CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-539

PHARMACOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21,539
Review number: 01
Sequence number/date/type of submission: June 27, 2002
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Cumberland Pharmaceuticals Inc.
Nashville, Tennessee

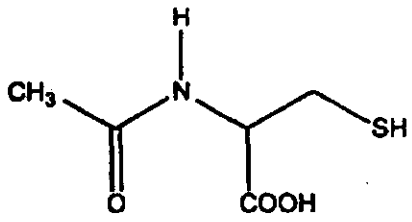
Manufacturer for drug substance:

[redacted]

Reviewer name: Ke Zhang
Division name: Division of Gastrointestinal and Coagulation Drug Products
HFD #: 180
Date of Receipt by HFD-180: July 3, 2002
Review completion date: November 29, 2002

Drug:

Trade name: Acetadote
Generic name: Acetylcysteine injection
Chemical name: N-acetyl-L-cysteine
Molecular formula/molecular weight: $C_5H_9NO_3S$ / 163.2
Structure:



Relevant INDs/NDAs/DMFs:
IND 442, [redacted] and NDA 13,601
Drug class: [redacted]
Indication: [redacted]

Clinical formulation:

Component	Grade	Amount (mg/mL)
Acetylcysteine	USP	200 mg
Edetate Disodium	USP	0.5 mg
Water for Injection	USP	[redacted]
[redacted]	NF	q.s.
Sodium Hydroxide	USP	q.s. to adjust pH

Route of administration: I.v. infusion.

Proposed use: Acetadote is used for treatment [redacted] A loading dose of Acetadote will be given by intravenous infusion at 150 mg/kg in 200 ml of 5% dextrose over [redacted] followed by an i.v. maintenance dose of 50 mg/kg in 500 ml of 5% dextrose over 4 hours and then 100 mg/kg in 1000 ml of 5% dextrose over 16 hours. The total i.v. infusion dose is 300 kg/kg over 21 hours.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Executive Summary

I. Recommendations

A. Recommendation on Approvability:

From a preclinical standpoint, approval of intravenous administration of acetylcysteine [redacted] is recommended.

B. Recommendation for Nonclinical Studies: None

C. Recommendations on Labeling

Sponsor should be asked to revise the labeling as recommended.

II. Summary of Nonclinical Findings

A. Pharmacologic Activity

Acetylcysteine is an antidote [redacted]
The *in vivo* studies in mice have demonstrated that coadministration of acetylcysteine with acetaminophen increased hepatic glutathione concentration, decreased the amount of covalent binding of acetaminophen to hepatic protein, and prevented acetaminophen-induced liver toxicity.

B. Brief Overview of Toxicology Findings

The results of 4-day toxicokinetic study in rats indicated that oral bioavailability of N-acetylcysteine was 24-29% for males and 37-43% for females. Treatment with oral dose of N-acetylcysteine did not produce any toxicity at oral doses up to 1000 mg/kg/day in the 4-, 12-, 28-week, and 18-month oral toxicity studies in rats. The target of organ toxicity was not identified in these studies. Based on the oral bioavailability of the total N-acetylcysteine in rats, the oral dose of 1000 mg/kg/day would be equivalent to 240-370 mg/kg/day of an i.v. dose.

The results of the 90-day i.v. toxicity study in dogs indicated that N-acetylcysteine at i.v. doses of 200 and 400 mg/kg/day produced transient clinical signs of toxicity including prolapse of the nictitating membranes, lacrimation, salivation, erythema of the ears, occasional restlessness, nervousness, and tremors.

Acetylcysteine was not teratogenic at oral doses of 500, 1000, and 2000 mg/kg/day in rats and 250, 500, and 1000 mg/kg/day to rabbits in the Segment II teratologic reproductive toxicity studies.

N-acetylcysteine was positive in the presence of metabolic activation in the *in vitro* mouse lymphoma cell forward mutation assay. N-acetylcysteine was not genotoxic in the Ames test and *in vivo* mouse micronucleus test.

C. Nonclinical Safety Issues Relevant to Clinical Use

None.

III. Administrative

A. Reviewer signature: _____

B. Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

cc: list:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Zhang
HFD-045/Dr. Viswanathan

Studies reviewed within this submission:

Type of Study	Study #	Lot	Lab	Page #
Pharmacology	Published reports			1-6
Absorption, Distribution, Metabolism, and Excretion (ADME):				
Pharmacokinetic studies in rats and dogs	Published reports			6-8
4-day toxicokinetic study in rats	693A-101-910-01		1	10-13
Acute Toxicity:				
Single i.v. dose toxicity studies in mice, rats, guinea pigs, rabbits, and dogs	Published report			8-10
Subacute, Subchronic, and Chronic Toxicity:				
4-week, 12-week, 28-week oral toxicity studies in rats	Published report			14-15
18-month oral toxicity study in rats	Published report			15
90-day i.v. toxicity study in dogs	Published report			15
Mutagenicity:				
Ames test	published report			16
Mouse lymphoma cell (L5178Y+/-) test	AA44TD.704 [redacted]	200319	2	16-19
In vivo mouse micronucleus test	AA44TD.123 [redacted]	200319	2	20-22
Reproductive Toxicity:				
Oral "fertility study" in male rats	Published report			23
Oral Segment II teratologic study in rats	Published report			23
Oral Segment II teratologic studies in rabbits	Published reports			23-24
Oral Segment III peri- and post-natal reproductive toxicity study in rats	Published report			23-24

1 = [redacted] 2 = [redacted]

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TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Mechanism of action:

Acetadote is indicated for treatment Acetaminophen is mainly metabolized in the liver by sulfation and glucuronidation, while a small percentage of acetaminophen (~4%) is converted to N-acetyl-p-benzoquinoneimine (NAPQI). NAPQI is a strong electrophile and oxidizing agent that is detoxified by reduced glutathione and excreted by the kidney (see Figure 1 from Flanagan R.J. et. Al., Am. J. Med., 91 (suppl. 3C): 3C-131S to 3C-139S, 1991).

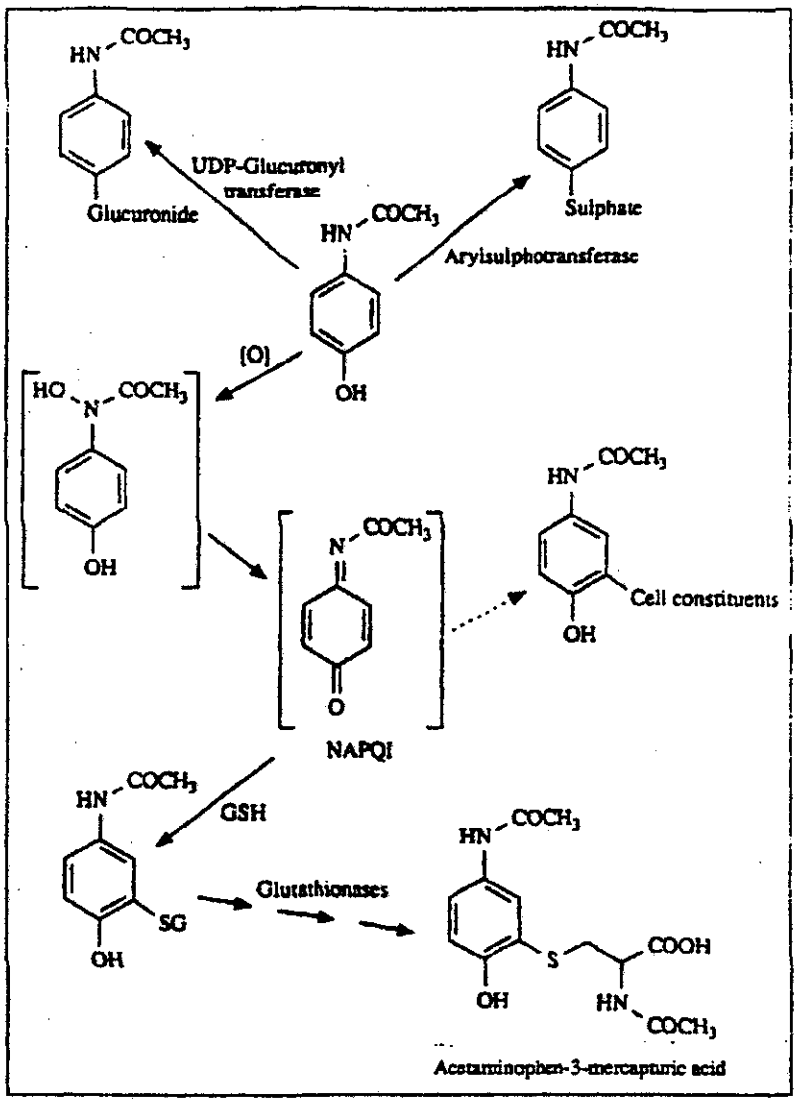

Figure 1. Summary of acetaminophen (paracetamol) metabolism.

