL PATIENTS COMBINED					
N	242	237	236	244	959
SEX M/F	152/90	144/93	143/93	136/108	575/384
AGE (years)*	56.0 ± 0.7	57.1 ± 0.7	56.9 ± 0.7	56.8 ± 0.7	56.7 ± 0.4
RACE (%)					
- Caucasian	98.3	98.3	97.9	96.7	97.8
- Black	0.4	0.4	1.3	1.2	0.8
- Oriental	0.4	1.3	0.4	0.4	0.6
- Other	0.8		0.4	1.6	0.6
WEIGHT (kg)*	68.1 ± 0.9 (N = 234)	67.6 ± 0.9 (N = 228)	68.1 ± 0.9 (N = 226)	67.1 ± 0.7 (N = 237)	67.7 ± 0.4 (N = 925)
PATIENTS WITH BULBAR ONSET ALS					
N	74	71	71	79	295
SEX M/F	42/32	33/38	34/37	42/37	151/144
AGE (years)*	59.6 ± 1.2	59.6 ± 1.3	58.6 ± 1.1	57.8 ± 1.2	58.9 ± 0.6
WEIGHT (kg)*	65.9 ± 1.5 (N = 72)	63.1 ± 1.5 (N = 68)	65.3 ± 1.6 (N = 69)	66.1 ± 1.4 (N = 77)	65.1 ± 0.8 (N = 286)
PATIENTS WITH LIMB ONSET ALS					
N	168	166	165	165	664
SEX M/F	110/58	111/55	109/56	94/71	424/240
AGE (years)*	54.4 ± 0.9	56.0 ± 0.8	56.2 ± 0.9	56.4 ± 0.8	55.7 ± 0.4
WEIGHT (kg)*	69.1 ± 1.0 (N = 162)	69.6 ± 1.0 (N = 160)	69.3 ± 1.1 (N = 157)	67.5 ± 0.7 (N = 160)	68.9 ± 0.5 (N = 639)

* mean ± s.e.m.

Table 4 : Disease features at baseline

	Placebo	R 50 mg	R 100 mg	R 200 mg	All
ALL PATIENTS COMBINED					
N	242	237	236	244	959
ALS diagnosis:					
definite (%)	120 (49.6)	103 (43.5)	108 (45.8)	107 (43.9)	438 (45.7)
probable (%)	122 (50.4)	133 (56.1)	127 (53.8)	136 (55.7)	518 (54.0)
possible (%)		1 (0.4)	1 (0.4)	1 (0.4)	3 (0.3)
Form of ALS at onset					
bulbar (%)	74 (30.6)	71 (30.0)	71 (30.1)	79 (32.4)	295 (30.8)
limb (%)	168 (69.4)	166 (70.0)	165 (69.9)	165 (67.6)	664 (69.2)
Duration of disease (years)*	1.8 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.8 ± 0.0
Familial (%)	11 (4.5)	11 (4.6)	10 (4.2)	8 (3.3)	40 (4.2)
Sporadic (%)	231 (95.5)	226 (95.4)	226 (95.8)	236 (96.7)	919 (95.8)
Vital capacity ratio†	87.6 ± 1.2	88.6 ± 1.2	88.4 ± 1.2	88.2 ± 1.2	88.2 ± 0.6
PATIENTS WITH BULBAR ONSET ALS					
N	74	71	71	79	295
ALS diagnosis:					
definite (%)	51 (68.9)	55 (77.5)	52 (73.2)	59 (74.7)	217 (73.6)
probable (%)	23 (31.1)	16 (22.5)	19 (26.8)	20 (25.3)	78 (26.4)
Duration of disease (years)*	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1
Vital capacity ratio†	82.9 ± 2.1	81.9 ± 1.7	85.6 ± 2.2	81.7 ± 2.0	83.0 ± 1.0
PATIENTS WITH LIMB ONSET ALS					
N	168	166	165	165	664
ALS diagnosis:					
definite (%)	69 (41.1)	48 (28.9)	56 (33.9)	48 (29.1)	221 (33.3)
probable (%)	99 (58.9)	117 (70.5)	108 (65.5)	116 (70.3)	440 (66.3)
possible (%)		1 (0.6)	1 (0.6)	1 (0.6)	3 (0.5)
Duration of disease (years)*	2.0 ± 0.1	2.1 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	2.0 ± 0.1
Vital capacity ratio†	89.7 ± 1.4	91.4 ± 1.6	89.7 ± 1.5	91.3 ± 1.5	90.5 ± 0.7

* mean ± s.e.m.

Table 5: Patient functional characteristics at baseline: Disease severity

	Placebo	R 50 mg	R 100 mg	R 200 mg	All
ALL PATIENTS COMBINED					
MUSCLE TESTING SCALE* (max. score = 110)	87.9 ± 1.1 (N = 242)	88.6 ± 1.0 (N = 233)	89.1 ± 1.0 (N = 235)	88.7 ± 0.9 (N = 241)	88.5 ± 0.5 (N = 951)
BULBAR NORRIS SCALE* (max. score = 39)	30.3 ± 0.6 (N = 241)	31.6 ± 0.6 (N = 237)	31.1 ± 0.6 (N = 235)	31.0 ± 0.6 (N = 241)	31.0 ± 0.3 (N = 954)
LIMB NORRIS SCALE* (max. score = 63)	44.1 ± 1.0 (N = 242)	44.4 ± 1.0 (N = 235)	44.7 ± 1.0 (N = 234)	45.8 ± 0.9 (N = 243)	44.7 ± 0.5 (N = 954)
PATIENTS WITH BULBAR ONSET ALS					
MUSCLE TESTING SCALE* (max. score = 110)	98.7 ± 1.2 (N = 74)	96.8 ± 1.4 (N = 71)	98.5 ± 1.3 (N = 71)	97.6 ± 1.3 (N = 78)	97.9 ± 0.6 (N = 294)
BULBAR NORRIS SCALE* (max. score = 39)	20.4 ± 1.0 (N = 74)	20.8 ± 1.0 (N = 71)	21.6 ± 1.0 (N = 70)	19.9 ± 0.8 (N = 77)	20.6 ± 0.5 (N = 292)
LIMB NORRIS SCALE* (max. score = 63)	54.4 ± 1.1 (N = 74)	53.8 ± 1.5 (N = 70)	53.4 ± 1.4 (N = 71)	54.6 ± 1.1 (N = 78)	54.1 ± 0.6 (N = 293)
PATIENTS WITH LIMB ONSET ALS					
MUSCLE TESTING SCALE* (max. score = 110)	83.1 ± 1.3 (N = 168)	85.0 ± 1.2 (N = 162)	85.0 ± 1.1 (N = 164)	84.4 ± 1.1 (N = 163)	84.3 ± 0.6 (N = 657)
BULBAR NORRIS SCALE* (max. score = 39)	34.7 ± 0.5 (N = 167)	36.2 ± 0.4 (N = 166)	35.1 ± 0.5 (N = 165)	36.2 ± 0.4 (N = 164)	35.5 ± 0.2 (N = 662)
LIMB NORRIS SCALE* (max. score = 63)	39.5 ± 1.2 (N = 168)	40.4 ± 1.1 (N = 165)	41.0 ± 1.1 (N = 163)	41.6 ± 1.1 (N = 165)	40.6 ± 0.6 (N = 661)

* mean ± s.e.m.

Table 6: Failures by treatment group

TYPE OF FAILURE/GROUP	R 50 mg (n=237)		R 100 mg (n=236)		R 200 mg (n=244)		Placebo (n=242)	
	N	%	N	%	N	%	N	%
Tracheostomy Intubation*	5	2.1	12	5.1	11	4.5	10	4.1
Death	101	42.6	90	38.1	92	37.7	110	45.5
Failure	106	44.7	102	43.2	103	42.2	120	49.6

*Patients who died after having been tracheostomized or intubated are counted in the tracheostomy / intubation category.

Table 7: Intent to treat analyses

	Incidence of failures	Survival Rate (%)	Stratified Logrank	Stratified Wilcoxon	Hazard Ratio 95% C.I.
Placebo	120 / 242	50.4			
R 50 mg	106 / 237	55.3	0.25	0.23	0.85 (0.66, 1.11)
R 100 mg	102 / 236	56.8	0.076	0.050	0.79 (0.60, 1.02)
R 200 mg	103 / 244	57.8	0.075	0.061	0.79 (0.61, 1.03)
All doses	311 / 717	56.6	0.048	0.038	0.81 (0.66, 1.00)

Table 8 : Bulbar stratum

	Incidence of failures	Survival Rate (%)	Logrank	Wilcoxon	Hazard Ratio 95% C.I.
Placebo	47/74	36.5			
R 50 mg	37/71	47.9	0.16	0.15	0.73 (0.47, 1.12)
R 100 mg	39/71	45.1	0.13	0.053	0.72 (0.47, 1.10)
R 200 mg	45/79	43.0	0.33	0.29	0.81 (0.54, 1.23)
All doses	121/221	45.2	0.10	0.06	0.75 (0.54, 1.06)

Limb stratum

	Incidence of failures	Survival Rate (%)	Logrank	Wilcoxon	Hazard Ratio 95% C.I.
Placebo	73/168	56.5			
R 50 mg	69/166	58.4	0.71	0.62	0.94 (0.68, 1.31)
R 100 mg	63/165	61.8	0.29	0.24	0.83 (0.59, 1.16)
R 200 mg	58/165	64.8	0.13	0.13	0.77 (0.54, 1.08)
All doses	190/496	61.7	0.22	0.18	0.85 (0.65, 1.11)

Table 9 : Cox model

	Estimate	p-value	Risk ratio	Lower 95% C.I.	Upper 95% C.I.
50 mg	-0.27	0.044	0.76	0.59	0.99
100 mg	-0.43	0.002	0.65	0.50	0.85
200 mg	-0.49	0.0004	0.61	0.47	0.80
age	0.04	0.0001	1.04	1.03	1.05
disease duration	-0.40	0.0001	0.67	0.61	0.74
vital capacity ratio	-0.02	0.0001	0.98	0.97	0.98
muscle testing scale	-0.02	0.0001	0.98	0.97	0.99
VAS fatigability	0.01	0.0001	1.01	.01	1.01
weight	-0.01	0.0046	0.99	0.98	1.00
other European countries	-0.38	0.0018	0.68	0.54	0.87
US / Canada	-0.36	0.0178	0.70	0.52	0.94
VAS stiffness	-0.005	0.0242	1.00	0.99	1.00
form at inclusion	0.28	0.0333	1.32	1.02	1.71
CGI severity	0.14	0.0441	1.15	1.00	1.32

Table 10: Death as endpoint

	Incidence of failures	Survival Rate (%)	Stratified Logrank	Stratified Wilcoxon	Hazard Ratio 95% C.I.
Placebo	116/242	52.1			
R 50 mg	104/237	56.1	0.29	0.26	0.86 (0.66, 1.13)
R 100 mg	94/236	60.2	0.029	0.023	0.74 (0.56, 0.97)
R 200 mg	100/244	59.0	0.075	0.054	0.79 (0.60, 1.03)
All doses	298/717	58.4	0.035	0.027	0.79 (0.64, 0.99)

Table 11: Norris Bulbar scale- Rate of deterioration (all measures)

	All			Bulbar				Limb			
	n	mean	sem	n	mean	sem	p	n	mean	sem	p
Placebo	233	-11.1	0.8	67	-11.7	1.5		166	-10.8	0.9	
R 50 mg	223	-9.7	0.8	67	-14.2	1.2	0.19	156	-7.7	1.0	0.02
R 100 mg	226	-9.6	0.7	66	-12.5	1.1	0.67	160	-8.5	0.9	0.08
R 200 mg	235	-10.1	0.8	74	-15.1	1.5	0.06	161	-7.8	0.9	0.02

Norris Limb scale- Rate of deterioration during treatment

	ALL			Bulbar				Limb			
	n	mean	sem	n	mean	sem	p	n	mean	sem	p
Placebo	230	-24.1	1.5	66	-28.4	4.1		164	-22.3	1.4	
R 50 mg	221	-22.7	1.6	67	-25.7	3.1	0.53	154	-21.4	1.8	0.67
R 100 mg	218	-20.1	1.3	65	-17.4	2.2	0.01	153	-21.3	1.5	0.67
R 200 mg	221	-22.1	1.5	70	-26.9	2.9	0.68	151	-20.0	1.7	0.31

Muscle testing - Rate of deterioration during treatment

	All				Bulbar			Limb		
	n	mean	sem	p	n	mean	sem	n	mean	sem
Placebo	231	-24.0	1.7		67	-23.4	3.7	164	-24.2	1.9
R 50 mg	220	-23.3	1.8	0.82	67	-22.1	3.4	153	-23.8	2.1
R 100 mg	219	-24.4	1.6	0.83	65	-20.7	3.0	154	-26.0	1.9
R 200 mg	221	-23.1	1.7	0.76	69	-21.9	3.1	152	-23.7	2.0

FIGURE 1

Kaplan-Meier survival curves

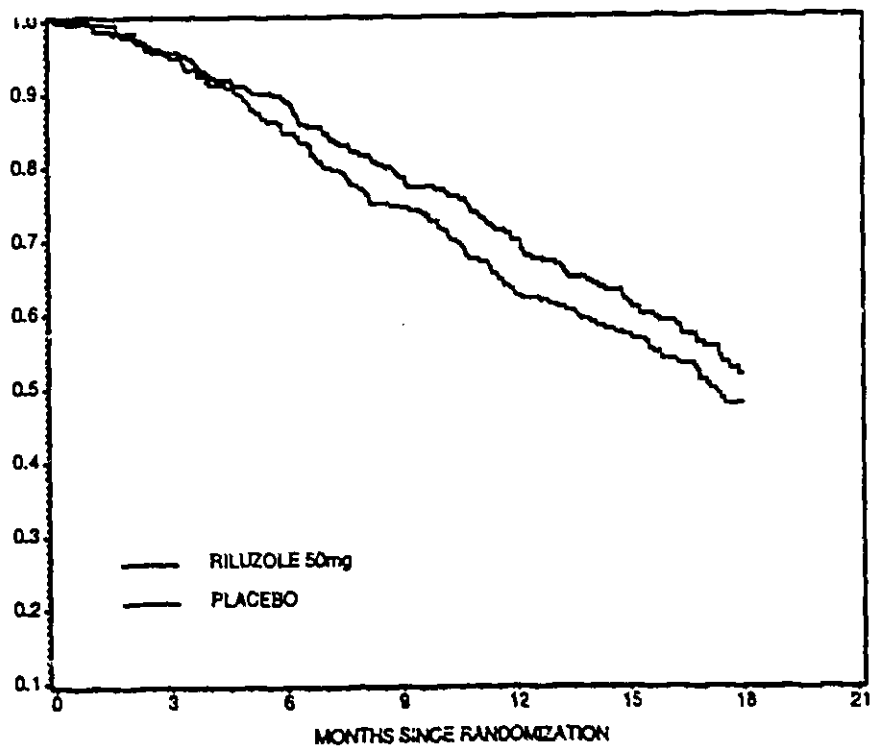
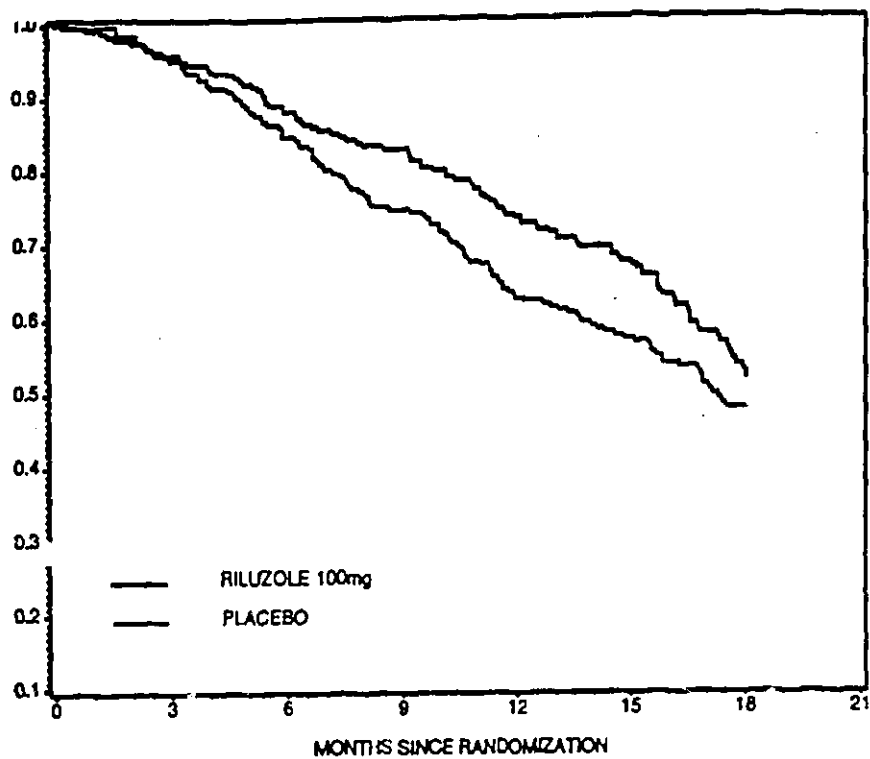


FIGURE . (cont)

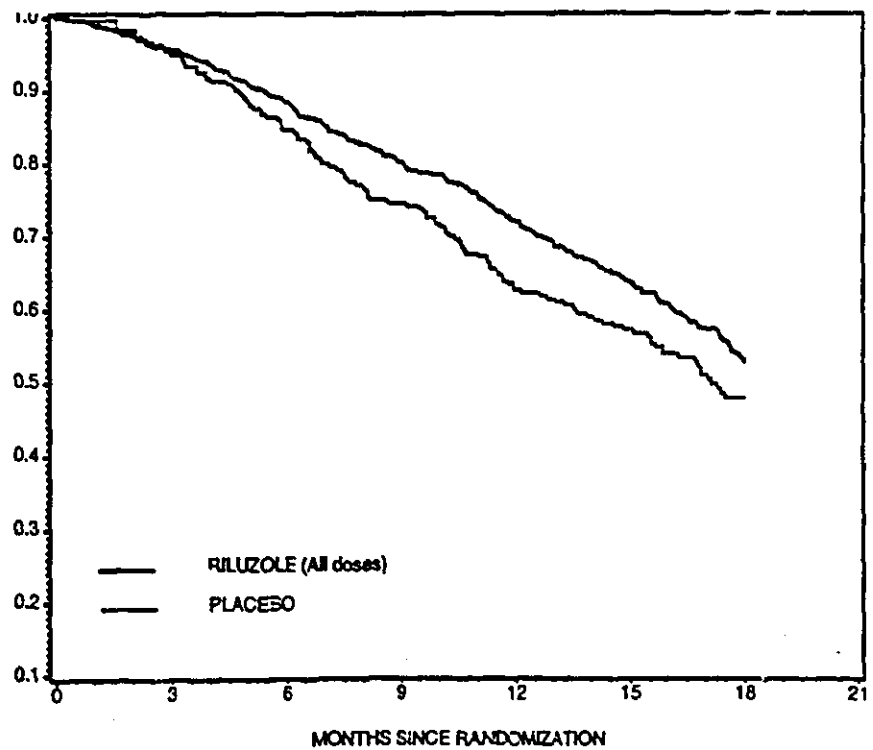
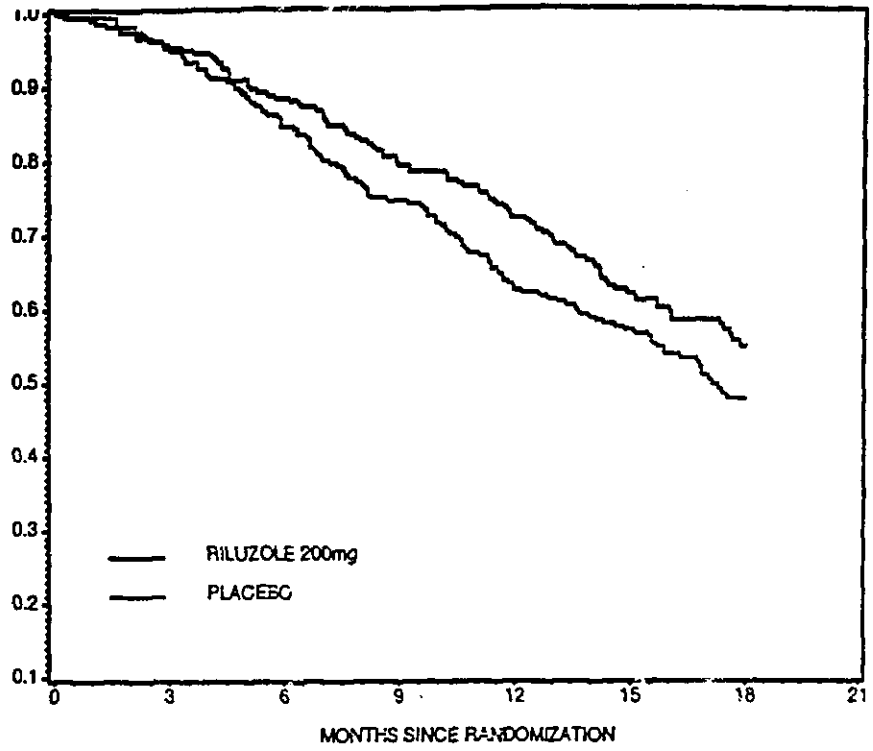


FIGURE 2

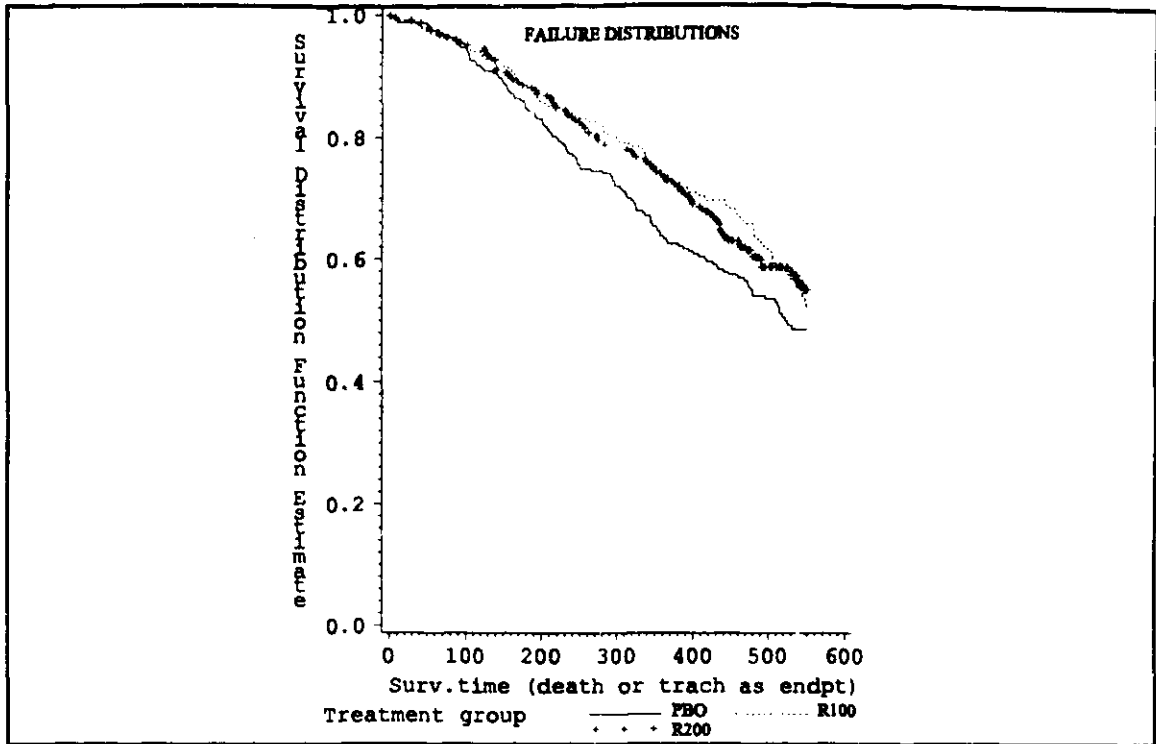


FIGURE 3

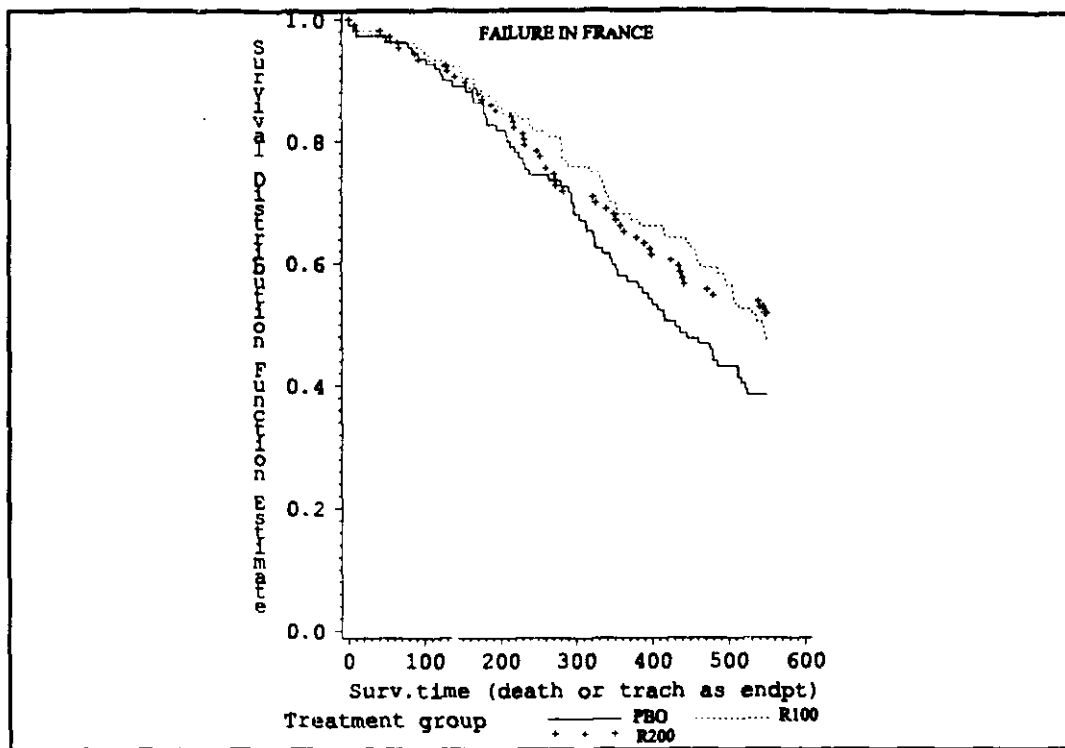


FIGURE 4

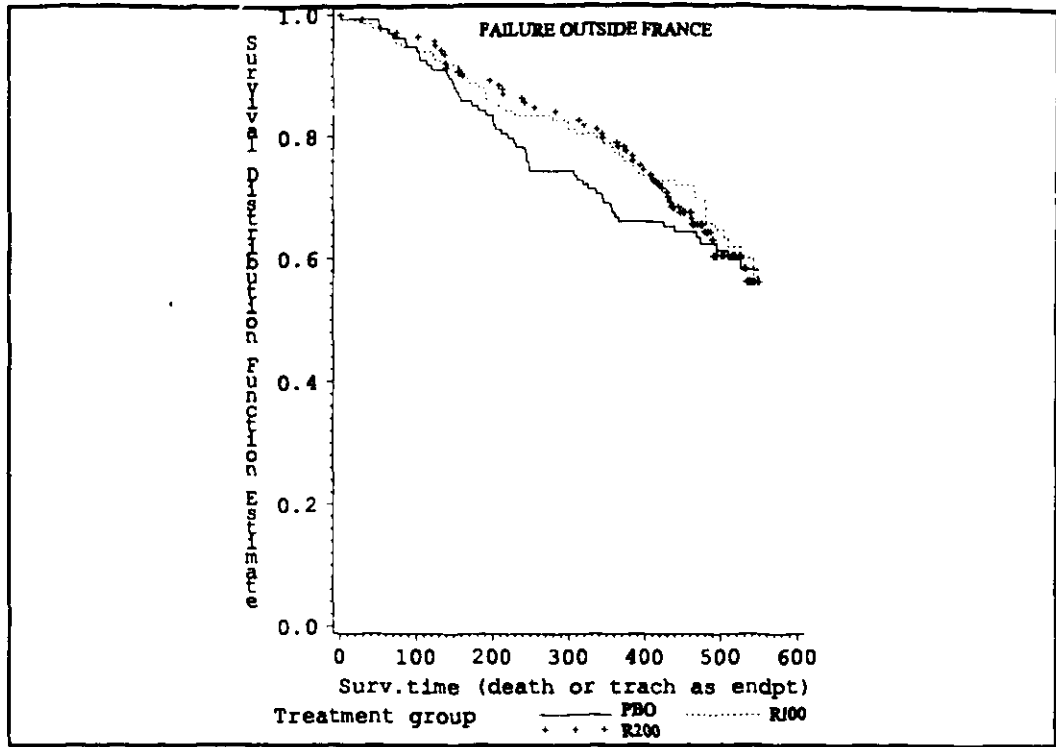


FIGURE 5

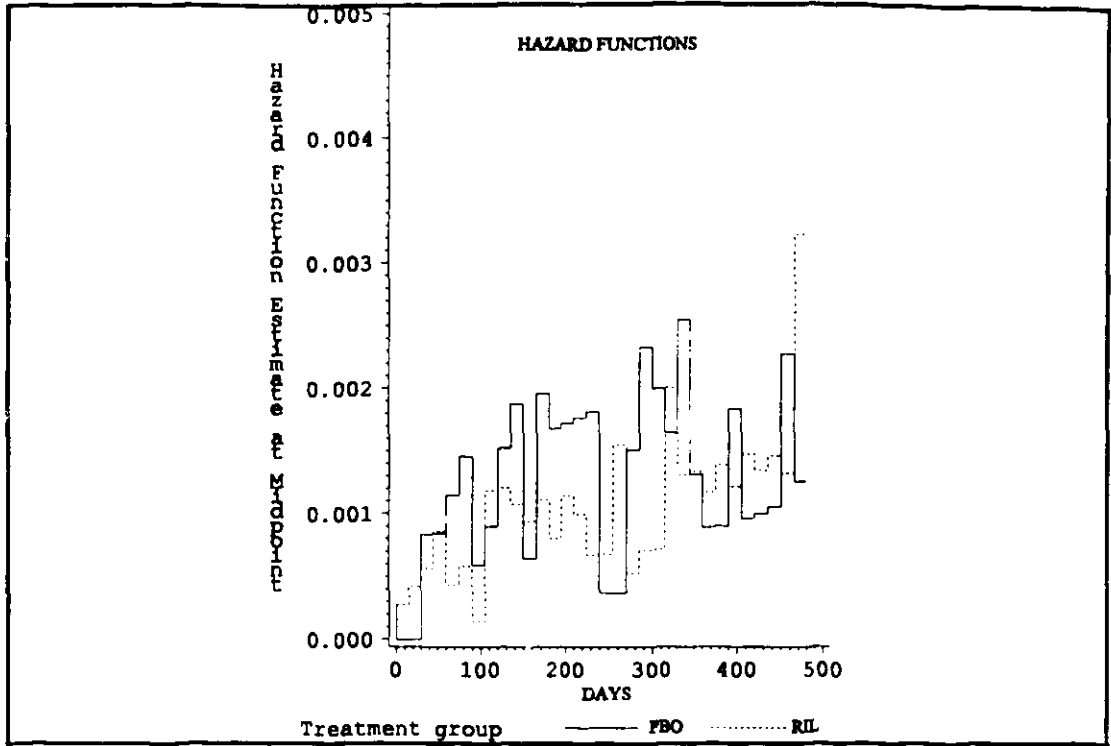


FIGURE 6

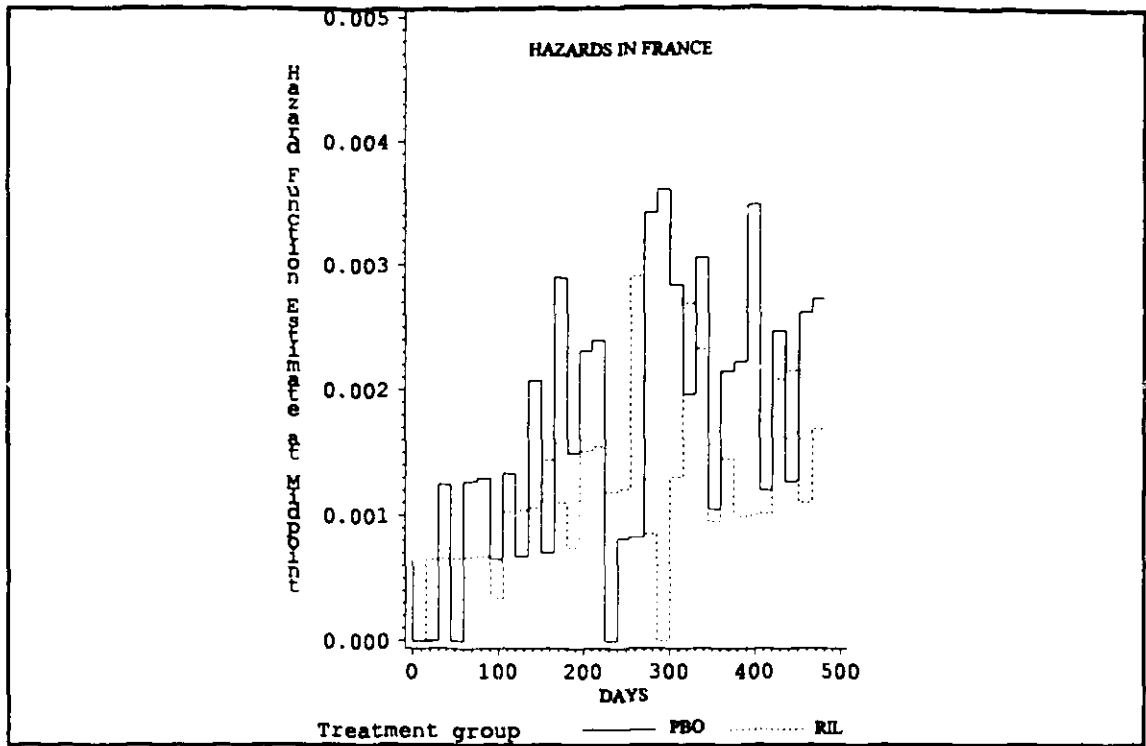


FIGURE 7

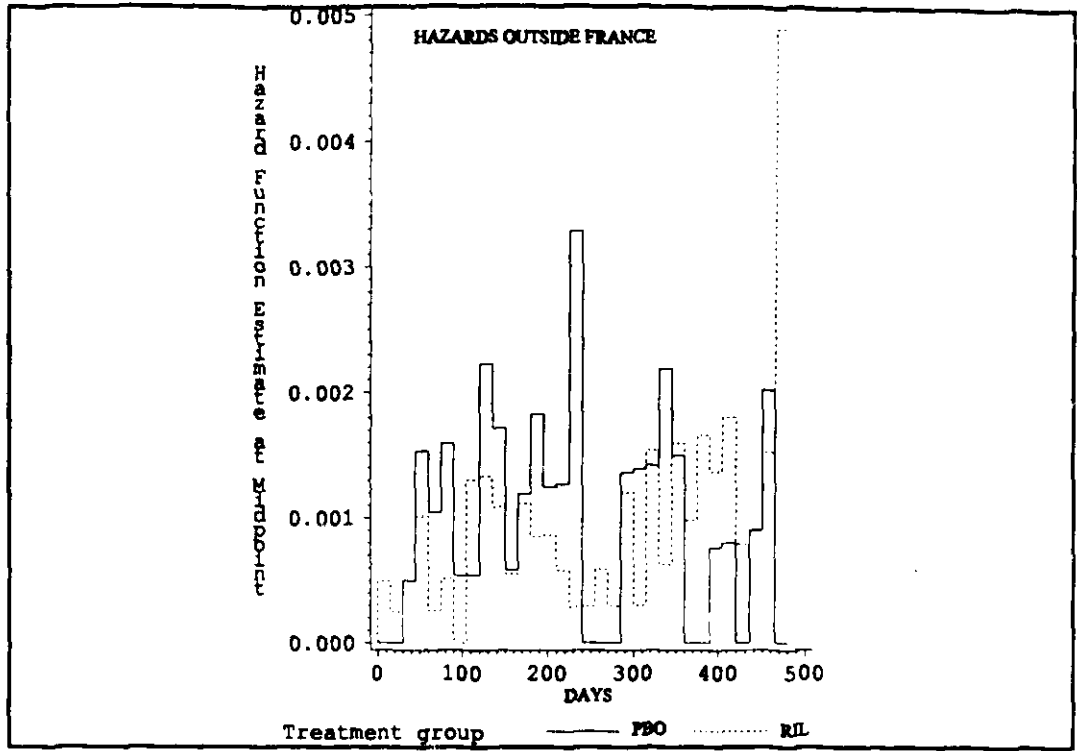
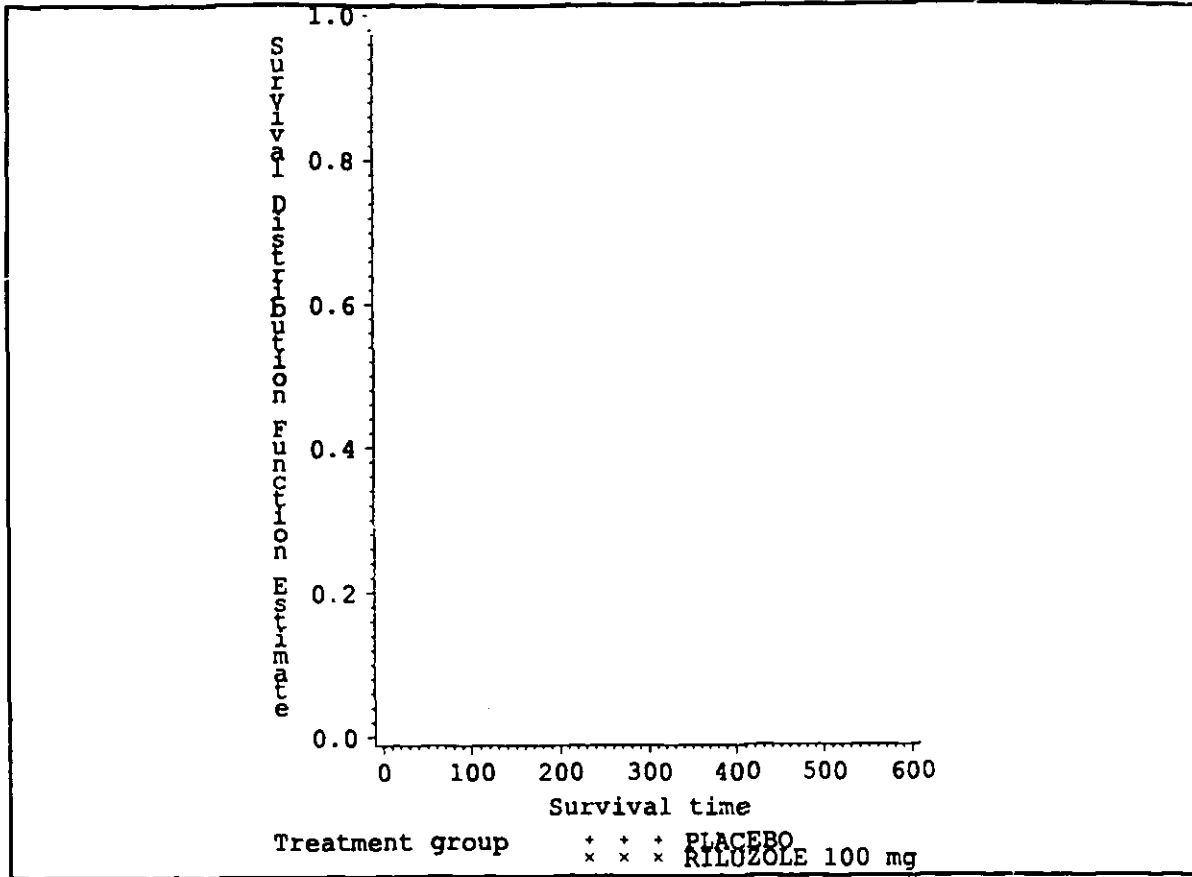


FIGURE 8
STUDY 216
LIMB AT BASELINE



Statistical Review and Evaluation

MAY 20 1994

IND#:

Applicant: Rhone-Poulenc Rorer

Name of Drug: Riluzole for treatment of ALS

Documents Reviewed: Vols 1-3 dated February 15, 1994

Medical Officer: John Feeney, M.D., HFD-120

Background

The sponsor has submitted a study report of a foreign trial (#216) which tested riluzole (100 mg/day for one year) against placebo in patients with Amyotrophic Lateral Sclerosis. This review summarizes the overall results of the primary endpoint 'failure' (death or tracheotomy) and the secondary endpoints Norris Bulbar Scale, Norris Limb Scale, Muscle Strength, Fev1, and Vital Capacity Ratio. It then examines in some detail the 'treatment' effect found in the stratum of patients who were classified with 'bulbar onset'.

Study Characteristics

Seven (7) investigators (6 French, 1 Belgian) randomized a total of 155 patients using permuted blocks of 4 to either riluzole or placebo in a double blind fashion. Patients were randomized and stratified on 'bulbar' or 'limb' onset within each center. Duration of disease before entry had to have been less than 5 years. Date of onset was determined by medical records or patient recollection, predominantly the latter. Patients were stratified on disease onset "in order to facilitate the interpretation of the riluzole effect on functional evaluation scales.... A differential effect of riluzole on survival, depending on site of disease onset, was not initially anticipated" (p. 139). Figure 1 displays the timing of study procedures. Functional scores were assessed every 2 months for 20 months maximum, while pulmonary tests were done every 6 months for 18 months maximum.

The protocol specified 110 patients in order to achieve 90% power to detect a one-year survival increase from 55% to 85% in the placebo and riluzole arms, respectively. The sponsor's design calls for one-sided tests since "this was a placebo-controlled study in a rare disease for which there is no approved treatment" (p.39), apparently a French regulatory requirement.

The protocol specifies the primary survival analysis at a cutoff date one-year after the last patient was enrolled. There was a six-month enrollment period. This corresponds to 30 Nov 1991 for a maximum follow up of 18 months. Additional analyses were done 4

months after the primary cutoff (12 March 1992) and another in which time on study was limited to each patient's first 12 months in the trial. The latter was done because "it appeared that the survival curves of the two treatments were not parallel and that the assumption of proportional hazards was questionable" in the 30 Nov 1991 analysis. A fourth analysis requested by the FDA analyzed time to premature discontinuation of study treatment.

Statistical Considerations

The analysis section of the protocol is very short. It says only that survival will be analyzed using a Cox Model and that repeated measures will compare slopes using the maximum likelihood method of Wu and Carroll, which incorporates a model of the censoring distribution. However, the sponsor states "As specified in the statistical analysis plan before unblinding, the primary survival comparison was the stratified logrank test (stratification on onset form of disease). The Cox model was also used to adjust for prognostic variables." (p.667 Appendix III)

Due to small sample sizes in each center, the sponsor pooled patients into two 'centers'. They then tested to see whether 'center' was a significant explanatory factor of failure in a Cox model, and then discarded 'center' as a stratum in the major analyses due to a non-significant p-value.

The actual report contains the stratified logrank tests, stepwise Cox regressions and three different longitudinal analyses of total functional and muscular testing scores: 1) a two-stage random effects model, 2) a simple analysis which compares the treatment groups with respect to the unweighted averages of patients' slopes of functional scores over time and 3) endpoint or LOCF. The second analysis is considered primary by the sponsor because it gives equal weight to slopes regardless of how long a patient was in the study. The reasoning here is that such censoring is informative in that a patient's slope was related to the reason for leaving the trial, namely failure (death or tracheostomy). Evidently, the Wu and Carroll MLE approach to informative censoring was not used because under that approach, a simple unweighted slopes analysis is the consequence of complete data where patients are assessed at the same time points.

Results

The sponsor states that no patients were lost to follow up for the survival analysis. However, one of the sponsor's statisticians told this reviewer that one patient's status was never actually confirmed at the end of the study. The increase from the designed 110 patients to 155 patients is due presumably to Amendment #1 which increased Dr. Meininger's enrollment from 36 to 72 patients.

Tables 1-5 display summary information about investigators, sample size by center, premature discontinuations, baseline demographics and selected baseline prognostic factors, respectively. Recall that patients were randomized from strata reflecting form of onset, not severity of any prognostic factor at the time of treatment.

Tables 6-8 display the survival results of the three different data cutoffs. Note that p-values are one-sided.

Figures 2-4 (note there is no Figure 1) display the Kaplan-Meier plots for the entire sample, the bulbar onset patients and the limb onset patients, respectively.

Figures 5-7 display the comparison of unweighted slopes as of one year of treatment, 12 Mar 1992, and the endpoint analysis for the three functional variables, respectively. Pulmonary functions were not analyzed in the study report, presumably due to missing data associated with only four time points over the course of the study. The only analyses which produce statistically significant differences between riluzole and placebo are unweighted for muscular testing. The dependence upon the unweighted analysis could well be due to the informative censoring (death or tracheostomy) which reflects rapid deterioration. By giving the steeper slopes (presumably reflecting shorter time in the trial) equal weight to those reflecting less serious disease, the statistic may have more power to detect a slight difference between the treatments.

Reviewer's Comments

In these comments p-values are reported as two-sided. In addition, the following results for failure are based on the 12 Mar 1992 data cutoff, four months after the protocol cutoff.

Overall analysis of failure

The p-values associated with the failure analysis at the protocol-specified data cutoff (30 Nov 1991), four months later (12 Mar 1992) and exactly one year of follow up on each patient were .116, .046 and .014, respectively using the stratified logrank test. The reason given for requiring the supplementary one-year analysis is that the assumption of proportional hazards did not seem to be apparent upon visual inspection of the two survival curves. However, the logrank test does not require proportional hazards in order to be a valid test. Furthermore, the sponsor does not present data to support the notion that the one-year data conforms to proportional hazards any more than the 30 Nov 1991 data. One possible reason that the sponsor may not have wanted to do the 30 Nov 1991 analysis is that they thought that the logrank test would be more powerful if the failure rates followed proportional hazards. Finally, if the 12 Mar 1992 analysis is taken seriously, it makes the 30 Nov 1991 analysis an unplanned interim analysis.

The sponsor reports a p-value of .058 for a stepwise Cox regression which ultimately retained baseline bulbar, age, vital capacity ratio, duration of disease and two visual analogue scales as covariates. However, baseline Fev1 was never even a candidate in the initial model. In fact, when bulbar score and Fev1 are the only predictors of survival, the p-value for Fev1 is .0002 and that for bulbar score is .16. Fev1 appears to be a stronger predictor of failure than bulbar score. When bulbar score, limb score, Fev1 and vital capacity ratio are covariates in a Cox model, the p-value for treatment is .11; in addition, the only significant covariates are the pulmonary functions. If Fev1 is the only covariate, the p-value .18. These analyses exclude 7 patients due to missing values on pulmonary tests.

Table 4 indicates that riluzole patients had higher vital capacity ratios on average (p=.10). Not reported is the fact that they also had a higher average baseline Fev1 (2.64 liters vs 2.34 liters, p=.02). Thus, the increase in the p-value using the Cox model with Fev1 as a covariate is due to the 'adjustment' for an imbalance in baseline Fev1 between the treatment groups.

Treatment Effect in "Bulbar at Onset" Stratum

There has been some speculation about the importance of the results in the patients designated as "bulbar at onset". Two of the three failure analyses result in statistically significant differences in failure using the logrank test (see tables 9, 10 and 11 and double the p-values), whereas the results in the "limb" stratum are weaker. However, this reviewer has found that the p-value for a treatment by stratum interaction is .75. On the other hand, the p-value for a treatment by baseline bulbar score interaction is .0063. 6, 7, 8.

In a teleconference with the sponsor, this reviewer was told:

- 1) Although it has been observed for a long time that people with more bulbar disease tend to have accelerated deterioration relative to those with less, nevertheless the study did not stratify on bulbar and limb for the express purpose of comparing failure rates.
- 2) The CRF's leave open the possibility of checking off both peripheral and bulbar onset. Patients who recalled both limb and bulbar symptoms at onset were classified as "bulbar" for purposes of stratification.

The second comment seems to be consistent with the fact that there is a wide range of bulbar scores in that stratum and that there is a substantial overlap of the two strata's baseline bulbar distributions. This confirms that there is not so much a "bulbar" patient in this stratum as someone who has at least some bulbar involvement.

Since there is some question about what identification of a treatment effect with the bulbar onset stratum really means in terms of the prognostic factors in that stratum, this reviewer examined this question.

I. The first method was to predict all the patients' probabilities of being in their respective strata using logistic regression with baseline total bulbar and limb scores as predictors in the model. Using a cutoff of .5, patients were 'switched' if their predicted probabilities fell below .5 for their assigned strata.

The result was that 5 patients were switched from bulbar to limb and 5 were switched from limb to bulbar. Figure 8 is a plot of bulbar vs limb total scores in the bulbar onset stratum, while figure 9 plots the same variables in the limb onset stratum. The circled observations represent patients who were switched into the other stratum as a result of the 'discriminant analysis'. The pattern of switches is plausible. Note that the 5 who were switched from bulbar to limb in Figure 8 had relatively high (good) bulbar scores, while those switched from limb to bulbar in Figure 9 had high limb scores but low bulbar scores. Upon recalculating a logrank test within the 'new' bulbar stratum, the p-value is .29. Controlling for total bulbar and limb scores within this stratum produces a p-value of .10 using a Cox model. In a third analysis, the actual predicted probability of stratum membership was used as the covariate in an analysis of all patients. The p-value was .08. The first two results are substantially due to the fact that 4 of the 5 patients switched from bulbar to limb were riluzole patients who were censored alive at the end of the study; the fifth was a placebo patient who died.

II. The second analysis reflected an analysis of two baseline pulmonary function tests: Fev1 and Vital Capacity Ratio. As noted above, when these two prognostic variables together with bulbar and limb baseline scores are tested for their influence on failure in a Cox model, only the pulmonary functions are significant. The notion that bulbar condition is 'aliasing' for pulmonary function, especially Fev1, the stronger of the two pulmonary predictors, is only partially borne out when examining the bulbar stratum. Although there is a statistically significant linear component between the two variables, the r-squared value is only .17. In the entire data set, the p-value for treatment by baseline Fev1 interaction is highly significant ($p=.0038$), in parallel with that of treatment by bulbar score interaction.

Having identified Fev1 as an important prognostic variable likely

related to bulbar condition, baseline Fev1 was divided into quartiles of patients and logrank tests were performed within each quartile . The table below describes the distributions of baseline Fev1:

	Limb (N=122)	Bulbar (N=27)
Median	2.54	1.97
75th %ile	3.15	2.47
25th %ile	2.03	1.32
Range	1.03-4.62	.75-3.65

The resulting p-values were .31 for the bottom quartile, .55 for the second, .61 for the third, and .21 for the top quartile. See Figures 10 to 13 for Kaplan-Meier plots of failure for the respective quartiles. Of the 27 patients in the bulbar onset stratum with available pulmonary data (out of a total of 32), 13 are common between the bottom quartile of Fev1 and bulbar onset (see Figure 14). One 'anomaly' in the results of these exercises is that Fev1 appears to be a stronger predictor of failure than bulbar scores, yet with approximately the same number of patients in the bottom quartile of Fev1 as the bulbar onset stratum, there is a trend (see Figure 10) but no statistically significant difference in failure rates between riluzole and placebo, unlike the result within the bulbar onset stratum. This pattern could be the result of factors not yet taken into account, 'random' patterns in the data, or more likely, both.

Another question of possible interest is the effect of duration of disease on treatment effect. Four strata were created: less than 1 year, between 1 and 2 years, between 2 and 3 years, and greater than 3 years. The p-value for treatment by stratum interaction was .08. Figures 15-18 display the Kaplan-Meier plots within the strata in ascending order of disease duration. Although only suggestive of a real trend to be confirm in Trial 301, there is no evidence of benefit in patients with less than 1 year duration, substantial benefit in patients with the longest duration, and intermediate results in patients with between 1 to 3 years duration.

Conclusions

Overall Analysis

1. Since the protocol and the sponsor agree that the 30 Nov 1991 cutoff is the primary analysis for failure, the p-value should be .116 if it is necessary to choose one analysis that is unadjusted by prognostic variables. Looking at the data to see whether or not the result conforms to some wished-for pattern is not a reason to

change to another (arbitrary) analysis.

2. It appears that the sponsor has not fully reported the role of pulmonary function, especially baseline Fev1, both in its role as a predictor of failure and as a covariate which was not balanced between the treatment groups at the time of treatment. A simple analysis which corrects for this imbalance suggests that the unadjusted results overstate the inference that riluzole was effective at the 5% level at the 12 Mar 1992 analysis.

Bulbar Onset Stratum Analysis

3. Exploratory analyses of the treatment effect in the bulbar onset patients do not indicate anything definitive. However, to the extent that baseline Norris functional scores are predictive of stratum membership, there is some suggestion that the effect cannot be associated with a well-defined cohort of patients with bulbar onset. Since patients were not stratified in order to anticipate differences in survival, the survival result in that stratum should be regarded, at this point, as an interesting finding, only. Trial 301 will be necessary to confirm this trial's result in a randomized stratum of patients who had at least some bulbar involvement according to the patient's recollection.


David Hoberman, Ph.D.
Mathematical Statistician

Concur: Dr. Nevius *SN* 5-20-94

for Dr. Dubey *SN* 5-20-94

This review consists of 7 pages of text, 17 figures and 8 tables.

cc: Orig IND
HFD-120
HFD-120/Dr. Feeny
HFD-120/Dr. Katz
HFD-120/Dr. Leber
HFD-120/Mr. Grilley
HFD-713/Dr. Nevius
✓ HFD-713/Dr. Hoberman
HFD-713/Dr. Dubey [File 1.3.1 IND]
chron

TABLE 1. INVESTIGATORS PARTICIPATING IN STUDY AG216

Investigator Name and Address	No. Patients Enrolled
	N= 68
	N=27
	N=12
	N=11
	N=14
	N= 9
	N=14

TABLE 2: DISTRIBUTION OF PATIENTS BY CENTER

CENTER	LIMB *				BULBAR **				ALL			
	RILUZOLE		PLACEBO		RILUZOLE		PLACEBO		RILUZOLE		PLACEBO	
	N	%	N	%	N	%	N	%	N	%	N	%
PARIS 1	27	44	25	41	7	47	9	53	34	44	34	44
PARIS 2	11	18	11	18	3	20	2	12	14	18	13	17
LIMOGES	6	10	6	10	0	0	0	0	6	8	6	8
MARSEILLE	5	8	5	8	0	0	1	6	5	6	6	8
TOULOUSE	5	8	6	10	2	13	1	6	7	9	7	9
CAEN	4	6	4	7	0	0	1	6	4	5	5	6
BRUSSELS	4	6	4	7	3	20	3	18	7	9	7	9
ALL	62	100	61	100	15	100	17	100	77	100	78	100

TABLE 3: DISPOSITION OF PATIENTS - PPEMATURE DISCONTINUATIONS FROM TREATMENT

REASON FOR TREATMENT DISCONTINUATION	LIMB				BULBAR				ALL			
	RILUZOLE		PLACEBO		RILUZOLE		PLACEBO		RILUZOLE		PLACEBO	
	N	%	N	%	N	%	N	%	N	%	N	%
ADVERSE EXPERIENCES	15	38	6	16	4	40	3	21	19	38	9	18
LACK OF EFFICACY	1	3	3	8	1	10	0	0	2	4	3	6
LOST TO FOLLOW-UP	3	8	2	5	0	0	0	0	3	6	2	4
TRACHEOSTOMY	1	3	2	5	0	0	0	0	1	2	2	4
DEATH	19	48	23	62	4	40	11	79	23	46	34	67
OTHER	1	3	1	3	1	10	0	0	2	4	1	2
ALL	40	100	37	100	10	100	14	100	50	100	51	100

TABLE 4: PATIENT CHARACTERISTICS AT BASELINE: DEMOGRAPHICS

	LIMB		BULBAR		ALL	
	RILUZOLE	PLACEBO	RILUZOLE	PLACEBO	RILUZOLE	PLACEBO
N =	62	61	15	17	77	78
SEX M/F	39/23	40/21	6/9	6/11	45/32	46/32
AGE (YEARS) MEAN ± SEM	56.5 ± 1.3	56.6 ± 1.5	57.9 ± 2.6	63.3 ± 1.7	56.8 ± 1.2	58.1 ± 1.2
WEIGHT (kg) MEAN ± SEM	67.0 ± 1.6	65.7 ± 1.4	61.9 ± 3.3	63.1 ± 2.9	66.0 ± 1.4	65.1 ± 1.3
DURATION OF DISEASE (YEARS) MEAN ± SEM	2.2 ± 0.2	2.4 ± 0.2	1.8 ± 0.4	1.6 ± 0.2	2.2 ± 0.2	2.3 ± 0.2
VITAL CAPACITY (RATIO) MEAN ± SEM	0.95 ± 0.02	0.87 ± 0.03	0.76 ± 0.07	0.82 ± 0.05	0.92 ± 0.02	0.86 ± 0.02

TABLE 5: PATIENT CHARACTERISTICS AT BASELINE : DISEASE SEVERITY

	LIMB		BULBAR		ALL	
	RILUZOLE	PLACEBO	RILUZOLE	PLACEBO	RILUZOLE	PLACEBO
N	62	61	15	17	77	78
LIMB NORRIS SCALE * (MAX. SCORE = 63) MEAN ± SEM	39.0 ± 2.1	36.5 ± 2.0	53.0 ± 2.6	55.9 ± 1.8	41.7 ± 1.8	40.8 ± 1.8
BULBAR NORRIS SCALE * (MAX. SCORE = 39) MEAN ± SEM	33.9 ± 0.9	34.6 ± 0.8	17.7 ± 2.5	14.1 ± 1.6	30.7 ± 1.1	30.1 ± 1.2
MUSCLE TESTING SCALE * (MAX. SCORE = 110) MEAN ± SEM	79.5 ± 2.1	75.5 ± 2.5	95.9 ± 2.9	92.3 ± 3.2	82.7 ± 1.9	79.1 ± 2.2
V A S STIFFNESS MEAN ± SEM	33.2 ± 4.3	34.0 ± 4.1	36.7 ± 8.2	25.7 ± 8.5	33.8 ± 3.8	32.3 ± 3.7
V A S TIREDNESS MEAN ± SEM	59.2 ± 3.6	56.6 ± 3.7	47.9 ± 7.0	42.9 ± 7.5	57.1 ± 3.2	53.7 ± 3.3
V A S FASCICULATIONS MEAN ± SEM	43.6 ± 3.7	37.7 ± 3.6	36.4 ± 5.3	42.8 ± 7.8	42.2 ± 3.2	38.7 ± 3.3
C.G.I. SEVERITY * MEAN ± SEM	4.3 ± 0.1	4.4 ± 0.1	3.9 ± 0.3	4.0 ± 0.2	4.2 ± 0.1	4.3 ± 0.1

* = see CRF pages 12-15, 34 in Appendix I.3 for a description of scales.

TABLE 6: RESULTS OF THE PRIMARY ANALYSIS: CUTOFF DATE 30-NOV-1991 *

	INCIDENCE OF FAILURE	SURVIVAL RATE	SURVIVAL MEDIAN (IN DAYS)	LOGRANK TEST P-VALUE **	COX MODEL P-VALUE **	COX MODEL RISK RATIO 95% CI
RILUZOLE PLACEBO	34 / 77 40 / 78	55.8% 48.7%	502 469	stratified p = 0.058	stratified p = 0.041	1.53 [0.95- 2.47]
BULBAR RILUZOLE PLACEBO	7 / 15 11 / 17	53.3% 35.3%	476 239	p = 0.036	p = 0.030	3.42 [0.95- 12.31]
LMB RILUZOLE PLACEBO	27 / 62 29 / 61	56.5% 52.5%	502 487	p = 0.21	p = 0.20	1.27 [0.74- 2.19]

* Interval from randomization to cutoff ranged from 12 to 17 months.

** All p-values are one-sided.

Source: Appendix III.

TABLE 7: RESULTS OF THE CONFIRMATORY ANALYSIS AT THE END OF THE DOUBLE-BLIND PHASE: CUT-OFF DATE 12-MAR-1992

	INCIDENCE OF FAILURE	SURVIVAL RATE	SURVIVAL MEDIAN (IN DAYS)	LOGRANK TEST P-VALUE **	COX MODEL P-VALUE **	COX MODEL RISK RATIO 95% CI
RILUZOLE PLACEBO	39 / 77 49 / 78	49.4% 37.2%	532 449	stratified p = 0.023	stratified p = 0.029	1.52 [0.985 - 2.36]
BULBAR RILUZOLE PLACEBO	7 / 15 11 / 17	53.3% 17.6%	>476 239	p = 0.007	p = 0.005	4.63 [1.45 - 14.75]
LMB RILUZOLE PLACEBO	32 / 62 35 / 61	48.4% 42.6%	531 523	p = 0.18	p = 0.22	1.27 [0.74 - 2.01]

* Interval from randomization to cutoff ranged from 16 to 21 months.

** All p-values are one-sided.

Source: Appendix III.

TABLE 8: RESULTS OF THE ANALYSIS USING THE FIRST 12 MONTHS (ONE YEAR) OF TREATMENT FOR EACH PATIENT

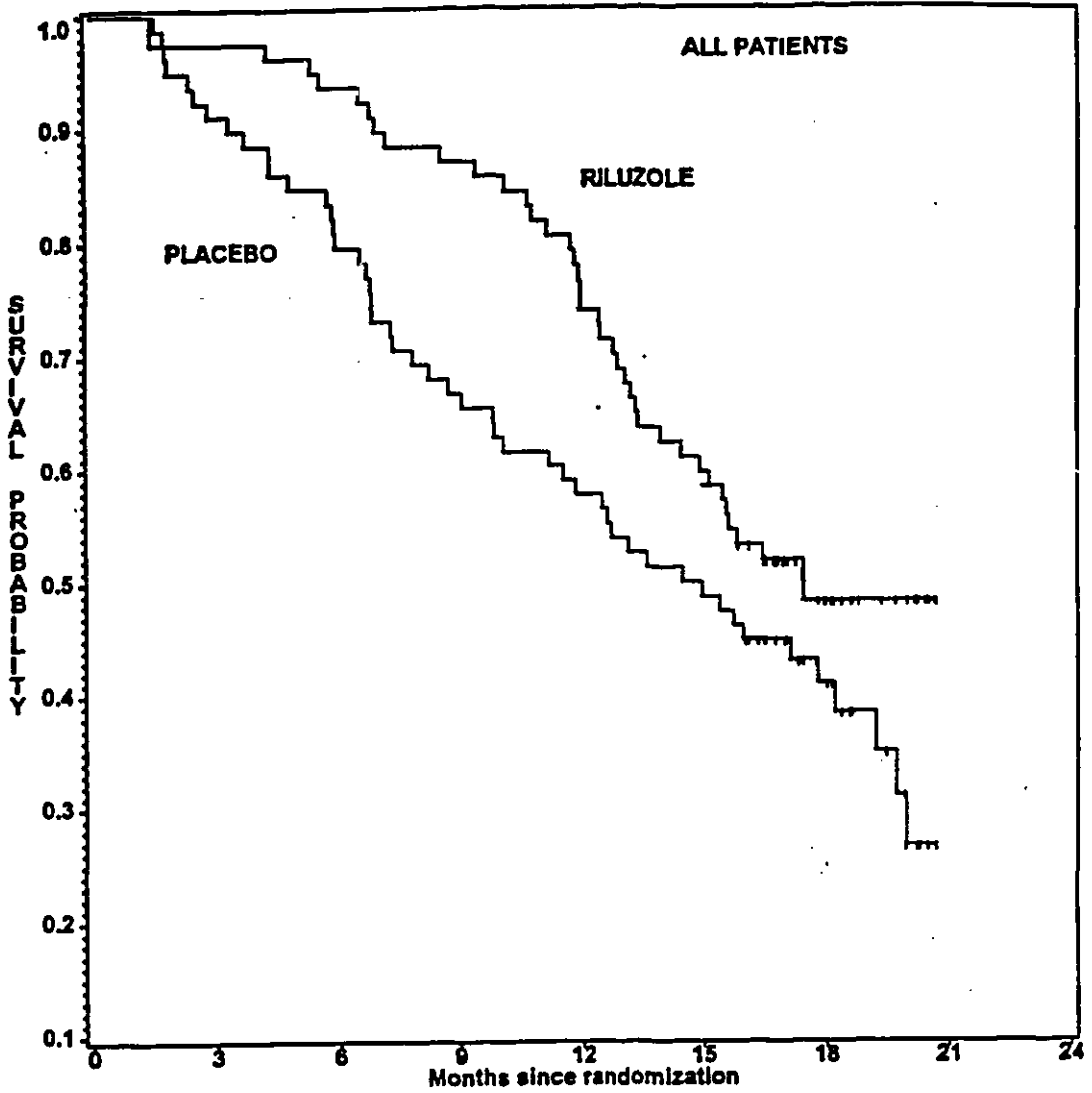
	INCIDENCE OF FAILURE	SURVIVAL RATE	LOGRANK TEST P-VALUE **	COX MODEL P-VALUE **	COX MODEL RISK RATIO 95% CI
RILUZOLE PLACEBO	20 / 77 33 / 78	74.0% 57.7%	stratified p = 0.007	stratified p = 0.002	2.30 [1.29 - 4.09]
BULBAR RILUZOLE PLACEBO	4 / 15 11 / 17	73.3% 35.3%	p = 0.007	p = 0.024	4.26 [1.01 - 17.97]
LMB RILUZOLE PLACEBO	16 / 62 22 / 61	74.2% 63.9%	p = 0.085	p = 0.039	1.84 [0.94 - 3.61]

* Interval from randomization to cutoff set at exactly 12 months.

** All p-values are one-sided.

Source: Appendix III.

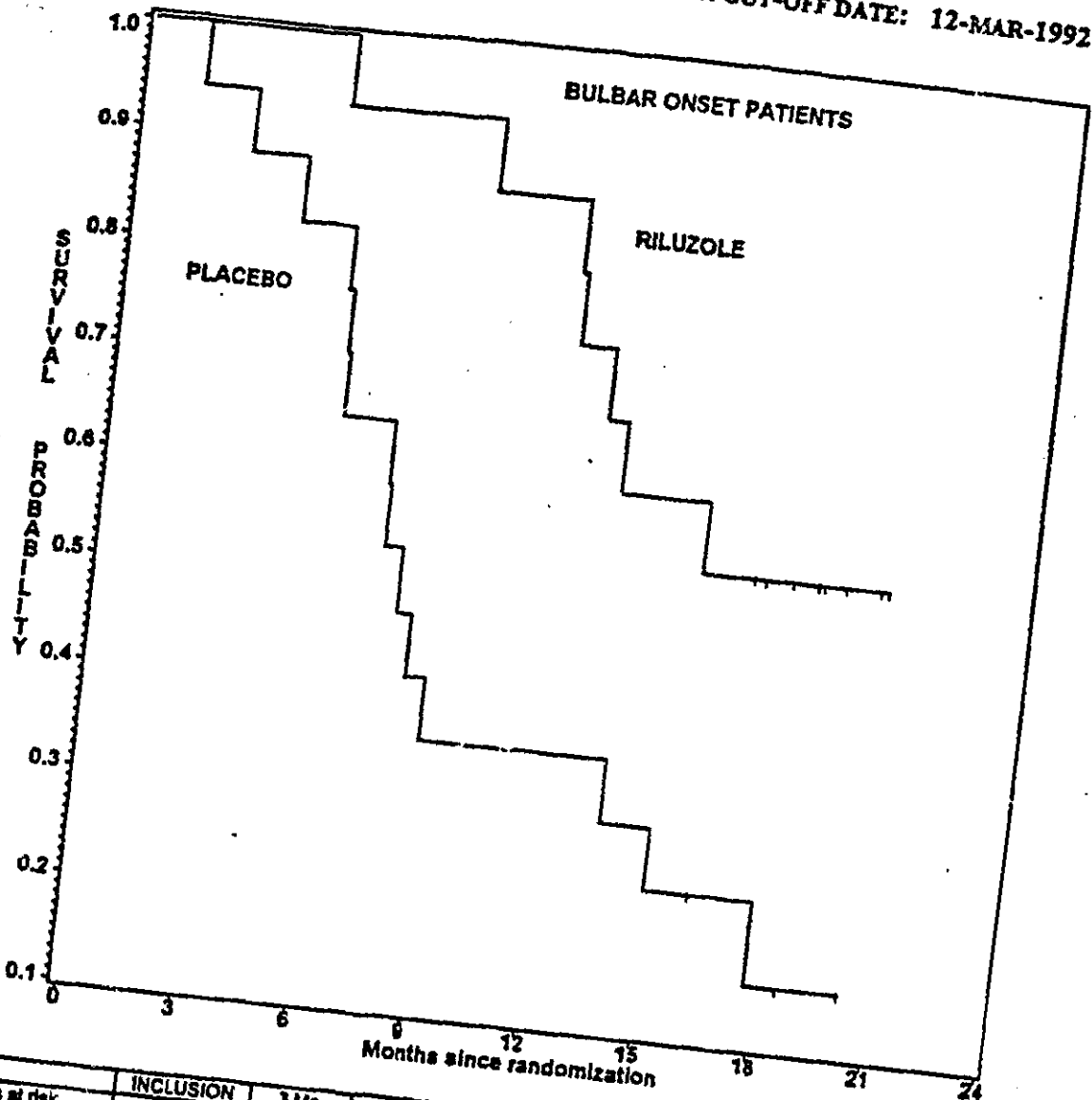
FIGURE 2. KAPLAN-MEIER SURVIVAL CURVES. CUTOFF DATE: 12-MAR-1992



	INCLUSION	3 Mo.	6 Mo.	9 Mo.	12 Mo.	15 Mo.	18 Mo.	21 Mo.
Patients at risk (placebo/riluzole)	78/77	71/75	62/72	52/67	45/57	38/45	19/23	0/0
Patients failed (placebo/riluzole)	0/0	7/2	16/5	26/10	33/20	40/32	45/39	49/39

Source: Appendix III

FIGURE 3. KAPLAN-MEIER SURVIVAL CURVES. CUT-OFF DATE: 12-MAR-1992



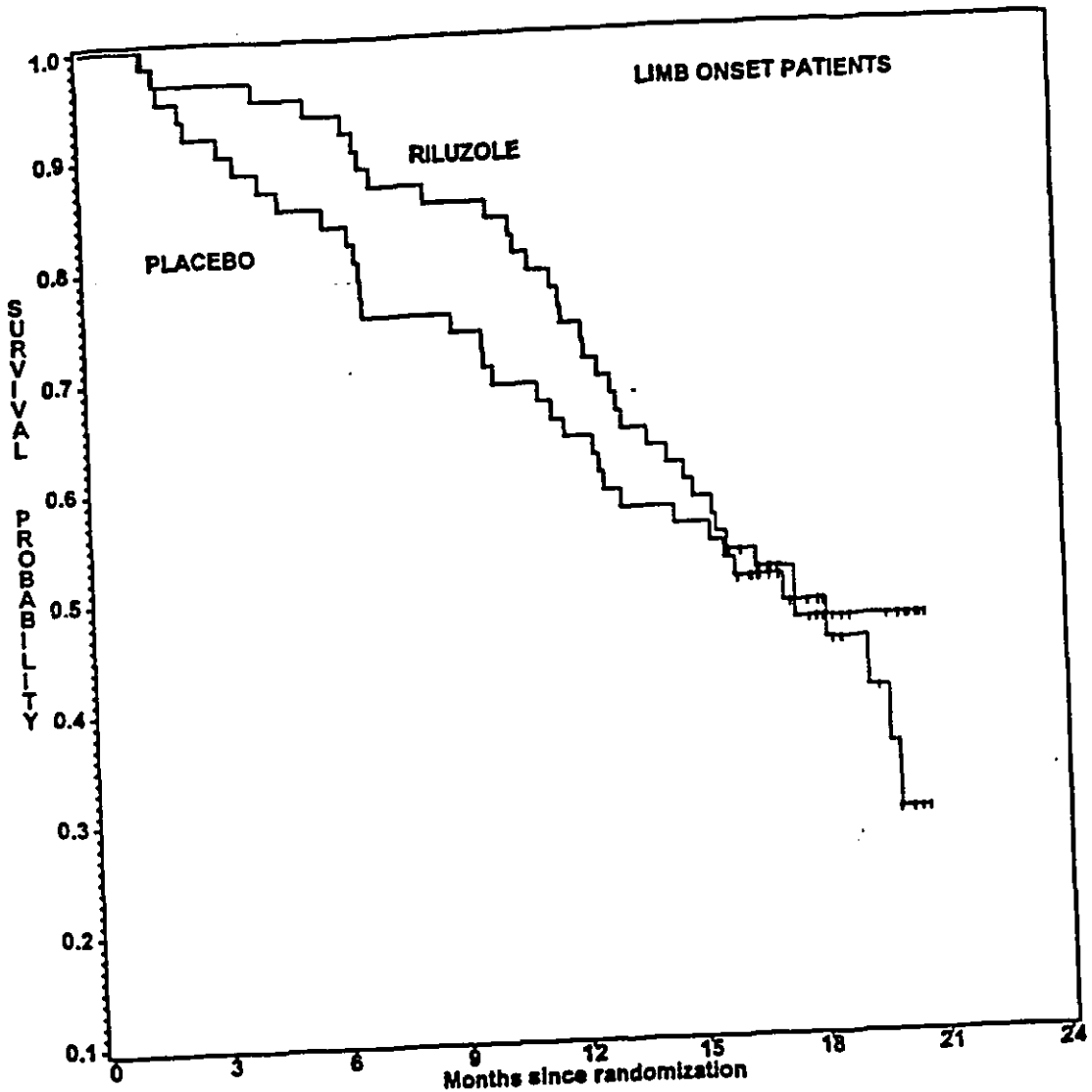
	INCLUSION	3 Mo.	6 Mo.	9 Mo.	12 Mo.	15 Mo.	18 Mo.	21 Mo.
Patients at risk (placebo/riluzole)	17/15	15/15	11/14	6/14	6/11	4/9	2/5	0/0
Patients failed (placebo/riluzole)	0/0	2/0	6/1	11/1	11/4	13/6	14/7	14/7

Source: Appendix III

CONFIDENTIAL

0058

FIGURE 4. KAPLAN-MEIER SURVIVAL CURVES. CUT-OFF DATE: 12-MAR-1992



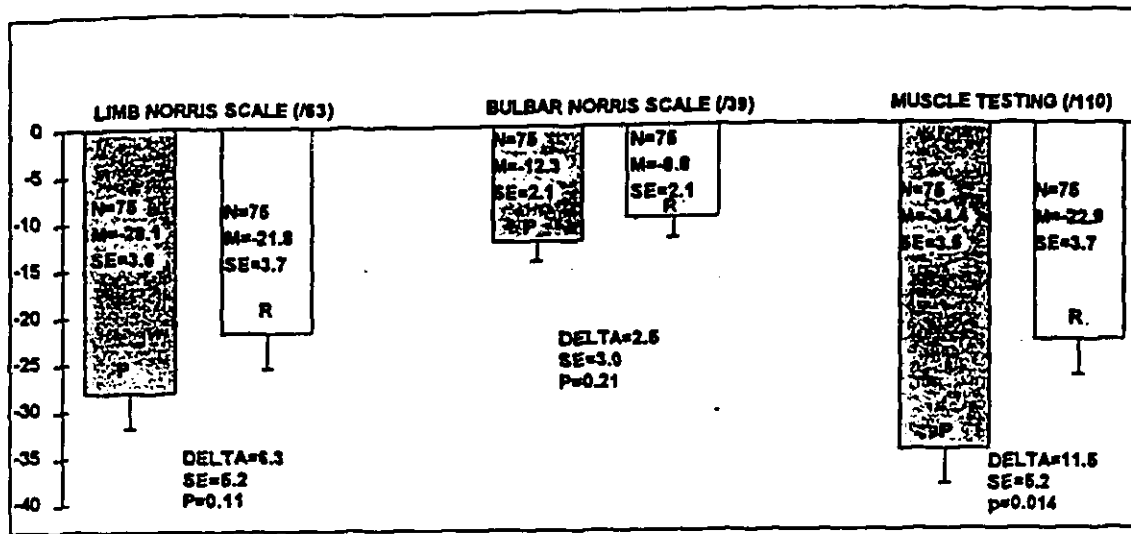
	INCLUSION	3 Mo.	6 Mo.	9 Mo.	12 Mo.	15 Mo.	18 Mo.	21 Mo.
Patients at risk (placebo/riluzole)	61/62	56/60	51/58	46/53	39/46	34/36	17/18	0/0
Patients failed (placebo/riluzole)	0/0	5/2	10/4	15/9	22/16	27/26	31/32	35/32

Source: Appendix III

CONFIDENTIAL

0060

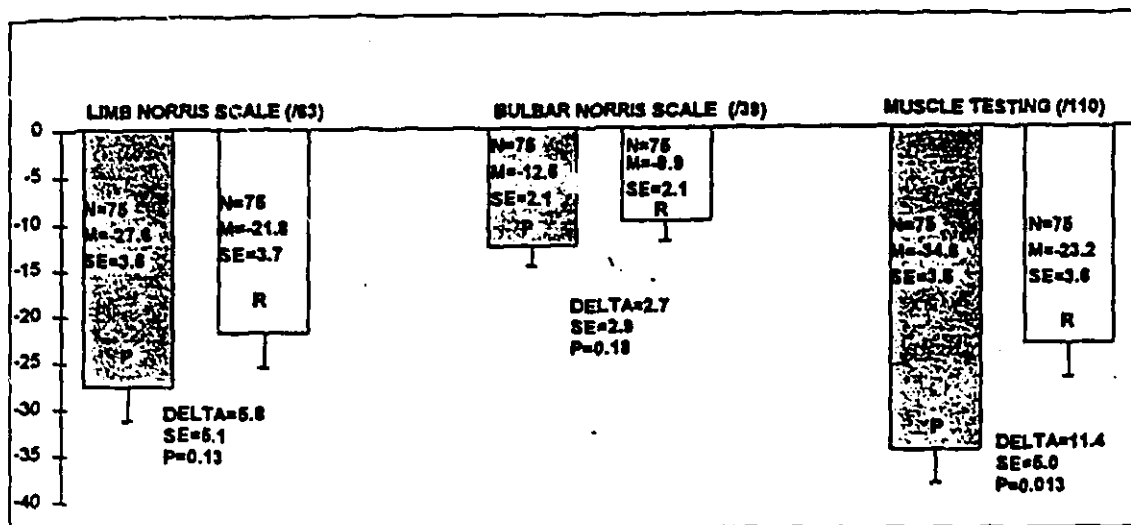
FIGURE 5. RATE OF DETERIORATION IN FUNCTIONAL EVALUATION AFTER ONE YEAR OF TREATMENT
 [unit = per year, unweighted estimates]



Source: Appendix III.
 P = PLACEBO
 R = RILUZOLE
 N = number of patients with at least 2 evaluations
 M = average slope (loss in total score over one year)

All p-values are one-sided.
 SE = Standard Error

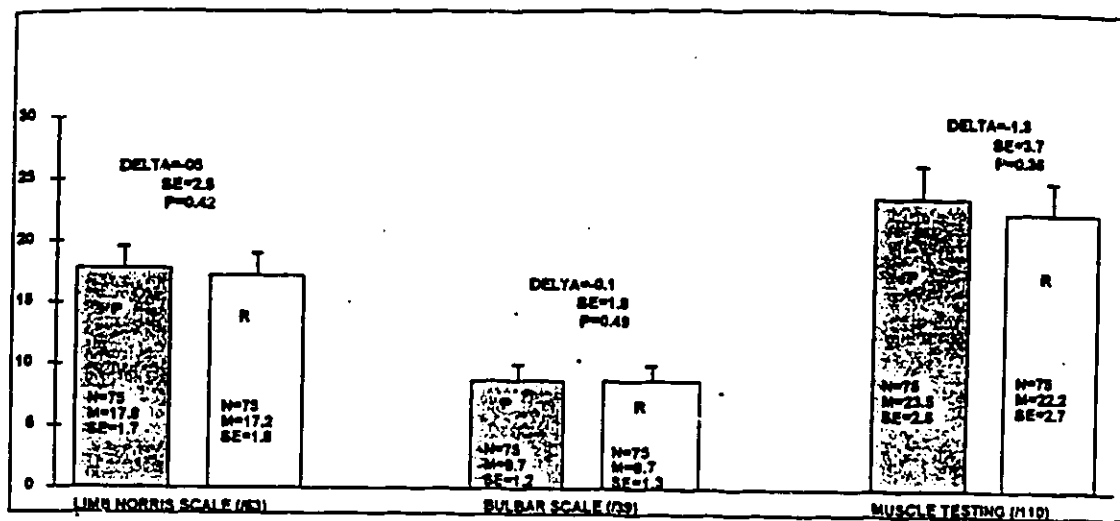
FIGURE 6. RATE OF DETERIORATION IN FUNCTIONAL EVALUATION AS OF 12-MAR-1992
 [unit = per year, unweighted estimates]



Source: Appendix III.
 P = PLACEBO
 R = RILUZOLE
 N = number of patients with at least 2 evaluations
 M = average slope (loss in total score over one year)

All p-values are one-sided.
 SE = Standard Error

FIGURE 7. ENDPOINT ANALYSIS OF FUNCTIONAL EVALUATION AS OF 12-MAR-1992
 (change between baseline and last evaluation)



Source: Appendix III.

P = PLACEBO

R = RILUZOLE

N = number of patients with at least 2 evaluations.

M = average change between baseline and last evaluation.

All p-values are one-sided.

SE = Standard Error

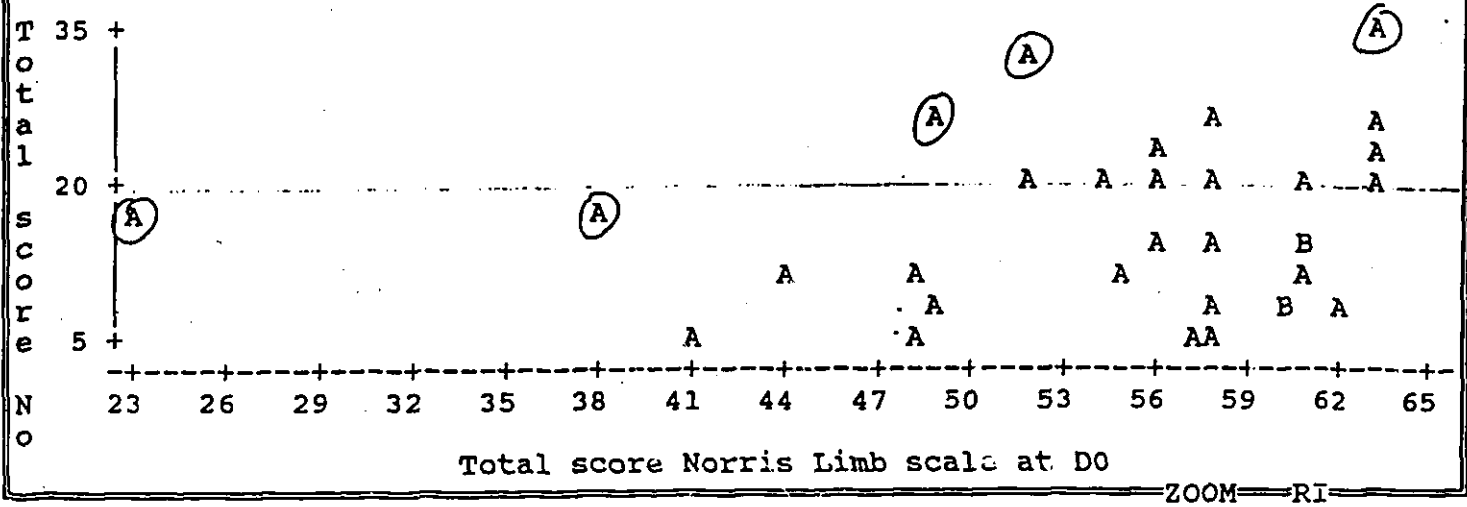
FIGURE 8 "BULBAR" Patients

OUTPUT
Command ==>

SAS 6:50 Tuesday, March 8, 1997 76

----- Onset Form of the disease=1 -----

Plot of BULNORO*LIMNORO. Legend: A = 1 obs, B = 2 obs, etc.



Total score Norris Limb scale at D0

ZOOM RI

Circled observations are predicted to be "LIMB"

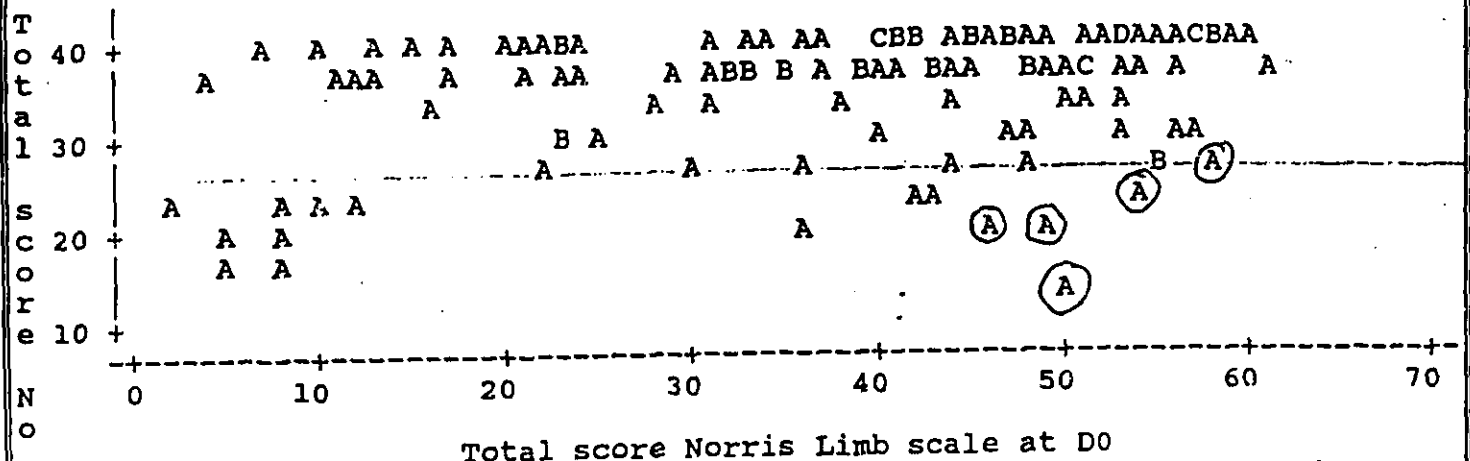
FIGURE 9 "LIMB" Patients

OUTPUT
Command ==>

sday, March 8, 1994 75

----- Onset Form of the disease=0 -----

Plot of BULNORO*LIMNORO. Legend: A = 1 obs, B = 2 obs, etc.



Circled observations are predicted to be "BULBAR"

FIGURE 10

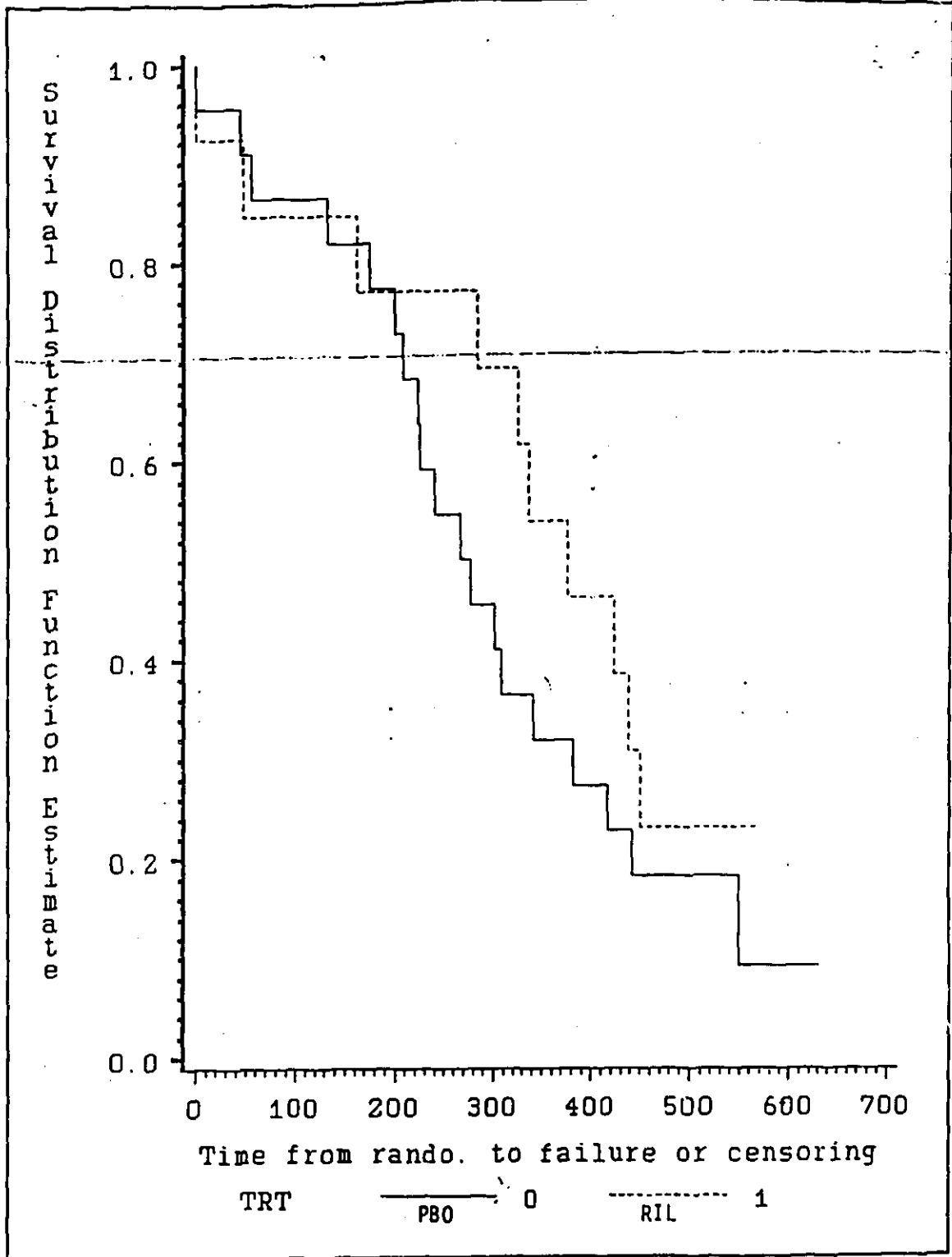


FIGURE 11

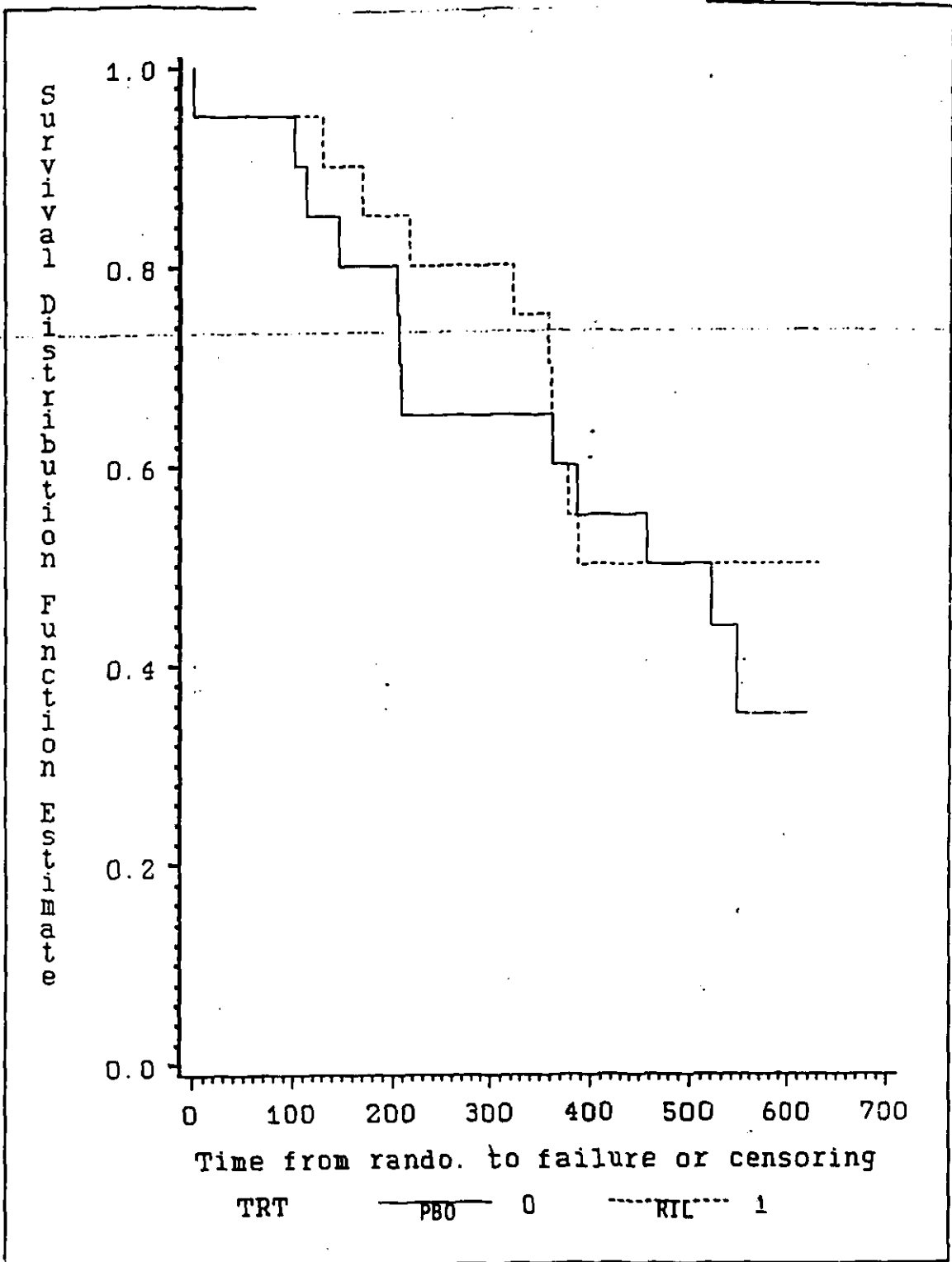


Figure 12

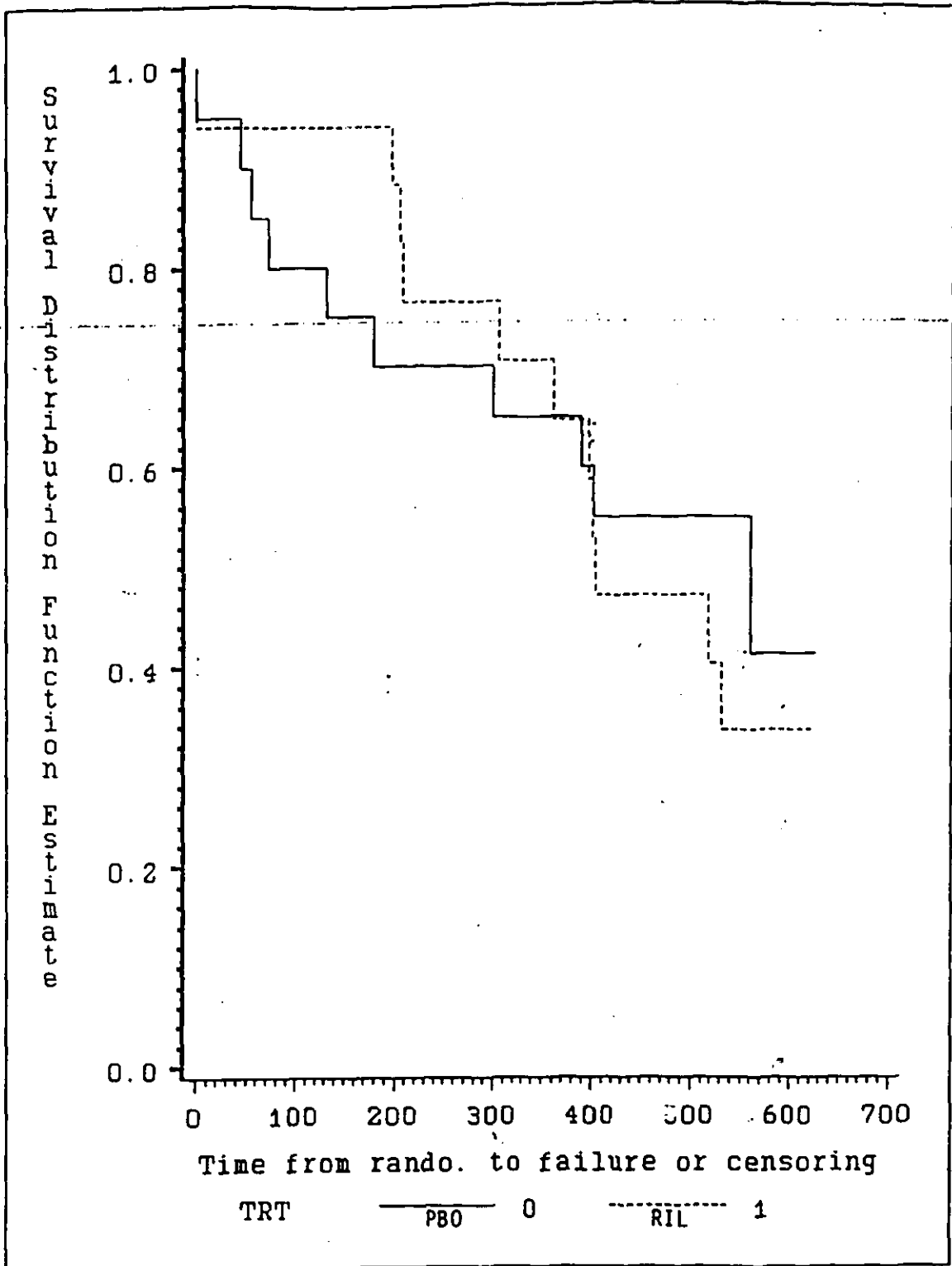


Figure 13

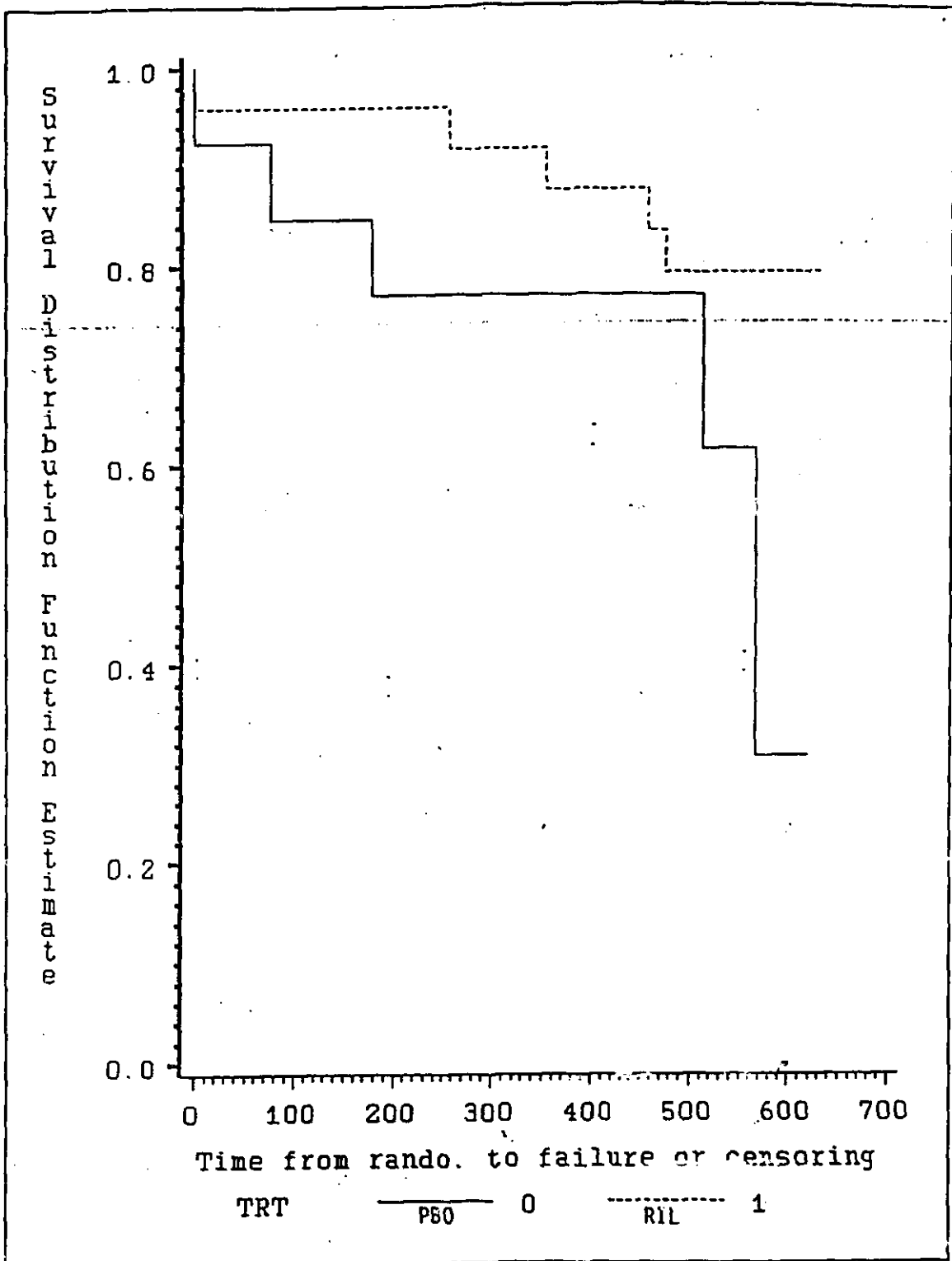
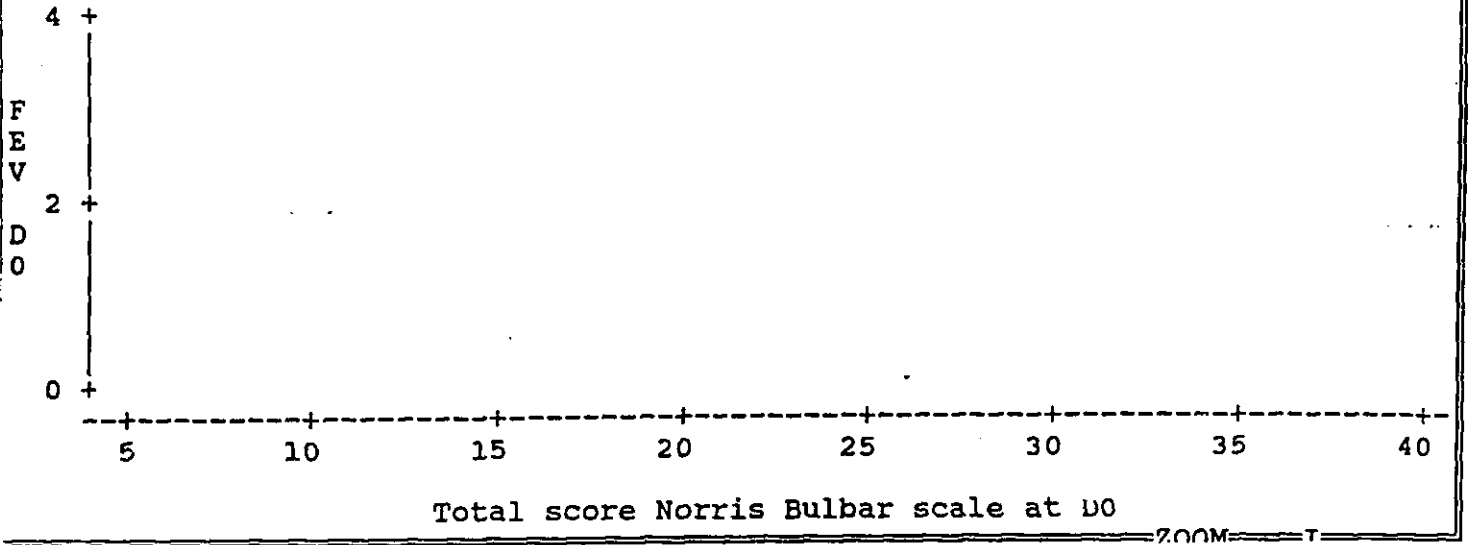


Figure 14 "BULBAR" Patients

OUTPUT
Command ==>

SAS 7:05 Thursday, March 10, 1994 2

Plot of FEV0*BULNOR0. Legend: A = 1 obs, B = 2 obs, etc.



Circled observations are below the 25th %ile for FEV1

Figure 15

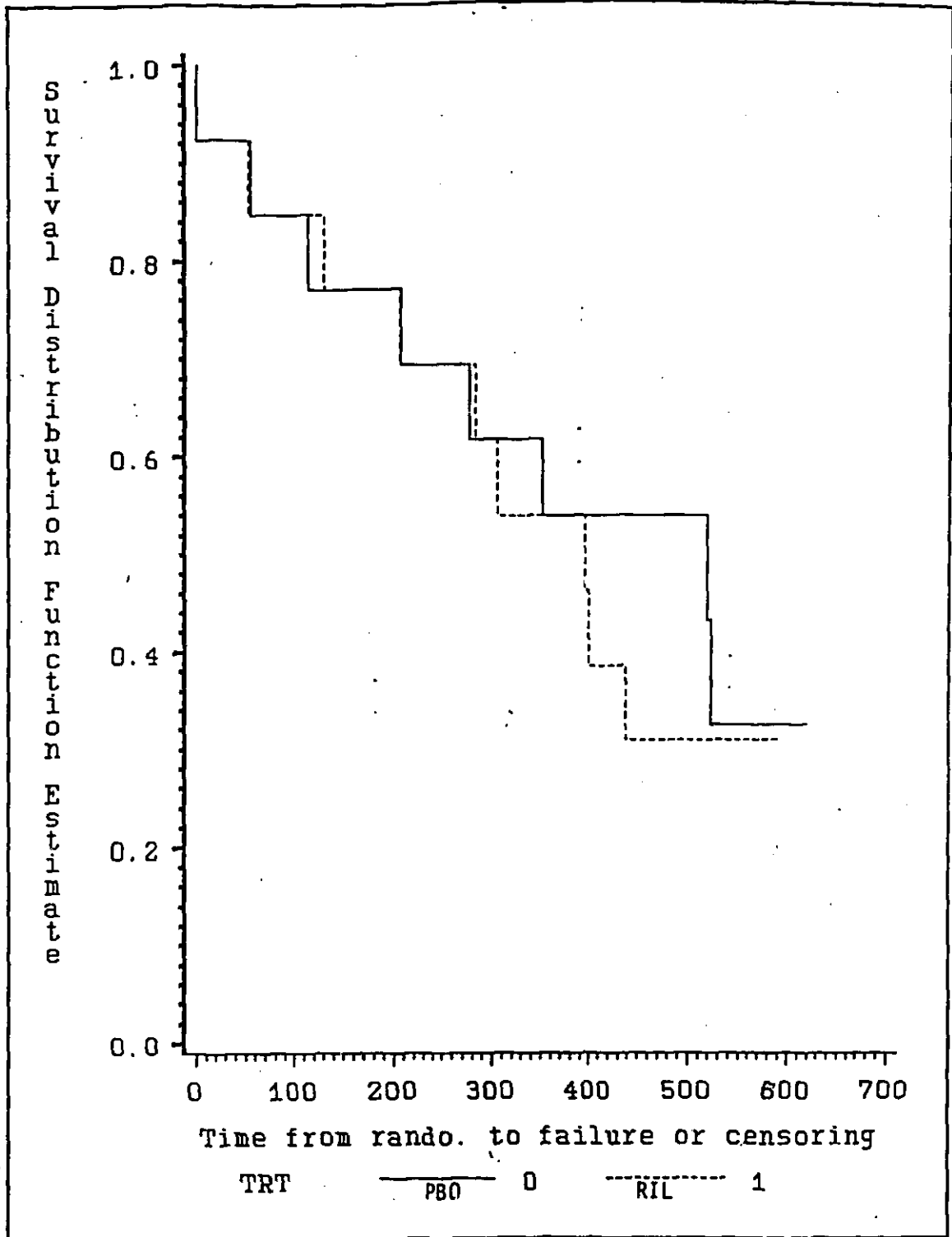


Figure 16

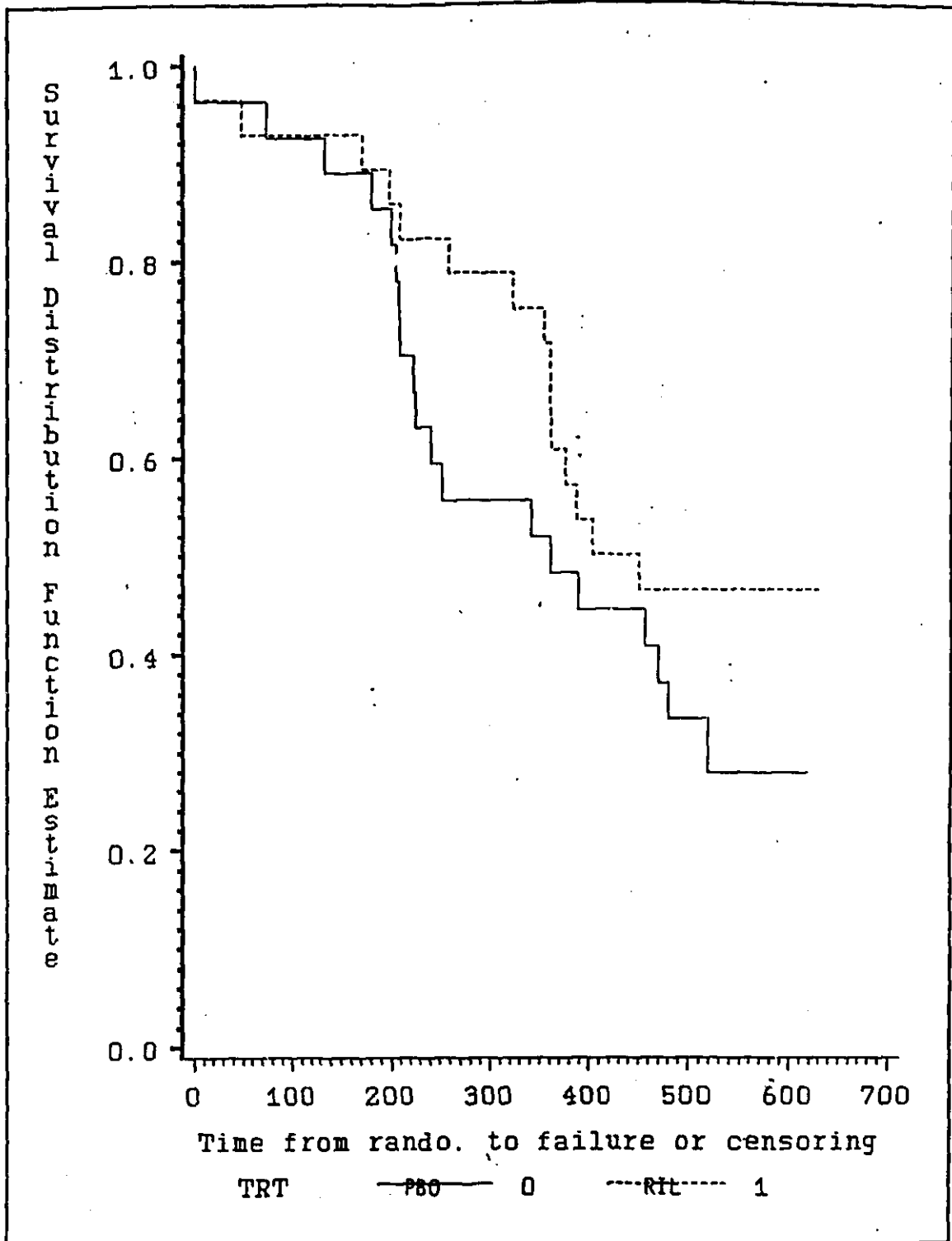


FIGURE 17

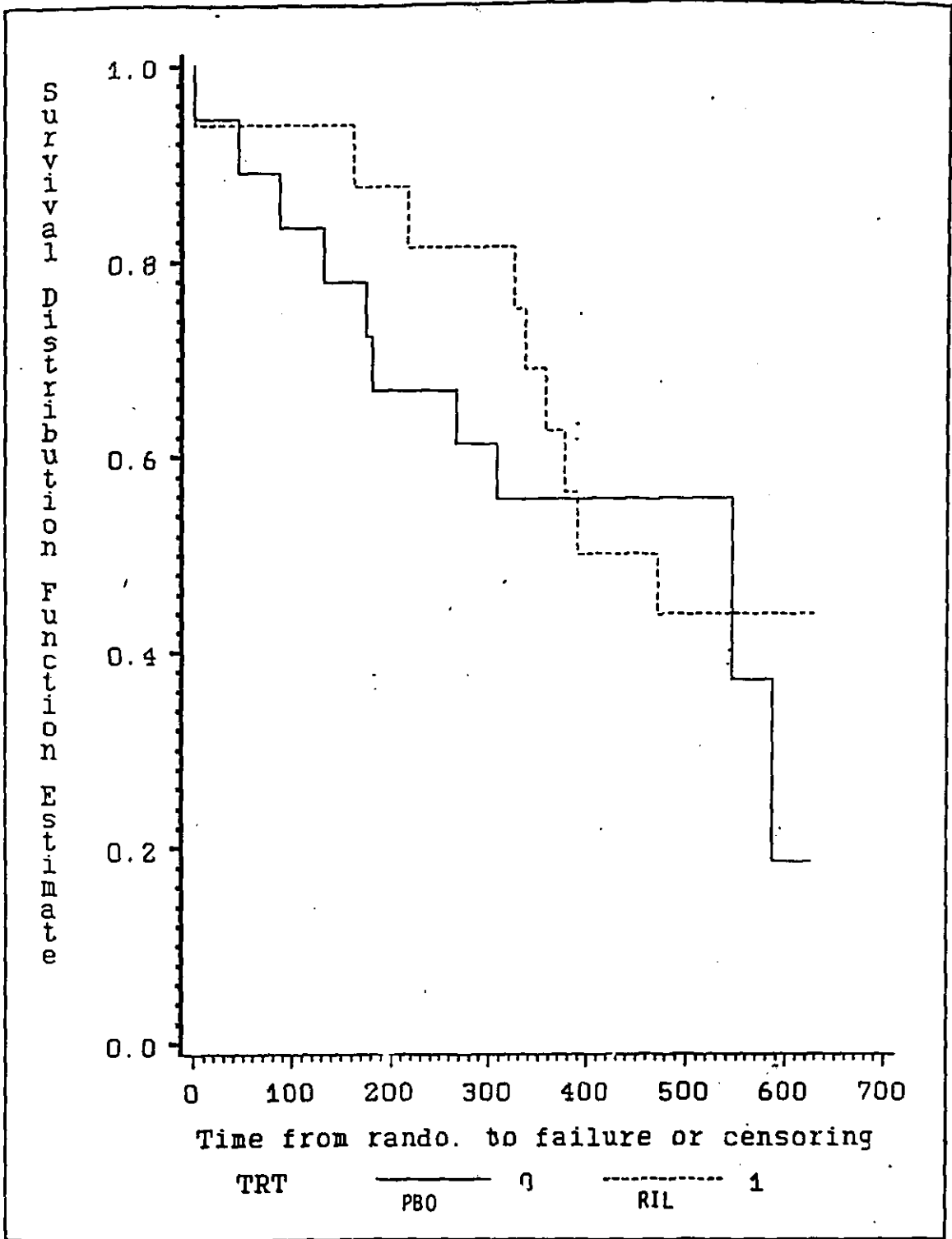
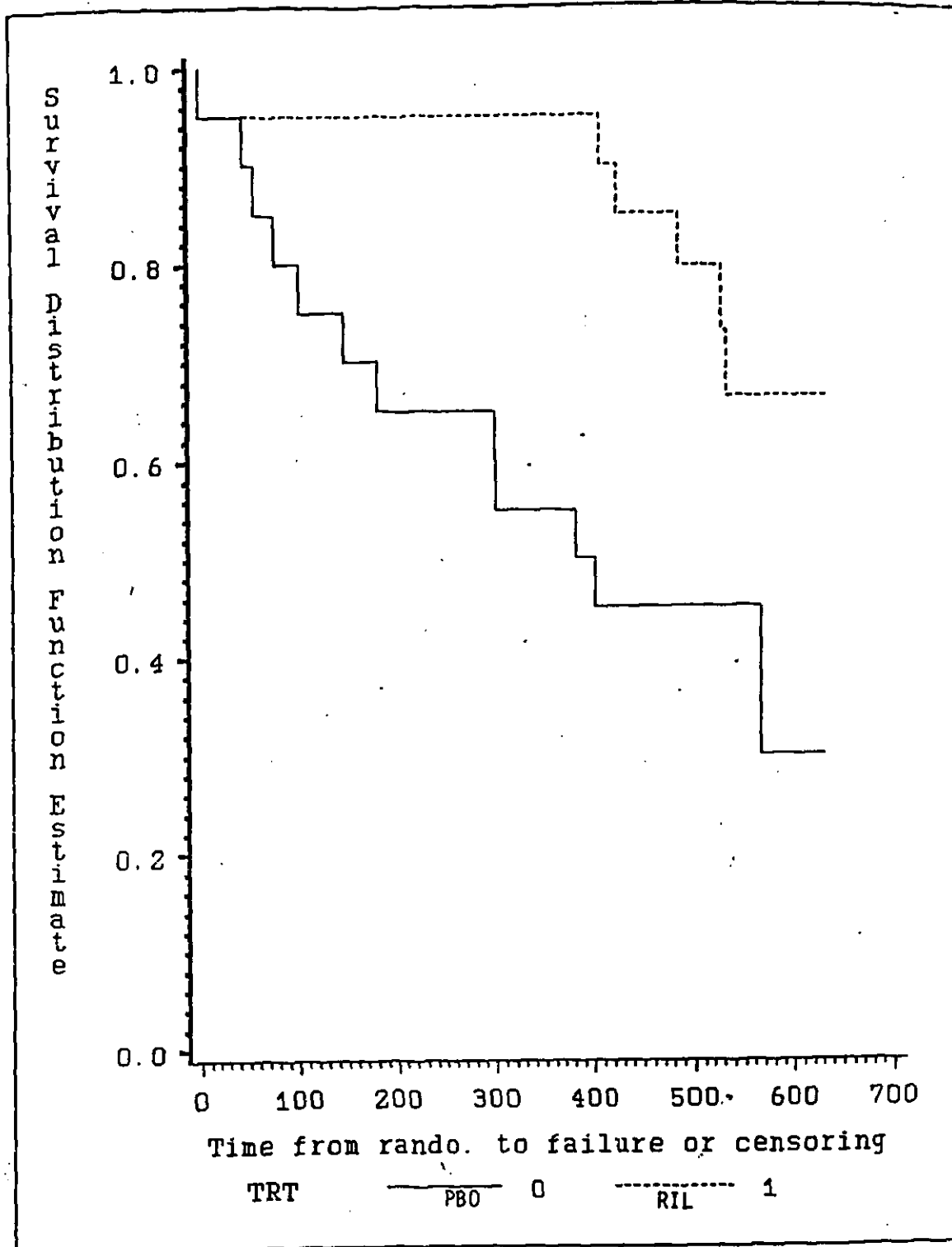


Figure 18



Statistical Review and Evaluation
Addendum

IND#

JUN 2 1995

Applicant: Rhone-Poulenc

Indication: ALS

Documents Reviewed: Fax dated May 3, 1994

Medical Input: John Feeney, M.D., HFD-120

Several issues about Study 216 have emerged since the review in 1994:

- 1) The p-value for failure reported in the New England Journal of Medicine article,
- 2) The fact that patients were lost to follow up (LTF) contrary to the assertion of no LTF in the NEJM article, together with the manner in which LTF was handled in the sponsor's three different analyses, and
- 3) The possibility that any survival advantage diminishes over the course of 18 months.

These 3 issues are discussed below.

1) The analysis specified in the protocol was Nov. 30, 1991, the point at which all patients had been followed for at least 1 year. The logrank p-value was .12 two-sided. According to the sponsor, the p-value of .014 reported in the NEJM article arose as a post-hoc analysis in which each patient had been followed for exactly one year. The reason given was that the 11/30/91 data showed evidence of non-proportional hazards. However, the logrank test is valid under this condition. As stated in my original review, this is not a valid reason to diminish the standing of the protocol-specified cutoff. **In fact, this analysis simply takes advantage of a slice of the data for which the survival curves are more widely separated than at the specified analysis. Thus, a statistically significant result is not surprising. See issue 3 below.**

2) Contrary to the article, there were 11 patients whose status (tracheostomy or death) was not known as of 11/30/91 and 3/12/92, the latter being approximately one week after the results of the 11/30/91 analysis were shown to investigators who then suggested that the follow up be curtailed. Of the 11 patients, 9 were in the riluzole group and 2 were in the placebo group. This imbalance is important because the sponsor assigned these patients survival durations equal to the time between randomization and a data cutoff, not the date at which status was last known. This procedure could result in a bias in favor of riluzole.

