FREEDOM OF INFORMATION

I. General Information:

Identification: NADA 55-099

Date: September 28, 1984

Name of Applicant: Beecham Laboratories
Div. of Beecham Inc.
501 Fifth Street
Bristol, TN 37620

Generic Name: Amoxicillin trihydrate/clavulanate potassium

Proprietary: SYNULOX™

Marketing Status – RX

II. Indications:

SYNULOX™ Tablets are indicated in the treatment of skin infections such as superficial/juvenile and deep pyoderma of dogs due to susceptible strains of the following organisms: Beta-lactamase (penicillinase) producing *Staphylococcus aureus*, non-Beta-lactamase *Staphylococcus aureus* and *Staphylococcus spp*.

Therapy may be initiated with SYNULOX™ prior to obtaining results from bacteriological and susceptibility studies. A culture should be obtained prior to treatment to determine susceptibility of the organisms to SYNULOX™. Following determination of susceptibility results and clinical response to medication, therapy may be re-evaluated.

III. Dosage:

SYNULOX™ (amoxicillin trihydrate/clavulanate potassium) is available as a film-coated tablet for oral administration to dogs. The recommended dosage is 6.25 mg per pound of body weight administered twice a day. This results in a therapeutic dosage of 5 mg of amoxicillin and 1.25 mg of clavulanic acid per pound of body weight per dose.

10 pound dog = 1 - 62.5 mg tablet b.i.d.
20 pound dog = 1 - 125 mg tablet b.i.d.
40 pound dog = 1 - 250 mg tablet b.i.d.

Dosage should be continued for 10-14 days or 48 hours after all symptoms have subsided. If no response is seen after 7 days of treatment, therapy should be discontinued and the case re-evaluated. The maximum duration of treatment should not exceed 30 days.

IV. EFFICACY:

A. Pivotal

SYNULOX™ is a formulation comprised of the broad-spectrum antibiotic, amoxicillin, and the β-lactamase inhibitor clavulanic acid. Amoxicillin exhibits a high level of bactericidal activity against a wide range of Gram-positive and Gram-negative bacteria. Clinical experience has demonstrated the efficacy of amoxicillin in the treatment of infection. However, amoxicillin is unstable to bacterial β-lactamases and an increasing proportion of isolates of bacteria responsible for infection are found to be resistant to amoxicillin as a result of β-lactamase activity. Clavulanic acid is a progressive inhibitor of the β-lactamase produced by certain Gram-positive and Gram-negative bacteria and is capable of protecting amoxicillin from inactivation by these bacteria. Consequently, the formulation of amoxicillin/clavulanic acid shows significant activity against many amoxicillin-resistant strains of bacteria.

1. Bioequivalency

A crossover blood level study was conducted to demonstrate the bioequivalency of amoxicillin in the serum following oral dosing with either AMOXI -TABS® (amoxicillin trihydrate, Beecham Laboratories) or SYNULOX™. This study involved a total of 12 dogs (6 of each sex). The dosage of amoxicillin was 5 mg/lb in each group.

Table #1 presents the results obtained in this bioequivalency study. The amoxicillin serum levels (mcg/ml) following dosing with SYNULOX™ were very similar to those of AMOXI-TABS®. The peak height was 6.41 for AMOXI -TABS® and 6.03 for SYNULOX™. The time to peak was 1.46 for AMOXI-TABS® and 1.67 for SYNULOX™. The area under the curve (mcg/ml X hrs.) was 18.09 for AMOXI -TABS® versus 15.56 for SYNULOX™.

A finding of bioequivalence with respect to the amoxicillin levels was apparent from this study. No statistically significant differences were noted between SYNULOX™ and AMOXI-TABS® for any of the key variables (peak concentration, time to peak and area under the curve). A lack of statistically significant differences for any of the nine sampling times further support these results. This study was conducted under the direction of Dr. T.J. Keefe, Beecham Laboratories, Bristol, Tennessee and carried out by Dr. Jeffrey Mehring, LRE, Kalamazoo, Michigan. The samples were assayed by Beecham Laboratories, Bristol, Tennessee.