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When acetaminophen overdose occurs, glutathione stores become exhausted, and the normal detoxification of NAPQI is interrupted. Under this condition, NAPQI is irreversibly bound to protein molecules within hepatocytes and disrupts the normal function of the liver, which leads to cell death. N-acetylcysteine prevents acetaminophen induced liver toxicity by promoting glutathione synthesis and furnishing glutathione store. Glutathione then reacts with acetaminophen to make them inert and excreted via the kidney.

Drug activity related to proposed indication:

In an *in vivo* study in mice (Corcoran GB., et. Al., J. Pharm. Exp. Ther., 232(3): 864-872, 1985), oral administration of acetylcysteine at 1200 mg/kg 20 minutes prior to or 60 minutes after treatment with acetaminophen at oral dose of 1000 mg/kg significantly prevented acetaminophen induced hepatotoxicity. In this study, acetylcysteine prevented elevation of serum glutamate pyruvate transminase (SGPT) and decreased the incidence of hepatic necrosis and mortality rate. The result presented in Tables 1 and 2 in this article. These tables are attached below.

TABLE 1

Time of p.o. N-acetylcysteine (NAC) administration vs. prevention of SGPT elevation after p.o. acetaminophen overdose in mice

N-acetylcysteine (NAC, 1200 mg/kg) was administered to male Swiss mice the specified number of minutes before or after 1000 mg/kg of acetaminophen. Surviving animals were sacrificed 6, 12 or 24 hr later. Control animals received water p.o. 60 min after acetaminophen.

Treatment	Time	Time of Sacrifice					
		6 Hr		12 Hr		24 Hr	
		Survival	SGPT activity*	Survival	SGPT activity*	Survival	SGPT activity*
	min						
Control		29/34	2945 ± 631	45/55	6090 ± 997	35/52	11686 ± 1461
NAC	−20	15/16	77 ± 13**	25/25	100 ± 10**	23/24	211 ± 86**
NAC	+60	14/14	114 ± 22**	14/15	76 ± 6**	16/18	64 ± 5**
NAC	+240	25/28	1451 ± 357	32/38	2224 ± 469**	32/38	912 ± 237**
NAC	+360			9/11	8156 ± 2409	9/11	3038 ± 1009
NAC	+480			10/10	9390 ± 2149	3/10	9812 ± 1280

* X ± S.E. of survivors reported in I.U./l.

** P < .01 vs. the control value at that time as determined by the Wilcoxon Rank Sum Test.

TABLE 2

Time of p.o. N-acetylcysteine (NAC) administration vs. prevention of hepatic necrosis after p.o. acetaminophen overdose in mice

Conditions as in table 1.

Treatment	Time	Relative Incidence and Extent of Hepatic Necrosis*											
		12 Hr						24 Hr					
		Survival	0	1+	2+	3+	4+	Survival	0	1+	2+	3+	4+
			% of Surviving Animals						% of Surviving Animals				
Control		45/55		49	49	2		35/52	6	11	37	37	9
NAC	−20	25/25	100					23/24	92	4	4		
NAC	+60	14/15	100					18/18	100				
NAC	+240	32/38		28	72			32/38	12	44	44		
NAC	+360	9/11		44	56			9/11		12	44	44	
NAC	+480	10/10		10	90			3/10			33	67	

* Hepatic necrosis in animals surviving at 12 and 24 hr was scored according to text. Data represent the percentage of surviving animals exhibiting the given levels of liver injury. Necrosis in the animals surviving at 6 hr was still developing and hence no attempt was made histologically to grade it.

In another *in vivo* study in mice (Hjelle, J.J., et. Al., J. Pharm. Exp. Ther., 236(2):536-534, 1986), coadministration of N-acetylcysteine (i.p, 4 mmol/kg) significantly prevented acetaminopohen induced hepatotoxicity and lethality. The results were presented in Figures 1 and 2 in this article. These figures are attached below.

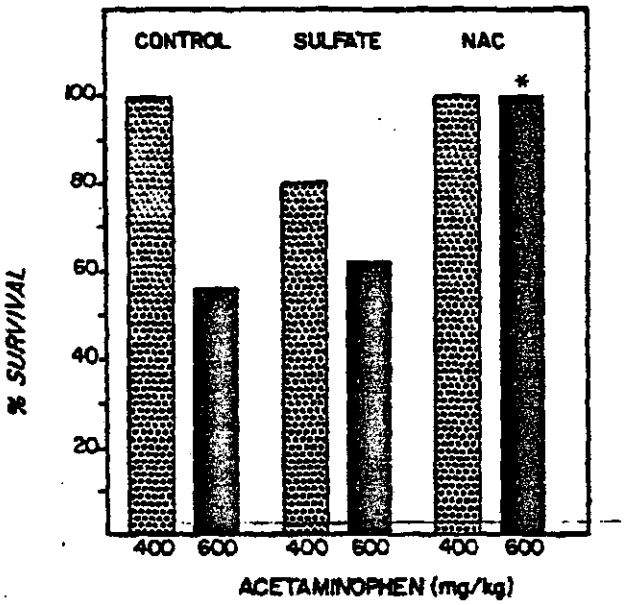

Fig. 1. Effects of sodium chloride (control), sodium sulfate or NAC on the lethality observed 48 hr after administration of AA. Each bar represents the percentage of mice that survived (groups of 10–16 mice). An asterisk denotes that the survival of mice treated with NAC and 600 mg/kg AA was significantly greater than that observed in control or sodium sulfate-treated mice administered 600 mg of AA per kg.

AA: acetaminophen given by i.p. injection, NAC: N-acetylcysteine

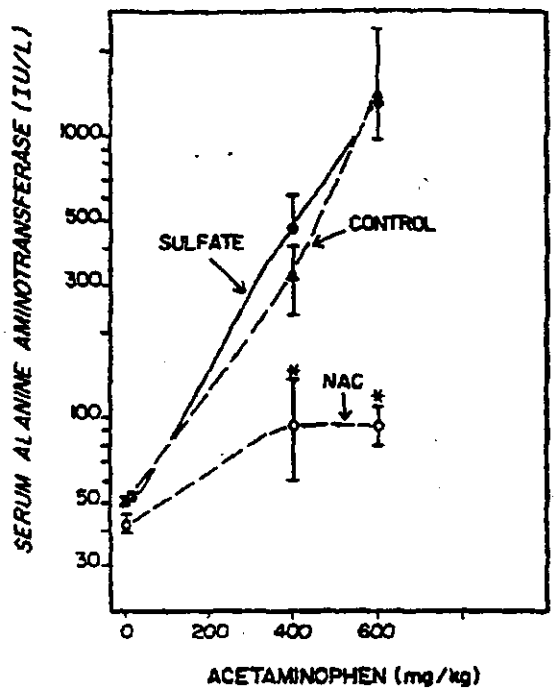

Fig. 2. Effects of sodium chloride (control), sodium sulfate or NAC on AA-induced increases in serum alanine aminotransferase activity 48 h after AA administration. Each point represents the mean ± S.E. of 6 to 11 mice. Asterisks denote significant differences between NAC-treated mice and control or sodium sulfate-treated mice given the same dosage of AA.

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It was also demonstrated that coadministration of acetylcysteine increased hepatic glutathione concentration, and decreased the amount of covalent binding of [^{3}H]-acetaminophen to hepatic protein, suggesting that the preventive effects of acetylcysteine on acetaminophen-induced liver toxicity are mediated via stimulating hepatic glutathione synthesis. These results were summarized in Figure 8 and Table 3 in this article which are attached below.