The sponsor has submitted the attached table with revised one-sided p-values resulting from the most recent follow up in which the statuses of 2 riluzole patients were still unknown. Note that the 3/12/92 p-value has increased to .062 two-sided from .048 which was originally submitted. The table makes clear why only the one-year analysis was reported to the NEJM.

3) The attached Figure 1 displays the plot of the hazard functions over time which correspond to the 3/12/92 analysis. Note that the hazards tend to increase with time and that the riluzole hazard tends to be greater than placebo's toward the end of follow up. The weakness of the logrank statistic indicates a tendency for mortality to converge near the end of follow up as in the other trial as in Study 301. See that review for further discussion. The attached Figure 2 showing the 3/12/92 Kaplan-Meier plots suggests a separation of the curves after November 1991. The attached Figure 3 displays the Kaplan-Meier plots for the 11/30/91 results. Median survival on riluzole was approximately 500 days while the median on placebo was approximately 440 days.



David Hoberman, Ph.D.
Mathematical Statistician

Concur: Dr. Nevius *SN* 6-2-95

for Dr. Dubey *SN* 6-7-95

cc:

Orig IND# 42,080

HFD-120

HFD-120/Ms Higgins

HFD-120/Dr. Leber

HFD-120/Dr. Katz

HFD-120/Dr. Feeney

HFD-713/Dr. Dubey [File: DRU 1.3.1]

HFD-713/Dr. Nevius

HFD-344/Dr. Lisook

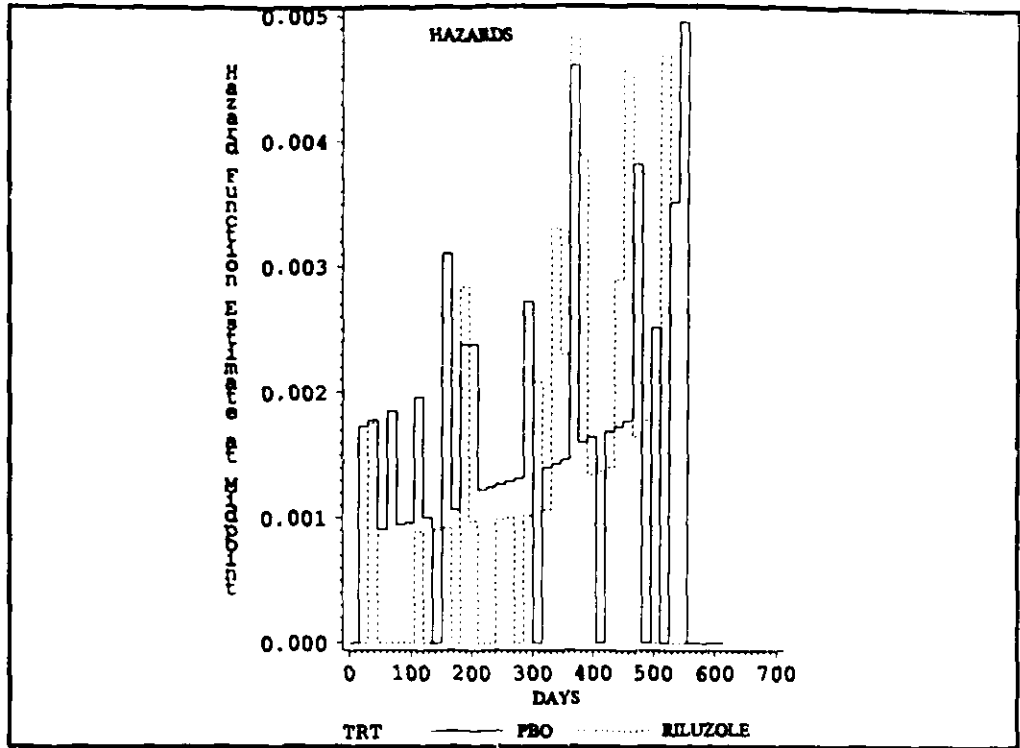
This review consists of 2 pages of text, 1 table and 3 figures

EFFICACY ANALYSES, RP 54274-216

ANALYSIS		STRATIFIED LOGRANK (p-value)*	STRATIFIED WILCOXON (p-value)*	COX MODEL (p-value)*
Nov. 30, 1991	ALL	0.070	0.029	0.044
	BULBAR	0.036	0.011	0.030
	LIMB	0.244	0.144	0.207
One Year	ALL	0.009	0.006	0.003
	BULBAR	0.007	0.005	0.024
	LIMB	0.099	0.078	0.041
March 12, 1992	ALL	0.031	0.020	0.033
	BULBAR	0.007	0.005	0.005
	LIMB	0.217	0.206	0.233

*All p-values are one-sided

FIGURE 1



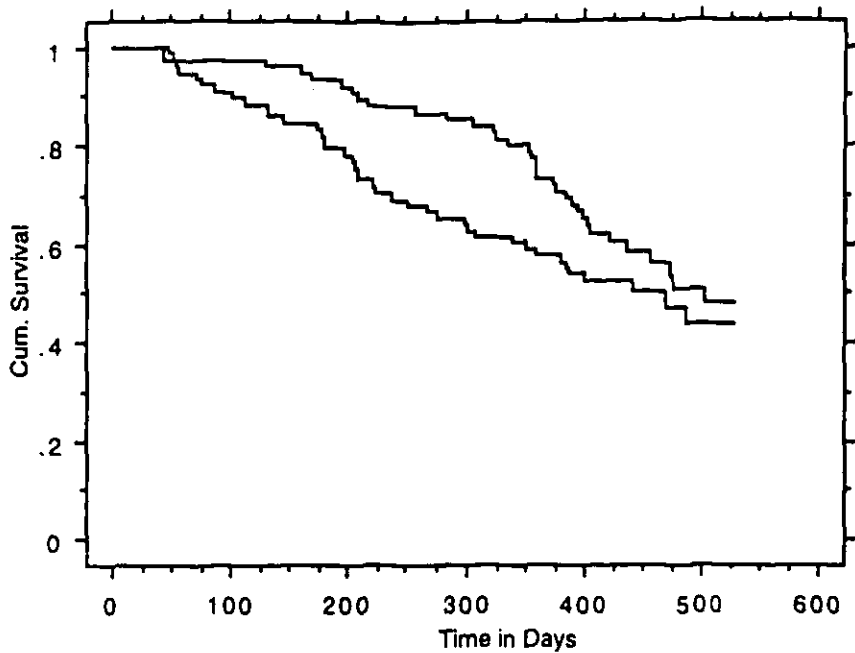


FIGURE 2

November 1991 Cutoff Date, riluzole vs placebo

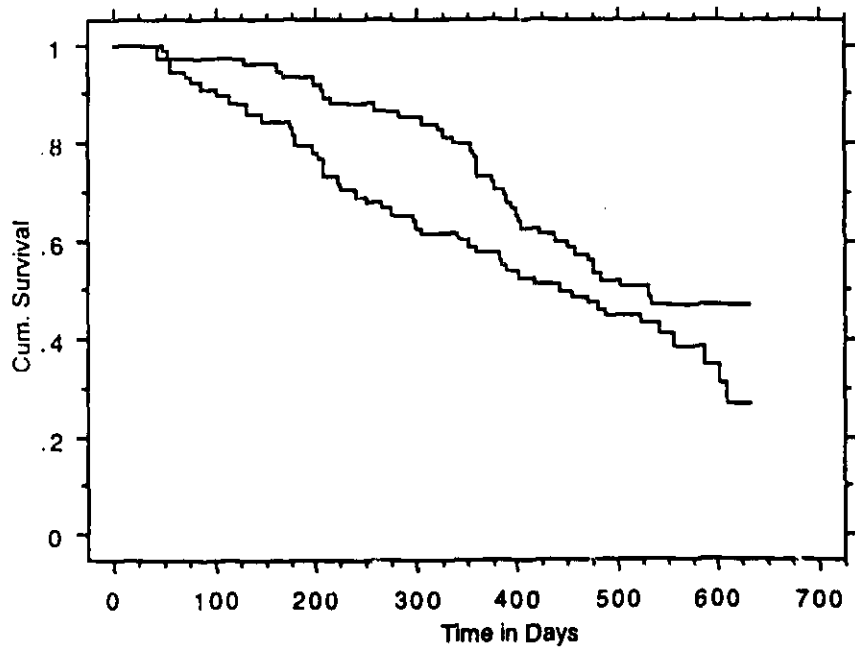


FIGURE 3

March 1992 Cutoff Date, riluzole vs placebo

Rilutek[®]
(Riluzole) Tablets

NDA 20-599

Chemistry

Volume ___ of ___

Division of Neuropharmacological Drug Products
Review of Chemistry, Manufacturing, and Controls

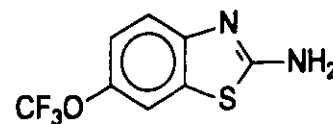
NDA: 20-599

	<u>Letter Date</u>	<u>Stamp Date</u>	<u>Rec'd by Chemist</u>	<u>Completed</u>
Initial Submission	29-Jun-95	30-Jun-95	05-Jul-95	16-Oct-95
Chemistry Review	#1	Sponsor	Rhône-Poulenc Rorer Pharmaceuticals INC	
Review Chemist	D. Scarpetti, Ph.D.	Address	500 Arcola Road PO Box 1200 Collegeville, PA 19426-0107	
Product Name				
Proprietary:	Riluzole			
Nonproprietary/USAN:	Riluzole			
Code Name/#:	RP 54274, PK 26124			

Chemical Name, Structural and Molecular Formula
2-Amino-6-(trifluoromethoxy)benzotriazole, $C_8H_5F_3N_2OS$ (MW 234.2)

OCT 25 1995

Dosage Form/Route of Administration
50 mg tablet / Oral



Pharmacological Category/Indication
Amyotrophic Lateral Sclerosis (ALS)

Supporting Documents

Remarks

Riluzole (Rilutek) is a new molecular entity synthesized in 5 steps not including an optional reupification step. The starting materials for the synthesis have adequate specifications and control over the manufacturing of the drug substance has been demonstrated by an extensive process validation campaign. The drug product is a 50 mg active, white, film-coated tablet that weighs 204 mg and is engraved with "RPR-R202". It has been formulated using a validated,

The analytical methods for detection of the drug substance neat and within the drug product matrix are adequate and validated. When the updated methods (validated) are submitted for the separation of the methods will be submitted to FDA labs for validation.

The tablets will be packaged 60 to a plastic bottle. A shelf life of 24 months was proposed by the sponsor but 18 months was suggested as a more reasonable period based on the available data

Conclusions and Recommendations

Recommendation for NDA 20-599 is approvable pending methods validation.

cc:

Original NDA 20-599
HFD-120/Division File
HFD-120/DScarpetti
HFD-120/KHiggins
HFD-120/SWBlum
Init by: SWB/

AMB 10/24/95

David Scarpetti 10/24/95
David Scarpetti, Ph.D., Chem Reviewer

Division of Neuropharmacological Drug Products
Review of Chemistry, Manufacturing, and Controls

NDA: 20-599

	<u>Letter Date</u>	<u>Stamp Date</u>	<u>Rec'd by Chemist</u>	<u>Completed</u>
Initial Submission	29-Jun-95	30-Jun-95	05-Jul-95	22-Aug-95
Amendments	BC 08-Sep-95	11-Sept-95	11-Sep-95	11-Oct-95
	BC 22-Sep-95	25-Sep-95	25-Sep-95	11-Oct-95

Chemistry Review #2 **Sponsor** Rhône-Poulenc Rorer Pharmaceuticals INC

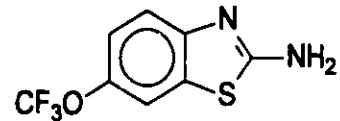
Review Chemist D. Scarpetti, Ph.D. **Address** 500 Arcola Road
PO Box 1200
Collegeville, PA 19426-0107

Product Name

Proprietary: Riluzole
Nonproprietary/USAN: Riluzole
Code Name/#: RP 54274, PK 26124

OCT 24 1995

Chemical Name, Structural and Molecular Formula
2-Amino-6-(trifluoromethoxy)benzotriazole : C₈H₅F₃N₂OS (MW 234.2)



Dosage Form/Route of Administration
50 mg tablet / Oral

Pharmacological Category/Indication
Amyotrophic Lateral Sclerosis (ALS)

Supporting Documents

Remarks

The information amendments to Original NDA 20-599 dated 08-Sep-95 provided information relating to
The information amendment
dated 22-Sep-95 provided information on the labeling for Rilutek.

Conclusions and Recommendations

The amendments submitted to original NDA are approvable.

cc:

Original NDA 20-599
HFD-120/Division File
HFD-120/DScarpetti
HFD-120/KHiggins
HFD-120/SWBlum
Init by: SWB/

AMB 10/23/95

David Scarpetti 10/23/95
David Scarpetti, Ph.D., Chem Reviewer

ENVIRONMENTAL ASSESSMENT
AND
FINDING OF NO SIGNIFICANT IMPACT

Rilutek®
(riluzole)
50 mg tablets

NDA 20-599

FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH
DIVISION OF NEUROPHARMACOLOGICAL
DRUG PRODUCTS (HFD-120)

FINDING OF NO SIGNIFICANT IMPACT

NDA 20-599

Rilutek®

(riluzole)

Tablets

The National Environmental Policy Act of 1969 (NEPA) requires all Federal agencies to assess the environmental impact of their actions. FDA is required under NEPA to consider the environmental impact of approving certain drug product applications as an integral part of its regulatory process.

The Food and Drug Administration, Center for Drug Evaluation and Research has carefully considered the potential environmental impact of this action and has concluded that this action will not have a significant effect on the quality of the human environment and that an environmental impact statement therefore will not be prepared.

In support of their new drug application for **Rilutek®**, **Rhône-Poulenc Rorer Pharmaceuticals, Inc.** has prepared an abbreviated environmental assessment in accordance with 21 CFR 25.31a(b)(3) (attached) which evaluates the potential environmental impacts of the manufacture, use and disposal of the product. **Rilutek®** qualifies for an abbreviated environmental assessment because the drug is used in the treatment of a rare disease and has been granted Orphan Drug Status by FDA.

Riluzole is a synthetic drug which is administered orally for the treatment of amyotrophic lateral sclerosis (ALS), a motor neuron disease. The drug substance manufacturing process will take place at several facilities identified in the environmental assessment. The drug product will be manufactured and packaged by Rhône-Poulenc Rorer, Republic of Ireland and packaged at Rhône-Poulenc Rorer, Manati, Puerto Rico. The finished drug product will be used in hospitals, clinics and by patients in their homes.

Disposal may result from production waste such as out of specification lots, returned or expired product, and user disposal of empty or partly used product and packaging. Pharmaceutical waste and returned or expired product will be disposed of by the manufacturer at licensed facilities. At U.S. hospitals empty or partially empty packages will be disposed according to hospital procedures. From home use, empty or partially empty containers will typically be disposed of by a community's solid waste management system which may include

landfills, incineration and recycling, while minimal quantities of unused drug may be disposed of in the sewer system.

Precautions taken at the sites of manufacture of the bulk drug product and its final formulation are expected to minimize occupational exposures and environmental release.

The Center for Drug Evaluation and Research has concluded that the product can be manufactured, used and disposed of without any expected adverse environmental effects. Adverse effects are not anticipated upon endangered or threatened species or upon property listed in or eligible for listing in the National Register of Historic Places.

10/20/95 Nancy B Sager
DATE PREPARED BY
Nancy B. Sager
Environmental Scientist
Center for Drug Evaluation and Research

10/20/95 R Williams
DATE CONCURRED
Roger L. Williams, M.D.
Center for Drug Evaluation and Research

Attachment: Environmental Assessment

REDACTIONS MADE
BY APPLICANT

NDA
Form FDA 356h
Item 3

Rilutek® (riluzole)

Item 3: *Chemistry, Manufacturing and Control*

Title: 3.6 ENVIRONMENTAL ASSESSMENT, FOI VERSION

Volume 1.10
Page

FOI Version

Report

Sections 1-4	3-9-85
Section 5	3-9-88
Section 6	3-9-113
Sections 7-15	3-9-155

REDACTIONS MADE
BY APPLICANT

New Drug Application
Form FDA 356h
Item 3

RILUTEK® (riluzole)

TITLE: Environmental Assessment for RILUTEK® NDA

SECTION 1: DATE: June 26th 1995

SECTION 2: APPLICANT: Rhône-Poulenc Rorer Pharmaceuticals, Inc.

**SECTION 3: ADDRESS: 500 Arcola Road
Collegetown, PA 19426-0107**

SECTION 4: DESCRIPTION OF THE PROPOSED ACTION:

4.A) Requested Action

Rhône-Poulenc Rorer is submitting a new drug application (NDA) for Riluzole for the treatment of amyotrophic lateral sclerosis (ALS), a motor neuron disease. The drug product is 50mg film-coated tablets packaged in HDPE bottles and will be marketed under the tradename Rilutek.

The drug substance, 2-amino-6-trifluoromethoxybenzothiazole, is one of a series of benzothiazoles synthesized by Rhône-Poulenc Rorer that appears to be an antagonist of glutamatergic transmission. Riluzole does not bind to any known receptors but it is known to exert a marked inhibitory effect on the spontaneous release of glutamate and on dopamine release induced by glutamate. The antagonism of glutamatergic transmission could be via interaction with voltage-dependent sodium channels or with G-proteins. However, the precise molecular target remains under investigation. The anti-glutamate activity of riluzole justified its evaluation in the treatment of ALS.

**REDACTIONS MADE
BY APPLICANT**

4.B) Need for the Action

Clinical studies have demonstrated that Rilutek offers a treatment for ALS. Approval of this NDA would make this treatment available to patients in the US.

4.C) Location Where the Product Will Be Produced

The drug substance will be manufactured at the following sites:

Steps 1, 2 and 3 are performed at:

Industrie Chimiques Mulhouse Dornach (ICMD)
72 Rue du Than
F 68 200 Mulhouse
France

Step 4 is performed at:

Rhône-Poulenc Rorer
Centre de Production Vitry
13 Quai Jules Guesde
94403 Vitry Sur-Seine Cedex
France

Step 5 is performed at:

Rhône-Poulenc Rorer
Villeneuve-La-Garenne Plant
35, Avenue Jean Jaures
92390 Villeneuve La Garenne
France

The Drug Product will be manufactured at:

Rhône-Poulenc Rorer
Linsbunny Industrial Estate
Nenagh, Co. Tipperary
Republic of Ireland

The tablets will be packaged at:

Rhône-Poulenc Rorer
Road 604 Coto Norte Industrial Park
Manati
Puerto Rico

4.D) Description of Facilities Where Rilutek® is Produced

The Industrie Chimique Mulhouse Dornach (ICMD) facility is a large industrial site located in an industrially zoned suburb of Mulhouse, France. The site is described in the DMF 11493-Type 1 referenced in Volume 3 of Item 3 of this application.

The Centre de Production Vitry (CPV) site is a large industrially zoned facility in Vitry-Sur-Seine, France. The site lies within the "Department de. Val de Marne".

The Villeneuve La Garenne Plant is located in an industrially zoned area approximately 5 km Northwest of Paris in the "Department des Hauts de Seine".

The Linsbunny Industrial Estate (Nenagh) is a small industrial area on the outskirts of Nenagh Town, Republic of Ireland. The Rhône-Poulenc Rorer facilities occupy about 9 acres of the 40 acre industrial estate.

The Rhône-Poulenc Rorer Puerto Rico facility occupies a site of about 10.5 acres North of the urban area of Manati Puerto Rico. The site is part of an existing industrial area in the San José Industrial Park in Manati.

All of the locations have soil that is free draining with good structure. All five sites are provided with appropriate potable water sources and sewage connections per the local requirements.

4.E) Locations Where the Product Will Be Used

Rilutek® will be used in hospitals for in-patient use and by patients for out-patient use through-out the United States.

**REDACTIONS MADE
BY APPLICANT**

RILUTEK®/RILUZOLE
Environmental Assessment Section of NDA

Section 5: Identification of Chemical Substances

5.A) Introduction

The physical and chemical properties of the drug substance Riluzole, the related isolated intermediates and raw materials are referenced in this section. The excipients for the drug product Rilutek®, the drug substance impurities and metabolites are also referenced.

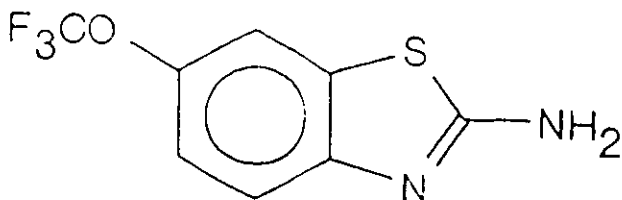
5.B) Drug Substance - Riluzole

5.B.I) Names and Codes

<u>Chemical Name:</u>	2-Amino-6-(trifluoromethoxy)benzothiazole
<u>INN/USAN:</u>	Riluzole
<u>RPR Code:</u>	RP 54274
<u>CAS Number:</u>	1744-22-5

5.B II) Structural Information

Chemical Structure



Riluzole, RP 54274

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REDACTIONS MADE
BY APPLICANT

Molecular Formula: C₈H₅F₃N₂OS

Molecular Weight: 234.2

5.B.III) Physical and Chemical Characteristics/MSDS

Appearance: White to slightly yellowish fine powder

Melting Point: Approximately 118°C

Water Solubility: 0.3 mg/ml

Partition Coefficient: Log P (Octanol/Water) = 3.5

pka: 3.8

MSDS: See Section 5, Attachment I

5.C) Drug Product Excipients

The drug product excipients for Rilutek[®] are identified in the Table I below with an indication of the amount of each excipient utilized relative to the amount of drug substance Riluzole. The drug product Rilutek[®] is an oral tablet which contains 50mg of the drug substance Riluzole. The CAS number for each of the excipients is also provided in Table I. Based upon the nature of the excipients, all of which are common compendial products, and the status of this filing as an abbreviated environmental assessment no further information is provided for the excipients. A MSDS for the Rilutek[®] drug product is provided as Section 5 Attachment II.

5 D) Synthetic Process

5 D.I) Process Description

The synthetic process to prepare Riluzole drug substance is described by the synthetic schematic provided as Section 5 Confidential Attachment III. A process schematic which describes the material flow related to the global synthesis schematic is provided as Section 5 Confidential Attachment IV. As noted in Section 4 C above the five step synthetic process is carried out in three parts at different sites in France

REDACTIONS MADE
BY APPLICANT

Table I

<u>Excipient Name</u>	<u>CAS Number</u>	<u>Amount Relative to Riluzole</u>
Dibasic calcium phosphate, anhydrous	7757-93-9	2.2
Microcrystalline cellulose	9004-34-6	0.59
Anhydrous colloidal silica	7631-86-9	0.02
Magnesium stearate	557-04-0	0.04
Croscarmellose sodium	74811-65-7	0.15

Film Coating Components

Methylhydroxypropyl cellulose	9004-65-3	-
Polyethylene Glycol 6000	25322-68-3	-
Titanium Dioxide	13463-67-7	-

5.D.II) Isolated Intermediates Names and Codes

In the five step process to prepare Riluzole, there are three isolated intermediates. These three process intermediates are described in Table II along with their CAS Number and amount required to prepare one kilogram of Riluzole drug substance.

5.D.III) Chemical Process Raw Materials

Provided in Table III is a listing of the chemical raw materials utilized in the preparation of Riluzole. In the Table III are included CAS Number, Molecular Formula, Molecular Weight and Appearance. Table IV presents the quantity of each chemical raw material required to produce one kilogram of Riluzole.

5.D.IV) Process Impurities

Certain synthetic process related impurities, specified at below 0.5% in the drug substance, have been identified. Information and chemical names for these process impurities are provided in Section 5, Confidential Attachment V. As none of the process impurities is present above 0.5% in the drug substance no further information or evaluation is provided for these materials.

5.E) Major Metabolites

Riluzole is extensively metabolized in man, with results after seven days from a single 150 mg dose of radiolabeled Riluzole indicating that about 91% of the radioactivity is excreted in the urine and about 5% in the feces. In man the primary route of metabolism is oxidative hydroxylation to give compounds with more polar character than Riluzole. In urine four hydroxylated species (RP 65077, RP 65110, RP 65331 and RPR 112512) have been identified along with a ureido derivative RP 69597. The four hydroxylated species appear in urine as glucuronosconjugates. Approximately 2% of the Riluzole dose is recovered in urine in an unconjugated form. The chemical names, code numbers and percentage recovered versus dose of Riluzole are provided in Section 5 Confidential Attachment IV. Under some conditions of glucuronidase cleavage of the RPR 112512 glucuronosconjugate, the material is converted directly back to the parent Riluzole. For the purposes of this filing no additional evaluation of individual metabolites has been undertaken.

TABLE II

Synthetic Intermediates

<u>Compound Name</u>	<u>MW</u>	<u>MF</u>	<u>CAS#</u>	<u>Description</u>	<u>Amount Required for 1 Kg Riluzole</u>
1-nitro-4-trifluoromethoxybenzene	C ₇ H ₄ F ₃ NO ₃	207.2	713-65-5	Light yellow to brown liquid	Confidential
4-trifluoromethoxy-benzenamine	C ₇ H ₆ F ₃ NO	177.2	461-82-5	Colorless to light brown liquid	Confidential
Riluzole crude (RP 54274)	C ₈ H ₅ F ₃ N ₂ OS	234.2	1744-22-5	Coarse yellow to yellow green powder	Confidential

REDACTIONS MADE
BY APPLICANT

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TAB III

RAW MATERIALS

<u>RAW MATERIAL</u>	<u>CAS#</u>	<u>MF</u>	<u>MW</u>	<u>APPEARANCE</u>
Trifluoromethoxybenzene	456-55-3	C ₇ H ₅ F ₃ O	162.11	Clear, Colorless Liquid
Sulfuric Acid	7664-93-9	H ₂ SO ₄	98.08	Clear to Slightly Opalescent Liquid
Nitric Acid	7697-37-2	HNO ₃	63.02	Clear liquid
Sodium Bicarbonate	144-55-8	NaHCO ₃	84.01	White Powder
Iron Filings	7439-89-6	Fe	55.85	Grey Magnetic Metallic Particles
Acetic Acid, Glacial	64-19-7	C ₂ H ₄ O ₂	60.05	Clear, Colorless Liquid
Potassium Thiocyanate	333-20-0	KSCN	97.18	Colorless Crystals
Sodium Carbonate	497-19-8	Na ₂ CO ₃	105.99	White Powder
Bromine	7726-95-6	Br ₂	159.82	Red/Brown Heavy Liquid
Aqueous ammonia (20%)	7664-41-7	NH ₄ OH	35.05	Clear Liquid
Methanol	67-56-1	CH ₃ OH	32.04	Clear Liquid
Charcoal L3S	7440-44-0	C	(12.01)	Fine Black Powder
Purified Water	7732-18-5	H ₂ O	18.02	Clear Liquid
Nitrogen (Gas)	7727-37-9	N ₂	28.02	Clear Oderless Gas

REDACTIONS MADE
BY APPLICANT

TABLE IV

Raw Materials Required for 1 Kg Riluzole

<u>Raw Material</u>	<u>CAS #</u>	<u>Quantity Required for 1 Kg Riluzole</u>
Trifluoromethoxy benzene	456-55-3	Confidential
Sulfuric Acid	7664-93-9	Confidential
Nitric Acid	7697-37-2	Confidential
Sodium Bicarbonate	144-55-8	Confidential
Iron	7439-89-6	Confidential
Acetic Acid, Glacial	64-19-7	Confidential
Potassium Thiocyanate	333-20-0	Confidential
Sodium Carbonate	497-19-8	Confidential
Bromine	7726-95-6	Confidential
Aqueous Ammonia (20%)	7664-41-7	Confidential
Methanol	67-56-1	Confidential
Charcoal L.A.S	7440-44-0	Confidential
Purified Water	7732-18-5	Confidential

**REDACTIONS MADE
BY APPLICANT**

REDACTIONS MADE
BY APPLICANT

SECTION 5: ATTACHMENTS

<u>ATTACHMENT #</u>	<u>ITEM</u>
I	MSDS Riluzole
II	MSDS Rilutek®
III	Chemical Process Schematic (CONFIDENTIAL)
IV	Process Schematic - Material Flow (CONFIDENTIAL)
V	Chemical Process Impurities (CONFIDENTIAL)
VI	Major Metabolites (CONFIDENTIAL)

REDACTIONS MADE
BY APPLICANT

ATTACHMENT I
MSDS - RILUZOLE

3-9-96

1. IDENTIFICATION**RILUZOLE****REDACTIONS MADE
BY APPLICANT****1.1 Manufacturer**

RHÔNE-POULENC RORER
BP 35 - 94403 VITRY-SUR-SEINE Cedex FRANCE

1.2 Emergency contact number

: Safety

Tel.: (33-1) 45 73 35 11

2. CHEMICAL CHARACTERIZATION/INFORMATION ON THE COMPONENTS

CAS Number : 1744-22-5

Component contributing to a hazard

: Riluzole

Impurities contributing to a hazard

: none

Other data

: Molecular Formula $C_8H_5F_3N_2OS$

: Molecular weight 234.2

3. HAZARDS IDENTIFICATION**TOXIC If swallowed****Risk of serious damage to eyes.****Dangerous for the aquatic environment.****4. FIRST AID MEASURES**

Skin contact

: wash immediately with plenty of water.

Eye contact

: wash immediately with plenty of water for at least 15 minutes.
Get medical help.

Ingestion

: consult a doctor immediately and show him the container or label.

5. FIRE-FIGHTING MEASURES

5.1 Fire extinguishing agents

: waterspray, foam, dry chemicals.

5.2 Fire extinguishing agents - not suitable

: hosepipe

5.3 Specific hazards fire or explosion

: nothing under 250°C

5.4 Special protective equipment for firefighters

: wear breathing apparatus.

6. ACCIDENTAL RELEASE MEASURES

6.1 Personal Precautions

: multi-purpose cartridge mask or protective hood with filtered breathing air, gloves, goggles, clothing.

6.2 Environmental precautions

: collect and burn in an approved installation

6.3 Methods for cleaning up

: to avoid dust, pick up with a damp rag and burn.

7. HANDLING AND STORAGE

7.1 Handling

: use with fume hood

7.2 Storage

: keep containers tightly closed and room temperature

8. EXPOSURE CONTROL/PERSONAL PROTECTIVE EQUIPMENT

- 8.1 Respiratory protection : multi-purpose cartridge mask or protective hood with filtered breathing air
- 8.2 Hand protection : gloves
- 8.3 Eye protection : chemical safety goggles
- 8.4 Skin protection : resistant protective clothing
- 8.5 Personal protection comments : shower after working.

9. PHYSICAL AND CHEMICAL PROPERTIES

- 9.1 Appearance : white to slightly yellowish fine powder
- 9.2 Odor : toxic material, will not be determined
- 9.3. pH : not applicable
- 9.4 Boiling point : not applicable
- 9.5 Melting point : approximately 118°C
- 9.6 Flash point : not applicable
- 9.7 Flammability (solid, gas) : upper to 250°C
- 9.8 Auto-ignition temperature : dust, E_{mi} 10-50 mJ
- 9.9 Explosion properties : dust, $T_{mi} > 600^\circ\text{C}$; $C_{mi} = 30\text{g/m}^3$ Class ST1
- 9.10 Oxidizing properties : no
- 9.11 Vapor pressure : not applicable
- 9.12 Density : 0.455 to 0.533
- 9.13 Solubility: – Water solubility : very slightly soluble
Aceton, Acetonitril, DMF : very soluble
- 9.14 Partition coefficient: n-octanol/water : log. P = 3.3 about

10. STABILITY AND REACTIVITY

- 10.1 Conditions to avoid : none to the best of our knowledge
- 10.2 Materials to avoid : an adsorption has been shown on PVC materials
- 10.3 Hazardous decomposition products : on combustion, toxic fumes are emitted: fluoride, nitrogen and sulphur compounds.

11. TOXICOLOGICAL INFORMATION

except for the micronucleus test, all studies reported here were performed with crude Riluzole

- acute LD 50 p.o. rat : 35 mg/kg ; note RPR/RD/CRVA/SM 93-0181 of 03/08/93
- Eye : irritant ; Pharmakon report n°7493 of 25/06/93.
- Skin : non irritant ; Pharmakon report n°77593 of 15/06/94.
- AMES test : negative; note RPR/RD/CRVA/SM 93-0155 of 15/10/93
- Micronucleus test : negative ref. 85/PHA/139/17

12. ECOLOGICAL INFORMATION

- 12.1 Mobility : target compartment, air : 43% and water : 48%
- 12.2 Persistence and degradability : not easy biodegradable (degradability = 0%)
- 12.3 Bio-accumulation : potentially bioaccumulable
- 12.4 Ecotoxicity : Daphnies CE 50/48h = 4.7 ppm (weight/weight)

13. DISPOSAL CONSIDERATIONS

Chemical product : incineration in approved center.
 Contaminated packaging : incineration in approved center.

REDACTIONS MADE
 BY APPLICANT

14. TRANSPORT INFORMATION

	RID/ADR	QMI	QACI/ATA	
Class	6.1	6.1	6.1	
Labelling	6.1 A	6.1-04	6 poisonous	
Group, Enumeration, Page	90° c	III 6236	III	
N° ONU		2811	2811	
Passenger Plane			P.I.: 619	100kg
Transport Aircraft			P.I.: 619	200kg

NOTE: P.I.: packing instruction

15. REGULATORY INFORMATION

European labelling : Toxic T and Dangerous for environment N
 R25 Toxic if swallowed
 R41 Risk of serious damage to eyes.
 H51-53 Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
 H22 Do not breathe dust
 S37/39 Wear suitable gloves and eye/face protection
 S61 Avoid release to the environment. refer to special instructions/Safety data sheets.
 N.B.: Comply with national legislation

16. OTHER INFORMATION

16.1 Warning statements : inform the personnel of the precautions to be taken and equip the concerned personnel with adequate personal protective equipment
 16.2 Use : pharmaceutical use, central nervous system diseases.
 16.3 Main literature references : Rhône-Poulenc Rorer in-house documentation .

IMPORTANT

The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication is in our opinion consistent with the state of general scientific and technical knowledge at that date but we cannot accept liability for any loss, injury or damage which may result from its use.

In compiling the Data Sheet we have taken into account all proper applications of the substance of which we are aware and any user of the substance should consult us before applying it to any novel or unusual use. We shall use our best endeavours to ensure that any revision of this Safety Data Sheet is sent to all customers to whom we have directly supplied this substance, but we must point out that it is the responsibility of any intermediate supplier to ensure that such revision is passed to the ultimate user. If any such ultimate user wishes to make arrangements for revisions to be sent to him direct, we shall, if so notified, be glad to make the necessary entry in our records.

The information given in this Safety Data Sheet is designed only as a guidance for the safe handling, storage and use of the substance. It is not a specification nor does it guarantee any specific properties.

REDACTIONS MADE
BY APPLICANT

ATTACHMENT II

MSDS - Rilutek®

Material Safety Data Sheet

Effective Date: 6/19/95 Date Printed: 6/19/95 MSDS: 181

Page 1

1. Chemical Product and Company Identification

Product Name Rilutek

Rhone-Poulenc Rorer

P.O. Box 1200

Collegeville, PA 19426-0107

24-Hour Emergency Phone: 610-454-8181

2. Composition/Information on Ingredients

<u>Component:</u>	<u>CAS</u>	<u>Exposure Limits</u>	<u>% by Wt.</u>
Riluzole	1744-22-5	NA	25
Dibasic calcium phosphate, anhydrous	7757-93-9	NA	55.1
Microcrystalline cellulose	9004346	NA	14.7
Silica	7631-86-9	10 mg/m ³	0.5
Magnesium stearate	557-04-0	10 mg/m ³	1
Croscarmellose sodium	74811-65-7	NA	3.7

3. Hazards Identification

Potential Health Effects

Emergency Overview

R25 Toxic if swallowed. R52 Harmful to aquatic organisms.

Eye

If tablet is crushed (R41) risk of serious damage to eyes from exposure to dust.

(Continued on Page 2)

Material Safety Data Sheet

Effective Date: 6/19/95 Date Printed: 6/19/95 MSDS: 181

Page 2

Product Name: Rilutek

Rhone-Poulenc Rorer

P.O. Box 1200

Collegeville, PA 19426-0107

24-Hour Emergency Phone: 610-454-8181

REDACTIONS MADE
BY APPLICANT

Skin Contact

As tablets -- None expected.

Skin Absorption

As tablets -- None expected.

Ingestion

R25 Toxic if swallowed.

Inhalation

As tablets -- None expected.

Chronic Effects/Carcinogenicity

Adverse potential reactions are detailed on the package insert and in the Physicians' Desk Reference.

4. First Aid Measures

Eyes

In case tablet is crushed (S25) avoid contact of dust with eyes S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Skin

No measures necessary for contact with intact tablets. If tablets are crushed, (S28) after contact with skin, wash immediately with plenty of soap and water.

Ingestion

In case of accidental ingestion, (S46) seek medical advice immediately and show this container or label.

Inhalation

Unlikely. If it occurs, seek medical attention.

(Continued on Page 3)

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Material Safety Data Sheet

Effective Date: 6/19/95 Date Printed: 6/19/95 MSDS: 181

Page 3

Product Name: Rilutek

Rhone-Poulenc Rorer

P.O. Box 1200

Collegeville, PA 19426-0107

24-Hour Emergency Phone: 610-454-8181

REDACTIONS MADE
BY APPLICANT

Note to Physician

Additional details are available on the package insert or in the Physicians' Desk Reference.

5. Fire Fighting Measures

FLAMABLE PROPERTIES:

Flash Pt: NA

Method:

FLAMABLE LIMITS:

Lower Flammable Limit: NA %

Upper Flammable Limit: NA %

Autoignition: NA

General Hazards

CO, CO₂ and oxides of nitrogen and sulfur may be generated in a fire.

Fire Fighting Extinguishing Media

S43 In case of fire use waterspray, foam or dry chemical.

Fire Fighting Instructions

S43 In case of fire, use full firefighting turnout (bunker) gear and self-contained breathing apparatus (SCBA). Keep personnel upwind and away from fire.

Hazardous Combustion Products

CO, CO₂ and oxides of nitrogen and sulfur may be generated in a fire.

6. Accidental Release Measures

Large Spill

Scoop up tablets. In case of crushed tablets, S36 wear suitable protective clothing

(Continued on Page 4)

Material Safety Data Sheet

Effective Date: 6/19/95 Date Printed: 6/19/95 MSDS: 181

Page 4

Product Name: Rflutek

Rhone-Poulenc Rorer

P.O. Box 1200

Collegeville, PA 19426-0107

24-Hour Emergency Phone: 610-454-8181

REDACTIONS MADE
BY APPLICANT

Small Spill

Scoop up tablets. In case of crushed tablets, S36 wear suitable protective clothing.

7. Handling and Storage

Special Handling

Protect package from physical damage. Wash thoroughly after handling.

Special Storage

S2 Keep out of the reach of children. S57 Use appropriate container to avoid environmental contamination.

8. Exposure Controls / Personal Protection

Eye Protection

As tablets, none required. If tablets are crushed, S39 wear eye/face protection.

Skin Protection

As tablets, none required.

Respiratory Protection

As tablets, none required. May be required for spill and clean-up or other situations in which product may become airborne.

Engineering Controls

As tablets, none required.

9. Physical and Chemical Properties

Boiling Point:		NA
Melting Point:	118	DEGREES C
Vapor Pressure:		NA at

(Continued on Page 5)

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Material Safety Data Sheet

Page 5

Effective Date: 6/19/95 Date Printed: 6/19/95 MSDS: 181

Product Name: Rilutek

Rhone-Poulenc Rorer
P.O. Box 1200
Collegeville, PA 19426-0107
24-Hour Emergency Phone: 610-454-8181

REDACTIONS MADE
BY APPLICANT

Vapor Density: NA
Soluble in Water: slightly
Specific Gravity: at NA
pH:
Odor: NA
Appearance: White film coated tablets

10. Stability and Reactivity

Stability

Product has a shelf life.

Incompatibility

No known incompatibilities. See package insert or Physicians' Desk Reference for more information on potential drug interactions.

Hazardous Decomposition Products

No known hazardous decomposition products.

Hazardous Polymerization

Will not occur.

General Information

S41 In case of fire and/or explosion do not breathe fumes.

11. Toxicological Information

Toxicology Tests

Test	LD/LC	Test Type	Route	Species	Results
50	LD	acute	p.o.	rat	35 mg/kg

(Continued on Page 6)

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Material Safety Data Sheet

Effective Date: 6/19/95 Date Printed: 6/19/95 MSDS: 181

Page 6

Product Name: Rilutek

Rhone-Poulenc Rorer

P.O. Box 1200

Collegeville, PA 19426-0107

24-Hour Emergency Phone: 610-454-8181

REDACTIONS MADE
BY APPLICANT

Toxicology Text

Dust is irritating to the eyes but not the skin in animal studies. Ames and micronucleus test negative.

12. Ecological Information

Ecological Information

R51 Toxic to aquatic organisms. Daphnia EC 50/48 hr = 4.7 ppm. Potentially bioaccumulable. Not easily biodegradable. Target compartment air (43%) and water (48%).

13. Disposal Considerations

Disposal Information

S35 This material and its container must be disposed of in a safe way. S61 Avoid release to the environment. Incineration is the preferred method.

Waste Disposal Methods

Waste must be disposed of in accordance with federal, state and local environmental regulations. Incineration is the preferred method.

14. Transport Information

15. Regulatory Information

TSCA

This material is a pharmaceutical agent and as such is regulated by the United States Food and Drug Administration (FDA).

(Continued on Page 7)

3-9-106

Material Safety Data Sheet

Effective Date: 6/19/95 Date Printed: 6/19/95 MSDS: 181

Page 7

Product Name: Rlutek

Rhone-Poulenc Rorer

P.O. Box 1200

Collegeville, PA 19426-0107

24-Hour Emergency Phone: 610-454-8181

CERCLA

SARA 302

SARA 313

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- BY APPLICANT

16. Other Information

Other Information

The information contained herein is based upon data considered true and accurate. Rhone-Poulenc Rorer makes no warranties, express or implied, as to the adequacy of the information contained herein. This information is offered solely for the user's consideration, investigation and verification. Prepared by RPRCR Department of Safety and the Environment - Collegeville. Report to the manufacturer any allegations of health effects resulting from handling or accidental contact with this material.

Revision Summary

3-9-107

Material Safety Data Sheet

Effective Date: 6/19/95 Date Printed: 6/19/95 MSDS: 181

Page 8

Product Name: Rilutek

Rhone-Poulenc Rorer

P.O. Box 1200

Collegeville, PA 19426-0107

24-Hour Emergency Phone: 610-454-8181

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BY APPLICANT

3-9-108

REDACTIONS MADE
BY APPLICANT

CONFIDENTIAL ATTACHMENT III

Chemical Process Schematic

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BY APPLICANT

CONFIDENTIAL ATTACHMENT IV

Process Schematic - Material Flow

REDACTIONS MADE
BY APPLICANT

CONFIDENTIAL ATTACHMENT V

Chemical Process Impurities

REDACTIONS MADE
BY APPLICANT

CONFIDENTIAL ATTACHMENT VI

Major Metabolites

RILUTEK®/RILUZOLE
Environmental Assessment Section of NDA

REDACTIONS MADE
BY APPLICANT

Section 6: Introduction of Substances into the Environment

6.A) Introduction

As noted by the listing of RPR production sites in Section 4 of this filing, the production of Riluzole drug substance and Rilutek® drug product involve a sequence of industrial sites. A schematic flow chart is provided (see Section 6, Confidential Attachment I) to clarify the correlation of production process stages with particular production sites. All of the activities with exception of the final drug product packaging take place in geographic areas not governed by the United States of America. All of the process chemistry related production sites are in receipt of governmental attestations of environmental compliance. The calculated EEC for Riluzole drug substance is provided.

6.B) Environmental Compliance for Non-US Sites

6.B.I) France - Governmental Attestations of Environmental Compliance

The appropriate governmental authorities have provided attestations of compliance with applicable environmental regulations for the three sites involved with Riluzole Production in France. The letters of attestation for these three sites are provided as Section 6 Attachments as follows:

<u>Site</u>	<u>Attachment</u>
ICMD (Mulhouse)	II
RPR-CPV (Vitry-Sur-Seine)	III
RPR-VLG (Villeneuve La Garenne)	IV

6 B. II) Republic of Ireland - Environmental Compliance for Nenagh Site

The bulk drug substance Riluzole is processed at the RPR Pharmaceuticals, LTD site in Nenagh to produce drug product tablets and the tablets are coated to prepare Rilutek®. The process is a normal pharmaceutical manufacturing process which employs pharmacopial excipients. The process does not include any chemical modification or treatment of the Riluzole drug substance.

REDACTIONS MADE
BY APPLICANT

6.B.II.1) Statement of Compliance

Provided as Section 6 Attachment V is a statement of compliance for the RPR-Nenagh site by the responsible Rhône-Poulenc Rorer official for the site. The statement applies to the production of Rilutek® drug product tablets at the site.

6.B.II.2) None of the excipients or other materials used in the production of the Rilutek® tablets at the Nenagh site are in the category of OSHA regulated substances as defined by SARA 313.

6.B.II.3) Information Related to Permits for Waste Disposal

Provided as Section 6 Attachment VI is a summary of the governmental permit and license numbers applicable to the disposal of waste water and solid waste streams from the Rilutek® tablet and tablet coating processes at the RPR Nenagh site. As a normal pharmaceutical tablet process the primary waste generated is the result of equipment cleaning. Damaged or rejected drug substance will be returned to the Riluzole production site for proper disposition via recovery or disposal. Rejected or returned drug product are expected to be minimal. Drug product that must be sent for disposal will be sent for incineration.

6.B.II.4) Engineering and Procedural Controls for Emissions

Provided as Section 6 Attachment VII is a description of the engineering and procedural controls in effect at the Nenagh site to insure minimization of emissions from the process to produce Rilutek® drug product tablets. As a part of the description of procedural controls there is included a listing of operating procedures which are relevant to the control of waste emissions from the site. A description of the employee training in operational procedures is included.

6.B.II.5) Impact on Utilities Consumption

The small volume of Rilutek® tablets produced on an annual basis will have an insignificant (<5%) impact on the utility requirements for the Rhône-Poulenc Rorer Nenagh site.

6.B.III) Puerto Rico - Environmental Compliance for the Manati Site

Bulk Rilutek® drug product tablets will be packaged and labeled at the Rhône-Poulenc Rorer Manati, Puerto Rico site for commercial

distribution. No chemical processing related to Riluzole drug substance or pharmaceutical processing related to the Rilutek® drug product will take place at the Manati site

REDACTIONS MADE
BY APPLICANT

6.B.III.1) Statement of Compliance

Provided as Section 6 Attachment VIII is a statement of environmental compliance for the Manati site related to activities with Rilutek® drug product. The statement is signed by the responsible Rhône-Poulenc Rorer official for environmental matters at the Manati site.

6.B.III.2) Use and Disposal of Product Materials

No Riluzole drug substance is processed at the Manati site and therefore disposal will not be required. Drug product bulk tablets will be packaged and labeled at the Manati site. The operations of packaging and labeling are strictly controlled by inventory and shipping procedures. All packaging and labeling materials are of standard pharmaceutical quality. As all bulk drug product utilized is previously released for commercial use the need for disposal of out-of-specification or rejected Rilutek® drug product is expected to be minimal.

6.B.III.3) Sewage and Waste Removal

Liquid wastes from the packaging of Rilutek® tablets will be from equipment washing, these wastes will be discharged to the process lines to be treated appropriately. The facility currently operates under extensions of the permit number GDA-92-210-037 with the Puerto Rico Aqueducts and Sewage Authority (Section 6, Attachment LX for a copy of the original permit cover and English translation of the permit) until completion of modifications to the pretreatment facility. Rhône-Poulenc Rorer Puerto Rico adheres to the National Pollutant Discharge Elimination System (NPDES) storm water discharge associated with industrial activity, general Permit No RPR 00A047 and Best Management Practices for pollution prevention and spill management

There is expected to be no hazardous waste material generated as a result of the Rilutek® packaging and labeling. Non-hazardous solid waste generated will be placed in the non-hazardous solid waste compactor Browning Ferris Industries, a duly authorized non-hazardous waste disposer, will periodically dispose of these wastes at the Vega Baja Municipal landfill. A significant

portion of the non-hazardous solid waste consists of cartons and plastic containers which Rhône-Poulenc Rorer currently recycles and will continue to do so.

REDACTIONS MADE
BY APPLICANT

6.C) Calculation of EEC

In the fifth year of commercial production the total quantity of Riluzole drug substance projected to be produced for use as Rilutek® in the United States is "Confidential" pounds. Based on human metabolism information for Riluzole the total drug substance would be expected to enter public and private sewer systems. With these assumptions a calculation of the total waste water treatment plant load of Riluzole, expressed in parts per million concentration gives a ppm value equal to "Confidential" ppm. See Section 6, Attachment X for the EEC calculation and assumptions.

SECTION 6: ATTACHMENTS

REDACTIONS MADE
BY APPLICANT

ATTACHMENT

ITEM

I	Production Sites (Confidential)
II	Certification ICMD
III	Certification Vitry-Sur-Seine
IV	Certification Villeneuve La Garenne
V	Compliance Statement - Nenagh, Ireland
VI	Waste Disposal Permits - Nenagh
VII	Engineering and Procedural Controls - Nenagh
VIII	Compliance Statement - Manati, Puerto Rico
IX	Industrial Discharge Permit - Manati
X	Calculation of EEC

REDACTIONS MADE
BY APPLICANT

CONFIDENTIAL ATTACHMENT I

Production Sites

REDACTIONS MADE
BY APPLICANT

ATTACHMENT II

Governmental Attestation of Environmental Compliance

ICMD (Mulhouse, France)

REDUCTIONS MADE BY APPLICANT

LE DIRECTEUR DE LA PRÉVENTION DES POLLUTIONS ET DES RISQUES

PARIS, le 21 JUIN 1994

Attestation de conformité à la réglementation en matière d'environnement

Le site de la Société INDUSTRIE CHIMIQUE MULHOUSE DORNACH (I.C.H.D.) de MULHOUSE, établissement classé, est selon la réglementation, sous le contrôle technique permanent des services administratifs compétents : Direction Régionale de l'Industrie, de la Recherche et de l'Environnement et Agence de Bassin.

Les résultats de ce contrôle montrent que les fabrications en place, destinées à la production de para-trifluoro-méthoxy-aniline, sont réalisées sur ce site conformément aux réglementations en vigueur, en matière d'environnement.

En foi de quoi, je soussigné, DEFRANCE Gustave, Directeur de la Prévention des Pollutions et des Risques, certifie que le site de la Société INDUSTRIE CHIMIQUE MULHOUSE DORNACH (I.C.H.D.) de MULHOUSE respecte les obligations de la législation française pour la protection de l'environnement.

En conséquence, la présente attestation a été rédigée pour servir et valoir ce que de droit.

Pour le ministre et par délégation le directeur de la prévention des pollutions et des risques, délégué aux risques majeurs

Fait à PARIS, le

21 JUIN 1994 Gustave DEFRANCE

The site of INDUSTRIE CHIMIQUE MULHOUSE DORNACH (I.C.H.D.) in MULHOUSE which is duly classified according to the french regulations, is under the permanent technical control of competent administrative departments : Regional Department of Industry, Research and Environment and Waste Water Treatment Agency.

The results of this control demonstrate that the manufacturing operations in place leading to the production of para-trifluoro-methoxy-aniline are conducted on this site in compliance with current environmental regulations.

In witness whereof, I undersigned, DEFRANCE Gustave, Director of the Department of prevention of pollutions and risks, certify that INDUSTRIE CHIMIQUE MULHOUSE DORNACH in MULHOUSE fulfills the requirements of the French regulations with respect to environment protection.

Consequently, the present certificate is issued to be worth and used for whatever purpose by whom it may concern.

21 JUIN 1994

PARIS, le

Pour le ministre et par délégation le directeur de la prévention des pollutions et des risques, délégué aux risques majeurs

20, avenue de Segur - 75302 PARIS 07 SP - Tél. (1) 42.15.20.21

REDACTIONS MADE
BY APPLICANT

ATTACHMENT III

Governmental Attestation of Environmental Compliance

Rhône-Poulenc Rorer - CPV (Vitry-Sur-Seine, France)

MINISTÈRE DE L'ENVIRONNEMENT

RÉPUBLIQUE FRANÇAISE

LE DIRECTEUR DE LA PRÉVENTION
DES POLLUTIONS ET DES RISQUES

REDUCTIONS MADE
BY APPLICANT

PARIS, le 21 Juin 1994

Attestation de conformité à la réglementation en matière
d'environnement

Le site RHONE-POULENC RORER de VITRY S/SEINE, établissement classé, est selon la réglementation sous le contrôle technique permanent des services administratifs compétents : Service Technique des Installations Classées, Direction Régionale de l'Industrie, de la Recherche et de l'Environnement, Agence de l'Eau.

Les résultats de ce contrôle montrent que les fabrications en place, destinées à la production de RILUZOLE BRUT sont réalisées sur ce site conformément aux réglementations en vigueur, en matière d'environnement.

En foi de quoi, je soussigné DEFRANCE Gustave, Directeur de la Prévention des Pollutions et des Risques, certifie que le site RHONE-POULENC RORER de VITRY S/SEINE respecte les obligations de la législation française pour la protection de l'environnement.

En conséquence, la présente attestation a été rédigée pour servir et valoir ce que de droit.

Pour le ministre et par délégation
le directeur de la prévention
des pollutions et des risques,
délégué aux risques majeurs

Fait à PARIS, le

Gustave DEFRANCE

The RHONE-POULENC RORER site of VITRY S/SEINE which is duly classified according to the French regulations, as under the permanent technical control of competent administrative departments : Technical Department for Listed Facilities, Regional Department of industry, Research and Environment, Waste Water Treatment Agency.

The results of this control demonstrate that the manufacturing operations in place leading to the production of RILUZOLE BRUT are conducted on this site in compliance with current environmental regulations.

In witness whereof, I undersigned DEFRANCE Gustave, Director of the Department of prevention of pollutions and risks, certify that RHONE-POULENC RORER site of VITRY S/SEINE fulfills the requirements of the French regulations with respect to environment protection.

Consequently, the present certificate is issued to be worth and used for whatever purpose by whom it may concern.

PARIS, le

Pour le ministre et par délégation
le directeur de la prévention
des pollutions et des risques,
délégué aux risques majeurs

21 Juin 1994
Gustave DEFRANCE

NDA20599

4 OF 7

REDACTIONS MADE
BY APPLICANT

ATTACHMENT IV

Governmental Attestation of Environmental Compliance

Rhône-Poulenc Rorer - VLG (Villeneuve LaGarenne, France)

REDACTIONS MADE
BY APPLICANT

LE DIRECTEUR DE LA PREVENTION
DES POLLUTIONS ET DES RISQUES

PARIS, le 21 JUIN 1994

Attestation de conformité à la réglementation en matière
d'environnement

Le site RHONE-POULENC RORER de VILLENEUVE LA GARENNE, établissement classé, est selon la réglementation sous le contrôle technique permanent des services administratifs compétents : Service Technique des Installations Classées, Direction Régionale de l'Industrie, de la Recherche et de l'Environnement, Agence de l'Eau.

Les résultats de ce contrôle montrent que les fabrications en place, destinées à la production de RILUZOLE PUR sont réalisées sur ce site conformément aux réglementations en vigueur, en matière d'environnement.

En foi de quoi, je soussigné DEFRANCE Gustave, Directeur de la Prévention des Pollutions et des Risques, certifie que le site RHONE-POULENC RORER de VILLENEUVE LA GARENNE respecte les obligations de la législation française pour la protection de l'environnement.

En conséquence, la présente attestation a été rédigée pour servir et valoir ce que de droit.

Pour le ministre et par délégation
le directeur de la prévention
des pollutions et des risques,
Gustave DEFRANCE

Fait à PARIS, le

21 JUIN 1994

Gustave DEFRANCE

The RHONE-POULENC RORER site of VILLENEUVE LA GARENNE which is duly classified according to the French regulations, as under the permanent technical control of competent administrative departments : Technical Department for Listed Facilities, Regional Department of Industry, Research and Environment, Waste Water Treatment Agency.

The results of this control demonstrate that the manufacturing operations in place leading to the production of RILUZOLE PUR are conducted on this site in compliance with current environmental regulations.

In witness whereof, I undersigned DEFRANCE Gustave, Director of the Department of prevention of pollutions and risks, certify that RHONE-POULENC RORER site of VILLENEUVE LA GARENNE fulfills the requirements of the French regulations with respect to environment protection.

Consequently, the present certificate is issued to be worth and used for whatever purpose by whom it may concern.

21 JUIN 1994

Pour le ministre et par délégation
le directeur de la prévention
des pollutions et des risques,
Gustave DEFRANCE

PARIS, le

Gustave DEFRANCE

REDACTIONS MADE
BY APPLICANT

ATTACHMENT V

Compliance Statement - Nenagh, Ireland



Rhône-Poulenc Rorer Pharmaceuticals Limited

Supplying the World from Ireland

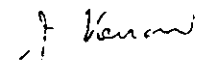
Lisbunny Industrial Estate, Nenagh, Co. Tipperary, Republic of Ireland
Telephone 067 32222 (National) +353 67 32222 (International) Fax +353 67 32249

REDACTIONS MADE
BY APPLICANT

Certification of Environmental Compliance

Rhone-Poulenc Rorer certifies that it is in compliance with, or on an enforceable schedule to be in compliance with, all emission requirements set forth in applicable local, national and European environmental regulations applicable to the formulation of Rilutek tablets at its facility in Nenagh, Ireland.

For and on behalf of
Rhone Poulenc Rorer



Jim Kernan
Engineering Manager

14-06-95
Date

Directors: G.J. Chemel (U.S.), D.M. Parker, J. Cawley
Registered Office: Lisbunny Industrial Estate, Nenagh, Co. Tipperary, Republic of Ireland
Registered in Ireland No. 52094 VAT No. IE 2740915V

REDACTIONS MADE
BY APPLICANT

ATTACHMENT VI

Waste Disposal Permits - Nenagh



Rhône-Poulenc Rorer Pharmaceuticals Limited

Supplying the World from Ireland

Uisbunny Industrial Estate, Nenagh, Co. Tipperary, Republic of Ireland
Telephone 067 32222 (National) +353 67 32222 (International) Fax +353 67 32249

REDACTIONS MADE
BY APPLICANT

Permits for the Disposal of Waste Water and Solid / Liquid Wastes

The following permits are applicable for the disposal of waste water from the Rhone Poulenc Rorer site at Nenagh, Co. Tipperary, Ireland.

- Nenagh Urban District Council, Licence To Discharge Trade or Sewage Effluent, Licence No. 20/108C(10) dated 28th February 1984.

The disposal of solid waste is contracted to a licenced waste disposal company. The company has been issued with the appropriate permit.

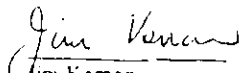
- Tipperary (North Riding) County Council, Permit To Collect Transport and Tip Refuse, issued to O'Brien Waste Disposal & Recycling Co.

Hazardous waste for incineration (principally organic liquids) is disposed of in the UK. The appropriate contractor registrations are as follows:

- Waste disposal contractor - Licence Number ESX/298003/B issued by East Sussex County Council (UK)
- Haulage contractor - Licence Number LAN/490364 issued by Lancashire County Council (UK)
- Transfer site operator - Toxic and Dangerous Waste Storage and Treatment Permit, dated 16th September 1994, issued by Clare County Council (IRL)
- Incineration Site - Authorization Number AG7946 issued by E.P.A. (UK)

The Nenagh plant does not as yet require an air emissions licence, however, in preparation for same a base line study has been carried out and indicates that the plant would satisfy the appropriate regulatory limits. It is expected that the Nenagh site will be required to apply for a new Integrated Pollution Control Licence within the next couple of years and this will include a stipulation on air emissions.

For and on behalf of
Rhône Poulenc Rorer


Jim Kernan
Engineering Manager

14 - 06 - 95
Date

REDACTIONS MADE
BY APPLICANT

ATTACHMENT VII

Engineering and Procedural Controls - Nenagh



Rhône-Poulenc Rorer Pharmaceuticals Limited

Supplying the World from Ireland

Lisburny Industrial Estate, Nenagh, Co. Tipperary, Republic of Ireland
Telephone 067 32222 (National) +353 67 32222 (International) Fax +353 67 32249

REDACTIONS MADE
BY APPLICANT

Nenagh Tablet Production Facility

Environmental Control Systems

Site Location

The site is located in a small industrial estate located on the outskirts of Nenagh Town (population approximately 6,000). Rhone Poulenc Rorer occupies about 9 acres of the 40 acre estate. The estate itself is approximately square in plan and its four sides are formed from the following features, (a) the primary road route from Dublin to the south-west of the country, (b) a local rail line, (c) a small tributary of the Shannon river and (d) rural country side.

The tablet production building in which Rilutek is manufactured is a multi-product solid dosage facility. Manufacturing takes place in various suites distributed over three floors. Associated technical service areas containing ventilation and dust collection systems, and other utilities, are arranged around the outside faces of the building on each of the three floor levels. The potential effects of the production processes on the environment are managed in the following ways :-

Aqueous Wastes

These waste streams, arising from sources such as equipment cleaning, clean-in-place systems, coating solution preparation, laundry etc. are transferred via stainless steel drains to a central effluent monitoring station. The temperature, flowrate and pH of the effluent is continuously monitored. It is pumped directly to the local municipal treatment plant. Monthly samples of the effluent are taken for full chemical analyses.

Liquid Organic Wastes

There are no organic wastes generated in the manufacture of Rilutek. Small quantities of organic waste are generated in the laboratory during testing of the product and such wastes are combined with other organic waste streams generated on the site. This type of waste is then disposed by means of incineration.

REDACTIONS MADE
BY APPLICANT

Emissions to the Atmosphere

The production is performed in a controlled environment. The general room ventilation system is fitted with appropriate filters on both the incoming air and where needed on the exhaust side. Where dust loadings are likely to be high, for example at the feeding station on a tablet compression machines or in the dispensary booths, dedicated local dust extraction systems are installed and these are independent of the main air handling units. Old filters from all air handling and dust extraction systems are disposed by means of landfill. An annual survey of all emission points from the building has been established and is used to identify any potential areas of risk. There are no organic vapour emissions associated with Rilutek processing.

Solid Waste.

Reject and waste materials including packaging are landfilled in a deep burial site. Active components such as reject tablets are first de-natured by shredding prior to landfill. Solid waste from the micro-laboratory is disposed of by means of incineration.

Procedural Controls

The following list of relevant SOP's are in operation on the site :

WH109/1	RETURN OF EMPTY SHIPPERS TO BOXMORE PLASTICS
WH111/1	RETURN OF EMPTY SHIPPERS TO EMPBANKMENT PLASTICS
WH114	CONTROL OF MATERIAL IN REJECT PAYS
CS-101	SHIPPING OF FINISHED PRODUCT
QC-003	NON-CONFORMING MATERIALS PROCEDURE
QC-004	RE-PROCESSING OF QUARANTINED GOODS
QC-019	DESTRUCTION OF REJECTED GOODS AND WASTE PRODUCT
QC-032	RETENTION AND STORAGE OF SAMPLES OF ALL MATERIALS, BOTH INGREDIENTS AND FINISHED GOODS
QC-040	VALIDATION OF CLEANING PROCEDURES WITHIN TABLET MANUFACTURING
QCM-080	USE OF LABORATORY GLASS WASHER
QC-C090	PROPER LABELLING OF REAGENTS
QCM-128	ENVIRONMENTAL MONITORING OF WORK AREAS
QC-A133	CLEANING PROCEDURE FOR THE SAMPLING ROOM
P-E038	DISPOSAL OF WASTE TABLETS, CAPSULES AND GRANULATIONS
P-023	GENERAL PLANT CLEANING
P-005	HANDLING OF GLASS IN PACKAGING AREAS
M-006	CHECKING AND EMPTYING OF DUST EXTRACTION UNITS
M-010	OPERATION OF HVAC SYSTEM

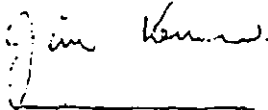
REDACTIONS MADE
BY APPLICANT

S-006	USE, CARE AND MAINTENANCE OF RESPIRATORY PROTECTION
T-001	EXECUTION, ASSESSMENT AND RECORDING OF TRAINING
GEN-001	CLOTHING PROCEDURE

Training

All employees at Nenagh are trained in basic GMP, housekeeping and equipment operation procedures. Those individuals involved in particular processes such as Rilutek are given training specific to the operations involved. Training records are maintained and updated regularly by the training officer. Throughout the training emphasis is placed on recognizing product hazards and on how to handle the process and any generated waste safely and properly. Material Safety Data Sheets are maintained for all components and reagents.

Apart from the information contained in SOP's, the Rilutek manufacturing and packaging formulas also address specific issues regarding safety, product handling and waste management.



Jim Kernan
Engineering Manager

REDACTIONS MADE
BY APPLICANT

ATTACHMENT VIII

Compliance Statement - Manati, Puerto Rico

3-9-133



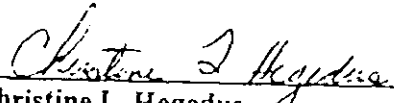
RHÔNE-POULENC RORER INC.

500 ARCOLA ROAD
P.O. BOX 1200
COLLEGEVILLE, PA 19426-0107
TEL. 610-454-8000

REDACTIONS MADE
BY APPLICANT

GENERAL COMPLIANCE STATEMENT

Rhône-Poulenc Rorer, Inc. states that it is in compliance with, or on an enforceable schedule to be in compliance with, all emission requirements set forth in permits, consent decrees and administrative orders applicable to the product Rilutek® at its facilities in Manati, Puerto Rico as well as emission requirements set forth in applicable federal, state, and local statutes and regulations applicable to the packaging of Rilutek® at its facilities in Manati, Puerto Rico.



Christine L. Hegedus
Director,
Health, Safety and Environment

REDACTIONS MADE
BY APPLICANT

ATTACHMENT IX

Industrial Discharge Permit - Manati

English translation
follows

ESTADO LIBRE ASOCIADO DE PUERTO RICO.
AUTORIDAD DE ACUEDUCTOS Y ALCANTARILLADOS
AREA DE PRETRATAMIENTO (MCCRACKEN)
APARTADO POSTAL 7066-ESTACION BARRIO OBRERO
SANTORCE, PUERTO RICO 00916

REDACTIONS MADE
BY APPLICANT

PERMISO DE DESCARGA INDUSTRIAL

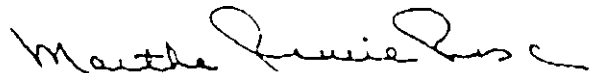
Permiso Num. GDA-2-210-037

En consonancia con el Reglamento para la Prestación de Servicios de Agua y Alcantarillado, así como con cualesquiera disposiciones de las Leyes o los Reglamentos del Gobierno Federal o del Estado Libre Asociado, la Autoridad de Acueductos y Alcantarillados (en adelante La Autoridad) autoriza a RONE POULENC RORER, INC., localizada en MANATI, a descargar en la Planta (Regional) de Tratamiento de Aguas Residuales de BARCELONETA, sujeto a todos los términos y condiciones que se especifican en el permiso.

Expedido hoy 17 de noviembre de 1992.

Efectivo el 1 de diciembre de 1992.

Expira el 1 de diciembre de 1993.



Martha Rivera Rosa.MEH
Directora
Area de Pretratamiento

**COMMONWEALTH OF PUERTO RICO
AQUEDUCT AND SEWER AUTHORITY
PRETREATMENT AREA (MCCRACKEN)
P.O. BOX 7066 - ESTACIÓN BARRIO OBRERO
SANTURCE, PUERTO RICO 00916**

REDACTIONS MADE
BY APPLICANT

INDUSTRIAL DISCHARGE PERMIT

Permit No. GDA-92-210-037

In accordance with the Regulations for the Provision of Water and Sewer Services, as well as with any provisions of the laws or the regulations of the Federal Government or of the Commonwealth, the Aqueduct and Sewer Authority (hereinafter the Authority) authorizes RHONE POULENC RORER, INC., located in MANATI, to make discharges into the BARCELONETA Waste Water Treatment Plant (regional), subject to all terms and conditions specified in permit.

Issued today, November 17, 1992
Effective December 1, 1992
Expires December 1, 1993

(Signature)
Martha Rivera Rosa, MEH
Director
Pretreatment Area

PERMIT USER GENERAL INFORMATION

PERMIT NUMBER - GDA-92-210-037

REDACTIONS MADE
BY APPLICANT

Name of Corporation: REONE POULENC RORER RP, INC.

Actual Address : CARR. SAN JOSE (PR)
PARQUE INDUSTRIAL SAN JOSE
MANATI, PR

Mailing Address : P. O. Box 345
MANATI, PR

Telephone Number : 854-1058

Emergency Telephone Number : 858-0524

Industrial Classification : SIC 2934
PHARMACEUTICAL PREPARATIONS

Category : 439.40 "MIXING AND COMPOUNDING
OF THE PHARMACEUTICAL POINT
SOURCE"

INDUSTRY: RHONE POULENC, PR, INC.
PERMIT NUMBER: 92-210-037

REDACTIONS MADE
BY APPLICANT

I. GENERAL CONDITIONS (attached)

II. SPECIAL CONDITIONS

A. DESCRIPTION OF DISCHARGES

Stream 001

Wastewater generated in the cream manufacturing area. Equipped with a grease trap. Sampling point 001 is located in this stream.

Stream 002

Wastewater generated in the existing products manufacturing area. Sampling point 002 is located in this stream.

Stream 003

Sewage from the four buildings. Equipped with a grease trap to collect grease from wastewater generated in the cafeteria. Sampling point 003 is located in this stream.

Discharge 001

A combination of streams 001, 002, and 003 which mix at the manhole located on the South side of building No. 2. This will be taken as sampling point 004.

B. GENERAL REQUIREMENTS APPLICABLE TO ALL DISCHARGES

The permit user shall comply with the general prohibitions and general provisions set forth in Section 1 of this permit

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Interconnections, relocation or mixing of streams between discharges are prohibited except with the prior written consent of the Authority. Likewise, any bypass of the pretreatment facilities are prohibited

Any anticipated facility expansions or process modifications which would result in new, different or greater discharges shall be notified to the Authority's Pretreatment Area prior to being executed.

C. SPECIFIC EFFLUENT LIMITATIONS AND SAMPLING REQUESTS FOR 001 DISCHARGE

During the period covered by this permit, the permit user is authorized to make discharges into the Authority facilities, limited and sampled by the user in accordance with the following:

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FINAL LIMITS AND SAMPLING REQUIREMENTS
001 DISCHARGE

<u>PARAMETER</u>	<u>DISCHARGE LIMIT</u>	<u>TYPE OF SAMPLE</u>	<u>FREQUENCY</u>
1. Temperature (°C)	40	Instant laboratory reading	1/month
2. pH (E.U.)	6.5-9.0	Continuous monitoring Instant laboratory reading	1/month
3. Total Copper (mg/l)	1.00	Compound/24 hours	1/month
4. Mercury (mg/l)	0.05	Compound/24 hours	1/month
5. Nickel (mg/l)	0.50	Compound/24 hours	1/month
6. Tot. chromium (mg/l)	1.0	Compound/24 hours	1/month
7. Silver (mg/l)	0.05	Compound/24 hours	1/month
8. Cadmium (mg/l)	0.10	Compound/24 hours	1/month
9. Zinc (mg/l)*	0.50	Compound/24 hours	1/month
10. Lead (mg/l)	0.20	Compound/24 hours	1/month
11. Aluminum (mg/l)	1.0	Compound/24 hours	1/month
12. Selenium (mg/l)	0.20	Compound/24 hours	1/month
13. Iron (mg/l)*	2.0	Compound/24 hours	1/month
14. Manganese (mg/l)	2.0	Compound/24 hours	1/month
15. Tin (mg/l)	5.0	Compound/24 hours	1/month
16. Tot. cyanides (mg/l)	0.10	Random	1/month
17. Oils and grease (mg/l)	50.0	Random	1/month
18. BOD5 (mg/l)	220.0	Compound/24 hours	1/month
19. Flow (gpd)	18,500	Continuous monitoring Laboratory reading	1/month

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FINAL LIMITS AND SAMPLING REQUIREMENTS
001 DISCHARGE

<u>PARAMETER</u>	<u>DISCHARGE LIMIT</u>	<u>TYPE OF SAMPLE</u>	<u>FREQUENCY</u>
20. COD (mg/l)*	425.00	Compound/24 hours	1/month
21. TSS (mg/l)	250	Compound/24 hours	1/month
22. TTO (mg/l)	2.13	Random/Compound 24 hours	1/month
23. Flash Point (°F)	>140	Random	1/month
24. Color (pt-Co)	15	Random	1/month
25. Phenols (mg/l)	0.50	Random	1/month

NOTE:

These discharge limits are not final. These may be more restrictive and depend on studies to be carried out in the area of Barceloneta. If any limits or prohibitions in the Certificate of Water Quality or in the "NPDES" Permit from the Treatment of Residual Waters Plant in Barceloneta are approved which would be more restrictive or which would control any contaminant not regulated by this permit, these will be incorporated herein. This provision is independent of any other conditions contained in this permit.

In the event more restrictive discharge limits are imposed such that the industry is unable to meet the technology used to comply with the current permit, such industry may apply for interim limits and submit a Compliance Schedule. Such schedule shall be evaluated and approved by the Aqueduct and Sewer Authority.

Pollutants classified as hazardous wastes in accordance with the Federal Recovery and Conservation of Resources Act (RCRA) are not accepted in the Authority sewer system. Such wastes shall be disposed of in accordance with current federal and state regulations.

* Parameters with interim limits. See part E of this permit.

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D. THE PERMIT USER SHALL LIKEWISE COMPLY WITH THE FOLLOWING CONDITIONS:

1. Analysis procedure:

Discharge analyses shall be carried out by a qualified laboratory and in accordance with methods approved by the Federal Environmental Protection Agency (EPA), 40 CFR, Part 136.

2. Sampling:

- a. Sampling of Discharge 001 shall be carried out during the period in which the plant is operating at a minimum of 70% capacity or at the maximum capacity foreseen for the period to be covered by the sampling.
- b. Unless otherwise specified, compliance with the permit shall be determined by means of compound samples taken during the complete period during which the discharge takes place. Whenever possible, the permit user shall obtain the samples by means of the compound sampling technique in proportion to the stream. Sampling shall be representative of the quality of the discharges for that day.
- c. All samples shall be preserved as indicated in the sample preservation procedures set forth by the EPA. The permit user shall maintain an appropriate file of chain of custody vouchers.
- d. Discharge analysis shall commence within a period of 10 days as of the date the permit becomes effective.

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- e. Sampling facilities and surrounding areas shall be kept free of obstacles or debris, with ample space to allow for an appropriate and accurate sampling.
 - f. The permit user is responsible for carrying out appropriate sampling, even when the sampling is performed by a third party. The Authority shall not accept, as a reason for violation of any limit set forth in this permit, the fact that an inappropriate sampling has been performed. No other sampling point shall be used except when duly approved in advance by the Authority.
 - g. The sampling point shall be labeled with the respective names.
3. Total toxic organics (TTO)

TTO samples shall be taken in be taken in March and August of each year.

TTO analyses shall be performed by means of a RANDOM (GRAB) SAMPLE for the volatile organics fraction and COMPOUND SAMPLE for the non-volatile organics fraction. The determination of the total amount shall take into account those organics in excess of or equal to 0.01 mg/l.

4. Requirements to comply with pretreatment

The permit user shall be responsible for complying with any revisions to 40 CFR and submit to the AAA the Preliminary Report for Industrial Users (PRIU) or any other report required in accordance with the General Pretreatment Regulation 40 CFR 403.12.

The limits established in this permit shall be considered pretreatment standards and any analysis rendering results in excess of such limits shall be considered a violation of the conditions of this permit. Any waste water exceeding the limits of the permit shall not be discharged into the Authority facilities

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5. Emergencies

Emergencies including, but not limited to, spills, operating abnormalities or any other event which may affect or modify the discharge quality or volume shall be notified to the Authority after they occur or after the permit user has become aware of their occurrence.

Notification shall be made to the AAA Pretreatment Area, telephone 250-6248 and to the Barceloneta Treatment Plant, telephone 846-4441, within 24 hours following the occurrence of the emergency. A written report shall be submitted no later than five calendar days following the emergency.

6. Permit renewal

The permit user shall apply for a renovation of the permit at least 120 days prior to its expiry. It shall further submit any new information or projected modification. This permit shall remain in force, including the analysis and discharge volume and quality requirements, until such time as the new permit takes force, unless the Authority revokes this permit.

7. AAA sampling

The Authority shall sample the discharges or used water streams within the complex each time it considers necessary in order to verify compliance with the permit or to determine the source of any specific pollutant.

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E. INTERIM LIMITS AND COMPLIANCE SCHEDULE

Compliance schedule

<u>ACTIVITY</u>	<u>COMMENCEMENT</u>	<u>TERMINATION</u>	<u>REPORT</u>
Treatability study	1-18-93	4-5-93	4-19-93
Plant design	4-1-93	6-31-93	7-15-93
Tender	9-1-93	9-15-93	9-30-93
Construction	9-16-93	3-30-94	4-15-94
Start-up and stabilization	4-1-94	5-1-94	5-15-94
Compliance	5-2-94		5-16-94

Interim limits

From December 1, 1992 through May 2, 1994, the permit user may discharge its waste waters with the following interim limits:

<u>PARAMETER</u>	<u>LIMIT</u>
BOD	850 mg/l
COD	2500 mg/l
A and G	150 mg/l
IRON	4.0 mg/l
ZINC	2.0 mg/l
*COLOR	45 (Pt-Co)

F. ADDITIONAL SAMPLING FACILITIES

1 The permit user shall provide a sampling point with a diameter equal to or greater than six inches, without angles or curves blocking the access line to the discharge or stream. The discharge sampling point shall be located outside the facility in order that representatives of the Authority have free and immediate access thereto

The permit user shall adapt the Compliance Schedule in order to achieve the final limit for the color parameter

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2. The permit user shall install a flow meter and pH meter with a continuous recorder for Discharge 001.
3. The above mentioned equipment facilities shall be acceptable to the Authority and be in operation by no later than 90 days following the effective date of the permit.
4. The flow meter shall have an accuracy of $\pm 5\%$, and the pH meter an accuracy of ± 0.1 S.U. The permit user shall maintain such equipment at all times in perfect working conditions.
5. All instruments shall be calibrated with sufficient frequency, in accordance with the manufacturer's specifications. The permit user shall keep records of such calibrations.
6. Each flow meter and pH meter register sheet shall indicate the date and time at which the sheet starts and ends. In the event the sheet refers to more than one day, each day shall be separately identified.

G. PENALTIES

1. The permit user shall pay penalties of up to \$5,000 per day per violation due to non-compliance or due to violations of the conditions set forth in this permit and/or the Authority Rules and Regulations. This condition does not apply to violations regarding interim limits and Compliance Schedule activities if specific penalties have been stipulated for such violations.
2. The permit user shall likewise pay to the Authority a surcharge, when applicable, even in the event the permit user has paid penalties for permit violations. Such surcharge shall be calculated in accordance with Section 5.02, Subpart F of the Authority Regulation.

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H. STIPULATED PENALTIES

1. The permit user shall pay to the Authority a stipulated penalty of-\$1,000 per day for delays in the completion of each activity agreed upon in the Compliance Schedule.
2. The permit user shall further pay a stipulated penalty of \$1,000 per day for each interim limit exceeded.
3. Payment of the stipulated penalties shall be included in the Monthly Auto sampling Report (MAR) corresponding to the violation.

I. PAYMENT OF PENALTIES

1. Checks for the payment of penalties shall be made payable to the Aqueduct and sewer Authority and shall be sent to the attention of:

Mrs. Ruth Rivera
Administrative Officer
Pretreatment Area (McCraken)
AQUEDUCT AND SEWER AUTHORITY
P. O. Box 7066 - Barrio Obrero
Santurce, Puerto Rico 00916

2. When sending the check, the permit user shall indicate:
 - a. Name of the industry
 - b. Permit number

J. PREPARATION OF REPORTS AND FILE MAINTENANCE

1. Auto sampling reports (ASR) and analyses shall be received at the AAA prior to the 28th of the month following the sampling. Each report shall contain the following information

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- a. Copy of the original laboratory report certified by a licensed chemist. This certificate shall clearly identify the discharge or stream sampled. Units of concentration shall be expressed in the same units set forth in the discharge limits indicated in this permit (mg/l, S.U., Pt-Co o GPD, C, F, etc., in accordance with the parameter) and shall further indicate the type of sample taken (random, compound, etc.).
 - b. Copy of the chain of custody sheet for the sample.
 - c. Copy of the pH and flow record sheets for the 001 discharge. Each sheet shall be properly identified, indicated the scale on which each parameter is determined.
 - d. A table summarizing the maximum, minimum and average daily values as obtained from the readings taken from the pH and flow record sheets.
 - e. Copy of all analyses performed during the sampling period, in addition to those required by this permit.
2. The permit used shall deliver to AAA, on or prior to December 31 and June 30 of each year, a six-month report indicating the compliance status during the preceding six months.

The six-month or periodic report to be submitted on December 31 shall include the compliance status for the period from June 1 through November 30, and the six-month report to be submitted before June 30 shall include the compliance status for the period from December 1 through May 31.

This report shall include:

- a. A summary of all analyses reported during the preceding six months, with maximum, minimum and average values for each parameter

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- b. Which parameters were the most difficult to control and the measures taken to improve control thereof.
 - c. Operating abnormalities, whether or not planned, and accidental discharges shall be notified and documented, including dates, causes, action taken to correct same and to prevent future occurrences.
 - d. Evidence of calibrations for all instrumentation during the period covered by the report.
 - e. Compliance Schedule status, if any. In the event of non-compliance, reasons for same and measures taken to become compliant.
3. The user shall comply with all reporting and file maintenance requirements set forth in section I of this permit.
4. All report shall be forwarded to:

Mr. Nelson Hernández (D-33)*
Head of the Compliance Division
Pretreatment Area, Barreras
Aqueduct and Sewer Authority
P. O. Box 7066, Estación Barrio Obrero
Santurce, Puerto Rico 00916

5. Division in charge

All reports sent to the AAA shall clearly indicate the permit number, the industrial facility and the division in charge. Division II of the Permits Department has been designated to handle this permit

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- h. "PRIU" signifies Preliminary Report for Industrial Users.
- i. "mg/l" signifies Milligrams per Liter.
- j. "MO" signifies Monitoring Only.
- k. "NSR" signifies No Sampling Required.
- l. "TTO" signifies Total Toxic Organics
- m. "SPCP" signifies Spill Prevention and Control Program.
- n. "WWTRP" signifies Waste Water Treatment Regional Plant.
- o. "WWTP" signifies Waste Water Treatment Plant.
- p. "SS" signifies Sedimentable Solids.
- q. "TSS" signifies Total Suspended Solids
- r. "S.U." signifies Standard Units.
- s. Pt-Co signifies color units in Platinum-Cobalt.
- t. "TDS" signifies Total Dissolved Solids.

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K. DEFINITIONS AND ABBREVIATIONS

1. DEFINITIONS

- a. "Discharge" signifies any connection from the plant to the AAA sanitary sewer system
- b. "Stream" signifies any flow or discharge passing through the internal plant sewer system.
- c. "Compound sample" means a series of random samples taken at equal time intervals, either in a quantity proportional to the flow or equal in volume.
- d. "Random sample" (grab) signifies an individual sample taken during a period of no more than 15 minutes.
- e. "Instant reading" means any individual reading taken during a single period.

2. Abbreviations

- a. "AAA" signifies The Aqueduct and Sewer Authority.
- b. "O & G" signifies Oils and Greases.
- c. "BOD" signifies Biochemical Oxygen Demand
- d. "COD" signifies Chemical Oxygen Demand.
- e. "EPA" signifies the Federal Environmental Protection Agency.
- f. "GPD" signifies Gallons per Day.
- g. "ASR" signifies Autosampling report

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ATTACHMENT X

Calculation of EEC

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RILUZOLE

CALCULATION OF EEC

Calculation of the maximum expected environmental concentration (MEEC) to be found for waste water treatment plants (WWTP) when the total fifth year production quantity (produced for United States use) of Riluzole is consumed and discharged to public and private sewers is shown below.

Basis for Calculation

A = Fifth year production of Riluzole = Kg/year (pounds)

B = Years/365 Days

C = Day Person/150 Gallons

D = 1/246,000,000 (US Population - Est)

E = Gallons/8.34 Pounds

F = 1,000,000

Calculation of EEC

ppm = A • B • C • D • E • F

ppm = () (1/365) (1/150) (1/246 x 10⁶) (1/8.34) (10⁵)

ppm = "Confidential"

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RILUTEK®/RILUZOLE
Environmental Assessment Section of NDA

SECTIONS 7 TO 11: SECTIONS NOT INCLUDED

The Sections 7 to 11 are not included in this item as the Rilutek® application is for treatment of a rare disease. (21 CFR 25.31(a)3.)

SECTION 12: LIST OF PREPARERS

William Studt (PhD Synthetic Organic Chemistry)
Process Chemistry & Biochemistry
Global Process Chemistry/Regulatory Liaison
Rhône-Poulenc Rorer
Collegeville, PA

Karen Quigley (BS Chemistry, MS Chemistry)
Sr. Regulatory Affairs Associate
Chemistry, Manufacturing and Controls Group
Rhône-Poulenc Rorer
Collegeville, PA

SECTION 13: CERTIFICATION

Appendix I contains the certification as to the veracity of this document.

SECTION 14: REFERENCES

There are no references for this document.

SECTION 15: APPENDICES

There are no additional appendices beyond the attachments included within this item and the Appendix I.

Appendix 1

Certification

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The undersigned certify that the information contained in this Environmental Assessment Report is true, accurate and complete to the best of our knowledge and that of Rhône-Poulenc Rorer Pharmaceuticals Inc.



Karen Quigley
Senior Regulatory Affairs Associate
Regulatory Affairs, USA



William Studt
Global Process Chemistry/Regulatory Liaison

3-9-83

Rilutek[®]
(Riluzole) Tablets

NDA 20-599

Biopharmaceutics

and

Pharmacology

Volume ___ of ___

SEP 27 1995

NDA: 20-599

IND:

SUBMISSION DATES: June 29, 1995
July 11, 1995 (2)
July 14, 1995
July 17, 1995
July 20, 1995
August 2, 1995
August 11, 1995 (2)
August 22, 1995
August 29, 1995
September 7, 1995
September 8, 1995 (2)
September 22, 1995

SUBMISSION DATES: April 25, 1995
May 10, 1995 (2)
June 14, 1995
June 23, 1995
August 7, 1995

OPTION

SEP 27 1995

GENERIC NAME: Riluzole

BRAND NAME: Rilutek®

DOSAGE FORM: Capsule-shaped Tablet

STRENGTH: 50 mg

ROUTE OF ADMINISTRATION: Oral

SPONSOR: Rhone-Poulenc Rorer

TYPE OF SUBMISSION: Original NME

DRUG CLASSIFICATION: 1 P

INDICATION: Amyotrophic Lateral Sclerosis (ALS)

REVIEWERS: Mohammad Hossain, Ph.D. (Project Leader)
Vijay Tammara, Ph.D.
Robert Harris, Ph.D.

SYNOPSIS:

Riluzole is well-absorbed with about 90% of an oral dose recovered in the urine mostly as glucuronides. The average absolute oral bioavailability of riluzole is 60% (CV=30%). Following oral administration, average peak plasma concentration occurs in about 0.75 hour. A high fat meal was shown to decrease AUC by 18% and Cmax by 44% while Tmax increased to 2 hours.

In the pivotal bioequivalence study, the 50 mg market tablet when compared to the 50 mg tablet used in the clinical trial NNY301 was found to be equivalent in terms of extent of absorption (AUC_{0-∞}) but not Cmax, which was 27% higher for the market tablet. However based on the clinical safety data collected with the 100 mg B.I.D. dosing regimen, the higher

C_{max} observed with the 50 mg market tablets may not be of any safety concern.

In healthy male Caucasian volunteers, the kinetics of riluzole is linear over the single oral dose range of 25-300 mg and over the multiple oral dose range of 25-100 mg when given every 12 hours. Riluzole is extensively distributed throughout the body. After I.V. infusion over 30 minutes, average V_{ss} and V_z were found to be 245 L (CV=28%) and 296 L (CV=38%) respectively. The mean apparent volume of distribution, V_z/F after a single oral dose was 893.5 L (12.8 L/kg; CV=36%). 96% of riluzole is bound to plasma proteins mainly to albumins and lipoproteins, but not to globulins over the *in vitro* concentration range of 125 to 5000 ng/mL, and binding does not appear to be saturable.

Steady-state was observed to be reached within 5 days. Following oral administration of 50 mg tablets every 12 hours, the average steady-state C_{max}^{ss} and average AUC^{ss} were 172.6 ng/mL (CV=42%) and 654 ngxh/mL (CV=43%), respectively. The mean elimination half-life of riluzole is 12 hours (CV=35%) after repeated doses. Upon multiple dose administration, riluzole accumulates in plasma by about 2 fold.

The metabolism of riluzole is mostly hepatic and consists of cytochrome P450-dependent hydroxylation (phase I reaction) and glucuronooconjugation (phase II reaction). The primary metabolic pathways of riluzole biotransformation in humans may involve direct glucuronidation of riluzole (involving the glucurotransferase isoform UGT-HP4) and oxidation of riluzole to N-hydroxyriluzole (involving primarily the cytochrome P450 (CYP) isoform CYP 1A2, (although CYP 2D6, CYP 2C19 and CYP 2E may also participate), followed by rapid glucuronidation. Based on exposure (AUC), it appears that riluzole accounts for a small proportion (<25%) of the circulating total radioactivity in plasma, indicating that there is significant exposure to metabolites, some of which may possess pharmacological activity. The marked inter-individual variability in the kinetics of riluzole (4-6 fold variability for C_{max} and AUC) is probably attributable to genetic and/or environmental factors that inhibit or induce CYP 1A2 activity. Phenotyping 12 healthy male subjects for CYP 1A2 activity from urinary metabolite patterns after an oral dose of caffeine revealed a 2-11 fold variability in CYP 1A2 activity.

Following a single 150 mg oral dose of ¹⁴C-riluzole to 6 healthy males, 90% and 5% of the radioactivity was recovered in the urine and feces, respectively, over a period of 7 days, for a mean combined recovery of 95%. Only 2% of a riluzole dose is recovered in the urine as unchanged drug. The elimination of total radioactivity in the plasma was described by a biexponential equation (two compartment model) with a long terminal half-life of 27 hours (CV=14%). After I.V. administration, the mean plasma clearance of riluzole was 795 mL/min (CV=18%) and the average apparent plasma clearance of riluzole (Cl/F) was found to be 1275 mL/min.

The pharmacokinetics of riluzole in the elderly, and in the renally and hepatically impaired, and the effects of race and gender on riluzole disposition in humans have not been studied.

The firm is planning to conduct formal studies in the future and a population pharmacokinetic analysis is currently being performed using the clinical trial NNY301 data to screen for important covariates (e.g. age, race, gender, drug interaction, renal and hepatic effects). Although not well-established, CYP 1A2 activity has been reported to be lower in women than in men. An *in vitro* study reported in the literature utilizing Japanese and Caucasian liver samples revealed several CYP 1A2-dependent activities to be significantly higher in Caucasians than in Japanese. An across study comparison of riluzole pharmacokinetics between Japanese and Caucasians at the proposed labeled dosing regimen of 50 mg given every 12 hours, suggest that on an average Japanese subjects have higher C_{max} and AUC values (47% and 60% increase, respectively) and a corresponding decrease in apparent systemic clearance (Cl/F) of 50% when normalized for dose and body weight as compared to Caucasians.

There have been no clinical studies to evaluate the interaction of riluzole with other drugs. *In vitro* studies using human liver preparations suggest that CYP 1A2 is the principal isozyme involved in the initial oxidative metabolism of riluzole. If this is an important metabolic pathway *in vivo*, then potential interactions may occur when riluzole is given concurrently with agents that affect CYP 1A2 activity. Potential inhibitors of CYP 1A2 (e.g. caffeine, grapefruit juice, phenacetin, 7,8-benzoflavone, theophylline, amitriptyline, enoxacin, acetaminophen, imipramine, clomipramine, quinolones, diclofenac, diazepam, nicergoline and quinine) could decrease the rate of riluzole elimination, while CYP 1A2 inducers (e.g. cigarette smoke, charcoal-broiled food, rifampicin, cimetidine, and omeprazole) could increase the rate of riluzole elimination. In addition, *in vitro* studies under the conditions of high riluzole and low substrate concentrations indicate that riluzole could inhibit the enzymatic activities of CYPs 1A2, 2A6 and 2E1 by 20% to 30%. Riluzole had minor inhibitory effect on the enzymatic activities of CYPs 2C9, 2C19, 2D6 and 3A4. Currently, it is not known whether riluzole has any potential for enzyme induction.

The manufacturing site change and scale-up ($\leq 10X$) was found to be acceptable based on the linear kinetics of the drug, identical formulation, identical manufacturing equipment and processing conditions, and on *in vitro* dissolution profiles in multiple media submitted for this immediate release product. The dissolution methodology and specification proposed by the firm have been found to be acceptable by the Agency (not less than _____ of the drug released in 30 minute:

RECOMMENDATION:

This submission (NDA 20,599) has been reviewed by the Division of Biopharmaceutics and has been found to be acceptable for meeting the Biopharmaceutics requirements. The sponsor is requested to incorporate all the labeling changes and to respond satisfactorily to all enclosed Comments.

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SUMMARY OF BIOAVAILABILITY AND PHARMACOKINETICS:

I. BIOAVAILABILITY:

A. Absolute Bioavailability:

In an open, randomized, 3 period, single dose, cross-over study involving 15 healthy male subjects, the average absolute oral bioavailability calculated by comparing bioavailability after oral intake of direct compression 2x50 mg riluzole market tablets with that observed after I.V. infusion of 50 mg (10 mL) for 30 minutes was found (Study NNY161).

B. Bioequivalence:

- (1) In the bioequivalence study NNY152, the 50 mg tablet (formulation used in the clinical trial NNY216 was compared with the 50 mg tablet (formulation B; used in the clinical trial NNY301. Using formulation A as the reference treatment for statistical comparisons, the two formulations were found to be equivalent in terms of extent of absorption (AUC_{0-12h} : 90% C.I. = 85-96%) but not Cmax (90% C.I. = 62-85%; 38% higher mean Cmax for formulation A). On an average, AUC_{0-12h} represents about 85% of AUC_{0-24h} . The observed bioinequivalence for Cmax could be due to differences in formulation, manufacturing process (particle size, shape (rod-shaped vs capsule-shaped), and final weight (200 mg vs 400 mg) between Formulation A and Formulation B, respectively. Since the inclusion/exclusion criteria for this study were not well-defined, the observed differences in bioavailability could also be attributable to environmental factors that inhibit or induce CYP 1A2 activity. Analysis of variance showed Tmax not to be statistically significant ($p=0.2$).
- (2) In the pivotal bioequivalence study NNY160, 2x50 mg tablets (formulation B used in the clinical trial NNY301 was compared to 2x50 mg tablets (formulation C; market formulation). Using formulation C as the reference treatment for statistical comparisons, the two formulations were found to be equivalent in terms of extent of absorption (AUC_{0-12h} : 90% C.I. = 87-99%) but not Cmax (90% C.I. = 69.5-94.5%; 27% higher mean Cmax for the market tablets). This could be due to differences in formulation, particle size and final weight (400 mg vs 200 mg) between Formulation B and Formulation C, respectively. Since the inclusion/exclusion criteria for this study were not well-defined, the observed differences in bioavailability could also be attributable to environmental factors that inhibit or induce CYP1A2 activity. Analysis of variance showed Tmax not to be statistically significant ($p=0.34$).

The C_{max} of Formulation B used in the clinical trial NNY301 is lower than Formulation A used in the clinical trial NNY216. The concern for lack of clinical efficacy due to the differences in C_{max} may be answered by the results of the dose-ranging clinical study NNY301 (25 mg, 50 mg and 100 mg B.I.D.). From a safety perspective, the C_{max} of the final Formulation C is higher than the C_{max} of the Formulation B used in NNY301. However based on the safety data collected with the 100 mg B.I.D. dosing regimen in the clinical study NNY301, the higher C_{max} observed with the 50 mg final Formulation C may not be of any safety concern.

C. Intrasubject Variability:

Using a 3-way cross-over design involving an I.V. and an oral dose repeated twice, the inter-subject variance for C_{max} and AUC was calculated by the sponsor to be 44% and 30% respectively, while the intra-subject variance for C_{max} and AUC was calculated to be 33% and 18%, respectively (NNY161). These estimates are based on the analysis of all the data, both I.V. and oral tablets. However, using only the repeated oral tablet treatments, the intra-subject variance for C_{max} and AUC was calculated to be 29.6% and 13.7%, respectively (calculated by the reviewer). If the intra-subject variability is greater than 30% for AUC, then a drug can be considered to be a "highly variable" drug. Therefore, riluzole would not be considered to be a "highly variable" drug. The marked inter-subject variability in the kinetics of riluzole (4-6 fold variability; C_{max}: CV=50-60% and AUC: CV=40-60%) is probably attributable to genetic and/or environmental factors that inhibit or induce CYP 1A2 activity. Phenotyping 12 healthy male subjects for CYP 1A2 activity from urinary metabolite patterns after an oral dose of caffeine revealed a 2-11 fold variability in CYP 1A2 activity. Genetic polymorphism has been reported for CYP 1A2 activity in humans.

D. Interaction with Food:

In a randomized open-label two period crossover study in which 2x50 mg tablets were administered to 16 healthy male subjects, a high fat, non-FDA recommended meal was shown to decrease the rate (C_{max} decreased by 44% and T_{max} prolonged by 2.5 fold; mean: 0.75 hour vs 2 hours), as well as the extent (AUC decreased by 18%) of absorption of riluzole compared to the fasted state (Study NNY159).

II. PHARMACOKINETICS:

A. Absorption:

Radiolabeled riluzole is well-absorbed with 90% of the radioactive oral dose recovered in the urine (Study AG114). Following oral administration, average peak plasma concentration occurs in about 0.75 hour (Studies NNY159, NNY160, and NNY161). T_{max} did not vary with the dose.

B. Distribution:

In Vivo

Riluzole is characterized by a large volume of distribution. The extent and degree of riluzole's distribution within various body compartments has not been systematically studied in humans. After 50 mg I.V. infusion over 30 minutes (N=15 healthy male subjects; Study NNY161), average V_{ss} and V_z were found to be 245 L (3.5 L/kg; CV=28%) and 396 L (5.7 L/kg; CV=38%) respectively. The mean apparent volume of distribution, V_z/F after a single oral dose was 893.5 L (12.8 L/kg; CV=36%) (Study NNY161).

In Vitro Protein Binding:

In whole blood, 35% of riluzole is bound to red blood cells (RBCs) and 65% to plasma proteins. In human serum, 96% of riluzole is bound to proteins mainly to albumin and to lipoproteins but not to globulins over the *in vitro* concentration range of 125 to 5000 ng/mL and binding does not appear to be saturable (Study UFR/PH 93/002).

C. Metabolism:

In Vivo

Riluzole is highly metabolized to six major and a number of minor metabolites (Study AG114). The metabolism of riluzole is mostly hepatic and consists of cytochrome P450-dependent hydroxylation (phase I reaction) and glucuronidation (phase II reaction). The primary metabolic pathways of riluzole biotransformation in humans may involve direct glucuronidation of riluzole and oxidation of riluzole to N-hydroxyriluzole (RPR 112512) followed by rapid glucuronidation. Only 2% of a riluzole dose is recovered in the urine as unchanged drug. The glucuronides account for more than 85% of the metabolites in urine. A single glucuronide of either riluzole or N-hydroxyriluzole (RPR 112512) accounts for approximately 40% of the administered dose. Oxidation of the trifluoromethoxybenzene nucleus into ortho and meta phenols yielding three phenolic metabolites (RP 65331, RP 65110 and RP 65077) followed by glucuronidation occurs to a small extent (combined 10% of dose). In addition, a urea containing derivative (RP 69597) and its glucuronide account for 10% of the dose. Over the first 6 hours after administration of radiolabeled riluzole, unchanged riluzole accounted for only 38% of the AUC_{0-6hr} for total radioactivity in plasma. Based on pharmacokinetic studies performed with cold drug, the estimated $AUC_{0-∞}$ for riluzole accounts for less than 25% of the $AUC_{0-∞}$ for total radioactivity. There is, therefore, significant exposure to metabolites, some of which may possess pharmacological activity (e.g. RPR 112512, RP 65077 and RP 65110).

In Vitro

In vitro experiments using liver microsomes show that hydroxylation of the primary amine group producing N-hydroxyriluzole (RPR 112512) is the main metabolic pathway in human, monkey, dog and rabbit (Study IBP/1887). In man, cytochrome P450 1A2 is the principal isozyme involved in the N-hydroxylation of riluzole (although CYP 2D6, CYP 2C19 and CYP 2E may also participate) (Studies IBP/1837 and IBP/1887). Whereas direct glucuronidation of

riluzole (involving the glucurotransferase isoform UGT-HP4) is very slow in human liver microsomes, N-hydroxyriluzole is readily conjugated at the hydroxylamine group resulting in the formation of O- (>90%) and N-glucuronides (IBP/1887). The rapid glucuronidation process for N-hydroxyriluzole, as observed *in vitro*, may explain why this metabolite has not yet been detected *in vivo*.

D. Elimination:

Riluzole and its metabolites are eliminated primarily via urinary excretion. Following a single 150 mg oral dose of ¹⁴C-riluzole to 6 healthy males, 91±5% and 4.5±1.5% of the radioactivity was recovered in the urine and feces respectively, over a period of 7 days, for a mean combined recovery of 95.5% (Study AG114). Over 90% of the radioactivity excreted in the urine was recovered in the first 24 hours. Only 2% of a riluzole dose is recovered in the urine as unchanged drug. The elimination of total radioactivity in the plasma was described by a biexponential equation (two compartment model) with a long terminal half-life of 27 hours (CV=14%). After I.V. administration, the plasma clearance of riluzole was 794 mL/min (CV=18%) (Study NNY161). Following single oral doses of 2x50 mg tablets in healthy male volunteers, the average apparent plasma clearance of riluzole, Cl/F, was found to be 1400-1866 mL/min (CV=35-52%) in Study NNY161 (N=15) and Study NNY160 (N=24).

E. Steady-State Kinetics:

Following oral administration of 50 mg tablets every 12 hours, the average steady-state C_{max}^{ss} and average AUC^{ss} were 172.6 ng/mL (CV=42%) and 654 ngxh/mL (CV=43%), respectively (Study NNY158). Steady-state was observed to be reached within 5 days (Study NNY158). Upon multiple dose administration, riluzole accumulates in plasma. Following 10 days of oral administration of 25 mg, 50 mg, and 100 mg dose given every 12 hours, an accumulation ratio of about 2 was obtained for C_{max} and AUC.

In Caucasian subjects, the elimination half-life of riluzole increased from 5.8 hours (CV=26%) after single oral dose administration, to 12.3 hours (CV=35%) after repeated doses (Study NNY158). A similar finding was observed in Japanese volunteer, in whom T_{1/2} increased from 5.5 hours (CV=27%) to 10.7 hours (CV=18%) (Study NNY157). The shorter half-life observed after the single dose must be viewed as approximate, due to the inability to measure plasma drug levels beyond 8-16 hours following a single oral dose. After multiple dose administration, about 20-30% of AUC was accounted for by the long terminal phase of the concentration-time profile compared to the single dose terminal profile.

III. DOSE PROPORTIONALITY:

In Caucasian subjects, the kinetics of riluzole is linear over the single dose range of 25-300 mg (Studies AG011, NNY101, NNY158) and over the multiple dose range of 25 to 100 mg when

given every 12 hours (Study NNY158).

IV. SPECIAL POPULATIONS:

The pharmacokinetics of riluzole in the elderly, and in the renally and hepatically impaired, and the effects of race and gender on riluzole disposition in humans have not been studied. The firm is planning to conduct formal studies in the future and a population pharmacokinetic analysis will be performed using the clinical trial NNY301 data to screen for important covariates (e.g., age, race, gender, drug interaction, renal and hepatic effects).

Literature report of an *in vitro* study utilizing Japanese and Caucasian liver samples revealed several CYP 1A2-dependent activities (phenacetin *O*-deethylation and 7-ethoxycoumarin *O*-deethylation) to be significantly higher in Caucasians than in Japanese. Although not well-established, based on *in vitro* and *in vivo* studies, CYP 1A2 activity has been reported to be higher in men than in women.

A. Effect of Race:

An across study comparison of riluzole pharmacokinetics was performed between studies conducted in Japanese (Study NNY157) and Caucasian (Study NNY158) subjects. In these two studies, identical study design, number of subjects (3 different groups of 12 subjects each), formulation, dose and dosing regimen (25 mg, 50 mg and 100 mg as single dose and multiple dose B.I.D.) were used. The Japanese subjects had an average body weight of 62.5 ± 5.5 kg and the Caucasian subjects had an average body weight of 78.8 ± 10 kg. In both Japanese and Caucasian subjects, <2% of unchanged riluzole is recovered in the urine. However, in Japanese subjects only 15-20% of the administered dose is recovered in the urine as a major glucuronide metabolite as compared to about 40% in Caucasians. This would suggest some differences in the qualitative and quantitative role of the different metabolic pathways involved in the metabolism of riluzole.

In Caucasian subjects, the kinetics of riluzole is linear over the single dose range of 25-300 mg and over the multiple dose range of 25-100 mg given every 12 hours. In Japanese subjects the kinetics of riluzole is linear over the single dose range of 25-200 mg, and over the multiple dose range of 25-100 mg given every 12 hours, both C_{max} and AUC appear to reach a plateau at higher doses.

Comparison of the pharmacokinetics between Japanese and Caucasians at the proposed labeled dosing regimen of 50 mg given every 12 hours, suggest that on an average Japanese subjects have higher C_{max} and AUC values (47% and 60% increase, respectively) and a corresponding decrease in apparent systemic clearance, Cl/F , of 50% when normalized for dose and body weight as compared to Caucasians. A statistically significant difference between both studies was found for C_{max} , AUC_{0-12hr} , Cl/F and Vd/F obtained at steady-state. Although it is not

clear if this difference is due to genetic or environmental differences (e.g., smoking, alcohol, coffee, and dietary preferences), it is possible that Japanese may possess a lower metabolic capacity (oxidative and/or conjugative) to metabolize riluzole when compared to Caucasian subjects.

V. PHARMACOKINETIC/PHARMACODYNAMIC RELATIONSHIPS:

No PK/PD study was performed by the sponsor. In clinical trials, elevations of serum alanine transaminase (ALT) and serum aspartate transaminase (AST) levels above the upper limit of normal range (ULN) were observed more in riluzole-treated than placebo-treated patients. The frequency of ALT and AST elevations appear to increase with an increase in dose. The single dose pharmacokinetic studies involving both oral and intravenous doses of riluzole did not reveal any trend of increase in serum transaminase levels (ALT/AST) with dose or plasma concentrations of riluzole. Also, in a multiple dose pharmacokinetic study where riluzole was administered as 50 mg, 100 mg and 200 mg daily dose, no apparent relationship was observed between plasma concentrations or dose of riluzole and laboratory safety measurements, including ALT and AST levels (Study NNY158).

VI. DRUG INTERACTIONS:

There have been no formal clinical studies to evaluate the drug interactions of riluzole. *In vitro* studies using human liver preparations suggest that CYP 1A2 is the principal isozyme involved in the initial oxidative metabolism of riluzole (Studies IBP/1837 and IBP/1887). If this is an important metabolic pathway *in vivo*, then potential interactions may occur when riluzole is given concurrently with agents that affect CYP 1A2 activity. Potential inhibitors of CYP 1A2 (e.g. caffeine, grapefruit juice, phenacetin, 7,8-benzoflavone, theophylline, amitriptyline, enoxacin, acetaminophen, imipramine, chlomipramine, quinolones, diclofenac, diazepam, nicergoline and quinine) could decrease the rate of riluzole elimination (Study IBP/1877), while CYP 1A2 inducers (e.g., cigarette smoke, charcoal-broiled food, rifampicin, cimetidine and omeprazole) could increase the rate of riluzole elimination. In addition, *in vitro* studies under the conditions of high riluzole and low substrate concentrations indicate that riluzole (10 μ M) could inhibit the enzymatic activities of CYPs 1A2, 2A6 and 2E1 by 20% to 30% (Study IBP/DD-95-067). Riluzole had minor inhibitory effect on the enzymatic activities of CYPs 2C9, 2C19, 2D6 and 3A4. Currently, it is not known whether riluzole has any potential for enzyme induction.

VII. PLASMA PROTEIN BINDING INTERACTIONS:

Riluzole is highly bound (96%) to plasma proteins (Study UFR/PH93/002). Drug-displacement studies have been performed *in vitro* for this highly bound drug (Study

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UFR/PH93/002). The effect of riluzole (up to 5 µg/mL) on warfarin (5 µg/mL) binding did not show any displacement of warfarin. Conversely, riluzole binding was unaffected by the addition of warfarin, digoxin, imipramine and quinine at high therapeutic concentrations.

VIII. IN VITRO DISSOLUTION:

Multiple media dissolution profiles using 50 mg tablets from the initial manufacturing site in Antony, France (involving the bio-batches) and manufacturing site in Nenagh, Ireland were generated with

Both disintegration and dissolution of the tablets in water and enzyme-free simulated intestinal fluid was very slow and incomplete with only about released after 60 minutes (UV spectrophotometry at while dissolution in gastric fluid was fast in 30 minutes]. The dissolution methodology and specification proposed by the firm have been found to be acceptable by the Agency (not less than of the drug released in 30 minutes in ; this is based on data submitted. Upon a request from the Division of Biopharmaceutics, the firm has also submitted dissolution data for a 25 mg strength riluzole tablet which is compositionally proportional to the proposed 50 mg strength commercial tablet of riluzole. The dissolution specification proposed for the 50 mg commercial tablet of riluzole could also apply to the 25 mg strength tablet if the sponsor wishes to market such a tablet in the future, or if it is deemed necessary by the clinical division to provide a lower strength of riluzole tablet for initial dosing in special populations (e.g. hepatic and renally impaired patients).

(Please see also Comment 1 under, "Comments To Be Sent To The Firm").

IX. ASSAY:

X. MANUFACTURING SITE CHANGE AND SCALE-UP:

All the clinical supplies were manufactured in the pilot plant of Rhône-Poulenc Rorer in Antony, France. The proposed commercial manufacturing site is Rhône-Poulenc Rorer in Nenagh, Ireland. A dissolution specification is being set based on the available comparative *in vitro* dissolution data for bio-batches manufactured in Antony, France and commercial lots manufactured in Nenagh, Ireland. The manufacturing site change (different campus) and scale-up for this immediate release product is found to be acceptable based on the linear kinetics of the drug, identical formulation, identical manufacturing equipment and processing conditions, and based on *in vitro* dissolution profiles generated in three different

(Please see also Comment 2 under, "Comments To Be Sent To The Firm").

GENERAL COMMENTS (Need not be sent to the firm):

1. The reviewing Chemist is requested to note that the commercial drug product will be manufactured by Rhone-Poulenc Rorer Pharmaceuticals in Nenagh, Ireland. Evidently, there is a change in manufacturing site involved. Also, the sponsor mentions in the labeling that patients should store riluzole tablets at temperatures up to 25°C (77°F). It appears that this may require refrigeration.
2. Upon a request from the Division of Biopharmaceutics, the firm has submitted dissolution data for a 25 mg strength riluzole tablet which is compositionally proportional to the proposed 50 mg strength commercial tablet of riluzole. The dissolution specification proposed for the 50 mg commercial tablet could also apply to the 25 mg strength tablet, if the sponsor wishes to market such a tablet in the future, or if it is deemed necessary by the clinical division to provide a lower strength of riluzole tablet for initial dosing in special populations (e.g. hepatic and renally impaired patients).
3. It was observed that riluzole was well tolerated up to the single I.V. dose of 50 mg in six healthy male subjects ($C_{max} = 800 \pm 163$ ng/mL). In the 100 mg dose group, 1/6 subjects had severe hypoglycemia, hypokalemia and ECG tracing disturbances (28% increase in QT_c) despite the fact that this subject had similar plasma levels ($C_{max} = 1450$ ng/mL and $AUC_{0-\infty} = 3500$ ng.hr/mL) as compared to other subjects in the same dose group. The sponsor claims that this subject when rechallenged with a 100 mg single I.V. dose did not appear to experience any such problem. In the same dose group (100 mg), an Oriental with maximum exposure ($C_{max} = 1572$ ng/mL and $AUC_{0-\infty} = 4592$ ng.hr/mL) to riluzole displayed no severe adverse events. However, all six subjects

reported of adverse events and the number of adverse events per subject were also higher in the 100 mg dose group as compared to the lower dose groups.

4. The following dosage and administration information is being suggested to the Medical Reviewer after a careful review of other drugs approved by HFD-120 utilizing similar dosing scheme:

Dosage in Special Populations:

A lower or less frequent dosage should be used in patients with severe hepatic or renal impairment, and in Japanese patients because of the expected decrease in clearance of riluzole. A lower or less frequent dosage should also be considered for the elderly and female patients, with concurrent diseases or who are on multiple medications. Serum transaminase levels (specifically ALT/AST) should be monitored weekly for at least the first three months in these patients.

Patients who smoke cigarettes are expected to eliminate riluzole faster. Therefore, these patients may require a higher or more frequent dose of riluzole.

COMMENTS TO BE SENT TO THE FIRM:

1. ***In Vitro* Dissolution:**

The Agency accepts the following dissolution methodology and specification for the 50 mg strength of Rilutek® Tablets proposed by the firm:

Method:

Assay:

Medium:

2. **Manufacturing Site Change and Scale Up:**

The change in manufacturing site (from RPR facility in Antony, France to RPR facility in Nenagh, Ireland) and scale up ($\leq 10X$) for the 50 mg Rilutek® Tablet has been found to be acceptable by the Agency, based on the linear kinetics of the drug, identical formulation, identical manufacturing equipment and processing conditions, and on *in vitro* dissolution profiles submitted in this application for this immediate release drug product.

3. **NONMEM Population Analysis:**

The sponsor is requested to submit the results of the population pharmacokinetic analysis of the NNY 301 clinical trial data to address special population issues (e.g. age, race, gender, and effect of cigarette smoking), drug-drug interaction, and pharmacokinetic/

pharmacodynamic (efficacy and/or safety) relationship within three months of receiving this comment from the Agency.

4. **Special Population Studies:**

In the absence of information from the population pharmacokinetic screen, the pharmacokinetics of riluzole in the elderly, and in the renally and hepatically impaired, and gender may need to be prospectively studied. Since CYP 1A2 has been suggested to be primarily involved in the metabolism of riluzole, the effect of cigarette smoking on the kinetics of riluzole may also need to be investigated.

5. **Drug Interactions:**

The firm is requested to conduct *in vivo* drug interaction study(ies) using clinically relevant drug combinations and drugs with a narrow therapeutic margin of safety. The *in vivo* study(ies) will also allow to confirm the findings from *in vitro* studies.

6. **Metabolism Study:**

- (i) The sponsor has provided AUC_{0-∞} values for total radioactivity in plasma. However, in order to determine the amount of exposure to parent drug and its metabolites, it is important to know their individual AUC_{0-∞} values.
- (ii) The *in vitro* studies utilizing human liver microsomes suggest that in addition to CYP 1A2, 20-40% of riluzole metabolism may be catalyzed by CYP 2C19, CYP 2E and CYP 2D6. *In vitro* studies are useful in that they suggest which enzymes may be involved in a drug's metabolism at a qualitative but not necessarily at a quantitative level. Because CYP 2D6 and CYP 2C19 displays a genetic polymorphism, the sponsor may consider ascertaining whether people deficient in these enzymes have an impaired ability to metabolize riluzole.

7. **Plasma Levels of Free Drug:**

Riluzole is highly bound (96%) to plasma albumin and lipoproteins. Therefore, changes in plasma protein binding resulting in changes in free drug levels may affect the pharmacokinetics and pharmacodynamics of the drug. Measurement of free drug levels in plasma is strongly recommended in selected pharmacokinetic studies (such as, in hepatic and renally impaired subjects where plasma protein levels are known to be variable). In addition, plasma samples from previously conducted pharmacokinetic studies using different doses could also be analyzed to obtain an estimate of free riluzole levels in plasma.

8. **Design of Food Effect Study:**

In the future, drug-food interaction study should be conducted using the FDA recommended "high fat meal".

LABELING COMMENTS:

The firm is requested to perform the following revisions on the submitted annotated draft labeling:

1. The bioavailability and pharmacokinetic information for Rilutek® Tablets provided under the "Pharmacodynamics, Pharmacokinetics and Metabolism" portion of the Clinical Pharmacology section of the labeling should be replaced with the following:

CLINICAL PHARMACOKINETICS

ABSORPTION

The average absolute oral bioavailability of riluzole is 60% (CV=30%). Following oral administration, riluzole is well-absorbed and average peak plasma concentration occurs in about 0.75 hour. A high fat meal was shown to decrease AUC by 18% and C_{max} by 44% while T_{max} increased to 2 hours. It is, therefore, preferable to administer riluzole tablets on an empty stomach to ensure rapid and complete systemic availability (see DOSAGE AND ADMINISTRATION).

In healthy male Caucasian volunteers, the kinetics of riluzole is linear over the single oral dose range of 25-300 mg and over the multiple oral dose range of 25-100 mg given every 12 hours. In the pivotal bioequivalence study, the 50 mg market tablet when compared to the 50 mg tablet used in the dose ranging clinical trial was found to be equivalent in terms of extent of absorption (AUC_{0-∞}) but not C_{max}, which was 27% higher for the market tablet. However based on the clinical safety data collected with the 100 mg B.I.D. dosing regimen, the higher C_{max} observed with the 50 mg market tablets may not be of any safety concern.

DISTRIBUTION

Riluzole is extensively distributed throughout the body. The extent and duration of riluzole's distribution within various body compartments has not been systematically studied in humans. After I.V. infusion over 30 minutes, average V_{ss} and V_z were found to be 245 L (CV=28%) and 396 L (CV=38%) respectively. The mean apparent volume of distribution, V_z/F after an oral dose was 894 L (CV=36%). Riluzole is 96% bound to plasma proteins mainly to albumin and lipoproteins but not to globulins over the concentration range of 125 to 5000 ng/mL, and binding does not appear to be saturable.

STEADY-STATE KINETICS

Steady-state was observed to be reached within 5 days. Following oral administration of 50 mg tablets every 12 hours, the average steady-state C_{max}^{ss} and average AUC^{ss} were 173 ng/mL (CV=42%) and 654 ngxhr/mL (CV=43%), respectively. The mean elimination half-

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life of riluzole is 12 hours (CV=35%) after repeated doses. Upon multiple dose administration, riluzole accumulates in plasma by about 2 fold.

METABOLISM

In Vivo

Riluzole is extensively metabolized to six major and a number of minor metabolites of which all have not yet been identified. The metabolism of riluzole is mostly hepatic and consists of cytochrome P450-dependent hydroxylation (phase I reaction) and glucuronidation (phase II reaction). The primary metabolic pathways of riluzole biotransformation in humans may involve direct glucuronidation of riluzole and oxidation of riluzole to N-hydroxyriluzole, followed by rapid glucuronidation. Based on exposure (AUC), it appears that in addition to riluzole, which accounts for a small proportion of the circulating total radioactivity in plasma (< 25%), there is significant exposure to metabolites, some of which may possess pharmacological activity.

The marked inter-individual variability in the kinetics of riluzole (4-6 fold variability) is probably attributable to genetic and/or environmental factors that inhibit or induce CYP 1A2 activity. Phenotyping 12 healthy male subjects for CYP 1A2 activity from urinary metabolite patterns after an oral dose of caffeine revealed a 2-11 fold variability in CYP1A2 activity.

In Vitro

In vitro experiments using liver microsomes show that hydroxylation of the primary amine group producing N-hydroxyriluzole is the main metabolic pathway in human, monkey, dog and rabbit. In man, cytochrome P450 1A2 is the principal isozyme involved in N-hydroxylation, although CYP 2D6, CYP 2C19 and CYP 2E may also participate. Whereas direct glucuroconjugation of riluzole (involving the glucurotransferase isoform UGT-HP4) is very slow in human liver microsomes, N-hydroxyriluzole is readily conjugated at the hydroxylamine group resulting in the formation of O- (> 90%) and N-glucuronides.

ELIMINATION

Following a single 150 mg dose of ¹⁴C-riluzole to 6 healthy males, 90% and 5% of the radioactivity was recovered in the urine and feces respectively over a period of 7 days, for a mean combined recovery of 95%. The glucuronides account for more than 85% of the metabolites in urine. Only 2% of a riluzole dose is recovered in the urine as unchanged drug. The elimination of total radioactivity in the plasma was described by a biexponential equation (two compartment model) with a long terminal half-life of 27 hours (CV=14%). After I.V. administration, the mean plasma clearance of riluzole was 800 mL/min (CV=18%), and the average apparent plasma clearance of riluzole (Cl/F) was found to be 1275 mL/min.