Table #1 Amoxicillin (mcg/ml) Serum Levels (Canine) - SYNULOX™ Vs. AMOXI-TABS®

		Time in Hours Post-Treatment								
Treatment	**Phase**	**0**	**1/2**	**1**	**1-1/2**	**2**	**3**	**6**	**9**	**12**
SYNULOX	I	0	.21	4.52	5.59	4.64	1.76	0.60	0.10	<0.05
SYNULOX	II	0	2.19	5.31	6.48	5.59	2.44	0.21	<0.05	<0.05
SYNULOX average	I & II	0	1.20	4.92	6.03	5.10	2.10	0.4	0.06	<0.05
AMOXI-TABS	I	0	1.21	5.73	6.15	6.28	2.28	0.68	<0.05	<0.05
AMOXI-TABS	II	0	1.39	5.82	6.66	6.12	2.57	0.38	0.07	<0.05
AMOXI-TABS average	I & II	0	1.30	5.78	6.41	6.20	2.42	0.53	0.06	<0.05

2. Field Studies

a. Study Design

A large multicentered clinical field study was conducted by Beecham Laboratories, Bristol, Tennessee under the direction of Thomas J. Keefe, D.V.M. The study design consisted of a blind comparison of SYNULOX™ and AMOXI-TABS® (NADA #55-078, Beecham Laboratories). SYNULOX™ was prepared to have a similar appearance to that of AMOXI-TABS®. Animals were assigned to their respective treatment group by use of a sponsor generated randomization schedule. Both groups received the approved recommended dosage level of amoxicillin, i.e. 5 mg/lb b.i.d. However, the SYNULOX™ group received an additional 1.25 mg/lb dose of clavulanic acid. This study was limited to skin infections in dogs. Pre- and post-treatment cultures were required in all cases unless healing precluded the availability of post-treatment culture. Clinical symptoms present at the time of pre-treatment and post-treatment cultures were described in detail and in a large number of cases photographs of the lesions were obtained.

b. Participating Investigators

A total of 16 investigators participated in this study. These investigators were located in 9 states and are listed below:

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c. Overall Clinical Response

A total of 296 cases were evaluated in the field study with 113 of these being acceptable for the efficacy assessment. The remaining 183 cases were excluded for failure to comply with the protocol or due to incomplete data collection.

Overall clinical response was judged according to the following criteria:

CURE - Clinical findings subsided in a reasonable period of time with no evidence of infection at the time drug was discontinued nor during follow-up.

IMPROVEMENT- Clinical findings subsided significantly in a reasonable period of time but with incomplete resolution of evidence of infection, or relapse, possibly related to underlying disease state.

FAILURE - No apparent response to therapy.

Of the 113 acceptable cases, 74 involved treatment with SYNULOX™ and 39 with amoxicillin. The SYNULOX™ cases demonstrated a 61% "cure" rate while a 41% "cure" rate was observed in the amoxicillin cases. Table #2 categorizes overall response by diagnosis. As shown, SYNULOX™ was more efficacious than amoxicillin in each indication. The 48% cure rate (71% cure/improvement) in deep pyoderma is a good response in light of the recognized difficulty in treating this disease condition (Muller/Kirk, Small Animal Dermatology, 2nd Edition, page 245).

Table #2 - SYNULOX™ Versus Amoxicillin – Overall Clinical Response by Diagnosis

		SYNULOX™						**Amoxicillin**					
Diagnosis	**Total # Cases**	**No. Cases**	**No. Cures**	**No. Imp.**	**No. Fail.**	**% Cure**	**% C/Imp.**	**No. Cases**	**No. Cures**	**No. Imp.**	**No. Fail.**	**% Cure**	**% C/Imp.**
Sup/Jv. Pyoderma	85	53	35	5	13	66	75	32	16	5	11	50	66
Deep Pyoderma	28	21	10	5	6	48	71	7	0	1	6	0	17
Total	113	74	45	10	19	61	74	39	16	6	17	41	56

d. Bacteriological Response

A total of 133 organisms were isolated during this clinical field study. Of the 83 organisms isolated in the group of dogs treated with SYNULOX™, 80% were eradicated by the time of the final examination. A 74% (37/50) elimination rate was observed in the animals receiving amoxicillin therapy. These data along with elimination rates for the specific organisms isolated are displayed in Table #3.