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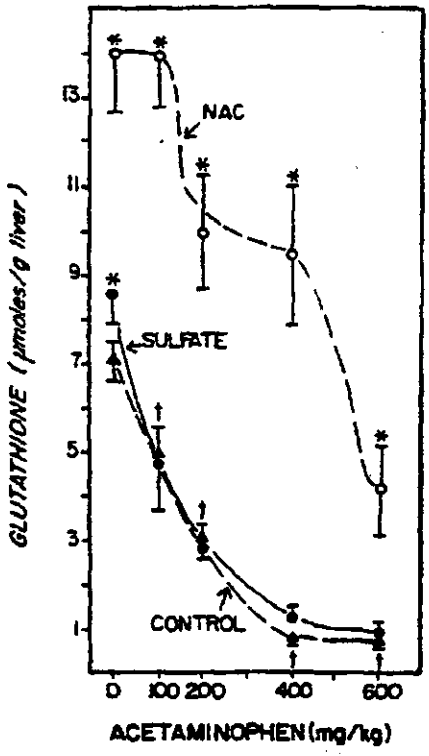

Fig. 8. Dosage-dependency of the effects of AA on hepatic glutathione concentration in sodium chloride (control)-, sodium sulfate- and NAC-treated mice. Glutathione concentration was determined in liver samples obtained 2 hr after injection of the various agents. The values represent the mean ± S.E. of five-or six mice. Daggers denote significance ($P < .05$) from the control group given no AA. Asterisks indicate significant differences from control mice that received the same dosage of AA.

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TABLE 3

Covalent binding of [^{3}H]AA in mouse liver 2 hr after sodium sulfate or NAC treatment

Group[a]	Liver/Body wt. Ratio (g/100 g body wt.)	nmol [^{3}H]AA Bound per mg Protein	μmol [^{3}H]AA Bound per kg body wt.
Control + AA	6.86 ± 0.18	0.657 ± 0.029	13.2 ± 0.7
Sodium sulfate + AA	7.02 ± 0.26	0.757 ± 0.086	15.5 ± 0.9
NAC + AA	6.45 ± 0.18	0.648 ± 0.112	9.9 ± 0.9[b]

[a] Values for each group represent the mean ± S.E. of five or six mice.
[b] Significantly different ($P < .05$) from the control and sodium sulfate groups.

Pharmacology conclusions:

Under normal condition, a small percentage of acetaminophen (~4%) is converted to N-acetyl-p-benzoquinoneimine (NAPQI) in the liver. NAPQI is a strong electrophile and oxidizing agent and detoxified by reduced glutathione and excreted by the kidney. However, when acetaminophen is overdosed, glutathione stores become exhausted, and the normal detoxification of NAPQI is interrupted. Under this condition, NAPQI is irreversibly bound to protein molecules within hepatocytes and disrupts the normal function of the liver, which leads to cell death. N-acetylcysteine prevents acetaminophen induced liver toxicity by promoting glutathione synthesis and furnishing glutathione store. Glutathione then reacts with acetaminophen to make them inert and excreted via the kidney. The *in vivo* studies in mice have demonstrated that coadministration of acetylcysteine with acetaminophen increased hepatic glutathione concentration, decreased the amount of covalent binding of acetaminophen to hepatic protein, and prevented acetaminophen-induced liver toxicity.

II. PHARMACOKINETICS/TOXICOKINETICS:

PK parameters:

Absorption:

In a 4-day toxicokinetic study in rats (693A-101-910-01), the oral bioavailability of N-acetylcysteine was 24-29% for males and 37-43% for females (this study is reviewed under toxicity section). The maximum plasma level of total N-actylcysteine was reached within 8 or 12 hours after i.v. infusion of N-acetylcysteine at 800 mg/kg/day in rats. The plasma level of total N-acetylcysteine declined with a half life of 6.4 hours or 8.7 hours in males or female rats, respectively.

Distribution and metabolism:

Following information was obtained from a published report (Biochemical Pharmacology, 15:1523-1535, 1966).

Methods: The solution of ^{35}S-acetylcysteine (specific activity = 8.81×10^4 count/min/mg) was given to ten fasted rats by stomach tube at 200 mg/kg or to female dogs by oral gavage at 200 mg/kg. The rats were sacrificed 2 or 24 hours after treatment. Urine samples were collected for 24 hours from both rats and dogs. The liver, kidneys, spleen, brain, adrenals, and femoral muscle were removed in rats for determination of radioactivity. The

radioactivity was determined using liquid scintillation spectrometer.

Results: The results indicated that following oral administration of ^{35}S-acetylcysteine in rats, N-acetylcysteine was found mainly in the kidney followed by the liver, adrenal gland, lung, spleen, blood, muscle, brain, and urine. The major metabolite in the urine was inorganic sulfate in both rats and dogs. The total sulfur excreted in the urine within 24 hours represented 38% and 71% of the dose administered in rats and dogs, respectively.

Excretion:

Following information was obtained from a published report (Eur. J. Respir. Dis., 61(suppl.111): 45-51, 1980).

Methods: The ^{35}S labeled acetylcysteine was given orally to rats and dogs at 100 mg/kg. ^{35}S-acetylcysteine was also given intravenously and intra-muscularly to rats at 50 mg/kg. Urine and feces were collected for 96 hours. The radioactivity in the urine and feces was then determined. Detail description of the methodology was not provided.

Results: The results were summarized in Table 2 in this article. This table is attached below.

TABLE 2. *Radioactivity (% dose) present in urine and feces of rats after i.v., i.m and oral administration, and of dogs after oral treatment. Collection time: 0-96 hours after treatment.*

Animal species	Route of administration	Radioactivity (% dose)		
		Urine	Feces	Urine + Feces
Rat	i.v.	38.55	3.39	41.94
	i.m.	28.16	5.20	33.36
	os	17.26	3.00	20.26
Dog	os	25.69	10.12	35.81

The results indicated that renal excretion is major route of excretion following oral, i.m., and i.v. administrations in rats and oral administration in dogs. The renal excretion of the radioactivity following oral administration represented 17% and 26% of the dose administered in rats and dogs, respectively. Following i.v. administration, the renal excretion of the radioactivity represented about 39% of the dose given in rats. The results also suggested that majority of the radioactivity

still remained in the body 96 hours after dosing in both rats and bogs.

PK/TK conclusions:

The oral bioavailability of N-acetylcysteine was 24-29% for male rats and 37-43% for female rats. When given by intravenous infusion, the maximum plasma level of total N-actylcysteine was reached within 8 or 12 hours in rats. In contrast, the maximum plasma level of total N-acetylcysteine was reached within 0.5-1.0 hours after oral dosing. The plasma level of total N-acetylcysteine declined with a half life of 6.4-8.7 hours in rats following intravenous infusion. N-acetylcysteine was found mainly in the kidney followed by the liver, adrenal gland, lung, spleen, blood, muscle, brain, and urine in rats. The renal excretion is major route of excretion following oral and i.v. administrations in rats and oral administration in dogs.

III. GENERAL TOXICOLOGY:

Single dose studies:

There were single i.v. dose toxicity studies with acetylcysteine in mice, rats, guinea pigs, rabbits, and dogs in a published report (Seminars in Oncology, 10(1), Suppl.1: 17-24, 1983).

In mice, the minimal lethal dose was 1000 mg/kg. Following clinical signs of toxicity were observed: ataxia, hypoactivity, increased respiration or labored respiration, and convulsion.

In rats, the dose of 2000 mg/kg was non-lethal dose. The minimal lethal dose was 2455 mg/kg. Following clinical signs of toxicity were observed: ataxia, increased depth of respiration, labored respiration, hypoactivity, cyanosis, and convulsion.

In guinea pigs, the dose of 1000 mg/kg was non-lethal dose. The minimal lethal dose was 1500 mg/kg. Following clinical signs of toxicity were observed: hypoactivity, increased and labored respiration, loss of righting reflex, and convulsion.

In rabbits, the dose of 1000 mg/kg was non-lethal dose. The minimal lethal dose was 1200 mg/kg. Following clinical signs of toxicity were observed: ataxia, increased respiration, loss of righting reflex, and convulsion.