SPECIAL POPULATIONS

The pharmacokinetics of riluzole in children and the elderly, and in the renally and hepatically impaired, and the effects of race and gender on riluzole disposition in humans have not been

studied. However, the following information could be useful to the prescribing physician. **Hepatic and Renal Disease:** Since riluzole is extensively metabolized and subsequently excreted in the urine, it is likely that functional hepatic and renal impairment may reduce the clearance of riluzole and its metabolites and higher plasma levels may occur (see **PRECAUTIONS** and **WARNINGS**).

Age: Age-related compromised renal and hepatic function may also cause a decrease in clearance of riluzole with possible higher plasma levels (see **PRECAUTIONS**).

Gender: Based on vitro and in vivo studies, CYP 1A2 activity have been reported to be lower in women than in men. Therefore, a gender effect on riluzole kinetics may be expected in women resulting in a lower clearance of riluzole and its metabolites (see **PRECAUTIONS**).

Smoking: Cigarette smoking is known to induce CYP 1A2 and patients who smoke cigarettes are expected to eliminate riluzole faster. Therefore, these patients may require a higher or more frequent dose of riluzole.

Race: Clearance of riluzole in Japanese subjects was found to be 50% lower as compared to Caucasians after normalizing for body weight.

Literature report of an in vitro study utilizing Japanese and Caucasian liver samples has revealed several CYP 1A2-dependent activities (phenacetin O-deethylation and 7-ethoxycoumarin O-deethylation) to be significantly higher in Caucasians than in Japanese. Although it is not clear if this difference is due to genetic and/or environmental differences (e.g. smoking, alcohol, coffee, and dietary preferences), it is possible that Japanese may possess a lower metabolic capacity (oxidative and/or conjugative) to metabolize riluzole when compared to Caucasian subjects. Therefore, a lower or less frequent dosage may be needed in Japanese patients.
(see **PRECAUTIONS**).

2. The following information should be included under the "PRECAUTIONS" and "DRUG INTERACTIONS" section of the proposed labeling:

DRUG INTERACTIONS

There have been no clinical studies to evaluate the interaction of riluzole with other drugs. As with all drugs, the potential for interaction by a variety of mechanisms is a possibility.

Drugs Highly Bound To Plasma Proteins:

Riluzole is highly bound (96%) to plasma proteins, binding mainly to serum albumin and to lipoproteins. The effect of riluzole (up to 5 µg/mL) on warfarin (5 µg/mL) binding did not show any displacement of warfarin. Conversely, riluzole binding was unaffected by the addition of warfarin, digoxin, imipramine and quinine at high therapeutic concentrations.

Effect of Other Drugs On Riluzole Metabolism:

In vitro studies using human liver preparations suggest that CYP 1A2 is the principal isozyme involved in the initial oxidative metabolism of riluzole, and therefore, potential interactions may occur when riluzole is given concurrently with agents that affect CYP 1A2 activity.

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Potential inhibitors of CYP 1A2 (e.g. caffeine, grapefruit juice, phenacetin, 7,8-benzoflavone, theophylline, amitriptyline, enoxacin, acetaminophen, imipramine, clomipramine, quinolones, diclofenac, diazepam, nicergoline and quinine) could decrease the rate of riluzole elimination, while inducers of CYP 1A2 (e.g. cigarette smoke, charcoal-broiled food, rifampicin, cimetidine and omeprazole) could increase the rate of riluzole elimination.

Effect of Riluzole On the Metabolism of Other Drugs:

In vitro studies under the conditions of high riluzole and low substrate concentrations indicate that riluzole at concentrations of 10 µM could inhibit the enzymatic activities of CYPs 1A2, 2A6 and 2E1 by upto 30%. These results suggest that riluzole may inhibit these enzymes in vivo, potentially resulting in drug interactions. Riluzole had minor inhibitory effect on the enzymatic activities of CYPs 2C9, 2C19, 2D6 and 3A4. Currently, it is not known whether riluzole has any potential for enzyme induction.

3. The following information should be included under the "WARNINGS" section of the proposed labeling:

WARNINGS

In clinical trials the use of riluzole has been associated with elevations of serum alanine transaminase (ALT) and serum aspartate transaminase (AST). Therefore, riluzole should be used with caution in patients who have active hepatic or renal disease.

4. The following information should be included under the "Special Populations" sub-section of the "PRECAUTIONS" section of the proposed labeling:

PRECAUTIONS

Special Populations:

Riluzole should be used with caution in patients who have active hepatic or renal disease (see WARNINGS), and in elderly patients whose hepatic or renal functions may be compromised due to age. Also, females and Japanese patients may possess a lower metabolic capacity to eliminate riluzole compared to Caucasian subjects. Therefore, all these patients should be closely monitored and serum aminotransferases including ALT and AST levels should be measured more frequently in these patients (see SPECIAL POPULATIONS).

5. The following information should be included under the "Laboratory Tests" sub-section of the "PRECAUTIONS" section of the proposed labeling:

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PRECAUTIONS

Laboratory Tests:

Serum transaminase levels (ALT/AST) should be monitored weekly for at least the first three months in patients with severe hepatic or renal impairment, in Japanese patients, and in the elderly and female patients, those with concurrent disease or on multiple medications because of the expected decrease in clearance and plasma accumulation of riluzole.

6. The following information should be included under the "DOSAGE AND ADMINISTRATION" section of the proposed labeling:

DOSAGE AND ADMINISTRATION

Riluzole tablets should be taken at least an hour before or two hours after a meal to minimize drug-food interaction, and to ensure rapid and complete systemic availability.

Biopharm Day 9/14/95 (10:30 am-12:00 noon) (Attendees: T. Ludden, Ph.D.; H. Malinowski, Ph.D.; N. Fleischer, Ph.D.; R. Baweja, Ph.D.; M. Hossain, Ph.D.; V. Tammara, Ph.D.; R. Harris, Ph.D.; A. Hussain, Ph.D.; M. Chen, Ph.D.; P. Hepp, Pharm.D.; A. Atrakchi, Ph.D.; G. Fitzgerald, Ph.D.; P. Leber, M.D.; R. Katz, M.D. and J. Feeny, M.D.)

Mohammad Hossain, Ph.D. (Project Leader)

M. Hossain 9/25/95

Vijay Tammara, Ph.D.

V. Tammara 9/25/95

Robert Harris, Ph.D.

R. Harris 9/25/95

Pharmacokinetics Evaluation Branch I

First Draft Prepared On August 25, 1995

RD initialed by Raman Baweja, Ph.D.

R.B. 8/31/95

FT initialed by Raman Baweja, Ph.D.

R. Baweja

9/27/95

NDA 20-599: RiLUTEK*
Riluzole Tablets

cc: NDA 20-599 (orig.), HFD-120, HFD-426 (Hossain, Tammara, Harris, Baweja, Fleischer), HFD-427 (Mei Ling Chen), HFD-340 (Viswanathan), Chron, Drug, Reviewer, FOI (HFD-19) and F files.

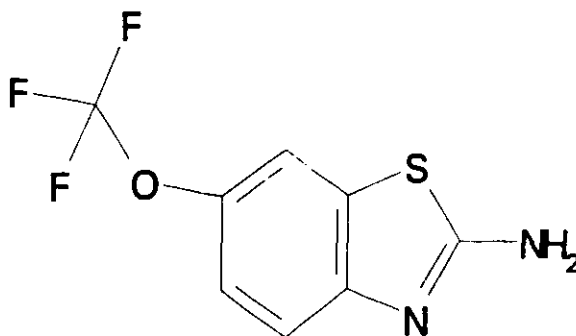
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APPENDIX I
INTRODUCTION

INTRODUCTION

Riluzole (RP 54274) is a compound synthesized by Rhône-Poulenc Rorer. Its chemical name is 2-amino-6-trifluoromethoxybenzothiazole. Its molecular formula is $C_8H_5F_3N_2OS$ and its molecular weight is 234.2.

The substance is in powder form. It is slightly soluble (0.3 mg/mL) in both water and 0.1N NaOH, sparingly soluble in 0.1N HCl (20 mg/mL), freely soluble in chloroform, and highly soluble in dimethylformamide, dimethylsulfoxide and methanol. It is a weakly alkaline substance with a pK_a of 3.8 and a $\log P$ of 3.5 (octanol-water partition coefficient). Its structural formula is as follows:



Riluzole - RP54274

DOSAGE FORMS AND ADMINISTRATION

Most of the pharmacokinetic studies were carried out after oral administration of riluzole in tablet form. A solution for intravenous administration has recently become available in dextrose solution, which has made it possible to clarify the pharmacokinetic characteristics of riluzole and to determine the absolute bioavailability of the final oral dosage form. Different tablet dosage forms were used in the clinical studies. The tablets were manufactured according to two processes: first by wet granulation (WG, 25- and 50- mg tablets) and then by direct compression (25-, 50-, and 100-mg tablets).

In the study involving [^{14}C]-riluzole administration, the active ingredient was administered in capsule form. Lastly, in a bioequivalence study, the final 50-mg tablet dosage form (DT - Batch No CB05921) was compared to the 50-mg tablet used in the Phase III studies.

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APPENDIX II
ANALYTICAL METHODS

3 Pages

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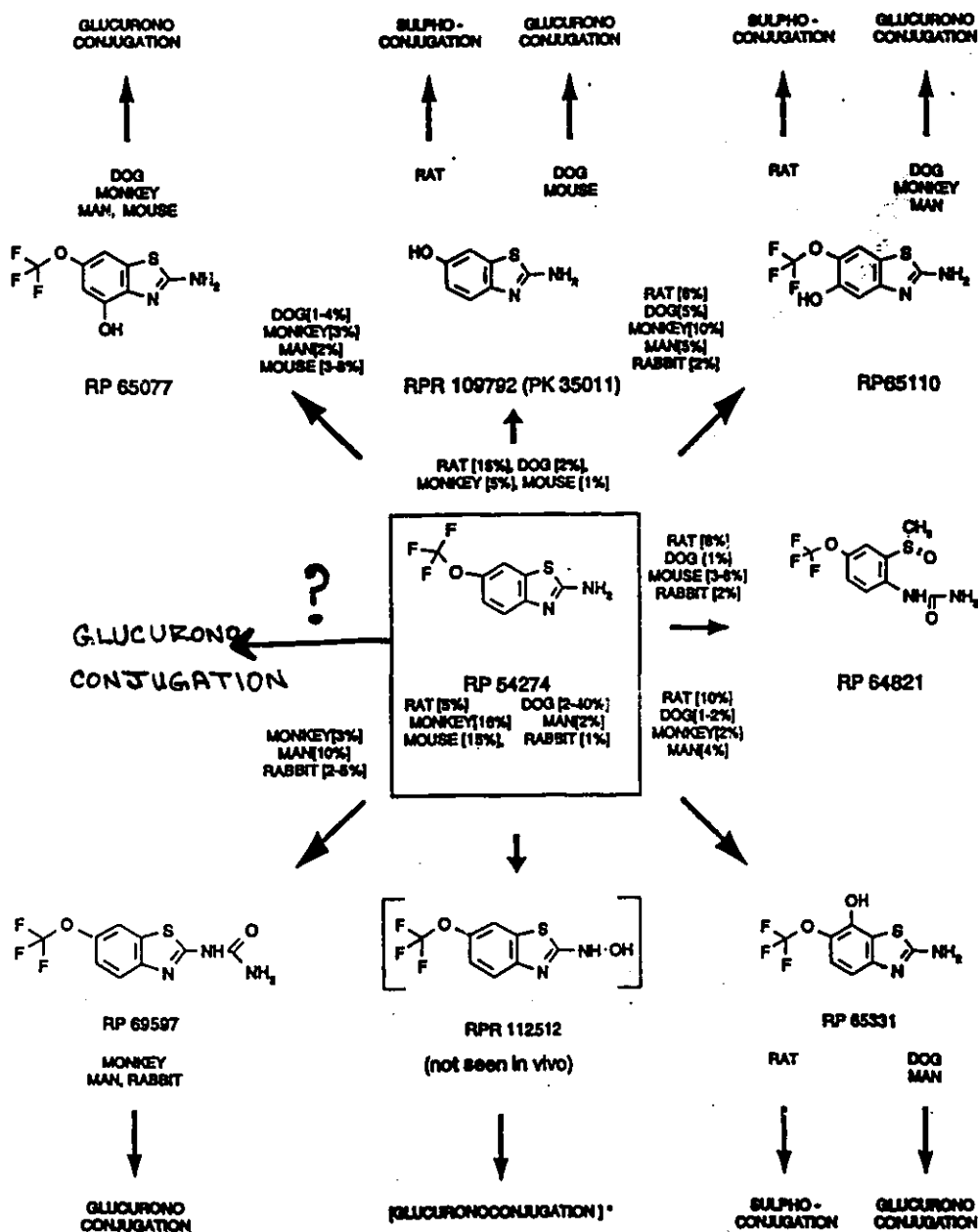
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APPENDIX III

***IN VIVO* ¹⁴C-ADME STUDY**

***In Vitro* Human Liver Microsome Study**

Metabolic pathways of riluzole



Percentages given correspond to fractions of dose found in urine following oral administration.

* Preliminary evidence indicates that this conjugate gives rise to riluzole following β -glucuronidase hydrolysis of urine (12% of dose in monkey, 10-20% in rabbit and 40% in man).

2 Pages

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IBP 1837, IBP 1887, IBP 1154**1.2 IN VITRO INTERACTIONS RELATED TO RILUZOLE METABOLISM IN HUMAN MICROSOMES**

The effects of various specific substrates/inhibitors of cytochrome P-450 on the hydroxylation of riluzole was studied *in vitro* in human liver microsomes. The initial concentration of riluzole was 15 μM . The substrates/inhibitors of CYP 1A were the only compounds found to inhibit the hydroxylation of riluzole. Among the cytochrome P450 enzymes studied, CYP 1A2 was the principal human liver microsomal enzyme responsible for the formation of RPR112512.

Table 1 - Effect of cytochrome P450 substrates/inhibitors on riluzole hydroxylation in human liver microsomes.

Compound	Main specificity	Concentration μM	Inhibition %	IC ₅₀ μM
Caffeine	CYP1A2	1000	37%	-
Acetanilide	CYP1A2	1000	21%	-
Alpha-naphthoflavone	CYP1A	1	80%	0.43
Tolbutamide	CYP2C8/9	50	5%	-
Sulfaphenazole	CYP2C8/9	100	-5%	1000
Mephenytoin	CYP2C19	1000	21%	1000
Omeprazole	CYP2C19	100	31%	-
Quinidine	CYP2D6	5	20%	-
Quinine	CYP2D6	5	19%	-
Aniline	CYP2E	50	-2%	1000
Chlorzoxazone	CYP2E	100	36%	280
P-nitrophenol	CYP2E	50	17%	1000
Isoniazid	CYP2E	1000	39%	1000
Troleandomycin	CYP3A	300	12%	-
Ketoconazole	CYP3A	1	2%	-

- Not determined.

IBP/DD-9C-067

Table 1: Summary of results for the *in vitro* drug interaction studies with riluzole.

Enzyme Activity	P450 Form	Low Substrate Conc.	Low Substrate Conc.	High Substrate Conc.	High Substrate Conc.
		1 μ M Riluzole	10 μ M Riluzole	1 μ M Riluzole	10 μ M Riluzole
phenacetin O-deethylase	CYP1A2	+5%, +5%	-21%, -17%	-1%, +1%	-13%, -11%
coumarin 7-hydroxylase	CYP2A6	-4%, -22%	-13%, -21%	0%, -4%	-8%, +5%
tolbutamide hydroxylase	CYP2C9	+9%, +3%	+5%, +1%	+16%, +3%	+5%, +2%
(S)-mephenytoin 4'-hydroxylase	CYP2C19	-2%, +4%	-4%, +1%	0%, -1%	+8%, -2%
bufuralol 1'-hydroxylase	CYP2D6	-9%, -8%	-14%, -14%	-11%, -10%	-14%, -14%
chlorzoxazone 6-hydroxylase	CYP2E1	0%, -10%	-28%, -27%	-9%, -5%	-22%, -17%
nifedipine oxidase	CYP3A4	+2%, -28%	+15%, -6%	-8%, 0%	+7%, +8%

TABLE 2. INHIBITION OF RPR 112512 FORMATION IN HUMAN LIVER MICROSOMES

[riluzole] = 15 μ M. Inhibitors at 1 mM, *except nicergoline and diazepam (0.5 mM).

IBP/1877

INHIBITOR	INHIBITION	IC ₅₀ μ M	INHIBITOR	INHIBITION	IC ₅₀ μ M
Clomipramine	99%	265 $\pm$ 25	Paracetamol	28%	
Diclofenac	99%	234 $\pm$ 19	Pyridoxine	18%	
Amisulpride	94%	210 $\pm$ 17	Enalapril	12%	
Imipramine	85%	356 $\pm$ 20	Thiamine	9%	
Enoxacin	71%	465 $\pm$ 77	Captopril	8%	
Nicergoline*	71%	256 $\pm$ 13	Pefloxacin	6%	nd
Quinine	63%	444 $\pm$ 62	Aspirin	6%	
Diazepam*	59%	240 $\pm$ 23	Amoxicillin	6%	
Theophylline	47%	937 $\pm$ 182	Metronidazole	5%	
Cimetidine	40%		Piracetam	5%	
Caffeine	37%		Baclofen	-3%	
Ranitidine	29%				

nd: not detectable.

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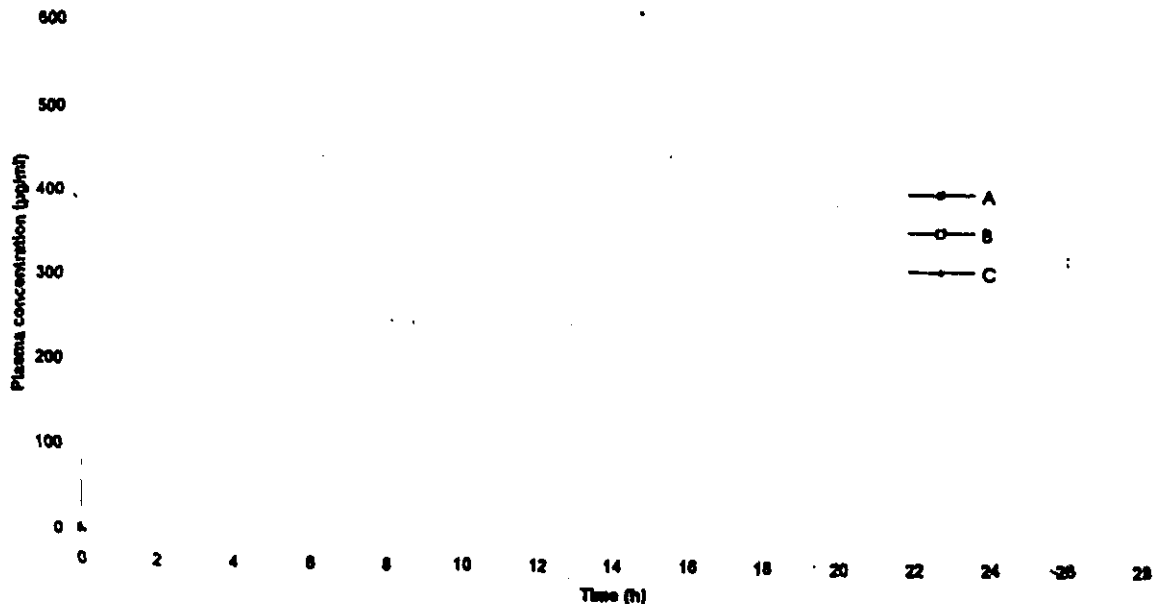
APPENDIX IV
BIOAVAILABILITY

NNX 161

STUDY SUMMARY

Title of the study	A phase 1, open, randomised, 3-period cross-over study to determine the absolute bioavailability of RP 54274 (riluzole) by comparing its kinetic profile after a single 50 mg I.v. dose and two single 100 mg oral doses administered to healthy male volunteers.
Investigator	
Study centre	
Publication	None
Study period	10 October 1994 (first subject enrolled) to 02 December 1994 (last subject completed)
Clinical Phase	1a
Objectives	1. To assess the pharmacokinetic profiles of single I.v. 50 mg dose and oral 100 mg doses of RP 54274, 2. To determine the absolute bioavailability of riluzole administered as two 50 mg tablets 3. To assess the intra-individual variability of the pharmacokinetic profile of the oral treatment following a single oral 100 mg dose of RP 54274.
Methodology	Open, randomised, cross-over trial comprising 3 separate periods, at least one week apart, when RP 54274 was administered as an I.v. (one occasion) or oral (two occasions) single dose.
Number of subjects (total and for each treatment)	16 subjects were initially enrolled. One was excluded after two periods (consumption of cannabinoids) and was replaced. A total of 15 completed the study as per protocol.
Diagnosis and criteria for inclusion	Caucasian, male, healthy volunteers, aged 18 to 35 years. No relevant clinical, haematological or biochemical abnormalities. No known hypersensitivity to caffeine-containing beverages.
Test product, dose and mode of administration, batch N°	RP 54274 : - oral 100 mg single dose : two 50 mg tablets, Batch CB05921, - I.v. 50 mg single dose : 50 mg/10 ml ampoules, Batch CB05757, administered as 30 min. infusion in 0.5% dextrose solution
Duration of treatment	1 day, at each of the 3 periods.

Figure 1: Mean plasma concentrations of Riluzole (ng.ml⁻¹) in healthy subjects given a single 50 mg IV 30 min. infusion (A) or two single 100 mg oral doses (B, C); c=ff(time) 0 to 28h



RESULTS:-

Parameter		Treatment A (50mg, IV)	Treatment B (100 mg oral)	Treatment C (100 mg oral)
C _{max} (ng/ml)	mean	584	318	430
	SD	137	200	244
	CV%	24	63	57
t _{max} (h)	median	0.5	1.0	0.75
	max	0.86	2.0	3
	min	0.25	0.5	0.5
AUC(0-lq) (ng·h/ml)	mean	1042	1206	1284
	SD	187	631	673
	CV%	19	44	45
AUC (ng·h/ml)	mean	1082	1328	1405
	SD	201	672	609
	CV%	19	43	43
t _{1/2} (h)	mean	5.9	8.0	7.3
	SD	2.1	2.8	1.8
	CV%	36	32	22
t _{1/2α} (h)	mean	1.1	1.1	1.2
	SD	0.3	0.3	0.4
	CV%	28	29	34
MRT (h)	mean	5.3	8.0	7.3
	SD	1.5	2.0	1.7
	CV%	28	25	23
CL/F (ml/min)	mean	794	1468	1333
	SD	140	580	409
	CV%	18	39	31
V _z /F (l)	mean	396	966	821
	SD	152	389	250
	CV%	38	40	30
V _{ss} /F (l)	mean	245	681	571
	SD	89	275	178
	CV%	28	40	31

Statistical data

Parameter	effects (GLM)	Pr > F	multicomparison test (α = 0.05) for treatments	• Lower C.I. x 100 (alpha=0.10)	• Upper C.I. x 100 (alpha=0.10)	Schirmann 90% C.I. (B _{inc})
Ln C _{max}	treatment	0.0001	A ≠ B ≠ C	22.23	34.46	60% - 91%
	period	0.0682		23.91	37.07	
	subject(sequence) sequence	0.0075 0.3828		82.28	140.89	
Ln AUC(0-lq) (ng·h·ml ⁻¹)	treatments	0.0001	A = B and C, B = C	51.33	88.75	
	periods	0.0455		47.18	61.35	
	subject(sequence) sequence	0.0002 0.0585		78.25	107.95	
Ln AUC (ng·h·ml ⁻¹)	treatments	0.0001	A = B and C, B = C	53.50	88.84	83% - 105%
	periods	0.2220		61.71	86.34	
	subject(sequence) sequence	0.0001 0.0568		82.97	112.58	
t _{1/2} (h)	treatments	0.0142	B = C A = B and C			
	periods	0.4858				
	subject(sequence) sequence	0.0186 0.4381				
t _{1/2α} (h)	treatments	0.3108	A = B = C			
	periods	0.2072				
	subject(sequence) sequence	0.2375 0.6547				
MRT (h)	treatments	0.0001	B = C A = B and C			
	periods	0.6199				
	subject(sequence) sequence	0.0020 0.6122				

Comparison of variances

study	parameter	inter-subjects	intra-subjects
AG161	Log C _{max}	0.073	0.103
	Log AUC(0-lq)	0.051	0.037
	Log AUC	0.050	0.033

STUDY NNY 161

34a

Figure 1: RP54274X161 - Scattergram of Cmax values by treatment, period 1, 2 and 3

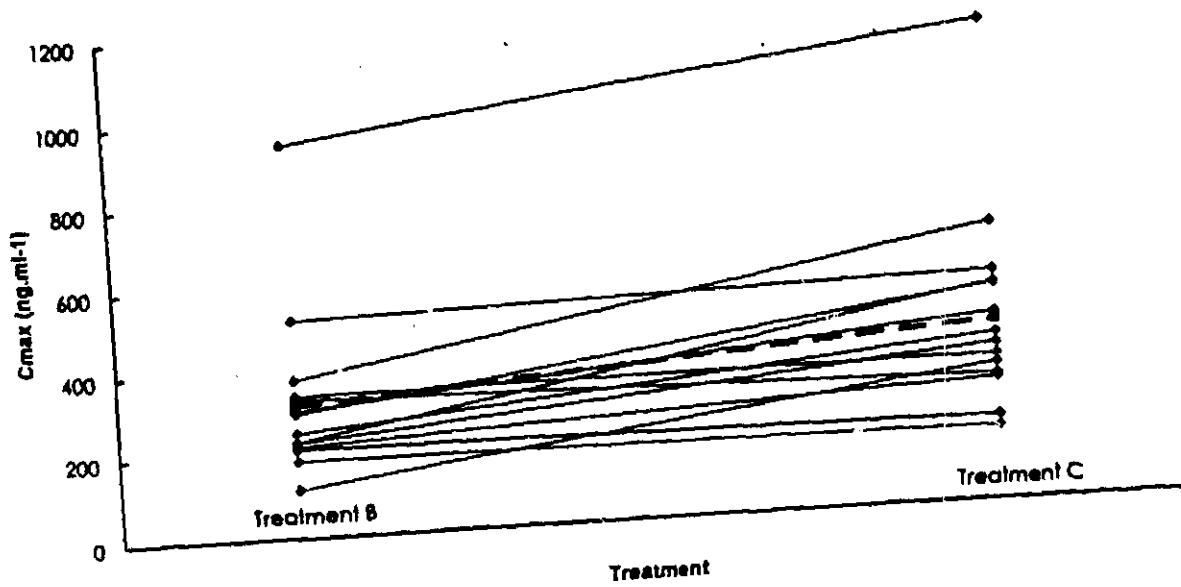
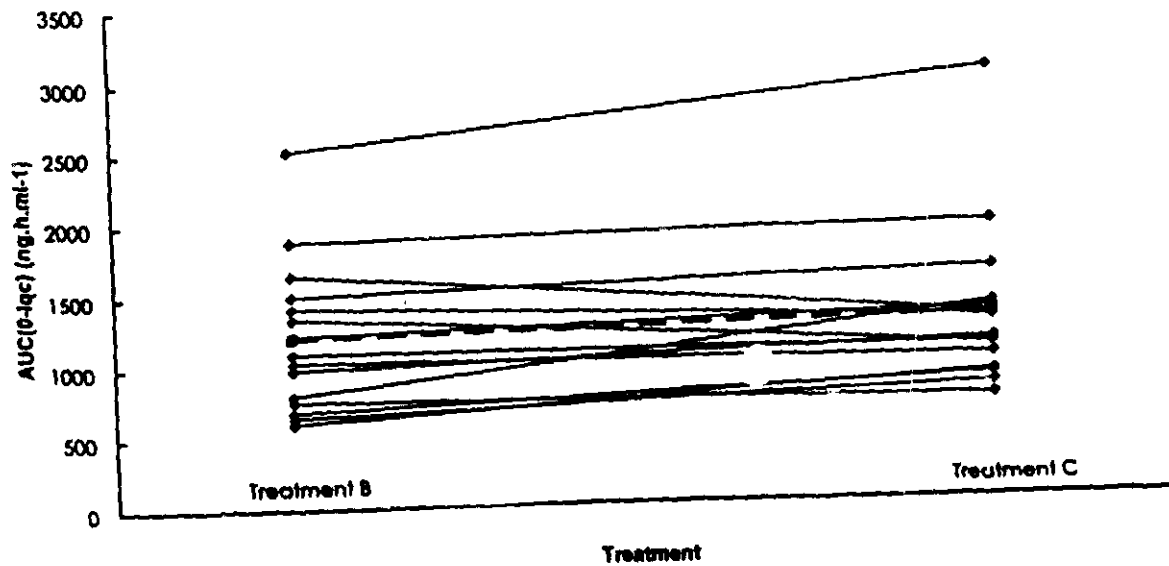


Figure 2: RP54274X-161 - Scattergram of AUC(0-lqc) values by treatment, period 1, 2 and 3



STUDY SUMMARY

Title of the study	RP 54274X-159 - A phase 1, open, randomised, cross-over study to investigate the effect of food intake on the disposition of rizole (RP 54274) administered as a single oral dose to healthy male volunteers. Protocol n° : RP 54274X-159.
Investigator	
Study centre	
Publication	None
Study period	Start date : 18 May 1994 (first subject enrolled) Completion date : 10 June 1994 (last subject completed)
Clinical Phase	1a
Objectives	- To assess pharmacokinetic parameters of RP 54274 after a high fat meal. - To compare pharmacokinetic parameters of RP 54274 after a high fat meal versus fasting condition.
Methodology	Open, randomized, 2-way crossover trial comprising 2 treatment periods separated by a wash-out period of at least 5 days. Assignment of subjects to one of the sequences "fasting/ high fat meal" or "high fat meal/fasting" was performed according to a computer-generated randomisation list.
Number of subjects (total and for each treatment)	16 subjects were enrolled and completed the study according to protocol. 8 received the sequence "fasting/ high fat meal", 8 the sequence "high fat meal/fasting".
Diagnosis and criteria for inclusion	Male, caucasian, healthy volunteers, aged 20 to 27 years, were recruited, with no relevant clinical, haematological or biochemical abnormalities.
Test product, dose and mode of administration, batch n°	RP 54274, single 100 mg oral dose at each of the 2 periods, provided as 2x50 mg tablets batch n° CB05021

Composition of the breakfast (microwave cooking)

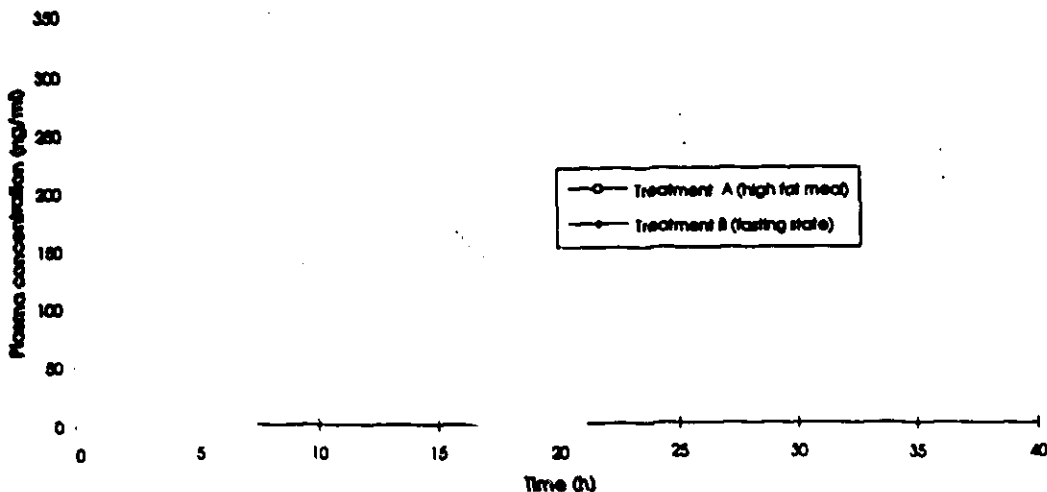
	Unit	Quantity
Eggs	piece	2
Butter	g	10
Sausages	g	100
Regular bread	g	60
French cheese "gruyère"	g	155

Composition Percentage	Proteins	Lipids	Glucides	Kcal
	47.6	67.4	40	864
	20.1	62.9	17	
	Amin./Veget. 1.5	SFA=48% MFA=42% PIFA=10%		

Sodium : 17.23 mg

SFA : Saturated Fatty Acids
MFA : Mono-unsaturated Fatty Acids
PIFA : Poly-unsaturated Fatty Acids

Figure 1 - RP 54274 -159 - Mean plasma concentration-time curves of RP 54274 in healthy subjects (n=16) after a single oral dose of 100 mg RP 54274



STUDY ABSTRACT

NNY 158

TITLE OF THE STUDY	A PHASE I DOUBLE-BLIND, PARALLEL GROUP, PLACEBO-CONTROLLED TOLERABILITY AND PHARMACOKINETIC STUDY OF RILUZOLE ADMINISTERED AS REPEATED DOSES (25 mg, 50 mg and 100 mg b.i.d.) TO HEALTHY MALE VOLUNTEERS
INVESTIGATOR	
STUDY CENTER	
PUBLICATION	None
STUDY PERIOD	Start date: 1 st July 1993 (first subject randomized) Completion date: 1 st september 1993 (last patient completed)
CLINICAL PHASE	Phase I
OBJECTIVES	<i>Primary objective</i> : To study the clinical and biological tolerability and the pharmacokinetic profile of 3 doses of riluzole (25 mg, 50 mg and 100 mg b.i.d.) for 10 days in healthy volunteers. <i>Secondary objective</i> : To assess the degradation of riluzole in plasma and serum samples after delayed freezing. <i>Third objective</i> : To assess the possible hemolytic potential of riluzole.
METHODOLOGY	Parallel group, placebo-controlled, double-blind study in healthy volunteers.
SUBJECTS	36 subjects (9 subjects for each treatment corresponding to 25, 50, 100 mg riluzole and placebo b.i.d.).
DIAGNOSIS AND CRITERIA FOR INCLUSION	Healthy male volunteers, caucasian, aged 18 to 35 years inclusive. Weight +/- 15% of ideal body weight Written informed consent.
TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH N°	Three dose levels of riluzole 25, 50 and 100 mg (1 tablet), orally, b.i.d. were consecutively studied in 3 different groups of subjects. Progression to the higher dose was only allowed when the investigator and the sponsor had determined that the preceding dose was safe and well tolerated. In each group, 9 subjects were allocated to receive riluzole and 3 placebo. The riluzole 25 mg (Lot N° CB 05635), 50 mg (Lot N° CB 05650) and 100 mg (Lot N° CB 05572) tablets used in this study were manufactured by direct compression.
DURATION OF TREATMENT	Treatment duration: 10 days repeated dose treatment preceded and followed by a single dose.
REFERENCE THERAPY, DOSE MODE OF ADMINISTRATION, BATCH N°	Placebo (1 tablet) orally, b.i.d. (Lot N° CB 05630P). Placebo tablets contained 0 mg riluzole. Identical appearance with riluzole tablets.
CRITERIA FOR EVALUATION	<i>Safety</i> : adverse events, physical examination, vital signs, ECG, laboratory screen. <i>Pharmacokinetics</i> : plasma samples at time 0, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 16, 24, 36, 48 hours after the first single dose (Day 1 to 3) and the last repeated dose (Day 13 to 15) and samples at times 0 (morning administration) and 1 hour on days 5 and 10. In addition, blood samples were taken for the evaluation of product stability (objective n°2) at time 1 hour on day 7.

The tablets were firm-coated strapped tablets with a core tablet finished to 400 mn.

Placebo was of identical appearance but contained no riluzole.

RESULTS:-

Table 1

PHARMACOKINETICS		Mean±SD pharmacokinetic parameters obtained after single and repeated oral administration of riluzole are:						
Dose (mg)	Day		C _{max} (ng/ml)	t _{max} (h)	AUC (ng/ml.h)	C _{min} (ng/ml)	t _{1/2β} (h)	
25	1	mean	51.51	1.11	207.36	1.98	5.16	
		SD	17.98	0.70	86.07	3.04	1.16	
	13	mean	77.40	0.78	295.54	6.83	9.24	
		SD	30.02	0.26	118.10	4.48	3.82	
50	1	mean	180.43	0.94	637.46	9.46	6.43	
		SD	121.54	0.46	272.54	7.57	1.91	
	13	mean	172.60	0.83	653.79	20.87	14.73	
		SD	72.37	0.50	279.72	11.69	4.88	
100	1	mean	282.49	1.56	1195.00	19.85	5.78	
		SD	126.12	1.07	600.46	11.42	1.46	
	13	mean	356.89	1.28	1483.24	41.81	13.00	
		SD	166.47	0.44	741.68	28.25	4.33	

Table 2 : Accumulation ratios of riluzole calculated after single and repeated oral administration of riluzole 25 mg, 50 mg and 100 mg

Subject	Dose 25 mg			Subject	Dose 50 mg			Subject	Dose 100 mg		
	R1	R2	R3		R1	R2	R3		R1	R2	R3

Mean	1.58	1.67	1.46	Mean	1.23	2.12	1.35	Mean	1.36	2.12	1.31
s.d.	0.51	0.63	0.34	s.d.	0.69	0.55	0.48	s.d.	0.45	0.59	0.36
CV%	32	38	23	CV%	56	26	36	CV%	33	28	27
Min.	0.94	1.06	1.04	Min.	0.43	1.56	0.92	Min.	0.83	1.40	0.86
Max.	2.65	2.32	2.19	Max.	2.51	3.27	2.47	Max.	2.32	3.09	2.03

: value not calculated because C_{min}(Day 1) is below the limit of quantification (5 ng.ml⁻¹)

with : R1 = C_{max}(Day 13)/C_{max}(Day 1)

R2 = C_{min}(Day 13)/C_{min}(Day 1)

R3 = AUC_{0-12h}(Day 13)/AUC_{0-12h}(Day 1)

$$R3 = \frac{AUC_{0-12hr}^{SS} (Day 13)}{AUC_{0-12hr}^{SD} (Day 1)}$$

Page
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Figure 4 : Mean $\pm$ s.d. plasma concentrations of riluzole ($\text{ng}\cdot\text{ml}^{-1}$) measured just prior to ($\square$) and 1 hour ($*$) after oral administration of riluzole 25 mg

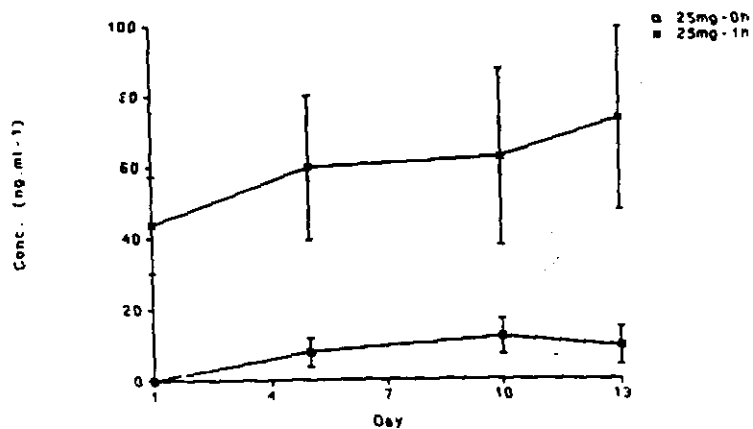


Figure 5 : Mean $\pm$ s.d. plasma concentrations of riluzole ($\text{ng}\cdot\text{ml}^{-1}$) measured just prior to ($\square$) and 1 hour ($*$) after oral administration of riluzole 50 mg

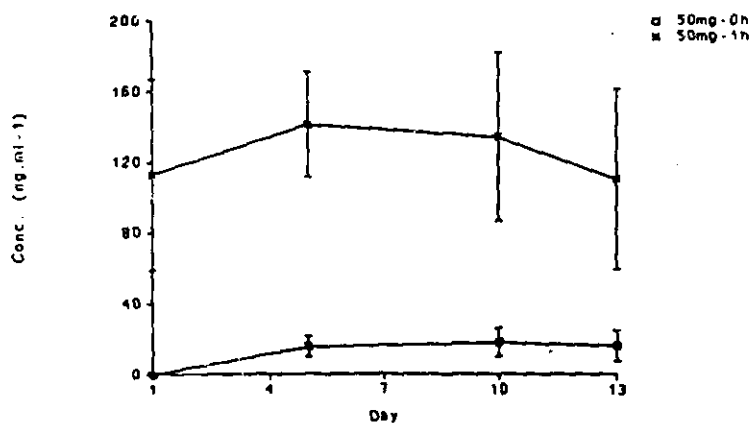
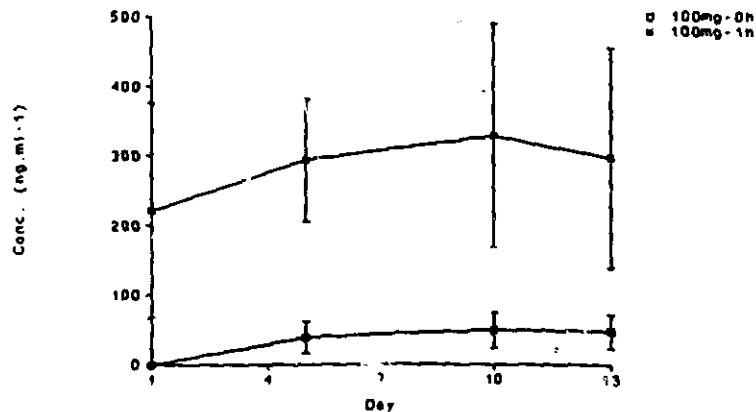


Figure 6 : Mean $\pm$ s.d. plasma concentrations of riluzole ($\text{ng}\cdot\text{ml}^{-1}$) measured just prior to ($\square$) and 1 hour ($*$) after oral administration of riluzole 100 mg



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APPENDIX V

DOSE PROPORTIONALITY/LINEARITY

STUDY NNY 101

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RP 54274 - (fluzole) - Rising intravenous doses to parallel groups of healthy male volunteers
Cmax levels vs dose

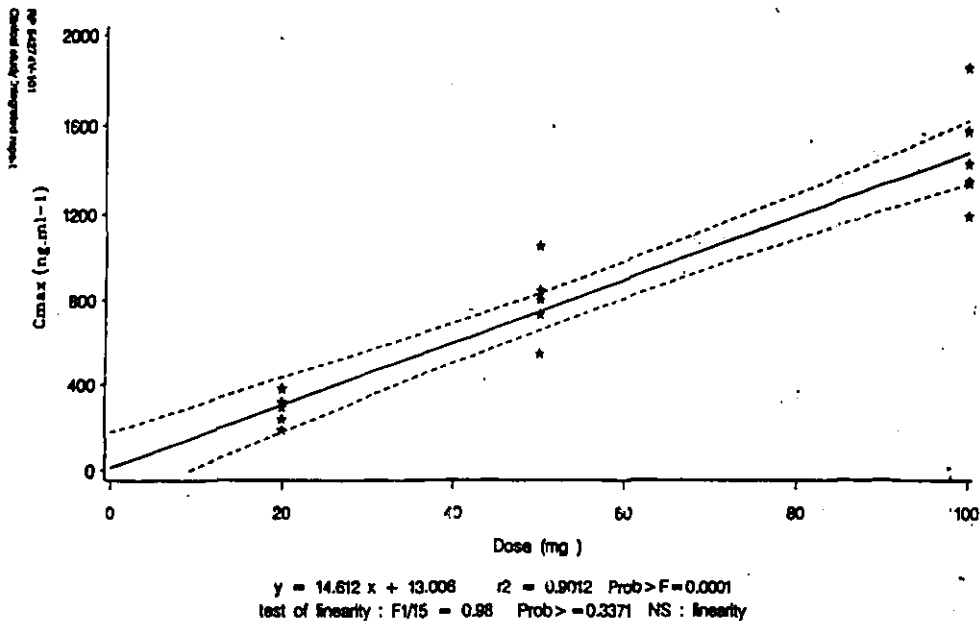


Figure 1

RP 54274 - (fluzole) - Rising intravenous doses to parallel groups of healthy male volunteers
AUC levels vs dose

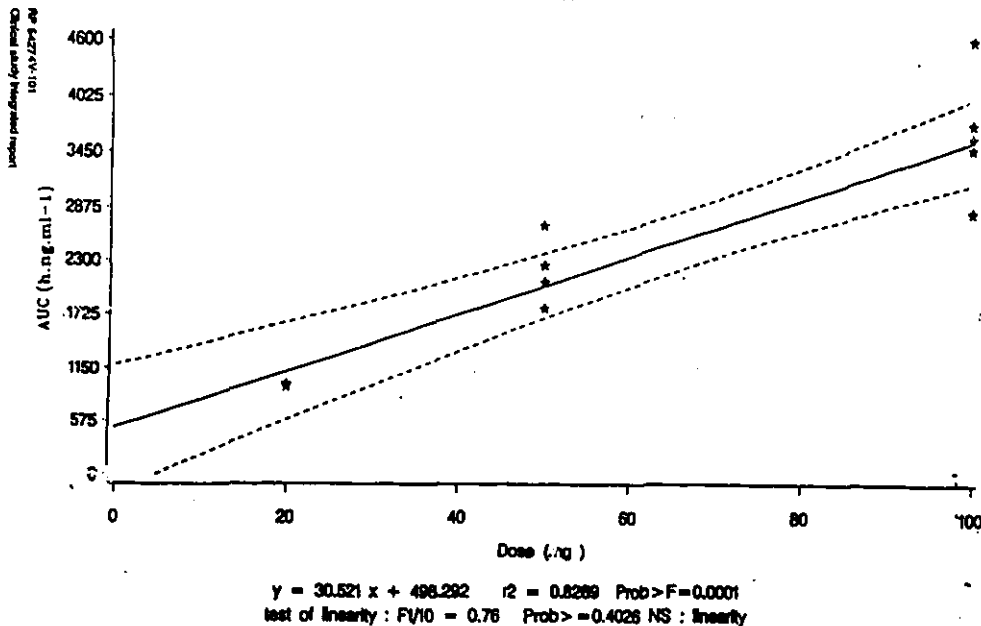


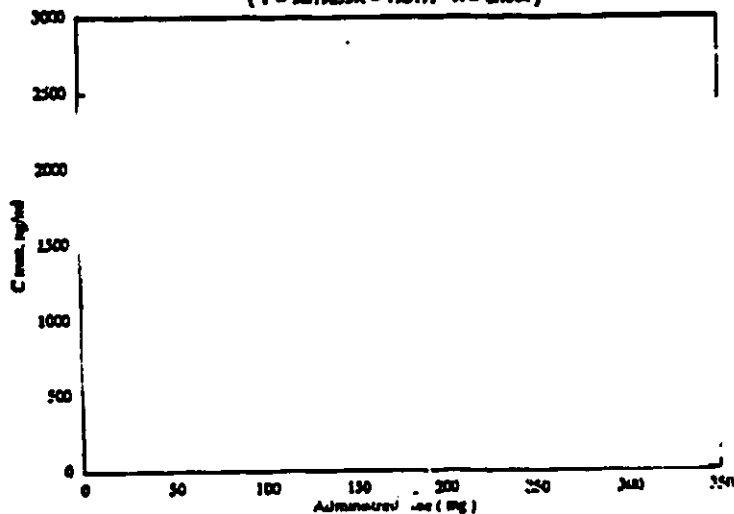
Figure 2

INVESTIGATOR'S BROCHURE
RILUTEK™/RILUZOLE/RP 54274

: Rilutek - AG011 - Single dose linearity study - Individual pharmacokinetic parameters in 12 healthy volunteers

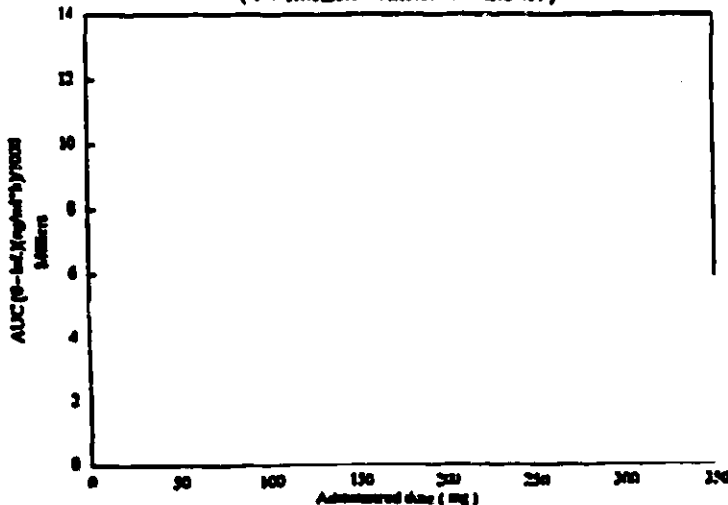
RP 54274 - C max versus dose

$$(Y = 5.019633X - 71.3191 \quad R = 0.7304)$$



RP 54274 - AUC (0-inf.) versus dose

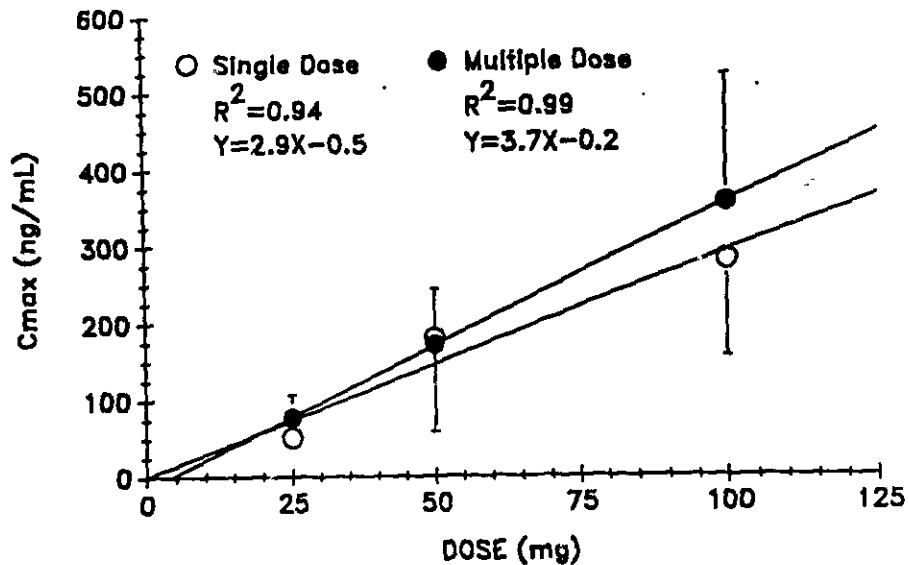
$$(Y = 19.73233X - 71.13907 \quad R = 0.724959)$$



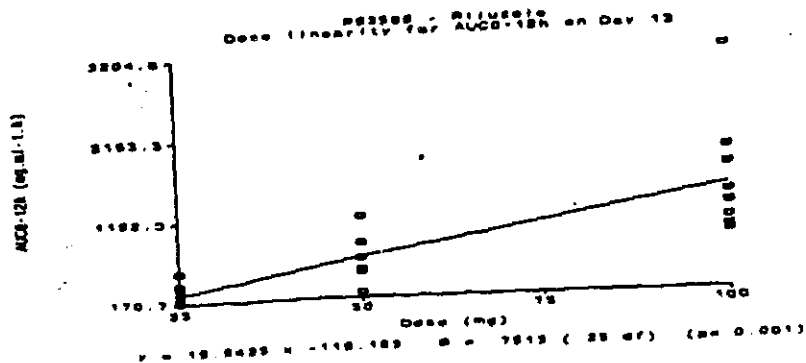
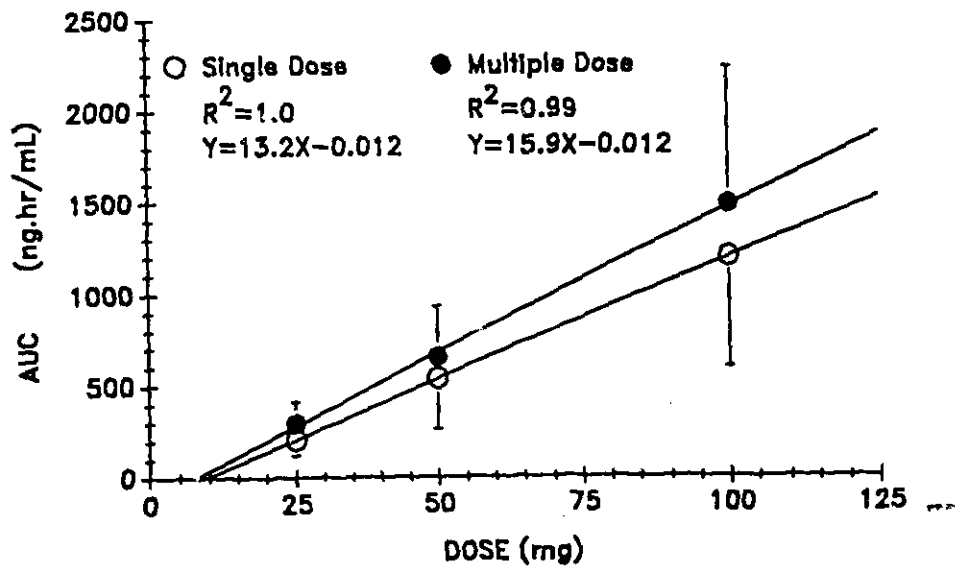
10 April 1995

CONFIDENTIAL

RILUZOLE DOSE PROPORTIONALITY
STUDY NNY158 (n=9)



RILUZOLE DOSE PROPORTIONALITY
STUDY NNY158 (n=9)



NDA 20-599: RILUTEK®
Riluzole Tablets

APPENDIX VI
SPECIAL POPULATIONS
JAPANESE VS CAUCASIAN PHARMACOKINETICS

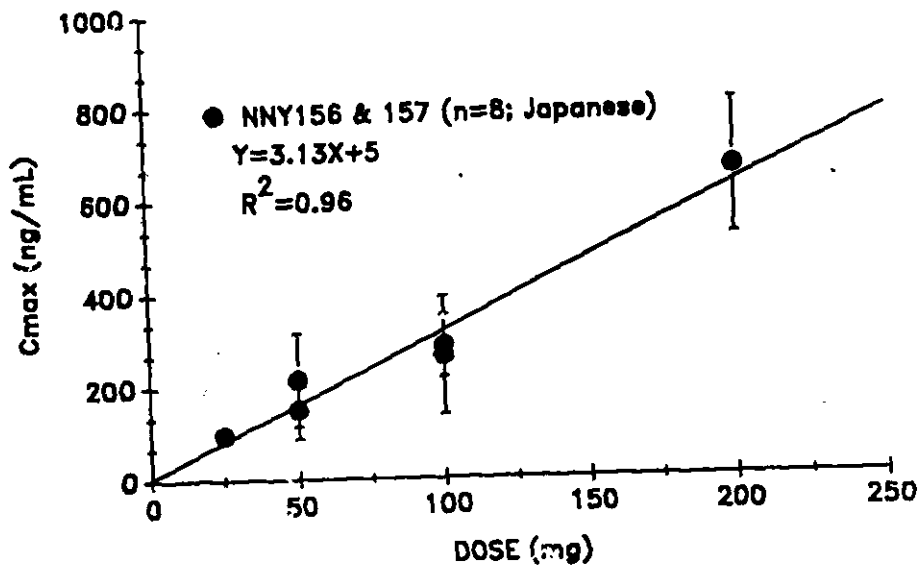
Title of the Study:	A Phase I, double-blind, parallel-group, placebo-controlled tolerability and pharmacokinetic study of rizuzole administered as repeated doses (25 mg, 50 mg and 100 mg bid) to healthy male Japanese volunteers (RP 54274X157)
Investigators:	
Study centre:	
Publication:	None
Studied period:	May - July 1993
Clinical Phase:	Phase I
Objectives:	To study the clinical and biological tolerability and the pharmacokinetic profile of 3 repeated doses of rizuzole (25 mg, 50 mg and 100 mg bid) in Japanese volunteers
Methodology:	Parallel-group, placebo-controlled, double-blind, study in healthy volunteers Single dose followed 48 hours later by 10-day repeated-dose; full kinetic profile after the single dose and at the end of the repeated dose period. The 3 dose levels were studied consecutively in different groups of subjects. The next higher dose was only given when the investigator and the sponsor had determined that the previously administered dose was safe and well tolerated.
Number of subjects:	n = 36; (9 subjects in each treatment group, i.e. 25 mg, 50 mg, 100 mg or placebo bid)
Diagnosis and criteria for inclusion:	Healthy male volunteers, aged 20 to 35 years, with weight $\pm$ 15% of ideal body weight (Japanese tables) having given their informed consent.
Test product, dose and mode of administration, batch N°:	Doses: 50 mg, 100 mg, 200 mg / day (25 mg, 50 mg and 100 mg bid) - Oral route Rizuzole 25 mg tablets: batch, CB 05635 Rizuzole 50 mg tablets: batch, CB 05571 Rizuzole 100 mg tablets: batch, CB 05572
Duration of treatment:	Single dose followed 48 hours later by 10-day repeated-dose
Reference therapy, dose and mode of administration, batch N°:	placebo capsules; Batch CB 05631P Oral route
Criteria for evaluation:	Safety: adverse events, neurological examination, physical examination, vital signs, ECG, laboratory tests Pharmacokinetics: samples at 0, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 16, 24, 36 hours after the first single dose (Day 1 to 2) and the last repeated dose (Day 13 to 14) and samples at 0 (morning administration), 1, 1.5 hours at day 5 and 8. Urine collection: 0-2 hrs, 2-4 hrs, 4-8 hrs, 6-8 hrs, 8-12 hrs, 12-24 hrs, 24-36 hrs, 36-48 hrs, after drug administration
Statistical methods:	Clinical data: descriptive analysis Pharmacokinetic parameters (C _{max} and AUC): ANOVA and linear regression (with dose)

The tablets were film-coated strapped tablets with a core tablet finished to 400 mg :
Placebo

mg

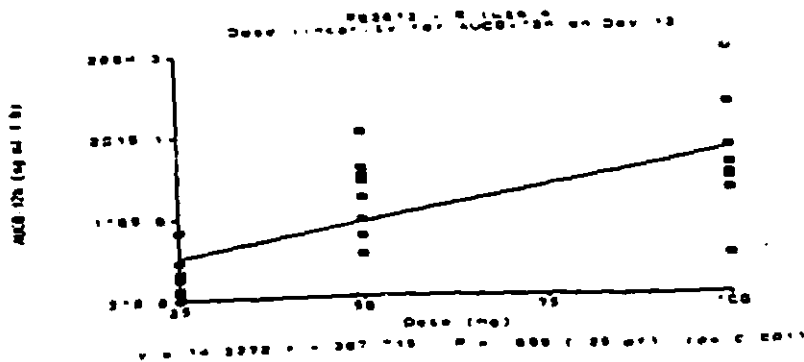
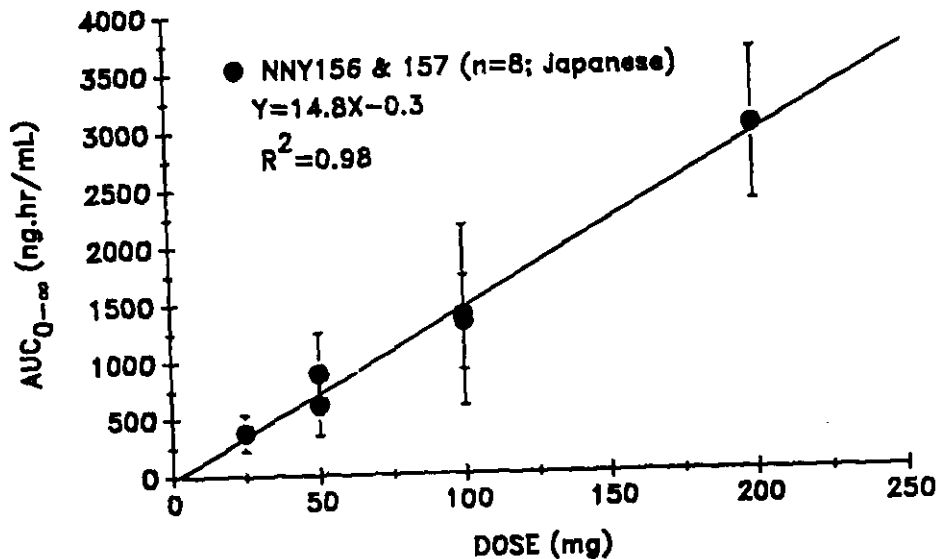
RILUZOLE DOSE PROPORTIONALITY

Single Dose

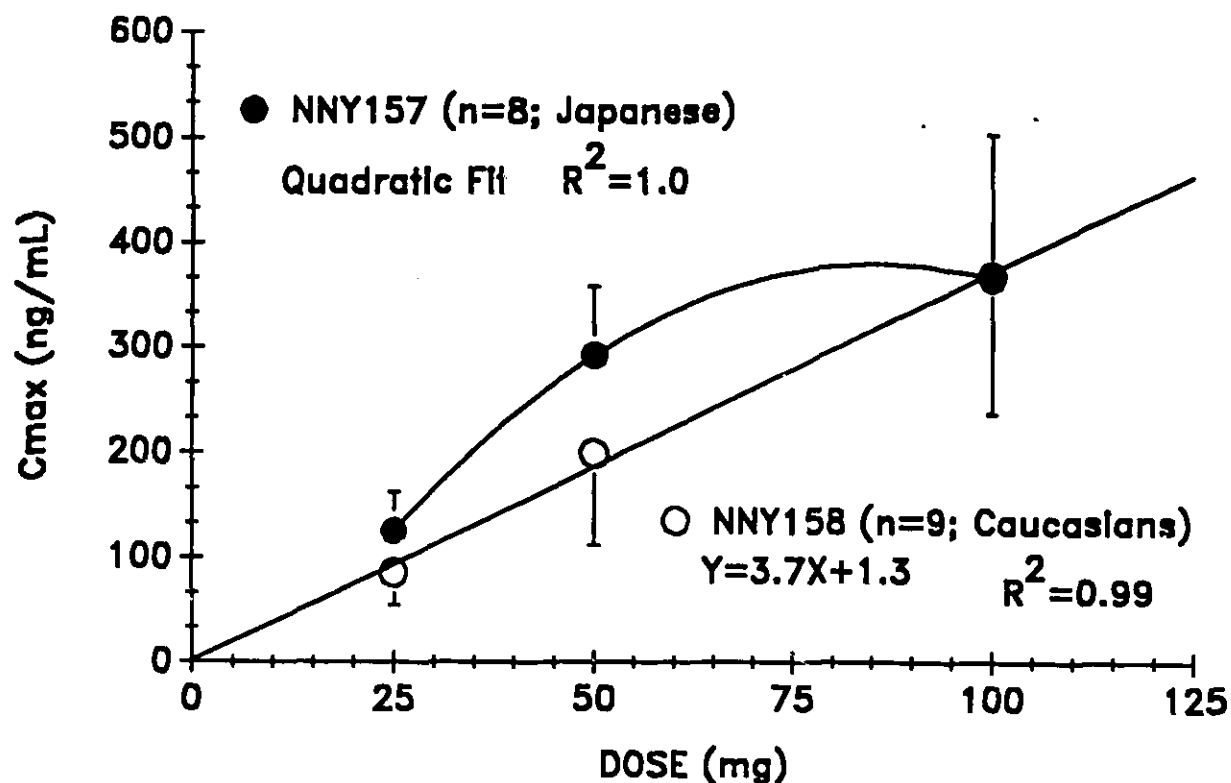


RILUZOLE DOSE PROPORTIONALITY

Single Dose



RILUZOLE DOSE PROPORTIONALITY (Multiple Dose)
Normalized To Mean Body Weight



RILUZOLE DOSE PROPORTIONALITY (Multiple Dose)
Normalized To Mean Body Weight

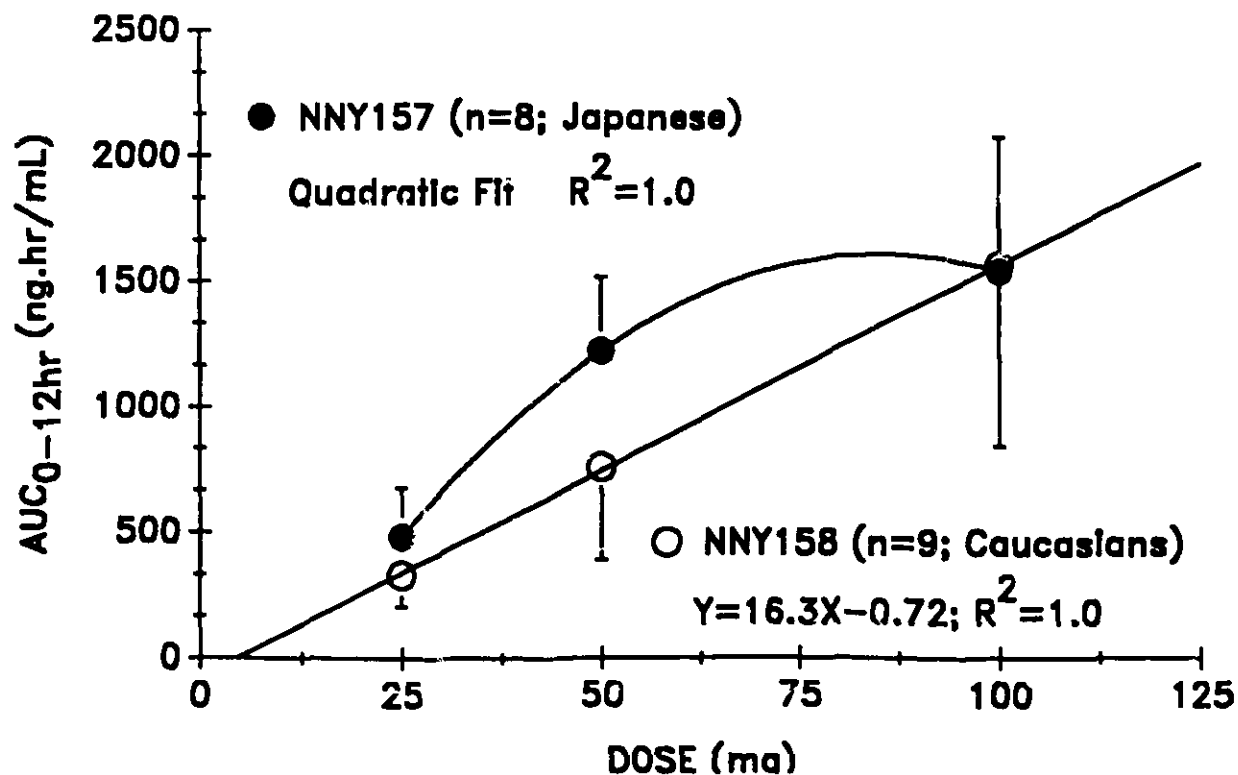


TABLE I

Mean kinetic parameters (mean $\pm$ S.D., normalized to the mean body weight) in Japanese subjects (study 157) and in healthy caucasian subjects (study 158) after single and repeated dose for 10 days of 25, 50 and 100 mg dose of riluzole b.i.d..

Dose (mg)	Study Day	Study	C _{max} ng.ml ⁻¹	T _{max} h	C _{min} ng.ml ⁻¹	AUC (0-12) h.ng.ml ⁻¹	AUC h.ng.ml ⁻¹	t _{1/2} h	CL/F L.k ⁻¹ .kg ⁻¹	V _{D/F} L.kg ⁻¹
25	1	157	83.5 $\pm$ 23.9	0.7 $\pm$ 0.3	5.4 $\pm$ 3.6	295 $\pm$ 130	330 $\pm$ 135	5.2 $\pm$ 1.5	1.2 $\pm$ 0.4	8.6 $\pm$ 3.3
		158	57.2 $\pm$ 19.0	1.1 $\pm$ 0.7	2.1 $\pm$ 3.3	202 $\pm$ 79	230 $\pm$ 89	5.2 $\pm$ 1.2	1.7 $\pm$ 0.6	8.7 $\pm$ 3.8
	13	157	126 $\pm$ 37	0.8 $\pm$ 0.4	13.1 $\pm$ 5.3	481 $\pm$ 193	685 $\pm$ 277	10.1 $\pm$ 3.3	0.8 $\pm$ 0.3	12.4 $\pm$ 6.9
		158	85.8 $\pm$ 31.0	0.8 $\pm$ 0.3	7.5 $\pm$ 4.9	327 $\pm$ 122	452 $\pm$ 203	9.2 $\pm$ 3.8	1.2 $\pm$ 0.4	14.2 $\pm$ 7.1
50	1	157	182 $\pm$ 61	1.1 $\pm$ 0.2	13.2 $\pm$ 3.7	707 $\pm$ 220	766 $\pm$ 229	5.6 $\pm$ 1.8	1.0 $\pm$ 0.3	7.8 $\pm$ 2.2
		158	215 $\pm$ 156	0.9 $\pm$ 0.5	11.0 $\pm$ 8.8	567 $\pm$ 318	623 $\pm$ 331	6.4 $\pm$ 1.9	1.6 $\pm$ 1.1	10.8 $\pm$ 6.8
	13	157	292 $\pm$ 66	0.8 $\pm$ 0.5	30.8 $\pm$ 8.5	1222 $\pm$ 294	1778 $\pm$ 448	11.9 $\pm$ 1.2	0.6 $\pm$ 0.2	10.6 $\pm$ 3.1
		158	199 $\pm$ 87	0.8 $\pm$ 0.5	24.4 $\pm$ 15.1	759 $\pm$ 369	1251 $\pm$ 688	14.7 $\pm$ 4.9	1.2 $\pm$ 0.6	20.1 $\pm$ 8.5
100	1	157	234 $\pm$ 110	1.4 $\pm$ 0.6	19.7 $\pm$ 13.6	1167 $\pm$ 669	1232 $\pm$ 701	5.9 $\pm$ 1.2	1.4 $\pm$ 0.7	11.9 $\pm$ 5.4
		158	296 $\pm$ 116	1.6 $\pm$ 1.1	20.8 $\pm$ 11.3	1183 $\pm$ 575	1259 $\pm$ 581	5.8 $\pm$ 1.5	1.4 $\pm$ 0.7	10.9 $\pm$ 4.8
	13	157	365 $\pm$ 138	1.2 $\pm$ 0.4	35.7 $\pm$ 13.5	1535 $\pm$ 538	2055 $\pm$ 737	10.0 $\pm$ 1.4	1.1 $\pm$ 0.5	15.8 $\pm$ 10.3
		158	368 $\pm$ 131	1.3 $\pm$ 0.4	44.4 $\pm$ 9.3	1557 $\pm$ 717	2386 $\pm$ 1243	13.0 $\pm$ 4.3	1.0 $\pm$ 0.3	19.5 $\pm$ 10.8

..: not calculated

mean body weight = 62.5 $\pm$ 5.6 kg (study 157)

mean body weight = 78.8 $\pm$ 10.0 kg (study 158)

* normalized to the mean body weight = 70.6 $\pm$ 11.5 kg (study 157 + study 158)

NDA 20-599: RILUTEK®
Riluzole Tablets

APPENDIX VII
BIOEQUIVALENCY

STUDY SUMMARY**NNY 152**

Title of the study :	RP 54274- Open randomised, crossover study of the comparative bioavailability of 50 mg of riluzole administered in 2 tablet formulations to healthy male volunteers
Publication :	None
Study period:	Start date : (first subject randomized) 19 January 93 Completion date : (last subject completed) 4 February 93
Clinical phase :	I
Objective :	To determine and compare the pharmacokinetic profiles of two (2), 50 mg tablet formulations of riluzole
Methodology	Open randomised two-way cross-over trial, single dose
Subjects	24 male, caucasian, volunteers, aged from 20 to 32 years (mean $\pm$ SEM): 25.71 ± 0.56 years), weight ranged from 60.8 to 91.8 kg (75.7 ± 1.74 kg) and height ranged from 167 to 191 cm (179.46 ± 1.36 cm).
Test product, dose and mode of administration, batch n°:	2 formulations of 50 mg riluzole tablets manufactured through: - wet granulation with polyvinylpyrrolidone in aqueous alcoholic solution (final weight: 200 mg) (batch CB 4567) - direct compression with anhydrous dihydrogenophosphate and microcrystalline cellulose (final weight: 400 mg) (batch CB 05571)
Duration of treatments :	1 day, (single dose) Administration of the two formulations one week apart.
Criteria for evaluation :	Pharmacokinetic drug evaluation include: blood sampling at 0, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 12, 16, 20, 24, 28, 32 and 36 hours post-dose and calculation of pharmacokinetic parameters. Safety drug evaluations include: side effects, physical examinations, vital signs, electrocardiogram (ECG) and laboratory assessments.

Statistical methods :		Comparison of pharmacokinetic parameters by the SAS GLM procedure. Assessment of the bioequivalence with 2 approaches: -calculation of Westlake's symmetrical 90% confidence intervals -calculation of the Schuirmann two one-sided tests procedure.							
Results									
Pharmacokinetics									
Treatment		C_{max} (ng/ml)	$\ln C_{max}$ (ng/ml)	t_{max} (h)	AUC(0-lqc) (h.ng/ml)	$\ln AUC(0-lqc)$ (h.ng/ml)	AUC (h.ng/ml)	t1/2 (h)	MRT (h)
A	mean	199	5.15	(0.5-3)*	629	6.32	746	8.9	9.0
	SD	106	0.58		291	0.53	270	2.8	2.2
	CV%	53	11		46	8	36	32	25
	median			0.75					
B	mean	144	4.83	(0.5-3)*	562	6.22	658	8.8	9.7
	SD	71	0.57		249	0.50	254	1.8	2.4
	CV%	50	12		44	8	39	21	24
	median			1					
* range									
Statistical analysis									
Parameter	effect (GLM)	Pr>F	C.I. Westlake	Schuirmann*	Schuirmann 90% C.I.				
C_{max}	treatment	0.0063	60%-140%	-24 - -87	-				
	period	0.7446	(p=90%)	(01:-40, 02:+40)					
	subject(sequence)	0.0047							
	sequence	0.7515							
AUC(0-lqc)	treatment	0.0049	85%-115%	-103 - -30	-				
	period	0.0749	(p=90%)	(01:-126, 02:+126)					
	subject(sequence)	0.0001							
	sequence	0.8279							
$\ln C_{max}$	treatment	0.0025	64%-156%	-0.16 - -0.48	62% -85%				
	period	0.5953	(p=90%)	(01:-0.223, 02:+0.223)					
	subject(sequence)	0.0001							
	sequence	0.7881							
$\ln AUC(0-lqc)$	treatment	0.0078	86%-116%	-0.02 - -0.16	85% - 96%				
	period	0.0742	(p=90%)	(01:-0.223, 02:+0.223)					
	subject(sequence)	0.0001							
	sequence	0.9313			90% -101%				
lqc= last quantified concentration; C.I.: confidence interval * the two forms are bioequivalent if confidence interval for $\mu_1 - \mu_2$ is completely contained in the equivalence interval (01, 02)									

FIGURE 1 RP 54274-152 - Mean plasma concentration-time curves of RP 54274 in healthy subjects (n=24) after a single oral dose of 80 mg RP 54274

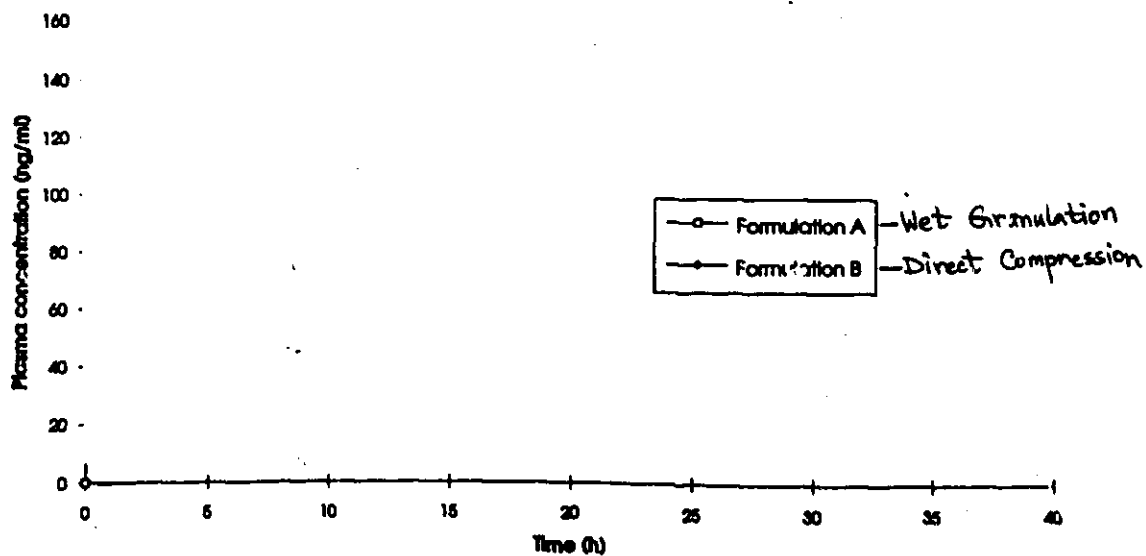


Figure 1: RP 54274 AG 152 - Scattergram of Cmax values by treatment, period 1 and 2

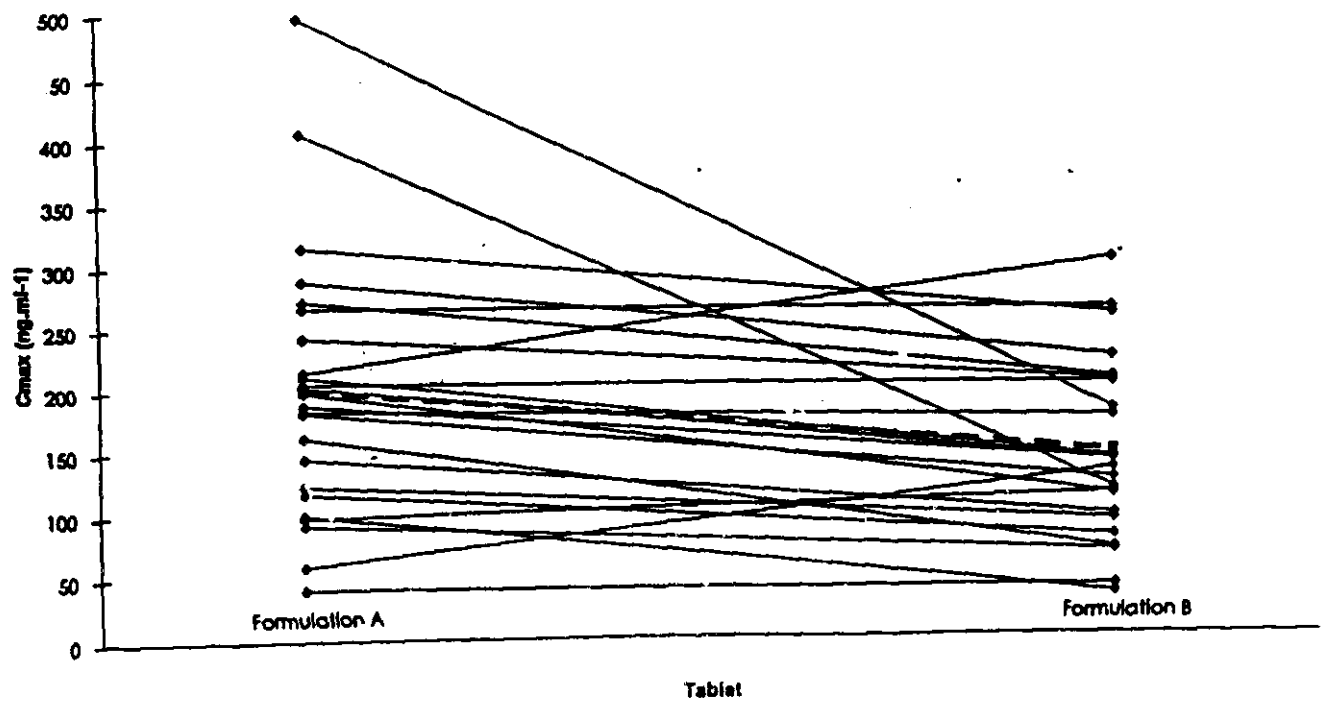
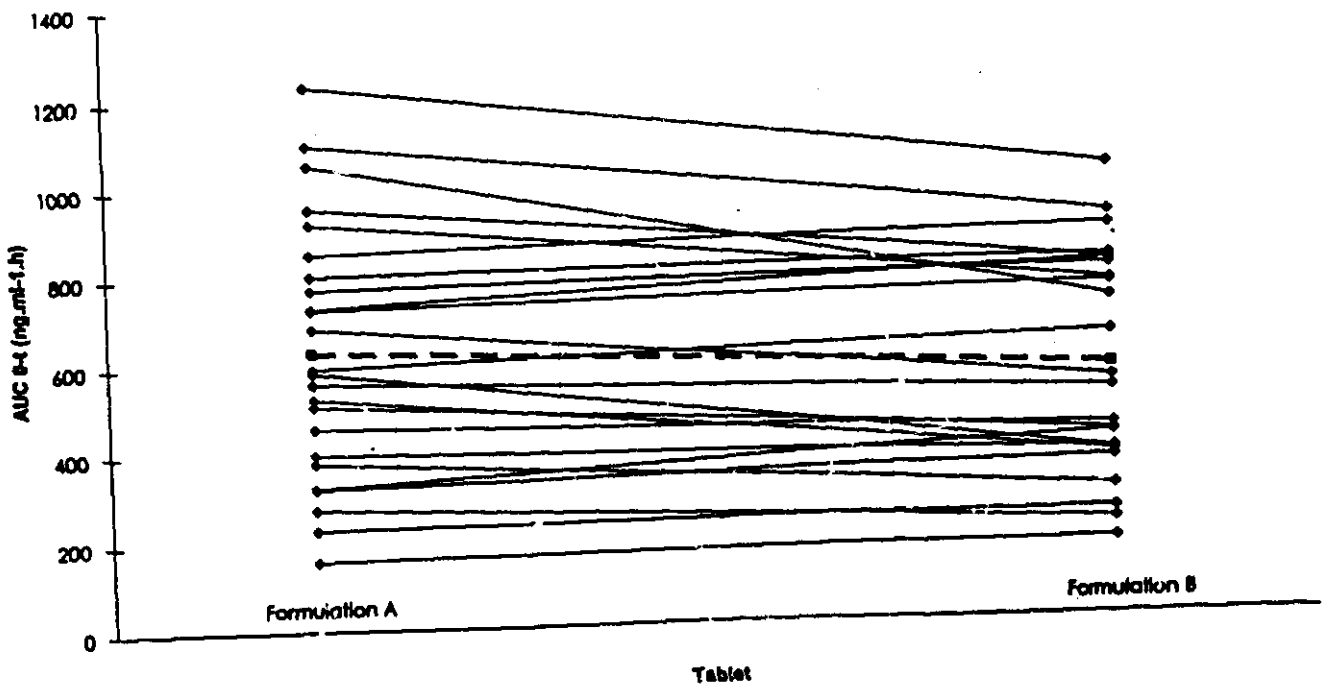


Figure 2: RP 54274 AG 152 - Scattergram of AUC(0-t) values by treatment, period 1 and 2



STUDY SUMMARYNNX 160

Title of the study	Open, randomised, cross-over study of the relative bioavailability of single dose riluzole 100 mg comparing 2 tablet formulations administered by oral route in healthy male volunteers.
Investigator	
Study centre	
Publication	None
Study period	Start date : 09 May 1994 (first subject enrolled) Completion date : 27 May 1994 (last subject completed)
Clinical Phase	1a
Objectives	- To determine and compare the pharmacokinetic profiles of two formulations (B and C) of riluzole.
Methodology	Open, randomized, 2-way crossover trial comprising 2 treatment periods separated by a wash-out period of at least 5 days. Assignment of subjects to a particular sequence "B/C" or "C/B" was performed according to a computer-generated randomisation list.
Number of subjects (total and for each treatment)	24 subjects were enrolled and completed the study according to protocol. 12 received the sequence "B/C", 12 the sequence "C/B".
Diagnosis and criteria for inclusion	Male, caucasian, healthy volunteers, aged 19 to 31 years, were recruited, with body weights within $\pm 10\%$ of the ideal weight for frame and height, and no relevant clinical, haematological or biochemical abnormalities.
Test product, dose and mode of administration, batch n°	RP 54274, single 100 mg oral dose at each of the 2 periods : - Formulation B : 2x50 mg tablets batch n° CB05702 - Formulation C : 2x50 mg tablets batch n° CB05921
Duration of treatment	1 day (single dose) for each period.

STUDY SUMMARY (Continued)

Criteria for evaluation	Safety : Physical examination, repeated vital signs, ECG, routine laboratory measurements and adverse events recording. Pharmacokinetics : Blood sampling from time 0 to time 36 h post-dose, calculation of pharmacokinetic and bioavailability parameters.
Statistical methods	Clinics : Descriptive statistics with tables. Pharmacokinetics : Comparison of pharmacokinetic parameters by the SAS GLM procedure. Assessment of the bioequivalence with 2 approaches: - calculation of Westlake's symmetrical 90% confidence intervals - calculation of the Schuimann two one-sided tests procedure

SUMMARY RESULTS**Safety :**

No specific adverse event profile was identified. Adverse events were minor and infrequent.

Pharmacokinetics :**Pharmacokinetic parameters:**

Treatment		C _{max} (ng/ml)	lnC _{max} (ng/ml)	t _{max} (h)	AUC(0-t _{pc}) (ng/ml.h)	lnAUC(0-t _{pc}) (ng/ml.h)	AUC (ng/ml.h)	t _{1/2} (h)	MRT (h)
B	mean	257	5.38	(0.5-4)*	1005	6.75	1081	7.1	7.3
	SD	94	0.43		638	0.56	644	1.3	2.0
	CV%	40	8		64	8	60	25	27
	median			0.75					
C	mean	300	5.59	(0.5-2)*	1087	6.84	1168	7.4	7.1
	SD	149	0.49		659	0.56	672	1.4	1.9
	CV%	50	9		61	8	58	19	26
	median			0.75					

* range, n=24

SUMMARY RESULTS (Continued)

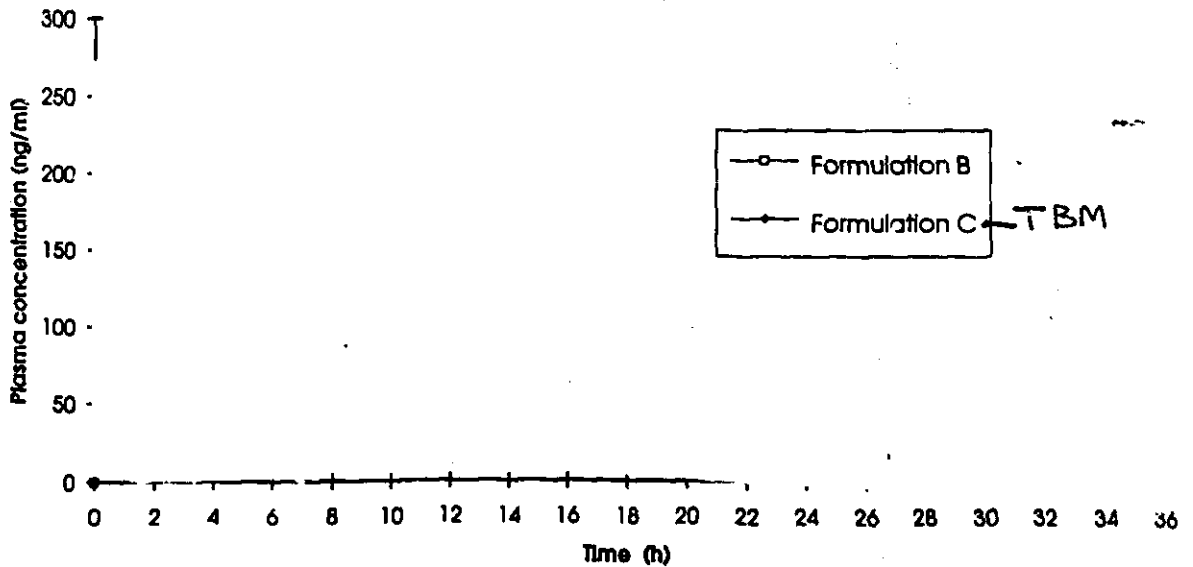
Statistical analysis					
Parameter	effect (G.M)	F>F	C.I. Westlake	Schulmann*	Schulmann 90% C.I.
lnC _{max}	treatment	0.0278	72%-139%	0.057 - 0.366 (θ1:-0.223, θ2:+0.223)	106%-144%
	period	0.0689	(p=90%)		
	subject(sequence)	0.0030			
	sequence	0.6821			
lnAUC(0-lqc)	treatment	0.0284	88%-114%	0.023 - 0.147 (θ1:-0.223, θ2:+0.223)	102%-116%
	period	0.0045	(p=90%)		
	subject(sequence)	0.0001			
	sequence	0.6745			

lqc: last quantified concentration; C.I.: confidence interval
 *the two formulations are bioequivalent if confidence interval *or $\mu_1 - \mu_2$ is completely contained in the equivalence interval (θ1, θ2)

CONCLUSION

The 2 formulations tested can be considered bioequivalent in terms of extent of absorption (AUC) but not in terms of C_{max}.

Figure 1 : Mean plasma concentration-time curves of RP54274 in healthy subjects (n=24) after a single oral dose of 100mg RP54274



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 pp 427/43-180

Figure 1

Scattergram of Cmax values by treatment, period 1 and 2

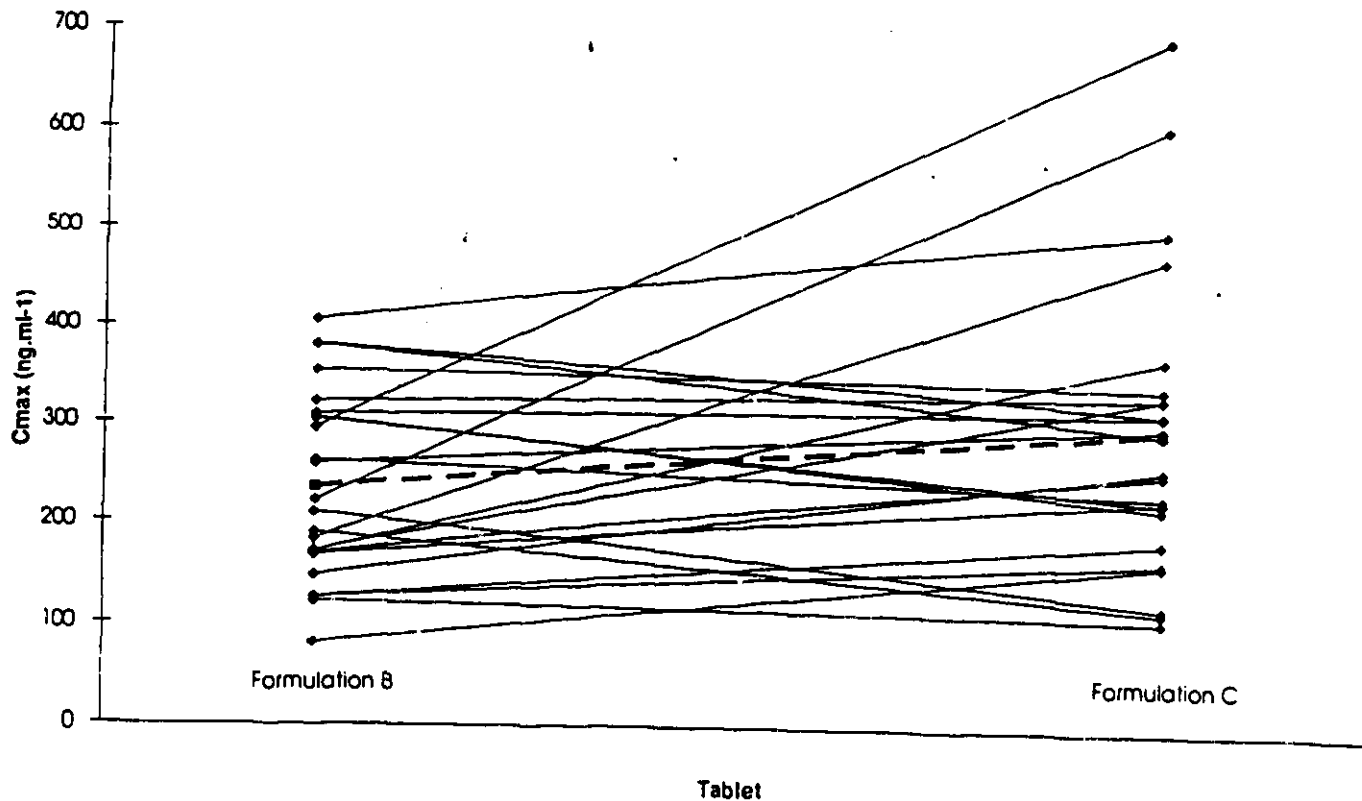
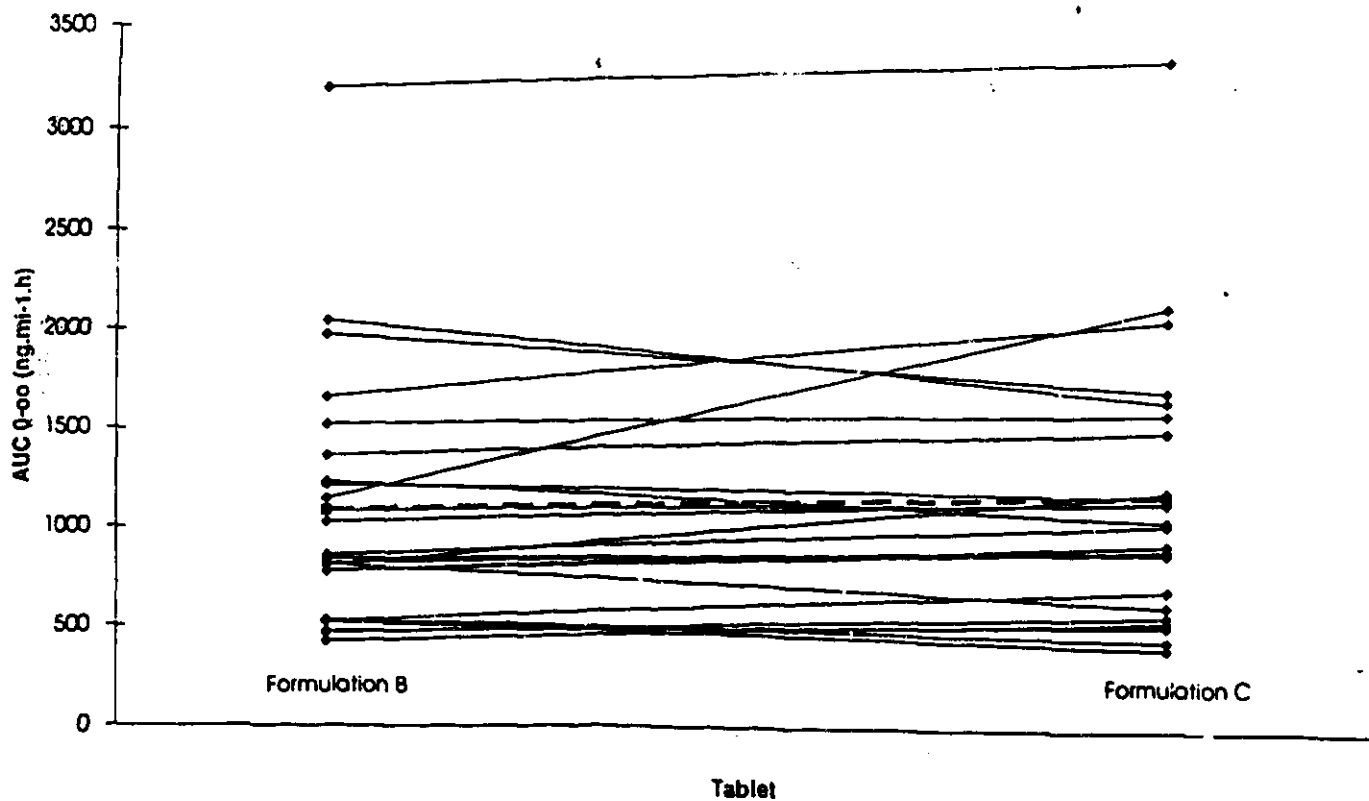


Figure 2

- Scattergram of AUC(0-∞) values by treatment, period 1 and 2



RILUTEK™ - 50 mg tablets

BACKGROUND

	"A"	"B"	"C"
Strengths	AG 216	AG 301	To-be-marketed formulation
Final mass	25 mg/ 60 mg tablets round/ rod shaped ab. 100 mg/ab.200 mg	25 mg- 50 mg- 100 mg capsule- shaped tablets ab. 400 mg	50 mg capsule shaped tablets ab. 200 mg
Formula per unit	- Riluzole 50 mg	- Riluzole 50 mg	
			ng
			9
			9

Coating

Process

Scale	RPR - Institut of Biopharmacy	RPR - Institut of Biopharmacy	RPR - Institut of Biopharmacy
S3e	152	152	160
Bioequivalence study:	CB4567	CB 05571	CB 05921
Batch #	15 min : % 30 min : %	15 min : % 30 min : %	15 min : 77 % 30 min : 88 %
Dissolution (50 rpm)	MUJ 441	1 PROC 92185	10 PROC 93049
Drug substance			
batch #			
Particle size			
Max			
D90			
D50			

..... in Japanese clinical trial / phase III

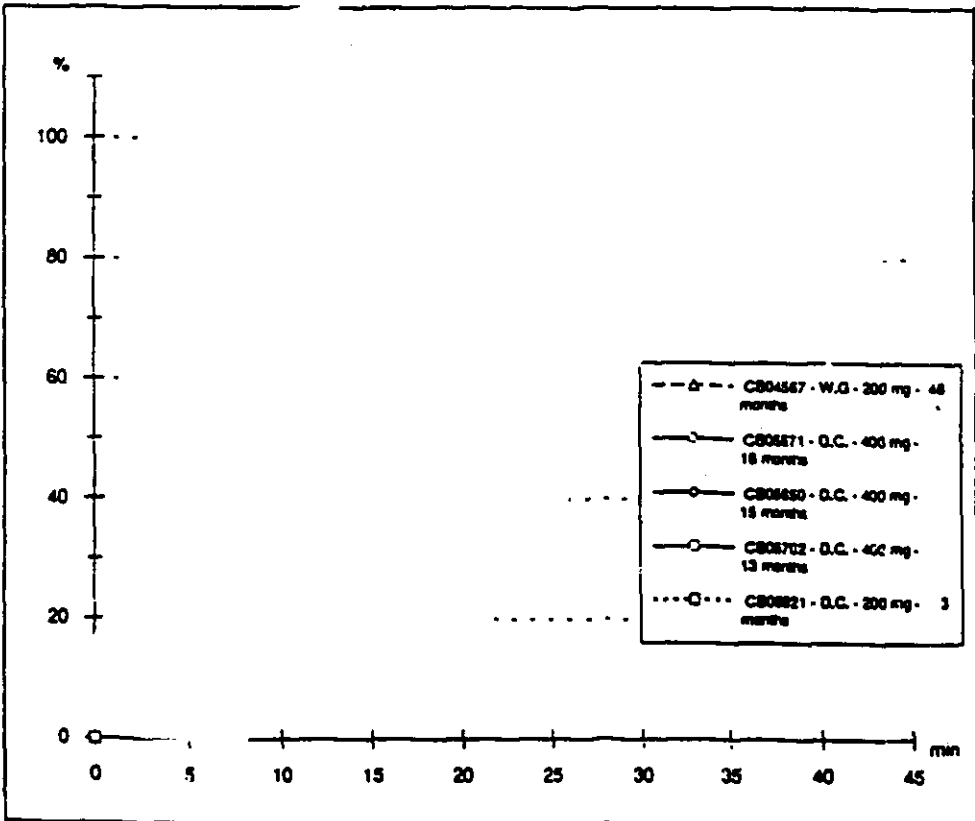
**Riluzole - RP 54724 - Coated Tablets 50 mg
COMPARATIVE DISSOLUTION PROFILES**

Batch CB04567
 Batches: CB05571, CB05650
 Batch CB05921

) A
 B
 C

Test Conditions

- Medium
- Volume (mL)
- Temperature (°C)
- Blade Rotation Speed (rpm)
- Assay



NDA 20-599: RILUTEK®
Riluzole Tablets

APPENDIX VIII

***IN VITRO* DISSOLUTION METHODOLOGY**

LIST OF FORMULATIONS AND BATCH SIZE

MANUFACTURING SITE CHANGE/SCALE UP (≤10X)

Calculation

The quantity of riluzole expressed in mg, contained in a tablet, is given by the equation:

$$\frac{M_R \cdot A_T}{A_R}$$

where:

M_R mass of riluzole reference substance in the reference solution (mg).

A_R absorbance of the reference solution.

A_T absorbance of the test solution.

Specifications

The requirements for content uniformity are met if the riluzole amount in each tablets lies within the range of _____ of the theoretical content and the relative standard deviation of the 10 individual results is less than or equal to _____ (USP specific standard). If not more than one individual content is outside the limits of _____ and none is outside the limits of _____ determine the individual contents of another 20 dosage taken at random. The requirements are met if not more than one individual contents of the thirty units is outside _____ of the average content, none is outside the limits of _____ of the average content and the relative standard deviation of the 30 dosage units does not exceed _____

3. Dissolution (Method Ref .)

The dissolution test is conducted on 6 tablets as per the procedure described in the USP23 <711> using an apparatus with a paddle stirring element.

The percentage of dissolution of riluzole is determined by

√ Conditions of the dissolution test

- Medium composition:
- Volume in each container:
- Paddle rotation speed:
- Sampling times:
- Sample volumes:

Spectrophotometric determinationExpression of results

The percentage of riluzole dissolved is given by the equation:

$$\frac{A_T \times 18 \times 10^5}{A_{1\text{cm}}^{1\%} \times T}$$

where:

$A_{1\text{cm}}^{1\%}$ = Specific absorbance of riluzole in

T = Theoretical unit content (mg of riluzole)

A_T = Absorbance of the test solution.

✓ Specifications

For each tablet, not less than (Q=) of the labeled amount of riluzole are dissolved in 30 minutes.

If necessary continue the test according to the USP < > monograph for stage until the requirements are met.

2.2. Secondary packaging

Cardboard box.

3. CLINICAL TRIAL FORMULAE

Names of ingredients	Unit formula	
	25mg (mg)	50mg (mg)
Core❖		
- Riluzole/RP54724	25.0	50.0

17 Pages

Purged

NDA 20-599: RILUTEK®
Riluzole Tablets

APPENDIX IX

***IN VITRO* PROTEIN BINDING AND INTERACTIONS**

NDR20599

5 OF 7

UFR/PH93/002**RILUZOLE *IN VITRO* DISTRIBUTION IN BLOOD AND PLASMA PROTEIN BINDING**

In whole blood, 35% of riluzole was bound to red blood cells (RBCs) and 65% to plasma proteins. In human serum, 96% of riluzole (Table 1) was bound to proteins (mainly to albumin and serum lipoproteins, Table 2). The percentage of serum protein binding remained unchanged over the concentration range of 125 to 5000 ng/ml.

Table 1 - Riluzole serum binding

C ng/ml	125	250	500	1000	2000	3000	4000	5000
% bound	96.3	96.7	96.7	97.0	96.6	96.5	96.6	96.6
SD	0.6	0.2	0.6	0.2	0.4	1.1	0.2	0.4

5 replicates per measurement, SD (standard deviation)

ANOVA, $p = 0.55$, no significant variations among binding percentages

Table 2 - Parameters of riluzole binding to isolated serum proteins (n and k, number of sites and binding constant, expressed in mM^{-1})

Protein	Binding Sites n	Binding Constants k (mM^{-1})
HSA	7.3 ± 0.8	3.82 ± 0.49
AAG	1.08 ± 0.14	10.5 ± 2.2
HDL	147 ± 49	2.8 ± 0.9
LDL	546 ± 155	3.7 ± 1.1
VLDL	2167 ± 819	1.6 ± 0.6

Estimation $\pm$ SD

HSA, human serum albumin (M_r 66,000); AAG, alpha-1 acid glycoprotein (M_r 40,000);

HDL, high-density lipoproteins

(M_r 300,000, 50% protein); LDL, low-density lipoproteins (M_r 3×10^6 , 20% protein);

VLDL, very-low-density lipoproteins

(M_r 20×10^6 , 5% protein).

There is only one binding site on alpha-1 acid glycoprotein, while there are seven on human serum albumin. On the other hand, lipoproteins have many binding sites (from about 150 to more than 2,000 as the density of the lipoproteins decreases). The differences in the binding constants are less marked, except for alpha-1 acid glycoprotein whose binding constant is about 10 mM^{-1} .

DRUG INTERACTIONS

1.1 *IN VITRO* INTERACTIONS RELATED TO PLASMA PROTEIN BINDING

Studies of drug interactions have been carried out *in vitro* on the displacement of protein-ligand binding. The effect of warfarin, digoxin, imipramine, and quinine on riluzole binding and conversely, the action of riluzole on warfarin binding, have been studied. The results, given in Tables 1 and 2, show no competition between riluzole and these compounds.

Table 1 - Effect of drugs on riluzole (1 µg/mL) serum binding

Test	Riluzole serum binding (%) mean ± SD
Control ¹	96.5 ± 0.2
+ Warfarin (5 µg/mL)	96.8 ± 0.4
+ Digoxin (3 ng/mL)	96.9 ± 0.5
Control ²	96.5 ± 0.5
+ Imipramine (100 ng/mL)	96.9 ± 0.6
+ Quinine (10 µg/mL)	97.1 ± 0.2

¹ 5 replicates per measurement, SD: standard deviation

¹ANOVA p = 0.27, ²ANOVA p = 0.25, no significant differences between riluzole serum binding percentages (versus control).

Table 2 - Effect of riluzole on warfarin (5 µg/mL) serum binding

Test	Warfarin serum binding (%) mean ± SD
Control	96.66 ± 0.74
+ riluzole (1 µg/mL)	97.08 ± 0.84
+ riluzole (3 µg/mL)	97.44 ± 0.51
+ riluzole (5 µg/mL)	97.42 ± 0.71

5 replicates per measurement, SD standard deviation

ANOVA p = 0.30, no significant difference between warfarin serum binding percentages (versus control)

Riluzole serum binding was not affected by warfarin, digoxin, imipramine or quinine at high therapeutic concentrations. In blood and serum, riluzole was bound mainly to human serum albumin and RBCs. In blood, a small fraction of riluzole was combined with RBCs, and most of the drug (more than 65%) was located in the plasma. The probability of riluzole interacting with drugs by competition via serum protein-binding is practically nil due to the high number of binding sites in proteins (lipoproteins and albumin).

NDA 20-599: RILUTEK®
Riluzole Tablets

APPENDIX X
LIST OF SUBMITTED STUDIES
SUMMARY TABLES

TABLE A
RILUZOLE - BIOPHARMACEUTICS STUDY SUMMARY

Study	Route of administration, Study Design	Dosage/Form (mg)	Dose (mg)	Batch	Number of subjects Enrolled	Conclusions	Document Number (Volume, Page)
101	injectable (single dose parallel groups)	10 mg/2 ml 2 ml P	20	CB05700 CB05701P CB05757 CB05758P	3 groups of 8 (2 P* per group)	t _{1/2} , MRT, Vz and Vss do not differ significantly between doses, whereas clearance is found lower at 20 mg compared to the 100 mg dose. There is a linear relation between AUC and dose, as well as between C _{max} and dose, over the tested dose range (20 to 100 mg)	6.13 (1.47, 6-11-1)
161	p.o. injectable (single dose cross over)	50 (DT tabl.) 50mg/10 ml	100 (2 tabl.) 10 ml	CB05921 CB05757	15	After i.v. administration, plasma clearance is about 60% of hepatic blood, a high volume of distribution is observed. Urinary excretion of the unchanged drug is negligible. The absolute bioavailability after oral administration of the to-be marketed is about 60%.	6.4 (1.39, 6-3-1)
152	p.o. (single dose cross over)	50 (WG tabl.) 50 (DT tabl.)	50 50	CB04567 CB05571	3 groups of 12 (3P* per group)	The two formulations can be considered bioequivalent in terms of extent of absorption (AUC) but not in terms of C _{max}	6.5 (1.41, 6-5-2)
156	p.o. (single dose cross over)	50 (DT tabl.) 100 (DT tabl.) P	50 100	CB05571 CB05572 CB05631P	8	The terminal half-life is about 6 hours for 50 mg, 100mg and 200 mg dose. t _{max} is not significantly different between the three doses, the C _{max} and the AUC are proportional to the administered dose. Urine recovery is very weak (0.5% to 0.8% of the dose) in comparison to that of conjugated riluzole (21.4% to 23.4% of the dose).	6.14 (1.49, 6-13-1)
157	p.o. (steady state parallel groups)	25 (DT tabl.) 50 (DT tabl.) 100 (DT tabl.) P	25 b.i.d. 50 b.i.d. 100 b.i.d.	CB05635 CB05571 CB05572 CB05631P	3 groups of 12 (3P* per group)	The elimination half-life of riluzole after single dose administration of 25 mg, 50 mg or 100 mg is about 5.5 h. The terminal half-life of riluzole tends to increase at steady state. The steady state is reached from day 5 onwards for the three dosage regimens. The C _{max} and AUC are significantly increased at steady state after the 100 mg dosage regimen. The bioavailability of riluzole at steady state is proportional to the dose. Urine recovery of free riluzole is very weak (about 1%) in comparison to that of conjugated riluzole which represents 16% to 20% of the dose.	6-15 (1.50, 6-14-1)

DT tabl.: Direct tableting or direct compression tablet
WG tabl.: Wet granulation tablet
P = placebo

TABLE A (continued)

RILUZOLE - BIOPHARMACEUTICS STUDY SUMMARY

Study	Route of administration Study Design	Dose/Form (mg)	Dose (mg)	Batch	Number of subjects Enrolled	Conclusions	Document Number (Volume, Page)
158	p.o. (steady state parallel groups)	25 (DT tabl.) 50 (DT tabl.) 100 (DT tabl.) P	25 b.i.d. 50 b.i.d. 100 b.i.d.	CB05635 CB05650 CB05572 CB05630P	3 groups of 12 (3P* per group)	The elimination half-life of riluzole after single dose administration of 25 mg, 50mg or 100 mg is about 6 h. The terminal half-life of riluzole tends to increase at steady state. The steady state is reached from day 5 onwards for the three dosage regimens. The C_{max} and AUC are significantly increased at steady state after the 100 mg dosage regimen. The bioavailability of riluzole at steady state is proportional to the dose.	6.11 (1.45, 6-9-124)
159	p.o. (single dose cross over, food inter action)	50 (DT tabl.)	50	CB05921	16	A high fat meal decreases the absorption rate of riluzole, resulting in a delay for T_{max} and a decrease for C_{max} : 44% on mean value as compared to fasting conditions. The extent of absorption is decreased by 17%.	6.12 (1.46, 6-10-1)
160	p.o. (single dose cross over)	50 (DT tabl.) 50 (DT tabl.)	2x 50 2x 50	CB05921 CB05702	24	The two formulations can be considered bioequivalent in terms of extent of absorption (AUC) but not in terms of C_{max} .	6.6 (1.42, 6-6-1)
IBP 1842/ AG 010	p.o. (steady state parallel groups)	25 (WG tabl.) P	25 o.d., 25 tid, 50 tid	CB3567 CB3569P	3 groups of 12 1 placebo group of 6	Steady state is achieved after the first dose of repeated administration for the 25 mg dose once daily administration. For the 25 mg tid and 50 mg tid dosage steady state is achieved on day 3 of treatment. $T_{1/2}$ does not change for the 25 mg o.d. but increases for the 25 mg tid and 50 mg tid, respectively from 6.1 h to 14.8 h and from 8.4 h to 19.4 h.	6.7 (1.43, 6-7-187)
IBP 1685/ AG 011	p.o. (single dose parallel groups)	25 (WG tabl.) 50 (WG tabl.) P	25, 50, 75, 100, 150, 200, 250, 300	CB3793 CB3432 CB2580P	12	The plasma levels increase with increasing dose. The maximal plasma concentration or area AUC were correlated with administered dose. A linear relationship between kinetic parameters (AUC and C_{max} are established in each group. The mean maximal concentration is reached after 0.9 to 1.5 hours and the apparent elimination half-life ranges between 9.2 and 14 hours.	6.8 (1.44, 6-8-1)

TABLE B
RILUZOLE - IN VIVO STUDY SUMMARY DATA
RILUZOLE DATA (MEAN S.D.) - single dose

Study	Route of administration dosage form	Dose (mg)	C _{max} (ng.ml ⁻¹)	T _{max} (h)	AUC or t (h.ng.ml ⁻¹)	t _{1/2} (h)	MRT (h)	Document Number (Volume, Page)
101	injectable (30min. infusion)	20	271 76	0.50	963 17	8.0 0.5	9.6 0.6	6.13
		50	799 163	0.50	2199 370	6.9 0.3	8.4 0.6	(1.47, 6-11-1)
		100	1453 232	0.50	3507 660	7.5 1.4	7.8 1.2	
161	injectable (30 min infusion) p.o. - D.T. tablets	50	584 137	0.50	1083 201	5.9 2.1	5.3 1.5	6.4
		100 ¹	318 199	1.00	1326 572	7.9 2.6	8.0 2.0	(1.39, 6-3-1)
152	p.o. - tablets bioequivalence (D.T. vs W.G.)	50 ^a	199 106	0.75	746 270	8.9 2.8	9.0 2.2	6.5
		50 ^b	144 71	1.00	658 254	8.8 1.8	9.7 2.4	(1.41, 6-5-2)
156	p.o. - D.T. tablets	50	150 63	1.00	613 263	5.6 2.2	5.7 2.0	6.14
		100	285 67	1.00	1330 409	5.8 0.9	5.7 0.9	(1.49, 6-13-1)
		200	663 145	1.00	3012 656	6.0 1.2	5.7 1.1	
159	p.o. - D.T. tablets (food interaction)	100 ^c	216 136	2	1047 482	6.6 1.5	8.1 1.5	6.12
		100 ^d	387 190	0.75	1269 631	7.1 1.7	6.7 1.7	(1.46, 6-10-1)
160	p.o. - D.T. tablets bioequivalence (400vs200)	2x 50 ^e	237 94	0.75	1081 644	7.1 1.8	7.3 1.9	6.6
		2x 50 ^f	300 149	0.75	1168 672	7.4 1.4	7.1 1.9	(1.42, 6-6-1)
IBP 1685/ AG 011	p.o. - W.G. tablets - 1st group	25	91 42	1.00	567 183	9.7 1.9	13.1 3.2	6.8
		75	247 138	0.90	1340 564	14.0 6.3	12.4 3.4	(1.44, 6-8-1)
		150	557 255	1.10	2525 1034	10.6 2.0	10.7 3.0	
p.o. - W.G. tablets - 2nd group	250	889 325	1.30	3885 1498	11.8 3.0	8.9 1.8		
	50	214 92	1.40	1337 426	10.6 2.0	17.3 7.8		
	100	428 241	1.40	2539 1619	10.4 3.3	13.6 2.7		
200	p.o. - W.G. tablets - 2nd group	200	1117 755	1.50	5236 2538	9.4 2.0	11.3 2.8	
		300	1611 665	1.30	7546 3360	9.2 2.9	9.8 3.4	

¹ : 1st oral administration, a = wet granulation (W.G.) tablet, b = direct tableting or direct compression (D.T.) tablet, c = fed, d = fasted, e = D.T. tablet, final weight : 400 mg, f = D.T. tablet, final weight = 200 mg

TABLE B (continued)
RILUZOLE - IN VIVO STUDY SUMMARY DATA
RILUZOLE DATA (MEAN S.D.) - repeated dose

Study	Route of administration Dosage form	Dose (mg)	C _{max} ^a (ng.ml ⁻¹)	T _{max} ^b (median) (h)	AUC(0-2)	T _{1/2} ^c (h)	C _{min} ^d ratio	C _{max} ^d ratio	AUC ^d ratio	Document Number (Volume, Page)
157	p.o. (b.i.d) D.T. Tablets	25	145.7 44.3	0.50	551.0 215.2	5.15 1.53	2.3 0.6	1.5 0.3	1.5 0.3	6-15 (1.50, 6-14-1)
		50	331.5 81.3	0.50	1397.3 394.9	5.57 1.76	2.5 0.8	1.8 0.9	1.7 0.6	
		100	410.8 157.6	1.00	1723.9 594.7	5.89 1.22	2.2 0.8	1.6 0.3	1.4 0.4	
158	p.o. (b.i.d) D.T. Tablets	25	77.4 30.0	1.00	295.5 118.1	5.16 1.16	1.7 0.6	1.6 0.5	1.5 0.3	6.11 (1.45, 6-9-12A)
		50	172.6 72.4	0.50	653.5 279.2	6.43 1.91	2.1 0.6	1.2 0.7	1.4 0.5	
		100	356.9 166.5	1.50	1483.2 741.7	5.78 1.46	2.1 0.6	1.4 0.5	1.3 0.4	

AUC(0-12) day 13/AUC day 1

a: D.T. = direct tableting or direct compression
 b: last day of dosing (Day 13)
 c: first elimination half-life
 d: C_{min} ratio = C_{min}day13/C_{min}day1, C_{max} ratio = C_{max}day13/C_{max}day1, AUC ratio = AUC(0-12) day13/AUC day1

Additional Submitted Study Reports:-

AG114 } in vivo } Metabolism
 IBP/1837 }
 IBP/1887 } in vitro } PK
 IBP/1154 }
 IBP/DD95-067 }
 AG015 } Analytical
 IBP/1817 }
 IBP/1844 } Protein Binding
 IBP/1845 }
 UFR/PH93/002
 DISSOLUTION

Pharmacology Review of NDA# 20-599

Drug: Riluzole/RP 54274
Category: Excitatory AA Antagonist and Na-channel Blocker
Indication: ALS
Sponsor: Rhone-Poulenc Rorer
Rev date: July 14 1995
Reviewer: Aisar H. Atrakchi, Ph.D. *AKA*
Supervisor: Glenna Fitzgerald, Ph.D.

ggf 10/12/95

CC.

/Div. File/NDA# 20-599
/G. Fitzgerald/A. Atrakchi/K. Higgins

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EXECUTIVE SUMMARY

Riluzole, a trifluoromethoxy benzothiazole, is indicated for ALS not as a cure, but to extend survival. The exact mechanism of neuroprotection of riluzole is unclear. A number of in vitro and in vivo animal models and radioreceptor binding assays have shown that riluzole acts as a non-competative EAA antagonist and a Na-channel blocker. Neuroprotection was investigated in various animal models both in vitro and in vivo. Riluzole in the in vitro models provided adequate neuroprotection against a given insult, some of these models included: hippocampal slice, primary neuronal cultures, and CSF from ALS patients. Riluzole did not show any affinity (100uM), towards all types of receptors used in the radioreceptor binding assays including NMDA, non-NMDA receptors, and metabotropic receptor sites. However, functional studies showed that riluzole inhibited responses to EAA. In electrophysiological studies, riluzole inhibited NMDA responses in *Xenopus* oocytes with an IC_{50} of 24uM; higher concentration was required to inhibit AMPA receptors (IC_{50} 174uM). Other functional experiments included brain slices or cultured neurons where responses such as calcium homeostasis, neurotransmitter release, and cGMP formation, were measured. Riluzole in these systems had IC_{50} s around 10uM. Table below from the sponsor shows IC_{50} values for riluzole and the parameter measured for some of the in vitro cell cultures:

Table 1.1.10. Inhibition by riluzole of the stimulatory effects of excitatory amino acids in a number of in vitro models

Model system	Parameter measured	Agonist (Concentration)	IC_{50} Riluzole	IC_{50} Reference compound
<i>Xenopus</i> oocytes	NMDA-evoked currents	NMDA (100 μ M)	24 μ M	5.6 μ M (2-APV)
<i>Xenopus</i> oocytes	Kainic acid-evoked currents	Kainic acid (50 μ M)	174 μ M	0.28 μ M (CNQX)
Rat cerebellar slices	cGMP formation	Glutamic acid (1 mM)	25 μ M	22 nM (MK 801)
Rat striatal slices	[³ H]-Acetylcholine release	NMDA (100 μ M)	14 μ M	ND
Rat olfactory tubercle slices	[³ H]-Acetylcholine release	NMDA (100 μ M)	29 μ M	ND
Rat striatal slices	[³ H]-Dopamine release	Glutamic acid (100 μ M)	3 μ M	ND
Rat cerebellar granule cells	D-[³ H]-Aspartate release	Glutamic acid (20 μ M)	10 μ M	ND
Rat cerebellar granule cells	D-[³ H]-Aspartate release	Ibotenic acid (100 μ M)	10 μ M	100 μ M (LSOP)
Rat cerebellar granule cells	Cytosolic calcium mobilization	Glutamic acid (20 μ M)	0.3 μ M	ND
Rat cerebellar granule cells	Cytosolic calcium mobilization	NMDA (100 μ M)	12.8 μ M	ND *

2-APV = 2-amino-5-phosphonopentanoic acid, CNQX = 6-cyano-7-nitroquinoxaline-2,3-dione, LSOP = L-serine-O-phosphate.