Table #2 - SYNULOX™ Versus Amoxicillin – Bacteriological Response by Organism

	SYNULOX™			**Amoxicillin**		
Organism	No. Isolates	No. Elim.	% Elim	No. Isolates	No. Elim.	% Elim
S. aureus Sen.	23	21	91	15	13	87
S. aureus Res.	26	17	65	15	7	47
Staph. spp.	13	12	92	7	6	86
Miscellaneous	21	16	76	13	11	85
Total	83	66	80	50	37	74

e. Establishment and Confirmation of Optimum Dose

(1) Amoxicillin

The dosage of amoxicillin trihydrate was previously established in NADA #55-078 at 5 mg/lb administered twice a day. A dose titration study was conducted as part of the NADA under the direction of Dr. George Brander, Beecham Pharmaceuticals Research, Walton Oaks, England. This study consisted of three treatment groups: amoxicillin administered at a dosage of 2-1/2 mg or 5 mg per pound of body weight twice a day, and an active control, ampicillin (Beecham, Brentford, England). A total of 302 dogs were treated. The 5 mg dose resulted in an 81% improvement in 100 cases, 77.5% improvement for the 2-1/2 mg dose in 102 dogs and 87% improvement for ampicillin in 100 dogs.

(2) Clavulanic Acid

The dosage of clavulanic acid was based upon the amount necessary to lower the MIC of the majority of *S. aureus* down to at least 2.5 mcg/ml.

In-vitro studies have demonstrated that 1.25 mg/lb of clavulanic acid in the presence of 5 mg/lb of amoxicillin (4:1 ratio) reduced the MIC's of *S. aureus* (veterinary isolates) tested to or below the target level (2.5 mcg/ml). Doubling the dose of clavulanic acid (2:1 ratio) increased the susceptibility by only 1%. These results support the conclusion that the 4:1 ratio is the optimal level. This study confirmed earlier studies which indicated that clavulanic acid was not antagonistic to the therapeutic activity of amoxicillin. The studies cited were conducted under the direction of Mr. Fred Barr, Microbiology Laboratory, Beecham Laboratories, Bristol, Tennessee and by David Wishart, Beecham Pharmaceuticals Research, Walton Oaks, England.

B. Corroborative Studies

Experimentally Induced Infection Study

An experimentally induced infection study conducted by Dr. Robin Bywater, Beecham Pharmaceuticals, Walton Oaks, England, was carried out in canine to demonstrate the efficacy of SYNULOX™. The procedure involved *S. aureus* isolated from a case of canine dermatitis. This organism had an MIC of >1000 for amoxicillin, however, the MJC decreased to 2.5 mcg/ml in the presence of amoxicillin/clavulanic acid. The pups were anesthetized and their abdomens clipped and surgically scrubbed. The skin was then inoculated with 5 microliters of a stock suspension (containing 10^{10} *S. aureus* suspended in a 1% casein hydrolysate with 10% DMSO) intradermally at 4 sites. Twenty-four hours after inoculation, the animals were randomly allocated to 1 of 2 treatment groups, SYNULOX™ at a dosage of 5/1.25 mg/lb twice a day or amoxicillin trihydrate (Beecham Laboratories) at 5 mg/lb twice a day. Both drugs were given for 14 days. A total of 6 dogs (3 in each treatment group) were used in Leg I of the study and then crossed over to the opposite treatment in Leg II, resulting in a total of 6 dogs per treatment group. The tablets were identical in appearance and the study was conducted on a blind basis.

The two criteria evaluated were lesion diameter and degree of inflammation. The size of the lesion treated with SYNULOX™ started decreasing on day 3 post-treatment and decreased steadily through day 14. Amoxicillin treated lesions started decreasing on day 4 and decreased more slowly and to a lesser degree than did the SYNULOX™ treated lesions.

With respect to inflammation, SYNULOX™ and amoxicillin provoked equivalent responses on days 1 and 2. However, on day 3 the inflammation peaked in the SYNULOX™ group while the amoxicillin group continued to climb through day 4. The inflammation in the SYNULOX™ group declined rapidly and steadily while the inflammation in the amoxicillin treated dogs evidenced a slower decline.

V. SAFETY:

During the preclinical investigations, extensive toxicology/pathology studies were conducted with clavulanic acid alone and in combination with amoxicillin. These studies demonstrated that clavulanic acid has a low order of acute oral toxicity. The results of studies with clavulanic acid formulated with amoxicillin have not indicated any synergistic or untoward toxicity either singly or in combination.