In dogs, the dose of 300 mg/kg was non-lethal dose. The minimal lethal dose was 500 mg/kg. Following clinical signs of toxicity were observed: emesis, ataxia, tachycardia, loss of righting

reflex, increased respiration or labored respiration, rectal bleeding, hemorrhages in GI mucosa and bloody fluid in intestinal tract, and convulsion.

The dosing information and results were presented in Tables 2 and 3 in this article. These tables are attached below.

Table 2. Acute Toxicity of NAC (Intravenous or Intraperitoneal Administration)

Species	Study No.	Dose (mg/kg)	No. Deaths/No. Dosed	LD_{50} (mg/kg)	Observations
Mouse (i.v.)	1	1000	0/5	1500*	Ataxia and increased respiration at 1000 and 2000 mg/kg. Convulsions and rapid death at 2000 mg/kg. (pH 1.5)
		2000	5/5		
	2	1000	2/10	1200	Convulsions and labored respiration immediately after dosing at all doses. (pH 1.5)
		1200	5/10		
		1500	9/10		
	3	1500	0/10	3725	Ataxia and hypoactivity all doses. Increased depth of respiration and convulsions at 3000 mg/kg or greater. (pH 7.0)
		3000	1/10		
		3750	5/10		
		4500	9/10		
	4	3160	1/10	4252	All deaths at 5000 and 6058 mg/kg occurred during the injection. Clonic convulsions at 4000 mg/kg or greater. Ataxia, hypoactivity, and labored respiration at all doses. (pH 7.0)
		4000	2/10		
		5000	10/10		
		6058	10/10		
Rat (i.v.)	1	2000	0/10	2675	Ataxia, increased depth and labored respiration, cyanosis, and clonic and tonic convulsions at 3000 and 4000 mg/kg. (pH 7.0)
		2500	2/10		
		3000	9/10		
		4000	10/10		
	2	2455	1/10	2550	Ataxia and increased depth of respiration at all doses. Cyanosis, hypoactivity, and convulsions at 2625 and 2925 mg/kg. (pH 7.0)
		2625	8/10		
		2925	10/10		
Rat (i.p.)	1	1500	0/10	2650	Ataxia and hypoactivity at all doses. Labored respiration preceded death. (pH 7.0)
		2000	1/10		
		2600	1/10		
		2800	8/10		
		3000	8/10		
	2	500	10/10	<500	Ataxia, hypoactivity, and labored respiration. (pH 1.7)
		750	10/10		
		1000	20/20		
		2000	10/10		
		3000	10/10		
	3	1700	0/10	>2500	Newborn pups 1–2 days old. No toxic effects. (pH 7.0)
		2500	0/10		

*Estimated value.

Table 3. Acute Toxicity of NAC (Intravenous or Intraperitoneal Administration)

Species	Study No.	Dose (mg/kg)	No. Deaths/No. Dosed	LD_{50} (mg/kg)	Observations
Dog (i.v.)	1	100	0/3	700	No toxic effects at 100 mg/kg. Emesis, ataxia, tachycardia, loss of righting reflex, increased respiratory rate, and rectal bleeding were major toxic signs. 4 ml/min. (pH 7.0)
		200	0/3		
		300	0/3		
		500	0/3		
		750	2/3		
		1000	1/1		
	2	250	0/4	664	Emesis and increased respiration at 250 mg/kg. Emesis, ataxia, deep and labored respiration, rectal bleeding, tachycardia, convulsions, and loss of righting reflex. At necropsy, hemorrhages in GI mucosa and bloody fluid in intestinal tract. 8 ml/min. (pH 7.0)
		500	2/4		
		750	1/4		
		1000	4/4		
	3	51	0/4	Nonlethal dose levels	No toxic effects at 51 mg/kg. Emesis at 66 mg/kg. Emesis and ataxia at both 86 and 112 mg/kg. 8 ml/min. (pH 7.0)
		66	0/4		
		86	0/4		
		112	0/4		
Rabbit (i.v.)	1	1000	0/6	1390	Ataxia, increased respiration, clonic convulsions, and loss of righting reflex at 1500 and 2000 mg/kg. (pH 7.0)
		1500	2/3		
		2000	4/4		
	2	800	0/4	1156	Ataxia, rapid-shallow respiration, loss of reflex and convulsions at 1200 and 1500 mg/kg. (pH 7.0)
		1000	0/4		
		1200	3/4		
		1500	3/4		
Guinea Pig (i.v.)	1	1000	0/10	1650	Ataxia at all doses. Clonic convulsions, loss of righting reflex, and labored respiration preceding death. (pH 7.0)
		1500	5/10		
		2000	8/10		
Guinea Pig (i.p.)	1	250	0/5	1500	No toxic effects at 250 mg/kg. Ataxia at 500 mg/kg or greater. Hypoactivity, increased and labored respiration, loss of righting reflex and clonic convulsions at 1500 and 1860 mg/kg. (pH 7.0)
		500	0/5		
		1000	0/10		
		1500	2/5		
		1860	5/5		

These studies were not GLP studies.

Repeated dose studies:

Study title: A 4-day comparative toxicokinetic study of oral and intravenous N-acetylcysteine in rats

Key study findings: The oral bioavailability of the total N-acetylcysteine was 24-29% for males and 37-43% for females. The maximum plasma level of N-acetylcysteinee was reached with 8 or 12 hours after i.v. infusion. N-acetylcysteine declined with a half life of 6.4 hours or 8.67 hours following i.v. infusion in males or females, respectively. The ratio of unchanged to total N-acetylcysteine was about 30-50% following oral and i.v. administrations.

Study no: 693A-101-910-01
Volume # 1.5, and page #: 271
Conducting laboratory and location:

[ ]

Pharmacokinetic analysis was conducted at

Date of study initiation: July 27, 2001
GLP compliance: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: Lot # 200319
Formulation/vehicle: 20% solution for injection

Methods:
Dosing:

Species/strain: Sprague Dawley rats
#/sex/group or time point (main study): 10
Age: 11-12 weeks old.
Weight: Males: 307.3-377.8 g, females: 213.7-267.8 g.
Doses in administered units: Oral: 458 and 2000 mg/kg/day, I.V.: 800 mg/kg/day given by continuous 24-hour i.v. infusion for 3 days at 33.3 mg/kg/hr.
Route, form, volume, and infusion rate: The target dose by i.v. infusion was 800 mg/kg/day at 2 ml/kg/h or 48 ml/kg/day for 16.66 mg/ml. However, during the second 24 hour period, male rats received 14.5 ml/rat or 707.4 mg/kg/day.
There was no control group.

Observations and times:

Clinical signs: Mortality and clinical signs of toxicity were observed daily.

Body weights: Body weight was determined prior to dosing and at termination.

Food consumption: Food consumption was measured daily.

Toxicokinetics: Blood samples were collected up to 48 hours after last dose. The plasma level of total and free N-acetylcysteine was deterimined using HPLC.

Results:

Mortality: One oral dose male was found dead about 12 hours after the last dose. The death occurred during blood collection and was not considered treatment related.

Clinical signs: Ocular and nasal discharge was noted in all i.v. dose animals.

Body weights: There was no control group in this study. The terminal body weight was decreased in all treatment groups as compared to the pretreatment values (Males: low oral dose: -7 g, high oral dose: -11 g, i.v. dose: -31 g and Females: low oral dose: -12 g, high oral dose: -7 g, i.v. dose: -16 g).

Food consumption: The average food consumption in males was 17.7-19.2 g/animal/day, 10.7-16.3 g/animal/day, and 5.8-14.1 g/animal/day for low oral dose, high oral dose, and i.v. dose groups, respectively. The average food consumption in females was 12-12.5 g/animal/day, 9.2-10.6 g/animal/day, and 4.8-13.3 g/animal/day for low oral dose, high oral dose, and i.v. dose groups, respectively.

Toxicokinetics: The toxicokinetic results were presented in a table on page 55 in Volume 1.5. This table is attached below.