* ND = not determined

Riluzole as a Na-channel blocker was studied indirectly in cerebellar granule cells (blockade of Ca entry induced by veratridine), or directly by electrophysiology. Riluzole showed good affinity (0.2uM) to the alpha subunit of the rat Na-channels expressed in *Xenopus* oocytes. Similarly, riluzole blocked responses evoked by Na-channel activation such as veratridine-induced DA release in rat striatal slices with an IC_{50} of 3.5uM, calcium mobilization induced by veratridine in rat cerebellar granule cell with an IC_{50} of 0.31uM, and aconitine-inhibition of active transport in rat striatal synaptosomes at an IC_{50} of 17uM.

Riluzole inhibited DA, Ach, GABA, and aspartate release with IC_{50} s ranging between 3-3.5 μ M, 14-29 μ M, 5-17 μ M, and 10 μ M respectively, in brain slices or neurons (cortical and cerebellar granule cells). Riluzole in other experiments, was shown to inhibit the release of aspartic and glutamic acids, a finding that is not mediated by Na-channels. The latter was demonstrated in rat cerebellar granule cell cultures where riluzole-induced inhibition of D-aspartate was reversed by pertussis toxin. Riluzole at 0.5-2mg/kg i.v. inhibited glutamic acid-induced release of DA in the rat caudate nucleus. There was about 50% decrease in spontaneous release of glutamate in the cat striatum after local perfusion of 10 μ M riluzole; no effect on aspartic acid release.

Effects of riluzole on neurodegeneration in vivo, were investigated in the genetic Mnd mouse model and on the sensitivity of motoneurons to EAA. The former represents a genetic strain of mice that progressively develop motoneuron degeneration of unknown etiology and the animals die by 11 months of life. Riluzole orally dosed at 8mg/kg 5d/wk for 15wks significantly improved mobility in these mice and improved neuronal cell count in the lumbar spinal cord but not other spinal areas or the hypoglossal nucleus. Riluzole at 0.6 or 1mg/kg i.p. or 0.6 and 1.5mg/kg i.v., completely abolished facial motoneuron excitation induced by NMDA, kainate, or quisqualate. The neuroprotective effects of riluzole were also assessed in models of cerebral ischemia (stroke models). In these models, the large increase in glutamic acid release and accumulation combined with all the sequelae of events that follow, seem to play a major role in neurodegeneration. Centrally acting drugs known to block glutaminergic neurotransmission provide adequate neuroprotection in these models. Riluzole was examined in some animal models: hypobaric hypoxia in the mouse (global ischemia), global ischemia in gerbils (transient bilateral carotid artery occlusion), and focal ischemia in the rat (occlusion of MCA). Riluzole in all these models provided protection (effect on survival, activity of EEG, histopathology) at doses between 4-8mg/kg i.p.

Riluzole was tested in a number of psychopharmacological test battery. The wake cycle was shortened by oral doses as low as 0.5mg/kg, riluzole prolonged REM and SWS at 1 and 2mg/kg, and at 2mg/kg, the wake latencies were reduced. These effects on sleep occurred at doses lower than those that caused sedation and anesthetic effects (20mg/kg i.p. in rats and 44mg/kg p.o. in mice). Riluzole is not a muscle relaxant, neuroleptic, or anti-depressant. Riluzole showed some anti-aggressive effect as demonstrated in the suppression of the fighting behavior in mice with an ED_{50} of 5mg/kg p.o. and 8.7mg/kg i.p. In spite of the anti-aggressive behavior, riluzole is not an effective anxiolytic as was seen in 3 tests for anxiety, but it was somewhat effective in one test (water lick conflict) where 0.5-2mg/kg p.o. antagonized the anxiogenic effect of FG7142 (a partial inverse benzodiazepine agonist), in the operant conflict test. Riluzole in the rat had an oral ED_{50} of 1mg/kg in generalization test where discriminatory stimuli were dose-dependent. Also, discrimination was not route-dependent since 90% of rats generalized after 2mg/kg i.p. dose. Stereotypy did not occur at doses upto 10mg/kg i.p.

Riluzole showed good anti-convulsant effects as it protected against seizures induced by: glutamate, aspartate (ED_{50} 2.5mg/kg i.p. or p.o.), dl-homocysteic acid (ED_{50} 5mg/kg i.p.); electroshock in mice (ED_{50} 4mg/kg i.p.), in rats (ED_{50} 0.8mg/kg i.p.); kindling in rats (<1.25mg/kg i.p.); inhibitors of glutamate uptake (ED_{50} 3mg/kg i.p.); inhibitors of glutamic acid decarboxylase (ED_{50} 6mg/kg); and audiogenic seizures in genetic DBA/2 mice (ED_{50} 1.2mg/kg i.p.). Riluzole's anticonvulsant effect does not cause tolerance with repeated dosing.

Interaction of riluzole with other drugs was examined in rats or mice. Riluzole did not modify the effect of alcohol in rats (ED_{50} >16mg/kg p.o. vs. 1.3mg/kg p.o. diazepam). Similarly, riluzole did not interact with the neuroleptic CPZ when tested in mice or rats. However, riluzole potentiated

hexobarbital sleeping time in mice dose-dependently and via oral and i.p. routes (ED₁₀₀ 13.5mg/kg p.o., 9.5mg/kg i.p. vs. 1mg/kg diazepam).

Cardiovascular effects of riluzole were studied in vitro and in vivo. Riluzole affected (shortened) APD, E_m, V_{max}, and amplitude in vitro electrophysiological recordings of papillary muscle and Purkinje fibers of g.pig at 100 and 3uM respectively. Oral single dose of 30mg/kg of riluzole administered to conscious dog did not modify EKG or HR but caused a mild effect on SBP and DBP; dogs showed sedation and salivation. At oral dose of 5mg/kg administered to conscious Spontaneously Hypertensive Rat, HR and MAP were decreased 5-7% but the 10mg/kg dose was without an effect on either parameter. Riluzole at low dose of 0.03mg/kg infused to anesthetized g.pigs decreased pulmonary resistance and, compliance was also decreased by the 2 higher doses of 0.3 and 3mg/kg; these changes were small and non-dose-dependent. Pulmonary compliance, SBP, and DBP were reduced at cumulative doses $\geq$ 2mg/kg and upto 8mg/kg administered to anesthetized dogs whereas, respiratory rate and HR were increased at 8mg/kg doses. In dogs with pre-existing myocardial ischemia, riluzole infusion at 3mg/kg caused an increase in sinus rate as early as 30sec of infusion and lasted 15min, and MAP was increased 11% of control values; HR generally was reduced. In a study that measured large number of CVS parameters, infusion of 0.03, 0.3, or 3mg/kg riluzole to anesthetized dogs (measurements done by telemetry), caused a small 3-7% but significant increase in SBP and DBP at the LD, 14-19% increase in TPR, and decrease in CO and SV at 3mg/kg. However, the vehicle, poloxamer, had many effects including significant 8-16% increase in HR and shortening of PR, QTc, and AVERP. The effects of riluzole in this study were similar to those of the vehicle.

The toxicity of riluzole following a single administration was studied in rats, mice, and monkeys. LD₅₀ values in the rat were 20-25mg/kg i.v., 45mg/kg p.o., and in mice, the minimum lethal oral dose was 125mg/kg in males and 300mg/kg in females, oral LD₅₀ was 85mg/kg and 34.5mg/kg i.v. Clinical signs in rats included piloerection, gasping, slow respiration, lethargy, ptosis, salivation, lacrimation, and hyperactivity in survivors. In mice, signs included hypoactivity, tremors, ataxia, dyspnea, and at high dose, loss of consciousness. Single oral dose of 100mg/kg to monkeys caused 100% death 48hr postdose with signs including hypoactivity, ataxia, tremors, hypothermia, drowsiness, dyspnea, and fixed stare. At 50 and 100mg/kg doses in monkeys, histopathological exam showed hepatic steatosis and renal microvacuolar steatosis and at 100mg/kg vacuolation of acinar cells of the pancreas with cytolysis was also seen. Following i.v. injection of 20mg/kg 1 male monkey died during infusion and at this dose and 30mg/kg, signs were severe and included dilated pupils, fixed stare, eyes flickering, unresponsiveness, and tremors. At 20mg/kg monkeys were also drowsy and breathing was labored and irregular. The minimum lethal oral dose in monkey is 100mg/kg and highest non-lethal oral dose is 50mg/kg, minimum lethal i.v. dose is 20mg/kg and highest non-lethal i.v. dose is 10mg/kg.

The potential toxicity of riluzole following repeated dosing was tested in rodents, dog, and monkeys. Doses tested in rodents ranged from 2.5 to 45mg/kg (p.o. and i.v.) (0.75-13.5x the max anticipated human dose of 200mg/d), in dogs only one dose of 30mg/kg was tested, and in primates 1-60mg/kg (p.o. and i.v.; 0.3-18x max anticipated human dose). Duration of testing ranged between 14days to 26months. The toxic effects of riluzole are discussed below by animal species and parameter:

Rat:

Mortality: at 45mg/kg p.o. all rats died between day1-3 of dosing.

Clinical signs: 10-45mg/kg less severe at 5mg/kg: ataxia, hypoactivity, hypothermia, sedation, lethargy, salivation, vomiting, impaired or loss of righting reflex, chromodacryorrhea, prostration, slow and shallow breathing.

At 20mg/kg dosed orally for 26wks, in addition to the above signs, head twitching, jerking, paddling, and seizures occurred.

Body wt and Food intake: in general, both parameters decreased at doses >5mg/kg relative to the control animals.

Hematology: some inconsistent and non-dose-dependent changes in blood parameters.

Clinical Chemistry: at 45mg/kg for 1male, urinary volume, Na and K excretion increased 1-2x the control, serum ALT, ALP, GPT, cholesterol, bilirubin, BUN, and glucose increased (some of these changes occurred at doses as low as 5mg/kg p.o.). After 4wk i.v. levels of BUN decreased, dose-dependent decrease noted in urinary K, Na, creatinine, and urine osmolality at doses >5mg/kg

Organ wt and Histopathology: target organ toxicity is the liver where both absolute and relative wts were found to increase at doses >10mg/kg. Also, after 15mg/kg i.v. dosing for 4wk, the absolute and relative wt of the prostate decreased; dose-dependent (10 and 15mg/kg) decrease in absolute and relative wts of the thymus and lungs, and salivary glands (latter only in females). Following 20mg/kg for 26wk and 14d drug-free period, the adrenal wt was reduced relative to the controls. The absolute and relative wt of one kidney (left) was increased dose-dependently in female rats dosed 5, 15, and 45mg/kg p.o. for 1month. Histopathology included absence of corpora lutea in females dosed 10 and 20mg/kg for 26wks including females during recovery period, liver vacuolation, necrosis, inflammation, liver and spleen hemosiderosis through recovery period, and 1 female dosed 5mg/kg died from lymphoblastic lymphosarcoma. The NOEL for repeat dosing in the rat is <5mg/kg p.o.

Dog:

Clinical signs: ataxia, hypoactivity, hyporeactivity, tremors, and vomiting.

Hematology: RBC, Hb, Hct, and haptoglobin concentration all decreased; the levels of MCV, thrombocyte, fibrinogen, and reticulocyte, increased. These changes are indicative of hemolytic anemia.

Clinical Chemistry: marked elevation in ALP and LDH levels.

Histopathology: liver and spleen hemosiderosis suggested to be secondary to anemia.

A NOEL can not be estimated for the dog because only 1 dose was tested.

Baboon/Monkey:

Mortality: all monkeys orally dosed at 20mg/kg for 26wks, died between wks 5-9 of dosing.

Clinical signs: following p.o. and i.v. at doses >5mg/kg: salivation, extreme sedation, ataxia, apathy, emesis, incoordination, hypothermia, vomiting, vacant expression, limb tremors, and pupil dilation. After 4wk i.v. injection of 2, 5, 10mg/kg monkeys showed gasping, ptosis, and incoordination. After 20mg/kg p.o. for 26wks, repetitive rocking movement and tremors occurred.

Body wt and Food intake: both decreased at doses >5mg/kg

Hematology: increased APTT, decrease in RBC, PCV, Hb, and WBCs at doses >10mg/kg.

Clinical Chemistry: increase in total proteins, Al, SGOT, and SGPT >5mg/kg. Cholesterol and inorganic phosphorous levels decreased and K increased at p.o. doses >5mg/kg.

Organ wt and Histopathology: following i.v. injection, at the injection site, there was perivascular reaction and partial to complete occlusion that prevented infusion (doses >2mg/kg). Following 3, 10, 25mg/kg p.o. for 13wks, subjective exam of the baboon's eyes on wks 3&6 showed optic and retinal disc defects with elevated intraocular pressure, however actual exam on wk 7 did not reveal any findings; the sponsor dismissed the previous effect suggesting it was an artefact and/or a transient finding. In one female that died after 30mg/kg dose, histopath. showed spleen and thymus involution and hemolymphoreticular changes in the adrenals. Liver seems to be the target organ of toxicity with increase in wt (dose-dependently from 5mg/kg upto 20mg/kg), and fat accumulation and vacuolation. The sponsor related these

findings to stress in monkeys dosed 20mg/kg. Thymus involution also observed and considered to be stress related. The NOEL in the monkey is <5mg/kg.

Single and repeated dose PK studies have been conducted in the rat, dog, monkey, and pregnant and non-pregnant rat and rabbit (single dose). Riluzole is highly protein bound at 80-90% in the rat. Riluzole is well absorbed as indicated by the rapid detection of the drug in blood and tissues between 15-30min after oral administration. Riluzole also crossed the BBB with brain levels 2-3x higher than blood levels upto 6hr postdose. Brain concentration ranged between 0.5-9.5ug/ml (mean±sd 4.4±3.7ug/ml) following 20mg/kg dose in monkeys. The drug is widely distributed with highest radioactivity detected in stomach, intestine, liver, melanin-rich tissues (eyes and skin), brain, and kidneys. Distribution within the brain was homogeneous except in the cerebellum where radioactivity was highest in the white matter. Peak radioactivity in the eyes of pigmented rats occurred at 1hr postdose and declined rapidly by 72hr but, slowly by 4wks with 0.0003% overall retention of total radioactive dose (2mg/kg). In the rat following 2mg/kg/d oral dose, the 21day tissue to plasma ratio was highest in stomach, Harderian glands, skin, liver, kidneys, adrenals, and spinal cord. In this study, plasma elimination half life (24-168hr) after the last dose was 39hr. Table below summarizes plasma concentration (min-max), in animals following repeated dosing of riluzole:

Species	Dose (mg/kg)	concent. (ug/ml)
Rat	2.5	0.23-0.54 (1hr/wk13)
	8	0.3-0.82 (1hr/wk13)
	25	1.1-3.3 (1hr/wk13)
	5	0.3-0.61 (1hr/wk13)
		0.4-0.82 (1hr/wk26)
	10	0.62-2.0 (1hr/wk13)
	20	1.0-1.6 (1hr/wk26)
Mouse 10		1.5-2.6 (1hr/wk13)
		1.7-1.7 (1hr/wk26)
Mouse 10	20	0.23-1.3 (1hr/wk13)
	30	0.73-2.0 (1hr/wk1)
		0.4-3.6 (1hr/wk13)
Baboon	3	1-1.4 (1hr/wk13)
	10	2.2-6.0 (1hr/wk13)
	30	3-10 (1hr/wk13)
Monkey	5	0.02-0.87 (8hr/wk13)
		0.08-1.0 (8hr/wk26)
	10	0.6-3.7 (8hr/wk13)
	<0.005-4 (8hr/wk26)	
	20	0.2-7.4 (8hr/wk4)
Human	1.7/d	0.147±0.128

* duration of dosing

In the rat, there seemed to be no drug accumulation after repeated dosing as indicated by low tissue retention of radioactivity with an average of 0.38% and 6% of accumulated and last dose of 2mg/kg respectively. However, in repeated-dose TK studies in primates and other rodent studies, marked accumulation of riluzole was seen both at low and high doses (3-20mg/kg) with

plasma levels 2-4x higher at end of study (cumulative ratios and concentration). First order kinetics were only seen in some studies and generally, plasma levels increased non-linearly with dose. Following 2mg/kg/d to the rat for 13wks, highest radioactivity concentration was localized in the Harderian gland, thyroid gland, sm. intestinal wall, and the liver. Radioactivity concentrations (mEqiv/kg tissue) were recorded at 0.5 and 24hr postdose on days 7, 14, and 21 of dosing. Blood and plasma radioactivity fell rapidly following the last dose but more slowly thereafter with elimination $t_{1/2}$ of 39hr (indicated above). In mice dosed 10 to 30mg/kg/d for 13wks, plasma levels ranged between 232-3636ng/ml measured 1hr postdose. Linear relationship between dose and concentration was observed only during wk1 at the 1hr blood samples, non-linear kinetics were observed during wk13 at the 1hr samples. Moreover, at wk13/24hr samples, plasma concentrations plateaued as if absorption, metabolism, and/or protein binding processes were saturated. Significantly higher plasma levels were seen at 30mg/kg measured on wk13 relative to concentration measured on wk1; no sex difference in plasma levels was observed in mice. Sex difference in exposure was demonstrated in some studies with generally higher levels in males.

Pregnant rabbits had generally higher plasma riluzole concentration and exposure than non-pregnant rabbits at doses between 3-60mg/kg administered during organogenesis. Concentration increased non-linearly with dose and drug accumulation was evident only in the 3mg/kg dose but not at higher doses (higher plasma concentrations on last day of dosing relative to day 1 values). Plasma concentration in non-pregnant rabbit ranged between 61-4587ng/ml and 55-7626ng/ml in pregnant rabbits for doses 3-60mg/kg.

Riluzole administered to pregnant rats during organogenesis at 3-27mg/kg, showed linear exposure (AUC_{0-8hr}) at 3 and 9mg/kg of 1519 and 5716ng.ml/hr respectively, but exposure was non-linear at 27mg/kg (31987ng.ml/hr). Mean maximum plasma concentration increased non-linearly with dose at all 3 doses reaching peak levels between 0.5 and 8hr postdose. When data normalized to 9mg/kg, a linear dose relationship was seen on days 6 and 15 upto 8hr postdose but statistically significant difference noted between dose, day, and sampling time. Exposure on the last day of treatment increased 8x disproportionately to dose when dose increased from 3-27mg/kg indicating drug accumulation. Mean plasma concentration on gd6 ranged between 287-2542ng/ml and on gd15 from 95-5590ng/ml. In another study, pregnant rats were orally administered a single dose of radiolabelled riluzole at 27mg/kg and autoradiography and dissection studies conducted on dams and fetuses. As indicated above, riluzole readily distributed to various tissues irrespective of gd (13 or 18 in this study) in the dam and crossed the placenta into the fetus. The lowest radioactivity in the dam was in the amniotic fluid, highest radioactivity was detected at 3hr on gd13 and at 24hr on gd18. Radioactivity in the whole fetus on gd13 was similar to that in plasma but was 2x higher than radioactivity in plasma on gd18. It was concluded that drug placental transfer increases with the progression in pregnancy and the process appears to be non-saturable.

Riluzole is transferred into the milk as demonstrated in lactating rats following a single oral dose of 15mg/kg labelled drug administered on gd10-11. Concentration was highest in milk at 7.5mEqiv/l with milk/plasma ratios increasing with time from 1.7 to 4.1 at 6hr. Milk AUC_{0-72hr} was 2.5x higher than that in plasma. Milk concentration decreased by 72hr to 2.5% and to 5% in plasma. Drug concentration showed 2 peaks at: 1hr (for milk, plasma, and blood) and at 6hr (for milk and plasma only), the sponsor indicated that this rebound in concentration might be due to enterohepatic recirculation.

Riluzole undergoes both phase I and phase II metabolism in humans, rodents, rabbit, dog, and monkey. It is metabolized by the P450 monooxygenase enzyme system in all of these species. In vitro studies in humans showed that CYP1A2 is the P450 isozyme subfamily responsible for metabolism of riluzole. Using

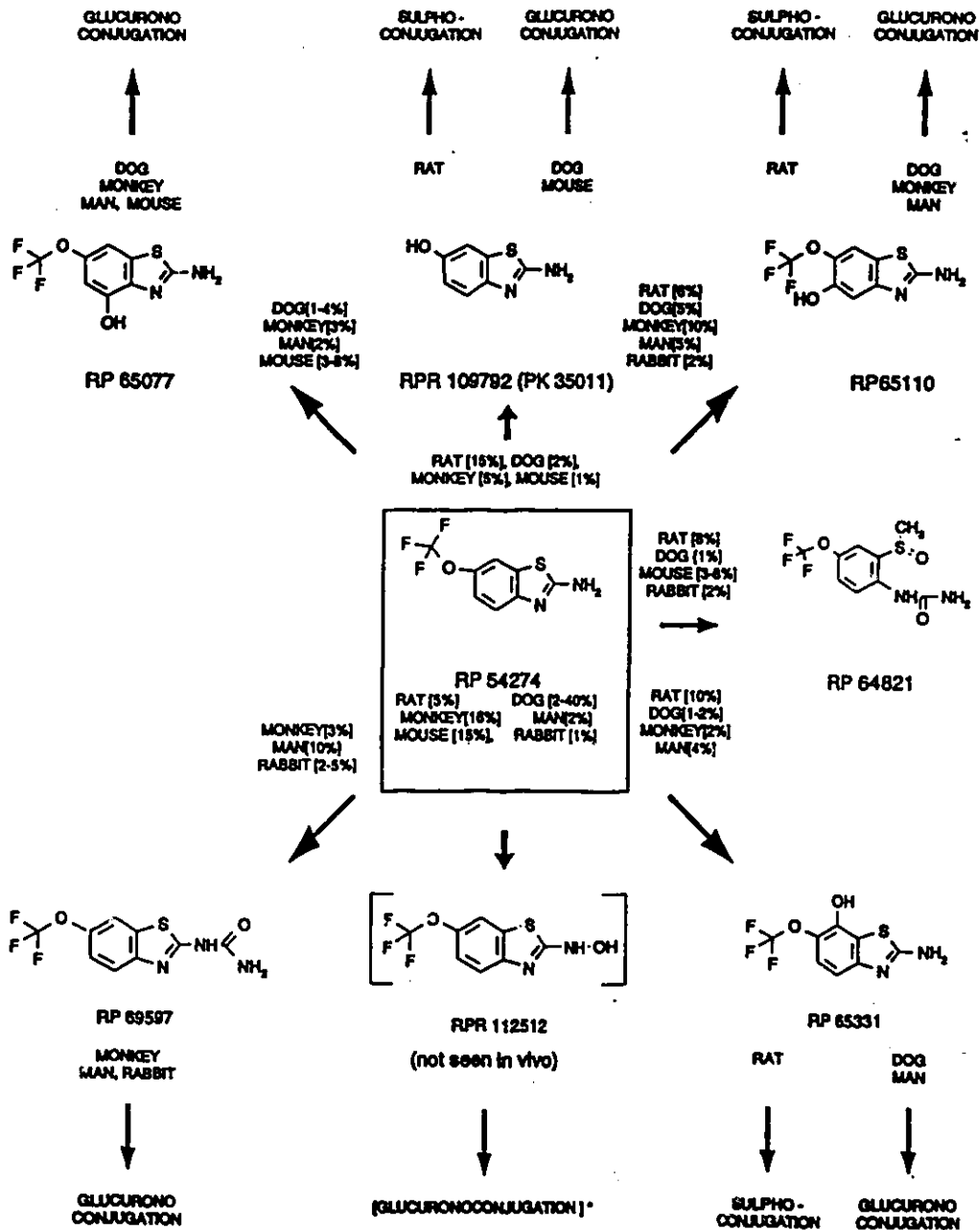
genetically engineered yeast cells that specifically expressed human CYP1A1, 1A2, and 3A4, it was shown that the rate of biotransformation of riluzole was about 5x higher with CYP1A1 than with CYP1A2. This finding indicated an extrahepatic metabolism since CYP1A1 is not expressed to a significant level in human liver. CYP1A2 catalyzed N-hydroxylation of the benzothiazole amine group to form RPR112512. This metabolite although is the major cpd in the dog, rabbit, and monkey and forms to lesser degree in other species, it was detectable only in vitro studies. Direct N- and/or O-glucuronidation of riluzole was seen in vivo studies in humans, a finding that is in contrast to that in vitro where direct conjugation was very slow. The sponsor suggested from a preliminary data that glucuronidation in humans in vivo occurs indirectly via RPR112512; no confirmation of this has been provided to date. In rabbit, dog, and monkey, the parent unchanged drug comprised the major cpd in liver microsomes. In mouse and rat liver microsomes, the unidentified metabolite A4, followed by the parent drug were the main metabolites. In plasma of rats, 8-10 metabolites were identified following a single oral dose of labelled riluzole (2mg/kg) with the parent unchanged drug being the predominant cpd. In the brain of these rats, the main metabolites included RP64821 (the methoxyphenylurea derivative), and unchanged drug. Brain levels were higher than plasma levels at all time periods upto 24hr postdose. There was sex difference in exposure with blood, plasma, and brain levels being higher in females than males. Also some qualitative differences between sexes were seen in plasma, blood, and brain metabolites but not those in urine or feces. Conjugation with both glucuronic acid and sulfates occurred in these rats. It is noted that sulfoconjugation constitutes 30% in the rat but very little in the dog which as in humans, glucuronidation is the main conjugation pathway in urine.

Major route of riluzole elimination is the urine with small amount excreted in feces. Most of the drug is eliminated by 48hr postdosing (90% in urine and 9-16% in feces). Following a single 2mg/kg oral dose of radiolabelled riluzole to fistulized rat, 56% of the administered dose was excreted through the bile which was higher than the 30% fecal elimination in non-fistulized rats. This latter finding was suggestive of first pass enterohepatic circulation.

Attached table from the sponsor illustrates the metabolic pathway of riluzole in animals and humans. It should be noted that to date, the major metabolite in humans and some animal species, the N-hydroxylated derivative RP112512, has only been indentified in in vitro animal and human studies.

The biological activity of the following metabolites was examined: RP64821, RP65077, RP69597, RPR109792, RP65110, RP65331, and RPR112512. Receptor binding study showed metabolite RP69597 at 10uM inhibited Na-channels by 58% and at higher (low affinity) concentration of 100uM it also inhibited Ca-channels (47%) and alpha2 receptors (48%). RP65331 at 10uM inhibited muscarinic receptors (53%), RP65110 inhibited NE uptake by 50% at 10uM. The other metabolites did not show any significant binding at any of the receptors tested. The cardiac electrophysiology of metabolites RP65077 and RP65110 was tested using isolated g.pig Purkinje fibers. At 3 and 30uM these 2 metabolites significantly and in a concentration-dependent manner reduced V_{max} and action potential duration supporting the Na-channel blocking effect of these metabolites noted on cardiac cells in vitro. Metabolites RP65110, RP65077, and RPR112512 inhibited veratridine-induced release of DA in the rat striatal slice with IC_{50} values of 1.8, 0.7, and 8.7uM respectively (riluzole is 3.5uM). Metabolites RP65110 and RPR112512 protected against MSG-induced seizures in the rat with ED_{50} values of 7.3 and 3.5mg/kg respectively, (riluzole value was 2.5mg/kg).

Table 25 : Metabolic pathways of riluzole



Percentages given correspond to fractions of dose found in urine following oral administration.

* Preliminary evidence indicates that this conjugate gives rise to riluzole following β -glucuronidase hydrolysis of urine (12% of dose in monkey, 10-20% in rabbit and 40% in man).

Riluzole caused several maternal and fetal toxicities including death, in rats and rabbits. The oral doses tested ranged between 2.5-60mg/kg and the maternal/fetal toxicities in the rabbit were observed at concentrations that were approximately 0.2-27x the human mean concentration and, at 30x in the rat. There were no drug-related malformation or teratogenicity.

Rats:

Doses 15, 25, 30, and 60mg/kg caused death in males and/or dams. Clinical signs included langor, ataxia, prostration, lethargy, tremors, hypo- or hyperactivity, muscle weakness, salivation. Some of these signs appeared within 1hr of dosing and persisted until the following day or later. Some of these clinical signs were observed at doses as low as 8mg/kg.

Mean body wt and/or wt gain, and/or food intake were reduced in rats dosed ≥ 3 mg/kg (significant wt loss in animals dosed ≥ 20 mg/kg). In more than one study, males were much more sensitive to wt loss and loss of appetite than females. Mean wt loss and/or reduced food intake in some studies occurred at 5, 8, and 15mg/kg during the pre-mating, gestation, lactation, and pre-weaning upto ppd21 in pups from dams dosed 15mg/kg (drug was given during pre-mating and throughout lactation in females).

In addition to the above, the following drug effects were recorded:

- pre-mating interval was prolonged at 15mg/kg.
- fertility index but not mating index, was reduced in males and females at 15mg/kg. At this dose, percent of females that became pregnant and reached term with viable fetuses was reduced. Necropsy of few dams at this dose showed absence of developed mammary tissue and lack or insufficient milk.
- growth retardation such as reduction in crown/rump length (27mg/kg), slight delay in eye opening of pups from dams treated with 25mg/kg during gestation. F1 pups showed very small delay in onset and completion of pinna detachment, incisor eruption, eye opening at 15mg/kg. Deficits in function included reduction in grip strength and some effect on behavioral tests such as water maze, memory and learning at doses as low as 3mg/kg; no adverse effects in F2 generation.
- small litter size or no., small fetal wt, or small pups at 9, 15, 25, and 27mg/kg.
- increase in pre-implantation loss at 9, 15, and 27mg/kg.
- no. corpora lutea decreased at 9 and 27mg/kg.
- decrease in no. of viable fetuses at 15mg/kg.
- 4 females dosed 15mg/kg did not litter, one dam had total resorption of its litter and, 2 had translucent fluid in uterus.
- in F1 generation, viability index was reduced on ppd 4, 7, and 21 (these pups came from F0 dams that were dosed 15mg/kg).
- male and female pups died pre-weaning between ppd 0-10, some were stillborn or found dead.
- mean wt and/or wt gain was reduced in rats upto ppd77 (F1 generation) however, no loss in wt during gestation period but during lactation days 1,4,7 mean wt was reduced in dams from F0 rats dosed 15mg/kg.
- mating performance assessed in a range-finding study in rats, was tested by the outcome of 2 pairings at 10mg/kg to determine any drug effect on male or female. The results showed no effect on mating performance but an effect on sperm quality and/or no. Testicular atrophy was also seen in these males together with flaccid and reduced testes/epididymis wt. In this study, pup loss was increased at 1st mate at 2.5mg/kg. Mean litter wt at 2nd mate and litter size at 15mg/kg were reduced at birth and ppd4-21.

Rabbit:

- No deaths upto 30mg/kg dosed during organogenesis.
- clinical signs included langor, ataxia, prostration seen at the low dose of 3mg/kg during organogenesis. Mean wt decreased in 60mg/kg group but no effect upto 30mg/kg.

In addition to the above, the following drug effects were recorded:

- crown/rump length (growth retardation) and fetal wt reduced in 27mg/kg group.
- 1 fetus from 27mg/kg dosed dam had cleft palate and a 2nd rabbit had scoliosis, 1 fetus from 3mg/kg had cephalic defects and a 2nd fetus had kidney agenesis.
- postimplantation loss recorded in 27mg/kg.
- half of dams dosed 60mg/kg were pregnant by gd28. Late uterine deaths/doe and percent of postimplantation loss was increased in dams dosed 3mg/kg.

VI. Mutagenicity:

Riluzole was non-mutagenic in the Ames assay and the in vivo bone marrow micronucleus assay in mice and rats. The clastogenic and mutagenic response of riluzole was equivocal in the human lymphocyte chromosomal aberration assay and the mouse lymphoma-ouabain locus assay. The mutagenic and clastogenic effects of riluzole in humans are unknown.

Recommendation:

This NDA is approvable. It is noted that a 1-year non-rodent toxicity study and a rodent (one species) carcinogenicity study have not been conducted at this time. It was discussed with and agreed by the sponsor that these studies be initiated as soon as possible. Below (in Bold) are the reviewer's recommended text for labelling:

Carcinogenicity - Mutagenesis - Impairment of Fertility
Page 2-1-15; 2nd line: replace "completed" with conducted.

2nd paragraph/2nd sentence/2nd line: replace: "these in vitro and in vivo assays" with the Ames test and the in vivo bone marrow micronucleus test in the mouse and rat. The clastogenic and mutagenic response was equivocal in the in vitro human lymphocyte and the mouse lymphoma mutation assays. The mutagenic and clastogenic potential of riluzole in humans is unknown.

Pregnancy: (Page 2-1-15)

First sentence line 2 replace: "to have an embryocidal effect" with to cause maternal and fetal lethality....

3rd line replace "in doses 10, 20, and 43 times" with at concentrations that were approximately 0.2-30 times the mean human plasma concentration.....

Page 2-1-16, 2nd paragraph replace the word "slight" with some; line 3 replace "15mg/kg/day" with 90mg/m²/day *...; line 4 replace "10 times" with 1.5 times...

* exposure data were not available at this dose.

Page 2-1-16, 3rd paragraph, lines 4&5 replace "27mg/kg/day and 60mg/kg/day (20 and 43 times the recommended human daily dose)..." with 162mg/m²/day and 708mg/m²/day (2.6 and 11.5 times the recommended human daily dose)..

Add the following at the end of 2nd paragraph:

Some of the effects at 90mg/m² included a decrease in fertility index, increase in pre-implantation loss, decrease in viable fetuses, and some females did not litter. The viability index of the first generation from females dosed 90mg/m², was reduced through post partum day (ppd) 21, some pups died pre-weaning on ppd 0-10, some were stillborn, or found dead. In another study in rats dosed 60mg/m², sperm count and/or quality was reduced and testicular atrophy was observed with flaccid testes. Mean litter wt and pup size were reduced at birth and during ppd 4-21. In rabbits, late uterine deaths per rabbit and percent of post-implantation loss was increased in animals dosed 35mg/m² (0.6 times the anticipated daily human dose). Teratogenic findings included cleft palate, scoliosis, and cephalic defects.

General note to the sponsor:

Human body weight of 60kg is used by this Division instead of 50kg (used by the sponsor) to calculate multiples of dose. Values such as those on page 2-1-7 top of the page line 5 should be 18 times instead of 20; line 7 should be 1.5 times instead of 2 times.

Riluzole labelling/New draft submitted Sep 27 1995:

Reviewer: Aisar Atrakchi
Date: Sep 27 1995
Supervisor: Glenna Fitzgerald

This NDA is approvable. It is noted that a 1-year non-rodent toxicity study and a rodent (one species) carcinogenicity study have not been conducted at this time; recently the sponsor informed us that a 1-year monkey toxicity study is to be initiated soon and a carcinogenicity study will also be conducted. Below in **Bold** are the reviewer's recommended text for labelling:

- Clinical Pharmacology/page 3/2nd paragraph/line 4: exposed to **excess glutamate.....**
- Mechanism of Action/page 4/1st paragraph/at end of point#3 add **without binding affinity to any of these receptors. I personally prefer to take this entire statement out, riluzole did not bind to any EAA recptor and its effect may be indirect through antagonism of Na- and/or Ca-channels and modulation of G-proteins.**
- Mechanism of Action/page 4/2nd paragraph/before end of 2nd sentence add **without affecting onset of the disease or motoneuron function. I would also take this statement out since it does not add any significance, if the sponsor think it does, then why not include the Mnd model as well?.**
- Carcinogenicity - Mutagenesis - Impairment of Fertility/Page 13/end of 1st paragraph: replace "completed" with conducted.
- Carcinogenicity - Mutagenesis - Impairment of Fertility/Page 13/2nd paragraph: replace "microbial" with bacterial and 2nd sentence of this 2nd paragraph: "there was no evidence of mutagenic potential in the in vitro bacterial Ames assay or the in vivo assays in the mouse and rat. The clastogenic and mutagenic response was however, equivocal in the in vitro human lymphocyte chromosomal aberration assay and the mouse lymphoma mutation assay. The mutagenic and clastogenic potential of riluzole in humans is unknown.
- Pregnancy/page 14/1st sentence/line2 replace: "to have an embryocidal effect" with to cause maternal and/or fetal toxicity including mortality in rats at doses 1.5 times the maximum human daily dose and in rabbits at 11.5 times the maximum human daily dose on a mg/m² basis.

Pregnancy section cont.

2nd paragraph replace the word "slight" with some; line 3 replace "at doses of 15mg/kg/day (approximately 10 times..." with at 15mg/kg/day which is approximately 1.5 times the maximum human daily dose on a mg/m² basis (exposure data at this dose were not available).

3rd paragraph/lines 4 and 5 replace "27mg/kg/day and 60mg/kg/day (20 and 40 times.... with 27mg/kg/day and 60mg/kg/day which are 2.6 and 11.5 times the recommended human daily dose on a mg/m² basis.

Add the following at the end of 2nd paragraph:

Some of the effects at 15mg/kg included a decrease in fertility index, increase in pre-implantation loss, decrease in viable fetuses, and some females did not litter. The viability index of the first generation from females dosed 15mg/kg, was reduced through post partum day (ppd) 21, some pups died pre-weaning on ppd 0-10, some were stillborn, or found dead. In another study in rats dosed 10mg/kg, sperm count and/or quality was reduced and testicular atrophy was observed with flaccid testes. Mean litter wt and pup size were reduced at birth and during ppd 4-21. In rabbits, late uterine deaths per rabbit and percent of post-implantation loss was increased in animals dosed 3mg/kg (1.8 times the anticipated daily human daily exposure/AUC basis). Teratogenic findings included cleft palate, scoliosis, and cephalic defects in one or 2 rabbits.

General notes to the sponsor:

1. Human body weight of 60kg is used by this Division instead of 50kg (used by the sponsor) to calculate multiples of dose. Therefore, please correct the following multiples:

Mechanis of Action/page 5/2nd paragraph/line 5: 18 times to replace 20 times and, the same paragraph line 7: 1.5 times to replace 2 times....

2. Please note that the above comments are also to be incorporated in the package insert (Attachment 4 of correspondance dated Sep 22 1995).

APPENDIX I

Pharmacology Review for IND#
Amendments 78 & 86

IND#

Drug: Riluzole/RP 54274

Category: Excitatory AA Antagonist and Na-channel Blocker

Indication: ALS

Sponsor: Rhone-Poulenc Rorer

Rev date: June 28 1995

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This report contains the review for all the studies that were submitted on March 13th and April 7th 1995.

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Pharmacology:

The mechanism of riluzole in ALS is unclear and complex. The pharmacology is divided into the following sections each in support of a given site of action.

Studies Supporting Antagonism of EAA:

There are 3 possible mechanisms for ALS: (1) autoimmune by producing antibodies for the N- and L-type calcium channels; (2) EAA hypothesis that may cause an increase in synaptic glutamate concentration via one or more mechanisms; and (3) oxidative stress hypothesis where a spontaneous genetic mutation occurs in the SOD (superoxide dismutase) enzyme seen in the familial form of the disease. In the EAA hypothesis, one proposed mechanism is the impairment or loss of glutamate transport. The latter has been found in brain membranes of some ALS patients and recently another specific astroglial transport subtype (Glt-1) is the affected parameter. The failure in glutamate transport was studied in postnatal rat spinal cord slices in culture using specific transport inhibitors. These inhibitors increase synaptic glutamate concentration and, through a non-NMDA receptor mechanism, cause a slow and relatively selective loss of ventral horn motoneurons after chronic application (2-4wks) (Rothstein et al., 1995). The selective glutamate inhibitor used in this study was threo-hydroxyaspartate (THA) tested at 500uM incubated with the slices for 14days (high toxicity), or 100uM incubated for 28days (low toxicity). Slices were analyzed for choline acetyltransferase (ChAt) activity; a change in the levels of this enzyme have been shown to correlate quantitatively with the loss of lumbar spinal motoneurons in this experimental culture. There was an 80% loss in ChAt activity in the high toxicity treatment and 65% loss in the low toxicity treatment. The cpds/drugs tested in this model included: non-NMDA antagonists, glutamate agonists (ionotropic and metabotropic), Na-K channel blockers (riluzole, phenytoin), anti-oxidants, Ca-channel blockers, Cl-channel blocker, Ca-endonuclease inhibitor (aurintricarboxylic acid, ATX), anticonvulsant (gabapentin), and diazepam. The most potent neuroprotectants were in decreasing order of potency: the non-NMDA antagonists>gabapentin>dantrolene-Ca channel blocker>riluzole only at 100uM (considered an inhibitor of presynaptic release of glutamate) (see table from the sponsor).

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Table 1. Effects of various putative neuroprotectants on chronic glutamate neurotoxicity.

Uptake Inhibitor	Weeks of Treatment	Mechanism or Site of Action	Putative Neuroprotectant (μM)	% Untreated Control (mean $\pm$ SE)	P
THA 500 μM	2	-	-	20.9 $\pm$ 1.6	-
* <i>non-NMDA antagonist</i>		Glutamate Receptor/Release	* GYKI-52466 100	96.0 $\pm$ 5.0	P < 0.01
			cyclothiazide 100	0.5 $\pm$ 0.5	P < 0.01
			<i>trans</i> -ACPD 50	22.0 $\pm$ 13.0	NS
			riluzole 10	27.2 $\pm$ 5.7	NS
			riluzole 100	51.7 $\pm$ 6.7	P < 0.01
		Ion Channels	gabapentin 100	55.4 $\pm$ 9.7	P < 0.01
			dantrolene 3	22.0 $\pm$ 3.7	NS
			dantrolene 50	44.2 $\pm$ 10.0	P < 0.05
			bepiridil 50	23.0 $\pm$ 2.0	NS
			nifedipine 1	16.0 $\pm$ 3.0	NS
			nifedipine 10	17.4 $\pm$ 2.2	NS
			ethacrynic acid 100	27.5 $\pm$ 3.5	NS
		Antioxidant	α -BPN 250	36.5 $\pm$ 0.7	P < 0.01
			U38383E 100	34.3 $\pm$ 8.5	P < 0.05
			vitamin E 100	19.5 $\pm$ 4.0	NS
			ascorbic acid 1000	19.0 $\pm$ 4.0	NS
		Other	ATX 100	30.8 $\pm$ 2.2	P < 0.01
			tofisopam 100	19.0 $\pm$ 4.0	NS
THA 100 μM	4	-	-	34.4 $\pm$ 5.3	
* <i>non-NMDA antagonist</i>		Glutamate receptor/synthesis	* PD144216 10	70.0 $\pm$ 5.0	P < 0.01
			* PD139977 10	63.0 $\pm$ 5.0	P < 0.05
			gabapentin 10	40.0 $\pm$ 12.0	NS
			gabapentin 33	43.2 $\pm$ 2.4	NS
			gabapentin 100	100.0 $\pm$ 21.3	P < 0.01
		Ion channel	phenytoin 100	39.0 $\pm$ 9.0	NS
		Antioxidant	L-NMMA 150	61.0 $\pm$ 12.7.0	P < 0.05
			mannitol 1000	33.0 $\pm$ 9.8	NS
		Other	ceftriaxone 1	45.0 $\pm$ 10.0	NS
			ceftriaxone 100	66.0 $\pm$ 8.0	P < 0.05

Spinal cord organotypic cultures were incubated for 2 weeks in the presence of 500 μM THA and the indicated drugs, or for 4 weeks with 100 μM THA and the indicated drugs. Data are expressed as % of

In another experiment (RPR-Biol 610; Nov 1994), the neuroprotective effect of riluzole was tested in fetal rat (gds 12-14) spinal cord enriched motoneurons in culture after long-term exposure (24hr), to glutamate, NMDA, kainate, or AMPA. It is shown that addition of glutamate upto 600uM did not induce acute neuronal damage/swelling, however, neuronal degeneration was seen within 24hr with an EC₅₀ of 300uM. NMDA at 100uM, kainate at 500uM, and AMPA at 10uM all induced cell loss during 24hr treatment. Riluzole at 0.1uM added simultaneously with glutamate (100uM), partially protected cell loss, and significant protection was seen with 10uM riluzole at 600uM glutamate (table below from the sponsor). Riluzole at 1uM also provided some protection against 100uM NMDA but no effect at AMPA or kainate. It is noted from the data that riluzole alone significantly and in a concentration-dependent manner increased neuronal death at 1, 10, and 100uM (100uM not shown) compared with the control. The sponsor stated that this was a surprising result however, it may be related to reduced neuronal activity which in turn affect cell differentiation in culture (Kalb & Hockfield, 1992).

Condition		Riluzole		
		0.1uM	1uM	10uM
None	100.0±3.5	94.4±4.4	84.0±4.9*	72.5±6.1*
Glutamate 100 uM	57.8±2.9*	77.7±5.2**	71.2±4.6**	ND
Glutamate 600 uM	22.6±2.6*	27.4±2.5*	37.2±4.4**	67.3±4.3**

Values are the mean ± S.E.M. from 3-6 experiments running in triplicate and expressed as percent of cell viability of untreated cultures. * p < 0.03 vs. control; ** p < 0.02 vs. glutamate 100uM; * p < 0.01 vs. glutamate 600uM. ND, not determined.

In another study, riluzole potentiated the anti-proliferative effect of cytosine-arabinoase on neural cells when both were added simultaneously to cultured motoneurons. Motoneuron-enriched cultures were treated with 10uM cytosine-A for 24hr and degeneration of glial cells was evident. Riluzole alone (1uM) had no effect but added together with cytosine-A, almost completely abolished glial cells and motoneurons. This high toxicity of riluzole in presence of cytosine-A on motoneurons could not be explained.

Cortical primary cultures of CSF from ALS patients was shown to have cytotoxic properties that could be blocked by AMPA or kainate antagonist CNQX (NeuroReport 5, 1994). Riluzole at 0.5uM partially enhanced neural survival in ALS CSF cultures compared with control and control CSF cultures but the protection was less than that produced by 20uM CNQX (60 vs. 80% survival respectively). This finding indicated that riluzole neuroprotective effect is mediated by glutamate AMPA/kainate non-NMDA receptor site.

Riluzole did not bind to NMDA, glycine site, dissociative site (PCP ref), or non-NMDA (AMPA and kainate) receptors upto 100uM when tested in radioligand binding assay using rat cortical membranes (Biol 586; Sep 1994). Using voltage clamp technique, riluzole effect on electrophysiological responses mediated by NMDA and AMPA receptors in Xenopus oocytes was examined (Biol 483; Jun 1994). Responses were assessed by measuring the amplitude of the inward currents induced by application of the agonists. Riluzole alone had no effect on basal current (holding potential of -60mV), however, responses induced by NMDA were inhibited with an IC₅₀ of 24uM. Inhibition of responses to kainate stimulation were less potent with an IC₅₀ of 174uM. It is noted that riluzole-inhibition of NMDA response was not use-dependent i.e. non-competitive.

Effect of riluzole on EAA release was tested using slices of adult rat striatum (Biol 494; Jun 1994). Riluzole inhibited NMDA-induced release of Ach with an IC₅₀ of 1.4x10⁻⁶ M (95% inhibition occurred at 1uM), 98% electrically-

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evoked release was inhibited at 10uM, and the electrically-evoked release in rat olfactory tubercle was also inhibited by riluzole with an IC₅₀ of 3x10⁻⁶M. The sponsor indicated that basal release was however, unaffected by riluzole (data were not provided). Riluzole also inhibited glutamate-induced release of newly synthesized DA with an IC₅₀ of 3uM (92% inhibition occurred at 0.1uM riluzole) and glutamate-induced release of aspartate from rat cultured cerebellar granule cells with IC₅₀ of 10uM. Riluzole had no effect on K-evoked release of DA. The inhibitory effect of riluzole on glutamate-induced release of aspartate was abolished by 1-10nM pertussis toxin, this may indicate an indirect mechanism through activation of G protein. It was concluded that riluzole inhibited neurotransmitter release irrespective of the EAA receptor subtype and the effect is selective since riluzole had no effect on k-evoked release. In another study (Biol 482; Mar 1994), riluzole inhibited glutamate-stimulated cGMP formation in neonatal rat cerebellar slices with an IC₅₀ of 25uM without affecting basal levels. EAA-evoked formation of cGMP in cerebellar slice is thought to be mediated by Ca-entry through NMDA receptor site. On the other hand, riluzole had no effect on inositol phosphate formation in either striatum or cerebellar granule cells from neonatal rat; an effect that is mediated by metabotropic EAA receptors. This is in contrast to previous finding where riluzole blocked transmitter release from cerebellar granule cells at concentrations used in this study (10⁻⁶ and 10⁻⁷M). This discrepancy could not be explained by the sponsor.

Using hippocampal slices from 7-9d old rat pups, riluzole protected against NMDA-induced neurotoxicity mediated by G-protein mechanism (59%/LDH release), and, against veratridine-induced neurotoxicity mediated by Na-channel (90%/LDH release) (Biol 467; May 1994). Neurotoxicity was assessed by LDH release and histopathology (neuronal cell count). The possible role of G-protein in riluzole's effect was demonstrated when 1ug/ml pertussis toxin pre-incubated with hippocampal slices for 16hr, completely abolished the protective effect of riluzole. Also, riluzole + TTX markedly reduced NMDA neurotoxicity (77% protection; 100uM riluzole), whereas, TTX alone did not (21%; 1uM). This finding supports an effect of riluzole via Na-channel. Effect on cell death was seen with 39-46% protection compared with NMDA treated slices.

Anesthetized rats and cats were implanted with push-pull cannulae into the caudate nuclei (Biol 531/Sep 1994). In the rat, basal DA levels were determined. Addition of glutamic acid to the perfusate increased DA release. Riluzole was administered i.v. to the rat at 0.1-2mg/kg, 40 or 20min before addition of glutamic acid. Complete inhibition of glutamic acid-induced DA release occurred at 1 and 2mg/kg riluzole at 40min and for the 0.5mg/kg at 20min; partial and delayed inhibition was seen at 0.5mg/kg at 40min. In the cat, levels of aspartic, glutamic acids, serine, glutamine, alanine, and tyrosine were measured. Direct application of riluzole at 10uM (into the perfusate), caused 50% decrease in basal glutamic acid release but did not affect the other cpds.

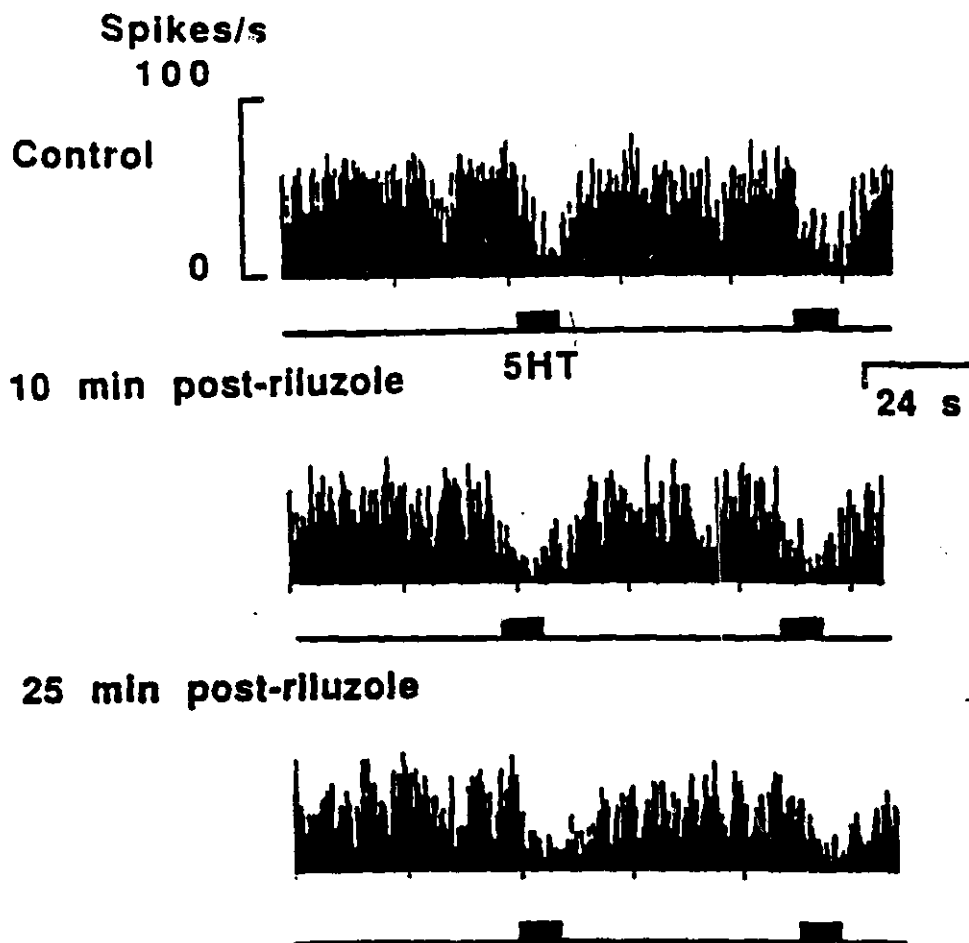
Effects Related to ALS:

Effect of riluzole on EAA was tested in anesthetized rat in vivo (Biol 487; June 1994). Male rats were implanted with multibarrelled recording electrodes positioned in the substantia nigra, facial nucleus, cortex, and raphe nucleus. Riluzole was injected either i.v. or i.p. Riluzole completely abolished the spontaneous activity/firing rate of DA A9 neurons in substantia nigra at 0.05-0.1mg/kg i.v. and at 0.1mg/kg i.p. Responses to application of NMDA, quisqualate, and kainate to the quiescent facial neurons were 100% abolished by 0.6mg/kg i.p. dose of riluzole but recovery did not occur. After i.v. injection of riluzole at 0.6mg/kg, responses were also depressed completely but at 0.3mg/kg where firing rate was depressed by only 40-70%, partial recovery in firing was seen. In the case of the usually spontaneously active raphe nuclei, responses to iontophoretically applied 5HT could be inhibitory

or excitatory. Riluzole at 0.6mg/kg i.v. decreased the background firing rate but the 5HT mediated response (inhibitory in this case) remained (see figure).

Figure 5

The response of a spontaneously active neurone in the rat raphe nucleus to microiontophoretically applied 5-HT. This neurone displayed an inhibitory response to 5-HT. The top trace shows the control record of spontaneous firing rate and two responses to 5-HT. Riluzole was administered after 5 control 5-HT applications. The middle trace illustrates the fall in spontaneous activity 10 minutes after injection of 0.6 mg/kg i.v. riluzole and the bottom trace the response at 25 minutes post injection. The response to 5-HT was still present at 25 minutes post injection, although the spontaneous firing rate had fallen, suggesting that riluzole does not block 5-HT receptors.



The same was seen in the cortical neurons: Ach-evoked responses in cortical neurons although were decreased by about 50% of control after 0.6mg/kg i.v. injection of riluzole, the spikes were still present. It was concluded that riluzole (i.v. or i.p.) depresses spontaneous firing rate of cells in substantia nigra and 5HT in raphe nuclei. However, the same doses of riluzole that completely abolished evoked responses in facial neurons to EAA did not affect evoked responses to Ach in cortex or to 5HT in raphe nuclei.

The Mnd mouse model was used to test the neuroprotective effect of riluzole (Biol 429; Dec 1993). These mice are a substrain of C57BL/6 that show genetic progressive degeneration of motoneurons with death at about 9months of age. Riluzole was orally dosed at 8mg/kg to 6months old Mnd mice for 5d/wk for 15wks. Mice were assessed weekly for motility in an open-field; crossings and rearings during 3min period. One wk after end of study, mice were killed and section of the spinal cord and brain were fixed and examined histopathologically. The areas examined included: hypoglossal nucleus, cervical, thoracic, and lumbar spinal cord, sections were stained with H&E and one section from each area was immunostained with GFAP antibodies to detect astrocytic proliferation. Total no. of sections examined was 3 in the hypoglossal nucleus and 12 each for the spinal cord regions. Motility was significantly increased in riluzole treated Mnd mice during wks 10,11,12,14, and 15 i.e. at 7.5months old; rearings also increased on wks 10 and 15.

Gr.	Treat. wk	Activity Sq.crossed/3min)	Rearings (events/3min)
cont	10	21.0±4.4	2.2±0.8
rilu		37.4±6.5	7.6±2.2
cont	11	21.3±4.5	3.2±1.1
rilu		41.0±6.1	6.0±1.3
cont	12	28.0±4.6	4.0±0.7
rilu		45.0±6.1	6.3±2.2
cont	14	20.0±2.5	18±1.0
rilu		32.1±3.3	4.0±1.2
cont	15	18.1±3.3	1.0±0.5
rilu		32.2±4.4	5.0±1.0

p<0.05 or 0.01 Student's t-test.

The sponsor indicated that there were no differences in motoneuron morphology between riluzole treated and control Mnd mice and, the patterns of anti-GFAP staining were similar. Criteria used included presence or absence of Nissel granules, lipofuscin, chromatolysis, eccentric nuclei, and distorted cell shapes. Although there were no differences in cell morphology, the number of lumbar spinal motoneurons was significantly higher in riluzole gr (9.1±2.5cells/section; p<0.05; n=94cells/mouse), vs. the control (5.7±2.0; n=88). No difference in motoneuron no. of the other spinal cord regions or the hypoglossal nucleus. It was concluded that treatment of Mnd mice with riluzole at 8mg/kg/d for 5d/wk/15wks improved motility and increased no. of lumbar motoneurons compared with control animals but had no effect on cell morphology or motoneurons in the medulla or other spinal cord regions.

Neuroprotection:

1. Neuroprotective effects in a model of focal cerebral ischemia in the rat, /June 1993/France.

Male Fischer rats underwent mid-cerebral artery occlusion (MCA) surgery. Two

groups of rats (11 and 13) were injected riluzole i.p. at 4 and 8mg/kg at 0.5 and 24.5hr post MCA occlusion. Two parallel groups (11 and 14) were used as the control under the same experimental conditions. Histopathological exam on serial coronal sections was done on these rats 72hr post MCA occlusion. Damaged brain areas were assessed by image analyzer. Table below from the sponsor shows the data:

Treatment			Infarct size (mm ²) 72 hours post occlusion		
Drug	Dose (mg/kg I.p.)	Number of rats	Cortex	Striatum	Total
CONTROL	0	14	89.2 ± 8.1	12.7 ± 1.2	111.9 ± 8.9
RILUZOLE	8	13	71.3 ± 8.8*	13.1 ± 0.8	84.4 ± 8.1*
CONTROL	0	11	94.8 ± 12.5	28.2 ± 2.9	122.8 ± 12.7
RILUZOLE	4	11	68.1 ± 10.1	22.4 ± 3.4	90.5 ± 10.5

* Significantly different from control : P ≤ 0.05 (Student's t test).

Riluzole at either dose had no effect on the striatum but significantly reduced cortical infarct area by 28% at 2x8mg/kg but had no effect on this area at the 2x4mg/kg. The total protection (cortex+striatum) at 8mg/kg reached statistical significance with infarct size reduced by 84% compared with the control.

2. Neuroprotection by i.v. in MCA occlusion in the rat/
1994/France.

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Experimental protocol was the same as above (Biol# 388); n=15/gr. Intravenous injection of riluzole at 4 and 8mg/kg 30min post MCA occlusion significantly and dose-dependently reduced infarct size of the cortex by 22-27% of the control measured 72hr post MCA occlusion. The total (cortex+striatum) at 8mg/kg only was also significantly different from the control (table below from the sponsor).

Effects of riluzole on focal cerebral ischaemia in the rat

Treatment administered 30 min
after middle cerebral artery occlusion

MEANS ± SEM			Infarct size (mm ²) 72 hours post occlusion		
Drug	Dose (mg/kg I.v.)	Number of rats	Cortex	Striatum	Total
CONTROL	0	15	112.9 ± 11.5	21.0 ± 1.4	133.3 ± 12.0
RILUZOLE	1	15	131.0 ± 9.5	23.0 ± 0.8	153.8 ± 10.1
RILUZOLE	2	15	130.7 ± 16.2	22.3 ± 1.3	152.3 ± 17.1
CONTROL	0	13	118.8 ± 13.8	18.8 ± 2.4	144.8 ± 12.4
RILUZOLE	4	15	90.8 ± 10.8*	18.8 ± 1.7	108.0 ± 11.8
RILUZOLE	8	14	85.3 ± 13.7*	18.3 ± 1.8	103.8 ± 15.1*

* Significantly different from control : P ≤ 0.05 (Dunn test).

3. Effect of riluzole on focal cerebral ischemia in rats/Article in Eur.J.Pharmacol. 230:209 1993.

Sprague-Dawley rats were used here in contrast to Fischer used in the above 2 studies. In this study, 6 grps were tested (n=12-15/grp): first 3 composed of sham-operated controls, rats dosed 4mg/kg, and rats dosed 8mg/kg; the other 3 grps included MCA occluded rats: control, 4mg/kg, and 8mg/kg rats. Riluzole was dissolved in HCl and diluted in saline and injected i.p. 30min post MCA or 30min post arachnoid removal in sham-operated rats. Neurological deficits were assessed on day 3 after MCA occlusion by the following parameters: equilibrium tests, grasping and righting reflexes, motility, limb flexion, and thorax twisting; normal rat gave global neurological score (GNS) of 22. Also, examined was passive avoidance test and histopathological evaluation of damaged brain areas. In sham-operated rats, riluzole had no effect on GNS or on the neurological parameters. Control MCA occluded rats showed a significantly less GNS than the sham-operated controls (12 ± 0.5 vs. 21 ± 0.7 respectively, $p < 0.01$) and ischemia markedly reduced the various neurological parameters. In the sham-operated rats, riluzole at either dose, did not affect the response latencies (acquisition and retention) in the passive avoidance test; a significantly shorter latencies were seen between the MCA occluded controls and sham-operated controls. Riluzole at 8mg/kg in the MCA occluded rats, significantly reduced (38 ± 24 sec; $p < 0.01$) the latency response to acquisition in the second test run compared with either the control MCA occluded rats or the 4mg/kg grp (184 ± 37 and 157 ± 38 sec respectively). The following rats died 1hr post surgery: 1 control MCA, 2 MCA rats injected 4mg/kg and 3 MCA rats injected 8mg/kg. The volume of cortical ischemic damage at various coronal levels in the MCA rats, was significantly reduced by the 4mg/kg riluzole (94 ± 12 mm³) and more efficient protection at 8mg/kg (73 ± 15 mm³ vs. 139 ± 8 mm³ for control). The protection was, similar to the above studies, only in the cortex without an effect on the striatum, in fact there was some worseninig of the infarct size in the striatum (61 ± 3 vs. 46 ± 3 and 48 ± 3 mm³ in the 4mg/kg and control MCA occluded rats respectively). Mean arterial pressure was measured and found to be 40% reduced after 80mg/kg riluzole in MCA occluded rats that lasted for only 3min. It was concluded that 4 and 8mg/kg i.p. riluzole injected 30min post-MCA occlusion in the rat, reduced significantly the cortical but not the striatal infarct. Striatal necrosis was in fact increased by the 8mg/kg riluzole (61 ± 3 mm³ vs. 49 ± 3 mm³ in the control). The neurological parameters and memory were not improved by riluzole.

4. Effects of post-ischemic administration of riluzole on hippocampal neuronal damage in gerbils/ /Sep 1989/France.

Transient bilateral carotid occlusion (BCO) was performed on female Mongolian gerbils (n=7-8). Riluzole was administered at 4mg/kg i.p. 30min, 4.5, 24.5, and 28.5hrs post-occlusion. The drug was prepared in HCl/saline solution. The control group received the vehicle in the same manner as the drug grp. It is noted that 1 riluzole-treated gerbil died after surgery. Animals were killed 15ds after the BCO and neuronal damage was assessed qualitatively by histopathology (serial coronal sections), and quantitatively by autoradiography of muscarinic and somatostatin receptors in the hippocampus. The BCO caused a marked necrosis of Hippocampal cells. Riluzole prevented CA1 cell necrosis (1.1 ± 0.6 vs. 4.8 ± 0.8 in controls; mean of pluses for each animal; intensity of necrosis increases from + to +++)(see figure from sponsor). Autoradiographic quantification of ³H-QNB binding sites in the control ischemic gerbils showed a remarkable decrease in muscarinic receptors. Riluzole significantly reduced the loss of muscarinic receptors (4.8 ± 0.17 vs. 4.1 ± 0.2 in ischemic and 5.6 ± 0.2 pmol/mg protein in control). Somatostatin receptors were also decreased markedly in the ischemic animals, riluzole reduced such loss in the CA1 area but not in other areas, compared with the

ischemic animals (0.074 ± 0.006 vs. 0.054 ± 0.012 optical densities) [see autoradiograph from the sponsor].

Comment:

Riluzole was administered at different times after BCO, the data presented did not indicate whether administration time played any role in riluzole protection.

Brid #95
post-ischaemic neuronal
damage in gerbils
Neuroprotecti- by
riluzole

FIGURE 1

**CONTROL
GERBIL**



**ISCHAEMIC
GERBIL**



**ISCHAEMIC
GERBIL
+ RILUZOLE
POST-OCCLUSION**



**CORONAL SECTIONS OF HIPPOCAMPAL FORMATION
Cresyl violet staining × 25**

5. ECoG of riluzole administered i.p. after induction of ischemia in gerbils/ /Aug 1990/France.

Male Mongolian gerbils were implanted with screw electrodes for ECoG recording in the right occipito-parietal regions of the cranium. Ischemia was induced by BCO for 6min that was followed by saline infusion. Riluzole was prepared in HCl/saline solution and administered by i.p. at 0.5, 4, 24, and 28hr post BCO. Grps tested included: ischemia control, sham operated, ischemia + riluzole at one of 3 doses: 2, 4, 8mg/kg, sham operated, and sham operated + riluzole 8mg/kg. ECoG was recorded at 1hr and upto 28d post BCO. Mean change in distribution of ECoG activity was recorded at frequency bands between 0-4, 4-7.5, 7.5-12, and 12-20Hz. Exaggeration of slow wave activity is indicative of cerebral infarction and EEG can be used (together with other clinical manifestations) to locate the lesion site. Riluzole showed some protection (preservation of a more normal EEG activity) at 4mg/kg on days 5-10 at low-mid frequencies (0-4, 7.5-12, 12-20Hz; $p < 0.05$). The effects were more remarkable at 8mg/kg at starting on day 1 to day 14 in slow wave of 0-4Hz and mid band 7.5-12Hz, at high frequencies of 12-20Hz, protection was seen on day 2-7; no effect at 4-7.5Hz. The sponsor indicated that protection at higher frequencies is indicative of improvement of learning, a proposal that was supported by histopathology in the CA1 area of the hippocampus observed after riluzole treatment in previous study. Also, protection at 7.5-12Hz (alpha band) has been reported to correlated with increase in blood flow and oxygen utilization (Nagata et al., 1989). Results were also evaluated by the total power of the ECoG signal, but interpretation was difficult due to technical problems.

Comment:

Riluzole was administered at different times after BCO, the data presented did not indicate whether administration time played any role in riluzole protection.

6. Neuroprotection by the oral route in a model of chorea in the rat/ /Aug 1994/France.

Male Sprague-Dawley rats were injected i.c.v into the striatum with 73nmol/ul quinolinic acid. Riluzole was dosed orally to rats (n=9-10/gr) 30min after injection of quinolinic acid at 1, 2, 4, and 8mg/kg (riluzole was prepared in HCl/water solution). Damage was assessed histologically and quantified by image analyzer to obtain area and then total infarction volume (with correction for edema). Control animals showed marked lesions after quinolinic acid injection that were limited to the striatum ($20 \pm 2.2 \text{mm}^3$); riluzole at 1 or 2mg/kg had no effect. Riluzole at 4 and 8mg/kg significantly, $p < 0.05$, reduced the size of the damaged area by 34-35% of the control ($17 \pm 2.3 \text{mm}^3$ for both doses vs. $26 \pm 3 \text{mm}^3$ in the control (2 sets of controls were used)).

7. Protection by i.p. route against KCN-induced histotoxic anoxia in mice/ /July 1994/France.

Riluzole produced a dose-dependant increase in survival of mice during the 30min observation period that followed 3mg/kg i.v. injection of KCN. Doses of 2 and 3mg/kg were not effective whereas, 4, 5, and 6mg/kg increased survival as follows: 3/6, 5/6 and 6/6 mice; ED_{50} was 4.6 (2.7-7.7mg/kg p.o.). Riluzole was prepared in HCl/water solution.

8. Protection by i.p. route against hypobaric hypoxia in mice/
541/July 1994/France.

CD-1 mice (3/sex/dose) were administered riluzole or the vehicle (HCl/water) i.p. 30min prior to placing them in a decompression chamber. The chamber was decompressed to 200hPa at 1.17hPa/sec for 6min. Survival was measured and a mouse surviving longer than 2s.d. above the mean survival of the controls, was considered to have been protected. Riluzole increased survival at doses $\geq 4\text{mg/kg}$ ($p < 0.05-0.001$) with ED_{50} of 3mg/kg (25.2mg/kg , i.p.)

Receptor Binding Studies:

RPR conducted a receptor binding assay using rat cerebral cortex membranes, hippocampus, striatum, brain w/out cerebellum and g.pig cerebellum and brain w/out cerebellum (Biol 452/Jan 1994). Receptor sites studied included: 5HT_{1&2}, DA₂, adrenergic, opiate/sigma, H₁, Ca-channels/verapamil-sensitive, and Na-channels/batrachotoxin-sensitive. Riluzole did not show affinity to any of these receptor sites (inhibition of specific binding was $\leq 30\%$) when tested at concentrations between 0.5 to 100uM. At 100uM, riluzole caused 82% inhibition of Na-channels in 1 of the 3 membrane preparations. NOVASCREEEN was performed on a total of 72 receptor sites including regulatory sites, immunological factors, 2nd messengers, etc. (Biol 597/Oct 1994). Up to 10uM, riluzole did not show high/specific affinities for any of the studied sites except as noted: at 100uM riluzole caused 52-60% inhibition of angiotensin II type 1, 49-53% NK₂ neurokinin, 82-88% DA uptake, 78% progesterone, 63-97% batrachotoxin-sensitive Na-channel, 84% Cl channels. Conflicting results noted at 10 and 0.1uM on the Na-channel, in one evaluation, these concentration caused 88 and 50% inhibition whereas, in the 2nd assay, riluzole was inactive at these levels. Riluzole was re-tested later by NOVASCREEEN and found to be inactive at these concentrations but showed 61 to 63% inhibition at 100uM.

Effects on Motor Activity:

Riluzole administered orally at 3mg/kg to Sprague-Dawley rats had no effect on spontaneous locomotor activity, slight but statistically significant effect at 10mg/kg and, a significant reduction at 30mg/kg which is considered as the minimum ED (RPR-Biol 590; Feb 1995). The locomotor activity was assessed by the total distance travelled and total time spent immobile for each min. during 30min period; results were compared with those obtained from the vehicle and diazepam-dosed rat groups. Riluzole's effect at 30mg/kg was comparable and paralleled that caused by diazepam at 10mg/kg p.o. The impairment of locomotion in the rat occurred at doses that are 6-18x the maximum anticipated human daily dose.

Effects on Behavior:

1. Effects on behavior in rats after i.v. (/Dec 1993), or oral administration (/Dec 1994)/France.

The Irwin (1968) check list for behavior and neurological effects was used to test the activity of riluzole. Riluzole given i.v. was prepared in poloxamer/saline and injected at 1ml/min into the tail vein of rats (n=6) at 1, 3, or 10mg/kg. In the oral study, riluzole was prepared in polysorbate 80/distilled water and dosed at 3, 10, or 30mg/kg. The behavioral test was a 50min observation of various parameters that lasted for 10min after i.v. and 30min after oral dosing. Riluzole at 10mg/kg i.v. caused slight dyspnea and sedation (ataxia and impaired gait). Following 30mg/kg orally, some myorelaxation was observed. Riluzole had no effect on any other parameter at the other doses tested in this paradigm.

Riluzole was tested in standard psychopharmacological tests in mice or rats (Biol 486/June 1994). Tests included the following:

1. anxiolytic: 4-plate, inhibition of PTZ discrimination, waterlick conflict, operant conflict.
2. sedative and muscle relaxant: electric foot shock-induced fighting, rotarod, grasping test, righting reflex.
3. sleep.
4. discriminative properties.
5. neuroleptic effects: hypothermia induced by low dose of apomorphine, stereotypy induced by amphetamine.
6. anti-depressant: potentiation of 5-HTP induced tremors, tetrabenazine ptosis.
7. appetite suppressant.

Riluzole shortened ($p < 0.05$) the wake cycle in rats at 0.5, 1, and 2mg/kg orally, and prolonged REM and SWS at 1 and 2mg/kg ($p < 0.05$ or 0.01). Also at 2mg/kg the sleep latencies were significantly reduced. These sleep effects occurred at doses that were less than those which caused loss of righting reflex (sedative and general anesthetic effects) (20mg/kg i.p. in rats and 44mg/kg p.o. in mice). Riluzole is not a muscle relaxant as indicated by the results in the rotarod or grasping tests. ED_{50} for rotarod in the mouse were 11mg/kg vs. 1mg/kg for diazepam and 0.22mg/kg for MK801 i.p., in rats the values after p.o. route were 8mg/kg vs. 6.8mg/kg for diazepam. For the grasping test, ED_{50} for riluzole in mice was 14mg/kg i.p. vs. 9mg/kg for diazepam. Riluzole showed some anti-aggressive effect in mice (suppression of fighting behavior) with ED_{50} of 5mg/kg p.o. and 8.7mg/kg i.p.. However, this effect was weaker after p.o. dosing with ED_{50} of 18mg/kg vs. 1mg/kg for diazepam. In spite of this anti-aggressive effect, riluzole was not anxiolytic in the 4-plate test in mouse, PTZ discrimination test in rat, or the operant conflict test in rat. However, riluzole showed some activity in the waterlick conflict test in rat that was non-dose-dependent, and low doses of 0.5-2mg/kg p.o. antagonized the anxiogenic activity of FG 7142, which is a partial inverse benzodiazepine agonist, in the operant conflict test. Trained rats discriminated between riluzole and saline. Eleven of 20 riluzole-treated rats maintained discriminative cue throughout the study. Results of generalization test showed that riluzole discriminatory stimuli were dose-dependent with an oral ED_{50} of 1mg/kg (Biol 486). Also, discrimination was not dependent on administration route as responses were also seen with 2mg/kg i.p. doses that showed 90% of rats generalized. Carbamazepine produced dose-dependent generalization with ED_{50} of 8mg/kg i.p. however, MK 801 did not generalize to riluzole at 0.1mg/kg i.p. Higher doses of MK 801 (0.3mg/kg i.p. showed PCP-type stereotypy (see table from the sponsor). This finding indicated that riluzole unlike MK801, is not a PCP-like cpd. Riluzole was not anti-depressant or neuroleptic and did not cause ataxia or stereotypy upto 10mg/kg i.p.

DISCRIMINATION TEST : DRUG GENERALIZATION TO RILUZOLE

Drug	Dose mg/kg i.p.	Rats selecting riluzole lever		Percentage control rate	Assay
		Rat number	Per cent		
Riluzole	1	1/8	12.5	98.6	MA 1828
	2	9/10	90	105.6	MA 1802
Carbamazepine*	2.5	2/8	25	103.4	MA 1863-1855
	5	6/11	54.5	101.8	MA 1852-1853
	10	4/10	40	93.3	MA 1836
	20	7/10	70	91	MA 1828
MK 801	0.1	0/8	0	91.5	MA 1849
	0.3	no response		0	MA 1825

* carbamazepine ED_{50} : 8.1 mg/kg i.p.

Anticonvulsant Effects:

Riluzole protected against seizures induced by:

- EAAs in rats such as glutamate, aspartate (ED₅₀ 2.5mg/kg i.p. or p.o.), DL-homocysteic acid (ED₅₀ 5mg/kg i.p.)
- electroshock in mice (ED₅₀ 4mg/kg i.p.), rats ED₅₀ 0.8mg/kg i.p.); or kindling in rats (<1.25mg/kg i.p.)
- inhibitors of glutamate uptake: ED50₅₀ 3mg/kg i.p. vs. ouabain and at ED₅₀ of 2.5mg/kg i.p. vs. harmaline.
- inhibitors of glutamic acid decarboxylase ED₅₀ 6mg/kg against tonic seizures induced by mercaptopropionic acid in mice and against seizures induced by isoniazid in rats ED₅₀ 4mg/kg i.p.
- audiogenic seizures in genetic DBA/2 mice with ED₅₀ of 1.2mg/kg i.p.

However, riluzole was ineffective against seizures induced by blockade of GABA ionophore complex; a finding unlike diazepam, phenobarb, or valproic acid. In another study (Biol 438/Dec 1993), i.v. injection of riluzole between 0.025-0.4mg/kg to rats protected against electroshock-induced convulsions with an ED₅₀ of 0.056mg/kg. Phenobarb used as a reference cpd produced 100% protection at 10mg/kg p.o. (ED₅₀ 7mg/kg). Tolerance to the anticonvulsant effect of riluzole was tested in mice orally dosed with riluzole at 8mg/kg for 8 and 16 days. These results were compared to those from a single dose administered to another set of rats. Riluzole following 8-day oral dosing to mice, protected against supramaximal electroshock with ED₅₀ of 7mg/kg compared with 6.6mg/kg after a single dose. Similarly, after 16-day, riluzole ED₅₀ was 8.5mg/kg vs. 8mg/kg for a single administration. These results indicate that riluzole does not induce tolerance to its anticonvulsant effect with repeated dosing in mice.

Drug Interactions:

Interaction with other drug:

June 1994/France.

Effect of riluzole on hypnotics, neuroleptics, or ethanol was tested at doses between 1-25mg/kg p.o. and 3-12.5mg/kg i.p.

- Hexobarbital sleeping time: male CD-1 mice were administered riluzole at 6.25-25mg/kg p.o. or 3-12.5mg/kg i.p; another set of mice received 1&3mg/kg diazepam i.p. These drugs were dosed 30min prior to 50mg/kg i.v. hexobarbital. Sleep time was assessed by the duration from onset of loss of righting reflex to recovery; the dose that produces 100% increase in mean duration of sleep over the control was the ED₁₀₀. Riluzole potentiated hexobarbital sleeping time dose-dependently and via both routes with ED₁₀₀ of 13.5mg/kg p.o. and 9.5mg/kg i.p. compared with 1mg/kg for diazepam.

- Effect on the neuroleptic CPZ in mice and rats: CD-1 mice (m+f), and male CD rats were dosed (p.o. or i.p.) 30min prior to 5-10mg/kg s.c. of CPZ. [note that this dose of CPZ did not induce loss of righting reflex]. Mice and rats were tested 90min post dosing; those that did not sit upright within 20sec were considered to not have an effect on the righting reflex. Riluzole was ineffective in either mice or rats in affecting the righting reflex. ED₅₀ for riluzole in mice was 13mg/kg p.o. vs. 1.8mg/kg p.o. diazepam, the value in rats was ≥16mg/kg p.o. for riluzole vs. 15mg/kg p.o. for chlordiazepoxide.

- Effect on alcohol/loss of righting reflex: riluzole did not potentiate the effect of ethanol assessed by righting reflex; ED₅₀ >16mg/kg p.o. vs. 1.3mg/kg p.o. diazepam.

Activity of Metabolites:

1. The sponsor conducted a receptor binding profile for the following riluzole metabolites (#RPR/Biol-CRVA 647; Mar 1995): RP64821 (methoxyphenylurea derivative), RP65077 (hydroxybenzothiazole derivative), RP69597 (benzothiazolyurea derivative), RPR109792A (hydroxybenzothiazole derivative). The concentrations tested were 0.5, 5, 10, and 100uM using 3 membrane preparations (cortex, striatum, and hippocampus). Also tested were RP65331 (hydroxybenzothiazole derivative) at 10uM in only 1 membrane preparation, and RP65110 (hydroxybenzothiazole derivative) and RP112512 (aminobenzothiazole derivative) each at 10uM. Metabolites RP65077, RPR109792A, and RP64821 did not show any binding to any of the receptors tested at concentrations between 0.5-100uM. Metabolite RP69597 showed 58% (SD was not reported) inhibition of binding at 10uM for Na-channel receptors and at 100uM it produced 62±13% inhibition of Na-channel, 47±7% inhibition of the Ca-channels, and 48±3% inhibition of alpha2 receptors. RP65331 inhibited muscarinic receptors by 53% at 10uM and RP65110 produced 50% inhibition of NE uptake at 10uM but RPR112512 did not show any inhibition at this concentration. It is concluded that the above 7 metabolites of riluzole did not show any significant binding to the following receptors at 10uM: adrenergic alpha1&2 and beta receptors, muscarinic, serotonergic1, D2, opiate, histamine, Na-channel (batrachotoxin-sensitive site), or Ca-channel (verapamil site) except for:

RP65331 and RP69597 for muscarinic receptors (53%) and Na-channel (58%) respectively, and RP65110 inhibited NE uptake by 50%.

2. In vitro transmembrane electrophysiology in g.pig purkinje fibers for RP65077 and RP65110 (RPR-Biol-CRVA 642 and 643; Mar 1995). Both metabolites at 0.3uM did not affect action potential amplitude, duration, upstroke velocity (V_{max}), or resting membrane potential of perfused g.pig purkinje fiber isolated from the cardiac left ventricle. However, at 3 and 30uM, both metabolites significantly and concentration-dependently reduced V_{max} and AP duration but these effects were independent on the pacing frequency (in this study 0.5 and 6Hz). These effects indicate that these 2 cpds similar to riluzole are Na-channel blockers in cardiac cells in vitro.

3. Activities of the following 7 metabolites of riluzole were tested in vitro and in vivo: RP64821, RP65077, RP65110, RP65331, RP69597, RPR109792A, and RPR112512. The Na-channel blocking effect was investigated in the classical in vitro model of inhibition of veratridine-induced release of DA in rat striatal slices, and the anti-epileptic activity was tested by the ability to antagonize seizures induced by intracerebral injection of MSG in rats. Metabolites RP65110, RP65077, and RPR112512 inhibited the veratridine-induced DA release at an IC_{50} of 1.76, 0.72, and 8.74uM respectively, (that for riluzole is 3.5uM), 80-95% inhibition occurred at concentrations ranging between 10-30uM of these 3 metabolites. Similar to riluzole, these metabolites were inactive when K was used as the depolarizing cpd indicating the Na-channel blocking effect. When riluzole 0.6-5mg/kg or its metabolites (2.5-20mg/kg) were injected i.p. to rats 30min prior to i.c.v. injection of MSG, the following cpds inhibited MSG-induced seizures: riluzole at 2.5 and 5mg/kg protected 3/6 and 5/6 rats respectively, with ED_{50} 2.5mg/kg, RP65110 protected 2/6 and 4/6 rats at 5 and 25mg/kg respectively, with ED_{50} 7.3mg/kg, RPR112512 showed 1/6, 5/6, 6/6 protection at 5, 10, and 20mg/kg respectively, with ED_{50} 3.5mg/kg. It is concluded that 3 metabolites blocked the voltage-dependent Na-channels in the rat striatal slices but only 2 of these metabolites blocked seizures induced by i.c.v. injection of MSG in vivo. In both tests, the potency of these metabolites were similar to that of riluzole.

Cardiovascular effects:

1. CVS effect after oral dosing in the conscious dog,
June 1994 report date but the experiment was done in Sep-Nov of
1983/France.
Batch# 4PC 164

Mongrel dogs (5/sex) were implanted with chronic catheters (via the femoral artery and channeled into the aorta) to measure systolic and diastolic pressures before and after a single oral gavage dose of 30mg/kg riluzole. Also measured were behavior, HR, and EKG (PR, QRS, and QTc) before and 3hr postdosing. Riluzole was dissolved in 0.1M HCl and distilled water. Data were collected at 5min predose and 15,30,60,120, and 180min postdose; animals were killed at end of study. It is noted that there was no vehicle control in this study, each dog acted as its own control.

Riluzole at 30mg/kg oral dose did not affect EKG or HR. Two dogs out of 5 showed an increase in SBP during the 15-120min and a 3rd dog the SBP increased during the 60-120min; values were normal at the other times. One dog showed higher DBP at 15,30,60, and 180min postdose. These changes for SBP ranged between 210-240mmHg vs. 190-198mmHg pre-dose, and for DBP values were 120-136mmHg vs. 84mmHg predose. It is concluded that a single oral dose of 30mg/kg riluzole has no effect on these CVS parameters upto 3hr postdose. The sponsor reported that salivation and sedation were seen in these dogs (data not provided).

2. CVS effect by i.v. route in conscious dogs with pre-existing myocardial ischemia,
June 1994 report date; experiment was done on
Jan-Mar 1984/France.
Batch# 4PC-164 or 3080-65

One male and 3 female Mongrel dogs were subjected to myocardial ischemia as follows: the anterior descending left coronary artery was partially occluded (80%) for 30min followed by complete ligation and intracardiac left ventricle injection of lidocaine to decrease the probability of early ventricular fibrillation. A catheter was inserted into the aortic arch to measure aortic pressure. Animals were allowed to recover for 16-24hr. Riluzole at 3mg/kg was infused into the saphenous vein over 5min and the aortic pressure was monitored by telemetry; the dog's behavior was also monitored. The parameters measured included EKG, HR, MAP, aortic pressure (AP), and sinus rhythm (SR) (QRS #/min. preceded by normal P wave).

Riluzole i.v. infusion caused HR to decrease in 2 females when measured at 1 and 2min postdose in one dog (132 & 138BPM vs. 156BPM in predose) and at 1-4min in the 2nd dog (105-129BPM vs. 168BPM in control). However, an increase noted in the male dog at 5,7.5, and 10min postdose (156-165BPM vs. 147BPM in predose) and at 0.5min of dosing in the 3rd female (204 vs. 162BPM in predose). The SR was increased in all 4dogs as early as 0.5min postdose and lasted till 15min (except for one dog till 7.5min). There was no change in MAP. It is concluded that riluzole administered by i.v. infusion at 3mg/kg to Mongrel dogs, slightly decreased HR and stimulated SR for a short period (in one dog only for 1min in another 2 dogs upto 15min). The SR increased from 18±12BPM initially to 86±34BPM at 0.5min of infusion. There was an 11% increase in MAP. The sponsor indicated that salivation (2dogs) and emesis (2 other dogs) were observed (data not provided).

3. Effects on bronchopulmonary resistance and compliance in anesthetized guinea pigs by i.v. route. /Dec 1993/France.
Batch# GRV 1044; MIJ 430.
This study was conducted within the "spirit" of GLP.

Male Hartley g.pigs (400-450g) were anesthetized, trachea removed and cannulated but animals were breathing spontaneously, left jugular vein catheterized for i.v. injections of drugs, and r.carotid cannulated for BP measurements and catheter introduced to the thoracic cavity for measurement of pulmonary pressure. The animal was then placed in a body plethysmograph fitted with a pneumotachograph and a differential pressure transducer. The vehicle used for riluzole was poloxamer 188. Riluzole at 0.03, 0.3, or 3mg/kg was infused at 0.2ml/kg/min over a 30min period to 4 pigs per dose. Baseline bronchopulmonary resistance, compliance, and respiratory rate were measured at 10min intervals for upto 90min postdosing. Same protocol followed for the vehicle and the saline control. Each parameter value was calculated as the mean of 20 signals over 2min and presented as mean±sem; pulmonary resistance and compliance presented as absolute values.

Riluzole caused a small but significant ($p < 0.05$; Student t-test) decrease (3-6%) in pulmonary resistance at 0.03mg/kg starting at 40min to 90min postdosing; HD also caused a decrease (2-6%) from 10-90min postdosing but the values did not reach statistical significance. The bronchopulmonary compliance was reduced by all 3 doses of riluzole compared with the vehicle but none reached statistical significance. In general, these effects were small and not dose-dependent therefore, it can be concluded that riluzole had no significant effect on bronchopulmonary parameters at these doses; however, a drug effect was seen at LD.

4. Respiratory effects by the i.v. route in anesthetized dogs,
June 1994/France.
Batch# 3080-65

Four female Mongrel dogs, 13-18kg, were anesthetized and the trachea and saphenous vein cannulated. A differential air pressure transducer was fitted to the trachea to measure pulmonary air flow (V), a 2nd cannula with a tip balloon was positioned in the oesophagus to measure pulmonary oesophageal pressure (EP). The signals from these 2 parameters, V and EP, were fed into a computer to compute dynamic pulmonary compliance (C)=(dV/dt) and total pulmonary resistance (R)=(EP/V). Both systolic and diastolic aortic pressures were also monitored as well as HR and respiratory rate (RR). Riluzole in 0.1M HCl was infused at cumulative doses of 1, 2, 4, and 8mg/kg or 1,1,2, and 4mg/kg, as a bolus over 1min with 10min intervals between injections.

Riluzole at all doses decreased compliance, SAP, and DAP (at cumulative doses >2 these changes were significant at $p < 0.05$; see table below from sponsor). Respiratory rate was significantly increased at 8mg/kg and HR was also increased significantly at this dose compared with the initial values. These changes were mild or minimal at the low dose. These effects were clear in the individual data from each dog; some more affected than others. It is concluded that riluzole infusion at 1mg/kg had no effect on any of the respiratory parameters however, at higher doses, compliance was markedly reduced and RR significantly increased specially at the highest dose. Also, the SAP and DAP were decreased and HR was moderately increased.

**Effects of riluzole on respiratory and cardiovascular parameters
in anesthetized dogs (n=4)**

Effects on the respiratory compliance (C) and resistance (R), the respiratory rate (RR), the systolic (SAP) and diastolic (DAP) aortic blood pressures and on the heart rate (HR).

MEAN±SEM					
Parameters	Initial Values	Maximal Variations from Initial Values (%)			
		Riluzole (mg/kg)			
		1	1	2	4
C (ml/cm H ₂ O)	133±30	-12±2	-12±2	-24±7*	-39±5**
R (cm H ₂ O/l/s)	2.40±0.21	22±7	14±8	25±13	64±18**
RR (insp./min)	7±0.05	15±12	19±12	47±25	70±40
SAP (mmHg)	140±15	-8±33	-2±2	-7±4	-14±6**
DAP (mmHg)	79±12	-5±3	-3±7	-5±2	-10±4*
HR (beats/min)	120±6	17±11	10±7	15±10	23±4*

Significant differences from initial values: *P<0.05 (paired Dunnett's t test).
Values are means ± SEM

5. Effects by i.v. on baseline BP and on hypotensive responses to Ach, salbutamol, and histamine in anesthetized rats/Biol 433/Mar 1994/France.

Male Sprague-Dawley (5-6/gr) were anesthetized with pentobarbital and mean carotid BP was measured every 5min for a total of 1hr postinfusion. Compounds were administered as i.v. infusion over 30min. Groups composed of control (saline), vehicle (Poloxamer), riluzole (0.03, 0.3, 3mg/kg), and another set of rats given saline, vehicle, and riluzole were injected i.v. with Ach (0.1ug/kg), salbutamol (0.6ug/kg), and histamine (2ug/kg). Riluzole at all doses had no effect on baseline BP at any time during or after infusion. However, the vehicle group showed a statistically significant increase in MAP compared with the saline control at 25 and 30min periods and, from time 0 (start of infusion). This increase (11-14%) persisted till 25-30min postinfusion. In the 2nd set of vehicle group, there was no change in MAP at any interval, therefore, the sponsor suggested that the increase in MAP in the 1st vehicle group was a transient finding and perhaps irrelevant. Riluzole did not change the hypotensive effects mediated by cholinergic (Ach), beta adrenergic (salbutamol), or histaminergic (histamine) at any dose.

6. Effects by i.v. on baseline BP and/or responses to electrical stimulation of spinal cord, NE, and angiotensin II (Ang II), in pithed rats, Mar 1994/France.

Male Sprague-Dawley rats were anesthetized briefly with ether and immediately pithed and maintained with artificial respiration. The left carotid was cannulated for BP measurements and the right was ligated and the vag. severed. Groups of 5-6 rats were administered saline, (control), vehicle (Poloxamer), or riluzole (0.03, 0.3, 3.0mg/kg) by i.v. infusion over 30min. Mean BP was measured every 5min for a total of 1hr. Another set of animals (control, vehicle, and riluzole), at 5min postinfusion, were exposed to a train of electrical impulses to the spinal cord followed by i.v. injection of NE (0.125ug/kg) or Ang II (0.125ug/kg) pressor agents. Riluzole did not affect baseline MAP nor the pressor responses mediated by NE or Ang II compared with

the control/vehicle group. However, at the 2 higher doses, riluzole reduced pressor responses to spinal cord stimulation (29-37% of vehicle control, $p < 0.05$; but not different from the saline control). This suggested an effect of riluzole on peripheral sympathetic neurotransmitter release. Reference cpds (prazosin, phentolamine, and losartan), reduced spinal cord pressor response by >50%.

7. CVS effects by the oral route in conscious SHR/ /June
1994/France.

Riluzole was orally administered to 12 male SHR (4-6month's old) at 5 or 10mg/kg dose (in 0.1N HCl) and MAP, ER, and behavior were monitored for upto 6hr postdose. Riluzole did not modify any of these parameters as evident from the mean±SD data (individual data were not provided). There was a small (5-7%) but not significant, decrease in BP and HR at the LD.

8. CVS effects by the i.v. route in anesthetized rabbit/Biol 519/June
1994/France.

Riluzole was injected i.v. to anesthetized rabbits (n=5) at cumulative doses starting with 0.1mg/kg and upto 10mg/kg. The following parameters were measured: mean systolic and diastolic BP, HR, and carotid blood flow (CBF). Riluzole had no effect on any of these parameters at doses upto 1mg/kg. However, at 3 and 10mg/kg, SBP, DBP, and HR were reduced relative to the predrug treatment, CBF was increased though not significantly, from initial readings:

Parameter	3mg/kg	10mg/kg
SBP (mmHg)	-10±9	-25±13
DBP (mmHg)	-18±11	-32±14*
HR (BPM)	-6±2	-20±15
CBF (ml/min)	42±30	52±24

* $p < 0.05$

It was indicated that all HD rabbits died due to respiratory arrest following injection.

9. Hemodynamic and electrophysiological effects by i.v. in anesthetized dogs/1
Mar 1994/France

Riluzole was infused over 30min to anesthetized dogs at 0.03, 0.3, and 3mg/kg (non-cumulative doses). Various CVS parameters were measured: SBP, DBP, HR, CO, SV, TPR, EKG, LVEDP (left ventricular end diastolic pressure), dLVP/dt_{max} (max rate of development of left ventricular pressure, measure of myocardial contractility), His bundle electrogram was recorded by endocavitary route to measure AH and HV time intervals; all parameters were assessed under electrical pacing of 360ms. Riluzole was dissolved in Poloxamer which was administered as the vehicle, and saline was used as the control group. Infusion of the control saline solution had no effect on any parameter except a significant ($p < 0.05$; Paired Student t-test) shortening of the PR interval (-6 to -13ms from initial reading). Infusion of the vehicle had no effect on BP, LVEDP, dLVP/dt_{max}, CO, or TPR. However, the vehicle caused a significant increase in HR (8-16%) compared with initial values and with the saline control (the latter noted during the end of the 3rd infusion), and a decrease

in SV (-11 to -20%), shortening of PR interval (-7 to -11%), slight shortening of AV node effective refractory period, QTc, whereas conduction times (AH, QRS, and HV) remained unchanged; these parameters were compared with the initial values. Riluzole, had no effect on HR, dLVP/dt_{max}, PR, QRS, QTc intervals. However, it slightly but significantly increased (3 to 7% of initial values) SBP and DBP at 0.03mg/kg at 15min of infusion. Riluzole also caused a significant increase in TPR (14-19%), decreased CO and SV (-11 to -15%) at 3mg/kg, although not statistically significant, LVEDP was increased at all doses (15-31%). Like its vehicle, riluzole shortened the AVNERP at the 3mg/kg dose by -7 to -8% of initial values (see tables from the sponsor).

6. TABLES

TABLE 1
Effects of 0.9% NaCl solution administered i.v.
on hemodynamics and surface ECG in anesthetized dogs (n=6)

Effects on heart rate (HR), systolic (SAP) and diastolic (DAP) aortic blood pressures, left ventricular end-diastolic pressure (LVEDP), maximal rate of development of left ventricular pressure (dLVP/dtmax), cardiac output (CO), stroke volume (SV), total peripheral vascular resistance (TPR), PR, QRS and QTc time intervals of the ECG

		MEAN ± SEM		% VARIATION AFTER 0.9% NaCl INFUSIONS					
PARAMETER (unit)		Initial values	1st inf.		2nd inf.		3rd inf.		
			5 min	15 min	5 min	15 min	5 min	15 min	30 min
HR	(beats/min)	121 ±5	2 ±3	3 ±3	3 ±4	5 ±4	3 ±4	6 ±3	5 ±4
SAP	(mm Hg)	99 ±3	6 ±2*	5 ±3	2 ±4	4 ±5	5 ±4	6 ±5	3 ±4
DAP	(mm Hg)	72 ±8	4 ±1	8 ±5	-1 ±5	2 ±5	1 ±5	6 ±8	-1 ±5
LVEDP	(mm Hg)	7.0 ±0.9	-8 ±5	-1 ±6	4 ±11	0 ±3	-5 ±8	-5 ±7	-13 ±6
dLVP/dtmax	(mm Hg/s)	1292 ±96	9 ±3*	3 ±4	3 ±5	4 ±5	8 ±6	9 ±7	10 ±7
CO	(ml/min)	797 ±67	7 ±3	-1 ±3	-2 ±5	0 ±4	5 ±5	3 ±6	2 ±6
TPR	(mm Hg/ml p.min)	0.11 ±0.01	-1 ±4	3 ±2	4 ±5	4 ±3	-3 ±4	2 ±4	-3 ±3
SV	(ml/min p.beat)	6.8 ±0.6	4 ±5	-4 ±3	-4 ±7	-5 ±7	2 ±8	-3 ±7	-3 ±7
PR	(ms)	100 ±6	-5 ±2*	-6 ±2*	-8 ±2*	-11 ±5	-10 ±3*	-12 ±4*	-13 ±4*
QRS	(ms)	38 ±1	0 ±0	0 ±0	0 ±0	0 ±0	1 ±1	1 ±1	2 ±2
QTc	(ms)	238 ±4	-1 ±1	0 ±2	-1 ±3	-1 ±2	-2 ±2	-2 ±2	-2 ±3

Intra-group (* P ≤ 0.05) significance of the deviations from initial values were assessed by the paired Student t test. The inter-group significance of the deviations from values of the 0.9% NaCl group (# P ≤ 0.05) and of the vehicle group (® P ≤ 0.05) were assessed by the non-paired Dunnett t test.

TABLE 2
Electrophysiological effects of 0.9% NaCl solution administered i.v.
in anesthetized dogs (n=6)

Effects on heart rate (HR), on effective (AERP) and functional (AFRP) refractory periods and on diastolic threshold (ADT) of the atrium, on conduction (AH) and effective (AVNERP) and functional (AVNFRP) refractory periods of the atrio-ventricular node, on conductions (HV, QRS, QTnc), effective (VERP) and functional (VFRP) refractory periods and on diastolic threshold (VDT) of the His bundle system and ventricle

MEANS $\pm$ SEM						
TISSUE PARAMETER (unit)	Initial values	% VARIATION AFTER 0.9% NaCl INFUSIONS				
		1st Inf.		3rd Inf.		
		5 min	5 min	5 min	15 min	30 min
SINO-ATRIAL NODE						
HR (beats/min)	121 ± 5	2 ± 3	3 ± 4	3 ± 4	6 ± 3	6 ± 4
ATRIUM						
>REFRACTORINESS						
AERP (ms)	125 ± 3	0 ± 2	-1 ± 1	0 ± 2	1 ± 4	1 ± 4
AFRP (ms)	145 ± 5	0 ± 2	-3 ± 2	0 ± 3	-2 ± 5	0 ± 5
EXCITABILITY						
ADT (V)	0.9 ± 0.1	7 ± 4	9 ± 4	11 ± 9	9 ± 9	10 ± 9
ATRIO-VENTRICULAR NODE						
CONDUCTION						
AH (ms)	82 ± 5	-2 ± 2	-3 ± 4	-10 $\pm 2^*$	-9 ± 4	-10 ± 5
REFRACTORINESS						
AVNERP (ms)	158 ± 5	1 ± 4	0 ± 4	-1 ± 6	-4 ± 5	-1 ± 9
AVNFRP (ms)	235 ± 8	-1 ± 1	-2 ± 1	-2 ± 1	-2 ± 2	-2 ± 2
HIS BUNDLE SYSTEM AND VENTRICLE						
CONDUCTION						
HV (ms)	23 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
QRS (ms)	38 ± 1	0 ± 0	0 ± 0	1 ± 1	1 ± 1	2 ± 2
QTnc (ms)	238 ± 4	-1 ± 1	-1 ± 3	-2 ± 2	-2 ± 2	-2 ± 3
REFRACTORINESS						
VERP (ms)	155 ± 5	2 ± 2	2 ± 1	4 ± 2	1 ± 3	0 ± 2
VFRP (ms)	168 ± 3	1 ± 1	0 ± 2	0 ± 2	-1 ± 1	-1 ± 1
EXCITABILITY						
VDT (V)	0.7 ± 0.1	-6 ± 5	-9 ± 4	-9 ± 4	-2 ± 7	0 ± 9

Intra-group (* $P \leq 0.05$) significance of the deviations from initial values were assessed by the paired Student t test. The inter-group significance of the deviations from values of the 0.9% NaCl group ($\# P \leq 0.05$) and of the vehicle group ($\oplus P \leq 0.05$) were assessed by the non-paired Dunnett t test.

TABLE 3
Effects of vehicle of riluzole (1.5% poloxamer 188 in 0.9% NaCl) administered i.v. on hemodynamics and surface ECG in anesthetized dogs (n=6)

Effects on heart rate (HR), systolic (SAP) and diastolic (DAP) aortic blood pressures, left ventricular end-diastolic pressure (LVEDP), maximal rate of development of left ventricular pressure (dLVP/dtmax), cardiac output (CO), stroke volume (SV), total peripheral vascular resistance (TPR), PR, QRS and QTnc time intervals of the ECG

MEAN $\pm$ SEM		Initial values	% VARIATION AFTER VEHICLE INFUSIONS						
PARAMETER (unit)			1st inf.		2nd inf.		3rd inf.		
			5 min	15 min	5 min	15 min	5 min	15 min	30 min
HR	(beats/min)	115 ± 8	5 ± 2	8 $\pm 2^*$	9 $\pm 3^*$	9 $\pm 3^*$	14 $\pm 3^*$	15 $\pm 3^*$	16 $\pm 3^* \#$
SAP	(mm Hg)	96 ± 3	3 ± 3	4 ± 4	2 ± 3	3 ± 4	4 ± 3	2 ± 3	2 ± 3
DAP	(mm Hg)	75 ± 3	3 ± 4	6 ± 4	2 ± 4	5 ± 5	4 ± 3	3 ± 4	2 ± 4
LVEDP	(mm Hg)	7.6 ± 0.9	0 ± 1.6	-3 ± 1.3	-3 ± 1.3	3 ± 1.3	5 ± 1.1	-3 ± 1.1	-12 ± 6
dLVP/dtmax	(mm Hg/s)	1237 ± 46	1 ± 5	-3 ± 5	-2 ± 6	-4 ± 6	1 ± 5	-1 ± 5	2 ± 6
CO	(ml/min)	775 ± 22	3 ± 4	-6 ± 3	-1 ± 5	-3 ± 4	2 ± 6	-5 ± 6	-7 ± 5
TPR	(mm Hg/ml p.min)	0.11 ± 0.01	4 ± 6	15 $\pm 5^*$	7 ± 9	7 ± 8	5 ± 8	13 ± 15	15 ± 11
SV	(ml/min p.beat)	7.2 ± 0.5	-3 ± 4	-12 $\pm 3^*$	-9 ± 5	-11 ± 6	-11 ± 6	-15 ± 6	-20 $\pm 5^*$
PR	(ms)	101 ± 5	-3 ± 3	-6 ± 5	-7 $\pm 3^*$	-8 $\pm 3^*$	-9 $\pm 2^*$	-11 $\pm 2^*$	-10 $\pm 3^*$
QRS	(ms)	38 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
QTnc	(ms)	253 ± 10	-2 ± 2	-4 ± 2	-5 ± 3	-5 ± 3	-8 ± 3	-8 ± 3	-8 $\pm 3^*$

Intra-group (* $P \leq 0.05$) significance of the deviations from initial values were assessed by the paired Student t test. The inter-group significance of the deviations from values of the 0.9% NaCl group (# $P \leq 0.05$) and of the vehicle group (® $P \leq 0.05$) were assessed by the non-paired Dunnett t test.

TABLE 4
Electrophysiological effects of vehicle of riluzole
(1.5% poloxamer 188 in 0.9% NaCl) administered i.v. in anesthetized dogs (n=6)

Effects on heart rate (HR), on effective (AERP) and functional (AFRP) refractory periods and on diastolic threshold (ADT) of the atrium, on conduction (AH) and effective (AVNERP) and functional (AVNFRP) refractory periods of the atrio-ventricular node, on conductions (HV, QRS, QTnc), effective (VERP) and functional (VFRP) refractory periods and on diastolic threshold (VDT) of the His bundle system and ventricle

MEANS ± SEM						
TISSUE PARAMETER (unit)	Initial values	% VARIATION AFTER VEHICLE INFUSIONS				
		1st Inf.		3rd Inf.		
		5 min	5 min	5 min	15 min	30 min
SINO-ATRIAL NODE						
HR (beats/min)	115 ±6	5 ±2	9 ±3*	14 ±3*	15 ±3*	16 ±3*#
ATRIUM						
REFRACTORINESS						
AERP (ms)	132 ±5	-2 ±3	-5 ±4	-5 ±3	-5 ±3	-3 ±5
AFRP (ms)	147 ±6	0 ±2	-1 ±1	0 ±2	-1 ±2	2 ±2
EXCITABILITY						
ADT (V)	0.9 ±0.1	0 ±0	-1 ±1	-3 ±2	-9 ±2*	-4 ±7
ATRIO-VENTRICULAR NODE						
CONDUCTION						
AH (ms)	83 ±9	-3 ±6	-6 ±7	-13 ±7	-14 ±7	-13 ±6
REFRACTORINESS						
AVNERP (ms)	167 ±5	-7 ±3	-7 ±4	-10 ±3*	-10 ±3*	-9 ±2*
AVNFRP (ms)	237 ±12	-3 ±1	-4 ±2	-6 ±3	-6 ±3	-6 ±3
HIS BUNDLE SYSTEM AND VENTRICLE						
CONDUCTION						
HV (ms)	20 ±2	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0
QRS (ms)	38 ±1	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0
QTnc (ms)	253 ±10	-2 ±2	-5 ±3	-6 ±3	-6 ±3	-6 ±3*
REFRACTORINESS						
VERP (ms)	163 ±5	-2 ±1	-2 ±1	-4 ±3	-4 ±2	0 ±3
VFRP (ms)	175 ±6	-3 ±1#	-4 ±2	-4 ±2	-4 ±2	-2 ±2
EXCITABILITY						
VDT (V)	0.6 ±0.1	-6 ±4	-6 ±4	-10 ±5	-10 ±5	-14 ±5*

Intra-group (* P ≤ 0.05) significance of the deviations from initial values were assessed by the paired Student t test. The inter-group significance of the deviations from values of the 0.9% NaCl group (# P ≤ 0.05) and of the vehicle group (® P ≤ 0.05) were assessed by the non-paired Dunnett t test.

TABLE 5
Effects of riluzole administered I.v.
on hemodynamics and surface ECG in anesthetized dogs (n=6)

Effects on heart rate (HR), systolic (SAP) and diastolic (DAP) aortic blood pressures, left ventricular end-diastolic pressure (LVEDP), maximal rate of development of left ventricular pressure (dLVP/dt_{max}), cardiac output (CO), stroke volume (SV), total peripheral vascular resistance (TPR), PR, QRS and QTnc time intervals of the ECG

MEAN ± SEM		Initial values	% VARIATION AFTER RILUZOLE INFUSIONS (mg/kg)						
PARAMETER (unit)			0.03		0.3		3		
			5 min	15 min	5 min	15 min	5 min	15 min	30 min
HR (beats/min)		130	3	6	1	4	3	3	4
		±4	±1	±3	±2	±3	±3	±3 [⊕]	±3 [⊕]
SAP (mm Hg)		96	4	6	3	6	4	6	2
		±3	±2	±2*	±1*	±2	±2	±3	±2
DAP (mm Hg)		71	3	7	1	6	1	5	-1
		±4	±2	±3*	±2	±3	±3	±4	±3
LVEDP (mm Hg)		5.1	8	15	21	31	21	21	15
		±1.0	±6	±12	±11	±12	±16	±14	±13
dLVP/dt _{max} (mm Hg/s)		1447	2	2	-2	2	-4	-2	-2
		±147	±2	±1	±2	±2	±5	±4	±4
CO (ml/min)		963	-1	-1	-8	-8	-9	-11	-11
		±67	±4	±2	±5	±4	±5	±4*	±4*
TPR (mm Hg/ml p.min)		0.09	6	6	11	12	14	19	15
		±0.00	±4	±4	±7	±6	±5*	±6*	±5*
SV (ml/min p.beat)		7.8	-4	-7	-9	-11	-11	-14	-15
		±0.3	±4	±4	±5	±3*	±4*	±3*	±3*
PR (ms)		89	-4	-4	-6	-5	-5	-6	-7
		±2	±2	±3	±3	±4	±4	±3	±3
QRS (ms)		40	-1	1	2	2	2	2	2
		±1	±1	±2	±2	±2	±2	±2	±2
QTnc (ms)		235	-3	-4	-2	-1	-2	-1	-3
		±4	±1*	±1*	±1	±1	±2	±2	±2

Intra-group (* P ≤ 0.05) significance of the deviations from initial values were assessed by the paired Student t test. The inter-group significance of the deviations from values of the 0.9% NaCl group (⊕ P ≤ 0.05) and of the vehicle group (⊙ P ≤ 0.05) were assessed by the non-paired Dunnett t test.

TABLE 6
Electrophysiological effects of riluzole administered i.v.
in anesthetized dogs (n=6)

Effects on heart rate (HR), on effective (AERP) and functional (AFRP) refractory periods and on diastolic threshold (ADT) of the atrium, on conduction (AH) and effective (AVNERP) and functional (AVNFRP) refractory periods of the atrio-ventricular node, on conductions (HV, QRS, QTnc), effective (VERP) and functional (VFRP) refractory periods and on diastolic threshold (VDT) of the His bundle system and ventricle

MEANS $\pm$ SEM		% VARIATION				
TISSUE PARAMETER (unit)	Initial values	AFTER RILUZOLE INFUSIONS (mg/kg)				
		0.03		3		
		5 min	5 min	5 min	15 min	30 min
SINO-ATRIAL NODE						
HR (beats/min)	130 ± 4	3 ± 1	1 ± 2	3 ± 3	3 ± 3	4 ± 3
ATRIUM						
REFRACTORINESS						
AERP (ms)	125 ± 3	2 ± 3	-2 ± 3	-5 ± 3	-5 ± 3	-4 ± 4
AFRP (ms)	143 ± 3	0 ± 2	-1 ± 2	-6 ± 3	-6 ± 3	-3 ± 2
EXCITABILITY						
ADT (V)	1.0 ± 0.1	-2 ± 2	3 ± 5	7 ± 7	3 ± 8	7 ± 7
ATRIO-VENTRICULAR NODE						
CONDUCTION						
AH (ms)	72 ± 5	-8 ± 4	-6 ± 3	-8 ± 4	-7 ± 4	-11 ± 5
REFRACTORINESS						
AVNERP (ms)	153 ± 2	-5 ± 3	-3 ± 2	-8 $\pm 2^*$	-8 $\pm 2^*$	-7 $\pm 2^*$
AVNFRP (ms)	220 ± 4	-1 ± 1	-2 ± 1	-4 ± 2	-4 ± 2	-4 ± 2
HIS BUNDLE SYSTEM AND VENTRICLE						
CONDUCTION						
HV (ms)	21 ± 1	2 ± 2	2 ± 2	2 ± 2	2 ± 2	2 ± 2
QRS (ms)	40 ± 1	-1 ± 1	2 ± 2	2 ± 2	2 ± 2	2 ± 2
QTnc (ms)	236 ± 4	-3 $\pm 1^*$	-2 ± 1	-2 ± 2	-1 ± 2	-3 ± 2
REFRACTORINESS						
VERP (ms)	160 ± 4	2 ± 2	1 ± 2	-1 ± 2	1 ± 2	-1 ± 3
VFRP (ms)	177 ± 2	0 ± 0	-1 ± 1	0 ± 0	0 ± 1	-1 ± 1
EXCITABILITY						
VDT (V)	0.6 ± 0.0	0 ± 4	0 ± 4	0 ± 4	0 ± 4	-5 ± 5

Intra-group (* $P \leq 0.05$) significance of the deviations from initial values were assessed by the paired Student t test. The inter-group significance of the deviations from values of the 0.9% NaCl group ($P \leq 0.05$) and of the vehicle group ($P \leq 0.05$) were assessed by the non-paired Dunnett t test.

10. Effects on the autonomic nervous system by i.v. in anesthetized dog
June 1994/France.

Riluzole (prepared in 0.1M HCl) was injected via the saphenous vein to anesthetized mongrel dogs (3/sex) at 3mg/kg over 1min. The HR and MAP (mean aortic pressure) were measured in response to vagal nerve stimulation and i.v. bolus of 10ug/kg NE, 10ug/kg Ach, and 10ug/kg histamine before and after riluzole. Pressor responses to 10ug/kg isoproterenol before and after riluzole were also recorded. Riluzole at 3mg/kg had no effect on HR or MAP in anesthetized dogs following cholinergic, adrenergic, or histaminergic stimulation.

11. CVS and behavioral effects by i.v. in conscious dogs,
/Sep, 1994/France.

A telemetry system was implanted in Beagle dogs (1m and 3f/gr) for measurement of MAP* and dogs were trained to stay quiet in a sling. Animals were deprived of food for 24hr prior to the experiment. Riluzole prepared in Poloxamer was infused via the cephalic vein over 30min at 0.03, 0.3, and 3mg/kg, MAP and HR were recorded every 5min over 10sec periods. EKG was recorded over 2sec every 5-6min for 60min, then every 15-16min for the next 4hr and additional recordings were made at 6, 23, and 24hr postinfusion. Hematology and clinical chemistry parameters were evaluated before initiation of the study, as well as clinical signs including tremors, abnormal behavior, defecation, vocalization, respiration, and salivation. Saline infusion had no effect early during infusion, however, at 45min from infusion, SBP, DBP, MAP, and HR decreased compared to initial values (-23, -22, -23, -13% respectively). These effects persisted till 24hr later. Maximum decrease in SBP was -25% at 1hr, and for DBP and MAP was at hr17 at -30 and -28% respectively, and for HR the max decline was -47% at hr19 (see tables from the sponsor). There was no effect on the QRS interval but the QT and PR intervals were prolonged starting at 1hr and lasting till 24hr postadministration; there was no change in animal behavior. The poloxamer vehicle, no effect during infusion but after end of infusion findings were more or less, similar to those of the saline control. SBP, DBP, MAP, and HR were decreased starting at 45min with maximum values reached at 2hr for DBP (-21%), MAP (-21%), at 2.5hr for HR (-40%), and at 19th hr for SBP (-17%); all values compared with initial readings. The PR and QT intervals were prolonged at 1.5hr (14 and 17% respectively), and lasting till 3hr for the PR and till the 4th hr for the QT; the interval was comparable to initial readings after the 3rd hr. Riluzole, had no effect on any parameter during infusion, however, after end of infusion, all 3 doses caused moderate-marked decrease in SBP, DBP, MAP, HR, and increase in PR and QT intervals that increased with dose (see tables). These changes lasted till 24hr postinfusion. The effects at 3mg/kg were maximum: at the 16th hr for SBP (-25%), DBP (-24%), MAP (-25%), and for HR at 2.5hr (-48%), and for the PR and QT intervals at 2.75hr (30 and 24% respectively; all values compared with initial readings). Riluzole effects were similar to those caused by the vehicle but occurred at different times. It was concluded that riluzole has no effect on these CVS parameters when measured by telemetry in conscious dog at i.v. doses upto 3mg/kg. The changes induced by the saline and the vehicle groups which were similar to those observed with riluzole, were contributed to experimental conditions due to restraining the animals in a sling for 30min; it is noted that these animals were previously trained to remain quiet during experimentation.

* MAP calculated as $DBP + (SBP - DBP) / 3$.