A. Pivotal

A chronic 6 month toxicity study was conducted with amoxicillin/clavulanic acid in 52 beagle dogs (26 male and 26 female) by Dr. Breckenridge at BioResearch Laboratories, Montreal, Canada. The duration of this study exceeds the 90 day requirement of 3X the maximum recommended treatment period. The following daily dose levels were evaluated: 0, 15 mg/kg (6.8 mg/lb), 30 mg/ kg (13.6 mg/lb), 60 mg/kg (27.3 mg/lb) and 150 mg/kg (68.2 mg/lb). These dosage levels are approximately 0.5X, 1.1X, 2.2X and 5.5X for a period of 180 days (6X the recommended duration). At the end of the 6 month treatment period, three males and three females from each of the high dose and vehicle control groups were maintained for 30 days off treatment before they were sacrificed in order to evaluate the regression of treatment related effects. Salivation and emesis were observed in the high dose group. Histological studies included examination of the following tissues: trachea, heart, lungs, thymus, cervical and mesenteric lymph nodes, liver, gallbladder, spleen, pancreas, kidneys, urinary bladder, uterus, prostrate, testes, ovaries, sciatic nerve, pituitary, spinal cord (cervical and lumbar), sternum, adrenals, thyroid, skin, skeletal muscle, mammary gland, tongue, eyes, optic nerve, brain (cortical, cerebellar and medullary), esophagus, salivary gland, stomach, duodenum, jejunum, ileum, cecum and colon. These histological studies revealed minor hepatic and renal changes in the form of cytoplasmic glycogen diminuation or disappearance and tubuler vacuolization via the high dose group which regressed during the 30 day post-treatment regression period.

B. Corroborative

1. Target Species Toxicity Study

   A target species toxicity study was conducted with SYNULOX™ tablets in 16 dogs (8 male and 8 female) by Dr. J. Mehring, LRE, Kalamazoo, Michigan. Three dosage levels of SYNULOX™ and a control group were included in this study. SYNULOX™ was administered at 0, 125, 375 and 625 mg per 20 lb. dog twice a day for 60 days. These dose levels corresponded approximately to 1X, 3X and 5X the recommended dosage for approximately 4X the duration for superficial/juvenile pyoderma and 2X duration for deep pyoderma.

   Based on the results obtained in this 60 day study, SYNULOX™ tablets appear to be safe and non-toxic at a 5X dosage. No differences were observed between the 3 treatment groups and the control. The histopathologist stated that he did not observe any significant microscopic changes between the treated and control dogs. Therefore, it can be concluded that SYNULOX™ can be administered safely to dogs for extended periods of time. The recommended dose is 6.25 mg/lb b.i.d. Maximum recommended duration of treatment is 30 days.

   The effect of SYNULOX™ on the testes and ovaries was also evaluated. No histological evidence of toxicity was noted at the 5X dosage after 60 days of treatment.

2. Acute Toxicity Studies

   Acute oral single dose toxicity studies were conducted with clavulanic acid in ten male and ten female mice and ten male and ten female rats at each of the

following dose levels: 3,163 (1,437.7), 4,218 (1917.3), 5,625 (2557), 7,500 (3409), and 10,000 (4545) mg/kg (mg/lb). The duration of the studies were 14 days. The LD_{50} was found to be in excess of 10,000 mg/kg (4545 mg/lb) body weight. No abnormal effects were observed immediately after dosing. No target organ toxicity was evident in the mice. There were indications that the kidney was the target organ in the rat. These studies were conducted under the supervision of Dr. T. L. Hardy, Beecham Pharmaceuticals Research Division, Harlow, Essex, England.

An acute subcutaneous toxicity study was conducted with clavulanic acid in ten male and ten female mice at each of the following dosage levels: 1,581 (719), 2,101 (955), 2,812 (1278), 3,750 (1705) and 5,000 (2273) mg/kg (mg/lb). The mice were examined for 14 days. The LD_{50} by the subcutaneous route was found to be 3,220 mg/kg (1464 mg/lb) in male mice and 4,480 mg/kg (2036 mg/lb) in female mice. Deaths were delayed, occurring up to 8 days after dosing. No immediate clinical signs were observed after dosing. The kidneys appeared to be the target organ. This study was conducted under the supervision of Dr. T. L. Hardy, Beecham Pharmaceuticals Research Division, Harlow, Essex, England.