Name of Sponsor Company:	*Individual Study Table Referring to Part of the Dossier*	*(For National Authority Use Only)*
Name of Finished Product:	*Volume:*	
Name of Active Ingredient: N-acetylcysteine	*Page:*	
• **Pharmacokinetics:**	For groups 1 and 2 animals, 9 blood samples were taken at: 0 (pre-dose), 0.5, 1, 2, 3, 5, 8, 12 and 24 hours post-dose, on study day 3. For group 3 animals, 9 blood samples were taken at : 0 (pre-dose), 4, 8, 12, 24, 26, 30, 36 and 48 hours after the start of the last 24-hour intravenous infusion on study day 3.	
Study Times Lines:	Analytical part: 11-Jun-2001 to 27-Nov-2001	

PHARMACOKINETIC RESULTS:

The pharmacokinetic parameters are presented in the following tables :

Total N-acetylcysteine

Gender	Treatment	Dose (mg/kg/day)	C_{min} (ng/mL)	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24} (ng*h/mL)	$t_{1/2}$ (h)	C_{max}/Dose	AUC_{0-24}/Dose	Fabs
Male	PO	458	347		1.0	103119*	2.13	84	225	0.24
	PO	2000	1396		0.5	540272	4.48	39	270	0.29
	IV inf.	800	295		8.0	737350	6.42	55	922	
Female	PO	458	335		0.5	177698	9.86	163	388	0.43
	PO	2000	1487		0.5	667792	3.93	70	334	0.37
	IV inf.	800	443		12.0	717854	8.67	43	897	

* : AUC_{0-12}

Unchanged (free) N-acetylcysteine

Gender	Treatment	Dose (mg/kg/day)	C_{min} (ng/mL)	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24} (ng*h/mL)	$t_{1/2}$ (h)	C_{max}/Dose	AUC_{0-24}/Dose	Fabs
Male	PO	458	121		0.5	41992*	0.88	37	92	0.25
	PO	2000	237		1.0	285450	3.35	27	143	0.39
	IV inf.	800	63		8.0	293606	NC	25	367	
Female	PO	458	233		0.5	74843**	0.48	91	163	0.65
	PO	2000	201		0.5	385572	2.82	44	193	0.77
	IV inf.	800	76		4.0	199658	NC	15	250	

*AUC_{0-8}
** AUC_{0-6}
NC : Not calculated, not enough points in the terminal phase

The oral bioavailability of the total N-acetylcysteine was 24-29% for males and 37-43% for females. The maximum plasma level of N-acetylcysteinee was reached with 8 or 12 hours after i.v. infusion. N-acetylcysteine declined with a half life of 6.4 hours or 8.67 hours following i.v. infusion in males or females, respectively. . The ratio of unchanged to total N-acetylcysteine was about 30-50% following oral and i.v. administrations.

There were oral toxicity studies in rats with acetylcysteine in a published report (Eur. J. Respir. Dis., 61, Suppl.III: 45-51, 1980). These studies were not GLP-studies. The dosing information was presented in Table 6 in this article and this table is attached below.

TABLE 6. *Subacute and chronic toxicity studies.*

Methods	Subacute and Chronic Tests in Rats			Chronic Test in Dogs
Number of Animals (50% males, 50% females)	80	192	160	16
Animal Strain	Sprague Dawley	Sprague Dawley	Sprague Dawley	Beagle
Dose Levels (mg/kg b.w./day)	0-500-1000-2000	0-250-500-1000	0-250-500-1000	0-50-100-300
Route of Administration	oral	oral	oral	oral
Duration of Dosing (weeks)	4	12	28	52

In the 4-week oral toxicity study in rats, rats were treated orally with acetylcysteine at 0, 500, 1000, and 2000 mg/kg/day for 4 weeks. The results indicated that treatment with acetylcysteine did not alter behaviour, body weight gain, hematology, hepatic and renal functions, prothrombin and bleeding times, and histopathological parameters at doses tested in this study. The oral dose of 2000 mg/kg/day would be equivalent to 480-740 mg/kg/day of an i.v. dose based on the oral bioavailability of the total N-acetylcysteine of 24-29% in male rats and 37-43% in female rats.

In the 12-week oral toxicity study in rats, rats were treated orally with acetylcysteine at 0, 250, 500, and 1000 mg/kg/day for 12 weeks. The results indicated that treatment with acetylcysteine did not alter behaviour, body weight gain, hematology, hepatic and renal functions, prothrombin and bleeding times, and histopathological parameters at doses tested in this study. The oral dose of 1000 mg/kg/day would be equivalent to 240-370 mg/kg/day of an i.v. dose based on the oral bioavailability of the total N-acetylcysteine of 24-29% in male rats and 37-43% in female rats.

In the 28-week oral toxicity study in rats, rats were treated orally with acetylcysteine at 0, 250, 500, and 1000 mg/kg/day for 28 weeks. The results indicated that treatment with acetylcysteine did not alter behaviour, body weight gain, hematology, hepatic and renal functions, prothrombin and bleeding times, and histopathological parameters at doses tested in this study. The oral dose of 1000 mg/kg/day would be equivalent to 240-370 mg/kg/day of an i.v. dose based on the oral

bioavailability of the total N-acetylcysteine of 24-29% in male rats and 37-43% in female rats.

There was a chronic oral toxicity study in rats in a published report (Seminars in Oncology, 10(1), Suppl.1: 17-24, 1983). In this study, rats (30/sex/group) were treated with acetylcysteine in diet at doses of 250, 500, and 1000 mg/kg/day for 18 months. Following parameters were determined: clinical signs of toxicity, body weight, food consumption, hematology, clinical chemistry, organ weight, gross and histopathology. The results indicated that the only possible treatment related change was increased kidney weight in the high dose group. There was no evidence of any gross or histopathological changes in the kidney. There was no other treatment related toxicity observed in this study. The oral dose of 1000 mg/kg/day would be equivalent to 240-370 mg/kg/day of an i.v. dose based on the oral bioavailability of the total N-acetylcysteine of 24-29% in male rats and 37-43% in female rats.

The results of a 90-day i.v. toxicity study in dogs were presented in a published article (Seminars in Oncology, 10(1), Suppl.1: 17-24, 1983). In this study, beagle dogs were treated with N-acetylcysteine at 100, 200, and 400 mg/kg/day by i.v. infusion into cephalic or jugular veins at 5 ml/min (0.5-2.0 ml/kg). The results indicated that N-acetylcysteine did not produce any treatment related changes in body weight, food consumption, hematology, clinical chemistry, urinalysis, organ weights, and histopathology. Dogs treated at 400 mg/kg/day had prolapse of the nictitating membranes, lacrimation, salivation, erythema of the ears, occasional restlessness, nervousness, and tremors. These changes were briefly observed during the infusion period. Slight prolapse of the nictitating membranes, lacrimation, occasional restlessness, nervousness, and tremors were also observed at 200 mg/kg/day. These changes were not seen at 100 mg/kg/day except for the slight prolapse of the nictitating membranes. This study was not GLP-study.

Toxicology summary:

In the single i.v. dose toxicity studies, the minimal lethal dose was 1000 mg/kg in mice, 2455 mg/kg in rats, 1500 mg/kg in guinea pigs, 1200 mg/kg in rabbits, and 500 mg/kg in dogs. Following clinical signs of toxicity were observed in all species tested: ataxia, increased respiration or labored respiration, and convulsion.

In the 4-day toxicokinetic study in rats, rats were treated orally at 458 and 2000 mg/kg/day or intravenously at 800 mg/kg/day at 2 ml/kg/h. The results indicated that oral bioavailability of the total N-acetylcysteine was 24-29% for

males and 37-43% for females. The maximum plasma level of N-acetylcysteinee was reached with 8 or 12 hours after i.v. infusion. N-acetylcysteine declined with a half life of 6.4 hours or 8.67 hours following i.v. infusion in males or females, respectively. The ratio of unchanged to total N-acetylcysteine was about 30-50% following oral and i.v. administrations.

In the 4-week oral toxicity study in rats, rats were treated orally with acetylcysteine at 500, 1000, and 2000 mg/kg/day. In the 12-week, 28-week, and 18-month oral toxicity studies in rats, rats were treated orally with acetylcysteine at 250, 500, and 1000 mg/kg/day. The results indicated that there were no treatment related changes in these studies. The target organs of toxicity were not identified. The oral bioavailability of the total N-acetylcysteine was 24-29% in male rats and 37-43% in female rats. Therefore, the oral dose of 1000 mg/kg/day would be equivalent to 240-370 mg/kg/day of an i.v. dose.