CVS

7. TABLES

CVS... v. P. (1/3) 20 in Conscious Dogs

TABLE 1
 Study of the effects of saline (NaCl 0.9 %, 0.6 ml/kg Lv.) in conscious dogs (n=4)
 Effects on systolic (SBP), diastolic (DBP), mean (MAP) aortic blood pressure,
 heart rate (HR), PR, QRS and QT time intervals of the ECG

GROUP 1 PARAMETER	INITIAL VALUE	% CHANGE FROM INITIAL VALUE																			
		INFUSION TIME (min)					TIME AFTER INFUSION (min or h and min)														
		5	10	15	20	25	30	35	40	45	50	55	1h	1h15	1h30	1h45	2h	2h15	2h30	2h45	3h
SBP (mmHg)	101 ± 5	-4 ± 3	-8 ± 0	-3 ± 3	-4 ± 3	-12 ± 2	-18 ± 3	-21 ± 3	-23 ± 3	-24 ± 2	-20 ± 2	-21 ± 3	-23 ± 3	-12 ± 2	-24 ± 2	-22 ± 2	-25 ± 2	-22 ± 2	-18 ± 2	-19 ± 2	-15 ± 2
DBP (mmHg)	111 ± 2	-4 ± 2	-8 ± 0	-2 ± 3	-4 ± 3	-12 ± 3	-13 ± 2	-18 ± 2	-19 ± 2	-19 ± 2	-19 ± 2	-14 ± 2	-22 ± 2	-6 ± 2	-18 ± 2	-28 ± 2	-26 ± 2	-22 ± 2	-14 ± 2	-22 ± 2	-10 ± 2
MAP (mmHg)	140 ± 2	-4 ± 2	-8 ± 1	-3 ± 2	-5 ± 2	-11 ± 2	-15 ± 2	-19 ± 2	-20 ± 2	-22 ± 2	-20 ± 2	-17 ± 2	-23 ± 2	-9 ± 2	-22 ± 2	-27 ± 2	-23 ± 2	-24 ± 2	-15 ± 2	-21 ± 2	-13 ± 2
HR (beats/min)	128 ± 5	0 ± 7	10 ± 8	10 ± 8	7 ± 7	-1 ± 8	12 ± 10	-2 ± 10	14 ± 11	-16 ± 11	-12 ± 11	-9 ± 11	-13 ± 14	0 ± 14	-20 ± 10	-20 ± 10	-30 ± 12	-34 ± 12	-22 ± 10	-28 ± 10	-18 ± 10
PR (ms)	68 ± 4	-3 ± 3	-4 ± 2	-4 ± 2	-4 ± 2	2 ± 3	4 ± 3	4 ± 3	2 ± 3	6 ± 3	6 ± 3	16 ± 2	16 ± 2	12 ± 2	ND	13 ± 2	24 ± 2	18 ± 2	14 ± 2	19 ± 2	26 ± 2
QRS (ms)	37 ± 2	5 ± 3	0 ± 3	0 ± 3	0 ± 3	5 ± 3	5 ± 3	5 ± 3	5 ± 3	0 ± 3	7 ± 3	5 ± 3	ND	5 ± 3	ND	13 ± 3	5 ± 3	5 ± 3	5 ± 3	5 ± 3	5 ± 3
QT (ms)	188 ± 10	0 ± 3	-2 ± 3	-2 ± 3	-3 ± 3	0 ± 3	3 ± 3	4 ± 3	9 ± 3	7 ± 3	15 ± 3	13 ± 3	ND	10 ± 3	ND	21 ± 3	25 ± 3	26 ± 3	16 ± 3	17 ± 3	22 ± 3

3/

#

TABLE 1 (continuation)
Study of the effects of saline (NaCl 0.9 %, 0.6 ml/kg i.v.) in conscious dogs (n=4)

Effects on systolic (SBP), diastolic (DBP), mean (MAP) aortic blood pressure, heart rate (HR), PR, QRS and QT time intervals of the ECG

GROUP 1 SEXES & GEN PARAMETER	INITIAL VALUE	% CHANGE FROM INITIAL VALUE TIME AFTER INFUSION (h and min)																										
		3h15	3h30	3h45	4h	4h15	4h30	4h45	5h	6h	7h	8h	9h	10h	11h	12h	13h	14h	15h	16h	17h	18h	20h	21h	22h	23h	24h	
		min	min	min	min	min	min	min	min	min	min	min	min	min	min	min	min	min	min	min	min	min	min	min	min	min	min	min
SBP (mmHg)	101	-17	-18	-16	-19	-21	-17	-17	-17	-21	-18	-24	-20	-19	-21	-24	-20	-20	-20	-21	-21	-21	-18	-16	-17	-12	-19	-16
DBP (mmHg)	26	±2	±3	±3	±2	±2	±3	±3	±3	±4	±1	±4	±4	±5	±3	±4	±4	±5	±5	±6	±3	±3	±3	±2	±2	±2	±2	±4
DBP (mmHg)	111	-18	-19	-18	-20	-25	-18	-20	-24	-17	-25	-24	-24	-18	-22	-25	-24	-23	-23	-28	-28	-28	-20	-16	-18	-11	-17	-22
MAP (mmHg)	140	-19	-19	-18	-21	-24	-19	-19	-24	-18	-26	-26	-24	-20	-25	-26	-24	-24	-24	-25	-25	-25	-20	-15	-19	-12	-19	-20
HR (b/min)	22	±3	±3	±6	±2	±3	±4	±2	±4	±3	±4	±4	±4	±5	±5	±4	±4	±4	±4	±5	±8	±5	±4	±3	±4	±4	±4	±6
HR (b/min)	128	-36	-34	-34	-30	-42	-31	-22	-28	-28	-30	-30	-28	-25	-27	-11	-35	-30	-30	-38	-31	-31	-29	-29	-31	-28	-32	-32
HR (b/min)	28	±7	±8	±13	±9	±10	±18	±14	±8	±8	±11	±8	±8	±8	±6	±18	±7	±8	±11	±10	±8	±7	±5	±5	±10	±12	±10	±13
PR (ms)	83	19	16	11	23	21	31	12	24	19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	27	28
PR (ms)	±4	±10	±6	±8	±8	±4	±6	±2	±8	±10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	±8	±18
QRS (ms)	37	6	6	6	6	6	6	6	6	6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	6	6
QRS (ms)	±0	±5	±5	±5	±5	±5	±5	±5	±5	±5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	±5	±5
QT (ms)	168	25	21	14	21	29	21	13	20	20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	29	21
QT (ms)	±10	±8	±10	±3	±7	±2	±4	±10	±8	±8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	±10	±7

TABLE 2
Study of the effects of the vehicle of riluzole (0.6 ml/kg i.v.) in conscious dogs (n=4)

Effects on systolic (SBP), diastolic (DBP), mean (MAP) aortic blood pressure, heart rate (HR), PR, QRS and QT time intervals of the ECG

GROUP 2 MEANS ± SEM PARAMETER Units	% CHANGE FROM INITIAL VALUE																			
	INFUSION TIME (min)										TIME AFTER INFUSION (min or h and min)									
	5	10	15	20	25	30	35	40	45	50	55	1h	1h15	1h30	1h45	2h	2h15	2h30	2h45	3h
SBP (mmHg)	180 ± 8	1	-3 ± 3	± 2	0 ± 2	± 2	-10 ± 5	-15 ± 2	-10 ± 4	-13 ± 7	-12 ± 5	-8 ± 5	-10 ± 2	-13 ± 2	-8 ± 5	-10 ± 2	-11 ± 2	-14 ± 1	-11 ± 2	-5 ± 6
DBP (mmHg)	101 ± 5	-2 ± 4	± 4	± 2	-1 ± 3	± 3	-6 ± 2	-12 ± 7	-2 ± 3	± 3	-11 ± 2	-10 ± 5	-11 ± 2	-11 ± 2	-8 ± 5	-17 ± 1	-11 ± 2	-16 ± 2	-2 ± 6	
MAP (mmHg)	128 ± 5	-1 ± 4	± 4	± 2	-1 ± 3	± 3	-6 ± 2	-11 ± 7	-5 ± 3	± 3	-10 ± 2	-10 ± 5	-12 ± 2	-12 ± 2	-10 ± 5	-10 ± 2	-10 ± 2	-15 ± 2	-7 ± 6	
HR (beats/min)	103 ± 8	-1 ± 4	± 4	± 2	-1 ± 3	± 3	-13 ± 2	-15 ± 7	-1 ± 3	± 3	-15 ± 2	-15 ± 5	-4 ± 2	-4 ± 2	-15 ± 5	-12 ± 2	-12 ± 2	-15 ± 2	-4 ± 6	
PR (ms)	96 ± 5	2 ± 4	± 4	± 2	-2 ± 3	± 3	± 1	± 1	± 1	± 1	± 1	± 1	± 1	± 1	± 1	± 1	± 1	± 1	± 1	
QRS (ms)	97 ± 2	-3 ± 3	± 3	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	
QT (ms)	217 ± 5	2 ± 3	± 3	± 2	-2 ± 2	± 2	-2 ± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	

TABLE 2 (continuation)
Study of the effects of the vehicle of rifuzole (0.5 ml/kg i.v.) in conscious dogs (n=6)

Effects on systolic (SBP), diastolic (DBP), mean (MAP) aortic blood pressure, heart rate (HR), PR, QRS and QT time intervals of the ECG

GROUP 2 DOSE & SEX PARAMETER (unit)	INITIAL VALUE	% CHANGE FROM INITIAL VALUE TIME AFTER INFUSION (h and min)																												
		3h15	3h30	3h45	4h	4h15	4h30	4h45	5h	6h	7h	8h	9h	10h	11h	12h	13h	14h	15h	16h	17h	18h	19h	20h	21h	22h	23h	24h		
		SBP (mmHg)	100 ±8	-4 ±4	-12 ±5	-2 ±2	-3 ±4	-5 ±4	-15 ±1	-5 ±4	-12 ±5	-2 ±4	-12 ±5	-14 ±5	-9 ±6	-18 ±6	-11 ±6	-11 ±6	-11 ±6	-11 ±6	-11 ±6	-11 ±6	-17 ±6	-13 ±6	-12 ±6	-17 ±6	-9 ±6	-10 ±6	-10 ±6	-6 ±6
DBP (mmHg)	101 ±5	-5 ±7	-18 ±8	-4 ±8	-2 ±5	-20 ±11	-6 ±8	-12 ±7	-2 ±7	-11 ±6	-19 ±6	-15 ±6	-19 ±6	-19 ±6	-19 ±6	-19 ±6	-19 ±6	-19 ±6	-19 ±6	-19 ±6	-20 ±6	-13 ±6	-14 ±6	-14 ±6	-14 ±6	-11 ±6	-14 ±6	-14 ±6	-14 ±6	-14 ±6
MAP (mmHg)	128 ±5	-6 ±6	-17 ±6	-7 ±6	-3 ±4	-8 ±6	-19 ±1	-5 ±4	-17 ±6	-15 ±6	-19 ±6	-19 ±6	-19 ±6	-19 ±6	-19 ±6	-19 ±6	-19 ±6	-19 ±6	-19 ±6	-19 ±6	-21 ±6	-15 ±6	-16 ±6	-17 ±6	-13 ±6	-10 ±6	-13 ±6	-4 ±6	-16 ±6	
HR (beats/min)	103 ±8	-28 ±8	-22 ±2	-18 ±11	-5 ±3	-30 ±7	-3 ±24	-34 ±4	-28 ±11	-31 ±11	-18 ±10	-19 ±10	-28 ±11	-18 ±13	-28 ±11	-28 ±11	-28 ±11	-28 ±11	-28 ±11	-28 ±11	-27 ±17	-27 ±15	-28 ±14	-29 ±14	-12 ±11	-18 ±19	-18 ±16	-2 ±16	-27 ±13	
PR (ms)	96 ±5	3 ±8	8 ±3	13 ±2	16 ±7	18 ±2	3 ±8	-2 ±5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-1 ±1	13 ±19	
QRS (ms)	37 ±2	2 ±2	0 ±0	0 ±4	2 ±2	2 ±2	0 ±2	2 ±2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2 ±2	2 ±2
QT (ms)	217 ±5	18 ±7	16 ±8	18 ±8	23 ±5	23 ±5	4 ±3	10 ±5	2 ±5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1 ±4	11 ±4	

TABLE 3
Study of the effects of rituzole (0.03 mg/kg i.v.) in conscious dogs (n=4)
 Effects on systolic (SBP), diastolic (DBP), mean (MAP) aortic blood pressure, heart rate (HR), PR, QRS and QT time intervals of the ECG

GROUP 3 MEANS ± SEM	PARAMETER INITIAL VALUE	% CHANGE FROM INITIAL VALUE																										
		INFUSION TIME (min)												TIME AFTER INFUSION (min or h and min)														
		5	10	15	20	25	30	35	40	45	50	55	1h	1h15	1h30	1h45	2h	2h15	2h30	2h45	3h							
SBP (mmHg)	100	3	-2	5	3	1	0	-10	-10	-9	-15	-13	-16	-13	-15	-14	-14	-14	-14	-14	-14	-13	-9	-13	-14	-12	-13	-9
DBP (mmHg)	29	23	22	25	24	24	24	22	25	25	26	24	24	24	24	24	24	25	25	25	25	25	25	27	24	25	25	24
MAP (mmHg)	102	0	-4	4	3	0	-1	-8	-15	-11	-13	-13	-15	-13	-15	-13	-13	-13	-13	-13	-13	-13	-12	-17	-13	-17	-8	-12
HR (beats/min)	24	23	23	25	27	25	26	23	23	24	23	24	23	24	23	24	23	24	23	24	23	24	23	24	23	24	27	29
MAP (mmHg)	129	0	-3	5	3	0	1	-8	-13	-8	-12	-13	-18	-14	-17	-13	-14	-14	-14	-14	-14	-14	-14	-14	-17	-16	-19	-10
HR (beats/min)	25	23	23	25	26	25	25	22	23	25	27	25	24	25	25	27	24	26	25	25	25	25	25	25	25	25	24	25
PR (ms)	100	1	3	1	-5	0	10	24	15	14	-4	-11	-11	-4	-11	10	-3	-10	-11	-15	-15	-15	-25	-31	-25	-46	-32	-23
QRS (ms)	23	28	29	29	22	24	29	29	21	21	21	20	20	20	24	18	11	14	14	15	15	11	17	17	14	13	14	18
QT (ms)	86	-4	6	2	11	-2	6	-3	-1	7	4	14	10	7	8	15	12	2	18	10	18	18	24	24	24	30	16	8
QTc (ms)	25	23	24	21	23	24	23	21	23	25	24	25	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22
QRS (ms)	30	0	3	0	0	0	0	0	0	0	0	0	0	0	2	3	3	3	3	3	3	3	3	3	3	3	3	3
QT (ms)	205	2	5	1	4	3	-5	-4	0	3	4	10	6	9	18	16	19	15	12	11	22	16	22	22	22	22	28	19
QTc (ms)	22	21	22	25	26	22	25	21	21	21	22	22	21	21	25	25	24	24	24	25	25	25	25	25	25	25	25	27

TABLE 3 (continuation)
Study of the effects of rizatriptan (0.03 mg/kg i.v.) in conscious dogs (n=4)
Effects on systolic (SBP), diastolic (DBP), mean (MAP) aortic blood pressure, heart rate (HR), PR, QRS and QT time intervals of the ECG

GROUP 3 MEANS ± SEM	% CHANGE FROM INITIAL VALUE																											
	PARAMETER INITIAL VALUE	TIME AFTER INFUSION (h and min)																										
		3h15	3h30	3h45	4h	4h15	4h30	4h45	5h	6h	7h	8h	9h	10h	11h	12h	13h	14h	15h	16h	17h	18h	19h	20h	21h	22h	23h	24h
SBP (mmHg)	180 ± 9	-12 ± 4	-19 ± 2	-7 ± 5	-4 ± 3	-10 ± 4	-5 ± 3	-7 ± 5	-15 ± 4	-17 ± 6	-12 ± 7	-15 ± 7	-20 ± 4	-16 ± 10	-18 ± 6	-16 ± 7	-21 ± 5	-19 ± 6	-12 ± 5	-19 ± 7	-20 ± 7	-12 ± 7	-17 ± 7	-10 ± 6	-14 ± 6	-13 ± 6	-13 ± 6	
DBP (mmHg)	102 ± 4	-14 ± 4	-19 ± 4	-8 ± 7	-8 ± 7	-17 ± 7	-6 ± 10	-14 ± 4	-14 ± 5	-19 ± 10	-13 ± 10	-16 ± 9	-19 ± 7	-17 ± 11	-19 ± 12	-23 ± 6	-24 ± 5	-20 ± 8	-11 ± 8	-22 ± 8	-24 ± 5	-11 ± 8	-24 ± 7	-19 ± 11	-15 ± 10	-10 ± 6	-12 ± 11	
MAP (mmHg)	129 ± 5	-17 ± 3	-19 ± 2	-9 ± 7	-8 ± 7	-15 ± 6	-5 ± 7	-11 ± 2	-21 ± 4	-19 ± 5	-13 ± 9	-16 ± 7	-23 ± 3	-17 ± 10	-18 ± 2	-21 ± 6	-24 ± 4	-22 ± 7	-11 ± 6	-22 ± 7	-24 ± 7	-11 ± 6	-22 ± 7	-11 ± 9	-14 ± 8	-11 ± 8	-13 ± 10	
HR (beats/min)	100 ± 3	-2 ± 7	-20 ± 6	-11 ± 10	-4 ± 11	-28 ± 8	-4 ± 11	-21 ± 4	-32 ± 4	-22 ± 11	-13 ± 9	-1 ± 11	-20 ± 6	-10 ± 9	-15 ± 7	-26 ± 7	-17 ± 9	-14 ± 6	2 ± 14	-12 ± 11	-23 ± 6	-6 ± 10	-6 ± 12	3 ± 11	-7 ± 14	-5 ± 14	-5 ± 15	
PR (ms)	86 ± 5	16 ± 6	27 ± 6	16 ± 13	15 ± 13	16 ± 13	20 ± 13	16 ± 13	22 ± 13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	25 ± 6	24 ± 7	
QRS (ms)	99 ± 2	6 ± 6	0 ± 6	0 ± 6	0 ± 6	0 ± 6	0 ± 6	0 ± 6	0 ± 6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0 ± 6	0 ± 6
QT (ms)	205 ± 2	24 ± 2	22 ± 2	22 ± 2	17 ± 2	16 ± 2	20 ± 2	19 ± 2	21 ± 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	14 ± 2	15 ± 2

TABLE 4
Study of the effects of nitroprusside (0.3 mg/kg i.v.) in conscious dogs (n=4)
Effects on systolic (SBP), diastolic (DBP), mean (MAP) aortic blood pressure, heart rate (HR), PR, QRS and QT time intervals of the ECG

GROUP 4 MEANS & SEM PARAMETER INITIAL VALUE	% CHANGE FROM INITIAL VALUE																							
	INFUSION TIME (min)						TIME AFTER INFUSION (min or h and min)																	
	5	10	15	20	25	30	5	10	15	20	25	30	35	40	45	50	55	1h	1h15	1h45	2h	2h15	2h45	3h
SBP (mmHg)	176 ± 10	-3 ± 1	-1 ± 3	-2 ± 1	-1 ± 4	-2 ± 2	-5 ± 3	-8 ± 2	-14 ± 4	-13 ± 3	-10 ± 3	-12 ± 4	-10 ± 5	-17 ± 6	-11 ± 7	-9 ± 5	-12 ± 4	-16 ± 5	-17 ± 6	-10 ± 5	-7 ± 4	-5 ± 3	-9 ± 4	-5 ± 4
DBP (mmHg)	97 ± 5	-6 ± 1	-3 ± 3	-3 ± 2	-3 ± 3	-4 ± 4	-4 ± 4	-5 ± 4	-15 ± 6	-11 ± 5	-10 ± 5	-2 ± 5	-5 ± 6	-17 ± 8	-10 ± 7	-9 ± 6	-9 ± 6	-18 ± 8	-18 ± 8	-9 ± 6	1 ± 7	2 ± 3	-10 ± 7	-7 ± 5
MAP (mmHg)	123 ± 7	-5 ± 2	-3 ± 3	-2 ± 2	-1 ± 4	-2 ± 3	-4 ± 4	-3 ± 3	-14 ± 6	-12 ± 5	-10 ± 5	-7 ± 6	-5 ± 6	-17 ± 8	-11 ± 7	-10 ± 6	-12 ± 7	-20 ± 9	-19 ± 8	-10 ± 6	-5 ± 5	-3 ± 4	-11 ± 7	-14 ± 6
HR (beats/min)	109 ± 10	1 ± 5	-15 ± 4	-16 ± 3	-8 ± 4	-8 ± 4	25 ± 7	-9 ± 8	-10 ± 8	-21 ± 7	-25 ± 4	-23 ± 4	-25 ± 5	-28 ± 8	-26 ± 9	-24 ± 12	-29 ± 8	-31 ± 18	-45 ± 6	-39 ± 7	-21 ± 12	-28 ± 9	-32 ± 10	-35 ± 13
PR (ms)	94 ± 7	-2 ± 2	-3 ± 2	-1 ± 1	-3 ± 1	-4 ± 1	-3 ± 1	3 ± 2	6 ± 2	6 ± 2	3 ± 2	-1 ± 2	4 ± 2	12 ± 3	14 ± 3	8 ± 4	17 ± 5	10 ± 4	18 ± 7	11 ± 9	17 ± 8	7 ± 6	14 ± 7	13 ± 8
QRS (ms)	90 ± 2	0 ± 0	0 ± 0	0 ± 0	3 ± 3	3 ± 3	0 ± 0	2 ± 2	0 ± 0	0 ± 0	0 ± 0	3 ± 3	5 ± 5	0 ± 0	2 ± 2	6 ± 6	2 ± 2	3 ± 3	6 ± 6	8 ± 8	8 ± 8	2 ± 2	6 ± 6	12 ± 12
QT (ms)	203 ± 10	6 ± 3	3 ± 4	3 ± 4	6 ± 6	10 ± 10	10 ± 10	10 ± 10	10 ± 10	6 ± 6	10 ± 10	13 ± 13	21 ± 21	19 ± 19	23 ± 23	24 ± 24	23 ± 23	24 ± 24	24 ± 24	25 ± 25	27 ± 27	15 ± 15	19 ± 19	23 ± 23

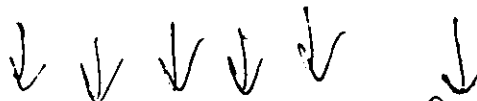
TABLE 4 (continuation)
Study of the effects of riluzole (0.3 mg/kg i.v.) in conscious dogs (n=4)
Effects on systolic (SBP), diastolic (DBP), mean (MAP) aortic blood pressure, heart rate (HR), PR, QRS and QT time intervals of the ECG

GROUP 4 MEANS ± SEM	% CHANGE FROM INITIAL VALUE																													
	PARAMETER (units)	TIME AFTER INFUSION (h and min)																												
		INITIAL VALUE	3h15	3h30	3h45	4h	4h15	4h30	4h45	5h	6h	7h	8h	9h	10h	11h	12h	13h	14h	15h	16h	17h	18h	19h	20h	21h	22h	23h	24h	
SBP (mmHg)	176 ± 10	-7 ± 6	-8 ± 4	-9 ± 6	-9 ± 4	-10 ± 1	-9 ± 4	-7 ± 6	-15 ± 3	-14 ± 3	-15 ± 3	-17 ± 2	-19 ± 1	-21 ± 2	-20 ± 4	-18 ± 4	-22 ± 2	-20 ± 2	-22 ± 2	-22 ± 2	-22 ± 2	-22 ± 2	-14 ± 4	-21 ± 4	-24 ± 3	-18 ± 4	-15 ± 4	-9 ± 4	-16 ± 2	
DBP (mmHg)	97 ± 5	1 ± 10	-7 ± 8	-7 ± 11	-9 ± 10	-8 ± 10	-14 ± 8	-5 ± 8	-9 ± 4	-4 ± 4	-9 ± 4	-14 ± 2	-15 ± 4	-23 ± 4	-16 ± 4	-14 ± 4	-19 ± 2	-20 ± 2	-21 ± 2	-21 ± 2	-21 ± 2	-21 ± 2	-10 ± 4	-24 ± 3	-14 ± 3	-10 ± 4	-6 ± 4	-6 ± 4	-11 ± 5	
MAP (mmHg)	123 ± 7	-11 ± 7	-8 ± 8	-13 ± 7	-10 ± 8	-13 ± 4	-9 ± 7	-9 ± 7	-17 ± 6	-15 ± 6	-18 ± 3	-16 ± 2	-18 ± 4	-23 ± 4	-18 ± 4	-17 ± 4	-19 ± 2	-21 ± 2	-21 ± 2	-21 ± 2	-21 ± 2	-21 ± 2	-13 ± 3	-22 ± 3	-22 ± 4	-19 ± 4	-12 ± 4	-7 ± 4	-12 ± 5	
HR (beats/min)	109 ± 10	-42 ± 5	-42 ± 4	-40 ± 5	-30 ± 14	-38 ± 7	-20 ± 17	-20 ± 10	-28 ± 14	-24 ± 11	-18 ± 11	-5 ± 10	-15 ± 13	-22 ± 9	-22 ± 9	-4 ± 10	-14 ± 6	-16 ± 10	-11 ± 9	-11 ± 9	-11 ± 9	-11 ± 9	-11 ± 9	-11 ± 9	-14 ± 10	-14 ± 10	-12 ± 11	-10 ± 12	-15 ± 13	
PR (ms)	94 ± 7	10 ± 13	9 ± 13	9 ± 13	3 ± 16	7 ± 13	-3 ± 13	12 ± 13	25 ± 13	25 ± 13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
QRS (ms)	98 ± 2	8 ± 11	0 ± 11	3 ± 11	0 ± 11	0 ± 11	3 ± 11	3 ± 11	8 ± 11	8 ± 11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
QT (ms)	205 ± 10	30 ± 28	26 ± 28	27 ± 28	21 ± 28	19 ± 28	17 ± 28	24 ± 28	26 ± 28	26 ± 28	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 5
Study of the effects of riluzole (3 mg/kg i.v.) in conscious dogs (n=4)

Effects on systolic (SBP), diastolic (DBP), mean (MAP) aortic blood pressure, heart rate (HR), PR, QRS and QT time intervals of the ECG

GROUP & PARAMETER INITIAL VALUE	% CHANGE FROM INITIAL VALUE																													
	INFUSION TIME (min)						TIME AFTER INFUSION (min or h and min)																							
	5	10	15	20	25	30	5	10	15	20	25	30	35	40	45	50	55	1h	1h15	1h30	1h45	2h	2h15	2h30	2h45	3h				
SBP (mmHg)	178 ± 13	-6 ± 3	-9 ± 3	-8 ± 4	-7 ± 2	-2 ± 1	-11 ± 4	-13 ± 8	-13 ± 8	-17 ± 4	-19 ± 8	-19 ± 8	-19 ± 8	-13 ± 10	-13 ± 8	-14 ± 8	-16 ± 8	-11 ± 8	-15 ± 8	-13 ± 8	-11 ± 8	-12 ± 8	-11 ± 8	-17 ± 8	-16 ± 8	-16 ± 8	-6 ± 5			
DBP (mmHg)	100 ± 6	-5 ± 7	-1 ± 6	-15 ± 6	-8 ± 3	0 ± 2	-10 ± 6	-12 ± 7	-11 ± 7	-15 ± 8	-13 ± 14	-20 ± 6	-20 ± 6	-9 ± 12	-11 ± 12	-11 ± 12	-17 ± 7	-8 ± 10	-13 ± 8	-16 ± 7	-10 ± 7	-11 ± 7	-17 ± 8	-15 ± 8	-17 ± 8	-17 ± 8	-3 ± 11			
MAP (mmHg)	128 ± 9	-6 ± 4	-3 ± 4	-12 ± 4	-9 ± 2	-2 ± 2	-11 ± 6	-12 ± 6	-14 ± 6	-19 ± 4	-23 ± 7	-22 ± 7	-22 ± 7	-14 ± 11	-18 ± 10	-15 ± 11	-19 ± 6	-11 ± 6	-19 ± 6	-16 ± 6	-12 ± 7	-10 ± 7	-18 ± 7	-22 ± 7	-22 ± 7	-22 ± 7	-7 ± 10			
HR (beats/min)	109 ± 7	-18 ± 4	7 ± 12	2 ± 4	2 ± 4	11 ± 11	0 ± 12	0 ± 12	-14 ± 6	-2 ± 11	-27 ± 6	-27 ± 6	-27 ± 6	-29 ± 6	-29 ± 6	-22 ± 13	-29 ± 4	-15 ± 12	-29 ± 11	-29 ± 11	-12 ± 19	-12 ± 19	-23 ± 18	-23 ± 18	-23 ± 18	-23 ± 18	-31 ± 18			
PR (ms)	91 ± 5	-3 ± 1	3 ± 1	3 ± 1	3 ± 1	-3 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1	0 ± 1			
QRS (ms)	41 ± 4	-2 ± 1	1 ± 1	1 ± 1	1 ± 1	-2 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1	-6 ± 1		
QT (ms)	213 ± 2	-3 ± 2	2 ± 2	2 ± 2	2 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2	-4 ± 2		



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TABLE 5 (continuation)
Study of the effects of .1urole (3 mg/kg i.v.) in conscious dogs (n=4)

Effects on systolic (SBP), diastolic (DBP), mean (MAP) aortic blood pressure, heart rate (HR), PR, QRS and QT time intervals of the ECG

GROUP 5 MEANS & SEM PARAMETER INITIAL	% CHANGE FROM INITIAL VALUE																												
	TIME AFTER INFUSION (n and min)																												
	3h15	3h30	3h45	4h	4h15	4h30	4h45	5h	6h	7h	8h	9h	10h	11h	12h	13h	14h	15h	16h	17h	18h	19h	20h	21h	22h	23h	24h		
SBP (mmHg)	173 ± 13	-12 ± 4	-7 ± 4	-6 ± 4	-9 ± 4	-12 ± 4	-14 ± 4	-6 ± 4	-16 ± 4	-18 ± 4	-19 ± 4	-19 ± 4	-18 ± 4	-18 ± 4	-19 ± 4	-23 ± 4	-18 ± 4	-25 ± 4	-13 ± 4	-21 ± 4	-17 ± 4	-20 ± 4	-9 ± 4	-13 ± 4	-19 ± 4	-17 ± 4	-18 ± 4		
DBP (mmHg)	100 ± 8	-4 ± 4	-7 ± 4	-1 ± 4	-11 ± 4	-14 ± 4	-20 ± 4	-6 ± 4	-12 ± 4	-18 ± 4	-22 ± 4	-22 ± 4	-18 ± 4	-18 ± 4	-20 ± 4	-24 ± 4	-21 ± 4	-24 ± 4	-9 ± 4	-22 ± 4	-17 ± 4	-21 ± 4	-7 ± 4	-10 ± 4	-21 ± 4	-16 ± 4	-17 ± 4		
MAP (mmHg)	128 ± 8	-10 ± 4	-8 ± 4	-8 ± 4	-12 ± 4	-14 ± 4	-19 ± 4	-7 ± 4	-14 ± 4	-20 ± 4	-21 ± 4	-22 ± 4	-19 ± 4	-19 ± 4	-21 ± 4	-25 ± 4	-21 ± 4	-25 ± 4	-12 ± 4	-23 ± 4	-18 ± 4	-22 ± 4	-6 ± 4	-14 ± 4	-21 ± 4	-16 ± 4	-18 ± 4		
HR (beats/min)	108 ± 7	-28 ± 11	-38 ± 11	-29 ± 8	-29 ± 8	-29 ± 8	-30 ± 10	-41 ± 8	-28 ± 8	-28 ± 8	-21 ± 4	-21 ± 4	-14 ± 4	-13 ± 4	-15 ± 4	-17 ± 4	-17 ± 4	-9 ± 7	-22 ± 8	-14 ± 8	-20 ± 14	-11 ± 14	-11 ± 14	-11 ± 14	-10 ± 8	-12 ± 8	-19 ± 17		
PR (ms)	91 ± 6	10 ± 6	11 ± 6	2 ± 6	8 ± 6	18 ± 6	18 ± 6	8 ± 6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
QRS (ms)	41 ± 4	5 ± 4	5 ± 4	-2 ± 4	-5 ± 4	1 ± 4	1 ± 4	1 ± 4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
QT (ms)	213 ± 2	12 ± 8	18 ± 8	8 ± 8	7 ± 8	18 ± 8	14 ± 8	18 ± 8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Effects on Bleeding Parameters:

1. Effect of riluzole on coagulation times after i.v. administration to rats, /Aug 1994/France
This study was conducted under GLP.
Batch# MIJ 430; 100% purity.

Male Sprague-Dawley rats (220-274g at study date) were used according to the following protocol provided by the sponsor:

Thrombin time and activated partial thromboplastin time (APTT) measurements were carried out in 7 independent groups of rats as follows :

Group	n	Treatment	Dose of active substance	Volume of administration (ml/kg i.v.)	Time (min) after administration at which blood is sampled
1	6	0.9 % NaCl solution	-	5 (1 ml/min)	5
2	6	Vehicle (1.5 % poloxamer 188 solution)	-		
3	6	RP 54274	0.03 mg/kg i.v.		
4	6		0.3 mg/kg i.v.		
5	6		3 mg/kg i.v.		
6	6	Vehicle of heparin (0.9 % NaCl solution)	-	1 (bolus)	30
7	6	Heparin	150 I.U./kg i.v.		

The 0.9 % NaCl solution, the 1.5 % poloxamer 188 solution, RP 54274 and heparin were administered into the penis vein during pentobarbital anesthesia.

Prothrombin time was measured in 7 other independent groups of animals as follows :

Group	n	Treatment	Dose of active substance	Volume of administration	Time (h) after administration at which blood is sampled
8	6	0.5 % NaCl solution	-	5 ml/kg i.v. (1 ml/min)	24
9	6	Vehicle (1.5 % poloxamer 188 solution)	-		
10	6	RP 54274	0.03 mg/kg i.v.		
11	6		0.3 mg/kg i.v.		
12	6		3 mg/kg i.v.		
13	6	Vehicle of warfarin (0.7 mM NaOH in 0.9 % aqueous NaCl)	-	5ml/kg i.p.	24
14	6	Warfarin	1 mg/kg i.p.		

Blood was collected from the abdominal aorta of each rat at the given times. Coagulation time, in sec., was determined ex vivo on plasma samples by a coagulometer and individual data were presented as means±sem. Riluzole did not produce any significant difference in TT or APTT measured 5min post i.v. infusion compared with the vehicle control (poloxamer 188). On the other hand, heparin significantly ($p < 0.05$; Student t-test) prolonged coagulation time measured 30min post injection. Similarly, riluzole did not change PT measured 24hr postinfusion compared to the vehicle. Warfarin as a reference compound, produced a significant ($p < 0.05$; Student t-test) increase in PT.

2. Effects on bleeding time in rats after i.v. administration,
'Mar 1994/France.

This study was conducted under the "spirit" of GLP.
Batch# GRV 1044

Male Sprague-Dawley rats (220-274g at study date) were used according to the following protocol provided by the sponsor (similar to the previous one #RPR-RD-CRVA-Biol 480):

Bleeding time was measured in the 7 groups of rats as follows:

Group	n	Treatment	Dose of active substance (I.v.)	Volume of administration	Time (min) of test after treatment
1	6	0.9 % NaCl solution	-	5 ml/kg (1 ml/min)	5
2	6	Vehicle (1.5 % poloxamer 188 solution)	-		
3	7	RP 54274	0.03 mg/kg		
4	6		0.3 mg/kg		
5	6		3 mg/kg		
6	6	0.9 % NaCl solution	-	1 ml/kg (bolus)	10
7	6	Heparin	250 I.U./kg		

RP 54274, the 1.5 % poloxamer 188 solution, heparin or the 0.9 % NaCl solution was administered into the penis vein during a brief period of diethyl ether anesthesia.

Riluzole caused a non-significant and non-dose-dependent increase in bleeding time compared with the vehicle as follows (mean±sem):

Group	Bleeding Time (sec)
vehicle	145±14 (90-190)
0.03	261±70 (105-540)
0.3	221±39 (105-375)
3	213±28 (160-330)
Heparin	>1200

Numbers in () are range values.

It is concluded that i.v. infusion of riluzole to rats caused non-significant and non-dose-dependent prolongation of bleeding time compared with the vehicle control (poloxamer) (47-80%) 5min post-administration.

3. In vitro hemolytic potential study with human, monkey, rat, and dog blood/SM 92-0107/Nov 1993/France.
This study was conducted under the French GLP.

This study was conducted to further investigate the hemolysis observed in vivo in the dog (SM 410) and to a lesser degree in other species. One male and one female animals from each species and 1/sex humans were used. The concentrations tested took into account the expected clinical levels recorded in previous studies. Hemolysis potential of riluzole was tested on animal and human blood incubated with or without glucose. It is suggested that riluzole may interact with enzymes that metabolize glucose (glucose 6-phosphate dehydrogenase) which can induce hemolysis. Interaction of riluzole with Hb was examined by measuring Hb levels and by checking for anisocytosis. Riluzole was prepared in DMSO at concentrations between 50-5000ug/ml, percent of hemolysis was compared to 100% hemolysis in the vehicle control and negative control (blood alone) samples. In absence of glucose there was no

significant hemolysis in any species at any of riluzole concentrations tested. In presence of glucose, marked hemolysis was seen in female monkey, female dog, and female rat, less hemolysis was seen in the rest of the animals; minimal hemolysis was seen in humans (tables below from sponsor):

RP 54274 - HEMOLYTIC POTENTIAL OF RP 54274 EXPRESSED IN % OF HEMOLYSIS

(incubation in the absence of glucose)

Tube n°	Final concentration of DMSO (%)	Final concentration of RP 54274 (µg/ml)	Human		Monkey		Dog		Rat	
			M	F	M	F	M	F	M	F
1	0.05	5	1	2	2	2	4	4	0	1
2	0.1	10	2	3	6	2	2	4	0	1
3	0.2	20	1	3	5	2	3	4	0	1
4	0.5	50	1	3	3	2	2	4	0	1
5	1	100	1	3	5	3	2	2	1	3
11	1	0	2	2	6	3	2	3	1	4
6	5	500	2	3	10	4	3	3	1	10
8	5	0	2	3	4	2	6	10	4	4
10	0	0	2	4	6	5	4	3	0	1

Tubes 7 and 11 were used for detection of anisocytosis and/or the reading of hemoglobin spectrum

Tube 9 was used as a 100% positive reference.

TABLE 3

RP 54274 - HEMOLYTIC POTENTIAL OF RP 54274 EXPRESSED IN % OF HEMOLYSIS AFTER INCUBATION WITH GLUCOSE

(after incubation with glucose)

Tube n°	Final concentration of DMSO (%)	Final concentration of RP 54274 (µg/ml)	Human		Monkey		Dog		Rat	
			M	F	M	F	M	F	M	F
1	0.05	5	3	3	1	2	2	10	20	3
2	0.1	10	4	4	2	2	5	15	20	6
3	0.2	20	3	4	2	2	5	13	11	4
4	0.5	50	4	4	2	2	4	13	8	4
5	1	100	4	4	2	2	4	8	8	4
11	1	0	3	3	1	2	11	11	12	3
6	5	500	6	11	10	9	4	9	12	26
8	5	0	14	4	12	9	14	24	18	27
10	0	0	3	4	2	2	11	19	16	6
12	0	0	1	1	0	0	2	6	0	0

Tubes 7 and 11 were used for detection of anisocytosis and/or the reading of hemoglobin spectrum

Tube 9 was used as a 100% positive reference.

It is noted that marked hemolysis was seen in the vehicle control in all species including humans. It is difficult to accurately conclude that riluzole hemolytic effect is intrinsic or related to DMSO. This is evident in tubes #6 (5%DMSO+500ug/ml riluzole) and 8 (5%DMSO alone), hemolysis was comparable between the 2 tubes therefore, contributing hemolysis to DMSO. On the other hand, marked hemolysis seen in f monkey at 50 and 100ug/ml riluzole in presence of 0.5 and 1%DMSO respectively, and at low concentrations in a rat (5&10ug/ml riluzole). Although the sponsor concluded that riluzole under the present experimental conditions, has little or no hemolytic potential, it is the opinion of the reviewer, that an accurate conclusion can not be made because of the variability of the data and the uncertainty of the effects of the vehicle.

Toxicology

Acute Toxicity Studies:

1. Acute i.v. in rats
conducted under GLP.

/Apr 1990/England. This study was

Preliminary study conducted with 5, 20, and 50mg/kg. The acute median lethal i.v. dose was around 20mg/kg. CD rats (5/sex/dose) were injected a single i.v. dose of 0 (saline), 16, 20, or 25mg/kg. Animals observed for clinical signs, time to death, and macroscopic exam of viscera and the cranium after 14d observation period.

	LD ₅₀ (mg/kg)
m+f	22 (20-24)
m	22 (20-25)
f	21 (19-24)

Time to death was within 15min of dosing in both sexes. Autopsy showed congestion of major blood vessels and liver. Clinical signs: piloerection, decrease in respiration, gasping respiration, collapsed state, pallor in extremities; all signs occurred during dosing and persisted throughout day1. Other signs noted during dosing: lethargy and ptosis in all rats dosed 16mg/kg and most of those dosed 20mg/kg, hyperactivity in surviving rats, increased salivation and lacrimation in groups 16 and 25mg/kg. Survivors were ataxic on day1; complete recovery on day5.

2. Acute oral study in monkeys,
This study was conducted under GLP.

'Sep 1990/France.

Macaca monkeys (1/sex/dose) were orally administered a single dose of riluzole at 50 or 100mg/kg in a capsule; control group received an empty capsule. Animals were observed for 2wks. Complete hematology, blood chemistry, and urinalysis were measured during the study and at end of study, both macroscopic and microscopic exam were done. Lead II EKG and HR were recorded. Both animals in the 100mg/kg died on day2 at 26-27hr of dosing, no drug-related deaths at the 50mg/kg (1f killed on d9 due to hand wounds). Clinical signs appeared 2hr postdose and in the LD complete recovery by 48hr, these signs included at both doses: hypoactivity, ataxia, tremors, hyporeactivity, hypothermia, drowsiness, dyspnea, decubitus, and fixed stare and death in HD. Hypothermia in LD were lower than normal even on day8. No drug effects on any of the parameters measured except as noted. Microscopic exam of LDf showed hepatic steatosis and renal microvacuolar steatosis localized in epithelial cells of proximal tubule. In the 2HD monkeys: significant liver and kidney steatosis, and vacuolation of acinar cells of the pancreas with cytolysis (non-steatotic).

3. Acute i.v. in monkeys,
This study was conducted under GLP.

/Jan 1991/England.

Cynomolgus monkeys (1/sex) were administered a single i.v. infusion of riluzole at 10, 20, or 30mg/kg and observed for 14days; control group injected with the vehicle (saline). The females in MD and HD were not dosed due to severe signs in HDm and death of MDm during infusion. Hematology and clinical chemistry parameters were determined prior to study initiation and 24hr after dosing. At sacrifice (14days later), bone marrow exam, macroscopic, and histopathology were conducted (not done on HDm). Clinical signs in the HDm that was dosed included those during infusion: fixed stare, dilated pupils, unresponsive, eyes flickering, muscle tremors, and no vital signs. After end of dosing: heart rate and respiration were measurable by 15min, tremors, salivation, shallow breathing, dilated pupil, and incoordination. In MDm, signs were less severe and included drowsiness, glazed eyes, laboured

breathing, irregular breathing, and by 29min the animal was unconscious and died. At the LD, both male and female monkeys were drowsy, eyes closed, unresponsive, ptosis, and uncoordinated, vomited by 5hr then became quiet till day14. There were no drug-effect on any of the parameters measured (note that no histopathology was done on the HDm).

4. Single oral dose in mice, /Apr 1994/France.
This study was conducted under GLP.

CD-1 mice (5/sex/dose) were orally administered a single dose of riluzole at 50, 125, and 300mg/kg given as a suspension in 0.5% MC; the control was dosed with the vehicle. Mice were observed for 14days. Clinical signs, B.wt, and macroscopic exam at necropsy (without the CNS), and only tissues with gross findings were examined microscopically. A preliminary study was done using 50, 300, and 500mg/kg administered to 3m mice, death occurred in 0/3, 2/3, and 3/3 respectively. Based on these results, the present doses were selected. No death was seen in control, LD, and MDf groups, 1/5MDm and all HD mice died between day2 (at 1-2hr of dosing) and day6 (at 1hr of dosing). Clinical signs at LD were limited to hypoactivity within 1hr of dosing and all animals were normal by 2hr, at MD signs seen on day1/30min included hypoactivity, tremors, ataxia, dyspnea, some of these signs were severe at 4hr postdose (hypoactivity and prostration), but absent by day2, except for one male that had dyspnea, loss of consciousness on days2&3 and death on day4. HD mice died within 1-5days with hypoactivity noted within 30min of dosing followed by prostration, dyspnea, tremors, and somnolence by 1hr. By 2hr all mice were dyspneic and lost consciousness. The minimal lethal dose is therefore, 125mg/kg for males, 300mg/kg for females; the highest non-lethal dose was 50mg/kg for males and 125mg/kg for females. There were no histopathological findings in any group.

Subchronic Toxicity Studies:

1. 4-wk oral toxicity study in rats /Sep 1982/France.
Batch# 4PC-164. This study was not conducted under GLP.

Unfasted male and female CD rats (10/sex/dose) were orally dosed with riluzole at 5, 15, or 45mg/kg/day for 28days. The control group was dosed with the vehicle, 1% methylcellulose. Parameters evaluated included clinical signs and mortality, body wt, urinalysis on day 21 (volume, Na, and K), RBC count on day29, clinical chemistry parameters on day29 (ALT, ALP, BUN, glucose, creatinine, and bilirubin), organ wt, gross exam, and histology of liver and kidneys (stained with Lillie-Pasternam dye, pH4).

Results:

Riluzole at 5 and 15mg/kg did not affect mortality or body wt. However, 6/10males and 7/10females of HD died starting on days 1-3 of treatment. Surviving males gained less wt than the control group starting on d11 (12%) and through d29 (14%) (significant at d29; statistical methods were not reported therefore, the P value is unknown. In HDf although a significant growth reduction was recorded on day21 (9%), this was caused by 1f and the wt gain was comparable on day29 relative to the control. Therefore, HDf growth was not affected by treatment. Data for clinical signs were not provided however, the sponsor stated that sedation occurred in HD rats that gradually decreased with subsequent dosing. There was no drug effect on RBC count. Urine volume was increased significantly in HDf (2.2 ± 0.5 vs. 1.1 ± 0.2 ml/100g control), and excretion of Na and K was increased in HDf nonsignificantly compared with the control values (151 ± 30 vs. 106 ± 31 uEq/100g for Na; 231 ± 44 vs. 147 ± 31 uEq/100g for K). Urinary K excretion was also increased in HDm (195 ± 18 uEq/100g vs. 169 ± 16 uEq/100g control). Serum ALT level was increased nonsignificantly in MD&HDf (37%), ALP level was significantly increased in MD and HD males and females (1.7-2x); see table below. Bilirubin was

significantly elevated in LD&MDm and HDf, BUN was significantly increased in HDm&f, and creatinine was significantly increased in all three doses of males and females relative with the control. A significant increase in glucose was measured in HDm and LDf.

Only values with significant difference are shown/p value and statistical test were not reported (values in () are for females):

	Cont	LD	MD	HD
ALP	207±23 (128±9)	- -	344±21 (218±23)	325±48 (247±41)
Bili.	4±0.1 (4±0.1)	5±0.1 -	5±0.2 -	- (5±0.6)
Creatin.	4.2±0.1 (4±0.1)	5±0.1 (5±0.1)	5.3±0.1 (5.2±0.1)	5.4±0.1 (5.3±0.1)
BUN	0.3±0.01 (0.3±0.01)	- -	- -	0.5±0.1 (0.4±0.1)

The increase in creatinine level was reported by the sponsor to be within the historical control data from Charles River (13.4mg/l for males and 13mg/l for females). Only in females was the absolute and relative wt of the left kidney significantly increased in all three drug groups and the right kidney wt in LD (only absolute) and MD (only relative wt). The absolute and relative wt of the liver was significantly increased in HDf but only the relative wt was increased in HDm. The relative wt of the adrenals and spleen were increased significantly in HDm but inconsistent changes in females. The absolute and relative wt of the heart were significantly elevated only in LDf. Histopathology exam was done only on the liver and kidneys and there were no drug-related findings. Hepatic lobular microgranuloma and nephrosis were observed in controls and drug groups; these are common in lab rats.

It is concluded that oral administration of riluzole to rats for 28 days caused 60-70% death at 45mg/kg and the remaining animals in this dose showed increase in liver wt and enzyme level. However, histopathological findings in the liver or kidney of any group were not evident. Some effects that reached statistical significance occurred in clinical chemistry and urinalysis but reported by the sponsor to be within the historical control data (not provided for review). The absolute and relative wt of the left kidney in treated females from all groups was significantly and dose-dependently increased but no such effect found in males; the toxicological significance of this finding is unclear. Drug effects in MD and LD were mild. The NOEL in this study is <5mg/kg based on organ wt changes and some clinical chemistry changes.

2. 7-day exploratory i.v. toxicity study in rats/

/Dec

1993/PA-USA.

Batch# MIJ 430; purity 97.4%

This study was an exploratory study and therefore not GLP.

It was conducted to test the toxicity of riluzole after i.v. dosing and to assist in selecting doses for a 4-week i.v. study.

Male and female Sprague-Dawley rats (6-7wks old; 130-202g at initiation) were used. There were 2 phases for the study:

phase 1 involved dosing 5/sex/gr rats with riluzole at:

2.5, 5, and 10mg/kg and the control dosed with vehicle (0.9% sodium chloride) once daily for 7 days.

[The dosing volume was 1ml/min except for all MDm that because of a dosing error were replaced and the new group was dosed for 6 days].

Phase 2 had an additional group of 4/sex dosed at 15mg/kg and a concurrent control of 4/sex dosed with the vehicle both for 7days. Another group of 2male rats was dosed with 20mg/kg as a 4min i.v. infusion and 1 each male and female dosed 20mg/kg as a 10min i.v. infusion.

All the above regimens had the same infusion volume of 20ml/kg.

Parameters analyzed were: mortality, clinical signs, body wt, food intake, hematology, clinical chemistry, gross morphology, and histopathology. The latter was done on the following tissues in control and HD only as well as any animals that died or killed while in moribund: liver, kidneys, lymph nodes (iliac), spleen, ovaries, post cava, aorta, and injection site. For the hematology and clinical chemistry blood was sampled from the retro-orbital sinus on necropsy day (day8). It is noted that organs were not weighed.

Results:

Mortality and Clinical signs: one LDf (2.5mg/kg) died after the 2nd dose the cause of death was not determined. No other deaths in any group that received multiple dosing. Two rats that received a single dose of 20mg/kg over 4min infusion died few min postdose and the dose was thus reduced to 15mg/kg. At 15mg/kg there were no deaths. Two rats injected 20mg/kg over 10min infusion survived but killed the next morning after dosing due to clinical signs including convulsions, excessive salivation, chromodacryorrhea, red-brown staining around the nose, and hypoactivity. Clinical signs observed at 10mg/kg included ataxia, dilated pupils, and lethargy, those at 15mg/kg were impaired righting reflex, prostration, slow and shallow breathing, and staining in the anogenital region. These signs had disappeared by the next morning prior to the next dose. Signs were mild in LD and noted in 1male that had increased respiration on a single occasion and 1MDm on a single occasion had dilated pupils, labored breathing, slight lethargy, and increased respiration. The signs in LD and MD may or maynot be drug-related because they occurred only on one occasion and in one animal.

Body wt: mean wt of phase 1 male and female rats was not affected by the drug upto 10mg/kg. However, mean wt gain of males and females in phase 2 (15mg/kg) was decreased 18 and 25% respectively, from study day1 till the end of day7.

Food intake: no effect on intake at doses <15mg/kg. At 15mg/kg, food was decreased in males 10-11% (p<0.05) and 17% in females (not significant) during day3 of the study but not on other days. The sponsor indicated that in some rats dosed 10mg/kg there was some apparent increase in appetite noted by the technician, immediately after dosing. This finding was verified by weighing the feeders before and few hours after dosing (data were not provided for review).

Hematology and Clinical Chemistry: no drug effect on any parameter upto 15mg/kg.

Gross Morphology and Histopathology: no findings and any observation was incidental and common in the rat. Histopathology was limited to the injection site including chronic phlebitis of tail veins and/or inflammation of the nearby veins; no findings observed at distal vasculature.

It is concluded that repeated i.v. administration of riluzole to male and female rats at doses upto 10mg/kg for 6-7days, did not cause death and did not affect body wt or food intake nor did it affect any of the other parameters. However, clinical signs were seen at 10mg/kg including ataxia, dilated pupils, and lethargy. There was one death at 2.5mg/kg but the cause was not determined.

Riluzole at 20mg/kg i.v. infused over 4 or 10min caused death and clinical signs including convulsions. When the dose was reduced to 15mg/kg, no animals died but clinical signs included impaired righting reflex, prostration, slow and shallow breathing, and staining in the anogenital region. These signs had disappeared by the next morning prior to the next dose. Clinical signs at 2.5 and 5mg/kg noted once and in one rat. The NOEL is therefore, 5mg/kg.

3. 4-wk i.v. in rats,
Batch# MIJ 430; purity 99.3%
This study was conducted under GLP.

/Apr 1993/PA USA.

Male and female Sprague-Dawley rats (43-45days old, 136-226g at study initiation) were injected with riluzole as an i.v. infusion over 4min at a volume of 10ml/kg/day. Rats 10/sex/gr, were dosed with riluzole at 5, 10, or 15mg/kg/day for 28days. Additional 3/sex/gr rats served as a satellite gr to determine plasma levels after the 1st dose i.e. rats were killed after one dose. Drug levels were analyzed at 0, 15min, and 24hr postdose. Plasma levels were also determined on last day of dosing, in 3/sex/dose of the main gr at 0min and 24hr postdose. Riluzole was injected as an i.v. infusion over 4min. The vehicle used was Poloxamer F-68 in 0.9% NaCl was used in the control gr.

Results:

Mortality and Clinical signs: the following deaths occurred:

cont. 1f day7 - due to dosing error.

LD 2m days 8&15 - due to dosing error or embolism from i.v. injection or suffocation due to restriction from the plastic bag that was placed over the animal to secure it for the injection at the tail vein.

HD 6m days 1,17,28 - drug related.

2f days 7&21 - drug related.

Clinical signs at LD were of low incidence and included ataxia (mainly during the first 2days), dilated pupils, impaired righting reflex, hypoactivity, and decreased respiration. The signs in LD occurred more in males than females. At MD signs were similar to those in LD but at much higher incidence and also included anogenital staining and loss of righting reflex. At HD signs included convulsions, respiratory depression, marked ataxia, impaired and lost righting reflex, and slight-marked hypoactivity. Convulsions noted only in HD and seemed to precede dosing and the incidence was much less in females than males (31 vs. 105 respectively). Other signs in HDf occurred at higher incidences than those in HDm.

Clinical signs that occurred 1 to several hrs postdose included:

- dose-dependent ataxia and anogenital staining at MD and HD.
- hypoactivity mainly in HD.
- impaired righting reflex in HDf.
- decreased respiration in HD rats.

Other drug related signs included lost of righting reflex, hyper-activity, ptosis, dilated pupils, salivation, and staining around the mouth.

Body wt and Food intake: mean wt and wt change in HDm was significantly less than the corresponding values in controls as of day4 of treatment and through study end ($p < 0.05$; wt change d4 28% to 39% d28; mean wt 9-15% of the control); no drug effect in females. Food intake in HDm was reduced 12% on day4 ($p < 0.05$) but similar to the control thereafter. HDf ate 12% less than the control on days 4 and 8 but intake was similar to the control after that.

Ophthalmologic Exam: conducted pre-drug and on wk3 of the study. There were no drug-related findings.

Hematology: there was no consistent drug effect on any parameter and any statistically significant finding was not dose-dependent or observed only in one gender and was within the normal variation for this species. The relative leucocyte count was increased 1-2fold in MD&HDm and MDf ($p < 0.05$), and the absolute and relative count of neutrophils was increased 1.8x ($p < 0.05$) in HDf relative to the mean count in controls.

Clinical Chemistry: similar to hematology, there were no dose-dependent and consistent findings, the following showed statistical significance or were present in males and females.

Mean ALT level was increased significantly ($p < 0.05$) in HDm (133%) and HDf (160%), cholesterol level was increased in HDm&MDm (1.2x; $p < 0.05$ only in HD),

glucose increased nonsignificantly in HDm&Hdf (1.1x), TG level was significantly increased in MDf (1.5x; p<0.05), and BUN was significantly (p<0.05) reduced in Hdf (31%) of the control level. Protein electrophoresis showed a significant decrease in alpha level in HDm&f (p<0.05; 17-25% of control).

Urinalysis: several parameters were affected: a dose-dependent decrease in urinary K of males (42-58%) and Hdf (78%), creatinine level was also decreased dose-dependently in males (37-51%; p<0.05) and females (6% and 81% in Hdf with p<0.05 only in HD), urinary Na was significantly reduced in HDm (61%) and Hdf (68%), urine volume was increased 388% in HDm from 8±5ml in controls to 31±23ml in HDm and 650% in Hdf from 6±2ml in control to 39±16ml in Hdf, and a significant decrease in urinary osmolality recorded in MD&HDm (532±103 MDm/477±534 HDm vs. 856±308mOsm/kg control) and Hdf (168±71 vs. 657±157mOsm/kg control).

Organ wts: there was no dose-dependet change in organ wt of any treated group, most of the findings were seen in HD. The following reached statistical significance:

Males/HD:

relative wt of brain increased in, absolute wt of kidneys, lungs, and prostate decreased, absolute and relative wt of the prostate was decreased, relative wt of salivary gland increased and the absolute and relative wts of thymus were decreased.

Females/HD:

absolute wt of the heart decreased, relative wt of the liver in MD&HD was dose-dependently increased, absolute and relative wts of the lung were decreased, and the absolute and relative wts of salivary glands of MD&HD were decreased dose-dependently.

Gross and Histopathology: there were no drug related findings in any group the following were found mainly in the animals that died or sacrificed in moribund and none of these results showed dose-dependency or seem to be related to treatment.

If no mention is made to a dose group or control it indicates a zero incidence or an incidence similar to or less than the control.

Organ/Tissue	Observation
liver	enlarged 2/10LDm, 1/10MDm, 3/10HDm; inflamm. cell infiltr. 1/1MDm, 4/10HDm, 2/10cont; centrilobular vacuolation 1/2LDm, 4/10HDm, 1/10Hdf; congestion 2/2LDm, 1/10HDm; hepatodiaphragmatic nodule 1/2LDm.
lung	discoloration 2/10LDm, 4/10HDm.
trachea	dilation and lymphocytic infiltrate 1/10Hdf.
thymus	enlargement and discoloration 1/10LDm, 1/10HDm.
urinary blad.	dilation 4/10HDm, 1/10MDf; lymphocytic infiltrate 2/10Hdf.
epididymis	acute inflamm. 1/10HD, cellul. debris in lumen 1/2LD, 2/10HD, absence of sperm 1/10HD.
lacrimal gl.	metaplasia of Harderian 2/10HDm.
ovary	congestion 1/10HD.
spinal cord	meningeal hemorrhage 1/10Hdf.
spleen	hyperplasia 1/10HDm.

Summary and Conclusion:

Intravenous infusion of riluzole over 4min to rats at doses upto 10mg/kg was non-toxic but at 15mg/kg it caused death, significant decrease in wt gain (28-39%) in males and clinical signs including convulsions. Other signs in HD were respiratory depression, marked ataxia, impaired and lost righting reflex, and slight-marked hypoactivity. The above signs except for convulsions, were also seen in MD and to much less incidence in LD. There were no consistent and dose-dependent findings in any of the parameters measured including histopathology. The i.v. NOEL is 5mg/kg although clinical signs were seen at this dose, they seemed to be an extension of the pharmacology of the drug.

Comment: the TK data were not presented in this study and the sponsor indicated that they will be presented in a seperate report/see PK-TK section.

4. Oral gavage MTD study followed by 13-day oral gavage range-finding toxicity study in baboons. 'Aug 1983/England. Batch# 4 PC 164.
This study was non GLP.

This study used 1 single animal per sex to examine the MTD for riluzole. Two phases for the study: 1 using escalating doses to determine MTD and phase 2, to dose with the MTD for 14days.

Riluzole was suspended in 1% methylcellulose and orally dosed by gavage to baboons (*Papio sp.*) at the following regimen provided by the sponsor:

Dose levels

3.2.3.1 Maximum tolerated dose (MTD) phase

Dose level (mg/kg/day)	Day of study
2.5	1 - 4
5.0	5 - 8
10.0	9 - 12
20.0	13 - 16
40.0	17 - 20
80.0	21 - 23*

* The study duration was increased by 3 days and the dose level was doubled to 80.0 mg/kg/day in order to identify the MTD.

3.2.3.2 14 day repeat dose phase

Dose level (mg/kg/day)	Day of study
60	1
40*	3 - 16

* The dose level of 60 mg/kg/day proved too high.
The study was stopped after one day and, after 1

There were no histopathology or TK done in this study.

50

Results:

There were no deaths. Vomiting postdosing or overnight occurred in all doses during the MTD phase i.e. starting at 5mg/kg to 40mg/kg (day5-20) and at 80mg/kg (days21-23) in addition to vomiting, animals salivated and were subdued/sedated upto 6hr postdosing. The sponsor indicated that during recovery from sedation, the baboons were ataxic but recovered by the following day. During phase 2 of the study i.e. repeat dosing at MTD for 14days, signs included salivation, vomiting, and sedation upto 6hr postdosing from day1 until end of study. It is noted that initially, dosing at 60mg/kg caused the male baboon to sleep almost continually for the next 24hr. The female vomited immediately after dosing but no other signs. The dose was reduced to 40mg/kg and at this dose vomiting and sedation were common findings as indicated above. The sponsor evaluated the effect on body wt and food intake by using the data from each animal pre-drug and after dosing i.e. animal acting as its own control. This is unacceptable assessment, concurrent control group should have been included. In general, wt and food intake during the MTD phase, decreased in the female and the male gained 0.15kg. During the 14day phase, the male lost wt but no change in female. Changes in wt and food intake were attributed to the episodes of vomiting and long sedation that deprived the animal from food and water. No drug effect on ophthalmoscopy, hematology, clinical chemistry, necropsy, or organ wt. It is concluded that oral dosing of riluzole to baboons at 40mg/kg is tolerable but caused extreme sedation, wt loss, and reduced food intake. Therefore, doses not greater than 30mg/kg can be used in a long term study.

5. Pilot i.v. infusion toxicity study in cynomolgus monkey
1993/Germany.

'Oct

This is a pilot study, therefore, it was non-GLP. It lacked detailed discussion of study conduct. Only one monkey/sex/dose was tested, the vehicle was unknown and a vehicle control group was not tested, animals were not killed at study end but returned to colony for general use, histopathology was not done. The objective of the study was to select doses for a 28day toxicity study.

Male and females cynomolgus monkeys (1/sex/dose) were infused by i.v. administration, riluzole at 10 or 15mg/kg at a total volume of 15ml/monkey and infusion rate of 0.5ml/min over 30min duration. The drug was administered once daily for 7days to chair-restrained animals.

Results:

There were no deaths. Both males and females in a dose-dependent manner showed apathy, ataxia/impaired mobility, and lateral position during infusion from day1-7 of the study. Emesis was recorded in 1Hdf and 1LDm on days 2 and 5 of study respectively. Animals gradually recovered (sluggish, hunched posture, and ataxia) with complete recovery by 0.5-5.5hr postdosing. Since there was no vehicle control group and the vehicle is unknown to the reviewer, an effect by the vehicle can not be excluded. A loss of 0.1-0.3kg of body wt occurred in males and females of both doses and food intake continued to decline but slightly recovered towards end of dosing. Again absence of a control group made an accurate conclusion difficult. It is concluded that 10 and 15mg/kg administered as an i.v. infusion to monkeys are well tolerated. They caused clinical signs, wt loss, and decreased food intake but no deaths. Doses upto 10mg/kg can be used in 30day study in monkeys.

6. 28-day i.v. toxicity study in cynomolgus monkeys/HLE# 198-066/June 1994/Germany. Batch# GRY 1061.
This study conducted under OECD guidelines.

Cynomolgus monkeys (*Macaca fascicularis*) were obtained from 2 sources: 5 from Moscow - Russia, and 27 from Hong Kong. Males and females (4/sex/dose) weighing 2.4-3.8kg were administered riluzole once daily as an i.v. infusion at 2, 5, 10mg/kg for 28days. The control group was dosed with the vehicle. Administration was done in the morning at infusion rate of 0.5ml/min and total volume per monkey of 15ml infused over 30min. All monkeys were quarantined for about 6wks after arrival and examined regularly. Riluzole was administered as an aqueous solution in 0.9% saline with Poloxamer.

Comments:

- Blood was collected after an overnight fast on day 1 and 2 at 5min before end of infusion, 15min after end of infusion, and 2hr after dosing. Drug level was analyzed by the sponsor therefore, data were not presented in this report.
- Histopathology was not conducted by Hazleton, it was sent to and assessed by the sponsor.
- It is unclear why there were 2 sources for the monkeys.

Results:

Mortality: there no deaths.

Clinical Signs: there were no signs in any monkey at LD or the control. Continuous infusion was impossible in 1MDm due to thickened veins at injection site during days23-28, and another MDm suffered from drowsiness during and after infusion with gradual recovery after 90min of dosing; there were no signs in the remaining 2 males. In MDf, one animal had no signs at any time, drowsiness during and after infusion was seen in the other 3females that gradually disappeared by 15-70min postdosing. Also in MDf, emesis was noted in a single female on day3 at 90min post dose, and thickened veins at injection site was seen in a single female during days22,23, and 28. Drowsiness occurred in all HD monkeys at higher incidence than that in MD; but similar to MD, animals gradually recovered by 3hr postdose. Other signs in HD were emesis in 2/4males and 3/4females during days 2-4 usually before dosing but also in 1HDf after dosing and overnight as well, ataxia after infusion in all females and most males, and thickened veins seen in 2/4males towards end of study but none in females. Mild loose stool/diarrhea was observed once in 1HDm.

Body wt and Food Intake: in general, there was no marked change in mean wt of treated and control animals. All male drug groups and HDf (wks 1-4) lost wt compared with the control; this wt change was however, nonsignificant and small (-0.1kg). There was no wt change in the other 2 female groups. Overall food intake and weekly mean food consumption were significantly less in MD&HDf (19&22%) than the control, throughout the study. In males, mean food was significantly reduced in MD during wk1 and nonsignificantly in MD and HD till end of study, overall consumption in MD&HDm was reduced but not significantly; all comparisons are relative to the control.

Ophthalmoscopy: eyes were examined prior to start and during the last wk of treatment. The ocular fundus with macula lutea, papilla, and ocular vessels were examined. There were no drug-related findings.

CVS: also tested before and during the last wk of study. The following were measured: EKG (PR, QRS, QT intervals), HR, and BP (SBP, DBP, MBP). There were no drug-related effects.

Hematology: there was no drug effect but some parameters reached statistical significance but none of the changes were dose-dependent. These changes included a decrease in HB (11%) in LD&HDf no change in MD and a 55-70% increase in platelets of MD&HDm.

Clinical Chemistry: although some statistical findings were observed, they were small and non-dose-dependent. These changes included an increase in total proteins in all 3 male drug groups (9-23%), an increase in albumin in

MD&HDm (10-12%), a 19% decrease in A/G ratio of HDm, and a decrease in LDH level in LD&MDf (30-33%). It is noted that significant changes in LDH of LD&MDf and all three male drug groups (the latter was nonsignificant decrease) were seen pre-dose which might have accounted for the significant finding in female groups. The LDH level in all three treated male groups was also decreased but nonsignificantly.

Urinalysis: no drug effect.

Organ wt: the following organs were weighed from all groups: heart, brain, kidneys, liver, adrenals, ovaries, pituitary, spleen, testes, and thyroid/parathyroid. There were no drug related effect on organ wts except for a 16 and 19% increase in absolute and relative liver wt respectively, of HDf and a relatively high but non-significant increase in absolute and relative wt of the testes in HDm. The latter was caused by a single high value in one male that was related to age since this male had histologically mature testes.

Gross Necropsy: the only finding was subcutaneous and/or intramuscular hematomas and edema at injection site in all groups including the control with higher incidence in drug groups. In HDm there was partly thickened corpuscles in the spleen and in another male there was thymus atrophy.

Histopathology: vascular occlusion and perivascular reaction were observed at injection site of MD&HD animals from both sexes. The vascular occlusion described in the pathology report as partial to complete occlusion of vascular lumen with proliferative smooth muscle and/or connective tissue. The perivascular reaction was characterized by one or more of the following: acute and/or chronic inflammation, granulation tissue, and fibrosis. All these changes correlated with thickened veins observed clinically.

Summary and Conclusions:

Riluzole was administered to cynomolgus monkeys once daily as an i.v. infusion at 2, 5, 10mg/kg for 28days. The control group was dosed with the vehicle. There were no deaths in any group. Clinical signs included drowsiness, emesis, and ataxia in MD and HD monkeys but the animals recovered by 3hr postdosing. Also noted was thickened veins at injection site towards end of study in all groups with increased incidence in drug groups vs. the control; these veins were partially to completely occluded and prevented infusion. Mean wt was not affected in any group but wt gain was decreased in all male drug groups and HDf. Overall food intake and weekly mean food consumption were significantly less in MD&HDf (19&22%) than the control, throughout the study. In males, mean food was significantly reduced in MD during wk1 and nonsignificantly in MD and HD till end of study, overall consumption in MD&HDm was reduced but not significantly. There were no other drug effect on any measured parameter. The NOEL is 2mg/kg.

7. Repeated i.v. administration in monkeys for 4 wks followed by a 15-day recovery period (final report) /Jan 1991

Batch# GRV 894.

This study was conducted under GLP.

Wild-caught cynomolgus male and female monkeys (*Macaca fascicularis*) 2-4yrs old at onset of study, weighed 2-5kg, were dosed with riluzole at 1,2,5mg/kg for 28days. There were 3/sex each in LD and MD and 5/sex each in HD and control group. The control group was dosed with the vehicle (0.9% saline). For logistical reasons, the sponsor indicated that the males were dosed for 4wks and subsequently the females were dosed for 4wks. The drug was administered as an i.v. infusion over a 30min period at a total volume of 20ml/kg through the saphenous or cephalic veins. The injection sites were rotated to reduce the incidence of hematoma and other vascular problems seen in previous studies.

Results:

Mortality: no deaths in any group.

Clinical Signs:

unsteadiness/incoordination	all HDm during dosing and, 3/5Hdf noted sometimes during wk1. [lasting <30min].
ptosis	2/5HDm during infusion on wk1.
tremors	1MDm during dosing day1.
gasping	2HDm during dosing on days1 and/or3
vomiting	2HD on isolated times.
reluctance to move	2/5HDm on few occasions and in 2/5 female controls on isolated occasions.

The sponsor indicated that these signs are not normally observed in lab monkeys however, they may not be drug related due to the small incidence and they may have been related to stress induced during dosing. Another sign, "quietness accompanied by huddled posture", observed both in treated and control animals with higher incidence in control females.

Body wt and Food Intake: In general, there was no drug effect on mean wt however, mean wt loss (wk0-4), of -43g was recorded in MDf. Also, the following individual animals showed moderate cumulative wt loss: 1LDm (-249g or 6% of predosing*), 2MDf (-24&-110g or 1-4% of predosing), 1HDm and 2Hdf (-13 or 3% and -64 & -133g or 3 & 4% of predosing). For the HD monkeys, wt loss also noted during recovery period in 1m (-204g from wk4 to recovery wk2) and 2f (-42 and -59g from wk4 to recovery wk2). Mean food intake was reduced 31% (p<0.05 William's test) in HDm from the control mean wt during wk1 of treatment, also, the overall intake was reduced (nonsignificantly) in LD and HDm, MD and Hdf. Individual animal intake was as follows: 2LDm (21-27% over predose), 1LDf (13% of predose), all 3MDf (2-31% of predose), and 2HDm (13-24%), 3Hdf (6-33% of predos). When comparing overall food intake during recovery in HD, 1/2HDm lost wt (-60g) compared to mean cumulative intake of predose but intake was higher (130g) when compared to mean overall intake during wks1-4. Corresponding results in Hdf, the 2/2 females ate less than the amount during predose but one monkey ate more and the other ate less than the cumulative food during wks1-4. Except for the mean decrease in food in HDm during wk1, all the above changes were non-significant.

* % relative to predose wt of the same animal not relative to the wt of the control.

Ophthalmoscopy: assessed before and during wk4 of dosing, and at recovery period. There were no drug related findings.

Hematology: blood was collected from the femoral vein of each monkey prior to dosing, during wk4 of dosing, and recovery wk2. There were no consistent or dose-dependent findings. The sponsor indicated that 1-2 animals from each group including the control, had malarial parasites in their blood. The sponsor indicated that this is a rare finding in lab monkeys and related to "stress" associated with infusion procedure. The sponsor also indicated that some of the abnormal hematological findings are related to the presence of the parasites however, when individual data were evaluated by the reviewer, this statement is not absolutely correct since the hematology data for some of these monkeys were comparable to those in the control.

Clinical Chemistry: blood was collected from the femoral vein of each monkey prior to dosing and during wk4 of dosing and recovery wk2. There were no significant drug related findings except for decrease in the following enzyme levels of HD monkeys: LDH, LAP (leucine amino peptidase), ALT, and AST. There is no toxicological significance of a decrease in enzyme level.

Urinalysis: urine was collected from all animals over a 16-hr period. There were no significant drug related findings.

Bone Marrow Smears: prepared from all animals prior to sacrifice. No significant finding.

Organ wt: there were some differences in absolute and/or relative mean organ wt of treated and control groups. These differences were due to 1 or 2 animals; the organs affected were: thymus, gonads, prostate, uterus, adrenals, thyroid, pituitary, liver, and spleen. Absolute wt: 2LDm had high wt of the gonads and prostate causing an increase in the mean values of these organs compared with the control. The mean and individual absolute wts of the gonads in MD&HD were also high relative to the control. During recovery, the mean absolute wt of the pituitary in HDf was increased over the control, the mean wt of the liver was decreased in HDf, the mean wt of the thyroid in HDm&f was reduced, and the mean as well as individual wts of the spleen in HDm&f were reduced compared with the controls. Relative wt: the mean wt of the spleen was decreased in LDm but increased in LD/MD/HDf, it was also decreased in HDm to about 50% of the control. The wt of the gonads was increased in LD&HDm relative to that in the control. During recovery, the only effect was a decrease in mean and individual relative wt of the spleen of HDm compared with the corresponding control wts.

Macroscopic and Histopathological Exam: no significant findings except at the injection site. There were hematomas/perivascular hemorrhage but histopathology exam did not show any difference in severity of lesions between the control and drug groups.

Comments:

It is noted that statistically significant values between the groups were found prior to study initiation (wk -2) in some parameters of hematology, clinical chemistry, and urinalysis.

Summary and Conclusions:

Riluzole was administered to cynomolgus monkeys once daily as an i.v. infusion at 1, 2, 5mg/kg for 28days. The control group was dosed with the vehicle. There were no deaths in any group. Clinical signs included unsteadiness/incoordination in all HDm during dosing and in 3/5HDf noted sometimes during wk1, ptosis in 2/5HDm during infusion on wk1, tremors in 1MDm during dosing day1, gasping in 2HDm and vomiting in 2HD on isolated times. There was no drug effect on body wt except for wt loss in few individual monkeys and mean food intake was reduced 31% (p<0.05) in HDm from the control during wk1 of treatment. There was no drug effect on hematology, clinical chemistry, urinalysis, ophthalmoscopy, or histopathology. Scattered findings were observed in: organ wt (absolute and relative wts of the spleen was decreased and those for the gonads were increased), liver, thyroid, thymus, and pituitary). Perivascular hemorrhage was seen at the injection site of all groups but there was no histopathology at these sites. The NOEL in this study is 2mg/kg.

Pharmacokinetics/Toxicokinetics:

Single Dose PK:

1. Pk in the rat after a single oral dose of 5, 10, 20mg/kg

Nov

1994/France.

This study was conducted under GLP.

Sprague-Dawley rats (16/sex/dose; 6-7wks old) were administered a single oral dose of 5,10,20mg/kg. Blood was sampled pre-dosing and upto 32hr postdose. Riluzole was prepared in 0.5% hydroxymethylcellulose aqueous solution. HPLC was used to determine plasma levels (quantification limit was 25ng/ml). Below are plasma concentration range and mean levels for the 3 doses in males and females; see attached tables for mean concentrations at different times.

Dose	Sex	Plasma conc. Range (mean±SD) (ng/ml)	T _{max} (hr)
5	m	26-1527 (475±114.5)	2*
	f	29-1755 (572±58)	0.5*
10	m	35-2805 (928±466)	0.5**
	f	32-3737 (1116±338)	3**
20	m	25-5280 (4169±1400)	3**
	f	50-6700 (4222±1134)	4**

Mean values are at 8hr unless indicated otherwise.

* drug was ND by 28hr based on individual data.

** still detectable by 32hr (45±32ng/ml).

In general, females had higher blood concentrations and AUC values than male rats at all doses. Drug levels increased linearly at 5 and 10mg/kg but more than proportional at 20mg/kg. Table below from the sponsor presents PK parameters.

MEAN PHARMACOKINETIC PARAMETERS IN THE RAT AFTER SINGLE ORAL ADMINISTRATION OF 5, 10 AND 20 MG/KG DOSES OF RP 54274

Dose mg/kg	MALE			FEMALE		
	C _{max} µg.ml ⁻¹	t _{max} h	AUC(0-lqc) µg.h.ml ⁻¹	C _{max} µg.ml ⁻¹	t _{max} h	AUC(0-lqc) µg.h.ml ⁻¹
5	1.20±0.31	2.00	5.65	1.38±0.31	0.50	11.60
10	2.22±0.90	0.25	21.43	2.66±0.87	3.00	26.26
20	4.50±1.05	3.00	76.80	5.87±0.54	6.00	95.85

lqc: last quantified concentration

lqc= 8h for 5 mg/kg dose, male; lqc=24h for 5 mg/kg dose, female; lqc=32h for 10 mg/kg and 20 mg/kg, male and female.



single dose - PK in the rat.

The plasma concentrations (ng.ml⁻¹) of riluzole (mean ± sd) in the 5mg/kg group is presented in the following table:

5 mg/kg group	Males	Females
0.25 hour		
0.5 hour		
1 hour		
2 hours		
3 hours		
4 hours		
5 hours		
6 hours		
8 hours		
24 hours		
28 hours		
32 hours		

NC : not calculated

The plasma concentrations (ng.ml⁻¹) of riluzole (mean ± sd) in the 10mg/kg group is presented in the following table:

10 mg/kg group	Males	Females
0.25 hour		
0.5 hour		
1 hour		
2 hours		
3 hours		
4 hours		
5 hours		
6 hours		
8 hours		
24 hours		
28 hours		
32 hours		

NC : not calculated



The plasma concentrations (ng.ml⁻¹) of riluzole (mean ± sd) in the 20mg/kg group is presented in the following table:

20 mg/kg group	Males	Females
0.25 hour		
0.5 hour		
1 hour		
2 hours		
3 hours		
4 hours		
5 hours		
6 hours		
8 hours		
24 hours		
28 hours		
32 hours		

This study has shown that following increasing oral doses of RP54274 to rats, the corresponding plasma concentration of riluzole increase proportionally. Plasma concentrations remain above the limit of quantification (LOQ) until 8 hours for males and until 24 hours for females after 5 mg/kg. After 10 mg/kg and 20 mg/kg the plasma concentrations remain above the LOQ until the last sampling time ie 32 hours. Riluzole concentrations seem to be lower in males than in females.

2. Radioactivity kinetics and excretion profile in the rat following a single oral dose of 2mg/kg /July 1994/France.
This study was conducted under GLP.

Sprague-Dawley rats (4/sex/time point) were orally dosed with 2mg/kg ¹⁴C-riluzole. Radioactivity was measured in blood, plasma, and brain between 0.25-72hr postdose, plasma protein binding was also measured at 0.25, 1, 3, and 6hr postdose. Total radioactivity excretion was determined in urine, feces, and lungs upto 72hr postdose (excepto for the lungs at 48hr). The drug was prepared in saline+1% HCl and dosed at 5ml/kg. Mean±SD for C_{max} of blood levels of total radioactivity in males and females were 1.2±0.33 at 0.5hr and 0.9±0.05mgEquiv/L at 3hr respectively, radioactivity declined rapidly by 72hr to 0.02mgEquiv/L. In plasma, radioactive levels in m and f were 0.7±0.17 at 0.5hr and 0.6±0.1mgEquiv/L at 2hr respectively. In the brain, max mean total radioactivity was 3± 0.8mgEquiv/L at 0.5hr in males and in females 2.2±0.36mgEquiv/L at 3hr. Brain levels as % of dose ranged between a maximum of 1.5% at 0.5hr to 0.01 at 72hr in males and 1.1% at 3hr to 0.013% at 72hr in females. Brain/plasma ratio ranged between 0.6-4.8 in m and f. Table below from the sponsor shows the PK parameters in these tissues.

Main Kinetic Parameters From Radioactivity Concentrations in the male and female rats following a single oral administration of ¹⁴C-RP 54274 at 2 mg.kg⁻¹

TISSUE	PARAMETER	MALE	FEMALE
BLOOD	t _{max} (h)	0.5	3
	C _{max} (mg eq.l ⁻¹)	1.17	0.87
	AUC _b (0-72h) (a)	9.4	11.5
	AUC _b (a)	10.2	12.6
	t _{1/2} (h) [24 - 72 h]	27	31
PLASMA	t _{max} (h)	0.5	2
	C _{max} (mg eq.l ⁻¹)	0.67	0.57
	AUC(0-72h) (a)	10.9	9.4
	AUC (a)	11.8	9.8
	t _{1/2} (h) [24 -72 h]	20	20
BRAIN	t _{max} (h)	0.5	3
	C _{max} (mg eq.kg ⁻¹)	2.99	2.22
	AUC _t (0-72h) (a)	16.5	23.1
	AUC _t (a)	17.2	24.1
	t _{1/2} (h) [24 -72 h]	22	25

(a)= expressed as mg RP 54274 eq.h.(l⁻¹ or kg⁻¹)

Mean plasma protein binding in male rats was between 62-89% and in females 79-91% measured upto 6hr postdose. Excretion of riluzole occurred mainly in urine at 62% in m and 75% in f. Excretion was less in feces at 32 and 25% in m and f respectively, minimal elimination recorded in the lungs as CO₂ at 0.44% in m and f. The drug does not seem to accumulate in the body as shown from the total retention of radioactivity by 72hr (1.7±0.31% in m and 2.4±0.60% in f). Mean tissue recovery was 96% in males and 103% in f. Among the tissues studied, tissue to plasma ratio was highest in the male and (female): the liver at 7(9), followed by the kidneys at 4.6(6), and the skin at 2.6(5).

3. PK in the monkey after a single oral dose of 5,10, or 20mg/kg,
 /Oct 1989/France.
 GLP status not reported.

Macaca fascicularis monkeys (2/sex/dose) were administered a single dose of riluzole at 5,10, and 20mg/kg in a capsule form. Biological samples analyzed included blood, urine, and feces for 24, 48, and 72hr respectively. HPLC/UV detector was the analytical method used at dl of 5ng/ml. The parent and glucuronide were measured. There was a large inter-animal variation in the data. Table below from the sponsor, shows PK parameters. Because of the small number of animals and the inter-animal variability in the data, an accurate conclusion regarding concentration-dose proportionality and exposure could not be made. Peak concn. reached in general, between 4-8hr except for 1 monkey it was 24hr. 24-hr urinary and feces elimination were low ranging between 0.05-3% for urine except for 1 monkey with high urine output at 8%, and 0.2-2% for feces. Such low excretion indicate high metabolism and presence of metabolites. Glucuronide conjugation seemed low as well with mean conjugated/free ratio between 1-2.

Females					Males				
Dose mg/kg	Monkey No.	C Max	T Max	AUC (0-24h) ng/mlxh-l.	Dose mg/kg	Monkey No.	C Max	T Max	AUC (0-24h) ng/mlxh-l
5	1419	1110	4	10286	5	1422	1623	4	22339
5	1421	694	4	9186	5	1424	799	4	8981
10	1425	1302	6	13582	10	1426	2545	6	30306
10	1429	2145	6	27434	10	1428	2521	4	32923
20	1431	2433	4	35031	20	1430	2014	8	42491
20	1433	3541	24	70990	20	1434	7869	6	91424

70
60

4. Kinetics and metabolic profile in the monkey after a single oral dose of 2mg/kg /Aug 1994.
This study was conducted under GLP.

Macaca fascicularis monkeys (2/sex) were administered a single oral dose of ¹⁴C-riluzole at 2mg/kg as a solution of HCl. Radioactivity was determined in blood, plasma, urine, and feces upto 7ds after dosing. Metabolite distribution was determined in blood and plasma from 0.25 to 24hr and in urine on days 1,2, and 3 before and after enzyme hydrolysis. There was no difference in radioactivity (uptake) between blood and plasma. Most of the radioactivity was excreted in urine at 74% by day7 and most (67%) of total radioactivity was eliminated during the 1st 48hr. Fecal elimination was small at 11% of administered dose. The parent drug accounted for most of plasma radioactivity upto 24hr (0.15ugEquiv RP54274/ml). Other plasma metabolites included metabolite O (RP 65331) detectable at 0.25hr (0.01 to 0.16ugEquiv/ml at 2hr) and p at max concentration of 0.11ugEquiv/ml at 1hr. Urinary metabolites before enzyme hydrolysis included the parent (16.5%), t1 (RP65110; 9.6%), u2 (8.1%), and metabolites O&P (together 15.7%; see tables from sponsor). After enzyme hydrolysis, a large increase noted in unchanged drug (45%).

single dose angles to monkey.

PK.

38
12

CODE OF ISOLEMENT	COLOR				MEAN	S.D.	C.V. (%)
	PINK	ORANGE	BEIGE	PURPLE			
(c)	0.40	ND	0.70	ND	0.28		
PK35011	3.30	4.70	7.90	3.40	4.83	2.15	44.50
(g)	0.30	ND	ND	ND	0.08		
(h)	ND	2.00	0.50	4.80	1.83	2.16	200.00
(k)	ND	7.20	0.30	ND	1.88		
(l)	ND	ND	ND	8.80	2.20		
RP64821	1.20	ND	0.30	0.40	0.48	0.51	107.86
(nl)	ND	ND	ND	2.00	0.50		
(n2)	7.50	11.50	6.00	7.30	-8.08	2.38	29.45
(o)	4.80	13.20	1.00	20.30	9.83	8.65	88.00
(p)	1.30	6.40	0.90	14.70	5.83	6.42	110.29
(q)	2.00	3.30	4.60	3.10	3.25	1.07	32.80
RP65331	2.10	1.90	2.10	0.50	1.65	0.77	46.81
(e) RP65110	10.10	11.60	16.20	0.60	-9.63	6.55	68.08
(td)	1.20	ND	0.20	ND	0.35		
RP65077	1.40	ND	4.80	ND	1.55		
RP69597	1.00	ND	3.70	0.40	1.28	1.67	130.83
--RP54274	-34.80	9.20	18.60	3.40	-16.50	13.71	83.11
(v)	0.10	3.30	1.00	0.10	1.13	1.51	134.29
unknown	0.10	0.30	0.30	0.40	0.28	0.13	45.76
TOTAL	71.54	74.60	69.05	70.10	71.32	2.41	3.38

TABLE 11 : 14C-RP54274. MONKEY STUDY
DISTRIBUTION OF URINARY METABOLITES . 0-3th DAY
(before enzymatic hydrolysis) , (expressed as % of administered dose)

S.D. : standard deviation C.V.(%) : coefficient of variation

N.D. : not detected (<0.1%)

Monkey PK single dose.



CODE OF ISOLEMENT	PINK MONKEY			CUMUL 1-3DAYS
	DAY 1	DAY 2	DAY 3	
PK35011	1.10	1.30	0.50	2.90
(g)	ND	ND	0.30	0.30
RP64821	0.60	0.50	0.10	1.20
(n2)	0.80	1.20	0.50	2.50
(o)	0.50	ND	ND	0.50
(p)	0.70	0.30	ND	1.00
(q)	0.80	0.50	0.30	2.00
RP65331	0.50	0.60	0.20	1.30
RP65110	4.20	4.20	1.20	9.60
(td)	ND	0.30	ND	0.30
RP65077	ND	0.80	0.50	1.30
RP69597	1.30	0.80	0.40	2.50
RP54274	-- 33.90	9.00	1.90	44.80
(v)	ND	0.70	0.10	0.80
unknown	ND	0.40	0.10	0.50
TOTAL	44.47	21.00	6.07	71.54

TABLE 13 : 14C-RP54274. MONKEY STUDY
 DISTRIBUTION OF URINARY METABOLITES (PINK MONKEY)
 (after enzymatic hydrolysis)
 SUBJECT: ROSE MONKEY (expressed as % of dose administered)

ND: not detected

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Repeats Dose Studies:

1. Quantitative distribution and excretion of ¹⁴C riluzole in male rats after 2mg/kg/day for 13wks/ 1797/June 1994/France.
Batch# for labelled drug: PX 6248-CEA 0690-1193; radiochemical purity 99.8%; chemical purity: >99%. Specific activity 56mCi/μmol.
This study was conducted under GLP

Male Sprague-Dawley rats (212-232g on day of study) were administered ¹⁴C riluzole at 2mg/kg/day for 13wks. The drug was prepared using (saline solution + 1%N HCL) and administered in the morning at 5ml/kg. Blood, plasma, and tissue radioactivity levels were measured on day 0, 7, 14, and 21 at specified times (starting at 0.5 and upto 168hr postdose). In addition, radioactivity in blood and plasma was monitored on day 21 at 0.08, 0.25 and upto 72hr postdose. Excretion of radioactivity was measured in urine and feces from 5rats upto 168hr postdose as well as tissue retention; total radioactivity was measured by liquid scintillation counting. [below is study design from the sponsor followed by sampling of blood and tissues].

GROUP	DAYS OF ADMINISTRATION	STUDY	NUMBER OF BATCHES	TOTAL
1	0	KINETICS AND DISTRIBUTION	3 batches of 3 male rats	9
2	0 to 7	KINETICS AND DISTRIBUTION	2 batches of 3 male rats	6
3	0 to 14	KINETICS AND DISTRIBUTION	2 batches of 3 male rats	6
4	0 to 21	KINETICS AND/OR DISTRIBUTION	13 batches of 3 male rats	39
5	0 to 21	EXCRETION	1 batch of 5 male rats	5

2.7.1. Blood

After prior anaesthesia with ether and laparotomy, 3 rats/time were killed by exsanguination from the abdominal aorta at the following times :

0.5, 3 and 24 hours after the administration on Day 0 (1 administration)

0.5 and 24 hours after the administration on Day 7 (8 administrations)

0.5 and 24 hours after the administration on Day 14 (15 administrations)

0.08 - 0.25 - 0.5 - 1 - 2 - 3 - 6 - 12 - 24 - 48 and 168 hours after the last administration on Day 21 (22 administrations).

The blood was individually collected by heparinized syringe.

2.7.2. Tissues

The following tissues : cerebrum, pituitary, eye, Harderian gland, thyroid, submaxillary gland, lymph node, thymus, heart, lungs, liver, kidneys, adrenals, spleen, white fat, brown fat, skeletal muscle, skin, bone marrow, testis, epididymis, prostate, urinary bladder, small intestine wall, large intestine wall, stomach wall, pancreas, aorta and spinal cord, were individually taken at each sacrifice time, on :

0.5, 3 and 24 hours after the administration on Day 0 (1 administration)

0.5 and 24 hours after the administration on Day 7 (8 administrations)

0.5 and 24 hours after the administration on Day 14 (15 administrations)

0.5 - 3 - 24 - 48 and 168 hours after the administration on Day 21 (22 administrations)

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Results: all values are means±S.D.

0.5hr during and after dosing:

Tissues	Radioactivity % of last dose			
	day0	day7	day14	day21
liver	3.1±0.6	9.1±4	6.4±0.1	7.4±1.3
sm.intest				
wall	3.3±1.0	6.0±1.0	5.0±1.0	5.0±1.0
skin	3.0±0.2	12.4±4.4	13.4±1.2	12.2±2.0
stomach				
wall	2.0±0.2	2.0±1.0	2.0±1.0	3.0±0.3
kidneys	1.0±0.1	1.2±0.4	1.0±0.1	1.0±0.1

Concentration (mg Equiv./kg fresh tissue)

stomach				
wall	5.4±6.2	6.2±2.4	7.0±3.2	11.3±1.3
urin.				
bladder	4.0±3.4	3.0±0.6	2.1±1.3	2.0±1.1
sm.intest				
wall	1.5±0.1	3.0±0.5	3.0±0.4	3.4±0.3
liver	1.2±0.2	3.2±1.1	3.0±0.1	3.1±0.2
kidneys	1.2±0.2	3.0±0.8	2.2±0.1	3.0±0.3
prostate	1.3±0.5	2.2±1.4	1.3±0.1	2.0*
adrenals	1.0±0.3	3.1±2.0	2.0±0.1	3.1±1.0
Harderian	1.0*	4.0±2.1	2.2±0.2	4.0±0.2

* mean of 2 rats only.

Other tissues showed high radioactive concentration at 0.5hr on day7 included: white and brown fat, epididymis, skin, aorta, lg intestine wall, lungs, heart, pancreas, thyroid, lymph node, submaxillary gland, spinal cord, and cerebrum.

24-hr during and after dosing:

Tissues	Radioactivity % of last dose			
	day0	day7	day14	day21
skin	1.3±0.1	8.0±1.3	6.0±1.0	10±0.5
liver	1.1±0.1	2.0±0.2	3.0±0.7	3.0±0.2
sm.intest				
wall	0.5±0.02	1.0±0.1	1.3±0.3	1.2±0.1

Concent. (mEquiv./kg fresh tissue)

Harderian	1.0±0.1	1.3±0.3	1.4±0.3	2.0±0.1
liver	0.4±0.02	1.0±0.1	1.0±0.1	1.2±0.1
kidneys	0.3±0.1	1.0±0.2	1.0±0.2	1.0±0.03
thyroid	0.2±0.2	0.4±0.1	1.0±0.3	1.4±1.0
sm.intest				
wall	0.2±0.03	0.4±0.1	1.0±0.3	1.0±0.2
skin	0.1±0.00	1.0±0.2	1.0±0.02	1.1±0.1

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Tissue/plasma ratios (in decreasing order) after 0.5hr on:

day0 stomach wall>urinary bladder>sm.intestine wall>prostate>kidney>liver>adrenals>Harderian gland.

day7 stomach wall>Harderian>liver>kidneys>adrenals>sm.intestine wall>urinary bladder>spinal cord>brown fat.

day14 stomach wall>sm.intestine wall>kidneys>Harderian>urinary bladder>adrenals.

day21 stomach wall>Harderian>sm.intestine wall>liver>adrenals>kidneys>spinal cord.

After 24hr:

day0 Harderian>liver>kidneys>sm.intestine wall>thyroid>pancreas>stomach wall.

day7 Harderian>liver>skin>kidneys>adrenals>stomach wall>pancreas>sm.intestine wall>thyroid.

day14 Harderian>liver>kidneys>urinary bladder>adrenals>thyroid>sm.intestine wall>skin.

day21 Harderian>thyroid>liver>skin>kidneys>sm.intestine wall>adrenals>urinary bladder>stomach wall.

Radioactivity (% of dose) following the first administration at:

0.5hr sm.intestine wall>liver>skin>stomach wall.

3hr skin>sm.intestine wall>skin>liver.

24hr skin>liver.

Concentration (mg Equiv./kg fresh tissue) following the first administration at:

0.5hr stomach wall>urinary bladder>sm.intestine wall>epididymis>liver>kidneys.

3hr kidneys>sm.intestine wall>liver>urinary bladder>Harderian>stomach wall>adrenals>spinal cord>white fat>prostate>brown fat>cerebrum>pancreasaorta.

24hr low in all tissues: Harderian>liver>kidneys>sm.intestine wall.

Tissue/plasma ratios following first dose at:

0.5hr stomach wall>sm.intestine wall>epididymis>liver>kidneys.

3hr kidneys>sm.intestine wall>liver>urinary bladder>Harderian>stomach wall>spinal cord>white fat>prostate>brown fat.

24hr Harderian>liver>kidneys>sm.intestine wall>thyroid>pancreas>stomach wall.

Following the last dose, highest radioactivity (%) was in the skin followed by the liver measured at 0.5, 3, 24, 48, and 168hr postdose. Concentration (mg Equiv./kg fresh tissue) on the other hand, was highest in stomach wall at 0.5hr and urinary bladder at 3hrs. Tissue/plasma ratio after last dose was highest in stomach wall followed by Harderian gland measured at 0.5hr, at 3hrs: urinary bladder>sm.intestine wall>Harderian>liver>prostate>stomach wall, at 24hr: Harderian>thyroid>liver>skin, at 48hr: skin>Harderian>liver>adrenals>kidneys; and at 168hr postdose the ratio was high in following tissues: adrenals (26±3)>thyroid (24.5±8.3)>liver (23.5±8)>skin (20.2±2)>kidneys (13.5±2)>Harderian (12.3±2)>brown fat (11±1)>spleen (10±1)>lungs (10±1). Blood tissue/plasma ratio was also moderately high with a mean of 7.3±0.4.

The mean drug blood levels were maximum at 1.2±0.2 mg Equiv./l at 3hr postdose and that in plasma was maximum at 0.78±0.02 mg Equiv./l measured at 6hr postdose (see table from the sponsor).

**TABLE 14 : BLOOD AND PLASMA CONCENTRATIONS
(mg eq RP/l) AFTER THE LAST ORAL
ADMINISTRATION AT 2 mg/kg**

HOURS	BLOOD		PLASMA	
	MEAN ± S.D.	S.D.	MEAN ± S.D.	S.D.
0.08				
0.25				
0.5				
1				
2				
3				
4				
6				
12				
24				
48				
72				
168				

Excretion of the drug (radioactivity) following the last dose was mainly through the urine at 78% of accumulated dose whereas, the feces accounted for 30% of the accumulated dose (see attached table from the sponsor). Total radioactivity excretion was on the average 109% of accumulated dose and 141% of the last dose over 168hr after last dosing. At 168hr from last dosing, total tissue retention was 0.38% of accumulated dose and 5.8% of the last dose. Summary of the calculated PK parameters and other relevant data are presented in the attached table from the sponsor. [also attached are tables from the sponsor for radioactivity retention in tissues].

Summary and Conclusion:

Oral administration of C¹⁴ riluzole to male rats for 13wks was detected in many tissues. Radioactivity tissue concentration did not increase overtime/repeated dosing except for the thyroid and Hardarian gland where concentrations were much higher. High radioactivity was recorded in the skin, liver, sm.intestine wall, kidneys, testes, stomach wall, cerebrum, lungs, lg.intestine wall, and pancreas. Highest concentrations were measured in the Harderain gland, thyroid, liver, skin, kidneys, sm.intestine wall, and adrenals. Whereas, lowest level was detected in the thymus, eye, and plasma. Major route of excretion was the urine at 78% and only 30% of accumulated dose was in feces. Total radioactivity excretion of 109% of accumulated dose and 141% of last dose measured over 168hr from last dose. There was no accumulation as seen from the small tissue retention measured after the last dose at 168hr with an average of 0.38% and 5.8% of accumulated and last dose respectively. Blood and plasma radioactivity fell rapidly following the last dose and then more slowly (terminal t_{1/2} of 39hr). At any time, blood concentration of radioactivity was higher than that in plasma indicating preferential uptake by blood cells.

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Quantitative distrib. & Exc. of C-14 in the rat. - PK section.

iv

Dose 2mg/kg for 13-wks

	MEAN		S.D.
TOTAL RECOVERY OF RADIOACTIVITY AT 168 HOURS AFTER THE LAST ADMINISTRATION (% of the accumulated dose)			
TOTAL RETENTION IN TISSUES	0.38	±	0.06
TOTAL RETENTION (TISSUES AND INTESTINAL CONTENTS)	0.40	±	0.07
FAECES	30.48	±	3.81
URINE	78.26	±	4.33
TOTAL EXCRETION	108.74	±	1.11
TOTAL RECOVERY	109.14	±	1.11

Value represent the mean and standard deviation of 5 animals

MAIN RADIOACTIVITY KINETIC PARAMETERS IN PLASMA		
(after the last administration)		
t_{max} (h)	6	Blood 3
C_{max} (mg eq.l ⁻¹)	0.78	1.16 ± 0.16
AUC(0-168h) (a)	21.9	41.4 -
AUC (a)	22.8	
$t_{1/2}$ (h) [24 - 168 h]	39	

(a) = expressed as mg RP 54274 eq.h.(l⁻¹)

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**TABLE 3 : RADIOACTIVITY RETENTION (% OF THE LAST DOSE) IN TISSUES
24 HOURS DURING AND AFTER REPEATED ORAL ADMINISTRATIONS AT 2mg/kg**

TISSUES	DAY 0		DAY 7		DAY 14		DAY 21	
	MEAN ±	S.D.	MEAN ±	S.D.	MEAN ±	S.D.	MEAN ±	S.D.
CEREBRUM	0.034 ±	0.005	0.071 ±	0.018	0.068 ±	0.017	0.069 ±	0.004
PITUITARY	N.S.		0.000 ±	0.000	0.001 ±	0.000	0.000 ±	0.000
EYE	0.003 ±	0.001	0.007 ±	0.002	0.008 ±	0.003	0.009 ±	0.001
HARDERIAN GLAND	0.031 ±	0.002	0.070 ±	0.013	0.089 ±	0.012	0.096 ±	0.006
SUBMAXILLARY GLAND	0.010 ±	0.002	0.024 ±	0.003	0.028 ±	0.006	0.031 ±	0.002
THYROID	0.001 ±	0.001	0.002 ±	0.000	0.003 ±	0.001	0.004 ±	0.003
THYMUS	0.008 ±	0.001	0.020 ±	0.003	0.023 ±	0.009	0.020 ±	0.003
PANCREAS	0.027 ±	0.004	0.085 ±	0.006	0.110 ±	0.017	0.079 ±	0.017
SPLEEN	0.011 ±	0.002	0.034 ±	0.011	0.050 ±	0.015	0.040 ±	0.006
HEART	0.016 ±	0.001	0.039 ±	0.007	0.050 ±	0.014	0.049 ±	0.003
LUNGS	0.035 ±	0.005	0.088 ±	0.019	0.100 ±	0.022	0.107 ±	0.002
ADRENALS	0.002 ±	0.000	0.006 ±	0.001	0.008 ±	0.000	0.008 ±	0.002
KIDNEYS	0.132 ±	0.027	0.294 ±	0.056	0.421 ±	0.085	0.365 ±	0.021
LIVER	1.105 ±	0.096	1.969 ±	0.179	2.770 ±	0.651	2.752 ±	0.167
STOMACH WALL	0.056 ±	0.005	0.121 ±	0.025	0.157 ±	0.020	0.155 ±	0.066
SMALL INTESTINE WALL	0.461 ±	0.027	0.785 ±	0.117	1.269 ±	0.322	1.153 ±	0.093
LARGE INTESTINE WALL	0.046 ±	0.011	0.095 ±	0.012	0.125 ±	0.016	0.103 ±	0.019
URINARY BLADDER	0.003 ±	0.002	0.004 ±	0.001	0.010 ±	0.010	0.007 ±	0.001
TESTES	0.068 ±	0.010	0.192 ±	0.070	0.212 ±	0.076	0.263 ±	0.025
EPIDIDYMIS	0.010 ±	0.001	0.028 ±	0.006	0.038 ±	0.007	0.044 ±	0.006
PROSTATE	0.005 ±	0.001	0.013 ±	0.004	0.029 ±	0.027	0.024 ±	0.004
SKIN	1.277 ±	0.145	7.989 ±	1.271	5.869 ±	0.716	9.839 ±	0.476

Values represent the mean and standard deviation of three animals

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TABLE 5 : RADIOACTIVITY CONCENTRATIONS (mg eq RP/kg fresh tissue) IN TISSUES
24 HOURS DURING AND AFTER REPEATED ORAL ADMINISTRATIONS AT 2mg/kg

TISSUES	DAY 0			DAY 7			DAY 14			DAY 21		
	MEAN ±	S.D.		MEAN ±	S.D.		MEAN ±	S.D.		MEAN ±	S.D.	
BLOOD	0.078 ±	0.082		0.214 ±	0.022		0.257 ±	0.042		0.322 ±	0.005	
PLASMA	0.082 ±	0.014		0.188 ±	0.023		0.205 ±	0.057		0.208 ±	0.004	
CEREBRUM	0.082 ±	0.007		0.207 ±	0.049		0.221 ±	0.061		0.276 ±	0.018	
SPINAL CORD	0.112 ±	0.013		0.311 ±	0.101		0.310 ±	0.110		0.416 ±	0.032	
PITUITARY	N.S.			0.201 ±	0.049		0.288 ±	0.086		0.340 ±	0.029	
EYE	0.053 ±	0.011		0.138 ±	0.027		0.164 ±	0.041		0.205 ±	0.013	
HARDERIAN GLAND	0.511 ±	0.057		1.267 ±	0.272		1.408 ±	0.307		1.753 ±	0.053	
SUBMAXILLARY GLAND	0.083 ±	0.023		0.234 ±	0.040		0.283 ±	0.067		0.298 ±	0.021	
LYMPH NODE	0.083 ±	0.009		0.235 ±	0.065		0.250 ±	0.043		0.327 ±	0.072	
THYROID	0.219 ±	0.200		0.395 ±	0.119		0.723 ±	0.251		1.351 ±	0.822	
PANCREAS	0.061 ±	0.013		0.152 ±	0.041		0.173 ±	0.033		0.228 ±	0.023	
SPLEEN	0.170 ±	0.007		0.439 ±	0.032		0.458 ±	0.081		0.417 ±	0.098	
HEART	0.078 ±	0.008		0.199 ±	0.035		0.261 ±	0.041		0.339 ±	0.037	
LUNGS	0.098 ±	0.012		0.222 ±	0.048		0.244 ±	0.074		0.304 ±	0.015	
ADRENALS	0.131 ±	0.027		0.341 ±	0.080		0.413 ±	0.089		0.508 ±	0.044	
KIDNEYS	0.146 ±	0.018		0.540 ±	0.100		0.766 ±	0.112		0.775 ±	0.233	
LIVER	0.253 ±	0.054		0.763 ±	0.170		0.969 ±	0.225		0.989 ±	0.031	
STOMACH WALL	0.116 ±	0.017		0.998 ±	0.085		1.033 ±	0.145		1.225 ±	0.072	
SMALL INTESTINE WALL	0.168 ±	0.025		0.454 ±	0.085		0.579 ±	0.070		0.631 ±	0.241	
LARGE INTESTINE WALL	0.215 ±	0.026		0.434 ±	0.100		0.752 ±	0.254		0.697 ±	0.161	
URINARY BLADDER	0.140 ±	0.019		0.358 ±	0.029		0.461 ±	0.049		0.485 ±	0.090	
BROWN FAT	0.168 ±	0.066		0.327 ±	0.076		0.783 ±	0.734		0.662 ±	0.028	
WHITE FAT	0.089 ±	0.020		0.263 ±	0.056		0.286 ±	0.084		0.432 ±	0.054	
SKELETAL MUSCLE	0.061 ±	0.015		0.158 ±	0.052		0.190 ±	0.029		0.201 ±	0.027	
AORTA	0.084 ±	0.014		0.224 ±	0.070		0.286 ±	0.097		0.395 ±	0.047	
BONE MARROW	0.072 ±	0.012		0.223 ±	0.021		0.304 ±	0.066		0.371 ±	0.008	
TESTES	0.054 ±	0.007		0.148 ±	0.016		0.166 ±			0.234 ±	0.016	
EPIDIDYMIS	0.123 ±	0.022		0.378 ±	0.115		0.412 ±	0.154		0.551 ±	0.057	
PROSTATE	0.128 ±	0.023		0.298 ±	0.055		0.358 ±	0.069		0.368 ±	0.045	
SKIN	0.075 ±	0.027		0.231 ±	0.022		0.414 ±	0.330		0.361 ±	0.063	
	0.141 ±	0.007		0.914 ±	0.163		0.588 ±	0.017		1.055 ±	0.144	

Values represent the mean and standard deviation of three animals or two animals (*)

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TABLE 6: RADIOACTIVITY DISTRIBUTION 0.5 HOUR DURING AND AFTER REPEATED ORAL ADMINISTRATIONS AT 2mg/kg TISSUE/PLASMA RATIO

TISSUES	0.5 HOUR			
	DAY 0 MEAN ± S.D.	DAY 7 MEAN ± S.D.	DAY 14 MEAN ± S.D.	DAY 21 MEAN ± S.D.
BLOOD	1.155 ± 0.051	1.279 ± 0.117	1.283 ± 0.093	1.512 ± 0.121
CEREBRUM	2.317 ± 0.085	2.494 ± 0.686	1.855 ± 0.281	2.912 ± 0.462
SPINAL CORD	2.940 ± 0.202	3.540 ± 1.366	2.679 ± 0.522	4.076 ± 0.765
PITUITARY	1.594 ± 0.074	1.767 ± 0.442	1.690 ± 0.354	2.158 ± 0.285
EYE	0.614 ± 0.055	0.769 ± 0.258	0.638 ± 0.089	1.008 ± 0.098
HARDERIAN GLAND	3.334 *	5.028 ± 1.181	4.745 ± 0.999	7.164 ± 0.367
SUBMAXILLARY GLAND	1.784 ± 0.032	2.015 ± 0.424	1.682 ± 0.171	2.431 ± 0.458
LYMPH NODE	1.581 ± 0.085	1.738 ± 0.276	1.412 ± 0.224	2.058 ± 0.215
THYROID	1.397 ± 0.113	2.042 ± 0.139	3.158 ± 0.417	3.598 ± 1.256
THYMUS	0.938 ± 0.022	1.047 ± 0.284	0.941 ± 0.076	1.482 ± 0.270
PANCREAS	2.548 ± 0.401	2.904 ± 0.532	2.506 ± 0.248	2.497 ± 0.718
SPLEEN	1.043 ± 0.022	1.308 ± 0.284	1.204 ± 0.165	1.770 ± 0.188
HEART	1.878 ± 0.035	1.989 ± 0.474	1.726 ± 0.150	2.413 ± 0.255
LUNGS	1.658 ± 0.115	1.813 ± 0.286	1.824 ± 0.300	2.486 ± 0.288
ADRENALS	3.716 ± 0.750	4.214 ± 0.978	4.113 ± 0.588	5.607 ± 0.865
KIDNEYS	5.038 ± 0.514	4.244 ± 0.365	4.774 ± 0.633	5.589 ± 0.201
LIVER	5.077 ± 0.312	4.653 ± 0.254	5.441 ± 0.546	5.746 ± 0.098
STOMACH WALL	23.365 ± 7.018	10.292 ± 5.928	20.72 ± 10.41	20.846 ± 2.626
SMALL INTESTINE WALL	6.358 ± 1.007	4.238 ± 1.362	5.686 ± 0.208	6.298 ± 0.585
LARGE INTESTINE WALL	1.147 ± 0.069	1.698 ± 0.243	1.686 ± 0.202	2.099 ± 0.051
URINARY BLADDER	18.47 ± 20.03	4.205 ± 1.847	4.415 ± 2.515	3.372 ± 1.988
BROWN FAT	2.947 ± 0.156	3.227 ± 0.610	2.373 ± 0.401	3.629 ± 1.074
WHITE FAT	2.405 ± 0.780	2.406 ± 0.725	1.555 ± 0.284	2.038 ± 0.275
SKELETAL MUSCLE	1.095 ± 0.420	0.988 ± 0.165	0.983 ± 0.156	1.512 ± 0.382
AORTA	1.878 ± 0.925	1.582 ± 0.162	1.501 ± 0.217	2.258 ± 0.655
BONE MARROW	1.037 ± 0.037	1.205 ± 0.333	1.009 ± 0.133	1.525 ± 0.218
TESTES	1.185 ± 0.469	1.344 ± 0.313	1.304 ± 0.263	1.393 ± 0.389
EPIDIDYMIS	2.768 ± 2.803	1.508 ± 0.265	2.000 ± 0.270	1.629 ± 0.144
PROSTATE	5.195 ± 1.288	2.904 ± 0.904	2.825 ± 0.431	3.514 *
SKIN	1.250 ± 0.217	1.982 ± 0.262	2.846 ± 0.478	2.314 ± 0.028

Values represent the mean and standard deviation of three animals or two animals (*)

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TABLE 7: RADIOACTIVITY DISTRIBUTION 24 HOURS DURING AND AFTER REPEATED ORAL ADMINISTRATIONS AT 2mg/kg TISSUE / PLASMA RATIO

TISSUES	DAY 0		DAY 7		DAY 14		DAY 21	
	MEAN ±	S.D.	MEAN ±	S.D.	MEAN ±	S.D.	MEAN ±	S.D.
BLOOD	0.953 ±	0.150	1.138 ±	0.054	1.276 ±	0.145	1.547 ±	0.030
CEREBRUM	1.012 ±	0.31	1.090 ±	0.138	1.078 ±	0.061	1.326 ±	0.077
SPINAL CORD	1.375 ±	0.31	1.628 ±	0.381	1.493 ±	0.121	2.001 ±	0.171
PITUITARY	N.S.		1.062 ±	0.138	1.299 ±	0.083	1.635 ±	0.157
EYE	0.653 ±	0.079	0.727 ±	0.056	0.804 ±	0.098	0.888 ±	0.079
HARDERIAN GLAND	6.290 ±	0.540	6.682 ±	0.692	7.042 ±	1.793	6.430 ±	0.226
SUBMAXILLARY GLAND	1.003 ±	0.120	1.238 ±	0.079	1.282 ±	0.032	1.432 ±	0.100
LYMPH NODE	1.023 ±	0.063	1.236 ±	0.207	1.241 ±	0.182	1.571 ±	0.344
THYROID	2.674 ±	2.433	2.094 ±	0.496	3.667 ±	1.555	6.468 ±	3.910
THYMUS	0.745 ±	0.032	0.799 ±	0.133	0.853 ±	0.088	1.098 ±	0.114
PANCREAS	2.105 ±	0.320	2.343 ±	0.139	2.270 ±	0.214	2.004 ±	0.445
SPLEEN	0.974 ±	0.165	1.053 ±	0.060	1.289 ±	0.142	1.631 ±	0.171
HEART	1.205 ±	0.053	1.171 ±	0.134	1.183 ±	0.084	1.484 ±	0.059
LUNGS	1.620 ±	0.351	1.797 ±	0.221	2.036 ±	0.175	2.442 ±	0.226
ADRENALS	1.769 ±	0.102	2.857 ±	0.205	3.827 ±	0.508	3.711 ±	1.060
KIDNEYS	3.535 ±	0.197	4.117 ±	1.116	4.756 ±	0.262	4.756 ±	0.097
LIVER	5.193 ±	1.067	6.327 ±	0.527	6.159 ±	0.766	5.893 ±	0.354
STOMACH WALL	2.076 ±	0.352	2.407 ±	0.319	2.960 ±	0.909	3.020 ±	1.099
SMALL INTESTINE WALL	2.675 ±	0.528	2.321 ±	0.514	3.646 ±	0.526	4.303 ±	0.688
LARGE INTESTINE WALL	1.737 ±	0.366	1.938 ±	0.418	2.385 ±	0.772	2.324 ±	0.387
URINARY BLADDER	1.969 ±	0.713	1.794 ±	0.668	4.070 ±	4.122	3.185 ±	0.193
BROWN FAT	1.085 ±	0.070	1.386 ±	0.156	1.400 ±	0.208	2.080 ±	0.271
WHITE FAT	0.742 ±	0.105	0.827 ±	0.190	0.952 ±	0.181	0.967 ±	0.116
SKELETAL MUSCLE	1.032 ±	0.034	1.169 ±	0.252	1.385 ±	0.217	1.898 ±	0.200
AORTA	0.887 ±	0.142	1.193 ±	0.080	1.498 ±	0.085	1.786 ±	0.032
BONE MARROW	0.665 ±	0.069	0.792 ±	0.026	0.964 ±	0.261	1.126 ±	0.058
TESTES	1.503 ±	0.120	1.969 ±	0.399	1.982 ±	0.281	2.655 ±	0.315
EPIDIDYMIS	1.576 ±	0.057	1.568 ±	0.107	1.768 ±	0.142	1.772 ±	0.227
PROSTATE	0.901 ±	0.194	1.243 ±	0.228	2.137 ±	1.871	1.735 ±	0.312
SKIN	1.747 ±	0.250	4.903 ±	0.954	2.987 ±	0.671	5.069 ±	0.621

Values represent the mean and standard deviation of three animals or two animals (*)

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TABLE 10: RADIOACTIVITY DISTRIBUTION AFTER
THE FIRST ORAL ADMINISTRATION AT 2mg/kg
TISSUE /PLASMA RATIO

TISSUES	0.5 HOUR		3 HOURS		24 HOURS	
	MEAN ±	S.D.	MEAN ±	S.D.	MEAN ±	S.D.
BLOOD	1.155 ±	0.051	1.007 ±	0.238	0.853 ±	0.050
CEREBRUM	2.317 ±	0.065	2.712 ±	1.120	1.012 ±	0.091
SPINAL CORD	2.840 ±	0.202	3.750 ±	1.843	1.375 ±	0.131
PITUITARY	1.594 ±	0.074	1.911 ±	0.868	N.S.	
EYE	0.614 ±	0.055	0.831 ±	0.255	0.653 ±	0.079
HARDERIAN GLAND	3.334 ±		4.308 ±	1.691	6.290 ±	0.540
SUBMAXILLARY GLAND	1.794 ±	0.032	1.920 ±	0.813	1.003 ±	0.120
LYMPH NODE	1.561 ±	0.065	1.665 ±	0.540	1.023 ±	0.063
THYROID	1.397 ±	0.113	1.771 ±	0.692	2.674 ±	2.433
THYMUS	0.939 ±	0.022	1.128 ±	0.450	0.745 ±	0.032
PANCREAS	2.548 ±	0.401	2.708 ±	0.967	2.105 ±	0.320
SPLEEN	1.043 ±	0.022	1.322 ±	0.501	0.974 ±	0.165
HEART	1.879 ±	0.035	1.979 ±	0.795	1.205 ±	0.053
LUNGS	1.656 ±	0.115	1.860 ±	0.523	1.620 ±	0.351
ADRENALS	3.716 ±	0.750	4.009 ±	1.815	1.789 ±	0.102
KIDNEYS	5.039 ±	0.514	6.169 ±	0.197	3.535 ±	0.197
LIVER	5.077 ±	0.312	4.638 ±	1.492	5.193 ±	1.067
STOMACH WALL	23.385 ±	7.018	4.113 ±	2.218	2.078 ±	0.352
SMALL INTESTINE WALL	6.356 ±	1.007	5.535 ±	1.062	2.675 ±	0.528
LARGE INTESTINE WALL	1.147 ±	0.069	1.448 ±	0.232	1.737 ±	0.369
URINARY BLADDER	18.47 ±	20.03	4.598 ±	3.440	1.989 ±	0.713
BROWN FAT	2.947 ±	0.156	3.096 ±	1.504	1.085 ±	0.070
WHITE FAT	2.405 ±	0.780	3.266 ±	1.591	0.742 ±	0.105
SKELETAL MUSCLE	1.095 ±	0.420	1.050 ±	0.358	1.032 ±	0.034
AORTA	1.878 ±	0.925	2.248 ±	1.567	0.887 ±	0.142
BONE MARROW	1.037 ±	0.037	1.114 ±	0.375	0.665 ±	0.069
TESTES	1.185 ±	0.469	1.232 ±	0.372	1.503 ±	0.120
EPIDIDYMIS	2.786 ±	2.203	1.835 ±	1.026	1.576 ±	0.057
PROSTATE	5.195 ±	1.288	3.104 ±	2.265	0.901 ±	0.194
SKIN	1.250 ±	0.217	1.628 ±	0.514	1.747 ±	0.250

Values represent the mean and standard deviation of three animals of two animals (*)

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TABLE 12: RADIOACTIVITY CONCENTRATIONS (mg eq RP/kg fresh tissue) IN TISSUES AFTER THE LAST ORAL ADMINISTRATION AT 2mg/kg

TISSUES	0.5 HOUR		3 HOURS		24 HOURS		48 HOURS		168 HOURS	
	MEAN ±	S.D.	MEAN ±	S.D.	MEAN ±	S.D.	MEAN ±	S.D.	MEAN ±	S.D.
BLOOD	0.823 ±	0.079	1.159 ±	0.163	0.322 ±	0.005	0.251 ±	0.008	0.118 ±	0.007
PLASMA	0.545 ±	0.036	0.710 ±	0.094	0.208 ±	0.004	0.128 ±	0.017	0.016 ±	0.002
CEREBRUM	1.587 ±	0.293	2.173 ±	0.392	0.276 ±	0.018	0.173 ±	0.034	0.057 ±	0.003
SPINAL CORD	2.221 ±	0.449	3.120 ±	0.533	0.416 ±	0.032	0.249 ±	0.053	0.081 ±	0.001
PITUITARY	1.173 ±	0.164	1.603 ±	0.313	0.340 ±	0.029	0.228 ±	0.034	N.S.	
EYE	0.549 ±	0.063	0.813 ±	0.154	0.205 ±	0.013	0.130 ±	0.018	0.036 ±	0.005
HARDERIAN GLAND	3.897 ±	0.215	4.559 ±	0.621	1.753 ±	0.053	0.886 ±	0.137	0.199 ±	0.041
SUBMAXILLARY GLAND	1.326 ±	0.277	1.718 ±	0.334	0.266 ±	0.021	0.211 ±	0.034	0.059 ±	0.003
LYMPH NODE	1.120 ±	0.131	1.483 ±	0.183	0.327 ±	0.072	0.209 ±	0.043	0.089 ±	0.005
THYROID	1.930 ±	0.563	1.313 ±	0.261	1.351 ±	0.822	0.396 ±	0.039	0.403 ±	0.168
THYMUS	0.810 ±	0.175	1.022 ±	0.192	0.228 ±	0.023	0.165 ±	0.038	0.077 ±	0.002
PANCREAS	1.347 ±	0.321	2.136 ±	0.470	0.417 ±	0.098	0.366 ±	0.015	0.126 ±	0.023
SPLEEN	0.982 ±	0.099	1.253 ±	0.157	0.339 ±	0.037	0.268 ±	0.039	0.163 ±	0.010
HEART	1.319 ±	0.207	1.786 ±	0.293	0.304 ±	0.015	0.217 ±	0.040	0.063 ±	0.006
LUNGS	1.355 ±	0.192	1.758 ±	0.227	0.508 ±	0.044	0.412 ±	0.098	0.159 ±	0.005
ADRENALS	3.053 ±	0.516	3.744 ±	0.508	0.775 ±	0.233	0.789 ±	0.199	0.416 ±	0.040
KIDNEYS	3.048 ±	0.294	4.055 ±	0.533	0.989 ±	0.031	0.670 ±	0.051	0.215 ±	0.006
LIVER	3.130 ±	0.212	4.387 ±	0.722	1.225 ±	0.072	0.816 ±	0.106	0.380 ±	0.145
STOMACH WALL	1.314 ±	1.314	3.885 ±	0.414	0.631 ±	0.241	0.382 ±	0.074	0.074 ±	0.002
SMALL INTESTINE WALL	3.423 ±	0.277	4.611 ±	1.391	0.697 ±	0.161	0.369 ±	0.006	0.069 ±	0.005
LARGE INTESTINE WALL	1.144 ±	0.094	1.839 ±	0.532	0.485 ±	0.090	0.295 ±	0.012	0.063 ±	0.007
URINARY BLADDER	1.818 ±	1.079	4.810 ±	3.680	0.662 ±	0.028	0.289 ±	0.025	0.082 ±	0.010
BROWN FAT	1.964 ±	0.628	2.636 ±	0.512	0.432 ±	0.054	0.339 ±	0.067	0.179 ±	0.012
WHITE FAT	1.103 ±	0.080	2.596 ±	0.582	0.205 ±	0.027	0.198 ±	0.063	0.088 ±	0.016
SKELETAL MUSCLE	0.825 ±	0.222	1.041 ±	0.156	0.395 ±	0.047	0.281 ±	0.092	0.101 ±	0.016
AORTA	1.234 ±	0.383	1.536 ±	0.328	0.371 ±	0.008	0.255 ±	0.060	0.129 ±	0.008
BONE MARROW	0.630 ±	0.124	1.045 ±	0.273	0.234 ±	0.016	0.157 ±	0.046	0.059 ±	0.002
TESTES	0.756 ±	0.203	1.247 ±	0.215	0.551 ±	0.057	0.386 ±	0.136	0.122 ±	0.013
EPIDIDYMIS	0.884 ±	0.034	1.428 ±	0.115	0.368 ±	0.045	0.219 ±	0.014	0.089 ±	0.013
PROSTATE	1.976 ±	0.096	3.931 ±	0.738	0.361 ±	0.063	0.198 ±	0.031	0.056 ±	0.005
SKIN	1.261 ±	0.096	1.983 ±	0.116	1.055 ±	0.144	0.957 ±	0.041	0.325 ±	0.033

Values represent the mean and standard deviation of three animals of two animals (?)