In the 90-day i.v. toxicity study in dogs, beagle dogs were treated with N-acetylcysteine at 100, 200, and 400 mg/kg/day by i.v. infusion into cephalic or jugular veins at 5 ml/min (0.5-2.0 ml/kg). The results indicated that the only treatment related changes were prolapse of the nictitating membranes, lacrimation, salivation, erythema of the ears, occasional restlessness, nervousness, and tremors briefly observed during the infusion period at doses of 200 mg/kg/day or higher.

IV. GENETIC TOXICOLOGY:

In a published report (Eur. J. Respir. Dis., 61 (Suppl.III): 45-51, 1980), acetylcysteine was not mutagenic in an Ames test. This was not a GLP study.

Study title: In vitro Mammalian cell gene mutation test (L5178Y/TK+/- mouse lymphoma assay)

Key findings: N-acetylcysteine significantly increase the mutant frequency as compared to the control group in the presence of S9 mix, suggesting that N-acetylcysteine was mutagenic in this test system.

Study no: AA44TD.704.
Volume #, and page #: volume 1.5, p354
Conducting laboratory and location: [redacted]
Date of study initiation: May 9, 2001
GLP compliance: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.
QA reports: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: lot no. 200319

Formulation/vehicle: 5% dextrose, 200 mg/ml.

Methods: To examine the potential mutagenic effects of N-acetylcysteine, the L5178Y TK+/- mouse lymphoma cell assay was conducted in the presence and absence of metabolic activation, S-9 mix from the rat liver. The following concentrations of N-acetylcysteine were used: 800, 1000, 1200, 1400, and 1640 µg/ml. Positive controls (Methyl methanesulfonate and 7,12 dimethylbenz(a)anthracene) were also tested. The TK+/- cell suspension was incubated with the test drug for 4 or 24 hours. After treatment period, the test article was washed out and the culture was allowed to grow for 24 or 48 hours. The number of TK+/- mutant colonies were then counted. Small and large colonies were also determined. The result is considered positive response if a concentration-related increase in mutant frequency was observed.

Strains/species/cell line: L5178 cells
Dose selection criteria:
Basis of dose selection: The dose selection was based on the results of preliminary toxicity assay.
Range finding studies: In the preliminary toxicity assay, L5178A cells were treated with test article at 100-1640 µg/ml. No visible precipitate was noted at any concentration. The suspension growth was 105% and 79% at the highest concentration tested (1640 µg/ml) without and with S9 mix, respectively at 4 hour exposure and 79% without S9 at 24 hour exposure.
Controls:
Vehicle: 5% dextrose.
Negative controls: 5% dextrose.
Positive controls: Methyl methanesulfonate and 7,12 dimethylbenz(a)anthracene.
Exposure conditions:
Incubation and sampling times: L5178A cells were exposed to the test article for 4 or 24 hours. After treatment period, the test article was washed out and the culture was allowed to grow for 24 or 48 hours.
Metabolic activation: Rat liver S9.
Doses used in definitive study: 800, 1000, 1200, 1400, 1640 µg/ml.
Study design: L5178 cells were exposed to N-acetylcysteine at 800, 1000, 1200, 1400, 1640 µg/ml in the presence of S9 mix for 4 hours or in the absence of S9 for 4 or 24 hours.
Analysis:
No. of replicates: Two
Criteria for positive results: The result is considered positive response if a concentration-related increase in mutant frequency was observed.

Results:

Study validity: Positive controls significantly increased the mutant frequency.
Study outcome: Treatment with N-acetylcysteine did not significantly increase the mutant frequency as compared to the control group in the absence of S9 mix. However, in the presence of S9 mix, N-acetylcysteine significantly increased the mutant frequency at all concentrations tested. The positive control also significantly increased it. The results were summarized in a table on page 360 in volume 1.5. This table is attached below.

Name of Sponsor Company: Cumberland Pharmaceuticals Inc.	Individual Study Table Referring to Part of the Dossier: Volume: Page:	(For National Authority Use Only)
Name of Finished Product: NAC		
Name of Active Ingredient: N-acetylcysteine		

Test Article	Dose (µg/mL)	Unactivated (4-hour exposure) Mutants[a]	Induced Mutants[b,e]	% Total Growth[c]	S9 Activated (4-hour exposure) Mutants[a]	Induced Mutants[b,e]	% Total Growth[c]	Unactivated (24-hour exposure) Mutants[a]	Induced Mutants[b,e]	% Total Growth[c]
Vehicle[d]	-	42	-	-	47	-	-	44	-	-
	-	60	-	-	38	-	-	48	-	-
NAC	800	49	-2	98	152	110	68	44	-2	79
	800	53	2	96	94	51	91	39	-7	90
	1000	38	-13	98	137	94	67	43	-3	74
	1000	47	-4	99	71	28	80	53	7	79
	1200	44	-7	96	97	54	94	38	-8	82
	1200	42	-9	90	121	78	68	40	-6	85
	1400	40	-11	102	145	102	71	62	16	84
	1400	40	-11	108	110	68	80	37	-9	94
	1640	43	-8	90	189	147	63	51	5	64
	1640	36	-16	102	132	90	80	54	8	80
MMS	10	287	236	51	-	-	-	-	-	-
	20	676	624	18	-	-	-	-	-	-
	2.5	-	-	-	-	-	-	145	99	74
	5	-	-	-	-	-	-	305	259	45
DMBA	1	-	-	-	268	225	66	-	-	-
	1.5	-	-	-	426	383	33	-	-	-

[a] Cultures containing <0.3x10[6] cells/mL on Day 1 and 2 were considered as having 0% total suspension growth.

[b] Total suspension growth = (Day 1 cell concentration/0.3x10[6] cells/mL) x (Day 2 cell concentration/Day 1 adjusted cell concentration.

[c] % total control suspension growth = (total treatment suspension growth/average vehicle control total suspension growth) x 100.

[d] Vehicle = 5% dextrose in sterile water.

[e] A result was considered positive if a concentration-related increase was observed and 1 or more dose levels with ≥10% total growth exhibited mutant frequencies ≥100 per 10[6] clonable cells over background.

The study met the validation criteria

Under the conditions of this study, 4 or 24 hour exposure to NAC did not provide a positive result in the L5178Y/TK[+/-] Mouse Lymphoma Mutagenesis Assay, whereas a positive result was obtained following 4-hour exposure in S9 activated cultures

CONCLUSION

The results of the L5178Y/TK[+/-] Mouse Lymphoma Assay indicate NAC was negative without activation following 4- and 24-hour exposures and positive with S9 activation.

The data on colony size distribution (not provided in the table above) indicated that the increase in the total mutant frequency was a result of increase in small colonies. The small colony mutants represent chromosome aberration.

In conclusion, N-acetylcysteine was positive in this test system.

Study title: In vivo mouse micronucleus test with N-acetylcysteine

Key findings: Treatment with N-acetylcysteine at i.v. doses of 187.5, 375, and 750 mg/kg did not significantly increase the incidence of micronucleated polychromatic erythrocytes in male or female mice. The results suggest that N-acetylcysteine was not mutagenic in this test system.

Study no: AA44TD.123. —
Volume #, and page #: volume 1.5, p321.
Conducting laboratory and location: [redacted]
Date of study initiation: May 9, 2001
GLP compliance: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.
QA reports: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: lot no. 200319
Formulation/vehicle: 5% dextrose, 200 mg/ml.

Methods: To determine the potential mutagenic effects of N-acetylcyteine, in vivo micronucleus test was conducted using mouse bone marrow cells. N-acetylcysteine was given by a single intravenous injection at 187.5, 375, and 750 mg/kg. Bone marrow cells were collected and examined for micronucleated polychromatic erythrocytes 24 and 48 hours after dosing. The test article was considered to induce a positive response if a dose-response increase in micronucleated polychromatic erythrocytes was observed and one or more doses were statistically increased relative to the vehicle control at any sampling time.

Strains/species/cell line: — mice.
Dose selection criteria:
Basis of dose selection: The dose selection was based on the results of dose ranging studies.
Range finding studies: In the first dose ranging study, mice were treated with test article at 1000 and 2000 mg/kg and the treated mice were found dead immediately after dosing. In the second dose ranging study, mice were treated intravenously at 5, 50, 100, 250, 500, and 750 mg/kg. There were no deaths. Clinical signs of toxicity were observed at doses of 500 and 750 mg/kg and these included hyperactivity and piloerection.
Controls:

Vehicle: 5% dextrose.
Negative controls: 5% dextrose.
Positive controls: cyclophosphamide.

Exposure conditions:

Incubation and sampling times: Bone marrow cells were collected 24 and 48 hours after dosing.

Doses used in definitive study: 187.5, 375, and 750 mg/kg.

Study design: Mice were treated with N-acetylcysteine intravenously at 187.5, 375, and 750 mg/kg. Bone marrow cells were collected and examined for micronucleated polychromatic erythrocytes 24 and 48 hours after dosing.

Analysis:

The incidence of micronucleated polychromatic erythrocyte per 2000 polychromatic erythrocytes was determined.

Criteria for positive results: The test article was considered to induce a positive response if a dose-response increase in micronucleated polychromatic erythrocytes was observed and one or more doses were statistically increased relative to the vehicle control at any sampling time.

Results:

Study validity: Positive control significantly increased the micronucleated polychromatic erythrocytes.

Study outcome: There were no deaths. All mice appeared normal ~4 hours after dosing. The number of micronucleated polychromatic erythrocyte per 2000 polychromatic erythrocyte in the treatment groups was not significantly increased as compared to the control group. However, the positive control, cyclophosphamide, significantly increased the micronucleated polychromatic erythrocytes. The results were summarized in Table 4 on page 336 in volume 1.5. This table is attached below.

Table 4

Summary of Bone Marrow Micronucleus Study Using N-acetylcysteine

Treatment	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes: Number per 1000 PCEs (Mean +/- SD)	Micronucleated Polychromatic Erythrocytes: Number per PCEs Scored*
D5W							
10 ml/kg	M	24	5	0.479 ± 0.04	—	0.3 ± 0.27	3 / 10000
	F	24	5	0.473 ± 0.04	—	0.5 ± 0.35	5 / 10000
N-acetylcysteine							
187.5 mg/kg	M	24	5	0.439 ± 0.06	-8	0.5 ± 0.35	5 / 10000
	F	24	5	0.510 ± 0.04	8	0.5 ± 0.35	5 / 10000
375 mg/kg	M	24	5	0.462 ± 0.04	-4	0.3 ± 0.27	3 / 10000
	F	24	5	0.521 ± 0.04	10	0.1 ± 0.22	1 / 10000
750 mg/kg	M	24	5	0.494 ± 0.02	3	0.4 ± 0.42	4 / 10000
	F	24	5	0.505 ± 0.01	7	0.3 ± 0.45	3 / 10000
CP							
50 mg/kg	M	24	5	0.507 ± 0.04	6	20.8 ± 5.44	*208 / 10000
	F	24	5	0.514 ± 0.04	9	27.0 ± 4.00	*270 / 10000
D5W							
10 ml/kg	M	48	5	0.449 ± 0.02	—	0.5 ± 0.35	5 / 10000
	F	48	5	0.493 ± 0.02	—	0.2 ± 0.27	2 / 10000
N-acetylcysteine							
750 mg/kg	M	48	5	0.464 ± 0.02	3	0.1 ± 0.22	1 / 10000
	F	48	5	0.537 ± 0.03	9	0.2 ± 0.27	2 / 10000

[1]*, p≤0.05 (Kastenbaum-Bowman Tables)

N-acetylcysteine was not genotoxic in this test system.

Genetic toxicology summary: Sponsor conducted an *in vitro* mouse lymphoma cell forward mutation test and an *in vivo* mouse micronucleus test with N-acetylcysteine. N-acetylcysteine was positive in the presence of metabolic activation in the *in vitro* mouse lymphoma cell forward mutation test. N-acetylcysteine was negative in the *in vivo* mouse micronucleus test. N-acetylcysteine was negative in an Ames test in the published report.

Genetic toxicology conclusions: N-acetylcysteine was positive in the presence of metabolic activation in the *in vitro* mouse lymphoma cell forward mutation test. N-acetylcysteine was not genotoxic in the Ames test and *in vivo* mouse micronucleus test.

V. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Following results of the reproductive toxicity studies were obtained from a published report (Eur. J. Respir. Dis., 61 (Suppl.III): 45-51, 1980) and these studies were non-GLP studies:

"Fertility study in male rats"

In a chronic toxicity study, male rats were treated orally with acetylcysteine at 0, 250, 500, and 1000 mg/kg/day for 15 weeks prior to mating and during mating. Each male mated with 2 untreated females. The results indicated that acetylcysteine did not produce any adverse effects on fertility in this study at dose of 250 mg/kg/day. The fertility was reduced at doses of 500 and 1000 mg/kg/day. No detail information was provided. Female rats were not treated in this study.

"Teratogenicity study in rats"

In this study, female rats were treated orally with acetylcysteine at 0, 500, 1000, and 2000 mg/kg/day from days 6 to 15 of pregnancy. The results indicated that acetylcysteine was not teratogenic in this study.

"Teratogenicity study in rabbits"

In this study, female rabbits were treated orally with acetylcysteine at 0, 250, 500, and 1000 mg/kg/day from days 6 to 18 of pregnancy. The results indicated that acetylcysteine was not teratogenic in this study.

"Peri- and post-natal study in rats"

In this study, female rats were treated orally with acetylcysteine at 0, 250, 500, and 1000 mg/kg/day from days 15 of pregnancy though day 21 post-partum. The results indicated that acetylcysteine did not produce "any adverse effects on delivery and lactation, physical development and maturation of the offspring" in this study.

The results of these studies were presented in Table 7 in this publication and this table is attached below.

TABLE 7. *Reproduction studies.*

Test	Animals	Doses mg/kg/day	No. of Animals per Group	Dosing Period	Remarks
Fertility study	Male rat	0-250-500-1000	12 (each mated with 2 untreated females)	15 weeks before pairing and during mating period	No adverse effects up to the dose of 250 mg/kg. For higher doses slight, non dose related, reduction of fertility
Teratogenicity	Rat	0-500-1000-2000	25	from day 6 to 15 of pregnancy	No teratogenic effect was observed related to the treatment
	Rabbit	0-250-500-1000	15	from day 6 to 18 of pregnancy	
Peri- and post-natal study	Rat	0-250-500-1000	20	from day 15 of pregnancy through day 21 post-partum	No adverse effects of NAC on delivery and lactation or on physical development and maturation of the offspring were noted

Following information was obtained from a published report (Seminars in Oncology; 10(1): 17-24, 1983):

Acetylcysteine was given orally at 500 mg/kg/day to pregnant rabbits during gestation days 6 to 16. The rabbits were sacrificed on gestation day 29 and fetuses and uterine horns were examined. The results indicated that acetylcysteine did not produce any adverse effects on litter size, pup weight, and "in utero survival". There were no evidence of treatment related malformations. Acetylcysteine was not teratogenic in this study. The results were summarized in Table 4 in this article. This table is attached below.

Table 4. Reproductive Toxicity of NAC (Rabbit Teratology Study)

Drug	Dose (mg/kg/day)	Average Litter Size	Average Pup Weight (g)	No. Grossly Normal	No. Malformed
Distilled H_2O	0 [16]	5.75	35.0	77	8*
NAC	500 [12]	5.92	31.8	61	6*

*Shortened tails (2 controls and 4 NAC-treated pups) and unossified spots on cranium (6 controls and 2 NAC-treated pups).
[ ] = number of pregnant rabbits.

Reproductive and developmental toxicology conclusions:

The results of the reproductive toxicity studies were obtained from published reports and no detail information was available in these reports. Following is a brief summary of these non-GLP studies. In the oral chronic toxicity study in male rats, acetylcysteine reduced fertility at doses of 500 and

1000 mg/kg/day. Female rats were not treated in this study. Acetylcysteine was not teratogenic in Segment II teratologic studies in rats at oral doses up to 2000 mg/kg/day and in rabbits at oral doses up to 1000 mg/kg/day. Oral administration of acetylcysteine did not produce any adverse effects on delivery and lactation, physical development and maturation of the offspring at oral doses up to 1000 mg/kg/day in the oral Segment III Peri- and post-natal reproductive study in rats.