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TABLE 13: RADIOACTIVITY DISTRIBUTION AFTER
THE LAST ORAL ADMINISTRATION AT 2mg/kg
TISSUE/PLASMA RATIO

TISSUES	0.5 HOUR		3 HOURS		24 HOURS		48 HOURS		168 HOURS	
	MEAN ±	S.D.	MEAN ±	S.D.	MEAN ±	S.D.	MEAN ±	S.D.	MEAN ±	S.D.
BLOOD	1.512 ±	0.121	1.638 ±	0.169	1.547 ±	0.030	1.976 ±	0.240	7.324 ±	0.390
CEREBRUM	2.912 ±	0.482	3.057 ±	0.380	1.328 ±	0.077	1.338 ±	0.088	3.508 ±	0.553
SPINAL CORD	4.076 ±	0.765	4.414 ±	0.750	2.001 ±	0.171	1.924 ±	0.168	5.084 ±	0.633
PITUITARY	2.156 ±	0.285	2.252 ±	0.308	1.835 ±	0.157	1.775 ±	0.091	N.S.	
EYE	1.008 ±	0.098	1.44 ±	0.158	0.988 ±	0.079	1.013 ±	0.042	2.211 ±	0.068
HARDERIAN GLAND	7.184 ±	0.367	6.451 ±	0.866	8.430 ±	0.220	9.986 ±	1.287	12.281 ±	1.730
SUBMAXILLARY GLAND	2.431 ±	0.458	2.409 ±	0.237	1.432 ±	0.100	1.641 ±	0.063	3.707 ±	0.472
LYMPH NODE	2.058 ±	0.215	2.095 ±	0.211	1.571 ±	0.344	1.622 ±	0.165	5.546 ±	0.312
THYROID	3.598 ±	1.256	1.898 ±	0.566	6.468 ±	3.910	3.147 ±	0.681	24.464 ±	8.320
THYMUS	1.482 ±	0.270	1.439 ±	0.210	1.096 ±	0.114	1.280 ±	0.182	4.805 ±	0.414
PANCREAS	2.497 ±	0.718	2.990 ±	0.302	2.004 ±	0.445	3.048 ±	0.472	7.843 ±	1.442
SPLEEN	1.770 ±	0.188	1.768 ±	0.158	1.631 ±	0.171	2.093 ±	0.174	10.132 ±	0.797
HEART	2.413 ±	0.255	2.515 ±	0.303	1.464 ±	0.059	1.689 ±	0.135	3.986 ±	0.539
LUNGS	2.486 ±	0.288	2.484 ±	0.269	2.442 ±	0.226	3.193 ±	0.468	9.884 ±	0.809
ADRENALS	5.607 ±	0.885	5.279 ±	0.459	3.711 ±	1.060	6.125 ±	1.058	25.923 ±	2.877
KIDNEYS	5.589 ±	0.201	5.746 ±	0.833	4.758 ±	0.097	5.257 ±	0.430	13.486 ±	1.907
LIVER	5.746 ±	0.098	5.167 ±	0.383	5.893 ±	0.354	6.366 ±	0.561	23.480 ±	7.795
STOMACH WALL	20.648 ±	2.826	5.564 ±	1.139	3.020 ±	1.099	2.966 ±	0.228	4.621 ±	0.463
SMALL INTESTINE WALL	6.298 ±	0.585	6.693 ±	2.770	4.303 ±	0.688	2.916 ±	0.409	5.531 ±	0.356
LARGE INTESTINE WALL	2.099 ±	0.051	2.557 ±	0.396	2.324 ±	0.387	2.333 ±	0.339	3.904 ±	0.279
URINARY BLADDER	3.372 ±	1.988	7.349 ±	6.199	3.185 ±	0.193	2.297 ±	0.475	5.061 ±	0.455
BROWN FAT	3.629 ±	1.074	3.713 ±	0.602	2.080 ±	0.271	2.615 ±	0.359	11.179 ±	1.223
WHITE FAT	2.038 ±	0.275	3.639 ±	0.589	0.967 ±	0.116	1.513 ±	0.308	5.561 ±	1.607
SKELETAL MUSCLE	1.512 ±	0.382	1.473 ±	0.216	1.898 ±	0.200	2.182 ±	0.562	6.224 ±	0.388
AORTA	2.258 ±	0.655	2.182 ±	0.506	1.788 ±	0.032	1.970 ±	0.232	6.062 ±	0.999
BONE MARROW	1.525 ±	0.218	1.456 ±	0.217	1.128 ±	0.058	1.223 ±	0.272	3.704 ±	0.287
TESTES	1.393 ±	0.399	1.751 ±	0.117	2.655 ±	0.315	2.976 ±	0.789	7.631 ±	1.136
EPIDIDYMIS	1.629 ±	0.144	2.028 ±	0.247	1.772 ±	0.227	1.742 ±	0.361	4.245 ±	0.547
PROSTATE	3.514 ±		5.679 ±	1.741	1.735 ±	0.312	1.541 ±	0.052	3.508 ±	0.341
SKIN	2.314 ±	0.026	2.783 ±	0.243	5.069 ±	0.621	7.593 ±	1.451	20.191 ±	1.616

Values represent the mean and standard deviation of three animals or two animals (?)

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2. TK of riluzole (RP 54274) in mice following daily oral dosing of 10, 20, and 30mg/kg for 13wks, /June 1994/France. This study is part of the toxicity study# Feb 1989 (discussed under the Subchronic Toxicology section). This study was conducted under GLP.

Male and female mice used in this study are part of 13-wk toxicology study (# listed above). The toxicity study was done at whereas, the drug plasma assay was done by RPR in France. There were 10 mice/sex/dose. The drug was prepared in 0.5% HMC and 5ml/kg dose was delivered. Blood was collected from the orbital sinus on day1 and wk13 from 5/sex/dose at 1 and 24hr postdosing. Plasma levels were measured by HPLC. Statistical analysis of the 24hr samples was not done because levels were below the quantitation limit on wk1 for both sexes.

Results:

Plasma concentrations in ng/ml:

At 1hr postdose:

	Dose (mg/kg)		
	10	20	30
wk1	557±96 (560±90)	1507±552 (1087±97)	1637±533 (1104±378)
wk13	429±139 (813±314)	1229±441 (1103±298)	1710±985 (2585±942)

At 24hr postdose:

wk13	133&132* (107±61)	136&128* (143±18)	168±27 (146±3)

* below quantitation limit of 25ng/ml for the other 3mice. As indicated above, no data could be obtained from either sex during wk1. Values are means±SD; No. in () are for females.

Below are the range of plasma levels in males and females (ng/ml):

At 1hr:	Dose (mg/kg)		
	10	20	30
wk1	431-697 (467-684)	945-2395 (994-1253)	1205-1866 (708-1608)
wk13	232-601 (536-1307)	904-1867 (729-1557)	355-2879 (1818-3636)

At 24hr:

wk13	133&132 (128-151)	136&128 (131-169)	135-198 (143-149)

The chromatogram at 30mg/kg sampling time 1hr on wk1, showed a major peak for [RP 61307] metabolite and a smaller peak for the parent. this may be a standard not a metabolite

A statistically significant difference (p<0.0001 by SAS) between concentrations measured at 1 and 24hr with levels much lower at 24hr. A linear relationship was seen between increasing dose and plasma levels at 1hr on wk1 (r=0.42). Blood levels were below quantitation limit for the 24hr

sample during wk1 for both sexes. During wk13 at 1hr, although plasma levels increased with dose, the increase was non-linear indicating some accumulation. On the other hand, at wk13/24hr concentrations did not increase with dose as if absorption, metabolism, and/or protein binding were saturated and drug levels reached a plateau. There was no significant difference in concentrations measured at 1hr between wk1 and wk13 except at 30mg/kg where concentrations seemed higher on wk13 relative to wk1. There were no sex differences in plasma levels. Maximum concentration reached at 1hr during wk1 was 1866ng/ml and that at wk13 was 3636ng/ml; maximum concentration at 24hr on wk13 was 198ng/ml.

3. TK in rats after repeated oral dosing at 2.5, 8, and 25mg/kg/day for 3months/ /June 1989/France. This study is part of the 13-wk oral gavage toxicity study in rats/HLE# 3764-81-30; protocol# P1883d (discussed under the Subchronic Toxicology section).

Riluzole was administered by oral gavage to male and female rats as a suspension in 1% MC, at 2.5, 8, and 25mg/kg/day for 13wks. Blood was sampled 1hr postdose on day1 and wks5 and 13.

Results:

Below are the range and mean plasma levels in male and female rats (provided by the sponsor):

- On Day 1 of treatment, plasma concentrations (for all animals) ranged between the following values:

2.5 mg/kg dose:

8 mg/kg dose:

25 mg/kg dose:

- During Week 5 (Wk5) of the study, the values for both sexes were as follows.

2.5 mg/kg dose:

8 mg/kg dose:

25 mg/kg dose:

- Lastly, in Week 13 (Wk13):

2.5 mg/kg dose:

8 mg/kg dose:

25 mg/kg dose:

There was no sex difference in plasma levels. Cumulative ratios for 2.5mg/kg ranged between 1-1.4 for wk5/d1, wk13/wk5, and wk13/d1; for 8mg/kg the ratios were 0.7-1.1 for these same periods; and for the 25mg/kg the ratios ranged between 1-2.5 for these cumulative time intervals. There was no linear relationship between dose and concentration on day1 however, a linear relationship was obtained during wks5 and 13 (r=0.89 and 0.87 respectively). There was no indication of drug accumulation.

4. TK in rats by the oral route after daily dosing at 5,10, and 20mg/kg for 26wks
'Nov 1994/France.

This study was conducted under GLP and is part of the 26wk toxicology study in the rat (see Subchronic Toxicology section).

CD rats (5/sex/dose) were dosed with riluzole by gavage at 5,10, and 20mg/kg/day for 26wks. The drug was suspended in 0.5% HMC and administered at a constant volume of 5ml/kg. Blood was collected on day1, wks13 and 26 at 1 and 24hr postdose and the concentration of unchanged riluzole was measured by HPLC with UV detection. Animals that died were replaced. Gender differences were analyzed by Wilcoxon rank test.

Results:

Mean±SD plasma concentrations for males and females () were as follows:

1hr			
Dose	wk1	wk13	wk26
5	355±28 (431±122)	467±87 (369±48)*	673±101 (489±90)*
10	574±50 (496±78)*	1234±437 (846±169)*	1321±209 (1067±65)*
20	809±197 (629±66)*	1864±249 (2158±320)	3009±690 (2716±663)
24hr			
5	NS (0)	173±48 (0)	256±62 (0)
10	75±6 (84±28)	487±162 (271±59)*	637±143 (236±105)*
20	358±263 (399±152)	1275±298 (1777±306)*	2027±446 (1399±869)

NS no sample.

There was no sex difference at 1hr/wk1 of the LD or any of the 3 doses at 24hr/wk1. However, at 1hr/wk1, a significant ($p<0.037$) difference was seen at MD&HD with mean values being generally lower in females than those in males. At 1hr/wk13, mean female values at LD&MD were significantly ($p<0.018&0.023$) less than the corresponding values in males; no effect at HD. The only significant effect noted at 24hr/wk13 was in MD&HD where females had lower values than those measured in males ($p<0.014&0.025$; table above). On wk26/1hr, a significant ($p<0.014&0.023$) increase in mean concentration was noted in LD&MD and a significant increase ($p<0.004$) at 24hr in MD.

Ratios of plasma levels to treatment duration were as follows: wk26/wk1 at 1hr increased with dose (in males: 1.9, 2.3, and 3.7 for LD, MD, and HD respectively, the corresponding ratios in females were: 1.13, 2.15, and 4.32), wk13/wk1 in females also increased with dose (0.86, 1.71, 3.43 respectively) but fluctuated with dose in males. At 24hrs, the ratio in males at MD and HD were 8.5 and 5.66 and in females: 2.81 and 3.51 respectively. Statistical significance was recorded on some of these comparisons.

There seem to be drug accumulation as evidenced by the higher plasma concentrations measured at end of study compared with those on day1 following 1 and 24hr measurements. The highest increase was in HDm at 1hr where mean plasma level was about 4x that at day1/wk1 (3009 vs. 809ug/ml). This was also

seen at 24hr postdose measurements where 5.7x increase noted in plasma levels at wk26 vs those at day1/wk1 (358 vs. 2027ug/ml).

Although, plasma concentration increased with dose, a linear dose-concentration relationship was seen only in males at 1hr on wk13 and 26 with $r = 0.881$ and 0.932 respectively.

Summary and Conclusion:

The 1hr plasma levels at all 3 time periods were higher in males than in females and the same is true at 24hr in LD&MD but levels were comparable between the 2 sexes in HD. Plasma levels increased with dose with linear correlation seen only in males at 1hr on wks13 and 26. Riluzole seemed to accumulate with repeated dosing as seen from higher concentrations measured at 24hr vs. 1hr postdose and the increase in plasma level to duration ratios. For example plasma level on wk26 at 1hr was 2-4x that after the first dose with the highest increase measured in HDm. At this dose, plasma concentration was higher than that in females.

5. TK in pregnant and non-pregnant rabbits after oral dosing of 3, 18, and 60mg/kg/day, /Sep 1990/France.
Batch# C8808501.
This study was conducted under GLP.

Riluzole was orally administered at 3, 18, or 60mg/kg to pregnant rabbits (n=7) from gd6-18 and non-pregnant rabbits (n=6) from d1-13. The drug was suspended in 1% MC and dosed by gavage at 2ml/kg. Blood was collected on the first and last days of treatment i.e. gd6&18 in pregnant rabbits and days1&13 for nonpregnant rabbits, before dosing and at 0.25, 0.5, 0.75, 1, 2, 3, 6, 8, and 24hr postdose. Blood was also collected on day9 of treatment in nonpregnant rabbits and gd14 for pregnant rabbits, before and 4hr after dosing. Plasma levels were analyzed by HPLC with quantitation limit of 5ng/ml.

Results:

below are plasma concentrations (mean±SD; ng/ml; values in () are for pregnant rabbits):

Day1/Day6 for pregnant rabbits:

Dose (mg/kg)	0.5	1	6	24
3	154±54 (229±110)	156±76 (178±56)	ND (63±7)	ND ND
18	1707±1136 (2113±724)	1666±770 (2173±927)	230±99 (265±215)	111±35 (90±27)
60	2080±1020 (3477±1486)	2723±527 (4072±1000)	1121±594 (1419±1306)	268±75 (256±91)

Day13/day18 for pregnant rabbits:

3	432±179 (238±94)	281±142 (214±97)	72±9 (75±30)	63±2 (65±11)
18	1822±838 (1507±738)	1782±694 (1496±867)	196±88 (209±130)	90±13 (105±56)
60	2427±488 (4061±1201)	2792±1155 (3153±883)	491±218 (519±139)	196±48 (221±100)

Pregnant rabbits in general, had higher plasma concentrations at all 3 doses on day1/day6 and on day13/day18, plasma levels for MD&HD were also higher in pregnant rabbits compared with the mean values in non-pregnant animals at the corresponding doses and all time periods. At the LD on last day of treatment, plasma level was comparable in pregnant and non-pregnant rabbits. There seem to be some accumulation at the LD when concentrations on first and last day of treatment were compared. However, no evident accumulation was noted at the higher doses. Peak plasma level in non-pregnant rabbits occurred between 0.5-1hr at the LD&MD and 0.75-2hr at HD, and in pregnant rabbits, the peak concentration was seen at 0.25-0.5hr. Plasma level was also measured on day9 and day14 in non-pregnant and pregnant rabbits respectively, before and 4hr postdosing. Plasma levels (mean and some individual values) were nondetectable in LD measured pre-dosing in both pregnant and nonpregnant rabbits, however, pregnant animals showed in general, higher levels at the 4hr period relative to the values in pregnant rabbits. The range of plasma levels on day9 in non-pregnant animals at the LD, MD, and HD were: 0-99, 80-1275, and 151-2047ng/ml respectively. The range of plasma levels on day14 in pregnant rabbits for the 3 doses were: 0-96, 56-878, and 137-1901ng/ml respectively.

The PK parameters calculated by the sponsor were as follows:

Day1/day6 for pregnant rabbits:

Dose (mg/kg)	C _{max} (ng/ml)	T _{max} (hr)	AUC _{0-4hr} (ng/ml.hr)
3	299±341 (315±248)	0.88 (0.5)	671±326 (1195±443)
18	2188±834 (2447±782)	0.63 (0.75)	9819±3081 (10267±5484)
60	3416±745 (4959±1769)	1.5 (0.75)	28748±9736 (33030±18012)

Day13/day18 for pregnant rabbits:

3	432±179 (285±119)	0.5 (0.5)	2122±417 (1930±808)
18	2070±724 (1770±921)	0.75 (0.75)	9907±5076 (7843±3312)
60	3289±652 (4397±839)	0.75 (0.63)	18085±6236 (23473±5455)

The above values were mean±SD for C_{max} and AUC and for T_{max} they were medians.

The mean peak level and AUCs were higher in pregnant than non-pregnant females at the start of treatment, however, the values seemed comparable when measured at end of study. Maximum time to reach peak level was similar in pregnant and non-pregnant rabbits.

Summary and Conclusion:

Oral administration of riluzole at 3, 18, or 60mg/kg to pregnant rabbits during organogenesis and to non-pregnant rabbits from day1-13 showed generally higher mean plasma levels and AUC in pregnant relative to non-pregnant rabbits. Concentrations increased non-linearly with dose in pregnant and non-pregnant rabbits. There was some accumulation of the drug particularly at the LD but no evidence of accumulation at the 2 higher doses. Peak concentration

were reached between 0.5-2hr. Range of plasma levels in pregnant and non-pregnant rabbits were as follows (ng/ml):

Dose (mg/kg)	Non-preg.	Preg.
3	61-989	55-763
18	73-3109	60-3658
60	131-4587	146-7626

6. TK in pregnant rats by the oral route/IBP-Biodyn 1829/Aug 1994/France. Batch# C88085-01

Pregnant Sprague-Dawley rats were treated orally with riluzole at 3, 9, 27mg/kg/day administered during gd6-15. Blood was collected on gd6 and 15 before dosing and at 0.5, 1, 3, 8, and 24hr postdose and on gd10 before dosing and 3hr postdose. Plasma levels were measured by HPLC at quantitation limit of 5ng/ml. The drug was suspended in 1% MC in water and dosed at 10ml/kg.

Results:

The AUC₀₋₂₄ values on day 15 for 3, 9, and 27mg/kg were 1519, 5716, and 31987ng/ml.hr and for 0-24hr was nondetectable at LD and 10427 and 79653ng/ml.hr for MD&HD respectively. Maximum concentration for 3mg/kg was 787ng/ml, for 9mg/kg was 2362ng/ml, and for 27mg/kg was 9731ng/ml. Generally, mean peak concentrations were reached at 0.5, 8, and 3hr for the 3 doses respectively. When dose was normalized (9mg/kg) to compare the plasma levels, a significant (p<0.0001-0.0027 Tukey's test) difference noted between dose, day, and sampling time. Plasma concentrations were constant upto 8hr on day6 and 15 but significantly reduced when measured at 24hr postdosing. There was a linear dose-concentration relationship on days 6 and 15 upto 8hr postdose with r=66 or 64. However, the AUC₀₋₂₄ on last day of treatment, was disproportionately increased relative to dose (about 8x with dose increase from 9-27mg/kg). There seem to be some drug accumulation at the HD as indicated by the higher 24hr levels on day15 vs. those on day 6.

Dose hrs	D6			D15		
	0.5	8	24	0.5	8	24
3	567±107	287±100	ND	306±55	95±24	ND
9	1062±95	1184±247	1111±377	616±163	542±155	47±46
27	2109±543	2542±660	679±383	5590±2981	4118±2054	1840±788

ND non-detectable.

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Distribution:

1. Autoradiography of tissue distribution in rats after 2mg/kg oral dose of ¹⁴C riluzole, /June 1988/France.
Specific activity 52mCi/mmol; Batch# PX5551 CEA383; unlabelled drug Batch# C1100.

Sprague-Dawley rats (male and female) were orally dosed with 2mg/kg ¹⁴C riluzole in a 5ml/kg volume (in HCl solution). One rat/sex was sacrificed and fixed at 0.5, 3, 6, 24, and 72hr. Sagittal sections (25microns) of the whole body were obtained.

Results:

Radioactivity was detectable by 0.5hr postdose in many tissues indicating rapid absorption and wide distribution of the drug. Radioactivity distribution was similar at 0.5 and 3hr postdose and maximum radioactivity in decreasing order was found in: stomach/intestinal contents, brain, spinal cord, liver, Harderian gland, fat tissue, kidney, urinary bladder, and submaxillary salivary glands. Some radioactivity was also detected in the heart, bone marrow, testes, and muscles. Radioactivity distribution in the brain was homogeneous except in the cerebellum where higher radioactivity was seen in white matter compared with grey matter. At 6hr postdose, marked reduction in radioactivity was seen in all tissues with the stomach/intestinal contents and bladder retaining maximum radioactivity followed by the liver, kidneys, Harderian gland, and the brain. Residual radioactivity was detectable at 24 and upto 72hr postdose only in the intestinal contents, liver, kidneys, Harderian glands, and brain. There was no sex difference in radioactivity tissue distribution and rate.

2. Radioactivity milk transport in lactating rat after a single oral dose of 15mg/kg, /April 1994/France.
This study was conducted under GLP.
Batch# CEA 0590-0193-PX6163 specific activity 56mCi/mmol; Batch# for unlabelled drug 10 Proc 93049.

Lactating Sprague-Dawley rats (gd 10-11), were orally administered a single radioactive dose of riluzole at 15mg/kg (as a suspension in MC; 5ml/kg). These female rats were allowed to deliver, and the litter no. was reduced to 3 pups on the 2nd day of lactation. Radioactivity was determined after 0.5hr of dosing and upto 72hr in milk, blood, and plasma (1 rat/time). Total radioactivity was measured by liquid scintillation counting.

Results and Conclusion:

Radioactivity was detectable in the 3 tissues by 0.5hr postdose with highest radioactivity recorded at this time in the blood. From 1hr and upto 72hr postdose, highest radioactivity was measured in milk ranging between 0.2 at 72hr to 7.5mgEquiv/l at 6hr postdose; whereas plasma levels ranged between 0.1 at 72hr to 1.9mgEquiv/l at 1hr. The milk/plasma ratio ranged between 1.7 to 4.1 indicating the higher drug-milk level than that in plasma; milk/blood ratios also indicated the higher level in milk (0.8-2). Table below from the sponsor presents PK parameters, as seen, exposure i.e. AUC was highest in milk at 187mgEquiv.hr/l. Attached table from the sponsor shows 2 peak drug concentrations, one at 1hr for all 3 tissues and the 2nd at 6hr for milk and plasma. This rebound in concentration might have been due to enterohepatic circulation which is seen in male rats.

BLOOD, PLASMA AND MILK LEVELS OF TOTAL RADIOACTIVITY ($\mu\text{g equivalent l}^{-1}$)					
TIME (h)	BLOOD	PLASMA	MILK	MILK / BLOOD	MILK / PLASMA
0.5	2.34	1.11	1.88	0.8	1.7
1	3.04	1.88	5.56	1.8	3.0
2	2.19	1.29	2.51	1.1	1.9
3	2.32	1.03	3.71	1.6	3.6
4	3.60	1.58	5.55	1.5	3.5
6	3.67	1.82	7.49	2.0	4.1
24	1.52	1.44	2.68	1.8	1.9
72	0.14	0.10	0.19	1.3	1.8

TISSUE	PARAMETER	
BLOOD	t_{max} (h)	6
	C_{max} (mg eq.l ⁻¹)	1.82
	$\text{AUC}_0(0-72 \text{ h})_{(a)}$	103
PLASMA	t_{max} (h)	1
	C_{max} (mg eq.l ⁻¹)	1.88
	$\text{AUC}(0-72 \text{ h})_{(a)}$	76
MILK	t_{max} (h)	6
	C_{max} (mg eq.l ⁻¹)	7.49
	$\text{AUC}_t(0-72 \text{ h})_{(a)}$	187

(a) = expressed as mg RP 54274 eq.h(l⁻¹ or kg⁻¹)

It was concluded that riluzole is readily distributed into milk of lactating rats after a single oral dose of 15mg/kg radiolabelled riluzole. Riluzole concentration was highest in milk at 7.5mgEquiv/l and milk/plasma ratios increased with time from 1.7 to 4.1 at 6hr. Concentration in all 3 tissues decreased by 72hr postdose to 2.5% in milk, 5% in plasma, and 3.8% in blood. Milk $\text{AUC}_{0-72\text{h}}$ was 2.5x higher than that in plasma.

3. Autoradiography tissue distribution of labelled riluzole after a single oral dose of 2mg/kg to monkeys /Oct 1989/France.
Batch# for unlabelled drug: C 88085 01; Batch# for labelled drug:

Three male *Macaca fascicularis* monkeys were orally administered by gastric intubation, 2mg/kg radioactive dose of riluzole in HCl solution (10 $\mu\text{Ci/kg}$). Animals were sacrificed at 0.5, 6, and 24hr postdose and standard procedure for autoradiography was followed.

Results and Conclusion:

Riluzole was detectable by 30min of dosing in many tissues indicating rapid absorption and good distribution. Melanin-rich organs such as the uveal tract, optic nerve, etc. showed high radioactivity that was slightly less intense by 24hr. The liver, gall bladder and hepatic ducts showed markedly high radioactivity that remained moderately high at 24hr postdose. The distribution of hepatic radioactivity was homogeneous at 0.5 and 3hr but heterogeneous at 24hr. Similar to the liver, markedly high homogeneously-distributed radioactivity was seen in the testes that remained high at 24hr. Radioactivity was slight in the accessory glands and penis. Moderate radioactivity was observed in the spleen and bone marrow at 0.5 and 3hr but the label was reduced markedly by 24hr. Other tissues/organs showed slight radioactivity. Within the brain, the substantia alba showed max labelling, and high radioactivity was seen in the thalamus, putamen, hippocampus, lateral geniculate and gyrus dentatus. The drug was eliminated mainly by the urine (high radioactivity in pelvis and urine). Fecal elimination was also present and related to marked-to-moderate elimination by the bile that lasted till the 24hr. The high radioactivity in the bile indicated enterohepatic circulation.

4. Tissue distribution by whole-body autoradiography and by dissection in pregnant rats on gds13 and 18 after a single oral dose of 27mg/kg, 1815/June 1994/France.

This study was conducted under GLP.

Batch# for radioactive cpd= CEA 0690-0193-PX 6163; specific activity 56mCi/mmol; batch# for unlabelled cpd= 1- Proc 93049.

Pregnant Sprague-Dawley rats received a single oral dose of ¹⁴C riluzole on gd13 and 18 as a suspension in 0.5% MC at 27mg/kg. The drug was administered by gastric gavage as a suspension in MC at 5ml/kg. In the autoradiographic study, rats were sacrificed at 0.5, 3, and 24hr postdose (2 rats/time point); and in the dissection study, 4 rats/time point were sacrificed. On gd13, radioactivity was determined in whole-fetuses and on gd18 some fetuses (n=6) were dissected and radioactivity was assessed in the brain, heart, lung, liver, and kidneys. Total radioactivity was assessed by scintillation counting.

Results and Conclusion:

Generally there was good correlation between dissection studies and autoradiography. The sponsor indicated that some of the difference was related to the method of killing the rat (exsanguination in dissection study, vs. asphyxiation in the autoradiography). Riluzole was distributed to various tissues in the dam and readily crossed the placenta into the fetus where it also showed wide distribution (attached tables from the sponsor). In the dam, whatever the gd, 13 or 18, radioactivity concentration was detected on gd13 in decreasing order as follows: adrenals, liver, brain, kidneys, mammary glands, ovary, and heart; and on gd18: adrenals, liver, brain, mammary glands, kidneys, and ovary. The lowest radioactivity in the dam was in the amniotic fluid. On gd13 in the dam, tissue radioactivity levels were generally higher at 3hr than those detected at 0.5hr and slightly lower at 24hr than levels at 0.5hr i.e. the highest radioactivity was at 3hr postdose. On gd 18, highest radioactivity was measured at 24hr and levels at 3hr were slightly higher than radioactivity measured at 0.5hr.

In the fetus, on gd13 radioactivity was similar to that in plasma whereas on gd18, radioactivity in fetus was 2x higher than that in plasma. It is concluded that placental transfer increases with the increase in day of gestation and is non-saturable process. In the fetus, maximum radioactivity was reached at 24hr postdose and was 2x higher than that in plasma. Fetal radioactivity levels were as follows in decreasing order: brain>liver>kidneys>heart>lungs.

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TABLE 7 : RADIOACTIVITY CONCENTRATION (mg eqv RP/kg fresh tissue) IN
TISSUES AFTER A SINGLE ORAL ADMINISTRATION OF ¹⁴C-RP 54274
AT 27MG/KG IN PREGNANT RATS ON DAY 18 OF GESTATION

TISSUES	0.5 HOUR		3 HOURS		24 HOURS	
	MEAN ±	S.D.	MEAN ±	S.D.	MEAN ±	S.D.
BLOOD	3.62 ±	0.42	3.55 ±	0.46	4.39 ±	0.83
PLASMA	1.85 ±	0.46	2.04 ±	0.18	2.92 ±	0.29
BRAIN	11.54 ±	1.74	10.65 ±	0.99	12.63 ±	3.04
HEART	8.23 ±	1.23	7.82 ±	0.82	8.37 ±	1.94
LUNGS	7.41 ±	1.16	6.93 ±	0.57	7.72 ±	1.17
LIVER	-12.60 ±	1.83	11.58 ±	0.95	13.53 ±	2.40
KIDNEYS	10.12 ±	1.08	10.37 ±	1.29	15.61 ±	1.81
ADRENALS	16.08 ±	2.42	15.21 ±	1.25	16.82 ±	3.90
UTERUS	3.56 ±	0.81	3.75 ±	0.59	4.71 ±	1.01
PLACENTA	5.44 ±	0.90	4.50 ±	0.30	6.47 ±	1.73
MAMMARY GLAND	10.95 ±	2.08	12.02 ±	1.61	13.88 ±	3.81
OVARY	0.41 ±	1.61	9.84 ±	1.64	10.80 ±	1.81
AMNIOTIC FLUID	0.84 ±	0.16	0.85 ±	0.17	1.44 ±	0.32
WHOLE FOETUS	32.40 ±	3.88	35.02 ±	3.82	75.32 ±	12.74-32
FOETUS BRAIN	4.80 ±	1.22	5.01 ±	0.46	6.87 ±	1.09
FOETUS HEART	5.14 ±	1.27	4.53 ±	0.41	5.50 ±	1.21
FOETUS LUNGS	4.32 ±	1.33	4.14 ±	0.39	5.29 ±	1.34
FOETUS LIVER	7.71 ±	2.48	5.22 ±	0.55	6.74 ±	1.70
FOETUS KIDNEYS	5.13 ±	1.43	4.35 ±	0.18	6.29 ±	1.20
FOETUS CARCASS	4.12 ±	0.72	3.84 ±	0.52	5.39 ±	1.18

Values represent the mean and standard deviation of four animals

TABLE 8 : RADIOACTIVITY CONCENTRATION (mg eqv RP/kg fresh tissue) IN
TISSUES AFTER A SINGLE ORAL ADMINISTRATION OF ¹⁴C-RP 54274
AT 27 MG/KG IN PREGNANT RATS ON DAY 13 OF GESTATION

TISSUES	0.5 HOUR		3 HOURS		24 HOURS	
	MEAN ±	S.D.	MEAN ±	S.D.	MEAN ±	S.D.
BLOOD	4.21 ±	1.13	8.79 ±	2.83	5.77 ±	1.89
PLASMA	2.08 ±	0.44	4.15 ±	1.15	4.01 ±	0.94
BRAIN	12.16 ±	3.51	24.15 ±	7.00	15.15 ±	5.74
HEART	9.86 ±	2.82	18.59 ±	5.17	10.18 ±	3.63
LUNGS	7.51 ±	1.79	15.35 ±	4.85	9.08 ±	3.33
LIVER	14.27 ±	4.35	27.55 ±	7.84	18.33 ±	5.72
KIDNEYS	11.95 ±	3.40	22.31 ±	6.84	21.70 ±	6.77
ADRENALS	18.85 ±	7.30	34.21 ±	10.18	20.30 ±	6.84
UTERUS	5.04 ±	1.94	12.09 ±	3.80	9.58 ±	3.08
PLACENTA	5.83 ±	1.40	14.87 ±	10.15	7.52 ±	2.74
MAMMARY GLAND	11.54 ±	1.98	25.84 ±	9.87	16.02 ±	6.18
OVARY	9.09 ±	2.83	21.77 ±	7.02	12.13 ±	6.34
AMNIOTIC FLUID	0.44 ±	0.14	1.04 ±	0.32	1.01 ±	0.30
FOETUS	2.43 ±	0.95	3.99 ±	0.95	3.31 ±	1.19

Values represent the mean and standard deviation of four animals

Metabolism:

In vitro Studies:

1. ¹⁴C-riluzole metabolism in liver microsomes/Inter-species comparison/IBP-
7/Dec 1994/France.

Lot# 8 PROC 92312. The radioactive riluzole had specific activity of
56mCi/mmol with radioactive purity >99%.

This study was conducted under GLP.

The protocol followed in this study was that reported in

Male and female Sprague-Dawley rats (180-200g; 7wks old) were used. Groups of
6 males were treated as follows:

- phenobarbital 80mg/kg/d i.p. in saline for 3days.
- 3MC 40mg/kg/d i.p. in corn oil for 2days.
- dexamethazone 50mg/kg/d i.p. in corn oil for 3days.
- pyridine 100mg/kg/d i.p. in saline for 3days.
- clofibrate 400mg/kg/d i.p. in corn oil for 3days. --

Six female rats received the same dexamethazone treatment and, a control group
was dosed with the vehicle.

Microsomes were prepared from pools of mice (OF1) and Sprague-Dawley livers,
from individual New Zealand rabbit, beagle dog, and monkey (Macaca
fascicularis) livers. All animals were males except for the rat where both
sexes were used. Human livers were obtained from organ transplant donors or
from excess of transplantable livers. A total of 6 livers were used (5 from
males and 1 from a female). Analyses were done by HPLC and LC/MS/MS.

Results:

Table 1, from the sponsor, compares metabolism between the 5 species and human.
All species showed phase I metabolic profile in the liver microsomes and no
biotransformation occurred in absence of NADPH. The main metabolic pathway of
riluzole in human liver microsomes was N-hydroxylation forming metabolite
RP112512 which is formed by CYP1A2 isozyme (see page 98). The parent was also
present at high levels in humans. RP112512 is also a major metabolite in the
monkey, dog, and rabbit and is formed to a lesser degree in the other species.
The main metabolite in the mouse and rat is A4 (unidentified) followed by
RP112512 (table below). In all of the species, the parent unchanged drug was
also present at moderate levels (attached table). Metabolite RPR109792 formed
by O-dealkylation, was present in all animal species. Aromatic hydroxylation
produced 2 metabolites, RP65077 and 65331, at low amounts in all species. There
seem to be no gender differences in the rat regarding metabolic profile except
for small quantitative differences. The highest mean biotransformation rate
(pmol/min/mg) was in humans>monkey>male rat>rabbit>dog>mouse>female rat at
171, 118, 93, 86, 76, 72, and 60pmol/min/mg respectively (attached table and
table below).

Table 2. Riluzole biotransformation and metabolite formation rates

SPECIES	RP54274 biotrans- formation pmol/min/mg	Metabolite formation rate % of total biotransformation rate					
		RPR 109792	A4	RP 65331	RP 65077	A2 RPR 112512	
MOUSE (n=2)	72.2	15	33	13	12	6	15
RAT MALE (n=6)	93.3	3	16	5	5	3	9
FEMALE (n=2)	60.3	3	27	4	7	5	25
RABBIT (n=2)	85.8	3	5	3	3	-	54
DOG (n=2)	76.1	6	5	4	9	7	38
MONKEY (n=2)	118.1	7	2	5	8	-	48
MAN (n=6)	171.3	-	-	-	-	-	81

- not detectable

The P450 isozymes responsible for riluzole metabolism were identified using 5 inducers of known specificities: 3MC for CYP1A, phenobarbital for CYP2B, pyridine for CYP2E, dexamethazone for CYP3A, and clofibrate for CYP4A. The highest enzyme induction of riluzole was that caused by 3MC followed by phenobarbital>pyridine>clofibrate and dexamethazone. 3MC induction caused highest formation of RPR109792 (o-dealkylation), RP65077 (aromatic hydroxylation), RPR112512 (N-hydroxylation), and RP65331 (aromatic hydroxylation). Whereas, phenobarbital induced the formation of A3, A4 (major metabolite), A5, and A2. Pyridine similar to 3MC, increased the formation of RPR112512, RP65077, RP65331, and RP109792 together with the other minor unidentified products. Dexamethazone and clofibrate inductions were intermediate between 3MC and phenobarb (table below). Therefore, CYP1A isozymes seem to be responsible for riluzole metabolism in these animal species since it induced not only N-hydroxylation, but also aromatic hydroxylation and O-dealkylation. Unlike human in vitro microsomal metabolism where only a single metabolite was formed (N-hydroxylation), all the 5 animal species examined showed more complex metabolic profile.

Table 4. Effect of enzyme induction on riluzole metabolism in the male rat

INDUCER	RP	RPR	A5	A4	RP	A3	RP	A2	RPR
	54274	109792			65331		65077		112512
	Rate in pmol/min/mg								
controls ¹	93.3	2.5	4.0	14.5	4.5	0.8	4.5	2.5	8.0
	Percentage increase								
3-MC	201%	1212%	213%	31%	240%	-	1040%	-12%	588%
PB	188%	-	553%	578%	-11%	963%	80%	296%	81%
PYR	136%	112%	210%	212%	127%	-	193%	188%	285%
DEX	90%	236%	85%	200%	11%	-	51%	204%	-59%
CLO	127%	40%	280%	323%	-60%	600%	49%	232%	-3%

¹ mean of six pools of control microsomes: one for each inducer and one untreated.
 -: not detectable. bold: greatest effect.

Direct glucuronidation of riluzole in humans in vitro was very slow compared with phase I reactions (7 vs. 171pmol/min/g at 15uM). This is in contrast to in vivo findings where riluzole in humans is mainly eliminated by direct conjugation with glucuronic acid and excreted in urine. However, in vitro when RPR112512 was incubated with liver microsomes in presence of UDPGA, both N- and O-glucuronides were formed as indicated by the peaks in LC/MS analysis. However, in vivo in humans, RPR112512 was not detected before or after enzymatic hydrolysis. These discrepancies were explained by the sponsor as possibly due to the difficulty in extrapolating quantitatively the relative activities of various enzymes from in vitro to in vivo. In addition, there was some indication that RPR112512 is unstable under specific incubation conditions with glucuronidase and therefore, difficult to detect in urine.

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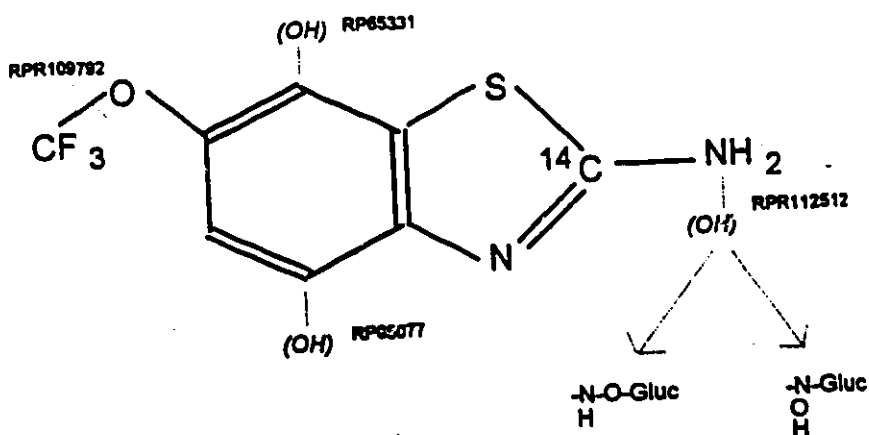
82

Summary and Conclusion:

This study compared microsomal metabolism of riluzole in 5 animal species and humans using ¹⁴C-riluzole. In all species and humans, riluzole was metabolized by P450 monooxygenase enzyme system. The main and only metabolite formed in human microsomes was RPR112512, the N-hydroxylated compound which was also the major metabolite in the dog, monkey, and rabbit and formed in the other species to a lesser degree. All 5 species showed more complex metabolism than humans with aromatic ring hydroxylation forming RP65077 and RP65331 and O-dealkylation producing the polar compound RPR109792. The latter was formed in relatively high amount in the mouse and monkey liver microsomes. Both in the mouse and rat, the major metabolite was A4, an unidentified compound. There was no gender difference in metabolic profile or rate in the rat. Riluzole metabolism was highest in the human microsomes at 171pmol/min/mg followed by monkey at 118pmol/min/mg and the rat at 93pmol/min/mg. The rate was around 80pmol/min/mg in the other species. Metabolite kinetics was first order/linear over 20min incubation time, which indicated absence of secondary metabolism under the present experimental conditions.

N-hydroxylation of riluzole was found to be catalyzed by CYP1A2 in human liver microsomes. In the induced rat liver microsomes, 3MC induced not only N-hydroxylation, but also O-dealkylation and aromatic ring hydroxylation. Phenobarbital induced significantly the formation of the unidentified A4 metabolite in the rat as well as other minor metabolites. The rest of the inducers had effect similar to that of phenobarb (but not to 3MC), but quantitatively less than phenobarb. All this indicated the role of other P450 isozymes in riluzole metabolism such as CYP2B or 2C families.

Direct glucuronidation of riluzole in human liver microsomes was shown to be slow. Nevertheless, the N-hydroxylated metabolite RPR112512, was readily glucuronidated forming an N- and O-glucuronides. Some of the discrepancies between the human in vivo and in vitro metabolism were explained by the sponsor to be due to the difficulty in extrapolating quantitatively the relative activities of various enzymes from in vitro to in vivo and the instability of RPR112512 under the specific incubation conditions with glucuronidase that hindered its detection in urine. [Below is the metabolic pathway of riluzole in vitro and attached are the structures for some of the metabolites].



identified riluzole metabolic pathways *in vitro*

Table 1. Interspecies comparison of riluzole metabolismParent drug and metabolite concentrations in μM , reaction rates in pmol/min/mg

Means of three experiments. Incubation time 20 minutes, protein 2 mg/ml.

-: not detectable

SPECIES	RPR109792	A4	RP65331	RP65077	A2	RPR112512	RP64274	CONTROL (no NADPH)
Batch								
Experiment								
MOUSE	0.32	0.60	0.29	0.16	0.17	-	12.58	12.21
921-S1	0.41	0.91	0.34	0.24	0.23	0.14	9.06	12.00
AGAX102	0.43	1.11	0.32	0.34	-	0.22	9.99	13.58
MEAN	0.39	0.87	0.32	0.25	0.20	0.18	10.54	12.60
911-S1	0.49	1.04	0.42	0.34	0.05	0.57	7.75	12.21
AGAX103	0.58	0.98	0.42	0.54	0.20	0.85	10.09	12.00
	0.44	1.03	0.50	0.42	0.12	0.58	8.79	13.58
MEAN	0.50	1.02	0.45	0.43	0.12	0.67	8.88	12.60
MEAN RATE	11.1	23.6	9.5	8.5	4.1	10.6	72.2	
RAT (values for six controls, table 3)								
AGAX58-70								
MEAN	0.19	0.58	0.18	0.18	0.10	0.32	9.22	12.95
MEAN RATE	2.5	14.5	4.5	4.5	2.5	8.0	83.3	
RABBIT	-	0.21	0.06	0.11	-	2.19	9.74	15.58
914-L1	-	0.21	0.07	0.08	-	2.27	11.83	15.89
AGAX105	-	0.23	0.16	0.13	-	2.22	11.18	15.30
MEAN		0.22	0.10	0.11		2.23	10.92	15.69
916-L1	0.07	0.15	0.06	0.12	-	1.11	12.93	15.58
AGAX107	0.10	0.16	0.11	-	-	1.69	13.85	15.89
	0.10	0.16	0.09	0.07	-	1.56	13.42	15.30
MEAN	0.09	0.16	0.09	0.10		1.45	13.40	15.69
MEAN RATE	2.5	4.7	2.3	2.5		46.0	85.8	
DOG	0.28	0.10	0.14	0.37	0.29	1.58	10.93	16.44
893-C1-2	0.11	0.14	0.07	0.33	0.40	1.70	11.21	16.71
	0.37	0.24	0.14	0.37	0.29	1.58	10.93	16.41
MEAN	0.25	0.16	0.12	0.36	0.33	1.62	11.02	16.52
891-C1-3	0.10	-	-	0.20	0.13	0.69	16.10	16.44
	-	-	-	0.11	0.10	0.76	15.84	16.71
	0.12	-	-	0.18	-	0.59	15.85	16.41
MEAN	0.11			0.16	0.12	0.68	15.93	16.52
MEAN RATE	4.5	4.0	2.9	6.5	5.5	28.3	76.1	

continued

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Table 1. Interspecies comparison of riluzole metabolism (continued)

SPECIES	RPR109792	A4	RP66331	RP66077	A2	RPR112512	RP64274	CONTROL
Batch								(no NADPH)
Experiment								
MONKEY								
914-SI1	0.29	0.09	0.22	0.41	-	2.55	11.65	17.90
AGAX110	-	-	0.24	0.52	-	4.17	10.74	18.56
MEAN	0.29	0.09	0.23	0.47	-	3.36	11.20	18.23
911-SI1-1	0.30	-	0.25	0.23	-	1.16	15.56	17.39
AGAX111	0.40	0.09	0.22	0.38	-	1.12	14.02	17.90
MEAN	0.43	0.06	0.25	0.40	-	1.16	17.04	18.56
MEAN RATE	8.3	2.1	5.9	10.0	-	56.4	118.1	
MAN								
881-H2-3	-	-	-	-	-	1.96	10.82	13.97
(female)	-	-	-	-	-	2.74	11.04	14.10
MEAN	-	-	-	-	-	2.29	10.12	13.82
883-H1-15	-	-	-	-	-	6.46	5.68	13.25
(male)	-	-	-	-	-	6.53	5.71	14.15
MEAN	-	-	-	-	-	6.75	5.56	14.13
884-H1-7	-	-	-	-	-	3.13	9.07	12.27
(male)	-	-	-	-	-	3.72	7.32	12.40
MEAN	-	-	-	-	-	4.13	8.08	12.82
921-H1-1	-	-	-	-	-	6.07	4.52	12.25
(male)	-	-	-	-	-	4.52	3.71	13.70
MEAN	-	-	-	-	-	6.07	4.51	12.49
922A-H1-4	-	-	-	-	-	7.33	3.98	12.38
(male)	-	-	-	-	-	8.01	3.77	11.07
MEAN	-	-	-	-	-	8.34	3.74	12.04
831-H1-1	-	-	-	-	-	7.27	3.19	9.54
(male)	-	-	-	-	-	6.92	2.96	12.15
MEAN	-	-	-	-	-	7.07	2.87	13.47
MEAN RATE						7.99	3.01	11.72
						137.9	171.3	

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2. Identification of human liver enzymes in vitro using ^{14}C -riluzole,
'Aug 1994/France.
Lot# 8 PROC 92312; specific activity 56mCi/mmol; radioactive purity >99%.
This study was conducted under GLP.

Protocol# was followed. It was indicated that additional enzyme inhibitors to those in the protocol were used. Human genetically expressed P450 isozymes were also tested. As indicated in the above study (Dec 1994), human livers (5m and 1f) were obtained from organ transplant donors or excess transplantable livers. Microsomes were isolated following standard procedures. Genetically engineered P450s were obtained from microsomes from human B-lymphoblastoid cell lines. Enzyme kinetics were tested at riluzole concentration range from 2-500uM. Sample analysis was conducted by HPLC/UV detection.

Results:

Incubation of ^{14}C -riluzole with human microsomes produced a single metabolite identified as RPR112512, the N-hydroxylated product. There was no metabolism of riluzole in absence of NADPH indicating the exclusive involvement of the P450 system. Formation of RPR112512 followed normal Michaelis-Menton kinetics/first order linear. The kinetic parameters for microsomes from one subject were: $K_{m(\text{app})} = 23 \pm 4 \mu\text{M}$ ($\pm$ sem), $V_{\text{max}} = 483 \pm 23 \text{ pmol/min/mg}$, and calculated Cl was $21 \mu\text{l/min/mg}$ protein. The mean rate of riluzole biotransformation at $15 \mu\text{M}$ was $171 \pm 60 \text{ pmol/min/mg}$ (range 93-218) and the mean formation rate for RPR112512 was $138 \pm 53 \text{ pmol/min/mg}$ (range 58-197).

Identification of the P450 isozyme family responsible for riluzole metabolism was done by (1) inhibition experiments using 12 substrates/inhibitors with different affinities to human P450 isozymes. They were as follows: alpha-naphthoflavone (ANF), acetanilide (ACD), and caffeine (CAF) for CYP1A, tolbutamide for CYP2C8/9, omeprazole (OMZ) for CYP2C19, quinidine for CYP2D6, aniline, p-nitrophenol (PNP), and chlorzoxazone (CZN) for CYP2E, and ketoconazole and troleandomycin for CYP3A. Riluzole concentration was $15 \mu\text{M}$ close to the $K_{m(\text{app})}$ value. The highest degree of inhibition was that seen with ANF with 80% decrease in rate of formation. The 2 substrates for CYP1A, ACD and CAF, also showed some inhibition at 21&13% respectively, (at 1000 and 400uM concentration). CZN inhibited formation by 36% at 100uM whereas, aniline and PNP (all 3 cpds for CYP2E) had no effect (-2 and 17% respectively). OMZ caused 31% inhibition at 100uM. Quinidine, tolbutamide, and ketoconazole all almost no effect (20% for quinidine and 2-5% for the other 2). It is noted that under all tests, there was a direct correlation between total riluzole biotransformation and formation of RPR112512. Dose-response inhibition studies were done using 7 substrates/inhibitors to confirm the potency of these cpds. ANF inhibited riluzole hydroxylation with a mean IC_{50} of $0.42 \pm 0.02 \mu\text{M}$ and that for CZN was $287 \pm 77 \mu\text{M}$. The mean IC_{50} for the other cpds was high and caused inhibition between 14-39% and sulfaphenazole caused no inhibition. (2) using genetically engineered microsomes from human cell lines: ^{14}C -riluzole incubated with these microsomes showed that only CYP1A2 capable of metabolizing riluzole and formation of RPR112512. There was no metabolism in absence of NADPH, or in microsomes containing CYP1A1, 2D6, 2E1, or 3A4.

Riluzole incubated with human liver microsomes (detergent-activated) in presence of UDPGA produced only one metabolite, RPR112512. Biotransformation did not occur in absence of UDPGA indicating that UDP-glucuronosyltransferase is needed for the reaction. Riluzole glucuronide conjugation showed first order kinetics with $K_{m(\text{app})}$ of $58 \pm 4 \mu\text{M}$, V_{max} of $98 \pm 16 \text{ pmol/min/mg}$, and intrinsic metabolic Cl of $1.7 \mu\text{l/min/mg}$ protein. Ten UDPGT substrates were tested on riluzole glucuronidation. The maximum inhibition was 70% with propofol (2,6-diisopropylphenol) and the inhibition was dose-dependent with an IC_{50} of

19±0.7uM. The rest of the cpds used also showed some degree of inhibition upto 40% for estradiol (100uM).

Summary and Conclusion:

Riluzole in human microsomes is biotransformed to only one metabolite, the N-hydroxylated RPR112512. There is also direct glucuronide conjugation that occurs at slow rate compared with the monooxygenase system. The $K_{m(app)}$ for P450 reaction was 23±4uM and V_{max} of 483±23pmol/min/mg protein. The mean rate of formation of this metabolite was 138±53pmol/min/mg at 15uM substrate concentration. The $K_{m(app)}$ for glucuronidation was 58±4uM and V_{max} 98±16pmol/min/mg. The P450 isozyme responsible for riluzole metabolism is CYP1A2.

3. Metabolism of riluzole by the "Bio Avenir" human CYP450 expressed in yeast-preliminary results and comparison with human liver microsomes /Nov. 1984/France.
Batch# 8 PROC 92312; specific activity 56mCi/mmol with radioactive purity >99%.

Human livers and microsome preparations are the same as those in #1&2 above. Human CYP1A1, 1A2, and 3A4 isozymes were expressed in genetically engineered yeast cells that also over-expressed yeast CYP450 reductase. Analyses were done by HPLC/UV detection and a radioactivity detector (LB507A, Berthold). UV detection for riluzole samples was linear in the range 1-20uM and radioactivity detection was linear at ¹⁴C-riluzole concentration mixture 1.35-27uM.

Results:

As shown previously (#2 above) incubation of 15uM riluzole with human microsomes for 20min produced a single metabolite with retention time identical to RPR112512. In the present study, 20uM ¹⁴C-riluzole incubated with the genetically engineered yeast expressing human CYP1A2 for 30min produced cpd identical with RPR112512. The rate of decrease in riluzole was quantitatively related to the formation of A1 at a reaction rate of 0.8pmol riluzole/min/pmol CYP1A2 or 27pmol riluzole/min/mg of microsomal yeast proteins. Incubation of 25uM ¹⁴C-riluzole with genetically engineered yeast expressing human CYP1A1 for 30min produced 4 metabolites, A1-4. A1 was identical to RPR112512, and A2, A3, and A4 corresponded to RP65077, RP65110, and RPR109792 respectively. The 1st two metabolites have been detected in human urine and the last one was found in monkey urine. The decrease in riluzole was exponential with time and formation of the 4 metabolites. The biotransformation rate was 3.8pmol riluzole/pmol CYP1A/min or 422pmol riluzole/min/mg yeast microsomal proteins. No metabolites were detected using CYP3A4. However, it is noted that the incubation was done without cytochrome B5, an electron donor necessary for maximum catalytic activity of CYP3A4 in presence of substrates.

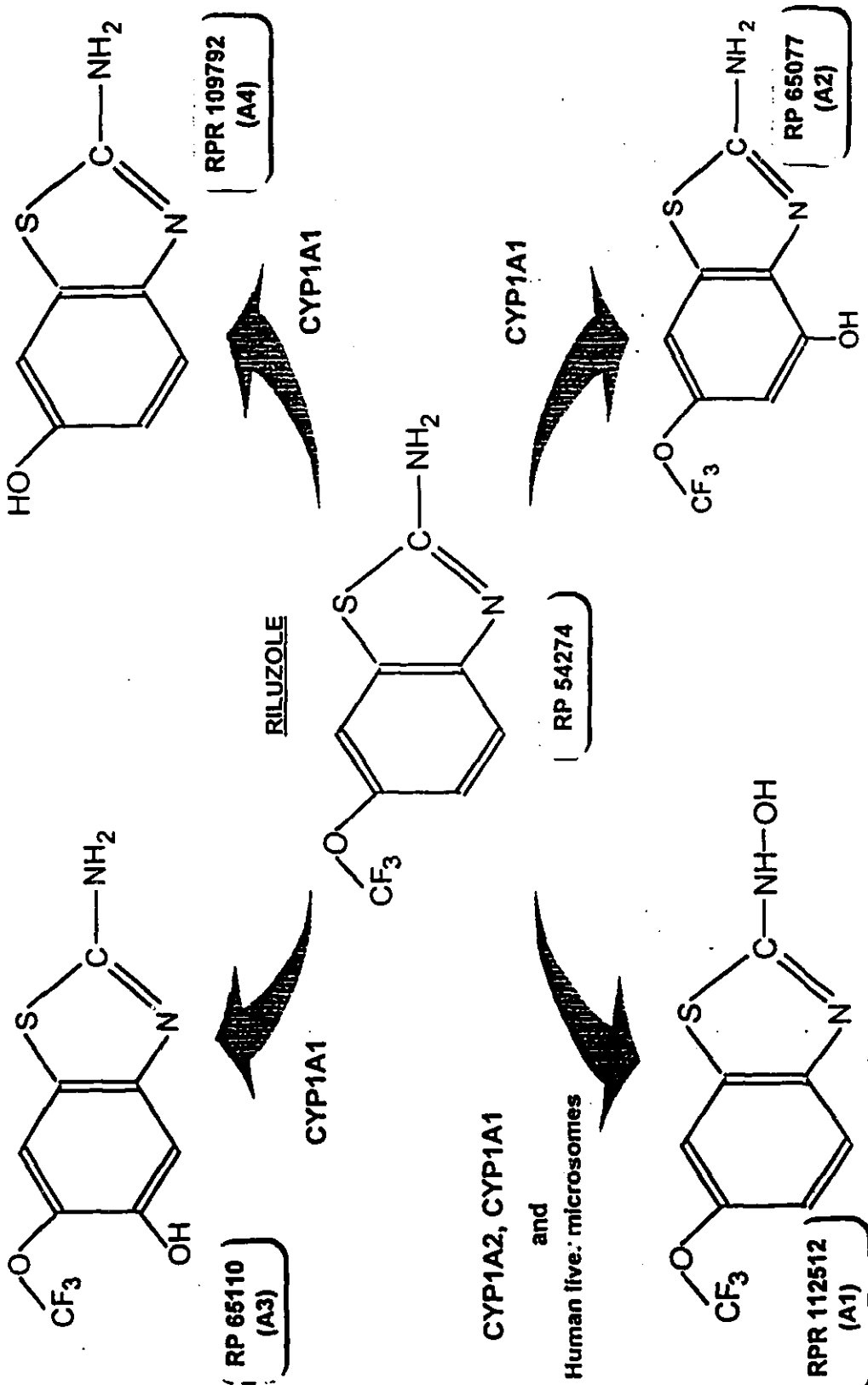
[attached figure from the sponsor presents riluzole metabolites formed by yeast cells].

Summary and Conclusion:

Using genetically engineered yeast cells that express human P450 isozymes, this study demonstrated that riluzole is metabolized mainly by CYP1A2 forming a single N-hydroxylated derivative RPR112512. Also, CYP1A1 isozymes were involved and formed 4 metabolites one of which was RPR112512, the other 3 consisted of O-dealkylation (RPR109792) and aromatic hydroxylation (RP65077 and 65110). From the rate of reactions, it appeared that riluzole biotransformation was 5x higher with CYP1A1 than with CYP1A2 (3.8 vs. 0.8) and the sponsor calculated 1pmol metabolite/min/pmol of CYP1A1 for production of RPR112512. The involvement of CYP1A1 in riluzole metabolism indicates

extrahepatic metabolism since this isozyme is not expressed to a significant level in human liver but to a great degree in the lungs.

FIGURE 3: Riluzole (RP54274) metabolism by yeast expressed human CYP450s and by human liver microsomes.



4. Riluzole metabolic drug-drug interactions in vitro
 1994/France.
 Lot# 8PROC 92312.
 This study was conducted under GLP.

/Oct

The study followed protocol IBP-Biodyn 93-04201. Human livers and microsomal preparation were the same as those reported above. IC₅₀ values (inhibitory concentration causing 50% reduction in riluzole hydroxylation) were calculated.

Results and Conclusion:

The effect of 24 drugs, some are known P450 inhibitors/substrates or used in 155 ALS patients as concomitant medication, were tested on riluzole metabolism. Table below from the sponsor summarizes the findings. Dose-response curves were constructed for the more potent inhibitors which included nicergoline, amitriptyline, diclofenac, diazepam, clomipramine, imipramine, quinine, enoxacin, and cimetidine. IC₅₀ ranged between 210-937µM. These results again indicate the role of CYP1A2 in human metabolism of riluzole and identify in vitro, those drugs that most likely will affect riluzole biotransformation.

TABLE 3. INHIBITION OF RPK 112512 FORMATION IN HUMAN LIVER MICROSOMES

[riluzole] = 15 µM. Inhibitors at 1 mM, *except nicergoline and diazepam (0.5 mM).

INHIBITOR	INHIBITION	IC ₅₀ µM	INHIBITOR	INHIBITION	IC ₅₀ µM
Clomipramine	99%	265 ±25	Paracetamol	28%	
Diclofenac	99%	234 ±19	Pyridoxine	18%	
Amitriptyline	94%	210 ±17	Enalapril	12%	
Imipramine	85%	356 ±20	Thiamine	9%	
Enoxacin	71%	465 ±77	Captopril	8%	
Nicergoline*	71%	256 ±13	Pefloxacin	6%	nd
Quinine	63%	444 ±62	Aspirin	6%	
Diazepam*	59%	240 ±23	Amoxicilline	6%	
Theophylline	47%		Metronidazole	5%	
Cimetidine	47%	937 ±182	Piracetam	5%	
Caffeine	37%		Baclofen	-3%	
Ranitidine	29%				

In Vivo Studies:

nd: not detectable.

1. Metabolic profile in the mouse and rabbit after a single oral dose of 2mg/kg/
 Aug 1994/France.
 Batch# for labelled drug= CRA 383-388-PX5624; Batch# for unlabelled drug= C8808501; specific activity 52mCi/mmol; radiochemical purity >99%.
 This study was conducted under GLP.

The study was based on protocol# The objectives were to measure drug elimination and metabolic profile in blood, plasma, urine, and feces in these species. The 2mg/kg dose was selected because it was used in other PK studies in the rat and dog and is close to the lowest dose in tox studies. OF1 male and females mice (n=5-16; 22-23g) and New-Zealand white rabbits (3/sex; 1.86-1.92kg) were used. The drug was prepared as a hydrochloride solution and dosed by oral gavage. Blood from rabbits and mice was collected at 0.5, 3, 6, and 24hr postdosing and urine and feces were collected between 0-24 and upto 96hr. Pulmonary ¹⁴CO₂ was measured only in mice. Total radioactivity was measured by liquid scintillation counting. HPLC was used to identify metabolites.

Results:

Rabbit: radioactivity was detected at 0.5hr in blood and plasma and decreased with time with only 0.5% of dose remaining at 24hr. Plasma had higher radioactivity than blood and female rabbits showed higher blood and plasma levels than male rabbits. Mean blood levels ranged between 0.02-0.6mgEquiv/L and mean levels in plasma ranged between 0.02-1.1mgEquiv./L. Most of radioactivity was excreted in urine by 24hr postdosing (70 and 84% in males and females respectively). Radioactivity in feces at 24hr accounted for 21 and 11% of dose in males and females respectively. Total radioactivity excreted by 96hr was 95-98% of dose.

Mouse: drug plasma levels were slightly lower than those in blood but there was no gender differences. Mean blood level ranged between 0.036 to 0.96mgEquiv/L and mean plasma level ranged between 0.025 to 0.70mgEquiv/L. Drug elimination was mainly through urine and most of the radioactivity was recovered by 24hr postdose (mean 76 and 72% in m&f respectively). Little radioactivity was eliminated by feces with the most elimination occurring within the 1st 24hr (mean 18&21% in m&f respectively). Total radioactivity excreted by 96hr was 95% of dose. ¹⁴CO₂ expired by the lungs was very low at about 0.36 and 0.41% of dose for male and female mice respectively.

Metabolism:

Rabbit: the main urinary metabolites were P (29% in m and 44% in f), Q (27% in m and 23% in f), and O (7% in m and 13% in f). A total of 6 metabolites were detected in both sexes (G, RP54274, O, P, Q, and T1) in addition to RP64821 and R2 in male urine. After enzyme hydrolysis by B-glucuronidase, N2, RP69597 were detected in both sexes, R2 in f and, in males, Rb, and R were detected. After enzyme hydrolysis the level of P&Q in male rabbit was reduced markedly (8&11% vs. 29&27% respectively), whereas, that of RP69597 and the parent (RP54274) were increased (23% vs. ND for RP54274). Such finding may indicate that metabolites P&Q could be conjugated products of the parent and RP69597. In females, there was no change in level of metabolite P, increase in O, and Q was non-detectable. In feces, the main products were the parent and RP65077 (7-11% of dose). The sponsor failed to identify most of these metabolites, it is unclear whether, metabolite Q or any one cpd is the glucuronide of another, since chemical structures were not provided.

Mouse: the common urinary metabolites in both sexes included: B,D,F,H,N1,N2,O,P,Q,R2,S,T1,V,RP64821, and RP54274. The main urinary metabolites included: the parent (21% in m/11% of dose in f), RP64821 (12% in m/13% in f), and O (12% in f). After enzyme hydrolysis, the levels of the parent and RP64821 in males was reduced but that of P increased. In females after enzyme hydrolysis, the level of RP64821 was decreased, but new metabolites (RP65077 and 69567) formed (the same confusion as with the rabbit regarding structure id). In feces, several metabolites were excreted such as: PK35011, RP65331, and others unidentified; the parent constituted the highest level in both sexes (11&6%).

2. ¹⁴C-riluzole metabolism in male and female rat,
1989/France.

May

Batch#C1100&C8808501 (cold cpd); Specific activity 52mCi/mmol with 99% purity.
This study followed protocol (or 01)/Aug 1987.

A single 2mg/kg dose of ¹⁴C-riluzole hydrochloride solution was administered to Sprague-Dawley rats (190-220g; 40/sex) by oral gavage at 5ml/kg. Drug distribution was studied in blood, plasma, brain, feces, urine, and lungs. For metabolism studies, 40m and 40f rats were orally dosed 15mg/kg/d for 3days and metabolites in urine and feces were determined 24hr postdose. Blood was collected between 0.5 to 72hr postdose, brains were isolated (4/sex/time

point) at 0.5, 3, 6, 24, and 72hr postdose, and samples of urine, feces, and expired ¹⁴C, were taken at 0-24, 24-48, and 48-72hr postdose. Total radioactivity was determined by scintillation counting, TLC/HPLC was used to analyze all biological samples, and metabolite identification was done by IR/NMR/MS. Both qualitative and quantitative analysis of metabolites was done and acid and enzyme hydrolysis methods were used; the enzymes included B-glucuronidase and arylsulfatase.

Results:

The following metabolites^(*) were identified (see attached figure from the sponsor for the proposed metabolic pathway in the rat):

Plasma: in male and female rat a total of 10 and 8 metabolites respectively, plus the unchanged riluzole were identified in plasma. The predominant cpd was riluzole at 0.38, 0.2, and 0.11mgEquiv/L in males and 0.39, 0.28, and 0.2mgEquiv/L in females at 0.5, 3, and 6hr postdose respectively. These metabolites were in male rat: a, c, l, m, n, p, q, r, t, and unknown, and in female rat: a, c, m, n, p, q, r, and unknown. In females, metabolites l and t1 were not detected.

Brain: male: m, unknown, and unchanged riluzole (u). Unchanged drug accounted for the highest radioactivity at 0.63, 0.38, and 0.14mgEquiv/L at 0.5, 3, and 6hr postdose respectively. Total radioactivity at 0.5hr was 0.68mgEquiv/L declined to 0.18mgEquiv/L at 6hr.

Female: m and unknown with unchanged riluzole accounting for the highest radioactivity at 0.88, 0.56, and 0.39mg.Equiv/L at 0.5, 3, and 6hr postdose respectively.

Urine: 20 metabolites in males and females plus the unchanged riluzole before enzyme hydrolysis. After enzyme hydrolysis, 14 metabolites were detected. Before enzyme hydrolysis, highest activity was contributed to metabolite c (16-17% in m and 13-15% in f), q (10-15% in m and 8-10% in f), m (5-7% in m and 4-8% in f), n (4-5 in m and 3-4% in f), h (nd-5% in m and 4-6% in f), l (3-9% in m and 6-8% in f), and unchanged drug accounted for 2-5%. Metabolites c, d, f, g, h, l, and s are sulfconjugated cpds and accounted for 30-32% of dose. After enzyme hydrolysis, c was nondetectable, q markedly reduced, m unchanged or slightly increased, n nondetected, t and unknown metabolite levels were increased 10-12% and 9% respectively, and r also increased 10-12% of dose (tables from sponsor), and parent accounted for 4-6% of dose. Metabolite e showed highest % of dose at 14% (mean), followed by t at 10-12%, and unknown at 6-12%.

Feces: 5 metabolites plus unchanged riluzole were detected in m&f rats with unchanged riluzole accounting for the highest activity at 12-17% in males and 3-7% in females.

^(*) m or RP64821: N-2-methylsulfonyl-4-trifluoromethoxyphenylurea .

e or PK 35011: 2-amino-6-hydroxybenzothiazole.

t1 (RP 65110), t2 (RP 65077), and r1 (RP 65331):

all are phenolic derivatives with OH-group at ortho, meta, or para position with respect to the OCF₃ group.

u (RP 54274): parent unchanged riluzole.

It is noted that metabolites c, l, m, n, p, and q have been identified in rat liver perfusate, a and q were identified in rat microsomes and, c and l were identified in rat S9 fraction.

Total radioactivity was similar in blood and plasma and in males and females except females had slightly higher plasma and blood levels at 6&24hr than the corresponding values in male rats. Total radioactivity in the brain of females was higher than that in males at all time periods upto 24hr postdose and, brain levels in both sexes were higher than plasma levels with

brain/plasma ratios between 1 and 3.3 (table below). Most of radioactivity was eliminated in urine within 24hr of dosing (63±5% in m&f) and the cumulative urinary excretion (0-72hr) was 67-68% of administered dose. There was small amount of radioactivity in feces (24%) within 24hr and 27-31% cumulative at 0-72hr postdose. Total excretion amounted to 87% by 24hr and 100% by 72hr.

DIFFUSION OF TOTAL RADIOACTIVITY INTO THE BRAIN						
RATS (n = 4/Time)		Time after administration (in hours)				
		0.25	3	6	24	72
MALE	% DOSE	0.69	0.41	0.19	0.026	0.0072
	eq. mg/kg	1.62	0.94	0.44	0.06	0.017
	B/P	2.2	1.9	1.8	1	1.3
FEMALE	% DOSE	0.9	0.59	0.47	0.042	0.0082
	eq. mg/kg	1.93	1.23	0.98	0.091	0.018
	B/P	3.3	2.8	2.7	1.2	1.3

B/P: Ratio Brain/Plasma

TABLE N° 4:

TOTAL RADIOACTIVITY KINETICS IN THE BRAIN OF MALE AND FEMALE RATS AFTER ORAL ADMINISTRATION OF 2 mg/kg 14C-RP 54274

Summary and Conclusion:

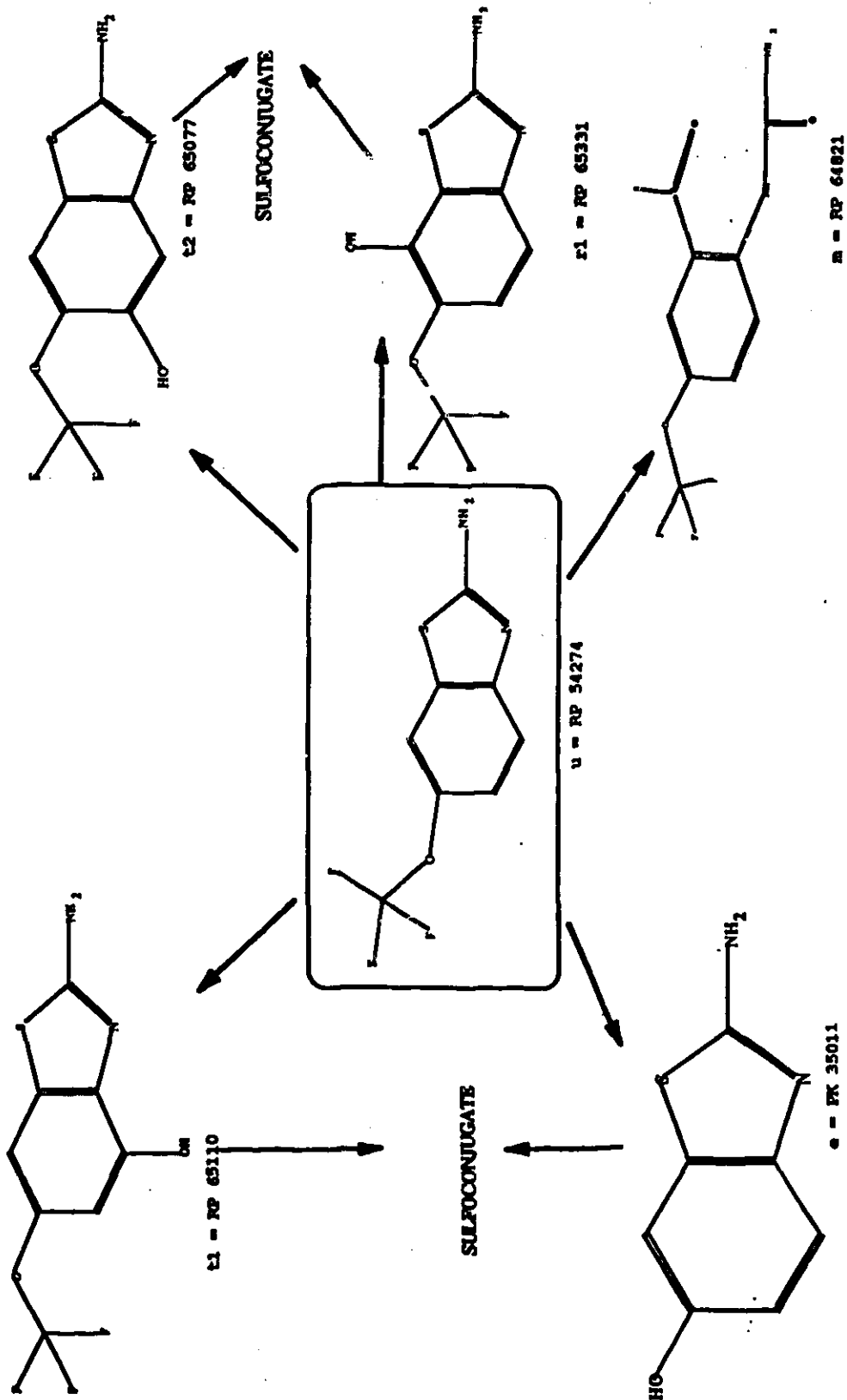
Single oral dose of radiolabelled riluzole to male and female rats at 2mg/kg was well absorbed and readily crossed the BBB. Major route of elimination was the urine at 68% and 30% in feces excreted within 24hr postdose. Total radioactivity was markedly reduced by 72hr postdose. Total radioactivity in the brain was higher than plasma levels upto 24hr postdose, females showed higher levels than males. Similarly, females showed higher blood and plasma radioactivity than males at 6&24hr postdose. There were qualitative differences between sexes in metabolites detected in brain and plasma, no such differences in urine and feces. The metabolic pathway in the rat included: oxidation of the benzene ring at the OCF, forming hydroxybenzothiazoles eliminated by sulfoconjugation (t1, t2, e, and r1), and oxidative break of the thiazole ring forming benzylurea derivatives (m). Some of these metabolites have been identified in previous rat studies such as liver perfusate, microsomes, and S9.

1165 ^{14} C-Ribuz. metabolism in ♂ & ♀ rats

APPENDIX 7

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PROPOSED METABOLIC PATHWAYS OF RP 54274 IN THE RAT



#1165 ¹⁴C-riluz. metabolites in ♂ & ♀ rat

ISOLATION CODE	RP or PK N°.	MALE N°1	MALE N°2	MALE N°4	FEMALE N°1	FEMALE N°2	FEMALE N°4
a		1.0	nd	nd	1.1	1.0	nd
b		nd	nd	nd	1.5	1.5	1.1
c		16.3	17.0	16.1	12.7	15.4	14.8
d		nd	0.9	nd	1.3	1.2	0.8
e	PK 35011	nd	nd	nd	2.2	2.6	4.1
f		nd	nd	nd	nd	nd	nd
g		nd	nd	nd	nd	1.7	2.2
h		nd	4.0	4.9	5.7	4.4	5.5
i		1.3	1.9	1.8	1.6	1.5	1.7
j		1.8	1.3	1.5	1.3	1.2	1.1
k		3.4	8.6	7.1	5.5	8.3	6.0
l		4.7	6.7	5.0	7.9	6.5	4.2
m		3.8	5.1	3.7	2.9	4.2	3.8
n		nd	nd	nd	1.4	nd	nd
o		1.3	2.4	1.5	nd	1.5	2.8
p		10.3	12.0	15.2	10.1	9.8	8.4
q		2.4	1.0	nd	1.7	1.4	nd
r		1.4	2.1	nd	1.0	1.0	nd
s		4.1	2.2	1.2	1.6	1.8	1.1
t		3.7	2.4	2.4	4.6	1.5	nd
u		0.9	nd	1.0	nd	2.8	1.2
unknown							

nd: not detected
results expressed as % of administered dose

TABLE 14:

METABOLITE DISTRIBUTION IN MALE AND FEMALE RAT (0-24h) URINE
AFTER A SINGLE ORAL ADMINISTRATION OF 2 mg/kg 14C-RP 54274

ISOLATION CODE	RP or PK N°.	MALE N°1	MALE N°4	FEMALE N°2	FEMALE N°4
a	PK 35011	1.6	1.2	nd	3.4
b		nd	1.8	nd	nd
c		nd	1.6	nd	nd
e		15.8	12.2	17.6	11.2
j		1.4	nd	nd	2.1
k		nd	nd	nd	2.9
m		4.4	6	10.2	5.9
n		nd	nd	1.3	nd
o		nd	1.2	nd	1
p		4.8	1.6	2.4	2.4
q		1.4	2.3	2	2.9
r		6	5.8	7.6	5.8
t		9.9	10.4	14	10.4
u		5	5.6	4.1	4.1
unknown	6.4	11.7	10.4	7	

nd: not detected

results expressed as % of administered dose

TABLE 15:

METABOLITE DISTRIBUTION AFTER ENZYMATIC HYDROLYSIS OF (0-24h) URINE OF MALE AND FEMALE RATS ORALLY TREATED WITH A SINGLE ADMINISTRATION OF 2mg/kg ¹⁴C-RP 54274

HO
100

ISOLATION CODE	RP or PK N°.	URINE BEH	URINE AEH	FECES
a	PK 35011	1.0	1.4	nd
b		nd	1.8	nd
~~c~~		16.5	1.6	nd
d		0.9	nd	nd
~~e~~		0.9	14.0	nd
f		0.9	nd	nd
g		0.9	nd	nd
~~h~~		4.5	nd	nd
j		1.3	1.4	nd
k		1.5	nd	nd
~~l~~		6.4	nd	nd
m		5.5	5.2	nd
~~n~~		4.2	nd	nd
o		nd	1.2	nd
p	1.7	3.2	nd	
~~q~~	12.5	1.8	nd	
~~r~~	1.7	5.4	3.0	
s	1.7		nd	
~~t~~	2.5	10.2	nd	
u	RP 54274	2.8	5.3	14.2
unknown		1.0	9.0	7.1

BEH: before enzymatic hydrolysis ;

AEH: after enzymatic hydrolysis

nd: not detected

results expressed as % of administered dose

TABLE 17:

MEAN METABOLITE DISTRIBUTION IN (0-24h) URINE AND FECES
 OF MALE RATS AFTER A SINGLE ORAL ADMINISTRATION
 OF 2 mg/kg 14C-RP54274

HT 101

3. Metabolic profile in the dog after oral administration of 10mg/kg ¹⁴C riluzole, /Dec 1989/France Batch# C 8808501 for cold riluzole and for labelled drug CEA 383-388 with specific activity of 52mCi/mmol.

¹⁴C riluzole at 10mg/kg was orally administered as a single dose of 2mg/kg to fasted male and female beagle dogs (2/sex). The drug was prepared in 1N HCl and dosed by intragastric tube at 2.5ml/kg. Blood was sampled at 1,2,4, and 6hr postdose, and urine and feces were collected at 0-24, 24-48, 48-72, 72-96, and 96-168hr postdose. Radioactivity was measured by scintillation counting. Enzyme hydrolysis of urine was done using pure and mixtures of beta-glucuronidase and arylsulfatase. Metabolites in urine, feces, and plasma were measured and identified using HPLC/UV detector.

Results:

Qualitative comparison of metabolism in plasma, urine, and feces: Plasma:
Male dog: parent (u) + 6metabolites: g,h,nl,o,p,s.
Female: parent + 9 metabolites: g,h,n,o,p,s,k,l,r1.

Urine:

enzymatic hydrolysis of urine showed one sulfoconjugated cpd (nl) and 5 glucuronconjugated cyds (a,g,h,l,q) which have not been detected in the rat urine. Three metabolites (e,q,t1) have been detected in dog microsomes. There was no qualitative difference in metabolism between sexes (see tables).

Feces:

metabolite t1 (RP65110; hydroxylated derivative of the benzene ring), constituted the highest radioactivity in males and females (0-48hr urine) followed by the parent and t2 (RP65077). (see tables).

Riluzole was well absorbed after oral dosing and there was no significant difference in total radioactivity between blood and plasma. Riluzole as unchanged molecule, accounted for most of the radioactivity in blood/plasma upto 6hr postdose (1.8 and 2.2mgEqu/L in nonmetabolizer and metabolizer dogs or 56-60% of total plasma radioactivity). After 1-2hr postdose, riluzole represented 74-85% of total plasma radioactivity. Metabolite (o) represented a mean of 15% of total plasma radioactivity at 6hr whereas the rest of the metabolites (g,h,p,s,h,k,nl,r1) at 6hrs accounted for 2.5-5%.

Total urinary radioactivity eliminated at 0-168hr postdose was 50.4% and 39.7% in feces. Total radioactivity eliminated (0-168hr) for urine and feces was 88-96% for males and females. Inter-animal variability in metabolism of riluzole was large and ranged between <0.1-45.3% of dose administered. In enzymatically hydrolyzed urine, riluzole represented 29 and 3% of administered dose in metabolizer and non-metabolizer dogs. Urinary metabolites e,m,t1,r1 accounted for 12-18% of administered dose in metabolizer and non-metabolizer dogs respectively, whereas in the rat urine, these metabolites represented 48% of administered dose. Quantitative comparison showed that sulfoconjugation in the dog (nl) accounted for 0.9-2% of urinary radioactivity relative to 30% in the rat. Glucuronidation represented 9-15% in the dog but in human urine it accounted for 70-75% of administered dose. Unchanged drug accounted for 3 and 29% in metabolizer and non-metabolizer dogs.

It was concluded that the metabolic profile of riluzole in the dog is different from that in the rat and humans as well as in the dog there is a large variability in the extent of metabolism. [see attached comparison table from the sponsor and other relevant tables].

DOG IDENTIFICATION (N°)		MALE DOGS						FEMALE DOGS						
		8089		8091		8089		8091		8094		8095		
ISOLATION CODE	N° RP	BEH	BEH	BEH	AFEH	AFEH	BEH	BEH	AFEH	AFEH	BEH	BEH	AFEH	AFEH
a		0.9	1.0	nd	nd	nd	0.7	nd	nd	nd	nd	nd	nd	nd
b		nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
c		0.5	0.8	0.9	0.9	1.5	0.9	0.5	1.4	1.4	0.5	1.4	1.0	nd
d		nd	0.8	1.4	1.4	3.0	nd	nd	1.9	1.9	nd	1.9	1.0	nd
e		nd	0.7	nd	nd	nd	nd	0.8	nd	nd	0.8	nd	nd	nd
f		1.2	3.4	nd	nd	2.5	1.7	1.8	1.2	1.2	1.8	1.2	1.3	nd
g		1.8	3.5	nd	nd	nd	1.7	1.0	nd	nd	1.0	nd	0.8	nd
h		1.1	1.2	1.3	1.3	1.1	1.7	nd	1.5	1.5	nd	1.5	1.0	nd
i		1.4	2.3	nd	nd	nd	1.2	0.8	nd	nd	0.8	nd	nd	nd
j		0.4	1.1	nd	nd	0.8	0.7	nd	nd	nd	nd	nd	nd	nd
k		0.9	2.0	0.6	0.6	1.3	1.2	nd	0.6	0.6	nd	0.6	0.8	nd
l		nd	0.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
m	RP 64821	1.0	1.3	1.1	1.1	1.5	1.9	1.0	nd	nd	1.0	nd	0.8	nd
n		5.1	8.8	3.8	3.8	3.3	6.2	3.4	nd	nd	3.4	nd	2.9	nd
o		0.6	nd	1.6	1.6	1.1	nd	nd	0.6	0.6	nd	0.6	0.8	nd
p		1.0	1.0	0.8	0.8	1.2	1.7	nd	1.4	1.4	nd	1.4	nd	nd
q		nd	0.7	3.3	3.3	6.1	0.5	0.8	4.5	4.5	0.8	4.5	3.6	nd
r		nc	nd	nd	nd	4.1	nd	nd	3.5	3.5	nd	3.5	0.8	nd
s		16.0	0.5	15.8	15.8	1.2	0.6	45.3	4.4	4.4	45.3	4.4	41.4	nd
t		nd	0.5	1.0	1.0	1.0	0.7	nd	nd	nd	nd	nd	0.2	nd
u		nd	0.5	1.0	1.0	1.0	0.7	nd	nd	nd	nd	nd	0.2	nd
5 to 8 metabolites < 0.1 %														
TOTAL		31.9	29.6	31.6	29.7	21.4	55.4	21.4	55.4	21.4	55.4	21.4	55.4	55.4
RADIOACTIVITY														

BEH: Before enzymatic hydrolysis; AFEH: After enzymatic hydrolysis

Results expressed as % of the dose administered
 nd: not detected

TABLE 6:
 METABOLITE DISTRIBUTION IN (0-24h) URINE OF MALE AND FEMALE DOGS
 ORALLY TREATED WITH A SINGLE DOSE (10 mg/Kg) OF 14-C RP 54274

MALE DOG N° 8090					
ISOLATION CODE	N° RP				
	TIME	1h	2h	4h	6h
g		nd	nd	nd	0.1
h		nd	nd	nd	0.1
nl		0.1	0.2	nd	nd
o		0.2	0.2	0.5	0.5
p		0.1	0.3	0.3	0.1
s		0.1	0.2	0.2	0.2
u	RP 54274	2.9	3.8	2.8	1.8
5 to 8 metabolites < 0.01,		nd	nd	nd	0.2
TOTAL RADIOACTIVITY		3.4	4.7	3.8	3.0

Results expressed in mg equivalent RP 54274/litre

nd: not detected

TABLE 4:

METABOLITE DISTRIBUTION IN THE PLASMA OF THE MALE DOG (N° 8089) ORALLY TREATED WITH A SINGLE DOSE (10 mg/Kg) 14C-RP 54274

non metaboliz.

114

103

FEMALE DOG N° 8094					
ISOLATION CODE	N° RP				
TIME		1h	2h	4h	6h
~~g~~		nd	nd	0.1	nd
~~h~~		nd	nd	0.1	0.1
~~k~~		nd	nd	0.1	nd
~~l~~		nd	nd	0.1	0.2
~~nl~~		0.1	0.2	0.4	0.2
~~o~~		0.2	0.3	0.5	0.6
~~p~~		0.2	0.2	0.1	0.2
~~r~~	RP 65331	nd	nd	0.1	0.2
~~s~~		0.1	0.3	nd	nd
~~u~~	RP 54274	3.2	2.9	2.7	2.2
5 to 8 metabolites < 0.01		nd	nd	0.1	0.2
TOTAL RADIOACTIVITY		3.8	3.9	4.3	3.9

Results expressed in mg equivalent of RP 54274/litre
 nd: not detected

TABLE 5:
DISTRIBUTION IN THE PLASMA OF THE FEMALE DOG (8094)
ORALLY TREATED WITH A SINGLE DOSE (10mg/kg) OF 14C-RP 54274

Dog 890 metabol. in 0.1%
75
paste

DOG IDENTIFICATION N°		MALE DOGS		FEMALE DOGS	
		8089	8091	8094	8095
ISOLATION CODE	N° RP				
r1	RP 65331	0.3	0.5		
r2		2.7	3.0	2.2	1.7
s		0.2	1.0	1.0	0.5
t1	RP 65110	-9.8	-13.0	-8.6	-6.3 ← $\bar{x} 9.4 \pm$
ta		2.5	4.2	4.9	1.0
tb		1.8	2.2	2.6	0.5
t2	RP 65077	6.2	10.5	6.3	4.1 ← $\bar{x} 6.8 \pm 2$
u	RP 54274	6.9	11.0	6.5	5.2 ← $\bar{x} 7.4 \pm 2$
5 to 8 metabolites < 0.1 %		0.8	0.5	1.0	0.1
TOTAL RADIOACTIVITY		31.2	45.9	33.1	19.4

Results expressed as % of dose administered
nd: not detected

TABLE 7: METABOLITE DISTRIBUTION IN (0-48h) FAECES OF MALE AND FEMALE DOGS ORALLY TREATED WITH A SINGLE DOSE (10 mg/kg) OF 14C-RP 54274

ISOLATION CODE	METABOLIZER DOGS	RATS
a	nd	1.5—
c	—1.5	nd
e	2.5	14.2
g	1.9	nd —
k	1.3	nd —
m	0.4	6.6
nl	1	nd —
o	0.7	nd—
p	—nd	2.8
q	1.6	2.1
rl	0.9	6.3
s	1.3	nd —
t1	5.3	11.1
t2	3.8	nd—
u	2.8	4.7

Results expressed as % of the dose administered
 nd: not detected

TABLE 9 :
COMPARISON OF THE METABOLITE DISTRIBUTIONS
AFTER ENZYMATIC HYDROLYSIS OF URINE OF
"METABOLIZER" DOGS AND RATS

4. Riluzole biotransformation in 6 healthy volunteers after a single oral dose of 150mg supplemented with 50uCi of ¹⁴C riluzole, /Dec
1989/France
This study was conducted under GLP.

Riluzole was administered to 6 overnight fasted healthy male volunteers (22-32yrs old) as a single oral dose in the form of capsules containing 150mg dose plus 50uCi ¹⁴C riluzole (lot# CB 3584). Blood was sampled at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, 24, 36, 48, and 72hr postdose. Urine was collected at these time intervals: 0-2, 2-4, 4-6, 6-8, 8-12, 12-16, 16-24, 24-36, and 36-48hr postdose, and 24hr urine for the next 5days. Feces was collected over 24hr periods for 7days postdose. The following were conducted: total radioactivity in blood, plasma, urine, and feces, and metabolism studies. Enzyme hydrolysis of urine was done using pure beta glucuronidase and arylsulfatase. HPLC with UV detector was the analytical method used and MS/IR/NMR spectroscopy were used to identify structures. In addition to the 6 volunteers that received a single dose, urine samples were also collected from 4 volunteers that received repeat dose of unlabelled riluzole during trial AG010; this urine was pooled and freeze dried for analysis.

Results:

Plasma levels of total radioactivity (parent+metabolites) were higher than those in blood (attached table from sponsor). Maximum concentration in blood was 3.7 vs. 5.8ugEquiv/ml in plasma, the corresponding values for AUC_{0-∞} were 48 and 60ug.hr/ml respectively. The higher concentration in plasma indicated small uptake of the drug into RBCs unlike the findings in some animals where drug levels were generally higher in blood. Absorption of riluzole was fast as demonstrated from the absorption t_{1/2} of 2.2hr and drug appeared in blood within 15min after dosing. Drug elimination t_{1/2} in plasma was 27hr with K_e of 0.128±0.0168hr⁻¹. The drug kinetics followed a 2-compartment open model. The highest radioactivity in plasma throughout the 6hr period was that of the parent (u) at 2.12ugEquiv/ml at 0.75hr, followed by (o) at 0.46ugEquiv/ml at 0.75hr, followed by (p) at 0.43ugEquiv/ml at 0.75hr and (k) at 0.34ugEquiv/ml at 0.75hr; these cpds were still measurable by 6hr postdose (except for k was detectable until 4hr at 0.14ugEquiv/ml). Other plasma metabolites included n1, r1 (RP65331), t1 (RP65110), ta, tb, t2 (RP65077), s, and unknowns (<0.1% of dose) (see table from sponsor). The blood/plasma used was that prior to enzyme hydrolysis, there was not enough plasma to carry out enzyme hydrolysis. Comparison with urinary metabolic profile (see below) it was found that k, o, and p are conjugated metabolites circulating in plasma.

Prior to enzyme hydrolysis, apparent urinary t_{1/2} of total radioactivity was calculated at 14.2hr prior to enzyme hydrolysis, which is shorter than that in plasma at 27hr. By 2hrs, urinary excretion of labelled riluzole accounted for 25% of dose administered and the cumulative excretion by 72hr was 92±8% of dose or 138±12 mgEquiv of riluzole. Within 7days, urinary excretion was 91% of dose indicating that the main route of riluzole elimination is the urine. About 82.7% of radioactivity was excreted within the first 24hr postdose. Excretion via feces was small and represented a mean of 4.7±1.4 of dose by 7days.

Urinary metabolites (urine 0-24hr) prior to enzyme hydrolysis composed of 8 main metabolites: k, n1, o, p, r1 (RP65331), t1 (RP65110), the parent (u), and unknowns. Metabolites r1 and t1 have been detected previously in rat urine and metabolites o and p in rabbit liver perfusate. After sulfatase hydrolysis, there was no change, a finding that is in contrast to that in the rat. After glucuronidase hydrolysis, metabolites k, o, and p disappeared therefore, corresponding to glucuroconjugated cpds. The main metabolites released after glucuronide hydrolysis were the parent (u) and t3 which seems to be specific to humans. Other metabolites released included in decreasing order: s, td, t1, tb, t2, v, tc, and ta. Metabolites r1, t1, and unknown

were partially conjugated; n1 on the other hand, did not seem to be a conjugated cpd (see attached figure from sponsor).

Quantitatively, after enzyme hydrolysis, the parent cpd was excreted rapidly accounting for 44% of dose by 24hr or 2.1% of dose before enzyme hydrolysis. This excretion however, was slow (1.1% at 0-2hr and 0.02% at 12-16hr postdose). Urinary elimination of unchanged riluzole after enzyme hydrolysis represented 53% of all cpds excreted at the 0-24hr interval (attached tables from the sponsor). This is different from the rat where unchanged urinary elimination of riluzole after enzyme hydrolysis accounted for 5%, 3-29% in the dog (interanimal variation), and 23% in monkeys.

The hydroxylated metabolites t1 and r1 were also excreted slowly. On the other hand, the conjugated metabolites o,p,k were excreted rapidly at 0-2 and 2-4hr (5.3, 10.7, and 4.5% at 0-2hr respectively) and accounted for 33, 23, and 13% of administered dose by 24hr. However, their excretion was relatively slow after 2-4hr period. These 3 conjugated cpds accounted for 69% of administered dose by 24hr indicating that their excretion was not complete by 24hr and they may have accounted for the apparent half life of 14hr. Also, these 3 metabolites at the period 0-24hr accounted for 84% of total radioactivity eliminated at this time. It is noted that excretion of the parent was not complete by 24hr after enzyme hydrolysis (44% of dose), it was suggested that metabolite o is a glucuronoconjugated form of the parent (see tables from the sponsor). The high contribution of o,p,k to urinary metabolite excretory pool in humans is different from that in dogs since only 13% of administered dose was excreted in the conjugated form and about 20% in the monkey.

Summary and Conclusion:

Oral administration of riluzole at 150mg to human volunteers showed that the drug is rapidly absorbed appearing in blood within 15min of dosing. Elimination $t_{1/2}$ in plasma of total radioactivity was 27hr and the drug kinetics followed a 2-compartment open model. Elimination $t_{1/2}$ in urine was shorter than that in plasma at 14hr (total radioactivity), this was probably due to the qualitative and/or quantitative difference in metabolites in urine and plasma. Fecal excretion was small (mean of 4.7% of dose during the 0-7days). Maximum concentration of riluzole in plasma was 2.36ug/ml reached at 1hr postdose.

The main metabolic pathway for riluzole is direct glucuronidation as well as metabolite t3 which is specific to humans and formation of metabolites o,p,k which were present in plasma and urine along with unchanged drug. Also, to a lesser extent, phase I P450 hydroxylation occurs, these hydroxylated metabolites are also detected in the rat.

Prior to enzyme hydrolysis at the 1st 24hr postdose, there were 6 main urinary metabolites together with unchanged drug, 2 of these (RP65331 and RP 65110) have been identified in urines from dog and rat. The other 4 metabolites seem to be specific to humans (k,n1,o,p). After enzyme hydrolysis, the unidentified metabolite n1 was not a glucuronide conjugate and metabolites o,p,k released mainly the parent and t3 (RP69597). Other metabolites that were released after enzyme hydrolysis included ta, td, te, t3 which are specific to humans. In the 1st 24hr, nonconjugated metabolites accounted for 9.5% of dose with unchanged drug representing 2% of this value. The glucuroconjugated metabolites (o,p,k) accounted for 69% of dose.

Metabolites
in plasma

SUBJECT	PLASMA LEVELS											BLOOD LEVELS	
	AUC 0-inf h.µg/ml	Cmax exp µg/ml	t½ h	t½β h	t½α h	Vc l	VdSS l	VSSC l	Cl ml/min	Kel h ⁻¹	K12 h ⁻¹	K21 h ⁻¹	AUC 0-inf h.µg/ml
MEN	60.09	5.77	30.30	1.86	20.35	78.85	106.70	40.67	0.1199	0.2039	0.0709	44.15	3.66
JMV	57.95	5.16	21.27	1.72	16.12	51.90	72.28	39.26	0.1461	0.1992	0.0898	48.29	3.36
FRA	52.89	4.29	26.41	2.69	23.98	67.51	99.22	43.41	0.1086	0.1130	0.0623	47.28	3.07
HAG	36.18	4.44	24.24	1.84	26.37	93.60	137.54	65.56	0.1491	0.1845	0.0724	35.01	2.57
PAC	47.51	4.49	29.48	1.69	25.05	94.90	128.86	50.50	0.1314	0.2283	0.0733	39.06	2.70
YER	39.51	3.76	30.40	3.37	32.37	90.05	160.61	61.02	0.1131	0.0738	0.0414	34.28	2.68
MEAN	49.02	4.65	27.02	2.20	23.71	79.47	117.54	50.07	0.1280	0.1671	0.0684	41.35	3.01
SD	9.61	0.71	3.67	0.68	5.43	16.81	31.26	10.89	0.0168	0.0593	0.0157	6.02	0.43
CV %	19.60	15.22	13.60	30.76	22.89	21.16	26.59	21.75	13.1073	35.4643	22.9949	14.57	14.27
maximum	60.09	5.77	30.40	3.04	32.37	94.90	160.61	65.56	0.1491	0.2283	0.0898	48.29	3.66
minimum	36.18	3.76	21.27	1.69	16.12	51.90	72.28	39.26	0.1086	0.0738	0.0414	34.28	2.57

ABLE 3A: 14C-RP 54274 PHARMACOKINETIC PARAMETERS FROM TOTAL RADIOACTIVITY

Blood and Plasma levels fitting according to a two-compartment open model
(pharmacokinetics parameters calculated for parent drug + radiolabeled metabolites)

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CODE	REF. NUMBER	0.5h	0.75h	1h	1.5h	2h	2.5h	3h	4h	6h
		k	ND	0.34	ND	0.42	0.31	0.27	0.37	0.14
n1		ND	ND	0.39	ND	ND	0.34	0.12	0.10	ND
(n)		0.24	0.46	0.52	0.67	1.26	0.91	0.72	0.92	0.55
P		0.26	0.43	0.50	0.50	0.40	0.27	0.20	0.15	0.13
r1	RP65331	0.23	0.34	0.39	0.59	0.21	ND	0.16	0.19	0.13
s		ND	ND	ND	ND	ND	ND	0.10	ND	ND
t1	RP65110	ND	ND	ND	0.61	ND	0.20	0.10	0.16	ND
ta		ND	ND	ND	ND	ND	0.23	ND	ND	0.13
tb		ND	ND	ND	0.51	ND	ND	ND	0.23	ND
t2	RP65077	ND	ND	ND	ND	ND	ND	0.22	ND	ND
(u)	RPS4274	1.90	2.12	2.36	1.09	1.40	1.26	0.87	0.72	0.75
unknowns < 0.1%		ND	ND	ND	ND	0.28	ND	ND	0.10	ND
TOTAL		2.63	3.69	4.16	4.39	3.86	3.48	2.86	2.71	1.69

TABLE 4A: 14C-RP 54274 DISTRIBUTION OF METABOLITES IN PLASMA

mean of six subjects

before enzymatic hydrolysis

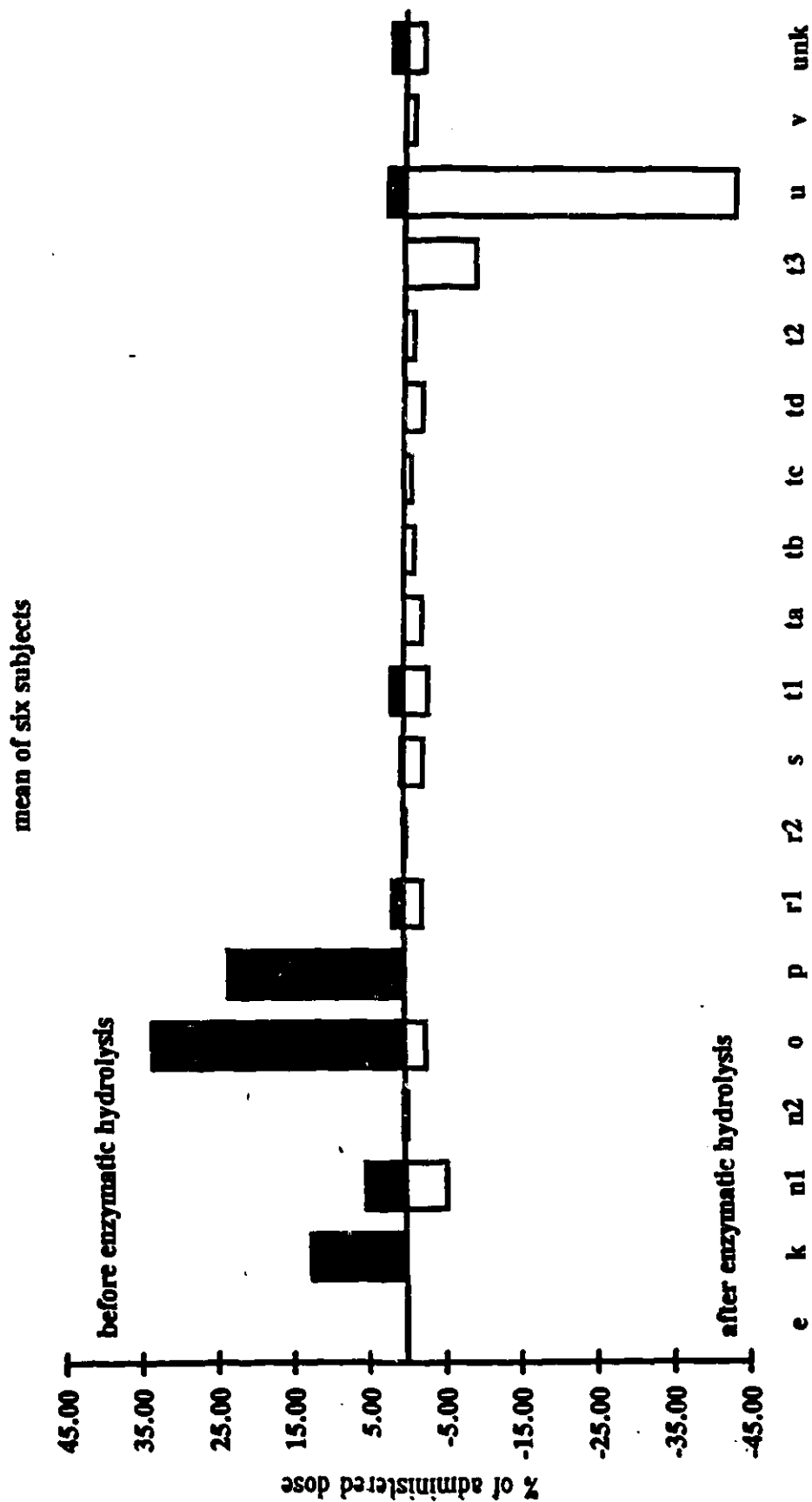
expressed as µg equivalent RP 54274/ml

ND: not detected

201

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FIGURE 11A: 14C-RP 54274 DISTRIBUTION OF (0-24 hours) URINARY METABOLITES



CODE	REF. NUMBER	MEAN							TOTAL
		0 - 2h	2 - 4h	4 - 6h	6 - 8h	8 - 12h	12 - 16h	16 - 24h	
k		4.45	2.90	1.87	0.87	1.12	0.82	0.58	12.61
n1		1.43	1.30	0.88	0.43	0.52	0.40	0.30	5.26
o		5.32	7.58	7.18	3.55	4.27	3.18	2.25	33.33
p		10.65	4.33	2.60	1.22	1.87	1.53	1.12	23.32
f1	RP 65331	0.18	0.33	0.23	0.12	0.20	0.20	0.15	1.41
o1	RP 65110	0.45	0.28	0.25	0.13	0.15	0.15	0.15	1.56
u	RPS4274	1.08	0.57	0.18	0.13	0.10	0.02	0.05	2.13

TABLE 9A: 14C-RP 54274 DISTRIBUTION OF MAIN URINARY METABOLITES

mean of six subjects
before enzymatic hydrolysis
expressed as % of administered dose

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CODE	REF. NUMBER	MEAN								TOTAL 0 - 24h
		0 - 2h	2 - 4h	4 - 6h	6 - 8h	8 - 12h	12 - 16h	16 - 24h		
n		1.88	1.40	1.03	0.47	0.18	0.23	0.15	5.35	
o		1.23	0.27	0.37	0.27	0.15	0.12	0.38	2.78	
r	RP65331	0.22	0.23	0.55	0.33	0.32	0.45	0.35	2.45	
s		0.65	0.52	0.57	0.32	0.22	0.22	0.07	2.55	
t	RP65110	0.57	0.48	0.80	0.32	0.38	0.53	0.22	3.30	
ta		0.42	0.55	0.63	0.12	0.23	0.27	0.18	2.40	
td		0.12	0.45	0.55	0.28	0.50	0.27	0.37	2.53	
l		3.33	0.57	1.68	0.62	1.08	1.47	0.72	9.47	
u	RPS4274	14.85	11.63	5.67	2.87	4.23	2.22	2.02	43.48	

TABLE 11A : 14C-RP 54274 DISTRIBUTION OF MAIN URINARY METABOLITES

mean of six subjects
after enzymatic hydrolysis
expressed as % of administered dose

1124 114

5. Biliary excretion and metabolic profile in conscious rats after oral dose of 2mg/kg
/Dec 1989/France.
Specific radioactivity was 5mCi/mmol; radioactivity per rat 11.9uCi.

Five conscious male Sprague-Dawley rats with biliary fistulas implanted 24hr previously were administered ¹⁴C riluzole in 1N HCl solution at 2mg/kg by oral gavage (5ml/kg). Bile was sampled at the following intervals: 0-0.5, 0.5-1, 1-3, and upto 24-48hr. Urine and feces were collected daily at 0-24 and 24-48hr. Animals were killed 48hr later and intestinal contents were recovered for residual radioactivity. Enzyme hydrolysis using pure sulfatase was done on both bile and urine samples and metabolites analyzed by HPLC with UV detector.

Results:

Large amount of radioactivity was excreted in bile accounting for 51±7% of administered dose by 48hr. Elimination was rapid with maximum radioactivity of 22±7% appearing in bile by 3hr(attached table from the sponsor). The 51% elimination of riluzole in bile in fistulized rats is higher than that in normal rats (about 30%), indicating that riluzole and/or its metabolites undergo enterohepatic circulating. Cumulative excretion in urine was 41% of dose by 48hr postdose and maximum excretion occurred within the 1st 24hr accounting for 40% of dose. Very little radioactivity was eliminated by feces (1.2% by 48hr). By 48hr postdose total excretion accounted for 94% of administered dose.

Metabolic profile in the bile (see tables from sponsor):

- the parent unchanged accounted for 18.9% only small fraction of 3.5% was sulfconjugated.
- the sulfconjugated cpds c,d,g,h,j,n1,n2, and s accounted for 8.5% of dose. These cpds have been identified in rat urine (IBP Biodyn# 1165).
- The other conjugated metabolites: e (PK35011), r1 (RP65331), t1 (RP65110), and m (RP64821) accounting for 13.3% of total radioactivity in bile before enzyme hydrolysis, have been identified in rat urine (IBP-Biodyn# 1165).
- Metabolites o and p of unknown structures have also been measured in rat urine (IBP-Biodyn# 1165).

Metabolic profile in urine:

- the sulfconjugated metabolites: c,l,n1,o,p, and q accounted for 23% of dose.
- unchanged parent drug accounted for 21% and 11% as a sulfconjugated cpd.
- the following metabolites were detected in urine before enzyme hydrolysis: c,l,n1,o,p,q,r1,t1,u, and 5-8 unknown metabolites at <1% of dose.

Summary and Conclusion:

Oral administration of 2mg/kg riluzole to conscious fistulized male rats showed the following:

- marked and rapid biliary excretion (51% of dose by 48hr).
- the biliary metabolic profile consisted of sulfconjugated metabolites accounting for 12% of dose. These have been identified in unfistulized rat urine.
- unchanged parent drug accounted for 19% and only 3.5% was sulfconjugated.

Biliary excretion & metabolic profile in conscious rat after single oral dose.

RADIOACTIVITY EXCRETION (% DOSE)							
Tracer: 14C-RP 54274		Study: GRN 28					
	RAT N°	1	2	3	4	5	Mean ± SD
BILE	0-0.5 h	2.2	0.04	4.5	0.6	0.1	1.5 ± 1.9
	0.5-1 h	11.4	6.1	15.2	4.4	6.1	8.6 ± 4.5
	1-3 h	27	17.5	29.7	12.7	21.3	(21.6) ± 6.9
	3-6 h	10.4	13.6	7.3	20.6	4.3	13.9 ± 5.4
	6-24 h	3.3	3.4	2.9	10.3	0.1	4.9 ± 3.1
	24-48 h	0.6	0.6	0.6	0.2	49.8	0.4 ± 0.2
	CUMUL	54.9	41.2	60.2	48.8	49.8	51.0 ± 7.1
	URINE	0-24 h	38.8	39.8	37.4	44.3	39.4
	24-48 h	0.9	1.4	1.2	1.1	2.4	1.4 ± 0.6
	CUMUL	39.7	41.2	38.6	45.4	41.8	41.3 ± 2.6
FECES	0-24 h	0.2	2.1	0.4	1	0.2	0.8 ± 0.8
	24-48 h	0.4	0.2	0.3	0.5	0.4	0.4 ± 0.1
	CUMUL	0.6	2.3	0.7	1.5	0.6	1.2 ± 0.8
INTESTINAL CONTENTS		1	0.3	0.1	0.1	0.1	0.3 ± 0.4
PROFILE		96.2	85	99.6	95.8	92.3	93.8 ± 5.5

TABLE 4:

MEAN AND INDIVIDUAL, BILIARY, URINARY AND FAECAL EXCRETION OF TOTAL RADIOACTIVITY IN CONSCIOUS BILE-DUCT CANNULATED MALE RATS AFTER ORAL ADMINISTRATION OF 2MG/Kg 14C-RP 54274

126 116

ISOLATION CODE	N ° RP or PK	0-0.5 h	0.5-1h	1-3 h	3-6 h	6-24 h	0-24 h	
a	PK 35011	ND	ND	ND	0.2	ND	0.2	
c		ND	ND	ND	0.2	ND	0.2	
d		ND	0.1	0.3	0.2	ND	0.6	
e		0.1	0.4	0.7	0.4	0.2	1.8	
f		0.1	0.6	1.5	0.8	0.4	3.4	
g		0.1	0.4	1.3	0.8	0.2	2.8	
h		ND	0.2	0.6	0.4	0.1	1.3	
j		ND	ND	ND	0.3	ND	0.3	
m		RP 64821	0.2	0.8	1.6	1.1	0.2	3.9
n1			ND	0.1	ND	0.5	ND	0.6
n2		ND	0.4	0.9	0.4	0.2	1.9	
o		0.4	1.7	1.1	1.0	0.2	4.1	
p		ND	0.4	0.5	ND	ND	0.9	
r1	RP 65331	ND	ND	0.5	0.8	0.5	1.8	
s		ND	ND	0.3	0.3	0.2	0.8	
u	RP 65110 RP 54274	ND	0.7	1.4	0.4	0.2	2.7	
5 to 8 metabolites <1 %		0	1.9	9.8	3.4	1.5	18.9	
		0.3	1.0	1.0	1.0	1.0	4.3	
% RADIOACTIVITY ELIMINATED		1.5	8.7	21.5	14.2	4.9	50.8	

results expressed as % of administered dose
 ND: not detected

TABLE 5: METABOLITE DISTRIBUTION BEFORE ENZYMATIC HYDROLYSIS IN BILE
 FROM MALE RATS ORALLY TREATED WITH 2 mg/Kg 14C-RP 54274

711
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ISOLATION CODE	N ° RP or PK	0-0.5 h	0.5-1h	1-3 h	3-6 h	6-24 h	0-24 h	
a	PK 35011	ND	0.2	0.2	0.3	0.7	0.7	
c		ND	ND	0.3	ND	ND	0.3	
d		ND	ND	0.2	0.1	ND	0.3	
e		ND	0.6	1.2	0.8	0.2	2.8	
f		ND	0.5	1.2	0.7	0.2	2.6	
g		ND	0.1	ND	0.1	0.1	0.3	
h		ND	0.2	0.1	0.1	ND	0.4	
m		RP 64821	0.1	0.6	0.4	0.4	0.2	1.7
o			0.4	0.8	2.1	1.2	0.2	4.7
p			ND	0.7	0.2	1.1	0.2	2.2
q		ND	0.1	ND	ND	ND	0.1	
r	RP 65331	0.2	0.9	1.8	1.3	0.5	4.7	
s	RP 65110	0.1	0.7	1.6	1.1	0.6	4.1	
t	RP 54274	1	2.8	11.0	6.1	2.0	22.4	
u		0.2	0.4	1.0	0.6	0.6	2.8	
5 to 8 metabolites <1 %								
% RADIOACTIVITY ELIMINATED		1.5	8.6	21.3	13.9	4.8	50.1	

results expressed as % of administered dose
 ND: not detected

TABLE 6: METABOLITE DISTRIBUTION AFTER ENZYMATIC HYDROLYSIS IN BILE FROM MALE RATS ORALLY TREATED WITH 2 mg/Kg 14C-RP 54274

8/8 1/8

6. Total radioactivity excretion in bile duct cannulated rat after a single i.v. dose of 5mg/.
July 1994/France.

This study was conducted under GLP.
Specific activity: 56mCi/mmol; Batch# PX 6163 CEA 0690-0193/Batch# for unlabelled cpd 10 Proc 93049.

Sprague-Dawley rats (6/sex) fistulized 18-24hr previously (mean bile flow 0.92-0.98ml/hr before injection), injected i.v. with a single dose of 5mg/kg ¹⁴C riluzole in saline solution + 1.4mg/ml Poloxamer 188. Infusion pump rate was 0.5ml/min and volume of 10ml/kg. Bile, urine, and feces were collected over 48hr; animals were sacrificed after 48hr. Excreta and total radioactivity was measured by liquid scintillation counting (direct counting was done on bile, urine, skin, intestinal contents, and feces 24-48hr; remaining carcass and intestine and 0-24hr feces). The 5mg/kg was selected based on 1-month tox study in rats and corresponds to the smallest dose used in that study.

Results and Conclusion:
Summarized in table below:

MEAN % DOSE RECOVERED AT 48 HOURS					
SEX	BILE	URINE	FAECES	TISSUE RETENTION	TOTAL
MALE	43.1 ± 9.5	44.8 ± 7.5	0.8 ± 0.2	2.0 ± 0.6	90.7 ± 4.2
FEMALE	49.0 ± 3.1	34.8 ± 2.5	1.9 ± 1.4	4.5 ± 0.7	90.3 ± 4.8

There was no sex difference in excretion. Maximum biliary excretion was 15±1.8% in males occurred at 3-6hr and that in females was 32±1.3% at 6-24hr postdose. Range of total biliary excretion in m+f was 28-50% of dose. Most of urinary excretion occurred by 24hr in both sexes representing 45±7.5% in males and 35±3% in females with a range from 32-55% of dose. Little radioactivity was eliminated in feces accounting for 1-2% of dose with a range from 0.6-4.5% of dose in both sexes. Total recovery by 48hr ranged from 85-97% (including skin, intestine, and carcass).

7. Enterohepatic cycle in male rat after a single oral dose of 2mg/kg.
Dec 1993/France.

This study was conducted under GLP.
Specific activity: 56mCi/mmol; Batch# PX 6163 CEA 0690-0193/Batch# for unlabelled cpd 10 Proc 93049.

Fistulized male rats were administered a single oral dose of ¹⁴C riluzole at 2mg/kg and bile collected for 0-3hr and 3-6hr. This bile of the "donor" rats was re-injected via intraduodenal infusion to another set of fistulized conscious rats (receptient). Bile, urine, and feces were collected from these receptient rats for 24hr. [The receptient rats had 2 cannulas: hepatic for collection of bile and duodenal for infusion of the donor bile into the

duodenum via the bile duct]. The recipient rats received 5ml of the bile pool donor sample, a volume that corresponded to about 31% of dose administered to donor rats.

Results and Conclusion:

This study assessed the contribution of enterohepatic circulation to riluzole metabolism and kinetics in the rat. The bile collected from the donor rats was pooled to standardize the sample infused to the recipient rats i.e.

recipient rats received the same bile sample. Table below from the sponsor summarizes the results:

RADIOACTIVITY EXCRETION IN THE RECIPIENT RAT (Mean % of infused dose recovered at 24 hours)				
BILE	URINE	FAECES	TISSUE RETENTION	TOTAL
47.1 ± 5.9	22.2 ± 1.6	15.9 ± 3.3	5.3 ± 4.4	90.4 ± 1.3

The 24hr mean radioactivity recovered from the skin was 0.8±0.5%, intestine 0.6±1%, carcass 4±3%, urine 22±1.6%, and bile 47±6% of infused dose to the recipient rat. The mean total (urine, bile, skin, intestine, carcass) radioactivity recirculating was 75±2.4% of infused dose and 90±1.3% when feces was added. This indicated that 74% of infused bile was reabsorbed which was re-excreted via the bile (47%) or urine (22%).

Other Studies:

1. Binding of ¹⁴C riluzole to melanin in pigmented rat, Dec 1988/France
Specific activity 52mCi/mmol; Batch# PX 5624

Long Evans male rats (280±15g) were administered orally a single dose of ¹⁴C riluzole solution (in 1N HCl) at 2mg/kg in a volume of 5ml/kg. Blood and eyes were collected from 4 rats per time point killed at 1, 24, 72hr and 1,2,3, and 4wks postdose.

Results and Conclusion:

Maximum radioactivity in eyes, blood, and plasma reached by 1hr postdose was: 0.51±0.26mgEquiv/kg, 0.76±0.27mgEquiv/ml, and 0.6±0.21mgEquiv/ml respectively. In the eyes, radioactivity accounted for 0.025±0.015% at 1hr and rapidly declined to 0.00062±10⁻⁴% at 72hr. Radioactivity in the eye declined slowly after 72hr and was similar at wk3 and wk4 retaining 25-27x10⁻⁴±1-2x10⁻⁴% of dose. Radioactivity in blood was slightly higher than that in the eye and plasma and by wk4 it accounted for 0.004mgEquiv/kg. Plasma radioactivity was nondetectable by 2wks. It is concluded that residual radioactivity in the eye of the rat following a single 2mg/kg dose was very low, an average of 0.003% of the dose at 24hr and 0.0003% or 3ppm at 4wks.

2. In vitro plasma protein binding in mouse, rat, monkey, and man
 Oct 1994/France.
 specific activity 56mCi/mmol; Batch# 0690-0193 and 0690-0394 CRA.

This study was conducted under GLP.

Plasma concentrations tested ranged between 0.125 to 5ug/ml (21uM). These plasma samples were obtained in animals from PK studies and those which were considered clinically effective. Equilibrium dialysis was the analytical method used and scintillation counting measured radioactivity.

Results:

Table 1: In vitro plasma binding in different species (% bound)

SPECIES	RP 54274 PLASMA CONCENTRATIONS			
	125 ng/ml (0.5 µM)	500 ng/ml (2.1 µM)	1000 ng/ml (4.3 µM)	5000 ng/ml (21 µM)
MOUSE	83.9 ± 3.6	85.8 ± 3.1	82.0 and 80.5 (*)	82.6 ± 3.1
RAT	75.5 ± 2.6	73.2 ± 2.2	72.6 and 74.2 (*)	70.0 and 70.9 (*)
MONKEY	90.3 ± 2.1	91.2 ± 1.0	90.0 ± 1.0	91.5 ± 1.3
MAN	91.7 ± 0.9	91.8 ± 0.9	91.5 ± 1.0	92.2 ± 0.3

m ± S.D.(n=3), except (*) where n=2

Radiochemical purity remained unchanged during assays.

The in vitro plasma (serum) protein binding of RP 54274 remained unchanged in all species over the concentration range 125 to 5000 ng/ml.

The fractions bound were similar in monkey (90 to 92%) and man (92%). However, they were lower in mouse (81 to 86%) and rat (70 to 76%).

It is concluded that in vitro protein binding to plasma was similar in monkey and humans, but lower in the mouse and rat.

3. Modification of cerebral glucose utilization after i.v. administration of riluzole in the conscious rat,
 1990/France.

May

Male Sprague-Dawley rats (n=17) were partially immobilized with a plaster cast from the lower abdomen to the hind limbs. This cast allowed movement of the head and forelimbs. The cast was taped to a lead brick and rats allowed to recover from anesthesia for 3hr before measurement of local cerebral glucose utilization (LCGU). Riluzole (prepared in HCl/saline) was injected i.v. over 5min at 3 or 9mg/kg at 10min prior to measurement; controls received equivalent amount of solvent at 1ml/kg. Both ABP and rectal temp. were monitored throughout the experiment. ¹⁴C-2-deoxyglucose (DG) was injected i.v. after riluzole injection and blood samples collected over 45min. The rats at end of study were killed, brain removed, and serial coronal sections were cut and autoradiograms were prepared. Local cerebral tissue concentrations of ¹⁴C were quantified by densitometry using image analysis system. The rate of glucose utilization was determined from concentrations of ¹⁴C and glucose in

plasma and, brain tissue concentrations determined by densitometry and the use of Sokoloff's original equation (Sokoloff et al., 1977). Riluzole did not affect ABP or temp. Riluzole caused muscle relaxation and sedation in a dose-dependent manner. At 3mg/kg only muscle relaxation was noted and it diminished within 10min of DG injection. However, at 9mg/kg both effects were seen and persisted till sacrifice. LCGU was assessed from 79 brain regions of control and riluzole rats. Riluzole had a differential effect on glucose utilization i.e. affecting some areas and no effect at others. Riluzole upto 9mg/kg had no effect on medullary regions, cochlear nucleus, and suprachiasmatic nucleus. A dose-dependent decrease in CGU (significant at 9mg/kg; $p < 0.05-0.001$), was observed in visual and auditory regions. The effect was more pronounced at 9mg/kg with a 35-45% decrease in auditory areas vs. 15-25% at 3mg/kg, and 30-35% decrease in visual structures at 9mg/kg vs. 12% at LD. Riluzole affected many structures in the limbic system with statistically significant decrease in HD, 40-45% in cingulate cortices, thalamic nuclei and some hypothalamic nuclei; only 15% in LD. No marked decrease (<10%) was seen at 3mg/kg on extrapyramidal and sensory motor system, however, a significant decrease (40-45%) was observed at 9mg/kg specially in motor nuclei, posterior area of caudate nucleus, and cerebellum (vermis but not much change in deep nuclei). Similarly, 9mg/kg significantly reduced GU in the thalamic nuclei of the reticular system (30-45%); slight non-significant effect was observed at 3mg/kg. The 4 areas of the white matter in the myelinated fiber tracts were significantly reduced by the HD (26-42%).

Reproduction and Developmental Studies:

1. Oral one-generation reproductive study in rats including F1 reproductive performance /June 1994/France.
Batch# 5Proc 92256; purity 100%.
This study was conducted under the French GLP guidelines.

Attached is the study chronology provided by the sponsor. Riluzole was prepared as a suspension in 0.5% methylcellulose and administered by oral gavage at 3, 8, or 15mg/kg (5ml/kg/day) to male (81days pre-mating) and female (14days pre-mating and throughout mating, gestation, and lactation) Sprague-Dawley rats (30/group). The control group was administered the vehicle following the same protocol. Observations included clinical signs, mortality, body wt, food intake, external exam and microscopical evaluation at necropsy. Only F0 rats were treated with riluzole. Half of F0 females were sacrificed on gd20 for evaluation of reproductive parameters: uterine content, resorptions, # corpora lutea, and soft and skeletal tissue examinations of fetuses. The rest of the dams were allowed to deliver and rear the young to ppd21 at which time they were sacrificed. F1 pups were examined for physical, functional, and behavioral performance. On ppd49±3, 1 male and 1 female were mated and allowed to deliver F2 generation that were examined at ppd7. Any animal (male or female) that died prior to scheduled sacrifice was examined macroscopically to identify cause of death and for females, implantation sites were recorded. For the F0 males, they were sacrificed on the day pregnancy was confirmed (presence of vaginal plug) and the absolute and relative wt of the testis and epididymidis were recorded. One female was paired with 1 male for 14days and if pregnancy did not occur, females were placed with another male for a second 14day. If by the end of this 2nd period, copulation did not occur, the females were kept for 2wks and sacrificed and ovaries removed and examined microscopically and presence of implantation sites was checked using Salewski staining. Attached information provided by the sponsor describe the calculations of the reproductive parameters as well as the statistical methods used.

STUDY CHRONOLOGY

F0 PARENTS

Dosing:	males females	81 days pre-pairing up to mating 14 days pre-pairing and throughout pairing, gestation and lactation
Necropsy:	males females	after successful mating <i>caesarean section groups</i> : day 20 of gestation <i>parturition groups</i> : day 22 ± 1 postpartum

F1 PUPS

Culling	day 4 postpartum (8 pups) day 21 postpartum (4 pups)
Weaning	day 21 postpartum

Developmental testsPhysical

• pinna detachment	from day 4 postpartum
• incisor eruption	from day 11 postpartum
• eye opening	from day 15 postpartum
• testes descent	on day 25 postpartum
• vaginal opening	on day 37 postpartum

Functional

• surface righting reflex	from day 4 postpartum
• negative geotaxis	from day 4 postpartum
• swimming development	on day 10 postpartum
• forelimb grip strength	from day 11 postpartum
• auditory startle reflex	from day 12 postpartum
• pupillary reflex	on day 19 postpartum
• rotarod	on day 25 postpartum

ability to turn & face up on
an inclined plane within 30 sec.

Behavioral

• homing on a level surface	on day 21 postpartum
• water maze - learning - memory	day 42 ± 3 postpartum 7 days after learning test
• open field exploratory and locomotor activities	on day 49 ± 3 postpartum

ability within 2 min to move
towards & return to its home cage.

Selection for pairing	day 49 ± 3 postpartum
Necropsy	day 49 ± 3 postpartum

F1 PARENTS

Pairing	1 to 28 day period from day 70 postpartum
Necropsy:	males females
	after mating period on day 8 ± 1 postpartum

F2 PUPS

Culling	day 4 postpartum (8 pups)
Necropsy	day 8 ± 1 postpartum

~~0 ml 1-gamma~~ - R/1430P
not study F1 pups. A7

2.6.1.5. F1 pups

After the second and third cull, non-selected young were euthanatized by CO₂ inhalation and subjected to macroscopic examination.

2.6.1.6. F2 pups

F2 pups were euthanatized on day 7 postpartum and subjected to macroscopic examination.

2.6.2. Microscopic examination

Ovaries from eight non-mated or non-pregnant females treated at 15 mg/kg/day (Nos. 353, 362, 363, 371, 374, 375, 377 and 379) and one control (No. 217) were processed, stained with hematoxylin, eosin and saffron and examined microscopically.

2.7. CALCULATIONS [11]

Calculations were performed using a SAS software package.

Group mean values were calculated for each recorded parameter.

Group mean values for the litter parameters were calculated as a mean of the litter averages.

Mating performance and fertility

For each sex and group the following were calculated:

$$\text{Male mating index} = \frac{\text{Number of males having mated}}{\text{Number of paired males}} \times 100$$

$$\text{Female mating index} = \frac{\text{Number of mated females}}{\text{Number of paired females}} \times 100$$

$$\text{Male fertility index} = \frac{\text{Number of males having produced at least 1 pregnancy}}{\text{Number of males having mated at least 1 female}} \times 100$$

$$\text{Female fertility index} = \frac{\text{Number of females with implantation sites}}{\text{Number of mated females}} \times 100$$

$$\text{Parturition index} = \frac{\text{Number of females with dead or live pups}}{\text{Number of females with implantation sites}} \times 100$$

2.7.1. Caesarean data

Group mean values for the size of litters, uterine deaths, early and late resorptions were calculated using either of the following means:

Mean 1: All pregnant females surviving to term bearing evidence of implantation (including total litter resorptions).

Mean 2: All pregnant females surviving to term bearing viable fetuses.

Mean 2 has more significance when group size is small as the data would be influenced by the presence of a single total litter resorption. Mean 1 is more accurate when several females exhibit total litter resorption.

For mean litter and fetal weights and the number of fetal observations, only Mean 2 was calculated.

R114306 -
Oral 1-guanthine rat @ F, repro.

Percentage pre-implantation loss was calculated from the formula:

$$\frac{\text{Number of corpora lutea} - \text{Number of implantations}}{\text{Number of corpora lutea}} \times 100$$

When the number of implantations exceeded the number of observed corpora lutea, percentage pre-implantation loss was taken as 0.

Percentage pre-implantation loss includes losses due to non-fertilization of ova and early post-implantation deaths (i.e. those occurring up to days 8-9 of gestation).

Percentage post-implantation loss was calculated from the formula:

$$\frac{\text{Number of implantations} - \text{Number of viable fetuses}}{\text{Number of implantations}} \times 100$$

Percentage post-implantation loss covers only the period between days 8-9 and 20 of gestation and does not include the first 2-3 days post-implantation as any death occurring in this phase would leave no remains visible on day 20.

Group mean implantation loss values were calculated as means of individual values.

Group fetal observation values (external, internal and skeletal) were expressed as a percentage and calculated from the formula:

$$\frac{\text{Number of fetuses with a particular observation}}{\text{Number of fetuses examined}} \times 100$$

2.7.2. Parturition data

Percentage post-implantation loss was calculated from the formula:

$$\frac{\text{Number of implantations} - \text{Total number of dead or live pups at birth}}{\text{Number of implantations}} \times 100$$

Percentage post-implantation loss covers only the period between days 8-9 and the end of gestation and does not include the first 2-3 days post-implantation, as any death occurring in this phase would leave no remains visible on day 21 postpartum.

Group mean implantation loss was calculated as a mean of individual values.

Live birth index was calculated with the following formula:

$$\frac{\text{Number of live pups at birth}}{\text{Number of implantations}} \times 100$$

Postnatal indices

$$\text{Viability index} = \frac{\text{Number of live pups on day 4 postpartum}}{\text{Number of live pups on day 1 postpartum}} \times 100$$

$$\text{Survival index on days 7 or 14} = \frac{\text{Number of live pups on day 7 or 14 postpartum}}{\text{Number of live pups on day 4 postpartum}} \times 100$$

$$\text{Lactation index on day 21} = \frac{\text{Number of live pups on day 21 postpartum}}{\text{Number of live pups on day 4 postpartum}} \times 100$$

INC Rinzok, Oral 1-generati on R FI rep.

The postnatal indices were calculated for each litter and then per group for these values. The calculation of the number of living pups on day 4 postpartum was established after culling, except for the viability index.

2.8. STATISTICAL METHODS NO TAG

Statistical analysis was performed on the individual values for dam parameters and by sex on the mean values for the litter and pup data.

Differences between the treated and control groups were tested using a decision tree to select an appropriate hypothesis testing procedure.

Parametric data, i.e., food consumption, body weight, body weight change, absolute and relative organ weight, gestation length, litter size, fetal weight, corpora lutea, number of implantation sites, percentages of pre- and post-implantation loss, physical and functional development and behavioral tests were analyzed in the following way:

A Bartlett's test was carried out in order to verify the homogeneity of variance, and then:

- when the variances were found to be homogeneous, an overall Analysis of Variance was carried out by means of the F test followed, when significant, by multiple and pairwise comparison of the group means using the Dunnett's test.
- when the variances were found not to be homogeneous, the overall comparison of the groups was carried out using the Kruskal-Wallis test followed, when significant, by multiple and pairwise comparison of the group means using the Wilcoxon Rank Sum test.

For the homing on level surface test, mean calculations by litter were performed taking into account censored data (failures at the end of the period of observation).

Categorical data, i.e. mating and fertility indices, gestation and parturition ratio, birth ratio, sex ratio, postnatal indices, number of failures in testes descent/vaginal opening, pupillary reflex test and the water maze test were analyzed by means of the Fisher's Exact test.

2.9. ARCHIVES

All raw data, specimens and other study documents pertaining to this study will be stored in the research center archives.

~~3. RESULTS~~

~~3.1. F0 GENERATION~~

~~3.1.1. Mortality~~

~~The F0 mortality incidence is summarized in the following table :~~

Results:

F0

Mortality: Treatment-related deaths were as follows:

Found dead 1/30Hdf and 3/30HDM

Euthanized 1/30Hdf and 5/30HDM

Death or euthanasia occurred between wks7-16 for males and wks3-6 for females. Clinical signs preceded death and included hyperactivity, tremors, muscle weakness, and loss of balance; these animals showed severe body wt loss.

Other deaths were due to either: gavage error (1/30 each in control and LD, 3/30MDM and 4/30HDM), euthanized due to procedural reasons such as having <6 pups/litter at 1st cull (1 female each in MD&HD), or due to insufficient or absence of milk (2/13Hdf) *see below under mating and fertility.

Clinical Signs: more frequent in HD males and females and MDM only. Signs included: tremors, muscle hypotonia, ataxia, hypoactivity, loss of balance, and/or salivation. The signs were observed from wk5 till end of treatment in males and from the 2nd wk of treatment (pre-mating) to wk5 (gd16) in females. Signs appeared within 1hr of dosing and remained till the next day in some rats. The incidence was higher in males: 19-20/30 HD vs. 3-6/30 in Hdf. No signs were noted in LD or controls.

Body wt and Food intake: There was no significant wt change or effect on food intake in males during pre-mating period. A significant ($p < 0.01$) decrease was noted in mean body wt of Hdf during pre-mating treatment days 8 and 15 (4% of the control) and the mean wt gain between treatment days 1-15 was also reduced dose-dependently ($p < 0.05$ only in Hdf, at 6, 7, and 29% in LD, MD, and HD respectively). Mean wt in Hdf was also reduced during gestation ($p < 0.001$; at 10% from the control on gd20 and 9% on gds12, 15, and 18). Also, during gestation, mean wt of Mdf was significantly reduced on gds12, 18, and 20 ($p < 0.05$ and 0.01; 4-5%). The mean wt gain between gds1 and 20 was reduced dose-dependently with statistical significance at MD (10%, $p < 0.05$) and HD (19%; $p < 0.001$). During lactation mean wt was also reduced in HD with statistical significance during lactation days 1, 4, and 21 ($p < 0.05$ & 0.01; 8-10% of the control). Changes in food intake in females correlated with the changes in body wt. A significant reduction in mean food in Hdf noted at: pre-mating wks1 and 2 at 6-8% of the control ($p < 0.05$ & 0.001), throughout gestation ($p < 0.05$ and 0.001) at 6-21%, and lactation days 7, 8, 10, and 12 at 13-18% ($p < 0.05$ & 0.01). Food was also reduced on other lactation days but did not reach statistical significance (upto 19%).

Mating and Fertility: a significant prolongation was noted in mating interval of HD compared with that in the control (mean $\pm$ SD in days: 7 $\pm$ 6 in HD vs. 3 $\pm$ 3 in control). Fertility index was reduced in males and females of HD though did not reach statistical significance (66 and 71% vs. 89 and 90% in male and female of the control respectively). No effect on mating index. The percent of females that got pregnant and those reaching term with viable fetuses/pups was less (20% in Hdf vs. the control but did not reach statistical significance. Four non-pregnant Hdf reached term without evidence of pregnancy vs. 1 in control. Necropsy of the 2Hdf that were euthanized due to lack of or insufficient milk, showed absence of developed mammary tissue, a finding considered to be drug-related. No drug-related macroscopical findings were observed in dams at terminal sacrifice. In F0 males, there was no drug-related macroscopical findings except for a smaller than normal size testes and prostate; a finding common in all groups including the control.

There was no difference in no. of corpora lutea or implantation sites. A slight and non-significant reduction in viable fetuses noted in HD (mean $\pm$ SD 14 $\pm$ 5 vs. 17 $\pm$ 3 in control). A slight non-significant increase was noted in pre-implantation loss of HD (12 $\pm$ 23% vs. 4 $\pm$ 7 in control), and post-implantation loss of MD (12 $\pm$ 8%) and HD (13 $\pm$ 16%) vs. 6 $\pm$ 9% in control. Also, the number of viable fetuses/litter size was less in HD than the number in the control (153 vs. 214 respectively). No drug effect on fetal wt or sex ratio. Gross examination of the viscera showed some findings in control and treated rats

that were slightly higher than the incidences reported in historical data.

There were 4 HDf that did not litter, one had total litter resorption, 2f showed translucent fluid in uterus, and 1f had mottled lungs. These findings did not seem to be drug related.

F1

Fetuses of treated F0 rats had unossified or incomplete ossification of the nose, thoracic vertebrae, sternbrae, and distal phalanx of forepaws and hindpaws. Some of these findings were also seen in the controls and frequently, the incidences were higher than that reported in the historical data. Association of these abnormalities to a drug effect is unclear. There were no malformed fetuses or pups. As indicated above, litter number at birth was reduced in HD (9 vs. 13 in control) as well as mean litter size (14±4 vs. 17±2 in control). Total number of dead pups on ppd1 was increased in HD (mean 14±4) vs. that in the control (1±1). No drug effect on fetal wt or sex ratio. Male survival on ppd4 also showed reduced litter no. in HD (7 vs. 13 in control), reduced viability index in HD (66% vs. 99%; $p < 0.001$) and viability index was still reduced on ppds 7 and 21 in HD compared with the control and other drug groups (92% vs. 98-100%). Similarly, female viability index was reduced ($p < 0.01$ or 0.001) on ppds 4, 7, and 21 (80-83% compared with the control (99-100%), as well as the number of litters on ppd4 was lower in HD (7) vs. the control (13).

Mortality/pre-weaning/males: 4 in control (from 3F0 dams), 2LD (from 2F0 dams), 5MD (from 4F0 dams), 28HD (from 6F0 dams). These pups died between ppds 0-10 and some were stillborn or found dead. Necropsy did not show any abnormalities, mainly organ autolysis, and in some MD and HD pups there was little or no milk in stomach. Pre-weaning/females: 5 in control (from 3F0 dams), 3LD (from 2F0 dams), 9MD (from 5F0 dams), 24HD (from 6F0 dams). Pups were found dead or stillborn but no abnormal necropsy findings mainly organ autolysis and in HD, many pups had no milk in stomach. One LD pup was euthanized due to presence of hematoma in head and thorax.

Body wt and Food intake: a significant reduction in mean wt occurred in HD males and females during pre-weaning period (14-25%; $p < 0.01/0.05$) on ppds 4, 7, 14, and 21 compared with the control. Mean wt gain was also reduced in HD (14-19%; $p < 0.01/0.05$). Similarly, mean wt was significantly reduced in HD males and females during ppds 28-77 (9-19%; $p < 0.05-0.001$; except towards the latter period it did not reach significance). Mean wt gain was reduced in HD males and females but reached statistical significance only in HDm during ppds 21-49 (14%); wt gain was also reduced in HDf during ppds 29-77 at 16% of the control but did not reach significant level. During gestation there was no significant loss in wt gain of any drug group compared with the control however, mean wt of HD was significantly reduced throughout gestation at 9-12% of the control ($p < 0.05$). During lactation days 1, 4, and 7, mean wt was reduced in HD dams, 9-10%, but reaching statistical significance only on ppd4 ($p < 0.05$).

There were some effects on the physical, functional, or behavioral performance of F1 pups. A very small delay was noted in time of onset and completion of pinna detachment, incisor eruption, and eye opening of HD male and female; these findings may have been contributed to the reduced body wt in this group of animals. Slight reduction in functional development reflected by the small reduction in the forelimb grip strength of HD rats which again may have been related to the reduced wt in this group. Behavioral performance was affected in HD as indicated by the small increase in the mean time recorded in the homing on a level surface test on ppd21 as compared with that of the control. The number of rats to successfully complete the water maze test for the learning parameter was slightly lower in LDm pups on ppd42 compared with the control (67% vs. 92% in controls), and females of HD showed slightly lower mean success rate on the 5th and 6th trial compared with the corresponding control scores (6±2 each trial vs. 11 in the control). The memory parameter of the water maze test on the other hand, was not affected in males but

slightly reduced in Hdf pups on the 3rd, 4th, and 6th trials compared with the control. There were no other findings in any drug group on any test.

F2

No adverse findings on any parameter.

Summary and Conclusion:

Oral administration of riluzole at 15mg/kg caused maternal toxicity including deaths in 8/30 males and 2/30 females, also clinical signs such as tremors, hypoactivity, postdose salivation, and muscle weakness predominantly in males. Mean food intake was significantly reduced during drug treatment mainly in females (pre-mating, gestation, and lactation) which might have caused the observed significant decrease in mean wt and wt gain in these animals. Fertility and reproductive parameters were also affected in this group as evidenced by prolongation of pre-mating interval, decrease in fertility index of males and females, decrease in the number of pregnant rats and dams that reached term with viable pups/fetuses. Litter size and number of implantations were reduced. Fetal viability was reduced and slight increase in pre- and post-implantation loss was also found. Four Hdf vs. 1 control reached term but were non-pregnant. The 2Hdf that were euthanized had no mammary tissue, a finding that was related to drug treatment. Postnatal development was affected perhaps due to the continued reduction in body wt of the dams during lactation. The reduced fetal development was minimal and fertility and other reproductive parameters of F1 generation were unaffected by drug treatment.

At 8mg/kg low incidence of clinical signs were also seen at this dose predominantly in males. Mean wt and wt gain and food intake were reduced during pre-mating, and gestation but not during lactation. However, this dose had no effect on fertility or development of F0 and F1 rats.

At the LD of 3mg/kg there was a non-significant decrease in pre-mating wt gain and food intake. However, there was no effect on any other fertility or reproductive parameters of F0, F1, or F2 generations.

There were no malformations of any sort and minimal macroscopic findings some of which were present in all groups including the controls but the incidences were higher than the historical data.

It is concluded that a dose of 3mg/kg of riluzole in the rat is the NOEL for reproductive and developmental performance and the 8mg/kg is the LOEL due to reduction in wt, food intake, and presence of mild clinical signs.

2. Range-finding in mature male and female rats and their offspring reared to weaning. /Nov 1990/England.

Batch# C88-085-01; purity 100.2%.

This was a preliminary study and was not conducted under GLP.

Doses were selected based on a 26-wk toxicity study in rats (RNP# 302-90178).

Male and female Sprague-Dawley rats (10/sex/dose) were treated with riluzole by oral gavage at 2.5, 5, and 10mg/kg. Males were dosed for 4wks and females for 2wks pre-mating, during mating, gestation, and through ppd21. The drug was suspended in 0.5% methocel (hydroxypropyl methylcellulose) and the control group received the vehicle. Animals were paired for 20days and vaginal smears were taken for 2wks prior to mating and during the 20day mating period. Collecting this information allows detection of any anomalies in the estrous cycle, determine the median pre-coital time, and whether or not pregnancy was interrupted after mating. Animals both males and females, were evaluated for clinical signs, mortality, body wt, and food intake. Fertility and reproductive parameters were evaluated for females as well as all standard

litter data. After ppd21, pups were killed and examined externally and internally for abnormalities. The females were mated for a 2nd time and allowed to litter and killed after weaning the pups. These dams were also examined externally and internally for any abnormalities. Uteri from non-pregnant females were fixed in ammonium sulfide to evaluate implantation sites and the following tissues were preserved: pituitary, ovaries, uterus, & vagina.

Results:

Clinical Signs and Mortality: post-salivation occurred in MD and HD males, in HDf, and in some HD rats, it was brown stained. No other drug related signs were noted. No deaths occurred in any drug treated group, one control female was sacrificed for humane reasons on wk19 during the 2nd littering due to hindlimb paralysis.

Body wt and Food intake: there was no drug effect on food intake of any group. Mean body wt gain in MD and HDm was significantly less than that of the control between wks0-23 (77&85% of control). Mean wt in females during 1st mate was not affected but wt gain was reduced in HDf during gestation and ppds 7 and 14 but not ppd21 (table below). During the 2nd mate, mean wt gain in HDf was similar to the control during gds7-14 but less on gd20 and ppds7 and 14 with actual loss of wt on ppd21.

Mean wt change in females in gms:

first mate:

Dose gr	gds			ppds		
	7	14	20	7	14	21
cont	29	62	124	22	37	12
HDf	18	50	112	15	24	18
% change	38	19	10	32	35	+33

2nd mate:

cont	-	-	105	16	19	5
HDf	-	-	90	9	15	-7
% change	-	-	13	44	21	-71

It is noted that the number of pregnant females in HD was reduced to 6/10 (60%) vs. 9/10 in control at 1st mate and to 5-4/10 (40%) vs. 7-6/10 in control at 2nd mate which according to the sponsor, contributed to the difference in wt gain in HDf. The pre-mating period was prolonged in HDf at 1st mate from 2days in the control to 9days in HDf however, there was no difference at 2nd mate, in fact, pre-mating period was superior in HDf (1day) over that in the control at 4days. One HDf in the 1st mate and 2HDf in the 2nd mate did not produce a litter and there was no indication of mating; this finding could not be attributed to a specific male. As indicated above, the mating performance was tested by the outcome of 2 pairings at 10mg/kg to establish a drug effect on the male or the female. The table below from the sponsor shows that 4 out of 10males had failed at the 2 matings, to induce pregnancy and a 5th male did not induce pregnancy at the 2nd mate.

Female number	First mate		Second mate		Male number	Result of	
	Male partner	Outcome	Male partner	Outcome		First mate	Second mate
71	31	P	32	NP	31	P	P
72	32	NP	31	P	32	NP	NP
73	33	P	34	NP	33	P	P
74	34	NP	33	P	34	NP	NP
75	35	NP	36	P	35	NP	NP
76	36	P	35	NP	36	P	P
77	37	NP	38	P	37	NP	NP
78	38	P	37	NP	38	P	P
79	39	P	40	P	39	P	NP
80	40	P	39	NP	40	P	P

NP Non-pregnant/failed to induce pregnancy
P Pregnant/inducing pregnancy

Comparison of mating performance for individual animals at 10 mg/kg/day between the two matings indicated that treatment at this dosage may have had an adverse effect on reproductive capacity in a number of males. Male nos. 32, 34, 35 and 37 failed to induce pregnancy at both pairings, and no. 39 failed to induce pregnancy at the second mating. There was no clear evidence of any treatment-related effect on the occurrence of mating, therefore it is possible that the lower pregnancy rate at 10 mg/kg/day arose as a result of effects on sperm quality and/or quantity associated with apparent testicular atrophy in some of the affected males.

A drug-related effect on mating performance/occurrence was not evident therefore, the decline in pregnancy rate in HD may be a drug effect on sperm quality and/or number. Testicular atrophy was found upon macroscopical exam of some of these rats. Testes in all groups with highest incidence in HD males, were small, blue, and flaccid and the wt of testis/epididymis was reduced compared with the controls. There was however, no effect on wt of pituitary or seminal vesicles/prostate.

There was no effect on gestation period at either mate. Litter data at 1st mate, showed slightly higher percent of pup loss in LD at birth (2.8%) and cumulative loss (%) at ppd4 (14.6%), ppd8 (15.6%), ppd12 (17.6%), and ppd21 (17.6%) vs. the control (0.6%±1.8%). Mean litter wt of LD was reduced throughout these periods. Cumulative pup loss was also high in HD during the above periods but not as high as that found in LD. At 2nd mate, LD showed a higher than the control pup loss (%) only during ppd8, 12, and 21 at 10.2% vs 3 and 3.9% in the control. However, mean pup wt was not affected. Cumulative pup loss was also increased in MD relative to the control in 2nd mate. HD litter wt at the 2nd mate was reduced at birth and ppd4-21 (8-17% of the control). Litter size of HD was also reduced as of ppd4 relative to the control. These findings were not dose-dependent and there were no difference on sex ratio nevertheless, they could well be drug related.

It is concluded that oral administration of riluzole at 10mg/kg is toxic to pregnant female rats causing post-salivation (also at 5mg/kg), and a decrease in wt gain during pre-mating, gestation, and lactation. At this dose riluzole

affected male fertility as evidenced by 50% reduction in number of males capable of inducing pregnancy in females and testicular atrophy. Pre-mating period was prolonged in HDf at 1st mate though was not different from that of the control during the 2nd mate. There seem to be a drug effect on pup survivability with highest percent loss in LD (2.5mg/kg) and mean litter wt. Cumulative pup loss was also high in MD and HD particularly during the 2nd mate. The NOEL in this study is <2.5mg/kg due to the findings on litter wt and pup survivability. The LOEL is 5mg/kg.

3. Oral gavage range finding in pregnant New-Zealand white rabbit (final report)/
/Sep 1983/England.

Batch# 4PC-164

This study was conducted under GLP.

Sexually mature female white rabbits (3-3.5Kg) were mated with one male rabbit and those females that mated successfully were given an i.v. of CGS to ensure ovulation. The day of mating was considered gd0. These pregnant rabbits (5/dose) were dosed with riluzole by oral gavage at 3, 10, or 30mg/kg from gd6-18 inclusive. The animals were maintained till gd28 then killed and examined. The drug was suspended in 1% methylcellulose and the control group was administered the vehicle following the same protocol. The following parameters were evaluated: clinical signs, mortality, body wt, and food intake. The ovaries and uteri were examined for: # of corpora lutea, implantation sites, live/dead fetuses, early and late resorptions (pre- and post-implantation loss/intrauterine deaths), fetal wt and sex, fetal crown/rump length, and any external macroscopical abnormalities.

Results:

There were no drug related deaths in any group except for 1HD and 1MD pregnant dams that were killed on gds9&8 due to respiratory distress. The MDf on gd8 was very lethargic, had scab-like yellow encrustation around and up the nostrils, and respiratory distress; necropsy showed pale liver and red patches on the lungs. The HDf on gd9 had nasal exudate, pale eyes, and respiratory distress; necropsy showed red congested lungs. Another HDf lost limb coordination postdosing for 1.5hr on gd13 and was lethargic on postdose days 16&17; it delivered normally on gd28. There was no drug effect on mean wt or food intake. All females showed 100% pregnancy rate. A small decrease was noted in no. of corpora lutea and implantation sites of treated females relative to the control:

	cont	LD	MD	HD
# corpora lutea	50	43	39	32
mean# per doe	10	8.6	9.8	8.0
# implant. sites	50	32	38	32
mean# per doe	10	6.4	9.5	8.0

The % of pre-implantation loss was high in LD (mean 25.6%) compared with the control that had none; HD had 0 loss and MD had 2.6% loss. There were no fetal abnormalities in any parameter measured.

It is concluded that riluzole orally administered to pregnant rabbits during organogenesis was nontoxic to dams or fetuses at doses upto 30mg/kg/day. The mild findings above were scattered, inconsistent, and non-dose dependent. The NOEL is therefore, 30mg/kg for maternal and fetal parameters.

4. Oral gavage range-finding study in pregnant New-Zealand white rabbit (final report), /Sep 1983/England.
Batch# 4PC-164.

This study was conducted under GLP.

This 2nd dose-range finding was conducted because the 1st (# 3400-81-37 above) did not show any reproductive effects at dose upto 30mg/kg.

The protocol for this study was similar to that in study# HLE 3400-81-37 above. Pregnant rabbits (4/dose) were administered riluzole by oral gavage at 45 or 60mg/kg during gds6-18 inclusive and killed on gd28. The drug was suspended in 1% methylcellulose. It is noted that there was no control group (vehicle-only) in this study therefore, comparison of results of drug treated groups could not be made. Lack of a control group invalidated the study.

Results:

There were no deaths in any group. Clinical signs in both groups included: lethargy, prostration, and hunched posture between 3-6hr postdose, also ataxia and limb incoordination. Two out of 4LD presented these signs during gds15-18, and all 4 dams in HD had these signs during gds5/6-18. All 4LD dams were pregnant by gd28 but only half of the HD were pregnant; no abnormalities were observed at necropsy. Evaluation of results of body wt changes and effect on food intake could not be conducted without a control group. The sponsor presented background historical data from Hazleton on some reproductive parameters. There was no drug effect on no. of corpora lutea, implantation sites, pre-implantation loss, or early uterine deaths. The mean no. of late uterine deaths per doe was high in LD (1.3) vs. the mean in historical data (0.6), also in this dose, % of post-implantation loss was high (13.2) relative to the control (10.6). No other drug effects were found on the dams or fetuses.

It may be concluded based on clinical signs in dams, that 60mg/kg dose could be the HD for the Segment II/teratology study of riluzole in rabbits.

Mutagenicity:

1. In vitro gene mutation of riluzole (RP 54274) in an E.coli strain,
/Nov 1993/France.

Batch# 8PROC92312.

This study was very well done and followed the European guidelines for GLP (OECD).

The E.coli strain WP2 uvrA was used in presence and absence of S9. This strain carries a mutation that affects one of the genes responsible for the synthesis of the AA tryptophane (Type E gene) in addition, mutation at the uvrA gene reduces the DNA excision-repair capacity. The negative control was DMSO, the vehicle for the test material and the positive control in presence of S9 was N-ethyl-N-nitro-N-nitrosoguanidine and in absence of S9 it was 2-anthramine. The plate incorporation assay was followed. A preliminary cytotoxicity assay was done with test material concentrations of 0, 1, 5, 10, 50, 100, 500, and 1000ug/plate (the 1000ug/plate was the solubility limit) on a 1/10⁶ dilution of E.coli strain; 3 plates per dose was used each +/- S9. The results were compared with those from the -ve control plates. At 500ug/plate there was 33 and 23% survival rate in + and - of S9 which is the acceptable value. Based on the preliminary assay, the concentrations of riluzole used in the mutagenicity assay (2 sets were done), were 31.3, 62.5, 125, 250, and 500ug/plate. Below are the assay acceptance criteria and the mutagenicity criteria excerpted from the sponsor:

2.8.1 Assay acceptance criteria

An assay is only considered valid if:

- the genetic characteristic of the strain showed that it possesses the *uvrA* mutation (sensitivity to ultra-violet radiation).
- the S9 mix and the highest dose level of treatment solution were proven sterile.
- the number of spontaneous revertants for each plate of negative controls remained within the range of our historical data calculated between January 16, 1990 and May 19, 1992 (see Table 6).
- the number of induced revertants for each plate of positive controls were above the lower limit of the range of our historical data calculated between January 16, 1990 and May 19, 1992 (see Table 6).

2.8.2 Mutagenicity assessment criteria

A test article is found to be:

- non mutagenic if, in the 2 independent assays, the value of the ratio "R" is less than 2,
- mutagenic if, in the 2 independent assays, the ratio "R" is greater than 2 (for at least one dose level) and demonstrates an effect-dose level relationship,
- doubtful in other circumstances. In this case, a third assay may be performed.

145

135

The results showed cytotoxicity at the 250 and 500ug/plate in presence or absence of S9 and there was no increase in the number of revertants at any concentration. A clear response was noted in the positive control plates validating the assay.

It is concluded that riluzole under the present experimental conditions and mutagenicity assessment criteria, is not mutagenic in E.coli uvrA strain in presence or absence of metabolic activation.

2. In vitro gene mutation test of RP 65176 on *S. typhimurium* and *E.coli* strain. 'Sep 1993/France.

Batch# CSG82.

This study was very well done and followed the European guidelines for GLP (OECD).

The following 5 bacterial strains were tested in presence or absence of S9: TA1535, TA1537, TA1538, TA98, and TA100 and the *E.coli* strain WP2 uvrA. The negative control was DMSO, the vehicle for RP 65176. The positive control in presence of S9 was 2-anthramine for both *Salmonella* and *E.coli* and in absence of S9 they were: sodium azide for TA1535 and TA100, 9-aminoacridine for TA 1537, 2-nitrofluorene for TA1538 and TA98, and N-ethyl-N-nitro-N-nitrosoguanidine for *E.coli*. The plate incorporation assay was followed. A preliminary cytotoxicity assay was done with test material concentrations of 0,1,5,10,50,100,500, 1000, 2500, and 5000ug/plate on a 1/10⁶ dilution of *Salmonella* strain TA100 and *E.coli* strain; 3plates per dose was used each +/- S9. The results were compared with those from the -ve control plates. Precipitate of the compound was seen in all plates of both strains at ≥ 1000 ug/plate and in soft agar tube at concentrations ≥ 500 ug/plate therefore, the highest concentration in the mutagenicity assays was 400ug/plate. Also, some cytotoxicity was observed in TA100 in absence of S9 at 100ug/plate (59% survival) and only very slight cytotoxicity was observed in +S9 of *E.coli* WP2 uvr A strain at >2500 ug/plate. Two sets of mutagenicity assay were done using the same concentrations in both assays. Based on the preliminary assay, the concentrations of RP 65176 used in the mutagenicity assay (2 sets were done), were 25, 50, 100, 200, and 400ug/plate in presence or absence of S9. Below are the assay acceptance criteria and the mutagenicity criteria excerpted from the sponsor:

2.8.1. Assay acceptance criteria

An assay is only considered valid if:

- the genetic characteristics of the strain showed that they possess the uvr B or uvr A mutation (sensitivity to ultra-violet radiation), the *rfa* mutation (cell wall deficiency) for *Salmonella typhimurium* strains and ampicillin resistance (strains TA98 and TA100) or sensitivity (strains TA1535, TA1537, TA1538 and WP2 uvr A).
- the S9 mix and the highest dose level of treatment solution were proven sterile.
- the number of spontaneous revertants for each plate of negative controls remained within the range of our historical data calculated between January 16, 1990 and May 19, 1992 for the *Salmonella typhimurium*, and between June 14, 1991 and May 19, 1992 for the *Escherichia coli* (see Tables 9 and 10).
- the number of induced revertants for each plate of positive controls were above the lower limit of the range of our historical data calculated between January 16, 1990 and May 19, 1992 for the *Salmonella typhimurium*, and between June 14, 1991 and May 19, 1992 for the *Escherichia coli* (see Tables 9 and 10).

2.8.2. Mutagenicity assessment criteria

A test article is found to be:

- non mutagenic if, in the 2 independent assays, the value of the ratio "R" is less than 2,
- mutagenic if, in the 2 independent assays, the ratio "R" is greater than 2 (for at least one dose level) and demonstrates an effect-dose level relationship,
- doubtful in other circumstances. In this case, a third assay may be performed.

The results in both mutagenicity assays showed cytotoxicity in almost all strains at 400ug/plate in absence of S9 and at 200ug/plate only in TA100. In presence of S9, cytotoxicity was seen only at 400ug/plate on all strains except TA98. There was no increase in the number of revertants at any concentration. A clear response was noted in the positive control plates validating the assay.

It is concluded that RP 65176 under the present experimental conditions and mutagenicity assessment criteria, is not mutagenic in any of the 5 bacterial strains of Salmonella or the E.coli WP2 uvr A strain in presence or absence of metabolic activation.

3. In vitro calstogenicity of PK 26124 (RP 54274/Riluzole) in cultured human peripheral lymphocyte. /Mar 1985/England.
Lot# C1100/Conducted under GLP

This study was conducted to re-examine the potential of riluzole to induce chromosomal aberrations noted in a previously conducted assay (LSR# 84-PHA128-257). Riluzole at 10 and 20ug/ml, similar to the previous assay, was dissolved in ethanol which was used as the negative control and tested in the presence or absence of S9. The lymphocytes were obtained from healthy non-smoking male volunteers. The positive control was cyclophosphamide both in presence and absence of S9; note that this compound is mutagenic only upon metabolic activation. The cells were cultured, harvested, and slides prepared and fixed according to the standard guidelines. Two slides from each culture were randomly assigned, coded, and scored; 100 metaphases were scored for chromosome #, chromosome aberrations, and type of aberrations. It is noted that a minimum of 200 metaphases per concentration and control are recommended by OECD to be analyzed and less is acceptable only if there are many aberrations. Cytotoxicity as reflected by the mitotic index (MI), was assessed from about 1000 cells as percent of cells in metaphases. Statistics were done using Fisher's Exact test. At 10ug/ml in absence of S9, mitotic index from each of the 3 cultures as well as the mean value was significantly reduced relative to the corresponding control value; no difference was found in presence of S9. At 20ug/ml both in -/+ S9, MI from each of the cultures as well as the mean value, was reduced relative to the control value (see table from the sponsor). At 10ug/ml, the % of cells with aberrations in presence of S9 in each culture plate was higher than that in the control (3,4,2 vs. 1,1,2 for drug and control cultures respectively), but did not show statistical difference. There was no difference in % of cells with aberrations excluding gaps between treated and control. One culture of 10ug/ml had a single endoreduplication that was absent from any group of the negative and positive controls. In all of the tests, CP produced a positive response specially in presence of S9. The sponsor pooled the data from the 10ug/ml obtained in this study with those at the same concentration from the previous study mentioned above (LSR# 84-PHA128-257), in order to compare large populations. The sponsor also pooled the data obtained in -/+ S9 when there was no significant difference between these cultures; the data from CP were however, analyzed separately. The pooled data for the 10ug/ml concentration showed a significant difference (increased frequency) in cells with chromosomal aberrations in presence of S9 and those of the control only when gaps were included.

It is concluded that riluzole in this assay and under these experimental conditions was cytotoxic at 10ug/ml (only in absence of S9) and 20ug/ml both in -/+ S9. There was a non-significant increase in percent of cells with aberrations in presence of S9 at 10ug/ml concentration. There was no significant increase in chromosomal aberrations in any treated group over the control. Based on these results, riluzole is not mutagenic.

Comment:

The sponsor in this and previous mutagenicity assay (LSR# 84-PHA128-257) pooled the data obtained in presence and absence of S9. This is an unacceptable scientifically, also though not essential, a positive control that does not require metabolic activation as CP should have been used.

IND R14302 Rhone-Poulenc Rome fcsR#85-PHA137-159

Human lymphocyte chrom. abs.

TABLE 1

Mitotic indices

Group	1	2	3	4
Compound	Ethanol	PK26124	-----	Cyclophosphamide
Concentration (µg/ml)	-	10	20	6

Group	1			2			3											
Culture number	1	2	3	19	20	21	10	11	12	22	23	24	13	14	15	25	26	27
S-9 mix	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
Lymphocytes	1093	1004	1026	1067	1032	1016	1043	1012	1019	1067	1041	1043	1025	1016	1054	1026	1004	1024
Metaphases	46	92	60	34	50	40	38	36	46	51	47	82	24	63	16	33	20	18
Mitotic index*	4.2	9.2	5.8	3.2	4.8	3.9	3.6	3.6	4.5	4.8	4.5	7.9	2.3	6.2	1.5	3.2	2.0	1.6
Mean mitotic index	6.4	4.0			(3.9)			5.7			(3.3)			(2.3)				
Group mean mitotic index	5.2			4.8			(2.8)											

* Mitotic index = $\frac{\text{number of metaphases}}{\text{number of lymphocytes}} \times 100$

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Human lymphocyte Chron. Abs

LSR # 85 - PHA 137-159
 APP -
 R: 1432x2

TABLE 1 - continued

Mitotic indices

Group	:	1	2	3	4
Compound	:	Ethanol	----- PK26124	-----	Cyclophosphamide
Concentration (µg/ml)	:	-	10	20	6

Group	4					
Culture number	16	17	18	28	29	30
S-9 mix	-	-	-	+	+	+
Lymphocytes	1031	1059	1032	1015	1007	1054
Metaphases	54	61	89	17	23	37
Mitotic index*	5.2	5.8	8.6	1.7	2.3	3.5
Mean mitotic index	6.5					
Group mean mitotic index	4.5					

* Mitotic index = $\frac{\text{number of metaphases}}{\text{number of lymphocytes}} \times 100$

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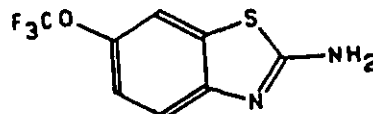
APPENDIX II

Pharmacologist Review of IND
Original Summary

Submission Date: 5/4/93
Stamp Date: 5/3/93
Review Date: 9/25/93
Safety Review Date: 6/2/93
Reviewer: Aisar Atrakchi

Sponsor: Rhone-Poulenc Rorer
500 Arcola Rd.
P.O. Box 1200
Collegeville, PA 19426-0107

Drug: Riluzole/RP 54274
Chemical Name: 2-amino-6-trifluoromethoxybenzothiazole
Molecular Weight: 234.2
Drug Category: Excitatory Amino Acid Inhibitor/Glutamate Antagonist
Indication: Amyotrophic Lateral Sclerosis (ALS)
Related IND/NDAs: None
Structure:



Molecular Formula: C₉H₇F₃N₂OS

Proposed Clinical Use:

Riluzole has multi-clinical indications, it has been studied in man since 1984 with indications in schizophrenia, depression, Parkinson's, anticonvulsion, as well as ALS. Clinical trials have been conducted in healthy volunteers and patients. This IND is submitted based on the beneficial findings in ALS patients. The proposed clinical trial is a multinational, double-blind, placebo-controlled, parallel-group, dose-ranging to investigate the efficacy and safety of Riluzole in ALS patients. A segment of this study will be conducted in the U.S.A with completion data by mid 1995. The drug will be administered orally as film-coated tablets with the following strengths: 12.5, 25, 50, and 100 mg; dose not to exceed 200 mg/day.

Pre-Clinical Pharmacology - Toxicology Studies Submitted by the Sponsor:

- I. Pharmacodynamics:
 - Neuropharmacology: anticonvulsant, anxiolytic, neuroleptic, antidepressant, sedative/muscle relaxant, anti-ischemic, psychostimulant, and spasmolytic effects.
 - Neurobiochemistry: radiobinding studies.
 - Other Effects
- II. Special Studies: sleep-wakefulness, cerebral glucose utilization, and drug interactions.
- III. Pharmacokinetics/ADME
- IV. Acute Toxicity Studies
- V. Subchronic Toxicity Studies
- VI. Reproductive: Segments I and II
- VII. Mutagenicity

The following pre-clinical studies have not been conducted:

- I. 1-year non-rodent
- II. Carcinogenicity
- III. Reproductive - Developmental Studies Segment III

MECHANISM OF ACTION:

Riluzole acts through a number of sites/mechanisms:

1. blockade of glutamate-induced excitatory neurotransmission affecting both the basal and evoked release (presynaptic), and postsynaptically by non-competitive binding to the receptors.
2. blockade of calcium mobilization in primary rodent neurons in culture.
3. inactivation of voltage-dependent Na-channels.
4. stimulation of G-protein dependent signal transduction.
5. blockade of excitatory amino acid(EAA)-induced convulsions (see anticonvulsant effects).

These effects are discussed below in more detail.

Riluzole inhibited ^3H -dopamine release induced by stimulation of glutaminergic thalamo-cortico-striatal loop in the cat caudate nucleus and it antagonized TTX-resistant stimulatory effect of glutamate or kainate on the release of newly synthesized ^3H -dopamine (Romo et al., 1986; Cheramy et al., 1986; Barbeito et al., 1990). In another study, Riluzole at 10^{-6}M applied locally to the caudate nucleus of the cat, significantly reduced (-57%) the spontaneous release of glutamate without affecting the release of aspartate or other excitatory neurotransmitters indicating the selectivity and specificity of this compound (Cheramy et al., 1992). Riluzole selectively blocked the NMDA-induced release of acetylcholine in rat striatum and olfactory tubercles with IC_{50} of 14 and $29\mu\text{M}$ respectively (Benavides et al., 1985). Riluzole antagonized the increase in cGMP induced by glutamate and quisqualate but not that of NMDA, kainate, cysteine sulphinate, and aspartate (Table 1). In *vitro* however, it antagonized the increase in cGMP induced by all these excitatory compounds (Table 2) (Benavides et al., 1985). These results are in contrast to those from binding studies (see below). Riluzole caused only weak antagonism of glutamate receptors as $100\mu\text{M}$ displaced only 30% of ^3H -glutamate receptors and at this concentration, Riluzole was inactive in displacing ^3H -L-cysteine sulphinate, a glutamate-like compound. Riluzole did not show affinity to GABA-(labelled with ^3H -muscimol), benzodiazepine- (^3H -flunitrazepam) receptor complex, or GABA_A binding. Riluzole at $100\mu\text{M}$ did not displace ^3H -apomorphine or ^3H -haloperidol, or glycine receptors labelled with ^3H -strychnine (Benavides et al., 1985). Therefore, binding studies on the NMDA receptor indicate that Riluzole does not bind to ion channel site, or glycine site, or the NMDA site. This discrepancy between binding studies and neuropharmacology is due to differences in excitatory amino acid receptors in cerebellar slices between adult and immature rats used in such experiments (Garthwaite 1982).

Pharmacology and binding studies of Riluzole were conducted in *Xenopus* oocytes injected with mRNA from rat whole brain or cortex (Benavides et al., 1985). Riluzole blocked responses to kainic acid with $\text{IC}_{50} = 167\mu\text{M}$ compared with $0.21\mu\text{M}$ for CNQX and $0.043\mu\text{M}$ for NBQX. Riluzole was more potent blocker to NMDA with IC_{50} of $18\mu\text{M}$ compared with $6\mu\text{M}$ for the competitive antagonist 2-APV (α -aminophosphonovaleric acid). Unlike the inhibition induced by MK-801, inhibition by Riluzole and 2-APV is reversible, and not use-dependent. Riluzole was shown not to act as a competitive or non-competitive NMDA antagonist in a number of species (Tsai et al., 1987; Koek and Woods 1988). Electrophysiological studies in contrast to binding studies showed that Riluzole at $50\mu\text{M}$ completely blocked NMDA responses but no inhibitory binding was noted at this concentration. Similarly, in the case of kainate responses, the currents are blocked at $>500\mu\text{M}$ at which no inhibition of binding was observed for up to 1mM . It is concluded that Riluzole has a direct but non-competitive binding to glutamate receptors.

Riluzole inhibited the release of L-glutamate and L-aspartate specifically from CA1 area of the hippocampus (Martin et al., 1991). At 10-100 μ M in presence or absence of Mg⁺⁺, Riluzole inhibited the release of these excitatory amino acids dose-dependently by 40% but only the highest concentration (100 μ M) inhibited GABA release. Riluzole was shown to be a specific blocker of inactivated Na-channels studied by voltage clamp of nodes of Ranvier of isolated nerve fibers of the frog (Benoit and Escande 1991).

To demonstrate the effects of Riluzole as an antagonist of excitatory amino acid neurotransmission, it is compared with known competitive and non-competitive antagonists and allosteric modulators of NMDA-type receptors as indicated in Tables 1&2 below (Benavides et al., 1985).

Table 1. Effect of PK 26124 on levels of cGMP in slices of cerebellum from 8-day-old rats: antagonism of the increase in cGMP elicited by several excitatory amino acid analogues

Drugs	cGMP (pmol/mg protein)		
	-	PK 26124 (25 μ M)	PK 26124 (100 μ M)
Saline	5.4 $\pm$ 2.1	3.2 $\pm$ 1.0	3.7 $\pm$ 1.3
L-Glutamate (1 μ M)	49.9 $\pm$ 5	31.3 $\pm$ 1.6***	18.9 $\pm$ 2.5***
L-Aspartate (1 mM)	41.8 $\pm$ 10.9	39.4 $\pm$ 7	39.4 $\pm$ 1.0
L-Cysteine sulphamate (1 mM)	40.6 $\pm$ 0.4	45.4 $\pm$ 6.2	39.2 $\pm$ 5.1
Quisqualate (0.1 mM)	28.5 $\pm$ 3.9	9.3 $\pm$ 1.3***	6.9 $\pm$ 1.0***
N-Methyl-DL-aspartate (1 mM)	78.2 $\pm$ 16.8	70.2 $\pm$ 11.4	83.6 $\pm$ 12.0
Kainate (1 mM)	35.1 $\pm$ 6.8	29.3 $\pm$ 2.6	28.5 $\pm$ 1.3

Slices of cerebellum were pre-incubated for 3 min with PK 26124 and then the excitatory compounds were added. Levels of cGMP were measured 15 min after this addition. Each group consisted of 5-10 experiments. Effect of PK 26124 was significant at ***P < 0.001.

Table 2. Effect of PK 26124 on levels of cGMP in the cerebellum: antagonism of the increase in cGMP induced by the intracerebroventricular administration of several excitatory amino acid analogues

Drugs	cGMP (pmol/mg protein)			
	-	PK 26124 (mg/kg)		
		0.5	2.5	5
Saline	4.0 $\pm$ 0.9	3.9 $\pm$ 0.7	2.8 $\pm$ 0.3	2.0 $\pm$ 0.4***
L-Glutamate (10 μ mol/kg)	7.7 $\pm$ 2.4	3.1 $\pm$ 1.4	3.3 $\pm$ 1.1**	2.9 $\pm$ 1.4**
L-Aspartate (5 μ mol/kg)	13.9 $\pm$ 2.3	7.7 $\pm$ 0.7**	3.0 $\pm$ 0.3**	4.8 $\pm$ 0.5***
L-Cysteine sulphamate (10 μ mol/kg)	11.5 $\pm$ 2.9	9.4 $\pm$ 2.5	3.7 $\pm$ 1.1***	1.9 $\pm$ 0.1***
Quisqualate (100 μ mol/kg)	9.3 $\pm$ 0.3	6.8 $\pm$ 1.9	3.9 $\pm$ 0.7**	2.7 $\pm$ 0.1***
N-Methyl-DL-aspartate (75 μ mol/kg)	9.4 $\pm$ 1.1	4.9 $\pm$ 0.7**	3.3 $\pm$ 0.6**	4.3 $\pm$ 0.4**
Kainate (3 μ mol/kg)	14.8 $\pm$ 2.7	12.0 $\pm$ 1.1	9.0 $\pm$ 0.8**	6.1 $\pm$ 0.3***

The PK 26124 was administered intraperitoneally 30 min before steroids. Excitatory amino acid analogues were administered by intracerebroventricular route 20 min after PK 26124 in a final volume of 20 μ l. Each group consisted of 3-10 rats. Effect of PK 26124 was significant at *P < 0.05, **P < 0.01, ***P < 0.001.

I. PHARMACODYNAMICS

Neuropharmacology: All studies were conducted under GLP unless indicated otherwise.

A number of articles have been published on the anticonvulsant, anti-anxiety, anti-depressant, and neuroleptic effects of Riluzole and its site(s) of action. Tables extracted from the sponsor's submission are presented with reviewer's comments.

Anticonvulsant Effect:

The attached table presents ED₅₀ of Riluzole with various compounds. From this table, Riluzole unlike benzodiazepines, is effective against convulsions induced by excitatory amino acids (glutamic, aspartic, and homocysteic acids), and maximum electroshock. Riluzole affects various neurotransmitters. However, it is partially active against convulsions induced by GABA antagonists (bicuculline) and synthesis inhibitors (3-mercaptopropionic acid). Riluzole is effective anticonvulsant through the oral and i.p. routes.

Sedative and Muscle Relaxant Activity:

Riluzole is a poor sedative and muscle relaxant in animals as compared with diazepam (Table below). It was more effective than diazepam only in the righting test in mice (26 vs. 72 mg/kg i.p. ED₅₀ respectively).

The anticonvulsant activity of RP 54274 was evaluated in various tests. The results of these tests are presented in table 1.

Table 1: Anticonvulsant profile of RP 54274

	Species	Route	ED50 (mg/kg)		
			Riluzole	Diazepam	Carbamazepine
L-glutamic acid	rat	i.p.	2.2	>20	12
L-aspartic acid	rat	i.p.	2.5	NA 10	ND
Homocysteic acid	rat	i.p.	5	>20	25
Kainic acid	rat	i.p.	NA 10	NA 10	ND
Quabain	rat	i.p.	3.1	8.2	ND
Quaralaine	rat	i.p.	2.5	2.2	ND
β-Mercaptopropionic acid	mouse	i.p.	6.4*	1.25	ND
Isoniazid	rat	i.p.	4	0.3	ND
Maximal electroshock	mouse	i.p.	4	3.8	10
Maximal electroshock	rat	p.o.	1.5	18.1	10
Audiogenic seizures	DBA ₂ mouse	i.p.	1.2	0.4	ND
Pentylentetrazole	mouse	p.o.	8*	0.57	14.1*
Bicuculline	mouse	p.o.	7.5*	0.46	11.9*
Picrotoxin	mouse	p.o.	4*	3.16	1.67*
4-aminopyridine	rat	p.o.	1.79	4.87	18.6

*: partial protection only (against clonic convulsions)

NA: inactive at the indicated dose

ND: not determined

Table 2. : Sedative and muscle relaxant activity of RP 54274

Drug	ED50 (mg/kg)			
	Footshock induced fighting		Rotarod	
	(male mice)		(rat)	(mice)
	p.o.	i.p.	p.o.	i.p.
RP 54274	5.0* (3.2-7.7)** (15 min)***	8.7 (6.8-11.1)	8.1 (4.1-16.1)	11.0 (7.8-15.5)
Diazepam	0.95 (0.6-1.6) (90 min)	0.8 (0.5-1.3)	6.75	1.03 (0.8-1.2)
Chlordiazepoxide	-	-	-	-
MK-801	-	-	-	0.22 (0.14-0.27)

Drug	ED50 (mg/kg)				
	Grasping		Righting		
	(mice)		(mice)	(mice)	(rat)
	p.o.	i.p.	p.o.	i.p.	i.p.
RP 54274	26.5* (20.5-34.0)** (15 min)***	14.7 (9.9-21.9)	44.0 (35.6-54.4) (15 min)	28.0 (2.6-34.7)	19.8 (18.7-23.6) (30 min)
Diazepam	-	5.8 (3.9-7.6)	-	72	-
Chlordiazepoxide	-	16.0 (12.0-21.0)	-	8.3 (8.0-11.5)	-
MK-801	-	-	-	-	-

* : minimal ED50 (when the ED50s were determined at several intervals of time after administration)

** : 95% confidence interval

*** : minimal time of ED50

Neuroleptic Activity:

Inactive when tested i.p. at 4 and 8 mg/kg in the following three neuroleptic tests:

1. inhibition of apomorphine-induced hypermotility in mouse (climbing test).
2. inhibition of amphetamine-induced stereotypy in mouse.
3. inhibition of apomorphine-induced hypothermia in mouse (only a slight decrease in temperature was noted after Riluzole).

Antianxiety Activity:

Riluzole had minimal or no anxiolytic effect in the following three tests:

1. 4-plate test: assessment of exploratory activity in the mouse placed in an anxiogenic condition. Riluzole at oral doses up to 30 mg/kg did not cause a significant increase in exploratory effect compared with the controls, whereas, chlordiazepoxide at 4 mg/kg induced a significant increase.
2. inhibition of PTZ discrimination test in rat: Riluzole at oral doses of 0.5 or 5 mg/kg did not have any effect, whereas zopiclone, a reference drug, had a marked effect at an oral ED₅₀ of 1 mg/kg.
3. water-lick conflict in unconditioned rat: Riluzole showed a small effect at 5 mg/kg oral dose but the response was not dose-dependent and no effect at higher doses. Chlordiazepoxide on the other hand, showed a significant effect at 16 mg/kg oral dose.

Riluzole produced a marked effect in the operant conflict test. Rats are conditioned to obtain water by pressing on a lever and in a subsequent phase they are given an electric shock therefore, creating a conflict. A pro-conflict is seen when there is a reduction in the number of activation of the lever. FG 7142 (8 carboline derivative) is a pro-conflict used as a reference (Table below). Riluzole caused a dose-dependent increase in inhibiting the anxiogenic effect of FG 7142. But Riluzole was not tested alone to evaluate its anxiolytic effect.

Table 4.3: Antagonistic activity of RP 54274 on the angiogenic effect of FG7142 in the operant conflict test in the rat

Drug	Dose (mg/kg p.o.)	Number of presses lever (mean (SEM))		
		Control period	Test period	% variation
FG7142	5	28.4 (2.3)	22.7 (6.3)	-20
	10	31.0 (1.7)	10.7** (3.5)	-65
	20	32.1 (2.5)	6.9** (2.5)	-78
FG7142(10gm/kg) + RP 54274	0	31 (1.7)	10.7** (3.5)	-65
	0.5	29.8 (2.9)	16.8* (6.3)	-44
	2	27.7 (2.9)	19.8 (7.1)	-29
	4	30.2 (3.0)	25.5 (3.0)	-16

Anti-ischemic and Neuroprotective effects:

1. Global cerebral ischemia in gerbils/Report# 104387
2. Focal irreversible ischemia in rats/Pratt et al., 1992.
3. Hypobaric hypoxia in mice.

1. Adult male Mongolian gerbils (4/gr) were implanted with screw electrodes in the right parietal and occipital regions of the cranium. Baseline measurements were made via bipolar recordings of the electrocorticogram (ECoG). Ischemia was induced by bilateral occlusion of the carotid arteries for 6 min. Riluzole was administered i.p. in acidified saline solution at 2, 4, or 8 mg/kg at 0.5, 4, 24, and 28 hr after induction of ischemia. ECoG was monitored before and after ischemia for up to 28 days. Sham operated animals served as the controls. Riluzole at 4 and 8 mg/kg produced neuroprotection compared with the brain damage seen in the untreated ischemic animals. It increased the proportion of higher frequency brain activity in the ECoG in a dose-dependent manner.

2. Male Fischer rats (11-14/gr) were used and the left middle cerebral artery (MCA) was occluded to induce focal cerebral ischemia. The main trunk of the MCA was exposed and observed with microscope under high magnification. Riluzole at 2x8 mg/kg significantly reduced the total volume and size of ischemia-infarcted areas in the cortex (25-28%) but was without an effect on striatal lesions. This is an additional supportive evidence for Riluzole's neuroprotection reported in the gerbils.

Glutamate is involved in neural damage and cell death following ischemia. From the above studies, it seems that Riluzole neuroprotection maybe mediated via indirect interaction with glutaminergic receptors perhaps by blockade of sodium channels (see other studies).

3. Male mice (n=12) were placed in a hypobaric tank simulating hypoxic conditions and mean survival time of these mice with or without Riluzole was determined. Riluzole (i.p.) produced a dose-dependent increase in survival time which was significant starting at 4 mg/kg (ED₅₀).

The neuroprotective properties of Riluzole (4 mg/kg i.p.) following ischemia were evaluated by a passive avoidance test in gerbils. Ischemic gerbils demonstrate a significant decrease in the time required to descend onto an electrified grid reflecting deterioration in memory due to ischemia. Riluzole did not show an effect on this parameter. However, histologically, Riluzole prevented necrosis of area CA1 pyramidal cells of the hippocampus and autoradiographic analysis showed a significant protection against the reduction in the number of muscarinic receptors in the CA1 area found in non-pretreated ischemic gerbils.

At 4 mg/kg, Riluzole showed a significant neuroprotection against cerebral lesions and 8 mg/kg administered at 30 min and 24 hr post occlusion significantly decreased the volume of infarcted cortex. A dose-dependent (up to 8 mg/kg), protection of high frequency component of the ECoG activity and a decrease in the predominance of slow wave was noted during the weeks that followed a 6-min bilateral occlusion of the common carotid in gerbils.

Anti-depressant Effects:

Riluzole did not demonstrate any anti-depressant activity in the mouse (Table below).

Drug	ED50 (mg/kg p.o.)	
	Potentiation of 5-HTP-induced tremor	Antagonist of tetrabenazine-induced ptosis
RP 54274	inactive at 25	inactive at 16
indalpine	18	-
imipramine	-	4.8

Cardiovascular Effects:

The following are cardiovascular effects noted in the rat, dog, and rabbit.

Table 7: Cardiovascular effects of RP 54274 in the rat, the rabbit and the dog

Species		Dose (mg/kg) (route)	Results
Rat	- anaesthetized, normotensive	(n = 5) 0.1 to 30 (i.v.)	Statistically significant decrease in SBP, DBP, MAoP and HR at 3 and 10mg/kg Death by respiratory arrest at 10 (2/5 rats) and 30mg/kg (3 remaining rats)
	- conscious, spontaneous hypertensive	(n = 6) 5 and 10 (p.o.)	Slight decrease (5-7% in MAoP and HR at 5mg/kg only)
Rabbit	anaesthetized	(n = 5) 0.1 to 30 (i.v.)	Significant decrease in SBP (-32%) and increase in carotid blood flow (+52%) at 10mg/kg Death from respiratory arrest at 30mg/kg
Dog	- anaesthetized	(n = 6) 0.3 to 10* (i.v.)	Significant decrease (8-9%) in HR at 2 and 7mg/kg Dose-dependent decrease in cardiac output from 1 mg/kg Decrease in dP/dt max (contractility index) (-34%) at 10mg/kg Dose-dependent increase LVEDP
	- conscious	(n = 5) 1 to 8* (i.v.)	Significant decrease in SBP (11 - 21%) and DBP (30-46%) at 4 and 8mg/kg (duration < 10min at 4mg/kg, < 30min at 8mg/kg)
		(n = 5) 30 (p.o.)	No significant modification in SBP, DBP, MAoP, HR and ECG
	- conscious with an exp. myocardial ischaemia	(n = 4) 3 (i.v.)	No significant modification in MAoP and HR. No antiarrhythmic properties, only a transient (about 5min) return of the sinus rhythm

*: administration of cumulative doses (the highest dose is the total dose)
 SBP: systolic blood pressure HR: heart rate DBP: diastolic blood pressure ECG: electrocardiogram
 MAoP: mean aortic pressure LVEDP: left ventricular and pressure

The data indicate that Riluzole had a significant effect on SBP and DBP in the conscious dog (i.v.), and in the anesthetized dog, it significantly decreased HR, contractility index, and CO but increased LVEDP. It significantly decreased HR and MAOP in conscious SHR (oral). The drug had no significant effect on any parameter in the conscious dog with or without experimental myocardial ischemia after i.v. administration.

Spasmolytic Effects:

Riluzole is not spasmolytic in vitro as noted in the table below.

Contractions induced by	Preparation	Reference product	Results (EC50 [mg/ml])	
			RP 54274	Reference
Acetylcholine	rat duodenum	atropine	7.8	0.014
Barium chloride	rat duodenum	papaverine	4.4	1.8
Histamine	guinea pig ileum	promethazine	> 10	0.003
Serotonin	rat uterus	methysergide	2.1	0.0014

Psychostimulant Amphetamine-like Activity:

Riluzole was in-effective as a psychostimulant in two tests: the decrease in food consumption (method of Le Douarec 1970), and induction of stereotypy tests at doses up to 10 mg/kg i.p. or oral.

Other Effects:

Riluzole significantly decreased urine volume and K excretion at 25 mg/kg oral dose. At 2 and 4 mg/kg it significantly reduced respiratory compliance and increased pulmonary resistance at 4 mg/kg (Table below).

System or type of effect studied	Species studied	Dose (mg/kg) Route	Reference product	Results
Autonomic nervous system	dog	3 i.v.	-	No effect
Respiratory function	dog	1.2 and 4 i.v.	-	Significant reduction in compliance at 2 and 4mg/kg. Increased pulmonary resistance at 4mg/kg
Effect on urine excretion	mouse	10 - 25 p.o.	-	Significant decrease in urine volume and potassium excretion at the highest dose
Local anesthetic effects Conduction anaesthesia - Platysma reflex	guinea pig	i.d.	lidocaine	EC50 RP 54274 = 0.8% EC50 lidocaine = 0.1%
Reaction to a mechanical stimulus	rat	i.m.	lidocaine	EC50 RP 54274 = 0.5% EC50 lidocaine = 0.1
Surface anaesthesia: - Corneal reflex	rabbit	ocular instillation	cocaine chlorhydrate	EC50 RP 54274 = 0.06% EC50 cocaine = 0.15%
Anticholinergic effects (antagonism of the effects of atropine)	mouse	p.o.	trihexyphenidyl	EC50 RP 54274 = 22mg/kg EC50 trihexyphenidyl = 5 to 22mg/kg

EC50 10min after administration

Neurobiochemistry:

Interaction of Riluzole with different receptors was examined by radiobinding assays:

- Adrenergic (α_1 , α_2 , β)
- Dopaminergic (D2)
- Serotonergic (5HT2)
- Glutamatergic (NMDA, AMPA, Kainate)
- Ion channels (calcium and potassium)
- Peptidergic receptors (CGRP, cholecystokinin B, galanin, neuropeptide Y, neurotensin, opiates, somatostatin, substance P, and VIP)

Riluzole did not demonstrate any marked affinity toward these receptor sites or ion channels; the IC_{50} was $>10^4 M$. However, as reported elsewhere, low concentrations ($10^{-4} M$) applied locally to the caudate nucleus of the cat inhibited the spontaneous release of glutamate.

II. SPECIAL STUDIES:

- Sleep-Wakefulness Cycle: Riluzole had no effect on sleep-wakefulness cycle in the rat at oral doses between 0.5-8 mg/kg. However, it significantly reduced the duration of the waking period at 1 mg/kg and significantly reduced the intermediate sleep and the frequency of onset of REM at 2 mg/kg. Also at 2 mg/kg, Riluzole, significantly increased slow wave sleep and REM sleep.

- Cerebral Glucose Utilization: administration of 3 and 9 mg/kg i.v. to the rat, produced a dose-dependent decrease in cerebral glucose consumption in almost all 79 structures analyzed. Analysis was conducted using the autoradiographic 2-deoxy-D-(1- ^{14}C)glucose technique. At 3 mg/kg, 10-25% reductions in glucose utilization was measured in the visual and auditory areas and at 9 mg/kg, this decrease was 30-45%. Alteration in glucose consumption was less marked in brain areas involving extrapyramidal and sensory-motor functions.

Drug Interactions:

Results of interaction of Riluzole with barbiturates (hexobarbital), neuroleptics (chlorpromazine), and ethanol are presented in the table below.

Table 4.10: Studies of pharmacodynamic interactions

Interaction with	Barbiturates (hexobarbital)	Neuroleptics (chlorpromazine)	Ethanol
Test	prolongation of hexobarbital-induced sleep	Loss of righting reflex	Loss of righting reflex
Results with diazepam	ED100 (i.p.) = 1 mg/kg	ED50 (p.o.) = 1.77 mg/kg	ED50 (p.o.) = 1.27 mg/kg (0.29/34)
Results with riluzole	ED100 (p.o.) = 13.5 mg/kg ED100 (i.p.) = 9.5 mg/kg	ED50 (p.o.) = 13 mg/kg	ED50 (p.o.) = 16 mg/kg
Conclusion	Potentiation at high doses	Potentiation at high doses	No interaction

ED100 is the dose required to produce an increase of 100 % of the mean duration of sleep as compared with the control group.

III. PHARMACOKINETICS/ADME:

Rat

1. Toxicokinetics in rats following repeated oral treatment for 3 months

Study #DN103451

GLP: not provided

Species/strain: not provided

Dose/Route/Duration: 2.5, 8, 25 mg/kg oral gavage for 3 months

Drug: RP 54274 in 1% methylcellulose aqueous solution

Batch/Lot#: not reported.

Results:

Blood/plasma collection times: 1 h post dosing on day 1, wks 5 and 13. Plasma drug concentrations were determined by HPLC with UV detector at dl of 0.17 µg/ml.

Dose (mg/kg)	Plasma Concentration (µg/ml)*		
2.5	0.2-0.3	0.2-0.3	0.2-0.5
8	0.2-0.9	0.2-1	0.3-0.8
25	0.5-2	1.3-3.5	1-3.3

* values are mean ranges from males and females (n=8-10/sex/gr.) one hr after dosing.

There was a great inter- animal variability ranging from 5-48% in males and 12-63% in females; there was no sex differences in plasma levels at all doses. Significant differences were noted between the doses and treatment weeks. Results from treatment wk13 differed from those for wks 1 and 5 at 2.5 mg/kg dose and at 25 mg/kg dose treatment wk1 and those from wks 5 and 13. However, no differences were found between treatment wks at 8 mg/kg.

Plasma concentrations varied with dose: linearly at wks 5 and 13 but nonlinearly on day 1. Results of plasma concentration as a function of time varied with increases and/or decreases. It is concluded that there are no sex differences in plasma levels over 3 month's treatment with Riluzole, linear relationship is present at 2-treatment wk5, and it appears that there is no accumulation of the drug.

2. 26-wk toxicokinetics in the rat by oral route following once-daily administration at 5, 10, 20 mg/kg.

Study #DN105368

GLP: not provided

Species/strain: Crl:Sprague-Dawley/5/sex/dose

Dose/Route/Duration: 5, 10, 20 mg/kg oral gavage for 26 wks (6.5 months)

Drug: RP 54274 in 0.5% hydroxypropyl methylcellulose suspension

Batch/Lot#: C8808501

Blood/plasma collection times: 1 and 24 hr post dose on day 1, any one day on wks 13 and 26. Drug levels were determined by HPLC with UV detector and dl of 5 ng/ml.

Three male rats died after wk13, and 5 died between wks 13 and 26; all dead animals were replaced. Two female rats died after wk13 and were replaced. Cause of death was not reported.

26wk PK in rat study # 105368

RESULTS : Results are summarized (mean $\pm$ standard deviation) in the following tables.

RP 54274 1-hour plasma levels (ng/ml)

Dose, mg/kg		5	10	20
Week 1	M	355 $\pm$ 28	574 $\pm$ 50	809 $\pm$ 197
	F	431 $\pm$ 122	496 $\pm$ 78	629 $\pm$ 66
Week 13	M	467 $\pm$ 87	1234 $\pm$ 437	1864 $\pm$ 249
	F	369 $\pm$ 48	846 $\pm$ 169	2158 $\pm$ 320
Week 26	M	673 $\pm$ 101	1321 $\pm$ 209	3009 $\pm$ 690
	F	489 $\pm$ 90	1067 $\pm$ 65	2716 $\pm$ 663

M = male animals

F = Female animals

RP 54274 24-hour plasma levels (ng/ml)

Dose, mg/kg		5	10	20
Week 1	M	ND	75 $\pm$ 6	358 $\pm$ 263
	F	ND	84 $\pm$ 28	399 $\pm$ 152
Week 13	M	173 $\pm$ 48	487 $\pm$ 162	1275 $\pm$ 298
	F	ND	271 $\pm$ 59	1777 $\pm$ 306
Week 26	M	256 $\pm$ 62	637 $\pm$ 143	2027 $\pm$ 446
	F	ND	236 $\pm$ 105	1399 $\pm$ 869

M = male animals

F = Female animals

ND = not detected

Attached Tables present plasma levels as function of time in males and females. The results indicate that there is sex differences with plasma drug levels higher in males than females. Also, drug levels could not be detected 24 hr post dose in LD female group. There seems to be some drug accumulation with time as plasma levels at end of study were higher than those at the start of study. For example drug levels after 1 hr during wk 26 are 2-4 times greater than values measured at beginning of study (wk1) with the highest increase noted in HD males. Plasma levels increased with dose but the relationship was nonlinear except in males at 1 hr postdosing during wks 13 and 26. All other concentration-dose relationships were nonlinear but concentration increased with dose.

3. Autoradiography of tissue distribution of radioactivity in rats following 2 mg/kg oral dose of ¹⁴C-RP54274

Study#DN103132

GLP: not provided

Species/strain: Sprague-Dawley/4/sex

Dose/Route/Duration: 2 mg/kg/oral gavage/single dose

Drug: ¹⁴C-RP 54274 labelled on C2 with specific activity of 52 mCi/mmol and 98.8% purity/prepared in HCl aqueous solution.

Batch/Lot#: PX5551 (C.E.A.383).

Results:

Radioactivity was detected within 30 min in all tissues of male and female rats indicating rapid absorption and wide distribution. The highest radioactivity in decreasing order at 0.5 and 3 hr postdose: stomach and intestine>brain>spinal cord>liver>Harder gland>adipose tissue>kidney>submaxillary salivary gland>heart>bone marrow>testicles-ovaries>muscle. Distribution was homogeneous in the brain except in the cerebellum where the levels in the white matter were higher than that in the grey matter. Six hr postdose radioactivity was markedly reduced with high levels remaining in the GIT, bladder and less in the liver, kidney, Harder gland, and CNS. At 72 hr postdose, very low levels were still detectable in the liver, kidneys, Harder gland, and CNS.

4. Study of binding of ¹⁴C-Riluzole to melanin in the pigmented rat.

animals: male Long Evans, 4/time point

Dose: 2 mg/kg orally

Time points: 1, 24, and 72 hr and 1, 2, 3, and 4 wks post administration.

Radioactivity was determined in blood, plasma, and eyes.

Results:

Peak levels in the eyes occurred at 1 hr and declined rapidly by 72 hr and slowly by 4 wks. Overall retention was low 0.0003% of total dose administered.

5. ADE Studies in the rat following 2 mg/kg oral dose of ¹⁴C-RP 54274

Study#DN103318

GLP: not provided

Species/strain: Sprague-Dawley/5/sex

Dose/Route/Duration: 2 mg/kg/oral gavage/single dose

Drug: ¹⁴C-RP 54274 labelled on C2 with specific activity of 52 mCi/mmol and >95% purity/prepared in HCl aqueous solution.

Batch/Lot#: C1100 of cold compound.

Results:

Blood samples collected from retro-ocular sinus prior to sacrifice and through aortic puncture following sacrifice at 0.25, 1, 3, 6, 24, and 48 hr postdose.

The following tissues were removed: liver, kidneys, heart, lungs, brain, adrenal glands, spleen, salivary glands, eyes, and testicles.

Absorption was rapid with T_{max} between 15-30 min and C_{max} around 1.2 μg equiv/ml decreasing to 0.03 μg equiv/ml by 48 hr (Table below for plasma parameters). [a rebound, more pronounced in males than females, occurred between 3 and 6 hr (AUC 11.5 and 9 $\mu\text{g}/\text{ml}\cdot\text{hr}$ respectively), however, none of the tables or figures show this rebound.]

Wide tissue distribution with C_{max} in liver > kidneys > brain. tissue/plasma ratio generally >1 in all tissues except the eye (0.4-0.5) with highest value in the liver (m:4-17), kidneys (4-12), and brain (1-4).

Strong protein binding 80-90%

Elimination $t_{1/2}$ about 12 hr

main excretion in urine with 90% elimination within 48 hr

fecal excretion was low 8.5 and 16% in male and female rats respectively.

Study DN103318 - Rat - oral

Oral route - 2 mg/kg		
Parameter	Male rat	Female rat
T_{max} (h)	0.25	0.5
C_{max} μg equiv/l	1.07	1.32
$[AUC]_{0-48\text{ h}}$ $\mu\text{g}\cdot\text{ml}^{-1}\cdot\text{h}$.	11.5	9
$T_{1/2}$ (8 to 48 h)	12	11.5

6. ^{14}C -Riluzole metabolism in male and female rats.

Study #DN103449

GLP: not provided

Species/strain: Sprague-Dawley/4/sex

Dose/Route/Duration: 2 mg/kg/oral gavage/single dose

Drug: ^{14}C -RP 54274 labelled on C2 with specific activity of 52 mCi/mmol and 99% purity/prepared in HCl aqueous solution.

Batch/Lot#: C1100 and C8808501

Results:

Blood samples drawn at 0.5, 3, 6, 24, 72 hr postdose/plasma samples were prepared.

Brains isolated/weighed/freeze-dried: from 4 rats/sex/time period (0.5, 3, 6, 24, 72)

Urine/feces/ $^{14}\text{CO}_2$ (0-48 hr) collected at: 0-24, 24-48, 48-72 hr postdose

Structural analyses of metabolite were done by IR, NMR, and MS

Nine metabolites were identified in males and 7 in females (Figure 1). Among the metabolites, 6 were also found in isolated rat liver perfusate, 2 in rat microsomes, and 2 in rat S9 fraction. The 6 metabolites are:

m = methoxyphenylurea derivative (RP 64821)

e = hydroxybenzothiazole derivative (PK 35011)

APPENDIX 7

Sulfoconjugate

+

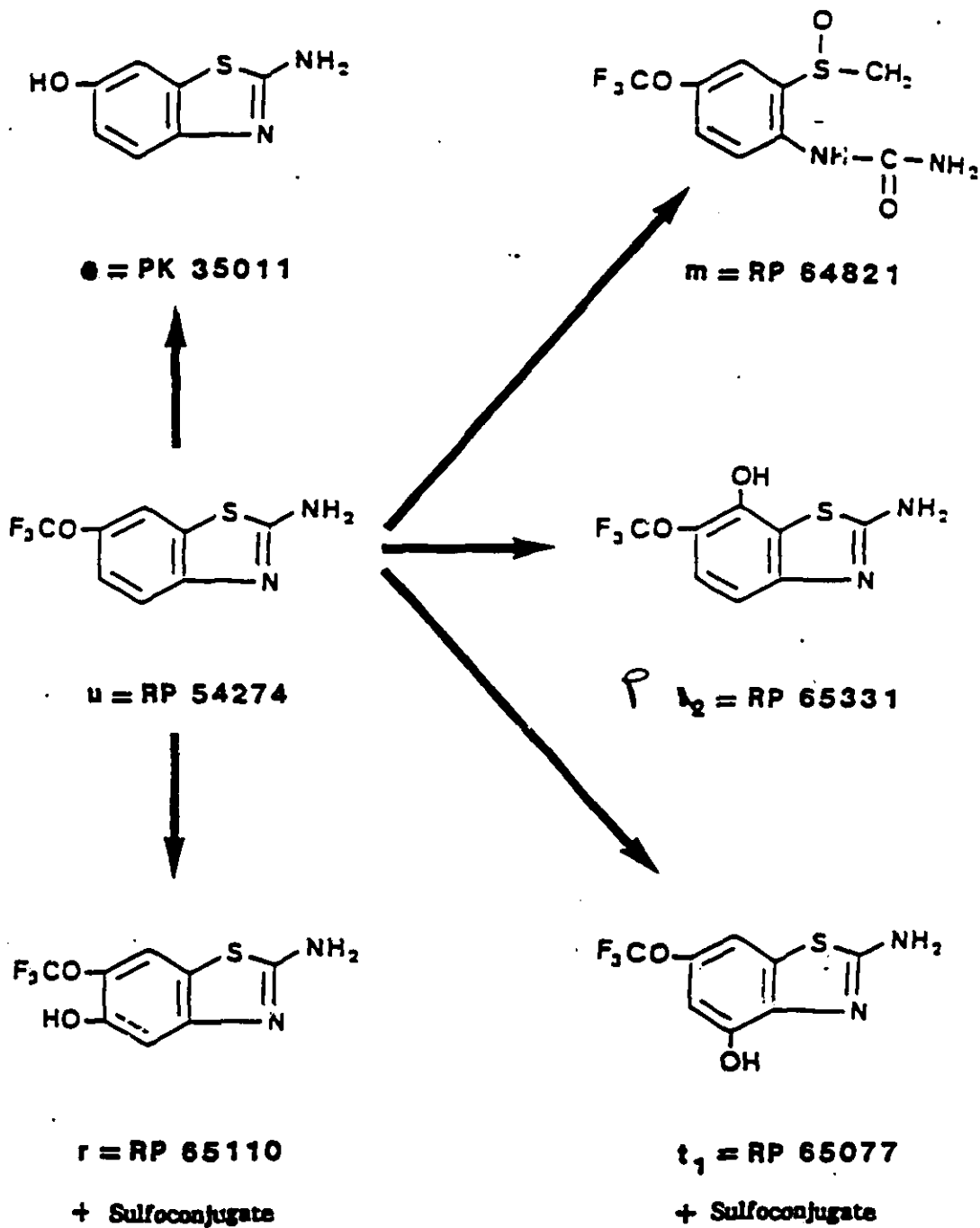


Figure 1

RP 54274 PROBABLE METABOLIC PATHWAY IN THE RAT

p, t1, and r = trifluoromethoxy 7 or 4 or 5 hydroxybenzothiazole respectively (RP 65331, 65077, 65110)
u = unchanged material (RP 54274)

The m and t1 were found in brains from males and only the m was detected in female brain. Urine hydrolysis showed 7 sulfoconjugated metabolites; no qualitative sex differences in urine or fecal metabolic profile.

Riluzole has good absorption and distribution as indicated by recovery of radioactivity. Readily crosses the BBB with brain levels 2-3 times higher than plasma levels for up to 6 hr postdose. Riluzole biotransformation involves oxidation by the hepatic cytochrome P450 enzyme system. The drug is eliminated within the first 24 hr. predominantly through the urine and some in feces. Plasma and brain metabolic profile: 50 and 80% of total radioactivity represents unchanged drug, and 30 and 17% respectively is metabolite m (methoxyphenylurea derivative).

Urinary metabolic profile: 30% sulfoconjugation: parent, e, m, r, and t1: 5, 14, 5, 5, and 10% respectively in males, and in females, the corresponding values are 4, 14, 8, 7, and 12%

fecal metabolic profile: 14% unchanged drug with no differences between sexes.

7. Biliary excretion and metabolic profile in conscious rats after 2 mg/kg oral dose.

Study #DN104565

GLP: not provided

Species/strain: Sprague-Dawley/5 males

Dose/Route/Duration: 2 mg/kg/oral gavage/single dose

Drug: ¹⁴C-RP 54274 labelled on C2 with specific activity of 52 mCi/mmol and 99% purity/prepared in HCl aqueous solution

Batch/Lot#: PX5624 (CEA383/388).

Results:

Sample collection:

Bile: 0-05, 0.5-1, 1-3, 3-6, 6-24, 24-48 hr

Urine and feces: 0-24 and 24-48 hr.

Urinary and biliary metabolic profile determined by HPLC.

51% of the dose was excreted rapidly in the bile (46% over the first 6 hr).

Simultaneous excretion occurred in urine (41% of dose) and feces 1.2%. Biliary excretion of 51% is higher than the ~30% fecal excretion in non-fistulized rats suggesting that the drug or its metabolites entered the enterohepatic circulation.

Biliary metabolic profile: parent unchanged drug at 19%. 4% sulfoconjugated, 3% metabolite e, 2% m, 5% r, and 4% t1. Two metabolites, o (5%) and p (2%) are of unknown structure but characterized in urine of non-fistulized rats. All sulfoconjugated metabolites constitute 12% of dose

Urinary metabolic profile: sulfoconjugated metabolites 23% of dose, 8% e, 4% r, 6% t1. Unchanged drug 21%, excreted in free form (10%) and conjugated form (11%).

8. Effect of Riluzole on metabolic hepatic enzymes in the rat and mouse.

Study #DN103

GLP: not provided

Species/strain: rat/Sprague-Dawley/8/sex/dose; mouse/IVFA-CREDO/12/sex/dose

Dose/Route/Duration: 10 and 30 mg/kg/oral stomach intubation/3 days

Drug: RP 54274 suspension prepared in 1% Tween 80

Batch/Lot#: HC5879

Results:

In mice and rats there was no change in liver weight, cytochrome p-450 content, and aminopyrine N-demethylase activity. However, Microsomal protein content was significantly increased in male rats and female mice of 30 mg/kg. Also Riluzole significantly inhibited aniline hydroxylase in both sexes of HD rat and mouse (and in LD male mice).

A similar study in the dog (Study #103131/male beagles treated orally for 3 days with 30 mg/kg) showed no effects on any of the hepatic metabolic enzymes with minimal increase in the weight of the liver.

Dog

1. Metabolic profile in the dog of ¹⁴C-Riluzole of 10 mg/kg oral administration
Study #DN104539

GLP: not provided

Species/strain: Beagle dogs/2/sex

Dose/Route/Duration: 10 mg/kg/oral gavage/single dose

Drug: ¹⁴C-RP 54274 labelled on C2 with specific activity of 52 mCi/mmol and 99% purity/prepared in HCl aqueous solution

Batch/Lot#: C8808501 cold compound.

Results:

Blood collection times: 1,2,4,6 hr postdose.

Urine and feces sampled over 168 hr and metabolic profile determined by gradient elution HPLC.

Plasma metabolic profile: 6 metabolites in males and 9 in female dog, 2 of these metabolites have been detected in plasma of the rat. Enzymatic hydrolysis of urine showed 1 sulfoconjugation and 5 glucuroconjugation, three metabolites (e,q,t1) have been previously detected in dog microsomes.

Irrespective of sex, absorption was good and radioactivity in blood and plasma was similar with unchanged drug constituting the main compound up to 6 hr postdose (57% of total plasma radioactivity; 75-85% in 1-2 hr postdose). 50 and 40% of total radioactivity was eliminated in urine and feces respectively over 168 hr. (urinary elimination almost completed by 24 hr, whereas fecal elimination up to 72 hr).

Urinary metabolic profile: sulfoconjugation 0.9-2% whereas in rat it is 30%, glucuronidation represented 9-15% compared with 70-75% in human urine.

Fecal metabolic profile: t1: 9%, t2: 7%, and unchanged drug 7%; no sex differences.

Comparison of metabolic profile in rat, dog, and human indicates that it is different in the dog. Large interanimal variation was noted in the dog, urinary elimination of unchanged drug varied between 0.6-45% of administered dose (attached Figure shows the metabolic pathways in man, dog, and rat).

2. Study of the effect of Riluzole on metabolic enzymes in the dog (Study #DN103131): See #8 above.

Rabbit

1. Toxicokinetics of RP54274 following once daily oral administration of 3, 18, and 60 mg/kg for 13 days in pregnant and non-pregnant rabbits

Study #DN107886

IBP-Biochem # 1590

Laboratory: IBP/Rhone-Poulenc/Miserey - France

GLP: signed and dated 10/9/90

Species/strain: New-Zealand white female rabbits/7/group (mean B.wt. 3-3.5 kg)

Dose/Route/Duration: 0, 3, 18, 60 mg/kg/oral gavage/daily from gds 6-18 for pregnant and gds 6-13 for non-pregnant rabbits.

Drug: RP 45274 in solution in 1% methylcellulose in water prepared daily.

Batch/Lot#: C8808501.

Results:

Blood samples collected on days 1 and 13 (non-pregnant) or day 6 and 18 in pregnant rabbits, before dosing and: 0.25, 0.50, 0.75, 1, 2, 3, 6, 8, 24 hr after dosing. Sampling was also done on day 9 for non pregnant and day 14 in pregnant females before dosing and 4 hr postdose. Parent and metabolites were identified by reverse phase HPLC with dl of 5 ng/ml plasma. Total of three animals died during the study: 2 non pregnant dosed 3 and 60 mg/kg and one pregnant dosed 60 mg/kg; the cause of death was not reported.

C_{max} ($\mu\text{g/ml}$):

Dose (mg/kg)	Non-Pregnant		Pregnant	
	Day 1	Day 13	Day 6	Day 18
3	989 (0.75)	729 (0.5)	763 (0.25)	473 (0.75)
18	3109 (0.5)	3007 (0.5)	3658 (1)	3310 (1)
60	4587 (2)	4063 (1)	7626 (2)	5533 (0.25)

numbers in () present the time (hr) to C_{max} .

On day 1 and 13/dose 3 mg/kg, C_{max} in nonpregnant females was 1.3-1.5 fold > than that in pregnant females measured at days 6 and 18. At HD, pregnant rabbits had 1.4-2 fold > C_{max} on days 6 and 18 compared with nonpregnant females at days 1 and 13. Also, C_{max} on day1 occurred earlier ($p < 0.01$) in 3 mg/kg dosed pregnant rabbits compared with nonpregnant female rabbits. In pregnant rabbits at LD, AUC on day1 was 1195 ± 443 vs. 1930 ± 808 on day 13 ($p < 0.03$), this indicated tendency of drug accumulation. However, at HD, C_{max} and AUC decreased on day13 vs. day1, this variation perhaps, is due to less optimal absorption of the drug as indicated by decreased C_{max} on day13. Similar finding of potential drug accumulation at LD was found in non pregnant rabbits with AUCs of 671 and 2122 ng/ml.hr on days 1 and 13 respectively ($p < 0.02$), without change in C_{max} . However, in nonpregnant females, a marked decrease (1.6 fold, $p < 0.02$) in AUC of HD was noted between days 1 and 13 without a change in C_{max} .

It is concluded that pregnancy in the rabbit has no significant systematic effect on Riluzole pharmacokinetic profile. On occasion, maximum concentration was significantly higher in pregnant vs. that in non pregnant rabbit; this was considered by the sponsor to be clinically irrelevant. Good plasma-dose correlation was found in pregnant rabbits however, the correlation was poor in non pregnant females. A tendency towards drug accumulation was found in pregnant and non pregnant females as noted from AUC results only of LD.

Baboon/Monkey

1. Toxicokinetics in Baboons in the Course of Repeated Oral Treatment with 3, 10, and 30 mg/kg for 3 months

Study #DN103450/report ref. 1220

GLP: study conducted under SOP/

: dated.

Species/strain: baboon/3/sex/gr

Dose/Route/Duration: 0, 3, 10, 30/25 mg/kg/oral gavage/daily for 3 months/on day 45 HD was reduced to 25 mg/kg due to signs of toxicity .

Drug: RP 45274 in solution in 1% methylcellulose in water prepared daily.

Batch/Lot#: HD 119/83-81

Results:

Blood sampled 1 hr after dosing on day 1, wks 6 and 13 and drug concentration determined by HPLC with UV detector. Interanimal variability was high (5-77% in males and females).

-Cumulative ratios: significant on wk13/day 1 at 3 and 10 mg/kg indicating a slight increase of concentration over time (attached Table).
 -Concentration-Dose correlation were not so good specially towards end of study:
 Day1 r=0.86
 wk6 r=0.77
 wk13 =0.62

Although a linear relationship was found between regression curve of plasma concentration and dose, the increase in concentration as a function of dose was poor as indicated by the r values above.

Dose (mg/kg)	Range Drug-Plasma Concentration (µg/ml)		
	Day 1	Wk 6	Wk 13
3	0.4-1.1	0.1-1.4	1-1.4
10	1.1-2.3	1-3	2.2-6
30	3-8	3-8	3-10

Plasma concentration measured 1 hr after gavage; the above values are for both sexes.

Conclusion: No sex differences in plasma levels, positive linear relationship between plasma level and dose existed however, there was a small but significant increase in accumulation ratios between start and end of study at the two low doses.

Comment: Amendment #2 (implemented 7/21/83) states that increase in intra-ocular pressure in treated animals was noted on wk 3 and confirmed on wk 6 by additional ophthalmic examination. The sponsor indicated that an examination is scheduled and other ophthalmic tests will be conducted on all animals.

Amendment #3 (implemented 8/2/83) noted that reducing the dose from 30 to 25 mg/kg was because of ataxia and incoordination noted in one HD female.

2. 26-wk Toxicokinetics in the monkey following once daily oral administration at 5, 10, and 20 mg/kg

Study # NR but a Report # is:1659

GLP: signed and dated 3/93
 Species/strain: wild-caught Cynomolgus monkey/5-7/sex/gr
 Dose/Route/Duration: 0, 5, 10, 20 mg/kg/oral/daily for 26 wks.
 Drug: RP 45274 gelatin capsule
 Batch/Lot#: C8908501/purity 100.2%

Results:

Blood sampled on days 1, 7, and 28, and wks 13 and 26 at specified times ranging from 4-24 hr postdose. Plasma drug concentrations determined by HPLC with UV detector/dl 5 ng/ml. Large interanimal variability was found with c.v.> 100%. AUC and C_∞ in general, increased with dose without differences between sexes. There were significant differences between concentration and treatment duration. Plasma levels at 8 and 24 hr postdose were significantly different when compared on wks 1,5,13, and 26 however, graphic presentation of normalized data indicated that values were similar at end of study but elevated during the first wks. Table 1 presents mean±S.D. PK data, Tables 2-5 show mean±S.D. plasma levels in m/f animals during different times for the 10, and 20 mg/kg doses. All animals on HD were sacrificed by wk8 due to poor condition; no further information was provided regarding the cause of death (Tables 1&2 for plasma levels). Significant drug accumulation at HD was found after day 28 specially in f with 2.7 fold higher levels at 8 hr postdose on day 28 vs. the level measured the same time on day 1 (Table 5). No significant accumulation was noted in LD or MD. Mean brain level of HD male



Table 4 - RP 54274 - Cumulative ratios

Baboon No.	SEX	DOSE mg/Kg	W6/Day1	W13/W6	W13/Day1
904	M	3	0.81	1.79	1.45
905			0.33	9.79	3.19
906			0.84	1.57	1.33
916	F	3	1.07	1.53	1.64
917			1.43	1.57	2.24
918			1.26	0.63	0.79
mean			0.96	2.81	1.77
S.D.			0.39	3.44	0.84
p*			N.S (W6vsD1)	N.S (W13vsW6)	N.S (W13vsD1)
907	M	10	1.59	0.72	1.14
908			1.79	2.15	3.84
909			2.71	0.78	2.11
919	F	10	1.29	1.33	1.71
920			2.36	1.33	3.13
921			0.63	5.17	3.28
mean			1.73	1.91	2.53
S.D.			0.75	1.68	1.04
p*			N.S (W6vsD1)	N.S (W13vsW6)	S (W13vsD1)
910	M	30	1.19	1.17	1.39
911			0.68	1.12	0.77
912			1.03	1.06	1.09
922	F	30	1.67	1.22	2.13
923			0.70	0.76	0.53
924			0.70	0.76	0.53
mean			1.05	1.08	1.18
S.D.			0.41	0.20	0.62
p*			N.S (W6vsD1)	N.S (W13vsW6)	N.S (W13vsD1)

* : (Significant or not Significant at Level of 5%)

Study # DN103450
Baboon oral 3month

study # 1659
26 wk. Toxicokinetic in monkey

Table # - Pharmacokinetic parameters (first day)

	Dose (mg/kg)											
	5				10				20			
Monkey No.	T Max	C Max	ADC	Monkey No.	T Max	C Max	ADC	Monkey No.	T Max	C Max	ADC	
	h	µg/ml	0-24h µg/ml.h		h	µg/ml	0-24h µg/ml.h		h	µg/ml	0-24h µg/ml.h	
Male	263	4	0.62	4.10	273	4	1.61	11.23	283	4	2.82	26.67
	265	4	0.65	5.28	275	4	3.13	45.42	285	4	5.33	72.53
	267	4	0.91	12.60	277	4	1.75	20.72	287	4	3.30	27.76
	269	4	1.47	14.42	279	4	1.29	9.99	289	4	1.22	9.68
	271	4	0.83	6.52	281	4	1.74	9.38	291	4	2.03	25.62
									293	4	1.39	12.32
									295	4	1.40	14.24
	Mean		0.90	8.54			1.90	19.35			2.50	26.97
	S.D.		0.34	4.62			0.71	15.28			1.47	21.42
	C.V.		38.31	53.91			37.31	79.01			59.01	79.41
Median	4			4				4				
Female	264	4	1.07	8.55	274	4	3.35	33.04	284	4	3.13	28.45
	266	4	0.92	6.24	276	4	2.28	15.46	286	8	5.71	77.32
	268	12	1.19	11.48	278	4	1.32	11.61	288	4	2.26	25.89
	270	4	0.92	8.40	280	12	1.52	22.32	290	8	1.94	25.27
	272	4	0.91	10.34	282	4	3.81	48.34	292	4	1.17	11.21
									294	4	1.70	23.27
									296	4	2.33	13.57
	Mean		1.00	9.00			2.46	26.15			2.61	29.35
	S.D.		0.12	2.01			1.10	14.83			1.50	22.14
	C.V.		12.41	22.31			44.71	56.71			57.41	75.41
Median	4			4				4				

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Table 2

RF 54274 - Plasma concentrations (µg/ml) in male monkeys after oral administration once a day for 26 weeks (dose = 5 mg/kg body weight)

Monkey No.	Day 1					Day 7			Day 28			Week 13				Week 26			
	0 h	4 h	8 h	12 h	24 h	8 h	24 h	8 h	24 h	8 h	24 h	8 h	24 h	4 h	8 h	12 h	24 h		
263	0.000	0.518	0.294	0.056	0.013	0.608	0.027	0.248	0.025	0.687	0.029	0.592	0.163	1.071	0.592	0.163	0.063		
265	0.000	0.649	0.396	0.137	0.008	0.382	0.021	0.479	0.025	0.741	0.023	0.719	0.218	0.947	0.719	0.218	0.062		
267	0.000	0.912	0.846	0.670	0.034	1.088	0.072	0.268	0.055	0.788	0.039	0.502	0.123	1.281	0.502	0.123	*		
269	0.000	1.468	1.015	0.546	0.020	0.715	0.038	1.266	0.050	0.026	*	0.083	0.066	0.114	0.083	0.066	0.077		
271	0.000	0.831	0.424	0.174	0.018	0.482	0.061	0.912	0.070	0.443	0.037	0.250	0.096	0.703	0.250	0.096	*		
Mean		0.896	0.395	0.316	0.019	0.655	0.044	0.634	0.045	0.537	0.025	0.429	0.133	0.823	0.429	0.133	0.040		
S.D.		0.343	0.316	0.273	0.010	0.273	0.022	0.442	0.020	0.315	0.016	0.259	0.059	0.448	0.259	0.059	0.037		
C.V.		38.3%	53.1%	86.7%	52.6%	41.7%	50.7%	69.7%	44.0%	58.7%	61.5%	60.3%	44.5%	54.5%	60.3%	44.5%	92.5%		
Minimum		0.618	0.294	0.056	0.008	0.382	0.021	0.248	0.025	0.026	*	0.083	0.065	0.114	0.083	0.065	*		
Maximum		1.468	1.015	0.670	0.034	1.088	0.072	1.266	0.070	0.748	0.039	0.719	0.218	1.281	0.719	0.218	0.077		

* : BLQ (below limit of quantitation : 0.005 µg/ml)

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Table 3

RP 54274 - Plasma concentrations (µg/ml) in female monkeys after oral administration once a day for 26 weeks (dose = 5 mg/kg body weight)

Monkey No.	Day 1				Day 7		Day 28		Week 13		Week 26				
	0 h	4 h	8 h	12 h	24 h	8 h	24 h	8 h	24 h	8 h	24 h	4 h	8 h	12 h	24 h
264	0.000	1.066	9.701	0.171	0.019	0.376	0.028	0.287	0.285	0.734	0.032	2.201	0.833	0.186	0.080
266	0.000	0.918	0.482	0.080	*	1.361	0.048	1.971	0.049	0.860	0.023	0.401	0.961	0.721	0.068
268	0.000	0.037	0.393	1.191	0.039	0.774	0.048	1.152	0.091	0.020	0.031	1.050	0.774	0.207	0.069
270	0.000	0.925	0.647	0.245	0.023	0.456	0.045	1.192	0.104	0.181	0.018	1.051	0.620	0.152	0.060
272	0.000	0.905	0.721	0.444	0.048	0.838	0.123	0.906	0.202	0.561	0.041	1.244	0.809	0.309	0.068
Mean		0.770	0.589	0.426	0.026	0.761	0.058	1.102	0.146	0.491	0.029	1.189	0.799	0.315	0.069
S.D.		0.415	0.144	0.448	0.019	0.390	0.037	0.606	0.096	0.368	0.009	0.649	0.123	0.234	0.007
C.V.		53.9%	24.5%	105.1%	72.0%	51.2%	63.7%	55.0%	65.5%	74.9%	30.7%	54.6%	15.3%	74.4%	10.3%
Minimum		0.037	0.393	0.080	*	0.176	0.028	0.287	0.049	0.020	0.018	0.401	0.620	0.152	0.060
Maximum		1.066	9.721	1.191	0.048	1.361	0.123	1.971	0.285	0.860	0.041	2.201	0.961	0.721	0.080

* : BLQ (below limit of quantitation) : 0.005 µg/ml

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26-wk toxicokinetics in monkey

Table 2

BP 54274 - Plasma concentrations (µg/ml) in male monkeys after oral administration once a day for 26 weeks (dose = 10 mg/kg body weight)

Monkey No.	Day 1				Day 7		Day 28		Week 13			Week 26		
	0 h	4 h	8 h	12 h	24 h	8 h	24 h	8 h	24 h	4 h	8 h	12 h	24 h	
273	0.000	1.611	0.779	0.209	*	3.275	0.057	2.359	0.047	2.392	0.058	*	0.065	0.062
275	0.000	3.130	2.978	2.517	0.142	3.447	0.146	2.651	0.111	1.920	0.070	3.800	2.685	1.038
277	0.000	1.753	1.406	0.977	0.044	4.003	0.195	1.299	0.046	2.005	0.052	3.365	2.351	0.930
279	0.000	1.295	0.592	0.305	0.022	2.666	0.220	1.841	0.189	1.939	0.123	2.426	1.766	0.792
281	0.000	1.743	0.391	0.105	*	2.139	0.083	0.847	0.026	2.881	0.117	3.546	1.835	0.304
Mean		1.906	1.229	0.823	0.042	3.106	0.140	1.799	0.084	2.228	0.084	2.628	1.730	0.626
S.D.		0.709	1.049	1.007	0.059	0.721	0.070	0.741	0.067	0.413	0.034	1.558	1.039	0.421
C.V.		37.2%	85.3%	122.4%	141.8%	23.2%	49.8%	41.2%	79.9%	18.5%	39.9%	59.3%	60.1%	67.2%
Minimum		1.295	0.391	0.105	*	2.139	0.057	0.847	0.026	1.920	0.052	*	*	0.065
Maximum		3.130	2.978	2.517	0.142	4.003	0.220	2.651	0.189	2.881	0.123	3.800	2.685	1.038

* : BLO (below limit of quantitation : 0.005 µg/ml)

26-wk toxicokinetics in monkey

Table 2

RP 54274 - Plasma concentrations (µg/ml) in female monkeys after oral administration once a day for 26 weeks (dose = 10 mg/kg body weight)

Monkey No.	Day 1					Day 7		Day 28		Week 13			Week 26			
	0 h	4 h	8 h	12 h	24 h	8 h	24 h	8 h	24 h	8 h	24 h	4 h	8 h	12 h	24 h	
274	0.000	3.350	3.931	1.421	0.091	3.013	0.140	1.865	0.080	3.679	0.050	4.088	3.393	0.933	0.072	
276	0.000	2.280	0.896	0.344	0.020	2.252	0.133	1.435	0.142	0.550	0.038	3.072	2.189	0.473	0.063	
278	0.000	1.322	0.988	0.275	0.028	1.859	0.055	0.685	0.047	1.668	0.063	2.421	1.640	0.482	0.068	
280	0.000	0.805	1.376	1.522	0.183	2.898	0.245	2.112	0.507	1.457	0.068	2.488	1.266	0.248	0.060	
282	0.000	2.806	3.134	2.234	0.452	5.007	0.950	3.857	0.375	2.685	0.079	3.043	1.780	0.479	0.084	
Mean		2.322	1.665	1.159	0.155	3.006	0.305	1.991	0.230	2.008	0.060	3.023	2.054	0.523	0.069	
S.D.		1.258	0.917	0.837	0.179	1.214	0.367	1.176	0.201	1.204	0.016	0.658	0.818	0.250	0.059	
C.V.		54.0%	55.1%	72.2%	115.3%	40.4%	120.5%	59.0%	87.2%	60.0%	26.7%	22.1%	39.8%	47.8%	11.3%	
Minimum		0.005	0.896	0.275	0.020	1.859	0.055	0.685	0.047	0.550	0.038	2.421	1.266	0.248	0.060	
Maximum		3.806	3.134	2.234	0.452	5.007	0.950	3.857	0.507	3.679	0.079	4.088	3.393	0.933	0.084	

26-wk Toxicokinetics in Monkey

Table 4

SP 54274 - Plasma concentrations ($\mu\text{g}/\text{ml}$) in male monkeys after oral administration once a day for 28 days (dose = 20 mg/kg body weight)

Monkey No.	Day 1					Day 7		Day 28	
	0 h	4 h	8 h	12 h	24 h	8 h	24 h	8 h	24 h
283	0.000	2.825	1.800	0.999	0.030	4.675	0.096	4.605	0.100
285	0.000	5.330	4.283	3.867	0.523	11.021	1.207	5.326	0.922
287	0.000	3.301	1.822	0.883	0.034	9.102	0.439	4.556	0.051
289	0.000	1.217	0.702	0.250	*	5.805	1.234	4.582	0.201
291	0.000	2.032	1.693	1.340	*	4.469	0.298	0.156	*
293	0.000	1.389	0.800	0.445	*	2.771	0.279	2.627	0.383
295	0.000	1.401	1.312	0.423	*	5.271	0.887	6.696	1.010
Mean		2.499	1.773	1.172	0.084	6.159	0.634	4.078	0.381
S.D.		1.475	1.198	1.248	0.194	2.879	0.469	2.108	0.419
C.V.		59.03	67.63	106.43	231.53	46.83	73.93	51.73	109.93
Minimum		1.217	0.702	0.250	*	2.771	0.096	0.156	*
Maximum		5.330	4.283	3.867	0.523	11.021	1.234	6.696	1.010

* : BLQ (below limit of quantitation : 0.005 $\mu\text{g}/\text{ml}$)

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26-wk toxicokinetics in monkey

Table 5

SP 54274 - Plasma concentrations ($\mu\text{g/ml}$) in male monkeys after oral administration once a day for 28 days (dose = 20 mg/kg body weight)

Monkey No.	Day 1					Day 7		Day 28	
	0 h	4 h	8 h	12 h	24 h	8 h	24 h	8 h	24 h
284	0.000	3.131	2.388	0.847	*	2.309	0.156	2.300	0.051
286	0.000	4.890	5.707	3.974	0.523	8.649	0.512	4.170	0.885
288	0.000	2.263	1.930	1.140	*	7.360	0.338	6.886	0.140
290	0.000	1.838	1.945	1.229	0.051	9.386	2.709	5.054	0.338
292	0.000	1.169	0.716	0.459	*	2.076	0.297	7.449	1.157
294	0.000	1.702	1.510	1.172	0.174	3.770	1.266	7.160	2.941
296	0.000	2.328	0.546	0.272	*	2.158	0.436	7.297	1.286
Mean		2.474	2.106	1.299	0.107	5.101	0.813	5.760	0.972
S.D.		1.227	1.724	1.236	0.194	3.251	0.911	1.976	0.995
C.V.		49.6%	81.9%	95.2%	181.6%	63.7%	112.0%	34.3%	102.3%
Minimum		1.169	0.546	0.272	*	2.076	0.136	2.300	0.061
Maximum		4.890	5.707	3.974	0.523	9.386	2.709	7.449	2.941

* : BLQ (below limit of quantitation : 0.005 $\mu\text{g/ml}$)

Table 5

RP54274 - Male monkey brain levels (µg/g), Cmax plasma levels (µg/ml)

and $\frac{\text{C brain}}{\text{Cmax plasma}}$ ratios after the 20mg/kg/day treatment

Monkey No.	Half brain weight g	Lyophilised weight g	Sample weight µg	C brain µg/g	Cmax plasma µg/ml	$\frac{\text{C brain}}{\text{Cmax plasma}}$
283	30.37	7.15	250 µg	4.52	2.82	1.60
285	31.62	7.64	250 µg	3.95	5.33	0.74
287	32.31	7.91	250 µg	9.49	3.30	2.88
289	32.82	8.09	250 µg	0.52	1.22	0.43
291	34.71	8.37	250 µg	7.43	2.03	3.66
293	34.33	8.49	250 µg	0.35	1.39	0.25
295	28.83	6.76	250 µg	*	1.40	
Mean				4.38	2.50	1.59
S.D.				3.65	1.47	1.40
C.V.				83.49%	59.03%	87.91%

* : Interference with another product

monkeys was $4.4 \pm 3.7 \mu\text{g/g}$ and C_{max} was $2.5 \pm 1.5 \mu\text{g/ml}$ with brain/plasma ratio of 1.6 ± 1.4 . C_{max} at 4 hr was: 1, 2-2.5, and 3 $\mu\text{g/ml}$ for 5, 10, and 20 mg/kg doses respectively (Table 1 for PK parameters for all doses and Table 6).

It is concluded that oral administration of Riluzole to monkeys for 26 wks caused death in HD and PK data were highly variable between the animals. Drug plasma levels increased with increase in dose and no accumulation with time in LD and MD. However, a significant accumulation occurred in HD after the 28-day treatment. Brain levels were similar to plasma levels after 28 days of HD treatment.

Comment for this and study #DN103450 above: according to the protocol, clinical signs, ophthalmoscopy, macroscopical examination, histopathology, hematology, clinical examination, and urinalysis were conducted and data are presented in the toxicology section.

3. Tissue Distribution of ^{14}C -Riluzole after oral administration in the monkey-Autoradiographic studies

Study #DN104569

GLP: not provided

Species/strain: 3 male *Macaca fascicularis* monkeys

Dose/Route/Duration: 2 mg/kg/single oral dose/gastric intubation

Drug: ^{14}C -RP 45274 purity 98.5% prepared in HCl/saline solution

Batch/Lot#: C8808501

Results:

30 min/monkey#1:

Radioactivity was distributed throughout the body except the bone. The uveal tract and the fundal mucosa of the stomach showed some of the highest radioactivity followed by, in decreasing order: hepatic paranchyma, cerebral regions, oesophageal mucosa, and adipose tissue. Intense labelling was found in certain areas of the liver (homogeneous distribution), gall bladder, and CNS. Highest levels were seen in the substantia alba, pulvinar thalamia, lateral geniculate bodies, corpus callosum, optic chiasm, and the gyrus dentatus in the cerebellum. Also high radioactivity were noted in the spinal cord and spinal bulb. High activity was also found in the kidneys and spleen with moderate levels in skeletal muscles, skin, blood and thymus. The label was also present in the myocardium, adrenal gland, bone marrow, and testes.

3 hrs./monkey#2:

Same as above but with less intensity. Highest intensity was also in the uveal tract and ocular structures as well as the skin.

24 hr./monkey#3:

By 24 hr, the label intensity was clearly decreased. The highest label remained in the uveal tract but less in the ocular melanins and skin melanin compared with the measurements in the above time periods. The next organ with high intensity is the hepatic paranchyma, gall bladder, and kidneys. Unlike the above two times, hepatic distribution was heterogeneous. High intensity (homogeneous distribution), similar to that in the liver was found in the testes, but no such high activity was found in other male reproductive system. High levels were noted in the bile with only moderate levels in the urine and contents of the GIT.

Conclusion: Highest activity occurred within 30 min in the blood and remained high after 3hr indicating rapid absorption. All melanin-rich organs were highly labelled with decreasing intensity by 24 hr which may indicate weak melanin affinity of the drug. High and homogeneous distribution was found in the liver and less was found in the kidneys at all 3 intervals and heterogeneous distribution was found at 24 hr. Moderately high levels were noted at the 30 min and 3 hr in the spleen and bone marrow with only traces

remaining by 24 hr. Moderate levels were found in the testes at all 3 times. The specific brain regions showed moderately high level of radioactivity indicating the drug's ability to cross the BBB. High radioactivity (similar to that in the liver) was also measured in the testis.

Comment: although it is clear that the drug is readily absorbed and distributed to various tissues, the drug-blood/plasma concentration was not quantified. The sponsor referred to it only under the conclusion/discussion. The first statement above in the conclusion section is as reported by the sponsor. It is understood that this study was a qualitative one but it is unclear how a conclusion about the blood can be made.

In Vitro

Preliminary study of the metabolism of ¹⁴C-RP 54274 in vitro with human and animal hepatic microsomes - comparison with isolated perfused liver of the rat and rabbit.

Study #DN10331;

GLP: not provided

Species/strain: mouse/OF1 Swiss IOPS, rat/Sprague-Dawley IOPS; rabbit/New Zealand, dog/beagle; monkey/Macaca. all animals are males; human/1 man and 2 women.

Dose/Route/Duration: in vitro/microsomal preparations in mouse, rat, dog, rabbit, monkey, and human, and perfused liver of rat and rabbit.

Drug: ¹⁴C-RP 45274

Batch/Lot#: IBP/GW870268 cold material

Results:

Gradient elution HPLC was used to study the metabolism of ¹⁴C-RP 54274. Noninduced hepatic microsomes and those induced with 3-methylcholanthrene or phenobarbital were used. Results indicate large differences both quantitative and qualitative, between species in Riluzole metabolism. Man hepatic microsomal capacity was highly variable since 2 of 3 preparations were incapable of metabolizing Riluzole. The hepatic monooxygenase enzyme system was responsible for biotransformation of Riluzole as evidenced by the induction studies. Induced preparations showed 22 and 18 fold increased rate of biotransformation compared with control microsomes from the rat. Qualitatively, the following metabolites were identified:

- 9 in the monkey/2 major metabolites: e and t,
- 5 in the rat/q most abundant
- 4 in mouse and dog/e and q most abundant in mouse and t and e in the dog
- 2 in rabbit/e and q (common to all species studied)
- 4 in man/g, q, r (specific to the monkey), and a (common to all species except the rabbit).

Using S9 fraction or perfused liver produced new metabolites. This is because some enzymes are absent in microsomal preparations such as sulfotransferase and glucuronyl transferase. Example: metabolite p is the main metabolite in rat liver perfusate and bile. Phenobarbital and 3-MC each induces different set of p-450 isozymes therefore different metabolites are produced. For example, phenobarbital pretreatment increases metabolite g whereas, 3-MC increases the synthesis of metabolites e and a and give rise to the new metabolites d, f, p, and r. The human microsomal metabolic activity on a quantitative basis, is intermediate between the rat and the monkey.

ADME Summary:

Single and repeat-dose studies have been conducted in the rat, dog, and monkeys. Riluzole after oral administration to the rat, was well absorbed with T_{max} between 15-30 min, and rapidly distributed throughout the body tissues with highest concentration in liver>kidneys>brain. It crosses the BBB with brain levels 2-3 fold higher than plasma levels. The following metabolites have been identified in the rat urine:

- methoxyphenylurea derivative (5% males and 8% females)
- hydroxybenzothiazole derivative (14% males and females)
- trifluoromethoxy 4 or 5 or 7 hydroxybenzothiazole (5-10% m+f)
- unchanged drug (4-5% m+f)

Plasma and brain metabolic profile: 50 and 80% unchanged drug respectively, and 30 and 17% respectively, methoxyphenylurea derivative. Elimination of 90% occurred within 48 hr mainly via the urine and some in feces. $t_{1/2}$ is 12 hr. 9-16% unchanged drug is eliminated in feces of males and females respectively. The metabolites excreted as sulfoconjugated compounds.

Both glucuronidation and sulfoconjugation is found in the dog. However, unlike that in the rat, sulfoconjugation accounted for 1-2% vs. 30% in the rat, and glucuronidation accounted for 9-15% vs. 70-75% in humans.

The drug appears to accumulate after long-term use with plasma levels 2-4 times at week 26 compared to day 1 of dosing. Drug-plasma levels were non-linear for female rats.

IV. ACUTE TOXICITY:

Studies conducted in Two species and Two routes: mouse and rat/oral and i.v. The following are study numbers:

- Acute oral toxicity study (LD_{50}) in the rat #DN101584
- Acute i.v. toxicity (LD_{50}) in the rat #DN101591
- Acute oral toxicity study (LD_{50}) in the mouse #DN101587
- Acute i.v. toxicity (LD_{50}) in the mouse #DN101592

LD_{50} values in males and females are as follows:

Mouse:

Route	oral	i.v.
No. animals/sex/dose	5	5
Dose (mg/kg)	31,44,63,75,89,106	25,29.7,35.3,42
Duration of Observation	14d	14d
Mortality	between days 1 and 3 after administration	within 15 min and one death on day 1
LD_{50} (mg/kg) (m+f) 95% confidence interval	85 (77-93)	34.5 (31.9-37.5)

NDP 20599

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Rat:

Route	oral	i.v.
No. animals/sex/dose	5	5
Dose (mg/kg)	31,44,63,89	17.8,19.4,21.2,25.2,30
Duration of Observation	14d	14d
Mortality	day of administration	30 min of injection
LD ₅₀ (mg/kg) (m+f) 95% confidence interval	45 (35-56)	21 (16.1-24.5)

Clinical signs after i.v. for both species: prostration or lethargy, deep/rapid respiration in most or all surviving animals. On the day of dosing, mice showed ataxia and hunched posture whereas rats had occasional piloerection and lacrimation. In both species, all surviving animals were normal after 24-48 hr.

Clinical signs after oral for both species: lethargy and/or prostration (from 44 mg/kg in mice and 31 mg/kg in rats), difficult or shallow, or slow and rapid breathing from 44 mg/kg in both species, hunched posture and piloerection in both species. Clonic convulsions in mice, ataxia, tremors, red stains around the nose and chromodacryorrhea in rats up to 4 days after administration. All mice were normal from day 6 onwards.

Necropsy: abnormal contents (small yellow lump) in bladder of one rat injected with 30 mg/kg, both species had fluid in the GIT after oral dosing. Change in color of lungs in orally administered rats (lighter or darker). No changes were noted in mice dosed via the i.v. route.

V. SUBCHRONIC TOXICITY:

The following studies were carried out:

1. 2-wk oral dog/#410
2. 13-wk oral rat/#DN101580
3. 13-wk oral mouse/#DN105148
4. 13-wk oral baboon/#DN101577
5. 26-wk oral cynomolgus monkey/#DN104374 and #DN105147
6. 26-wk oral rat/#DN104426
7. 26-wk oral rat/#DN105369

13-wk oral rat:

Doses (mg/kg)	Mean Plasma Levels* µg/ml (m/f)	
	wk 5	wk 13
2.5	0.3/0.2	0.3/0.4
8	0.5/0.5	0.7/0.4
25	2.5/1.9	2.0/2.0

26-wk rat:

Doses	wk 13	wk 26
5	0.5/0.4	0.7/0.5
10	1.2/0.9	1.3/1.0
20	2.0/2.2	3.0/2.7

* 1-hr postdose

13-wk Rat:

GLP: signed and dated 10/1985

Species/strain: Sprague-Dawley/15/sex/gr

Dose/Route/Duration: 0, 2.5, 8, 25 mg/kg/oral gavage/daily for 13 wks

Drug: RP prepared daily in 1% methyl cellulose aqueous suspension

Batch/Lot#: 4PC164/100% pure

Results:

Mortality: 5/15 HD rats died, 1 male killed on day 10 and 2 each males and females died under anesthesia during blood collection during wks 4-13. The male that died showed ataxia, hypothermia, and hypoactivity. All other animals survived. These deaths are considered drug-related based on the effects of the drug on respiratory center and heart rate noted in the rat and baboon (HLE DN #3723-81-31), effect on thermoregulation, and sedation.

Clinical Signs: excessive salivation in HD animals observed first in one male on wk 2 then in 7 animals on wk 6 (5m, 2f); salivation was reduced thereafter. Initially, salivation occurred after dosing but later animals salivated in anticipation of dosing. The frequency was less in females and the other treatment groups.

Body Weight and Food Consumption: mean body weight gain in HD males was significantly reduced starting from wk 6 till end of study (8% compared with the control). Reduced food consumption accompanied the decreased body weight (maximum reduction compared with the control intake was 17%). Food intake in the male and female control rats, HD male, and all treatment female groups was reduced on wk 13 by 10-20%. The decline in food intake in the control groups was not observed on wk 12 (explanation was not provided for this effect in the control groups). Food loss in the controls and treated females did not affect body weight gain.

Hematology: the only change was a 12-22% reduction in APTT (sec) in HD animals on wk 14 relative to the control values.

Clinical Chemistry: GPT and glucose levels were 45-50% reduced in HD females compared with the control values pre-drug. On treatment wk 13, glucose level was 22% decreased in males but not in females compared with the control values. Although apparently, GPT and glucose levels were comparable between HD and control later on during the study, the marked decrease noted pre-drug was not explained. Also in HD males and females during wk 13, ALP levels were 16 and 36% elevated compared with the control values respectively.

Organ Weights: mean relative liver weight was 12 and 9% increased in HD males and females relative to the controls. This increase may be responsible for the increase in plasma ALP noted in HD females.

Histopathology: no marked changes were noted except the following: 5/15 HD females had congested ovaries compared with 1/15 in controls; thyroid cyst found in 7/15 males and 6/15 females of HD vs. 1/15 and 2/15 in male and female controls respectively.