LABELING:

The labeling is according to 21 CFR, Subpart B. The following revisions in the labeling are recommended:

1. Sponsor's Version:

2. Sponsor's Version:

2 page(s) of draft labeling has been removed from this portion of the review.

VI. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Acetaminophen is mainly metabolized in the liver by sulfation and glucuronidation, while a small percentage of acetaminophen is converted to N-acetyl-p-benzoquinoneimine (NAPQI) which is a strong electrophile and oxidizing agent. Under normal condition, NAPQI is detoxified by reduced glutathione and excreted by the kidney. However, when acetaminophen is overdosed, glutathione stores become exhausted, and the normal detoxification of NAPQI is interrupted. Under this condition, NAPQI is irreversibly bound to protein molecules within hepatocytes and disrupts the normal function of the liver, which leads to cell death. The *in vivo* studies in mice have demonstrated that coadministration of acetylcysteine with acetaminophen increased hepatic glutathione concentration, decreased the amount of covalent binding of acetaminophen to hepatic protein, and prevented acetaminophen-induced liver toxicity.

In the present NDA, sponsor is seeking for approval to market Acetadote for treatment _____ The repeat dose toxicity studies with acetylcysteine available from literature were oral studies. Sponsor was asked to conduct a 4-week i.v. toxicity study in rats or alternatively, a comparative toxicokinetic study by oral and intravenous administrations in rats in the pre-NDA meeting on December 15, 2000. Sponsor was also asked in this meeting to conduct genetic toxicity studies including *in vitro* human lymphocyte chromosomal aberration test or a mouse lymphoma cell forward mutation test and *in vivo* micronucleus test in mice or rats. In support of this NDA, sponsor conducted the comparative toxicokinetic study by oral and intravenous administrations in rats, *in vitro* mouse lymphoma cell forward mutation test, and *in vivo* micronucleus test in rats and submitted the reports of these studies. In addition, following preclinical studies obtained from literature were also submitted in this NDA: pharmacological studies, pharmacokinetic studies in rats and dogs, single i.v. dose toxicity studies in mice, rats, guinea pigs, rabbits, and dogs, 4-week, 12-weerk, 28-week, and 18-month oral toxicity studies in rats, 90-day i.v. toxicity study in dog, and reproductive toxicity studies: oral chronic toxicity study in male rats, oral Segment II teratologic studies in rats and rabbits, and oral Segment III peri- and post-natal reproductive toxicity study in rats. Some of these studies were reviewed in NDA 13,601 and the pharmacology review of this NDA was consulted.

The oral bioavailability of N-acetylcysteine was 24-29% for male rats and 37-43% for female rats. When given by continuous i.v. infusion, the maximum plasma level of total N-actylcysteine was reached within 8 or 12 hours in rats. In contrast, the maximum plasma level of total N-acetylcysteine was reached within 0.5-1.0 hours after oral dose. The plasma level of total N-acetylcysteine declined with a half life of 6.4-8.7 hours in rats. The volume of distribution of N-acetylcysteine was 0.471l/kg and its plasma clearance was 0.11 l/hour/kg in humans. The covalent protein binding was up to 50% in humans. N-acetylcysteine was found mainly in the kidney followed by the liver, adrenal gland, lung, spleen, blood, muscle, brain, and urine in rats. The renal excretion is major route of excretion following oral and i.v. administrations in rats.

In the single i.v. dose toxicity studies, the minimal lethal dose was 1000 mg/kg in mice, 2455 mg/kg in rats, 1500 mg/kg in guinea pigs, 1200 mg/kg in rabbits, and 500 mg/kg in dogs. Following clinical signs of toxicity were observed in all species tested: ataxia, increased respiration or labored respiration, and convulsion. These studies were obtained from published reports.

Since repeat i.v. dose toxicity studies with acetylcysteine in rats were not available, sponsor was asked to conduct a comparative toxicokinetic study by oral and intravenous administrations in rats. The results of the comparative toxicokinetic study can be used to assess the repeat oral dose toxicity in rats using the published oral toxicity studies.

In the 4-day comparative toxicokinetic study in rats, rats were treated orally at 458 and 2000 mg/kg/day or by continuous i.v. infusion at 800 mg/kg/day. The results indicated that oral bioavailability of the total N-acetylcysteine was 24-29% for males and 37-43% for females. The maximum plasma level of N-acetylcysteinee was reached with 8 or 12 hours after i.v. infusion. N-acetylcysteine declined with a half life of 6.4 hours or 8.67 hours following i.v. infusion in males or females, respectively.

Followings are the oral toxicity studies in rats obtained from literature.

In the 4-week oral toxicity study in rats, rats were treated orally with acetylcysteine at 500, 1000, and 2000 mg/kg/day. In the 12-week, 28-week, and 18-month oral toxicity studies in rats, rats were treated orally with acetylcysteine at 250, 500, and 1000 mg/kg/day. The results indicated that there were no treatment related changes in these studies. Therefore, the target organs of toxicity were not identified. The oral dose of 1000 mg/kg/day would be equivalent to 240-370 mg/kg/day of an

i.v. dose based on the oral bioavailability of the total N-acetylcysteine of 24-29% in male rats and 37-43% in female rats.

In the 90-day i.v. toxicity study in dogs, beagle dogs were treated with N-acetylcysteine at 100, 200, and 400 mg/kg/day by i.v. infusion into cephalic or jugular veins at 5 ml/min (0.5-2.0 ml/kg). The results indicated that the only treatment related changes were prolapse of the nictitating membranes, lacrimation, salivation, erythema of the ears, occasional restlessness, nervousness, and tremors briefly observed during the infusion period at doses of 200 mg/kg/day or higher.

In the oral chronic toxicity study in rats, male rats were treated at 250, 500, and 1000 mg/kg/day for 15 weeks before mating and during mating. The treated male rats mated with untreated female rats. Acetylcysteine did not produce any adverse effects on fertility at dose of 250 mg/kg/day. Fertility was reduced at doses of 500 and 1000 mg/kg/day (no data were provided).

In the oral Segment II teratologic reproductive toxicity studies, acetylcysteine was given orally to rats at 500, 1000, and 2000 mg/kg/day from gestation day 6 to day 15 and at 250, 500, and 1000 mg/kg/day to rabbits from gestation day 6 to day 16 or 18. Acetylcysteine was not teratogenic in these studies.

In the oral Segment III peri- and post-natal reproductive toxicity study in rats, rats were treated orally at 250, 500, and 1000 mg/kg/day from gestation day 15 through day 21 post-partum. Acetylcysteine did not produce any adverse effects on delivery and lactation, physical development and maturation of the offspring.

N-acetylcysteine was positive in the presence of metabolic activation in the *in vitro* mouse lymphoma cell forward mutation assay. N-acetylcysteine was not genotoxic in the Ames test and *in vivo* mouse micronucleus test.

Conclusions:

In the present NDA, sponsor is seeking approval to market Acetadote for treatment of _____. A loading dose of Acetadote will be given by intravenous infusion at 150 mg/kg in 200 ml of 5% dextrose over _____ followed by an i.v. maintenance dose of 50 mg/kg in 500 ml of 5% dextrose over 4 hours and then 100 mg/kg in 1000 ml of 5% dextrose over 16 hours. The total i.v. infusion dose is 300 mg/kg over 21 hours. In support of this NDA, the recommended preclinical studies were conducted and adequate preclinical studies were submitted in this NDA. Therefore, from a

preclinical standpoint, this NDA is approvable. Relevant findings of the preclinical studies should be included in the labeling as recommended. Sponsor should be asked to revise the labeling as recommended.

Recommendations:

1. From a preclinical standpoint, this NDA is approvable.

2. Sponsor should be asked to revise the labeling as recommended.

Ke Zhang Date

Jasti B. Choudary, B.V.Sc., Ph.D. Date

Cc:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Zhang
HFD-045/Dr. Viswanathan

R/D Init.: J. Choudary 11/11/02

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