No changes were noted in ophthalmoscopy, urinalysis, or necropsy.

Summary:

Riluzole orally administered to rats for 13 wk at 2.5, 8, and 25 mg/kg caused a total of 4 deaths. The deaths of the animals in this study were related (by the sponsor), to previously reported effects of the drug (sedation, effects on thermoregulation, respiratory center and cardiovascular hemodynamics in rats and baboon (HLE#3723-81-31). Riluzole caused excessive salivation in HD animals observed on wks 2 and 6 but salivation was reduced thereafter. The increase in mean relative liver weight may reflect the elevation of ALP. Five of 15 HD females had congested ovaries and thyroid cyst identified in 7/15 males and 6/15 females of HD. The NOEL in this study is 8 mg/kg.

26-wk Rat:

Study# DN104426

GLP: signed and dated 9/1990

Species/strain: Sprague-Dawley/25/sex/gr

Dose/Route/Duration: 0, 5, 10, 20 mg/kg/oral gavage/daily for 26 wks, 5/sex retained for a 4-wk recovery period.

Drug: RP 54274 suspension prepared daily in 0.5% hydroxypropyl methylcellulose.

Batch/Lot#: C8808501/purity 100.2% pure

Results:

Mortality: Table below presents the incidence of mortalities throughout the study. A total of 17 deaths (including 1 control f), occurred during treatment wks 1-13 and one control female during recovery wks 27-30. These animals were sacrificed due to deterioration of clinical condition with toxicity signs persisting for up to 24 hr postdose. Autopsy showed no histopathology however, these deaths are considered to be drug-related.

Weeks	Group/dosage (mg/kg/day)							
	1 ^o	2 ^o	3 ^o	4 ^o	1 ^o	2 ^o	3 ^o	4 ^o
	Control	5	10	20	Control	5	10	20
Treatment period								
1-13	0	0	0	4	0	0	0	1
14-26	0	0	0	4(2)	1	1	0	4
1-26	0	0	0	8(2)	1	1	0	5
Recovery period								
27-30	0	0	0	0	1	0	0	0

() Rats originally designated to recovery group

Clinical Signs: all HD rats (m+f) showed lack of coordination and unsteady gait which lasted for several hours postdose. These signs occurred during treatment wk 1 (severe) and till end of wk 26 (moderate intensity). On occasions these signs were also observed in MD m and both LD and MD females. HD rats lacked muscle tone starting wk 12 (loss of grip, transient recumbancy with slow recovery to normal posture). HD males also were lethargic, and together with LD and MD f, showed head twitching and jerking of forelimbs and paddling all are signs of convulsion. Convulsions lasted up to 60 sec. Postdose salivation was noted in all m and f MD and HD starting wk1 and throughout the study, salivation was occasionally seen in some LD rats. Salivation was accompanied by brown peri-oral staining. During recovery wk1, 3/4 HD m were hyperactive and 1 of these rats showed tremors. No clinical signs of drug toxicity were observed during recovery wk2.

Body Weight, Food Consumption, and Water Intake: dose- and time-dependent significant decrease in body weight gain (g/rat) was noted in MD and HD m. Body weight gain was also significantly decreased in HD females though not dose-dependently, with significant difference throughout the study. Food consumption was decreased significantly in HD males during the study period but no difference was found in other treatment groups. During recovery period, food intake in HD m remained lower than that of the control. Mean water consumption was increased in HD f throughout the study but the increase was not dose dependent; no change in males.

Ophthalmoscopy: performed during wks 13 and 26 on controls and HD rats showed no drug-related effects.

Hematology: random and dose-independent effects were recorded. Significant decrease in RBC, PCV, and Hb was noted in all treated f groups during wk12 and in MD and HD f during wk26; no changes in male groups. These hematology parameters were comparable to the controls during the recovery period. Lymphocyte count and total WBC were significantly lower for HD males during the study (significant from control values on wks 4, 26, and recovery wk 4). Similar effects were not found in any other group.

Clinical Chemistry: changes were inconsistent. The table below show some of the changes that were significant. ALP levels were significantly increased in all 3 m groups during wk26 and only in HD f during wk12. LDH levels were highly inconsistent, significantly increased in HD m wk12 and not different from the control on wk26. LDH level showed a significant dose-dependent decrease in f during wk26 and during wk12 the decrease was dose-independent and noted only in MD and HD f. GPT level was significantly increased in HD m and f during wk12 (als wk4 for HD f), but not different on wk26 in either sex. GOT levels did not differ at any time period. Cholesterol was significantly increased in MD and HD m and f during wk26 and wk12 (not significant for HD m). Other effects included changes in globulin but none were dose-dependent and values were comparable to those in the control during the recovery period.

wk12

Dose mg/kg/d	glucose mg/dl	ALP mU/ml	GPT mU/ml	GOT mU/ml	LDH mU/ml	chol mg/dl
control	132 (106)	144 (94)	33 (26)	67 (61)	194 (234)	88 (84)
5	127 (104)	185 (111)	38 (25)	74 (60)	220 (186)	88 (86)
10	134 (115)	175 (112)	38 (25)	71 (56)	249 (149)**	113 (93)
20	117* (112)	191 (136)*	46** (39)*	63 (56)	366** (163)**	105 (105)**

wk 26

Dose mg/kg/d	glucose mg/dl	ALP mU/ml	GPT mU/ml	GOT mU/ml	LDH mU/ml	chol mg/dl
0	139 (103)	96 (60)	31 (33)	67 (76)	284 (519)	103 (98)
5	123 (105)	154* (62)	46 (26)	72 (61)	241 (312)**	114 (108)
10	128 (106)	134* (63)	41 (37)	69 (74)	252 (238)**	138* (127)**
20	113** (109)	126* (78)	39 (31)	53 (59)	286 (200)**	123* (127)**

Values are means, * is $p < 0.05$, ** is $p < 0.01$. Numbers in () are for females.

It is concluded that the clinical chemistry data are inconsistent throughout the study which made it difficult to assess.

Urinalysis: increase in water intake partially contributed to increase in urine volumes noted in HD males towards end of study; another factor was a decrease in the specific gravity of urine.

Organ Weight: mean ovary weight of HD females and to a lesser degree MD females (significant for HD only), was lower (26% of control) than that of the control group at end of study but no differences were found during recovery period. Also in HD females, mean adrenal weight was significantly decreased (16% of control) and remained lower than the control during the recovery period; however, no microscopical finding could be detected.

Necropsy: corpora lutea were absent in HD females (at end of study), and in MD as well as HD females (during recovery period). No other changes.

Histopathology: some liver histopathology was detected in all treated male groups with the following incidence (control 7/20, LD 15/20, MD 10/20, HD 8/10. The liver changes included centrilobular hepatocyte vacuolation and enlargement, hepatocyte necrosis/inflammation, and ballooned cells with brown pigmented macrophages in portal triad. In HD f 11/15 had occasional kupffer cells with positive pigment compared with none in the control. Spleen: hemosiderosis in MD and HD males rats killed at end of study and only in HD group killed at end of recovery period. In all treated females hemosiderosis was also seen in 5/19 LD, 13/20 MD, and 14/15 HD with 5/19 in the control at end of study and although the incidence was less, all three treated f groups killed during recovery period had hemosiderosis. Kidneys: minimal brown pigmentation in cortical tubular epithelium in HD m rats killed at end of study and in one LD female killed at end of recovery period. HD m had vacuolated cells in the anterior pituitary (2/10 vs. none in control). All treatment f groups killed during recovery, had uterus luminal dilatation with incidence in HD of 3/5 compared with 0/5 in the control. The cause of death in one LD f was due to lymphoblastic lymphosarcoma. No other significant drug-related histopathology was detected in any group.

Summary:

Oral administration of Riluzole at 5, 10, and 20 mg/kg to rats for 26 wks caused a total of 17 deaths in HD animals and one in LD. The cause of death was related to worsening of clinical signs and in the LD animal to lymphoblastic lymphosarcoma. Both the HD and MD are considered toxic therefore, a NOEL could not be determined in this study; the 5 mg/kg may be considered a LOEL based on presence of clinical signs and histopathology in liver, kidney, and spleen.

13-wk Baboon:

Study #DN101577

GLP: signed and dated 10/1985

Species/strain: Papio Baboons/3/sex/gr

Dose/Route/Duration: 0, 3, 10, 30/25 mg/kg/oral gavage/daily for 13 wks. Dose reduced to 25 mg/kg on day 45 of study due to toxicity at 30 mg/kg.

Drug: RP 54274 suspended in 1% methyl cellulose*.

Batch/Lot#: 4PC164/99.5% pure

* analytical results on wks 1, 6, 9, and 13 showed only 50-69% of the nominal concentration for the LD. This finding was associated with settling out of the drug material from the vehicle. Results were acceptable for the MD and HD. The sponsor stated that these results are of no significant outcome.

Results:

Mortality: one HD female on day 16.

Clinical Signs: only in HD animals included: piloerection, ataxia, and heavy sedation for several hr; these signs were more common in females than males. In the female that was sacrificed, 2 hr after dosing the animal was hypothermic, showed tachycardia, slow shallow respiration, and was heavily sedated. The animal was not dosed on days 12, 13, and 14 and recovery was almost complete. However, when dosing resumed on day 15, the signs returned and the animal was sacrificed on day 16.

Body Weight and Food Consumption: both parameters were reduced in all treated animals and body weight was decreased in females only. None of the effects were significantly different from the control.

Ophthalmoscopy: subjective examination on wks 3 and 6 showed retinal and optic disk defects, and increase in intraocular pressure in all treated animals. However, actual examination/measurement on wk 7 revealed no treatment related

changes in any parameter. The earlier observations were considered by the sponsor to be artifacts or transitory.

Hematology, Clinical Chemistry*, Urinalysis, and Necropsy: no drug related effects.

* the only change is that in the HD female killed on wk 3: marked increase in SGOT (12.6 fold > predose), and SGPT (3.7 fold > predose).

Histopathology: no treatment related effects. The following were found in the HD female that was sacrificed: thymus involution and spleen follicular involution (both marked), also hemolymphoreticular changes in the adrenals. These changes were considered stress related and non-specific therefore, of no consequence.

Summary:

Oral administration of 3, 10, and 30/25 mg/kg Riluzole to baboons for 13 wks caused death in one HD female. Signs of toxicity were observed only in HD and included piloerection, ataxia, and heavy sedation for several hr; these signs were more common in females than males. Drug-related changes in ophthalmoscopy and tissue histopathology were observed only in HD animals and fell within the normal range for these animals. The NOEL in this study is 10 mg/kg/day.

26-wk Monkey

Study #DN104374

GLP: signed and dated 1/1990

Species/strain: wild-caught Cynomolgus monkey/5-7/sex/gr

Dose/Route/Duration: 0, 5, 10, 20 mg/kg/oral/daily for 26 wks.

Drug: RP 54274 gelatin capsules.

Batch/Lot#: C8808501/100.2% pure

Results:

Mortality: all HD animals were sacrificed between wks 5 and 9 due to toxic signs.

Clinical Signs: one or more of the following signs was noted in all treatment groups: quietness, huddled posture, lethargy* (reluctance and unwillingness to move), incoordination/unsteadiness*, vacant expression* (characterized by staring and unawareness of surroundings), vomiting within 1 hr of dosing, salivation, piloerection (including the controls), and isolated incidences of limb tremors. Single incidence of ptosis, pupil dilation, REM, erratic movement, and repetitive rocking movements were noted only in HD. * occurred within 1 hr of dosing during wks 1-8.

Body Weight and Food Consumption: no significant difference in body weight between controls and LD and MD animals. Food consumption however, decreased in these groups during the first few weeks of treatment, subsequently, consumption was similar to that in the control animals. Food intake decreased in LD animals between wks 1 and 11 and wk 6 for MD. HD animals showed variations in body weight and food intake compared with the controls and predose levels. Food intake was reduced in all HD animals till sacrificed on wk 8. Clinical signs such as lethargy and quietness affected appetite (decreased) which contributed to the loss in body weight.

Organ Weight: dose-dependent increase in relative liver weight. Mean kidney weight in MD group was slightly higher than that of the control.

Ophthalmoscopy: conducted pre-study, and weeks 6, 13, and 26 using Keeler direct ophthalmoscope. No drug-related effects.

Electrocardiography: recorded twice before study, and 1, 3, 6, 13, and 25 wks of study. No changes due to treatment.

Hematology: RBC, PCV, and Hb levels in all groups were slightly but significantly decreased (6-8%), on wk 5, 12, and 25; some of these parameters were significantly reduced prior to dosing. APTT in MD group was increased 14-15% over the control values during wks 12 and 25. Lymphocyte count was significantly reduced in MD during wks 5 and 25 (23%), and in HD (34%) during wk 5.

Clinical Chemistry: during wks 12 and 25 there was a slight decrease in inorganic phosphorous (significant only in MD during wk 12), and cholesterol in all treated groups compared with the control. Potassium levels were significantly increased 11-23% in MD and HD during wk5. Cholesterol level was decreased in all treated groups compared with the control values throughout the study with significant difference only in HD during wk5.

Urinalysis: no drug-related effects.

Necropsy: no treatment related changes.

Histopathology: fat droplets in hepatocytes with vacuolation were found in 2/5 MD females and 1/7 male and 2/7 female HD however, this was related to poor appetite and drug-unrelated. HD animals had thymic involution in 3/7 males and 5/7 females related to stress. Other findings in HD included marked lung fibrosis 2/7 females and 1/7 males, moderate to marked thymus involution 2-3/7 animals, sinus histiocytosis in 3/7 males and 1/7 females, cortical scarring in the kidneys in 1/4 females, 3/5 MD males had moderate cortical kidney scarring, 3/5 LD and 1/5 MD females had parovarian and multilocular cysts, and one female had dilated ovarian bursa.

Summary:

Oral administration of 5, 10, and 20 mg/kg Riluzole to monkeys for 26 wk caused death in all HD animals and toxicity in the other two doses consequently, a NOEL could not be identified. Signs of toxicity included quietness, huddled posture, lethargy (reluctance and unwillingness to move), incoordination/unsteadiness, vacant expression (characterized by staring and unawareness of surroundings), vomiting within 1 hr of dosing, salivation, piloerection (including the controls), and isolated incidences of limb tremors. Single incidence of ptosis, pupil dilation, REM, erratic movement, and repetitive rocking movements were noted only in HD. No drug related changes in ECG, ophthalmoscopy, urinalysis, and minimal and dose-independent changes in hematology, clinical chemistry, body weight, and food consumption. The liver appears to be the target organ for toxicity as indicated by the increase in relative weight and the accompanied histopathology. A second study was conducted to determine NOEL in monkeys (#DN105147). The NOEL is 2 mg/kg/d for 26 wk oral dosing.

2-wk dog:

Report #410

Laborat

GLP: signed and dated 3/1993

Species/strain: pure-bred beagle dogs/3/sex/gr

Dose/Route/Duration: 0 and 30 mg/kg/oral gavage/daily for 14 days.

Drug: RP 54274 in gelatin capsules

Batch/Lot#: C8808501/100% pure

Results:

Mortality: none

Clinical Signs: vomiting in all 3 treated males and females 1-3 hr postdose, CNS signs included ataxia, hypomotility, hyporeactivity, tremors, and somnolence. These signs disappeared within 5-24 hr postdose.

Body Weight and Food Consumption: no marked changes except in one male, body weight decreased 8% on days 7 and 14 and similarly, food intake in this animal was reduced 50-95% on days 9, 10, and 11.

Hematology: RBC count is decreased in males (15-29%) and females (15-43%) on days 8 and 14, Hb and Hct were also reduced (10-25% in males and 19-33% in females. MCV was slightly increased 10-13% in both sexes and thrombocyte (39-60%) and reticulocyte (8-27 fold) counts were elevated as well. Fibrinogen levels increased in males and females on day 8 (53 and 44%) and day 14 (135 and 123%) respectively. Haptoglobin concentration was decreased on day 8 (77 and 80% m and f), and on day 14 it decreased 100% for both sexes. This change as stated by the sponsor, is indicative of hemolytic process as it correlated with hemogram changes. Myelogram parameters were also increased as follows for males and females: basophilic (50-54%), polychromatophilic (93-77%), and

acidophilic normoblasts (108-41%); these changes also correlated with hemogram changes and associated with regenerative anemia.

Clinical Chemistry: no significant changes, however, the following were found: ALP levels were increased in males and females on days 5 (51,11%), 8 (39,13%), and 14 (53,17%) respectively; LDH level was increased in males on days 8 (169%) and 14 (106%) and in females only on day 14 (88%), and total bilirubin was increased 167-200% on days 8 and 14 in both sexes.

Urinalysis: no change except one male had increased urinary bilirubin at end of study; considered secondary to increase in serum bilirubin.

Necropsy: no effect.

Histopathology: hemosiderosis of the liver and spleen noted in all treated animals; considered secondary to drug-induced hemolytic anemia.

Summary:

Riluzole at 30 mg/kg oral dose caused no mortalities. At this dose it produced vomiting, tremors, ataxia, hypomotility, hyporeactivity, and somnolence in both sexes. These signs disappeared within 5-24 hr postdose. Changes in hematology parameters included decrease in RBC count, Hb and Hct, MCV was slightly increased, and thrombocyte and reticulocyte counts were elevated. Collectively these changes were reported by the sponsor, to be secondary to hemolysis as supported by the increase in bone marrow normoblasts and hemosiderosis found in the liver and spleen. No gross or histopathology was observed.

In a PK study, 10 mg/kg oral dose produced peak plasma level that ranged between 2.8-4.8 µg equivalents/ml at 2 hr postdose.

VI. REPRODUCTIVE AND DEVELOPMENTAL STUDIES:

The following studies were conducted:

- Oral Fertility and Range-Finding study
Rat
- Oral Teratology study - Segment II
Rat
Rabbit
- No Segment III studies were submitted.

Oral Fertility and Range-finding study in the rat.
Study #DN101588/Report #3466-R1-36

GLP: signed and dated 10/1985

Species/strain: Sprague-Dawley m and f rats, 6/dose; rats were 12 wks of age at study initiation.

Dose/Route/Duration: 0, 3, 10, 30*/25 mg/kg/oral gavage/d, males dosed 2 wks prior to mating and during mating and females dosing continued throughout gestation, lactation, and till day 14 post-partum.

* dose reduced to 25 mg/kg/d on day 3 of study due to toxic signs.

Drug: RP 54274 in 1% aqueous solution of methyl cellulose.

Batch/Lot#: 4PC-164

Results:

Mortality and Clinical Signs: 4 of 6 HD m were sacrificed and 1 died due to severe toxic signs. Because of these adverse effects, HD was reduced to 25 mg/kg on day 3. The rest of HD m were killed or died during treatment wk3. No deaths occurred in treated f or LD and MD m. Clinical signs in HD m included languid and ataxia starting 2-3 hr postdose in all rats, 2 rats did not recover and the rest recovered but the signs recurred within 45 min of the 2nd dose. Decreasing the dose to 15 mg/kg helped reduce the severity of these signs but during wk 3, animal condition deteriorated and 2 rats were killed and one died, the remaining 3 survived till study ended (one HD m was killed on wk 1 but was replaced). Ataxia was noted in 5/6 MD m within 3 hr of

dosing, all but one recovered and no signs occurred thereafter; one rat was aggressive for 3 days on wk3. No clinical signs noted in LD m or controls. Similar to HD m, HD f showed toxicity signs but they were less severe following 30 mg/kg, and reduction of dose to 25 mg/kg caused no clinical signs. One of 6 MD f had ataxia 3 hr after the 1st dose but no other signs. Clinical signs were absent in LD and controls f.

Body Weight and Food Consumption: Body weight gain was significantly reduced in HD m over the entire treatment period relative to the controls (11 and 18% respectively). Insignificant decrease is also noted in LD and MD m during wks 1 and 3 but by necropsy, all weights were comparable to the controls. No significant changes in body weight was observed in HD f (small decline during pre-mating, gestation, and lactation). Body weight is generally higher in MD and LD f relative to the controls. Reduced body weight in HD m was accompanied by significant reduction in food intake. No change was noted in LD and MD m or f. HD f ate 7-15% less during lactation than the controls.

The following Table lists the parameters examined and their findings:

Parameter	Findings
mating	no effect except 3 HD m could not mate due to clinical condition. Consequently the other 3 HD m were used twice. Only 1/6 HD f required 2 estrus cycles prior to insemination, the rest required only one.
Fertility index/Fecundity index	the same in all groups (83%), all f in each group gave birth to live pups*; no effect on duration of gestation (21-22 qd).
sex ratio	no effect, all results were comparable.
clinical condition of pups	no drug effect and no effect on incidence of abnormalities in the dead or live pups.
growth development/pinna unfolding/hair growth/incisor eruption/eye opening*	none was affected by treatment except the eye opening could not be fully assessed due to early termination but it seems that the onset was slightly delayed in HD rats relative to the other groups and the controls.
mean pup weight	at birth and post-partum day 4 was comparable in all groups. On post-partum days 7 and 14 weight in HD was less than the controls and only 85% of the weight of the controls on day 14.
necropsy	no abnormalities were detected. A high incidence of hydronephrosis occurred in all groups including the control.

* % pre-weaning loss in the control is 26% (43±8.6 alive d14 vs. 58±11.6 born), which is high and no explanation was provided; the corresponding values in LD/MD/HD are 28/26/23%, respectively. The pre-weaning loss in all treatment

groups is high although not dose-dependent and is similar to that in the control. No conclusion can be made however, irrespective of the control data, the loss in treatment groups is high and must be addressed by the sponsor.

Data in Tables 3.2, 5.1, and 5.2 are misrepresented, they must be resubmitted (Tables are attached).

Summary and Conclusion:

Riluzole administered orally to rats at 3, 10, 30/25 mg/kg caused death and severe clinical signs in HD animals, signs also occurred in MD but were less severe and there were no deaths reported in MD. The 3 mg/kg can be considered a NOEL since the drug had no effect in this group. Most of the toxic effects occurred in HD. Males of HD were more sensitive to the drug than females. There were no effect on mating index, fertility index or gestation index (3/6 HD m were unable to mate due to clinical signs therefore, the other 3 m were used twice). Body weight and food consumption were significantly reduced in HD m. Dams in all groups gave birth to live fetuses, the pups in HD weighed less than the controls on post-partum days 7 and 14 and some indication of slower growth was noted in HD as assessed by opening of the eyes compared with the control and other treatment groups. No drug effect on necropsy.

The sponsor proposed using two different HD doses in m and f in the fertility study based on the sex differences noted in the present study with doses not to exceed 30 mg/kg in f and 20 mg/kg in m.

This study can not replace Segment I and a standard Segment I must therefore, be submitted.

Comments:

This is a "range-finding" study; a Segment I study was not submitted. If this study is considered by the sponsor to be a Segment I, it can not be accepted by the reviewer because it failed the FDA guidelines as indicated below:

- According to the guidelines, males are dosed 60-80 days prior to mating. However, male rats in this study were dosed for only 2 wks prior to mating.
- The number of m and f must be at least 20 per group, only 6/sex/dose was used in this study.
- The guidelines suggests sacrifice of one half of the dams on gd 13 and the other half after weaning of offspring. In this study all dams were allowed to litter and rear their offspring and were killed on postpartum day 14.
- Number of corpora lutea and implantation sites were not assessed (they were evaluated in Segment II).

The following are general comments:

- Data in Tables 3.2, 5.1, and 5.2 are misrepresented, they must be resubmitted/see detail above.
- Dose formulation was not analyzed in this study; no explanation was provided.
- Stability of dose formulation was not conducted. The sponsor indicated that this was unnecessary because the drug was prepared daily and administered to the rats shortly after preparation.
- Proof of absorption of the drug was not performed; no explanation was provided.

oral fertility in the rat
study 101588

TABLE 3 (continued)

3.2 Group mean indices

Parameter	Group 1	Group 2	Group 3	Group 4
Mating index %	100	100	100	83.3
Fertility index %	83.3	83.3	83.3	83.3
Secundity index %	83.3	83.3	83.3	83.3
Gestation index %	100	100	100	100
Live birth index %	98.3	100	98.5	100
Viability index 1 %	87.7	100	98.5	98.2
Viability index 2 %	100	100	100	100
Viability index 3 %	100	100	100	97.7
% pre-weaning loss	15.7	0	3.8	4.9

incorrect data

this isn't zero

that many controls died?

study #101588

58-43
58

APPENDIX 5

Individual littering data

5.1 Pup numbers

Group number	Sire number	Duration of gestation (days)	Number of pups born		Number of pups alive day 1		Number of pups alive day 4		Number of pups alive day 7		Number of pups alive day 16		
			M	F	M	F	M	F	M	F	M	F	
1	25	22	5	5	5	5	4	5	4	5	4	5	
	26	22	5	0	5	0	5	0	5	0	5	3	
	27	23	2	2	2	2	2	2	2	2	2	5	
	28	22	9	5	10	5	8	5	8	5	4	5	
	29	Net pregnant											
	30	21	7	10	3	4	7	4	7	4	3	4	
Total Mean		22.0	58	11.6	57	11.4	50	10.0	50	10.0	43	8.6 pup/dam	
2	31	22	5	10	5	10	5	10	5	10	4	6	
	32	Net pregnant											
	33	22	4	5	4	5	4	5	4	5	4	3	
	34	21	6	9	6	9	6	9	6	9	5	5	
	35	22	7	5	7	5	7	5	7	5	2	5	
	36	22	5	9	5	9	5	9	5	9	5	5	
Total Mean		21.0	69	13.0	69	11.0	69	13.0	69	13.0	50	10.0	
3	37	22	5	8	5	8	5	8	5	8	5	5	
	38	21	4	9	4	9	4	9	4	9	4	6	
	39	Net pregnant											
	40	21	6	9	6	9	6	9	6	9	6	5	
	41	22	3	10	4	8	4	8	4	8	4	6	
	42	22	10	4	10	4	10	4	10	4	10	6	
Total Mean		21.6	60	13.6	67	13.4	66	13.2	66	13.2	50	10.0	
4	43	21	7	7	7	7	6	7	6	7	5	4	
	44	Net pregnant											
	45	22	3	10	3	10	3	10	3	10	3	7	
	46	21	7	8	7	8	7	8	7	8	5	5	
	47	22	3	1	3	1	3	1	3	1	3	1	
	48	21	4	6	4	6	4	6	4	6	4	6	
Total Mean		21.4	56	11.2	56	11.2	55	11.0	55	11.0	43	8.6	

6 one pup wh.-sired day 0

Study 101588

APPENDIX 5 (continued)

5.2 Individual indices

Group number	Sam number	Live birth index	Viability index 1	Viability index 2	Viability index 3	Pre-weaning loss	
1	25	100	90	100	100	10.0	
	26	100	100	100	100	0.0	
	27	100	100	100	100	0.0	
	28	92.9	100	100	100	9.1	
	29	Not pregnant					
	30	100	64.7	100	100	37.5	
2	31	100	100	100	100	0.0	
	32	Not pregnant					
	33	100	100	100	100	0.0	
	34	100	100	100	100	0.0	
	35	100	100	100	100	0.0	
	36	100	100	100	100	0.0	
3	37	100	100	100	100	9.0	
	38	100	100	100	100	0.0	
	39	Not pregnant					
	40	100	93.3	100	100	9.1	
	41	92.3	100	100	100	9.1	
	42	100	100	100	100	9.0	
4	43	100	92.9	100	90.0	19.2	
	44	Not pregnant					
	45	100	100	100	100	0.0	
	46	100	100	100	100	0.0	
	47	100	100	100	100	0.0	
	48	100	100	100	100	0.0	

Oral Teratology study in the Rat - Segment II

Study #DN101589/Report #3875-81-38

GLP: signed and dated 10/1989

Species/strain: Sprague-Dawley sexually mature virgin f rats, 24/dose; rats were 222-269 g at mating.

Dose/Route/Duration: 0, 3, 9, 27 mg/kg/oral gavage/d, 1-2f mated with 1m over a period of 6 days, mated f were dosed daily from gd 6-15 (inclusive) and maintained without dosing till gd 20 then sacrificed.

Drug: RP 54274 in 1% aqueous solution of methyl cellulose.

Batch/Lot#: C1100

Results:

Mortality: 2/24 HD were killed on gd 7 due to clinical signs (prostration, lethargy, and cold to touch). No other deaths were reported.

Clinical Signs: langour for upto 50 min observed in 14/25 HD rats 1 hr postdose and in most of the animals it occurred only on the 1st day of dosing. No other significant drug-related signs noted in any other treatment group.

Body Weight and Food Consumption: a significant dose-related decrease noted in MD and HD relative to the control over gd 6-15 (dosing period). The reduction in weight was also noted throughout the gestation period as well as when adjusted for gravid uterus weight ($p < 0.05$ gd 0-20). No weight change noted in LD except a significant reduction noted pre-dosing on days 0-6 this has no toxicological significance. Weight was similar in all groups after end of dosing and until sacrifice. Reduced food intake accompanied the decrease in weight noted in HD but intake was similar to the control after the end of dosing. No change noted in LD or MD.

Litter and Fetal Weights and Crown/Rump Length: insignificant and dose-independent decrease in mean litter weight was found in MD and HD. This decrease was related in MD to the smaller litter size of this group and to significantly smaller fetal weight in HD ($p < 0.05$). The crown/rump length was also less in HD than the control but the difference was not significant (see attached Table).

Necropsy: no drug-related findings were detected in any group.

The attached Table present maternal and fetal data. No significance in post-implantation loss, but a significant increase in pre-implantation loss noted in MD and HD relative to the control. Number of corpora lutea in MD and HD was less than that in the control but the difference is insignificant.

Necropsy as indicated above, did not show any differences in fetal defects in treated vs. control group. One major malformation of blood vessels (preductal coarctation of the aorta), and 5 animals had enlarged atria (minor malformation) in MD (attached Table). High incidence of skeletal ossification was found in all groups including the controls but in general, the minor defects were higher in MD and HD relative to the control. However, this difference was not dose-related and not statistically significant. Historical control table is also attached.

Summary and Conclusion:

Riluzole administered orally to f rats at 3, 9, or 27 mg/kg during gd 6-15 caused 2 deaths in HD animals. Clinical signs were noted only in HD and included langour. A significant dose-related decrease in body weight was noted in MD and HD relative to the control over gd 6-15 (dosing period). The reduction in weight was also noted throughout the gestation period and when adjusted for gravid uterus weight ($p < 0.05$ gd 0-20). No weight change noted in

Study # DN101589 Rat

TABLE 4
Group mean caesarian data

Parameter	Group 1	Group 2	Group 3	Group 4
a) Uterine/implantation data				
Number of pregnancies	21/24	22/24	20/24	20/24
Percentage pregnancies	87.5	91.7	83.3	83.3
Number of pregnant animals surviving to day 20	21	22	20	19
Number of corpora lutea	332	356	316	301 ←
Mean number per dam	15.0	16.2	15.8	16.7
Number of implantations	317	322	200	265 ←
Mean number per dam	15.1	14.6	14.0	14.7
% pre-implantation loss	4.5	9.6	11.4*	12.0** ←
Number of early intrauterine deaths	10	13	9	0 ←
Mean number per dam	0.48	0.59	0.45	0.44
Number of late intrauterine deaths	4	1	3	1
Mean number per dam	0.19	0.05	0.15	0.06
% post-implantation loss	4.4	4.3	4.3	3.4
Number of foetuses	303	308	268	256 ←
Mean number per dam	14.4	14.0	13.4	14.2
% of implantations	95.6	95.7	95.7	96.6

Significantly different from control: * p<0.05
** p<0.01

Study # DN101589 Rat

TABLE 4 (continued)

b) litter data

Parameter	Group 1	Group 2	Group 3	Group 4
Mean number of fetuses per dam	14.4	14.0	13.4	14.2
Number of male fetuses	166	155	149	146
Number of female fetuses	137	153	119	125
Sex ratio: females:males	0.83	0.99	0.80	0.86
Mean litter weight (g)	49.6	48.8	45.8	46.3
Mean foetal weight (g)	3.4	3.5	3.4	3.3* ← ?
Mean foetal weight (g) - males only	3.5	3.6	3.5	3.3
Mean foetal weight (g) - females only	3.4	3.4	3.3	3.2
Mean crown/rump length (mm)	37.2	37.5	37.3	36.6

* significantly different from controls: p<0.05

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Study # 101589 Rat

TABLE 4 (continued)

c) Foetal defect data

Parameter	Group 1	Group 2	Group 3	Group 4
EXTERNAL AND VISCERAL DEFECTS				
Number of foetuses examined	303	308	268	271
Number showing minor defects only	46	30**	40	33
% of foetuses examined	15.2	9.7	14.9	12.2
Number showing major defects	2	2	2	1
% of foetuses examined	0.7	0.6	0.7	0.4
SKELETAL DEFECTS				
Number of foetuses examined	211	213	185	187
Number showing minor defects only	144	145	140	132
% of foetuses examined	68.2	68.1	75.7	70.6
Number showing major defects	0	0	0	0
% of foetuses examined	0.0	0.0	0.0	0.0
VARIANTS				
Number of foetuses examined	211	213	185	187
Number showing variants	171	162	160	160
% of foetuses examined	81.0	76.1	86.5	85.6

** significantly different from control: p<0.01

TABLE 5

Fetal defects (key, nature and incidence)

Key	Type of defect	Classification	Group 1	Group 2	Group 3	Group 4
EXTERNAL/VISCERAL DEFECTS						
Body						
1	Subcutaneous haemorrhagic areas	minor	10 (5.9)	7 (2.3)	23 (8.6)	12 (4.4)
Head						
2	Micropthalmia, left eye	major	1 (0.3)	1 (0.4)	1 (0.4)	1 (0.4)
4	Bilateral lens opacities	variant	14 (4.6)	10 (3.2)	9 (3.4)	4 (1.5)
5	Bilateral lens opacity	variant	3 (1.0)	1 (0.3)	1 (0.4)	
Thorax						
3	Major malformation of great vessels: preductal coarctation of aorta, abnormal route of left common carotid, innominate artery not branched	major			1 (0.4)	
4	Atria enlarged	minor	1 (0.3)	1 (0.3)	5 (1.9)	1 (0.4)
5	Atrium enlarged	minor		1 (0.3)	1 (0.4)	2 (0.7)
6	Atrium reduced	minor				2 (0.7)
Abdomen						
7	Bilateral hydronephrosis	major		1 (0.3)		1 (0.4)
8	Unilateral hydronephrosis	major	2 (0.7)			
9	Bilateral slight hydronephrosis	minor	1 (0.3)			
10	Right kidney elongated	minor			1 (0.4)	
11	Right kidney reduced in size	minor		1 (0.3)		
12	Haemorrhagic appearance of adrenal	minor			1 (0.4)	
13	Bilateral increased renal pelvic cavitation	minor	8 (2.6)	11 (3.6)	1 (0.4)	10 (3.7)

Figures in brackets denote the percentage of affected foetuses/foetuses examined. A foetus with multiple defects will appear more than once in this tabulation.

study 101589 Rat

TABLE 5 (continued)

Key	Type of defect	Classification	Group 1	Group 2	Group 3	Group 4
14	Unilateral increased renal pelvic cavitation	minor	3 (1.0)		3 (1.1)	
15	Bilateral dilated ureters	minor	18 (5.9)	12 (3.9)	6 (2.2)	10 (3.7)
16	Unilateral dilated ureter	minor	2 (0.7)			
SKELETAL DEFECTS						
<u>Skull</u>						
17	Hyoid not ossified	minor	1 (0.5)		1 (0.5)	
18	Basals incompletely ossified	minor	21(10.0)	13 (6.1)	5 (2.7)	30(16.0) ↓
19	Frontals incompletely ossified	minor	23(10.9)	18 (8.5)	32(17.3) ↓	34(18.2) ↓
20	Parietals incompletely ossified	minor	10 (4.7)	18 (8.5) ↓	21(11.4) ↓	14 (7.5)
21	Cleft in parietal	minor	1 (0.5)			
22	Interparietal incompletely ossified	minor	103(48.8)	97(45.5)	85(45.9)	78(41.7)
23	Cleft in interparietal	minor	6 (2.8)	6 (3.0)	10 (5.4)	13 (7.0) ↓
24	Fissure in interparietal	minor	1 (0.5)	1 (0.5)		
25	Occipital incompletely ossified	minor	63(29.9)	66(31.0)	61(33.0)	66(35.3)
26	Zygomatic arch incompletely ossified	minor		1 (0.5)	3 (1.6)	
26	All skull bones incompletely ossified	minor		4 (1.9)	4 (2.2)	2 (1.1)
<u>Vertebral arches/centrae</u>						
27	Thoracic vertebral centra 1 incompletely/not ossified	minor	4 (1.9)	4 (1.9)	1 (0.5)	4 (2.1)

Figures in brackets denote the percentage of affected fetuses/foetuses examined. A fetus with multiple defects will appear more than once in this tabulation.

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Table 5 (continued)

Key	Type of defect	Classification	Group 1	Group 2	Group 3	Group 4
28	Thoracic vertebral centra 1 bipartite	minor			1 (0.5)	
29	Thoracic vertebral centra 2 incompletely/ not ossified	minor	1 (0.5)			1 (0.5)
30	Thoracic vertebral centra 3 bipartite	minor	1 (0.5)			
31	Thoracic vertebral centra 11 asymmetrically ossified	minor	1 (0.5)			
32	Thoracic vertebral centra 12 asymmetrically ossified	minor	1 (0.5)			
c	Thoracic vertebral centra in region 10-13 bipartite	variant	1 (0.5)			1 (0.5)
33	Lumbar vertebral arches 5 and 6 R incompletely ossified	minor			1 (0.5)	1 (0.5)
34	Sacral centra 4 not ossified	minor				1 (0.5)
35	Ribs					
35	Wavy ribs	minor		1 (0.5)	2 (1.1)	
36	Kinked ribs	minor		2 (0.9)	2 (1.1)	3 (1.6) ←
37	Incompletely ossified ribs	minor	1 (0.5)	2 (0.9)	2 (1.1)	
61	13th rib not ossified	minor			1 (0.5)	
38	13th rib reduced in size	minor	1 (0.5)	4 (1.9) ←	5 (2.7) ←	3 (1.6) ←
39	13th pair of ribs reduced in size	minor			4 (2.2) ←	1 (0.5)
40	Extra rib	minor	4 (1.9)	4 (1.9)	2 (1.1)	4 (2.1)
41	Extra pair of ribs	minor	3 (1.4)	1 (0.5)	2 (1.1)	
42	Extra pair of ribs on 7th cervical vertebra	minor		1 (0.5)		
	<u>Sternum</u>					
43	Only 2 sternbrae ossified	minor			1 (0.5)	
44	Sternum 1 incompletely ossified	minor		5 (2.3) ←	1 (0.5)	3 (1.6) ←
45	Sternum 1 asymmetrically ossified	minor			1 (0.5)	
45	Sternum 2 incompletely/not ossified	minor	13 (6.2)	10 (4.7)	7 (3.8)	11 (5.9)

Figures in brackets denote the percentage of affected fetuses/fetuses examined. A fetus with multiple defects will appear more than once in this tabulation.

TABLE 5 (continued)

Key	Type of defect	Classification	Group 1	Group 2	Group 3	Group 4
47	Sterebra 2 asymmetrically ossified	minor	5 (2.4)	4 (1.9)	5 (2.7)	4 (2.1)
48	Sterebra 3 incompletely/not ossified	minor	1 (0.5)	0 (3.0) ←	1 (0.5)	3 (1.6)
49	Sterebra 3 asymmetrically ossified	minor	8 (3.8)	9 (4.2)	12 (6.5)	2 (1.1)
50	Sterebra 4 incompletely/not ossified	minor	2 (0.9)	5 (2.3)	3 (1.6)	3 (1.6)
51	Sterebra 4 asymmetrically ossified	minor	26 (12.3)	19 (8.9)	27 (14.6)	11 (5.9)
52	Sterebra 5 bipartite	minor	3 (1.4)		2 (1.1)	1 (0.5)
53	Sterebra 5 asymmetrically ossified	minor	7 (3.3)	5 (2.3)	7 (3.8)	1 (0.5)
4	Sterebra 5 incompletely/not ossified	variant	100 (47.4)	112 (52.6)	94 (50.8)	112 (59.9)
6	Sterebra 6 incompletely/not ossified	variant	127 (60.2)	115 (54.0)	118 (63.8)	117 (62.6)
Pelvic girdle						
54	Pubis incompletely/not ossified	minor	15 (7.1)	4 (1.9)	5 (2.7)	8 (4.3)
55	Ischium incompletely/not ossified	minor	3 (1.4)	1 (0.5)		5 (2.7)
56	Bilateral insertion of pelvic girdle on 2nd sacral vertebra	minor		1 (0.5)	2 (1.1)	1 (0.5)
Limbs						
57	All limb bones foreshortened	minor		1 (0.5)		
58	Metacarpals bipartite	minor		1 (0.5)		
59	Metatarsals incompletely ossified	minor	2 (0.9)	3 (1.4)	3 (1.6)	3 (1.6)
f	Metacarpals incompletely ossified	variant	119 (56.4)	105 (49.3)	110 (59.5) ←	116 (62.0) ←
g	Forelimb phalanges not ossified	variant	98 (46.4)	111 (52.1)	94 (50.8)	98 (47.1)

Figures in brackets denote the percentage of affected fetuses/foetuses examined. A fetus with multiple defects will appear more than once in this tabulation.

81/38
 more # fetuses examined?
 since it's larger than 664)
 listed under CNTR. gr.?
 Because the affected fetuses are
 more in this gr. than in control!

Study 101589

study 101589 Rat

APPENDIX 16

NLE cumulative data on CD Sprague-Dawley derived rats supplied by Charles River (UK) Ltd., Margate, Kent. Correct to February 1984

a) General data

Parameter	Control (vehicle tested)	Animals from inactive treatments	Combined vehicle treated animals and animals from inactive treatments	Positive control (Acetyl-salicylic acid 250 mg/kg/day)
Number of pregnant dams	345/377	684/744	1007/1125	25/26
% pregnancy incidence	91.5	91.4	90.5	96.2
<u>Corpora lutea</u>				
Mean number per dam	15.2	15.2	15.2	15.7
Total number	5231	10405	15436	392
Range of individual values	4-24	1-29	1-29	13-23
<u>Implantations</u>				
Mean number per dam	13.6	13.9	13.8	14.0
Total number	4702	9531	14233	350
Range of individual values	1-19	1-29	1-19	5-16
<u>Pre-implantation loss</u>				
Mean percentage loss	10.1	8.6	9.0	10.7
Range of individual values	0.0-88.9	0.0-90.9	0.0-90.9	0.0-68.8
Range of group values	6.4-19.0	0.8-15.3	0.8-19.0	10.7
<u>FOETUSES</u>				
Mean number per dam	13.1	13.4	13.3	7.9
Total number	4504	9160	13664	197
Range of individual values	1-19	1-19	1-19	0-15
Percentage of implantations	95.8	96.1	96.0	56.3
<u>Early intrauterine deaths</u>				
Mean number per dam	0.5	0.4	0.5	5.3
Total number	169	297	466	132
Range of individual values	0-12	0-6	0-12	0-15
Percentage of implantations	3.6	3.1	3.3	37.7
<u>Late intrauterine deaths</u>				
Mean number per dam	0.1	0.1	0.1	0.8
Total number	27	72	99	21
Range of individual values	0-2	0-6	0-6	0-7
Percentage of implantations	0.6	0.8	0.7	6.0
<u>Total intrauterine deaths (post-implantation loss)</u>				
Mean number per dam	0.6	0.5	0.5	6.1
Total number	196	369	565	153
Range of individual values	0-12	0-10	0-12	0-15
Range of group % values	0.6-8.0	0.3-7.8	0.3-8.0	
Percentage of implantations	4.2	3.9	4.0	43.7

LD. Food consumption was significantly decreased in HD only. Mean litter weight was slightly and insignificantly lower in MD and HD relative to the control. The smaller weight in MD was attributed to the smaller litter size of this group and in HD it was associated with the smaller fetal weight ($p < 0.05$). Fetal crown/rump length was also less in HD (insignificant) relative to the control. There were no drug-related significant abnormalities in fetuses or pups, only some scattered findings. Incidence of skeletal incomplete ossification particularly interparietal and occipital bones, was found in all groups including the control but was higher than the background data. The sponsor indicated that this finding maybe related to the smaller fetuses and therefore drug-unrelated. The 3 mg/kg can be considered as NOEL. Based on these findings, Riluzole at the doses tested is not teratogenic in the rat.

Comments:

- analysis of the test article on days 1, 4, 16, and 20 showed concentrations below acceptable limits (73%). This was attributed to "settling out" of the drug from suspension and filtering by "fibers" of methyl cellulose. The formulations were agitated vigorously throughout administration and the sponsor indicated that animals received homogeneous doses.
- stability of the test article formulation was not measured because, as indicated by the sponsor, it was prepared daily and administered shortly after preparation.
- based on the guidelines, pregnant f are examined at least 2x/daily for signs of ill being, toxicity, and behavior. In this study, f were examined only once/day.

Oral Teratology Study in the Rabbit

Study #DN101576/Report #3888-81-42

GLP: signed and dated 10/1985

Species/strain: New-Zealand white sexually mature f (3-4.1 kg)/n=16/dose

Dose/Route/Duration: 0, 3, 18, 60 mg/kg/d/oral-gavage/dosed daily from gd 5-18 (inclusive), without drug until gd 28 and sacrificed on this day. Each lf mated with 1m over a 6-day mating period.

Drug: RP 54274 in 1% aqueous solution of methyl cellulose.

Batch/Lot#: C1100

Results:

Mortality: no drug-related mortalities were reported. The following were contributed to difficulty in dosing or lung abnormalities. 1LD, 1MD aborted, 2cont, 2LD, 1MD, and 1HD died or killed. One additional control animal was killed due to delivery prior to scheduled necropsy. It was found that this was due to incorrect assessment of mating time.

Clinical Signs: all HD had drug-related signs mainly langour and/or ataxia and some prostration. Signs occurred immediately after dosing and gds 14-18. Only 1MD and on single occasion showed similar signs to those in HD. 1LD showed langour and another was prostrate, both rabbits were killed due to poor condition.

Body Weight and Food Consumption: all treated groups had lower body weight than the control from the start of dosing, but the weight was similar in all groups after end of dosing. Statistically reduced weight occurred only in HD during gd 6 and 15. Weight loss in LD and MD was noted only during the first few days of dosing. Slightly reduced weight (not significant) also noted after adjustment with gravid uterus weight in MD and HD. There was a marked intragroup variation in food intake therefore, significant differences could not be shown. Food consumption in general was reduced in a slight dose-dependent manner at initiation of dosing. In LD and MD food intake was

similar to the control by gd 12 but in HD it remained lower till end of dosing.

Fetal Weight and Crown/Rump Length: both parameters were significantly lower in HD relative to the control and background incidence (the data for the latter were not available). Both parameters were also lower than the control in LD and MD but not significantly different and they were similar or higher than the background mean values.

Attached Tables presents maternal and fetal parameters.

In part (a) of the Table, pre-implantation loss in MD and HD was lower than that in the control and background incidence, the incidence in LD and control groups was high and the sponsor indicated that this was due to the high number of unilateral pregnancies in these 2 groups. A dose-independent/insignificant increase in post-implantation loss was noted (16% HD vs. 7.8% in control). The number of early intrauterine deaths was higher in the control than any of the treated groups. However, late intrauterine deaths increased insignificantly, in HD vs. the control (19 vs. 1 respectively). The percent implantation was similar in all groups as well as fertility index.

Fetal Defects: (attached Table), number of fetuses with major incidences is 6 cont, 2LD, 1MD, and 2HD. Five of the 6 abnormal fetuses in the control were from the same litter and showed absence of interparietal. In LD one fetus had cephalic defects and the 2nd had kidney agenesis. The MD fetus had interrupted aortic arch and one malformed HD fetus had cleft palate and the 2nd had scoliosis. Higher incidence of minor defects occurred in all treatment groups (significant only in MD). However, these defects were commonly occurring in rabbits. No increase in fetuses with minor skeletal defects or variants.

Summary and Conclusion:

Riluzole did not cause drug-related death when administered orally to female rabbits during gd 6-18 at 3, 18, and 60 mg/kg. Maternal toxicity occurred at all doses (significant only in HD) and demonstrated by decreased weight gain and food consumption. However, weight was similar to the control after dosing ended. Small but significant decrease in fetal development (weight and crown/rump length) was noted in HD. Post-implantation loss increased insignificantly in HD relative to the control (16% vs. 7.8% respectively). The control group had higher number of early intrauterine deaths than any of the treated groups. This was reported by the sponsor to be drug-unrelated and is a natural standardization of litter size as the number of live fetuses per dam in HD was higher than that in the control. Late intrauterine deaths on the other hand, increased insignificantly, in HD vs. the control (19 vs. 1 respectively). Fetal defects were observed as follows: cephalic defects in 1LD and kidney agenesis in a second LD, interrupted aortic arch in 1MD fetus, and 1HD fetus had cleft palate and a 2nd had scoliosis. Higher incidence of minor defects occurred in all treatment groups (significant only in MD). However, these defects were commonly occurring in rabbits. No increase in fetuses with minor skeletal defects or variants. Based on the results in this study, Riluzole is a maternal toxic agent but not teratogen.

Comments:

- the guidelines suggest sacrificing the rabbits on day 29 of gestation. Closure of the heart palate occurs on gd 28 or 29 therefore, sacrifice on gd 28 may produce high incidence of fetuses with incomplete closures. In the present study, sacrifice was done on gd 28.
- Head sections must be done on 1/3 of all rabbits as suggested by the guidelines. None was done in the present study.
- The doses in this study were chosen based on "Range Finding" studies DN#101574 and 101585 (this latter study did not test a control group).

TABLE 4

Group mean caesarian data

a) Uterine/implantation data

Parameter	Group 1	Group 2	Group 3	Group 4
Number of pregnancies	14/16	13/16	14/16	14/16
Percentage of pregnancies	87.5	81.3	87.5	87.5
Number of females surviving to day 28	12	10	12	13
Number of corpora lutea	120	94	118	136
Mean number per doe	10.0	9.4	9.8	10.5
Number of implantations	97	74	113	124
Mean number per doe	8.1	7.4	9.4	9.5
% pre-implantation loss	19.2	$\frac{21.5}{20.5}$	$\frac{22.0}{4.2}$	$\frac{9.0}{2.0}$
Number of early intrauterine deaths	7	3	2	1*
Mean number per doe	0.6	0.3	0.2	0.1
Number of late intrauterine deaths	1	6	3	19
Mean number per doe	0.1	0.6	0.3	1.5
% post-implantation loss	7.8	11	49.5	16.1
Number of foetuses	89	65	108	104
Mean number per doe	7.4	6.5	9.0	8.0
% of implantations	91.8	87.8	95.6	83.9

* significantly different from control group: p<0.05

** significantly different from control group: p<0.01

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Riluzole

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TABLE 4 (continued)

b) Litter data

Parameter	Group 1	Group 2	Group 3	Group 4
Mean number of foetuses per doe	7.4	6.5	9.0	8.0
Number of male foetuses	48	35	59	60
Number of female foetuses	41	30	49	44
Sex ratio males:females	1:0.85	1:0.86	1:0.83	1:0.73
Mean litter weight (g)	285.8	231.8	315.7	281.4
Mean foetal weight (g)	39.4	37.3	36.4	35.5**
Mean foetal weight (g) - males only	39.6	37.7	37.1	36.0
Mean foetal weight (g) - females only	39.3	36.9	35.8	34.7
Mean crown/rump length (mm)	98.8	97.6	96.1	95.9*

* significantly different from control group: p<0.05

** significantly different from control group: p<0.01

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TABLE 4 (continued)

c) foetal defect data

Parameter	Group 1	Group 2	Group 3	Group 4
<u>EXTERNAL AND VISCERAL DEFECTS</u>				
Number of foetuses examined	89	65	108	104
Number showing minor defects only	9	11	27	24
% of foetuses examined	10.1	16.9	25.0*	23.1
Number showing major defects	0	2	1	1
% of foetuses examined	0.0	3.1	0.9	1.0
<u>SKELETAL DEFECTS</u>				
Number of foetuses examined	89	65	108	104
Number showing minor defects only	32	18	31	29
% of foetuses examined	36.0	27.7	28.7	27.9
Number showing major defects	6	1	0	2
% of foetuses examined	6.7	1.5	0.0	1.9
<u>VARIANTS</u>				
Number of foetuses examined	89	65	108	104
Number showing variants	76	56	97	92
% of foetuses examined	85.4	86.2	89.8	88.5

* significantly different from control group: p<0.05

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TABLE 5
Foetal defects (key, nature and incidence)

Key	Type of defect	Classification	Group 1	Group 2	Group 3	Group 4
<u>EXTERNAL AND VISCERAL</u>						
<u>Head</u>						
1	Multiple major defects:- exencephaly; unilateral exophthalmos; unilateral microphthalmia; agnathia; bilateral cleft lip	major		1 (1.5)		
2	Cleft palate	major				1 (1.0)
3	High arched palate	minor		1 (1.5)	2 (1.9)	
4	Bilateral lens opacities	minor		1 (1.5)		
<u>Body</u>						
5	Subcutaneous haemorrhagic areas (haematomas)	minor	1 (1.1)		3 (2.8)	1 (1.0)
<u>Thorax</u>						
6	Abnormal route of right pulmonary artery; pulmonary arch dilated; lungs abnormally small	major		1 (1.5)		
7	Interrupted aortic arch	major			1 (0.9)	
8	Abnormal common carotid	minor	2 (2.2)		4 (3.7)	5 (4.8) ←
9	Atrium/atria enlarged	minor	5 (5.6)	4 (6.2)	2 (1.9)	2 (6.2)
10	Atrium/atria reduced in size	minor	1 (1.1)	1 (1.5)	1 (0.9)	1 (1.0)
11	Post caval lung lobe agenesis	minor		1 (1.5)	1 (0.9)	1 (1.0)
12	Post caval lung lobe reduced in size	minor				3 (2.9)

Figures in brackets denote the percentage of affected foetuses/foetuses examined.
A foetus with multiple defects will appear more than once in this tabulation.

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TABLE 5 (continued)

Key	Type of defect	Classification	Group 1	Group 2	Group 3	Group 4
<u>Abdomen</u>						
13	Omphalocele	major		1 (1.5)		
14	Unilateral kidney agenesis	major		1 (1.5)		
15	Liver pale	minor				7 (6.7)
16	Gall bladder agenesis	minor		2 (3.1)	6 (5.6)	
17	Gall bladder enlarged	minor				3 (2.9)
18	Gall bladder reduced in size	minor		1 (1.5)	8 (7.4)	
19	Contents of gall bladder pale	minor				1 (1.0)
20	Haemorrhagic area on surface of kidney	minor			1 (0.9)	
<u>Limbs</u>						
21	Malrotation of right hindpaw	major		1 (1.5)		
<u>SKELETAL</u>						
<u>Skull</u>						
22	All skull bones malformed/absent	major		1 (1.5)		
23	Interparietal absent	major	6 (6.7)			
24	Cleft palate	major		2 (3.1)		1 (1.0)
25	Island of bone between frontals	minor		1 (1.5)		
26	Nasals incompletely ossified	minor		1 (1.5)	1 (0.9)	
27	Cleft in nasals	minor			4 (3.7)	
28	Abnormal suture line between frontals	minor	→ 5 (5.6)			2 (1.9)
29	Abnormal suture line between parietals	minor	1 (1.1)			
30	Parietals incompletely ossified	minor	3 (3.4)	3 (4.6)	1 (0.9)	1 (1.0)
31	Fissure in parietals	minor	1 (1.1)			
32	Interparietal incompletely ossified	minor	6 (6.7)		3 (2.8)	
33	Cleft in interparietal	minor	2 (2.2)		1 (0.9)	
34	Fissure in interparietal	minor				1 (1.0)
35	Occipital incompletely ossified	minor	1 (1.1)	1 (1.5)		
a	Frontals incompletely ossified	variant	21 (23.6)	22 (33.8)	22 (20.4)	22 (21.2)
b	Cleft in frontals	variant	23 (25.8)	18 (27.7)	41 (39.0)	39 (37.5)

Figures in brackets denote the percentage of affected foetuses/foetuses examined. A foetus with multiple defects will appear more than once in this tabulation.

TABLE 5 (continued)

Key	Type of defect	Classification	Group 1	Group 2	Group 3	Group 4
Vertebral arches/centrae						
36	Only 6 cervical vertebral centrae ossified; centra 2 not ossified; centra 3, 4, 5 and 6 asymmetrically ossified	major		1 (1.5)		
37	Thoracic vertebral centra 1 and 2 asymmetrically ossified/fused; centra 5, 6 and 7, and arches 5 and 6 asymmetrically ossified, causing curvature of spine	major				1 (1.0)
38	Caudal vertebrae misaligned	major		1 (1.5)		
39	Cervical vertebral centra 1 and 7 incompletely ossified	minor		1 (1.5)		
40	Cervical vertebral centra 4, 6 and 7 incompletely/asymmetrically ossified; thoracic vertebral centra 3, 4 and 6 asymmetrically ossified	minor	1 (1.1)			
41	Thoracic vertebral centra 4 asymmetrically ossified	minor				1 (1.0)
Ribs						
42	Clotted ribs	minor	7 (7.9)	2 (3.1)	3 (2.8)	
c	Single extra rib	variant	14(15.7)	10(15.4)	14(13.0)	9 (8.7)
d	Extra pair of ribs	variant	28(31.5)	23(35.4)	29(26.9)	26(25.0)
Sternum						
43	Sternum 1 malformed	minor	1 (1.1)			
44	Sternum 1 asymmetrically ossified	minor				1 (1.0)
45	Sternum 2 asymmetrically ossified	minor	1 (1.1)	2 (3.1)	5 (4.6)	5 (4.8)
46	Sternum 2 bipartite	minor				1 (1.0)

Figures in brackets denote the percentage of affected foetuses/foetuses examined.
A foetus with multiple defects will appear more than once in this tabulation.

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TABLE 5 (continued)

Key	Type of defect	Classification	Group 1	Group 2	Group 3	Group 4
<u>Sternum (continued)</u>						
47	Sternum 2 incompletely ossified	minor		1 (1.5)	1 (0.9)	
48	Sternum 3 asymmetrically ossified	minor	3 (3.4)		2 (1.9)	1 (1.0)
49	Sternum 4 asymmetrically ossified	minor	1 (1.1)	2 (3.1)	3 (2.8)	4 (3.8)
50	Sternum 4 bipartite	minor	10(11.2)	6 (9.2)	1 (0.9)	8 (7.7)
51	Sternum 5 asymmetrically ossified	minor		1 (1.5)	7 (6.5)	
52	Sternum 5 bipartite	minor		1 (1.5)	1 (0.9)	1 (1.0)
53	Sternum 6 asymmetrically ossified	minor	31(34.8)	11(16.5)	41(38.0)	50(48.1)
e	Sternum 5 incompletely/not ossified	variant	3 (3.4)		4 (3.7)	3 (2.9)
f	Sternum 6 incompletely/not ossified	variant				
<u>Pectoral girdle</u>						
54	Clavicles malformed; right scapula malpositioned and malformed; radius and ulna rotated (both forelimbs)	major		1 (1.5)		
<u>Pelvic girdle</u>						
55	Pubis incompletely/not ossified	minor	6 (6.7)	3 (4.6)	4 (3.7)	3 (2.9)
g	Bilateral insertion of pelvic girdle on 2nd sacral vertebra	variant	21(23.6)	13(20.0)	22(20.4)	12(11.5)
<u>Limbs</u>						
56	Metacarpals incompletely ossified	minor	3 (3.4)	7(10.8)	8 (7.4)	15(14.4)
57	Astragal not ossified	minor			2 (1.9)	2 (1.9)
h	Forelimb phalanges incompletely ossified	variant	19(21.3)	17(26.2)	33(30.6)	15(14.4)
j	Hindlimb phalanges incompletely ossified	variant		5 (7.7)	2 (1.9)	3 (2.9)

Figures in brackets denote the percentage of affected foetuses/foetuses examined.
A foetus with multiple defects will appear more than once in this tabulation.

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VII. MUTAGENICITY:

1. A. Ability of Riluzole to induce mutation in 4 histidine-requiring strains of *S. typhimurium*/3535-81/32.
B. Ames Test/ST/CRVA/IRSM452/DN 105223.
2. Mouse Lymphoma Fluctuation Assay/3536-81/33.
3. In vitro human peripheral lymphocytes/report #84PHA128/257.
4. In vivo micronucleus test in the mouse/bone marrow erythrocytes/report #85PHA139/17.
5. In vivo micronucleus test in the rat/chromosome assay/report #1714-81/35.

1A. The plate incorporation assay was used and 4 bacterial strains were tested. The vehicle, DMSO, served as the negative control and cyclophosphamide, benzo[a]pyrene 4-nitro-ophenylenediamine, 3,3-dichlorobenzidine, and 3-methylcholanthrene, served as the positive controls for TA1535, TA97, TA98, and TA100 respectively. Five drug concentrations were tested: 0.01, 0.05, 0.1, 0.5, and 1.0 mg/plate. Riluzole was not mutagenic in the *Salmonella typhimurium* in presence or absence of metabolic activation (Tables attached).

1B. Riluzole was not mutagenic in Ames *S. typhimurium* assay as indicated in the results presented in attached tables. In a preliminary test to determine concentration range, Riluzole at ≥ 500 $\mu\text{g}/\text{plate}$ was cytotoxic as no bacteria (TA100) were able to grow.

2. Riluzole at 6.25, 25, and 100 $\mu\text{g}/\text{ml}$ caused an increase in ouabain locus mutation frequency, and at 50 $\mu\text{g}/\text{ml}$ it increased 6TG resistant mutation in presence of S9 (Table 1a). When repeated, results were similar to the control except at 50 $\mu\text{g}/\text{ml}$ where the frequency of ouabain mutation was still slightly (not significantly) higher than the control (Table 2a). In the absence of S9, ouabain mutation frequency was increased over the control at 100 $\mu\text{g}/\text{ml}$, and at 25 $\mu\text{g}/\text{ml}$ 3TG frequency of mutation was also higher than the control. When the assay was repeated, results were similar to the control except at 12.5 $\mu\text{g}/\text{ml}$ for the ouabain locus and at 100 $\mu\text{g}/\text{ml}$ for 3TG locus. None of these findings reached statistical significance and they were nondose-dependent therefore of no biological significance. Also, the sponsor indicated that these positive results are unreliable because of the low viability in these cultures. It was concluded that Riluzole is not mutagenic in this test.

3. Human peripheral blood was collected from healthy, non-smoking, male volunteers not taking any medication at time of test. Drug concentrations were chosen based on a preliminary toxicity assay; concentrations tested ranged between 6-3825 $\mu\text{g}/\text{ml}$. Concentrations in the main assay were 0.4, 2, and 10 $\mu\text{g}/\text{ml}$ and incubated with peripheral blood for 24 hr. Ethanol served as the negative control (vehicle for Riluzole), and cyclophosphamide was the positive control with and without metabolic activation. The results are shown in attached tables. Riluzole compared with the negative control, induced small reductions in mitotic index at 0.4 and 2 $\mu\text{g}/\text{ml}$ but at 10 $\mu\text{g}/\text{ml}$ a small increase was found in presence of S9 and a reduction in absence of S9 (Table 1&2-specific aberrations and group totals). Table 3 presents the number and types of aberrations, the only statistically significant difference is that at 10 $\mu\text{g}/\text{ml}$ in absence of S9 and when gaps are excluded from the analysis; compared with the negative control (Table 3&5). A significant difference was noted within the 10 $\mu\text{g}/\text{ml}$ of activated and nonactivated cultures in chromosome breaks and fragments (Table 4). It is concluded that Riluzole is equivocally or marginally mutagenic in this test. This assay was repeated (DN 101596) using 10 and 20 $\mu\text{g}/\text{ml}$ Riluzole concentrations. The data in this study were analysed separately and with data pooled from the previous one to compare large populations. Riluzole at 10 $\mu\text{g}/\text{ml}$ caused no difference in mitotic index but 20 $\mu\text{g}/\text{ml}$ caused 45% reduction in mitotic index relative to the negative/vehicle control (Table 1). There was no significant increase in number of aberrations relative to the control at either dose and with or without gaps (Table 2&3). Tables 4&5 present statistical analyses and a significant difference is noted within group 10 $\mu\text{g}/\text{ml}$ between activated and

nonactivated cultures. When pooled data were examined at 10 µg/ml, frequency of aberration was significantly increased between activated and non-activated cultures whether gaps were included or excluded (Tables 6,7,8). When comparisons were made with the control culture, a significant increase in aberration incidence occurred only with S9 and only when gaps are included (Table 8). The sponsor indicated that since aberration frequency did not increase in 20 µg/ml and biological significance of gap-type aberration is unclear, Riluzole is not mutagenic in this test but is cytotoxic.

Comments:

- A positive control that is mutagenic in absence of S9 was not used. CFP was used as a positive control in presence and absence of S9 but is mutagenic only after metabolic activation as clearly indicated by the sponsor. Another positive control must be used that is mutagenic in this test in absence of S9.
- The clastogenic effect of CFP in the first study (#84PHA128/257) was weak compared with the results obtained in the repeat study. This made comparisons with the negative control data and drug-treated cultures difficult.

4. Male and female mice (15/sex/dose) were administered a single oral dose of 40 mg/kg Riluzole, or 1% methylcellulose (negative control), or 30 mg/kg chlorambucil (positive control, 5/sex). The dose was selected based on MTD of 44 mg/kg. Treated and negative control m and f mice were sacrificed 24, 48, and 72 hr after dosing (5/sex/time period). Femur marrow cells were isolated and processed and cell suspension smeared on slides, fixed, stained, and mounted. Attached Tables indicate that Riluzole did not induce chromosomal or other damage indicated by an increase in polychromatic erythrocytes, in mice at any time period examined.

5. Single oral doses of Riluzole (2, 7, 20 mg/kg), were administered to Sprague-Dawley rats (20/sex/dose). Animals were sacrificed at 6, 12, 24, and 48 hr after dosing (5/sex/dose at each time). Methyl cellulose 1% served as the negative control and cyclophosphamide 40 mg/kg served as the positive control (5/sex). Bone marrow cells were processed according to the modified technique by Evans (1976) and Kilian et al. (1977). Animals of HD were slightly depressed and 1 animal was prostrate. All vehicle control rats were ataxic at variable times of inspection; it is unclear why this group would be such a high incidence of ataxia specially in the evening when the rats are most active. One vehicle control f lost 59 g in 2 days and body weight in rats treated with the positive control was significantly reduced during the 24 hr postdose relative to the control. No other changes in body weight were noted in drug treated animals. Table 1 present the results of aberration, no significant difference was found between negative control and Riluzole-treated cultures. However, at 12 and 48 hr post-treatment, an increase in aberration was found in 7 mg/kg group and at 24 hr at 20 mg/kg dose group (1.3% vs. 0.9% control, 0.4% vs. 0% control, and 0.9% vs. 0.3% control of aberrant cells per group, respectively). It was concluded that single oral administration of 2, 7, 20 mg/kg Riluzole to rats did not significantly increase the frequency of chromosomal aberrations.

Summary and Conclusion of Mutagenicity:

Riluzole was not mutagenic in the in vitro Ames Salmonella assay and in vivo bone-marrow micronucleus assay in mice and rats. In the mouse lymphoma chromosomal aberration assay, Riluzole increased frequency of mutation over the negative control incidence at 100 and 25µg/ml for ouabain locus and 3TG respectively, in the absence of S9. Because of these equivocal findings the assay was repeated and results were similar to the control except at 12.5µg/ml for the ouabain locus and at 100µg/ml for 3TG locus. However, none of these findings reached statistical significance and were nondose-dependant

therefore of no biological significance. The sponsor also indicated that these positive results are unreliable because of the low viability in these cultures. It is concluded that Riluzole is not mutagenic in this test. Effect of Riluzole on chromosomal aberrations were tested using human peripheral blood/lymphocytes. The only statistically significant difference was noted at 10 $\mu\text{g/ml}$ in absence of S9 when gaps were excluded from the analysis; compared with the negative control. A significant difference was noted within the 10 $\mu\text{g/ml}$ of activated and nonactivated cultures in chromosome breaks and fragments. It is concluded that Riluzole is equivocally or marginally mutagenic in this test. This assay was repeated using 10 and 20 $\mu\text{g/ml}$ Riluzole concentrations. The data in this study were analysed separately and with data pooled from the previous one to compare large populations. Riluzole at 10 $\mu\text{g/ml}$ caused no difference in mitotic index but 20 $\mu\text{g/ml}$ caused 45% reduction in mitotic index relative to the negative/vehicle control. There was no significant increase in number of aberrations relative to the control at either dose with or without gaps. A significant difference is noted within group 10 $\mu\text{g/ml}$ between activated and nonactivated cultures. When pooled data were examined at 10 $\mu\text{g/ml}$, frequency of aberration was significantly increased between activated and non-activated cultures whether gaps were included or excluded. When comparisons were made with the control culture, a significant increase in aberration incidence occurred only with S9 and only when gaps were included. The sponsor indicated that since aberration frequency did not increase in 20 $\mu\text{g/ml}$ and biological significance of gap-type aberration is unclear, Riluzole is not mutagenic in this test but is cytotoxic. From the negative findings in the Ames assay and the in vivo bone-marrow micronucleus test in mice and rats, Riluzole is not clastogenic. However, the results were equivocal in the mouse lymphoma and human peripheral lymphocyte tests. These latter tests were repeated and although the results were not clearly negative, the mutagenic/clastogenic potential of Riluzole is small.

TABLE 1 Raw plate counts for PK 26.124

Compound (ug/plate) <u>PK 26.124</u>	Liver	<u>TA02</u>	<u>TA100</u>	<u>TA1535</u>	<u>TA97</u>
10	+	10,15,17, 14,14,19	100,82,86, 72,81,39	9,8,6, 7,9,4	67,69,70, 72,75,74
	-	7,10,6, 7,9,10	81,72,67, 71,93,72	11,10,8, 5,5,6	87,72,70, 65,55,72
50	+	16,17,18, 18,18,18	97,85,79, 29,75,78	9,9,8, 7,6,10	74,72,67, 87,73,88
	-	10,9,9, 11,10,9	92,72,70, 70,86,79	9,12,6, 10,10,9	73,79,67, 69,73,72
100	+	19,16,16, 14,17,15	79,86,82, 51,86,88	14,19,9, 8,10,4	69,67,67, 8,74,76
	-	11,10,11, 10,8,10	74,74,80, 69,8,74,86	11,7,8, 8,8,12	84,79,61, 68,69,70
500	+	17,14,14, 16,19,15	91,96,74, 83,85,88	9,5,6, 3,10,7	74,72,78, 65,73,75
	-	9,9,17, 18,9,10	80,81,76, 62,81,83	7,12,10, 9,9,7	77,68,65, 51,70,70
1000	+	9,8,11, 14,18,16	73,50,80, 73,75,34	3,2,4, 2,6,7	54,26,37, 32,7,7
	-	0,3,6, 8,8,6	72,61,63, 65,46,48	7,7,7, 7,7,7	7,7,7, 7,7,7
Controls (100ul DMSC)	+	16,15,18, 18,18,19	79,81,85, 92,87,89	8,8,9, 9,13,12	84,85,79, 88,83,79
	-	12,10,16, 13,16,13	85,81,79, 90,74,73	6,10,12, 8,12,11	97,72,89, 79,79,75
3,3'-Dichloro- benzidine (50ug)	+	1298,1179,1332			
	-	1298,1679,1533 58,48,62,68,48,65			
3-Methylchol- anthrene (50ug)	+		95,922,769 269,986,1097		
	-		106,111,83, 86,104,127		
Cyclophospho- amide (200ug)	+			105,139,137	
	-			127,83,79 31,49,25 23,26,32	
Benzo(a) pyrene(25ug)	+				529,621,665 624,591,538
	-				
4-Nitro-c- phenylene diamine (50ug)	+				544,571,655, 697,501,537
	-				

+ liver present T toxic
 - liver absent C Contaminated

TABLE 2

Compound	Concntr. (ug/plate)	Liver/ type	His ⁺ revertants/plate				
			T498	T4100	T41535	T497	
PK 26.124	10ug	+	15 ± 3.1	85 ± 9.3	7 ± 1.8	71 ± 3.0	
		-	8 ± 1.7	73 ± 13.9	8 ± 2.3	70 ± 10.8	
	50ug	+	18 ± 0.8	82 ± 5.6	8 ± 1.5	77 ± 8.6	
		-	10 ± 0.8	76 ± 6.7	9 ± 2.0	72 ± 4.0	
	100ug	+	16 ± 1.4	84 ± 4.4	9 ± 3.2	73 ± 4.0	
		-	10 ± 1.1	75 ± 6.3	9 ± 2.2	74 ± 8.4	
	500ug	+	16 ± 1.9	86 ± 7.5	7 ± 2.6	72 ± 5.6	
		-	12 ± 4.3	75 ± 8.6	9 ± 1.9	67 ± 9.7	
	1000ug	+	13 ± 4.0	69 ± 17.5	4 ± 1.7	34 ± 12.4	
		-	6 ± 2.0	59 ± 10.1	Toxic	Toxic	
	Controls-DMSO	100ug	+	16 ± 1.6	86 ± 4.9	10 ± 2.1	83 ± 3.5
			-	13 ± 2.3	80 ± 6.5	10 ± 2.4	52 ± 9.4
3,3'-dichloro-benzidine	50ug	-	<u>1387 ± 183.6</u>				
		-	<u>58 ± 8.5</u>				
3-Methylchol-anthrene	50ug	-		<u>932 ± 110.5</u>			
		-		103 ± 16.4			
Cyclophosphamide	200ug	+			<u>112 ± 26.7</u>		
		-			<u>31 ± 9.5</u>		
4-Nitro-ortho-phenylene diamine	50ug	-				<u>584 ± 75.7</u>	
Benzo (a)pyrene	25ug	-				<u>601 ± 45.0</u>	

+ Aroclor liver present
 - Aroclor liver absent

Figures underlined indicate significant mutagenicity

Riluzole / Ames Assay
 Dn 105223

TABLES 3A and 3B

RP 54274 - RESULTS OF THE SECOND MUTAGENICITY TEST
WITHOUT AND WITH METABOLIC ACTIVATION

REVERTANT COUNTS : MEANS

STRAINS	S9	Controls		Test compound concentrations (ug/plate)				
		Negative	Positive	15.6	31.2	62.5	125	250
TA1535	-	10	236	10	12	8	6	4
	+	13	203	12	9	11	10	7
TA100	-	132	452	141	138	133	131	109
	+	126	1393	140	135	123	127	96
TA1537	-	9	201	8	12	10	11	8
	+	9	171	9	9	9	8	8
TA1538	-	13	291	16	13	15	13	12
	+	21	1084	19	20	16	22	20
TA98	-	30	192	32	34	34	32	27
	+	28	1406	31	30	32	30	25

REVERTANT COUNTS : R RATIO

STRAINS	S9	Test compound concentrations (ug/plate)				
		15.6	31.2	62.5	125	250
TA1535	-	1.03	1.24	0.86	0.62	0.41
	+	0.92	0.74	0.87	0.79	0.53
TA100	-	1.07	1.04	1.01	0.99	0.82
	+	1.11	1.07	0.97	1.01	0.76
TA1537	-	0.89	1.25	1.07	1.14	0.82
	+	1.00	1.00	1.08	0.96	0.88
TA1538	-	1.24	1.05	1.16	1.05	0.97
	+	0.90	0.98	0.79	1.06	0.98
TA98	-	1.05	1.13	1.13	1.05	0.88
	+	1.11	1.07	1.14	1.05	0.87



Ri 14306
 Report 3536-81-33
 Mouse Lymphoma

MLP
 Results of PK 26.124 in presence of rat liver S9

3.1 Table 1A

Exposure time	0 hr			48 hr			168 hr		
	PM 10 (survival)	Relative survival	PM 10 (viability)	DM ^a (10 ⁻³ M)	Mutation frequency/10 ⁶ viable cells	PM 10 (viability)	DM ^a (1.8x10 ⁻⁴ M)	Mutation frequency/10 ⁶ viable cells	
No. of cells/well	2		2	4x10 ⁴		2	1 x 10 ⁴		
No. of 96 well plates	1		1	4		1	4		
Treatment									
DMSO (-vs control)	68	100.00	68	0,0,3,1	0.42	64	6,10,6,12	18.97	
Test compound 6.25 ug/ml	66	100.00	50	3,0,2,1	1.07	68	13,16,12,8	22.16	
12.5 ug/ml	67	85.19	58	1,0,0,2	0.42	60	4,3,4,1 C	6.57	
25 ug/ml	48	65.65	73	3,3,1,2	0.83	74	15,16,11,6	17.72	
50 ug/ml	61	85.42	62	0,1,0,0	0.13	50	16,13,12,13	40.38	
100 ug/ml	51	71.65	66	3,3,1,1	0.85	75	12,9,16,14	18.76	
Positive Control 1.5 x 10 ⁻⁵ MX(a)P	2	1.88	18	1,2,1,2	3.79	60	74,73,60,70	280.56	
							C = control		

Riluzole
Report 3536-81-33
Mouse lymphoma

Results of PK 26.124 in absence of rat liver Sq
PAGE 10 OF 14

3.1 Table 1b

Expression time	0 hr		48 hr			168 hr		
	FM 10 (survival)	Relative survival %	FM 10 (viability)	OUR (10 ⁻³ M)	Mutation frequency/10 ⁶ viable cells	FM 10 (viability)	6TC ^R (1.8x10 ⁻⁴ M)	Mutation frequency/10 ⁶ viable cells
No. of cells/well	2		2	4x10 ⁴		2	1 x 10 ⁴	
No. of 96 well plates	1		1	4		1	4	
Treatment								
DMSO (-ve control)	74	100.00	68	0,0,0,0	0.00	76	14,4,14,6	14.3
Test compound 6.25 ug/ml	63	79.73	58	0,0,1,0	0.15	72	10,10,10,4	13.37
12.5 ug/ml	67	89.37	68	0,0,0,1	0.11	74	12,11,15,10	19.34
2.5 ug/ml	56	65.36	55	0,1,0,0	0.15	70	16,12,16,15	26.48
50 ug/ml	60	73.23	49	1,0,0,1	0.37	75	12,19,13,15	21.95
100 ug/ml	63	79.73	56	2,3,3,3	1.57	76	16,12,16,11	19.71
Positive control 1 x 10 ⁻³ M NQO	42	42.96	59	5,11,5,8	4.12	75	46,40,39,37	72.11

Riluzole
Report 3536-81-53
Mouse lymphoma

Repeated study - in presence of S9

3.2 Table 2a

Expression time	0 hr			48 hr			168 hr		
	PM 10 (survival)	Relative % survival	PM 10 (visibility)	GM _N (10 ⁻³ M)	Mutation frequency/10 ⁶ viable cells	PM 10 (visibility)	GM _N (1.8x10 ⁻⁴ M)	Mutation frequency/10 ⁶ viable cells	
No. of cells/well	2		2	4x10 ⁴		2	1 x 10 ⁴		
No. of 96 well plates	1		1	4		1	4		
Treatment									
DMSO (-ve control)	68	100.00	70	0,0,0,0	0.00	63	4,4,3,0	6.44	
Test compound 6.26 ug/ml	53	71.70	65	1,0,2,0	0.35	65	4,4,4,1	6.08	
12.6 ug/ml	50	65.66	71	0,0,0,0	0.00	54	0,1,2,2	3.17	
25 ug/ml	42	51.36	61	0,2,1,0	0.38	58	3,2,0,1	3.40	
50 ug/ml	49	63.76	62	1,1,1,2	0.63	57	1,2,1,0	2.32	
100 ug/ml	40	48.12	66	0,1,1,0	0.22	57	1,1,0,2	2.32	
Positive control 1.5 x 10 ⁻⁶ M B(a)p	23	12.89	36	9,14,11,18	14.74	67	22,26,22,30	50.40	

Riluzole
 Report 3536-81-33
 Mouse lymphoma

Repeated study in Absence of S9

3.2 Table 2b

Exposure time	0 hr		48 hr			168 hr		
	PM 10 (survival)	Relative % survival	PM 10 (viability)	QmR (10 ⁻³ M)	Mutation frequency/10 ⁶ viable cells	PM 10 (viability)	QmR (1.8x10 ⁻⁴ M)	Mutation frequency/10 ⁶ viable cells
No. of cells/well	2		2	4x10 ⁴		2	1 x 10 ⁴	
No. of 96 well plates	1		1	4		1	4	
Treatment								
DMSO (-ve control)	74		61	0,0,0,0	0.00	60	5,3,2,1	5.93
Test compound 6.25 ug/ml	62		62	0,0,1,1	0.25	60	2,3,1,3	4.84
12.5 ug/ml	55		66	3,1,0,3	0.79	62	3,4,0,1	4.06
25 ug/ml	56		58	0,0,0,0	0.00	64	4,1,4,3	5.78
50 ug/ml	50		59	0,0,0,0	0.00	56	1,2,0,1	2.20
100 ug/ml	44		68	0,0,0,1	0.11	58	6,3,5,2	9.78
Positive control 1 x 10 ⁻⁶ M NQO	48		71	7,9,5,7	2.81-	54	9,9,8,5	20.36

TABLE 2
 Lymphocyte cytogenetic test - mitotic indices

Group : 1 2 3 4 5
 Compound : Ethanol ----- PK 26124 ----- Cyclophosphamide
 Concentration : 0 0.4 2 10 6
 (µg/ml)

Group	1					2					3						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Culture number	1081	1057	1053	1063	1063	1040	1079	1023	1042	1009	1056	1064	1069	1019	1057	1058	1006
S-9 mix	-	-	-	+	+	-	-	-	+	+	+	+	-	-	+	+	+
Metaphases	102	89	75	77	82	26	53	73	88	65	28	63	57	64	52	68	63
Mitotic index*	9.4	8.4	7.1	7.3	7.7	2.4	5.1	6.8	8.6	6.2	2.8	6.0	5.4	6.0	5.1	6.4	6.0
Mean mitotic index	5.8					6.8					5.0					5.2	
Group mean mitotic index	7.1					5.9					5.3						

* Mitotic index = $\frac{\text{number of metaphases}}{\text{number of lymphocytes}} \times 100$

a = bacterial contamination present.

In Vitro human lymphocytes
 Report 84-PHA 1280-257

TABLE 2 - continued
 Lymphocyte cytogenetic test - mitotic indices

Group	1	2	3	4	5
Compound	Ethanol		PK 26124		Cyclophosphamide
Concentration (µg/ml)	0	0.4	2	10	6
Culture number	10 11 12 25 26 27 13 14 15 28 29 30				
S-9 mix	- - -	-	+	-	+
Lymphocytes	1019 1004 1051 1021 1033 1013 1045 1066 1048 1020 1043 1055				
Metaphases	99 82 36 92 96 97 79 104 72 95 115 66				
Mitotic Index*	9.7 8.2 3.4 9.0 9.3 9.6 7.6 9.8 6.9 9.3 11.0 6.3				
Mean mitotic index	7.1	9.3	8.1	8.9	
Group mean mitotic index		8.2	8.5		

* Mitotic Index = $\frac{\text{number of metaphases}}{\text{number of lymphocytes}} \times 100$

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TABLE 3

Lymphocyte cytogenetic test - group totals of specific aberrations

Group	1	2	3	4	5			
Compound	Ethanol		PK 26124					
Concentration (ug/ml)	0	0.4	2	10	6			
	Cyclophosphamide							
Group* S-9 mix	Single strand gap (chromatid)	Double strand gap (chromosome)	Single strand break (chromatid)	Double strand break (chromosome)	Fragment	Exchange	Multiple aberration	Other aberration
1 -	2	1	0	0	0	0	0	0
1 +	2	0	3	0	4	0	0	1a
2 -	7	1	2	0	4	0	0	1a
2 +	9	0	1	0	4	0	0	2a
4 -	12	0	3	0	12	0	1	2a 1c
5 -	7	0	1	0	7	1	0	2a
5 +	17	3	5	1	23	0	0	2a

* Values obtained with and without S-9 mix are pooled for Groups 2 - 4; see text.
a, Endoreduplication.
c, Pulverised metaphase.

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TABLE 1
Lymphocyte cytogenetic test - group totals

Group	S-9 mix	Numbers of cells scored	Mean mitotic index	Cells with aberrations*		Cells with aberrations other than gaps*	
				Total	Mean % [†]	Total	Mean % [†]
1	-	300	8.3	3	1.0	0	0.0
1	+	300	5.8	10	3.3	8	2.7
2	-	600	5.9	14	2.3	6	1.0
2	+	600	5.3	16	2.7	7	1.2
4	-	600	8.2	29	4.8	18	3.0
4	+	300	8.1	14	4.7	7	2.3
4	+	300	8.9	49	16.3	30	10.0

* Values obtained with and without S-9 mix are pooled for Groups 2 - 4; see text.

† Mean % aberrations = $\frac{\text{No aberrant cells}}{\text{No cells scored}} \times 100$

in vitro human lymphocytes
 Report 84-PHA128-257

TABLE 2
Lymphocyte cytogenetic test - number and types of chromosomal aberrations.

Group	1	2	3	4	5
Compound	Ethanol	-----	PK 26124	-----	Cyclophosphamide
Concentration (µg/ml)	0	0.4	2	10	6
Group	1	2	3		
Culture number	1 2 3 16 17 18 4 5 6 19 20*	21 7 8 9 22 23 24			
S-9 mix	- - - + + +	- - - + + +	- - - + + +	- - - + + +	- - - + + +
Number of cells scored	100 100 100 100 100 100 100 100 100 100 100 100 100 100 100	100 100 100 100 100 100 100 100 100 100 100 100 100 100 100	100 100 100 100 100 100 100 100 100 100 100 100 100 100 100	100 100 100 100 100 100 100 100 100 100 100 100 100 100 100	100 100 100 100 100 100 100 100 100 100 100 100 100 100 100
% cells with aberrations	1 1 1 2 6 2 1 2 5 3 3 0 3 2 2 4 3 2				
% cells with aberrations other than gaps	0 0 0 2 5 1 0 2 1 2 1 0 1 1 1 2 0 2				
SSG	1 1 1 1 1 1 1 3 1 2	2 1 1 2 3	1 1 1 2 3	1 1 1 2 3	
DSG	1				
SSB	3	1 1 1	1	1	1
DSB					
E					
F	1 2 1 1 1 3	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1			
OA	1a	1a	1a	1a	1a

SSG Single strand (chromatid) gap.
 DSG Double strand (chromosome) gap.
 SSB Single strand (chromatid) break.
 DSB Double strand (chromosome) break.
 * Bacterial contamination present.
 E Exchange.
 F Fragment.
 OA Other aberrations.
 a Endoreduplication.

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TABLE 3 - continued

Lymphocyte cytogenetic test - number and types of chromosomal aberrations												
Group	1	2	3	4	5							
Compound	Ethanol					----- PK 26124 -----						
Concentration (µg/ml)	0	0.4	2	10	6							
Group	4					5						
Culture number	10	11	12	25	26	27	13	14	15*	28	29	30
S-9 mix	-	-	-	+	+	+	-	-	-	+	+	+
Number of cells scored	100	100	100	100	100	100	100	100	100	100	100	100
% cells with aberrations	3	4	1	11	2	8	3	3	8	17	18	14
% cells with aberrations other than gaps	(3)	(2)	(1)	5	2	5	1	2	4	8	12	10
SSG	2	2	(7)	3	2	1	4	8	6	3		
DSG												
SSB		2		1			1	1	2	2		
DSB												
E										1		
F	2	1	3	2	4	1	6	7	9	7		
OA	1a	1b	1a		1c	2a	2a					

Number and specific types of chromosomal aberration

SSG Single strand (chromatid) gap.
 DSG Double strand (chromosome) gap.
 SSB Single strand (chromatid) break.
 DSB Double strand (chromosome) break.
 E Pulverised metaphase.
 F Exchange Fragment.
 OA Other aberrations.
 a Endoreduplication.
 b Multiple aberrations.
 * In addition, one severely damaged metaphase noted - chromosome count impossible due to damage.

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TABLE 3

Lymphocyte cytogenetic test - statistical analysis of the frequency of chromosomal aberrations - comparison between treated and control groups and the effect of S-9 mix

Group	1	2	3	4	5
Compound	Ethanol	----- PK 26124 -----			Cyclophosphamide
Concentration (ug/ml)	0	0.4	2	10	6
Group	1	2	3	4	5
S-9 mix	- +	- +	- +	- +	- +
Difference between cultures treated with and without S-9 mix (two-tailed test)	p-value 0.8885x10 ⁻¹	0.7881	0.8016	0.2086x10 ⁻¹	0.3601x10 ⁻⁵
Significance	NS	NS	NS	*	***
Difference between treated groups and control groups ^a (two-tailed test)	p-value 0.5000 b	0.7084	0.2224	0.6359x10 ⁻¹	0.1155x10 ⁻¹ 0.6783x10 ⁻⁷
Significance	NS	NS	NS	NS	* ***

NS Not Significant, p > 0.05
 * Significant, 0.01 < p < 0.05
 *** Significant, p < 0.001
^a Comparison based on pooled group data for Groups 1 - 3; activated and non-activated treatments compared separately in Group 4 and 5.
^b One-tailed probability.

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TABLE 2

Lymphocyte cytogenetic test - statistical analysis of the frequency of chromosomal aberrations excluding gaps - comparison between treated and control groups and the effect of S-9 mix

Group	1	2	3	4	5
Compound	Ethanol	----- PK 26124 -----			Cyclophosphamide
Concentration (ug/ml)	0	0.4	2	10	6
Group	1	2	3	4	5
S-9 mix	+	-	+	-	+
Difference between cultures treated with, and without S-9 mix (two-tailed test)	p-value	0.7455×10^{-2}	0.6572^b	0.5003^b	0.2308
	Significance	**	NS	NS	NS
Difference between treated groups and control groups* (two-tailed test)	p-value	0.2489	0.2224	0.2489	0.3046
	Significance	NS	NS	NS	NS
					0.1509×10^{-1}

NS Not Significant, $p > 0.05$
 * Significant, $0.01 < p < 0.05$
 ** Significant, $0.001 < p < 0.01$
 *** Significant, $p < 0.001$
 † Activated and non-activated treatments compared separately in Groups 1-5.
 b One-tailed probability.

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2nd. assay

TABLE 3

Group totals of chromosomal aberrations

Group	S-9 mix	Numbers of cells scored	Mean mitotic index	Total	Range %	Mean %	Total	Range %	Mean %
1	+	600	5.2	13	1-6	2.2	7	0-3	1.2
2	-	300	4.8	11	0-4	1.8	5	0-0	0.0
3	+	300	2.8	9	0-3	1.5	6	1-2	1.7
4	-	300	6.5	4	0-4	1.3	2	0-3	1.0
	+	300	2.5	92	18-45	30.7	73	16-36	24.3

1
 2
 3
 4
 6
 PK26124
 Ethanol
 Cyclophosphamide

* Values obtained with and without S-9 mix are pooled for Groups 1 - 3; see text.
 a Values obtained with and without S-9 mix are pooled for Groups 1 and 3; see text.
 + Mean % = $\frac{\text{No. aberrant metaphases}}{\text{total cells scored}} \times 100$

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and assay

In vitro human lymphocytes
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TABLE 2
Number and types of chromosomal aberrations

Group	1			2			3											
Compound	Ethanol			PK26124			Cyclophosphamide											
Concentration (µg/ml)	1	2	3	4	5	6	7	8	9									
Culture number	1	2	3	19	20	21	10	11	12	22	23	24	13	14	15	25	26	27
S-9 mix	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
Number of cells scored	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
% cells with aberrations	2	6	1	1	1	2	1	0	1	3	4	2	0	3	1	3	0	2
% cells with aberrations other than gaps	0	3	1	1	1	1	0	0	0	1	2	2	0	3	0	2	0	1
SSG	2	3				1	1	1	2	2			1	1	1	1		1
DSG																		
SSB										1	1	1						
DSB																		
E										1								
F			3	1	1	1	1	1	1	1	1	1	1	2	1	1		
OA																		

SSG Single strand (chromatid) gap.
 DSG Double strand (chromosome) gap.
 SSB Single strand (chromatid) break.
 DSB Double strand (chromosome) break.
 E Exchange.
 F Fragment.
 OA Other aberrations.
 a Endoreduplication.

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TABLE 2 - continued

Number and types of chromosomal aberrations

Group	1	2	3	4
Compound	Ethanol	-----	PK26124	-----
Concentration (µg/ml)	-	10	20	6
Group	4			
Culture number	16	17	18	28
S-9 mix	-	-	-	+
Number of cells scored	100	100	100	100
% cells with aberrations	4	0	0	45
% cells with aberrations other than gaps	2	0	0	36
SSG	2	13	15	4
DSG	3	1	1	1
SSB	24	7	5	
DSB	4	1	1	
E	6	6	6	2
F	2	19	8	9
OA				

SSG Single strand (chromatid) gap.
 DSG Double strand (chromosome) gap.
 SSB Single strand (chromatid) break.
 DSB Double strand (chromosome) break.
 E Exchange.
 F Fragment.
 OA Other aberrations.

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 TABLE 3

Statistical analysis of the frequency of chromosomal aberrations - comparison between treated and control groups and the effect of S-9 mix

Group	1	2	3	4
Compound	Ethanol	----- PK26124 -----		Cyclophosphamide
Concentration (µg/ml)	-	10	20	6
Group	1	2	3	4
S-9 mix	- +	- +	- +	- +
Difference between cultures treated with and without S-9 mix (double-sided test)	p-value 0.2617	0.0630	1.0001	< 0.0005
Significance	NS	NS	NS	* * *
Difference between treated groups and control groups (single-sided test)	p-value -	0.4185	0.2595	0.1308
Significance	-	NS	NS	NS

NS Not Significant $p > 0.05$
 *** Significant, $p < 0.001$
 a Comparison based on pooled group data for Groups 1 - 3; activated and non-activated treatments compared separately in Group 4.

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Human lymphocytes
and assay
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TABLE 6

Statistical analysis of the frequency of chromosomal aberrations excluding gaps - comparison between treated and control groups and the effect of S-9 mix

Group	1	2	3	4
Compound	Ethanol	PK26124	Cyclophosphamide	
Concentration (µg/ml)	0	10	20	6
Group	1	2	3	4
S-9 mix	- +	- +	+ - +	- - +
Difference between cultures treated with and without S-9 mix (double-sided test)	p-value 1.0005	0.0307	1.0003	< 0.0005
	Significance NS	*	NS	* * *
Difference between treated groups and control groups ^a (single-sided test)	p-value -	0.0619	0.3625	0.3430
	Significance -	NS	NS	NS
				< 0.0005

NS Not Significant, $p > 0.05$
 * Significant, $0.01 < p < 0.05$
 *** Significant, $p < 0.001$
^a Comparison based on pooled group data for Groups 1 and 3; activated and non-activated treatments compared separately in Groups 2 and 4.

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22

and assay

Human lymphocytes
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TABLE 3

Number and types of chromosomal aberrations - pooled data

Treatment	Solvent control (Ethanol)												PC26124 (10 µg/ml)												
	1	2	3	1*	2*	3*	10	11	12	10*	11*	12*	1	2	3	1*	2*	3*	10	11	12	10*	11*	12*	
S-9 mix	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Number of cells scored	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	
% cells with aberrations	2	6	1	1	1	1	1	1	1	0	1	3	1	0	3	1	0	0	0	0	0	0	3	2	1
% cells with aberrations other than gaps	0	3	1	0	0	0	0	0	0	0	0	3	2	1	0	3	1	0	0	0	0	0	3	2	1
SSG	2	3		1	1	1	1	1	1	1	1	2	2	3		1	1	1	1	1	1	1	2		
DSG																									
SSB																									
DSB																									
E																									
F																									
OA																									

SSG Single strand (chromatid) gap.
 DSG Double strand (chromosome) gap.
 SSB Single strand (chromatid) break.
 DSB Double strand (chromosome) break.
 E Exchange.
 F Fragment.
 OA Other aberrations.
 a Endoreduplication.
 * From previously reported study.
 b Multiple aberrations.

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DND assay

Human lymphocytes
Report 85 PHA 137-159

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TABLE 7 - continued

Number and types of chromosomal aberrations - pooled data

Treatment	Solvent control (Ethanol)											PK26124 (10 µg/ml)					
	19	20	21	16*	17*	18*	22	23	24	25*	26*	27*					
Culture number	19	20	21	16*	17*	18*	22	23	24	25*	26*	27*					
S-9 mix	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Number of cells scored	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
% cells with aberrations	1	1	2	2	6	2	3	4	2	11	2	8					
% cells with aberrations other than gaps	1	1	1	2	5	1	1	2	2	5	2	5					
SSG	1	1	1	1	1	1	2	2	2	7	3						
DSG																	
SSB					3				1	2	1						
DSB																	
E																	
F	1	1	1	1	2	1	1	1	1	3	2	4					
OA																	

SSG Single strand (chromatid) gap.
 DSG Double strand (chromosome) gap.
 SSB Single strand (chromatid) break.
 DSB Double strand (chromosome) break.
 E Exchange.
 F Fragment.
 OA Other aberrations.
 α Endoreduplication.
 C Pulverised metaphase.
 * From previously reported study.

Human Lymphocytes
in vitro
Report 85FMA137/159

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TABLE 8

Group totals of chromosomal aberrations - pooled data

Treatment	S-9 mix	Numbers of cells scored	Cells with aberrations			Cells with aberrations other than gaps		
			Total	Range %	Mean %	Total	Range %	Mean %
Solvent control (Ethanol)	-	600	12	1-6	2.0	4	0-3	0.7
	+	600	14	1-6	2.3	11	1-5	1.8
PK26124 (10 µg/ml)	-	600	10	0-4	1.7	6	0-3	1.0
	+	600	30	2-11	5.0	17	1-5	2.8

† Mean % = $\frac{\text{No. aberrant metaphases}}{\text{Total cells scored}} \times 100$

TABLE 9

Group totals of specific aberrations - pooled data

Treatment	S-9 mix	Single strand gap (chromatid)	Double strand gap (chromosome)	Single strand break (chromatid)	Double strand break (chromosome)	Fragment	Exchange	Other aberration
	+	3	0	3	0	7	0	1 ^a
PK26124 (10 µg/ml)	-	4	0	0	0	3	0	2 ^{a,1b}
	+	14	0	4	0	11	1	1 ^{a,1c}

^a Endoreduplication.
^c Pulverised metaphase.
^b Multiple aberration.

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TABLE IV

Statistical analysis of the frequency of chromosomal aberrations: comparison between treated and control groups and the effect of S-9 mix. Pooled data

Treatment	Solvent control (Ehrlich)	
	analysis including gaps	analysis excluding gaps
S-9 mix		
Difference between activated and non-activated solvent control cultures of both experiments (double-sided test)	p-value 0.1420	p-value 0.1745
	Significance NS	Significance NS
Difference between cultures treated with and without S-9 mix (double sided test)	p-value 0.8447	p-value 0.1163
	Significance NS	Significance NS
Difference between treated and control groups (single-sided test)	p-value -	p-value -
	Significance -	Significance -

NS Not Significant, $p > 0.05$ * Significant, $0.01 < p < 0.05$ *** Significant, $p < 0.001$

TABLE 2

The micronucleus test - group means and standard deviations by sex after 24 hours

Group	:	1	2			
Compound	:	1% methyl cellulose	PK25124			
Dosage (mg/kg)	:	-	40			
Group		1	2			
Sex		M	F	M	F	M
Total polychromatic cells scored		5089	5248	5112	5381	5144
Total micronucleated polychromatic cells		14	6	10	6	222
Mean micronucleated cells per 1000 and standard deviation		2.7±1.4	1.1±1.2	2.0±1.2	1.1±0.7	43.2±16
Total mature cells scored		5303	5251	5101	5074	5373
Total micronucleated mature cells		15	9	2	6	12
Mean micronucleated cells per 1000 and standard deviation		2.8±1.5	1.8±1.3	0.4±0.9	1.2±0.8	2.3±2.
<u>Polychromatic cells</u> Mature cells		1.0	1.0	1.0	1.1	1.0

TABLE 2 - cont inued

The micronucleus test - group means and standard deviations by sex after 48 ho

Group	1	2
Compound	1% methyl cellulose	PK26124
Dosage (mg/kg)	-	40
Group	1	2
Sex	M	F
Total polychromatic cells scored	4966	5222
Total micronucleated polychromatic cells	11	9
Mean micronucleated cells per 1000 and standard deviation	2.4±1.7	1.7±2.0
Total mature cells scored	4906	5080
Total micronucleated mature cells	5	4
Mean micronucleated cells per 1000 and standard deviation	1.0±1.4	0.8±0.8
<u>Polychromatic cells</u> <u>Mature cells</u>	1.0	1.0
		1.2±0.8
		0.
		2.

TABLE 2 - continued

The micronucleus test - group means and standard deviations by sex after 72 hours

Group : 1 2
 Compound : 1% methyl cellulose ----- PK26124 -----
 Dosage (mg/kg) : - 40

Group 1 2

	M		F	
	N	F	N	F
Total polychromatic cells scored	5196	5194	5475	5369
Total micronucleated polychromatic cells	5	5	13	5
Mean micronucleated cells per 1000 and standard deviation	1.0±1.2	1.0±1.2	2.5±2.1	0.9±0.
Total mature cells scored	5087	5058	5149	5134
Total micronucleated mature cells	0	5	3	3
Mean micronucleated cells per 1000 and standard deviation	0.0±0.0	1.0±1.2	0.6±0.9	0.6±0.
Polychromatic cells Mature cells	1.0	1.0	1.1	1.0

TABLE 3

Statistical analysis of data after 24 hours of exposure

Group : 1
 Compound : 1% methyl cellulose
 Dosage (mg/kg/day) : 40

PK26124
 40

Group	1	
Sex	M	F
Difference between sexes within a group	R 36.5	35.5
	Sig. NS	NS
Difference between treated and control groups	R -	113
	Sig. -	NS

The R value given is the higher of the two obtained by the Mann-Whitney calculator

Sig. Significance
 N.S. Not significant, $p > 0.05$
 ** Significant $0.01 > p > 0.001$

TABLE 3 - continued

Statistical analysis of data after 48 hours of exposure

Group : 1 2
 Compound : 1% methyl cellulose ----- PK26124 -----
 Dosage (mg/kg) : - 40

Group	1	2
Sex	H F M	H F M

Sex	H	F	M
Difference between sexes within a group	24	30	NS
	Sig.	NS	NS

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Difference between treated and control groups	R	101.5
	Sig.	NS

The R value given is the higher of the two obtained by the Mann-Whitney U test.

Sig. Significance
 N.S. Not significant, $p > 0.05$

TABLE 3 - continued

Statistical analysis of data after 72 hours of exposure

Group : 1 2
 Compound : 1% methyl cellulose
 Dosage (mg/kg/day) : - 40

PK26124
 40

Group	M	F	M
Sex			
Difference between sexes within a group	R	27.5	32.5
	Sig.	NS	NS
Difference between treated and control groups	R	-	119
	Sig.	-	NS

The R value given is the higher of the two obtained by the Mann-Whitney U

Sig. Significance
 N.S. Not significant, $p > 0.05$

Table A
 Summary of Aberration Data
 In vivo Bone Marrow Chromosome Study with PK 26124
 6-hour

Group Number	Treatment	Number of Animals Per Group	Number of Metaphases Analyzed Per Group	Number of Aberrant Cells Per Group	Number of Aberrant Cells Per Group	P Value	Total Number of Aberrations Per Group
1	Methyl Cellulose (1%)	10	350	1	0.3	NS	1
3	PK 26124 (2 mg/kg)	10	350	2	0.6	NS	2
4	PK 26124 (7 mg/kg)	10	350	2	0.6	NS	2
5	PK 26124 (20 mg/kg)	10	350	0	0	NS	0

NS = Not Significant
 *Statistical analysis performed with mean numbers per animal.

Table 2 - Continued
 Summary of Aberration Data
 In vivo Bone Marrow Chromosome Study with PK 26124
 (12/12) Hour

Group Number	Treatment	Number of Animals Per Group	Number of Metaphases Analyzed Per Group	Number of Aberrant Cells Per Group	Percent Aberrant Cells	P* Value	Total Number of Aberrations Per Group	Average Number of Aberrations Per Cell
1	Methyl Cellulose (1%)	10	450	4	0.9	-	7	0
3	PK 26124 (2 mg/kg)	10	350	1	0.3	NS	1	0
4	PK 26124 (7 mg/kg)	10	450	6	1.3	NS	7	0
5	PK 26124 (20 mg/kg)	10	500	0	0	NS	0	0

NS = Not Significant

*Statistical analysis performed with mean number per animal.

26-

Table 2 - Continued
 Summary of Aberration Data
In vivo Bone Marrow Chromosome Study with PK 26124
 24-hour

Group Number	Treatment	Number of Animals Per Group	Number of Metaphases Analyzed Per Group	Number of Aberrant Cells Per Group	Percent Aberrant Cells Per Group	P Value	Total Number of Aberrations Per Group	Ave Num Abe Per
1	Methyl Cellulose (1%)	10	400	1	0.3	-	1	1
2	Cyclophosphamide (40 mg/kg)	10	330	57	17.3	0.0006(S)	197	19.7
3	PK 26 (2 mg/kg)	10	400	0	0	NS	0	0
4	PK 26124 (7 mg/kg)	10	350	1	0.3	NS	2	2
5	PK 26124 (20 mg/kg)	10	442	4	0.9	NS	8	8

NS = Not Significant

S = Significant

*Statistical analysis performed with mean numbers per animal.

Table 2 - Continued
 Summary of Aberration Data
 In vivo Bone Marrow Chromosome Study with PK 26124
 48-Hour

Group Number	Treatment	Number of Animals Per Group	Number of Metaphases Analyzed Per Group	Number of Aberrant Cells Per Group	Percent Aberrant Cells Per Group	P ^a Value	Total Number of Aberrations Per Group	Average Number of Aberrations Per Cell
1	Methyl Cellulose (1x)	10	450	0	0	-	0	0
3	PK 26124 (2 mg/kg)	10	350	0	0	NS	0	0
4	PK 26124 (7 mg/kg)	10	450	2	0.4	NS	2	0.4
5	PK 26124 (20 mg/kg)	10	350	0	0	NS	0	0

NS = Not Significant

^aStatistical analysis performed with mean numbers per animal.

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Executive Summary:

Riluzole a benzothiazole, is a glutamate inhibitor with multiple sites of action. A single mechanism of Riluzole antiglutamate effect is unknown at this time. Riluzole produced the following effects:

1. blockade of glutamate-induced excitatory neurotransmission affecting both the basal and evoked release (presynaptic), and postsynaptically by non-competitive binding to the receptors.
2. blockade of calcium mobilization in primary rodent neurons in culture.
3. inactivation of voltage-dependent Na-channels.
4. stimulation of G-protein dependent signal transduction.
5. blockade of excitatory amino acid (EAA)-induced convulsions.

Electrophysiological studies showed that Riluzole at 50 μ M completely blocked NMDA responses but did not inhibit binding. Similar responses noted with kainate where currents were blocked at >500 μ M without an effect on binding for concentration up to 1mM. Therefore, Riluzole may bind directly but non-competitively with EAA receptors. The drug does not possess neuroleptic, antianxiety, antidepressant, spasmolytic, or psychostimulant amphetamine-like effects. In some models of global or focal ischemia, Riluzole showed some degree of neuroprotection, histologically it decreased the area of necrosis. Cerebral glucose utilization was reduced dose-dependently after i.v. injection of Riluzole to rats. Riluzole affected the cardiovascular system parameters as follows: a significant effect on SBP and DBP in the conscious dog (i.v.), and in the anesthetized dog it significantly decreased HR, contractility index, and CO but increased LVEDP. It significantly decreased HR and MAOP in conscious SHR (oral administration). The drug administered i.v. had no significant effect on any parameter in the conscious dog with or without experimental myocardial ischemia.

Pharmacokinetic studies (single and repeat dose) have been conducted in the rat, dog, and monkey. Riluzole is rapidly absorbed in the rat (T_{max} 15-20 min), and widely distributed with highest concentration in liver > kidney > brain. Brain levels were 2-3 fold higher than plasma levels. Mean plasma levels in the rat (m+f) ranged between 0.23 $\pm$ 0.04 to 3.10 $\pm$ 0.7 μ g/ml. About 50 and 80% unchanged drug is found in the plasma and brain respectively, with 30 and 17% of methoxyphenylurea derivative, respectively (see chart for metabolic pathways). The drug is also well absorbed in the dog and rabbit but slowly absorbed in monkeys. Mean plasma levels in the dog ranged between 3-5 μ g equivalent/ml. Metabolites are excreted mainly via the urine and in the rat sulfconjugation predominates, whereas glucuronidation is predominant in the dog. Pregnant rabbits unlike nonpregnant ones, showed good plasma-dose relationship and on occasion, C_{max} was significantly higher in pregnant vs. nonpregnant rabbits. The sponsor indicated that this difference in concentration was clinically irrelevant. Some tendency toward drug accumulation was noted at 3mg/kg dose in both pregnant and nonpregnant rabbits. Mean C_{max} in the nonpregnant rabbit ranged from 0.3 $\pm$ 0.34 to 3.4 $\pm$ 0.7 μ g/ml whereas the corresponding values in pregnant rabbit are 0.3 $\pm$ 0.1 to 11.8 μ g/ml. Toxicokinetics in monkeys indicated a significant increase in accumulation ratios between the start and end of 1st and 26th studies. In the latter study, Riluzole orally administered to 5 monkeys was well absorbed and good correlation was found between plasma and brain levels.

In the 26th study, Riluzole orally administered to 5 monkeys was well absorbed and good correlation was found between plasma and brain levels. In the 26th study, Riluzole orally administered to 5 monkeys was well absorbed and good correlation was found between plasma and brain levels.

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and jerking of forelimbs, and paddling i.e. convulsion. These clinical signs were observed in rats dosed 20 or 25mg/kg as well as in some animals dosed 8 or 10mg/kg in both sexes. Body weight gain in general was significantly reduced mainly in male rats and the decrease was dose- and time-dependent in the 26wk study. In HD f rats body weight was also reduced significantly but the decrease was dose-independent. The only common clinical chemistry finding in the 13- and 26-wk studies, is a significant elevation in ALP (in the 26wk ALP in all 3 dose groups was elevated in m). Other changes included a significant increase in cholesterol of 10 and 20mg/kg m and f rats. LDH levels was significantly reduced in 10 and 20mg/kg dosed f during wks 12 and 26 of study. No significant drug-related changes were noted in ophthalmoscopy, hematology, or urinalysis. In the 13wk study, 5/15 HD f had congested ovaries and thyroid cyst was found in 7/15 m and 6/15 f of HD. Histopathology examination of the 26wk rats showed liver changes including centrilobular hepatocyte vacuolation, enlargement, necrosis, and ballooned cells with brown pigmented macrophages in portal triad. In 11/15 HD f had occasional Kupffer cells with positive pigment; none was observed in the control. Treated m and f rats of 10 and 20mg/kg dose killed at end of study, had hemosiderosis (hemosiderosis was noted only in animals of HD killed at recovery period), all treated f killed during recovery had uterus luminal dilatation. A NOEL could not be determined in the 26wk, 5mg/kg can be considered the LOEL based on histopathological findings. In a 26wk cynomolgus monkey study, animals dosed 20mg/kg died or were sacrificed between wks 5 and 9 due to toxicity. Clinical signs noted in 5 and 10mg/kg dosed monkeys and included huddled posture, lethargy, incoordination, salivation, piloerection, isolated incidences of limb tremors, and vomiting. Only in HD animals erratic movement and repetitive rocking movements were also noted. There were no drug-related changes in EKG, urinalysis, and minimal dose-independent effects on body weight, hematology, and clinical chemistry. A 2mg/kg dose is the NOEL for monkeys. In a 2wk dog toxicity study, no deaths noted at 30mg/kg, clinical signs included vomiting, tremors, ataxia, hypomotility, hyperreactivity, and somnolence in both sexes. All signs were absent by 24hr postdose. Hematological changes were noted and considered (by the sponsor), secondary to hemosiderosis of the liver and spleen. These changes include decrease in RBC count, Hb, and Hct, increase in thrombocytes, reticulocyte, and myelogram parameters such as normoblasts. A marked decrease in haptoglobin concentration was noted on day 8 and 14 (up to 100%) in both sexes. These changes were contributed to hemolysis/anemia. All treated dogs had hemosiderosis of the liver and spleen, and as indicated above, the sponsor considered this finding secondary to drug-induced hemolytic anemia. No statistically significant effects in clinical chemistry were found however, the following changes were measured: ALP levels were increased in m and f on days 5, 8, and 14 (11-53%); LDH level was increased in m on days 8 (169%) and 14 (106%) and in f only on day 14 (88%); and total bilirubin was increased 167-200% on days 8 and 14 in both sexes.

Segment II reproductive studies were conducted in rat and rabbit however, a standard Segment I was not done. A range-finding study in rats may have substituted for Segment I however, this study is deficient and did not comply with the guidelines. In general, oral administration of Riluzole at 30/25mg/kg caused death in primary toxicity signs include anquid and ataxia, males more affected than females and less severe. At 10mg/kg animals were observed to have tremors, vomiting, and incoordination. At 5mg/kg animals did not show any signs of toxicity. At 2mg/kg animals showed no signs of toxicity. Significant changes in body weight and food intake were observed in the 10mg/kg group.

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The NOEL in this study is 3mg/kg. Oral administration of Riluzole to pregnant rabbits from gd 6-18 caused no deaths and clinical signs such as languor and ataxia occurred mainly in 60mg/kg (HD) rabbits. Significant weight loss was noted only in HD during gd 6-15. Small but significant decrease in fetal weight and crown/rump length reported in HD. Small but insignificant increase was noted in post-implantation loss in HD relative to the control. High incidence of minor fetal defects occurred in all treated groups (significant only in 18mg/kg dosed animals). The sponsor indicated that these defects are common in rabbit. In summary, Riluzole was not teratogenic in rat or rabbit but caused maternal toxicity.

Riluzole was not mutagenic in the Ames Salmonella assay or the in vivo bone-marrow micronucleus assay in rats and mice. An equivocal result was obtained in the mouse lymphoma chromosomal aberration assay but when repeated the results were negative. Potential induction of chromosomal aberration by Riluzole was tested in human peripheral blood. A significant increase in chromosomal aberration was found in absence of S9 at 10µg/ml concentration and only when gaps were excluded from the analysis. A significant difference was also noted between activated and nonactivated cultures at the same concentration. The assay was repeated and data analyzed separately and pooled with the previous assay. Similar results obtained, but since the positive findings noted only in one concentration, the significance of gap-type aberration is unclear. From the results of the 4 assays, the mutagenic/clastogenic potential of Riluzole to humans is small.

Recommendation:

Based on the submitted pre-clinical pharmacology/toxicology studies, the proposed clinical trial for this IND may proceed. The following comment section contain some pharmacology issues that need to be provided/explained by the sponsor.

Comments:

The pharmacology reviewer requests clarification and/or information on the following preclinical issues:

1. Analysis of the drug formulation in the rat reproductive study #DN101589 on days 1, repeat day 1, 4, 16, and 20 produced unacceptable low concentrations of the drug material. The sponsor indicated that this was due to "settling out" of the drug material from the suspension and fibers of methyl cellulose interfered with the analytical method. As reported by the sponsor, this finding had no effect on the doses administered. What were the actual doses administered? Evidence or supportive data to this statement is requested; the explanation provided is inadequate.
2. In the same rat reproductive study mentioned above, the temperature and/or the humidity in the rooms housing the rats was exceeded on several occasions. The exact duration and extent of this elevation were not mentioned. This information is requested since large fluctuation in room temperature can affect the well-being of the animals, fertility and abortion rates.

See chronic toxicity studies conducted on rat and mouse. See also...

In the rat, the toxicity of the drug was observed in the pregnant female animals...

...

6. The amino acid taurine is markedly elevated in brain and spinal cord of ALS patients. There are animal models for studying taurine levels; do you know if Riluzole affects taurine levels in any way? Again this is merely a scientific curiosity.

Aisar Atrakchi

Aisar H. Atrakchi, Ph.D.

cc.
IND ORIG (#42,080)
Div. File
HFD-120

/G. Fitzgerald/A.H. Atrakchi/Paul David

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Amendment
NDA# 20-599

Drug: Riluzole
Sponsor: Rhone-Poulenc Rorer, PA
Corsp. Date: Sep 22 1995
Rec. Date: Sep 27 1995
Rev Date: Sep 28 1995
Reviewer: Aisar H. Atrakchi, Ph.D. *NHA*
Supervisor: Glenna Fitzgerald, Ph.D. *997 10/30/95*

Report Title: Riluzole effect on survival in FALS-SOD transgenic mice/RPR-RD-DISC-Biol 748/Sep 11 1995/GLP.

Background:

Familial ALS accounts for about 10% of the ALS population. Rosen et al., 1993 showed mutations in the gene coding for Cu⁺⁺/Zn⁺⁺ superoxide dismutase (SOD) in about 20% of the 10% FALS population. Deficiency in this enzyme may trigger free-radical toxic mechanisms in the body. Because of the role of free radical-induced injury in neurodegenerative diseases, transgenic mice were used to study the role of the SOD gene in the pathogenesis of FALS. Transgenic mice (B6SJL-TgN(SOD1-G93A)1Gur) were raised to express human SOD gene that carries the Gly⁹³ to Ala mutation. These mice develop progressive paralysis of limbs starting at 3months of age and die by 5-6months from respiratory failure and/or inability to eat. This animal model expresses both functional and histopathological (loss in spinal cord and brain-stem motoneurons), properties of ALS.

Study Results and Discussion:

Riluzole (Batch# MIJ 436) was administered to mice (20/gr) in drinking water at 100ug/ml starting at age 50days (water was replaced every 3days). The overall condition of the mouse was monitored 3x/wk, onset of disease was assessed by testing tremors in limbs when the animal was held from its tail and suspended in the air. At later stages of the disease the mouse laid on its side and did not attempt to move when touched. Mice were killed if they could not: (1) groom themselves and one or both eyes develop severe infections, (2) right themselves within 30sec of being placed on their sides. Length of survival was determined and compared between treated and control animals. Survival was evaluated by Kaplan-Meier analyses and differences in mean age at onset of disease or age at death were compared using 2-tailed Student's t-test.

One treated mouse escaped from the cage and another mouse showed severe adverse effects (not identified by the sponsor); both mice were not included in the data analysis. Table 1 from the sponsor presents individual survival data and Table 2 provides the mean survival and disease onset. Mice treated with riluzole on the average, lived longer (11%) than the controls however, onset of the disease and muscle function were not affected by treatment.

Comments/Questions to the Sponsor:

1. It is clear from these results that riluzole had no effect on onset or muscle function the only effect was increase in survival. It would have been valuable to have conducted histopathology on riluzole-treated mice and compare the findings with those in the control.
2. Please report the serious adverse effects noted in mouse# 6 and onset of these symptoms.
3. Of the 20 mice, how many were males and how many were females?
4. Please provide certificate of analysis for riluzole batch used in this study.

cc.

/Div File/Orig NDA# 20-599
/G. Fitzgerald/A. Atrakchi/K. Higgins

TABLE 1

Individual survival data

CONTROL		RILUZOLE	
Mouse N°	Age at death (days)	Mouse N°	Age at death (days)
1	116	1	146
2	127	2	120 (<i>escaped</i>)
3	140	3	143
4	142	4	134
5	120	5	139
6	136	6	99 (<i>killed</i>)
7	140	7	142
8	125	8	175
9	136	9	148
10	140	10	148

TABLE 2

Effect of riluzole on mean survival and mean onset

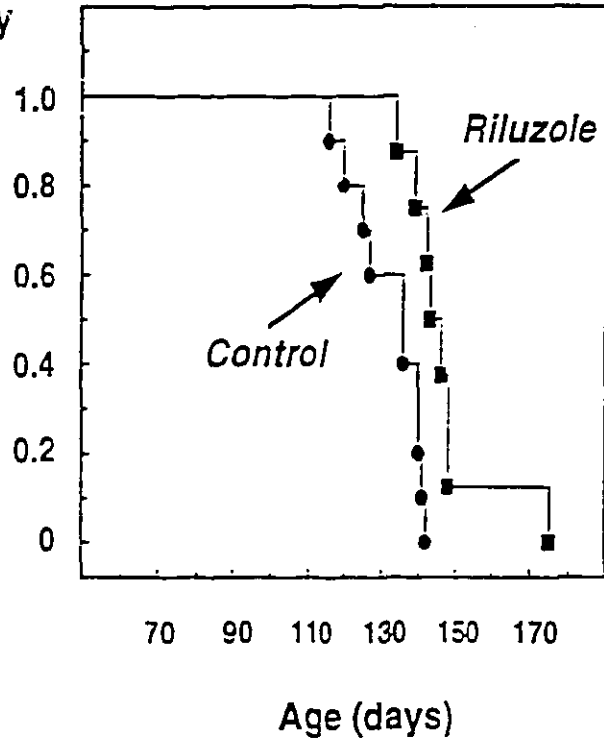
Data are presented as the mean $\pm$ SEM. The asterisk indicates a significant ($p < 0.05$; Student's *t*-test) difference between the control and riluzole groups.

Treatment	Mean age at onset	Mean age at death
Control (n = 10)	95 $\pm$ 12 days	132 $\pm$ 9 days
Riluzole (n = 8)	98 $\pm$ 11 days	147 $\pm$ 12 days*

FIGURE 1

Effect of riluzole on survival

Survival probability



END

SD.