The LD_{50} of clavulanic acid administered by the subcutaneous route was found to be 1,999 mg/kg (909 mg/lb) in male rats and 2,331 mg/kg (1050 mg/lb) in female rats. This study was conducted by Dr. T. L. Hardy, Beecham Pharmaceuticals Research Division, Harlow, Essex, England. Twenty rats (ten female and ten male) received a single dose at the following levels: 1,250 (568), 2,500 (1136) and 5,000 (2273) mg/kg (mg/lb). These rats were observed for 14 days. The kidney was considered to be the possible target organ.

The toxicity of clavulanic acid when administered by the intravenous route was evaluated in 100 mice (50 male and 50 female). The mice were observed for 14 days. The drug was administered at the following dosages: 960 (436), 1,372 (624), 1,960 (891), 2,800 (1273) and 4,000 (1818) mg/kg (mg/lb). The LD_{50} of clavulanic acid by the intravenous route was found to be 2,701 mg/kg (1228 mg/lb) in the males and 2,622 mg/kg (1192 mg/lb) in the females. Immediately after dosing at all levels except 960 mg/kg (436 mg/lb), convulsions, increased urination, dyspnea and collapse were noted. No target organ toxicity was evident on post-mortem examination. This study was conducted by Dr. T. L. Hardy, Beecham Pharmaceuticals Research Division, Harlow, Essex, England.

The LD_{50} of clavulanic acid by the intravenous route in rats was found to be 1,850 mg/kg (841 mg/lb) in 50 males and 1,569 mg/kg (713 mg/lb) in 50 females. The drug was given at the following dosages: 1,200 (545), 1,715 (780), 2,450 (1114), 3,500 (1591) and 5,000 (2273) mg/kg (mg/lb). Similar immediate clinical effects as in the above mouse study were observed. The kidney appeared to be the target organ. This study was conducted under the direction of Dr. T. L. Hardy, Beecham Pharmaceuticals Research Division, Harlow, Essex, England.

A single dose acute toxicity study was conducted with the sodium salt of clavulanic acid in 220 (110 male and 110 female) 4, 22 and 33 day old rats. The drug was administered by the oral route at the following dosages: 0, 500

(227), 1,000 (455), 2,000 (909), 3,160 (1436), 4,000 (1818), 4,220 (1918), 5,620 (2555), 7,500 (3409) and 8,000 (3636) mg/kg (mg/lb). Animals were observed for 14 days. Among the 4 day old rats, the LD_{50} was less than 500 mg/kg (227 mg/lb). The acute median lethal dose was 6,200 mg/kg (2818 mg/lb) for the 22 day old rats and in excess of 7,500 mg/kg (3409 mg/lb) for the 33 day old rats. This study was conducted by A. K. Palmer and P. A. Allen, Huntingdon Research Center, Huntingdon, England.

A second acute oral toxicity study with sodium clavulanate was conducted by the same individuals in sixty (30 male and 30 female) 4 day old rats. The dosages were 0, 125 (57), 250 (114), 500 (227), 1,000 (455) and 2,000 (909) mg/kg (mg/lb). These young rats were observed for 14 days. The LD_{50} in this group of rats was calculated to be 1,073 mg/kg (488 mg/lb). There were no obvious treatment related macroscopic findings on post-mortem examination.

The oral acute LD_{50} of the clavulanate potassium was determined in eight male and six female mice. The dosage levels used were 5,000 mg/kg (2273 mg/lb) and 10,000 mg/kg (4545 mg/lb), the animals being observed for 14 days. The LD_{50} was found to be in excess of 5,000 mg/kg (2273 mg/lb) but less than 10,000 mg/kg (4545 mg/lb). This study was conducted under the supervision of Dr. T. L. Hardy, Beecham Pharmaceutical Research Division, Harlow, Essex, England. Dr. Hardy repeated the study utilizing 24 rats (12 male and 12 female) at the same dosages. The LD_{50} was found to be in excess of 10,000 mg/kg (4545 mg/lb).

An acute subcutaneous single dose toxicity study was conducted with the clavulanate potassium in 72 mice (36 male and 36 female) and 72 rats (36 male and 36 female). The animals were observed for 14 days. The drug was given at the following dose levels: 840 (382), 1,200 (545), 1,715 (720), 2,450 (1114), 3,500 (1591) and 5,000 (2273) mg/kg (mg/lb). The LD_{50} was found to be 2,050 mg/kg (932 mg/lb) for male mice and 2,719 mg/kg (1236 mg/lb) for the females. Deaths occurred up to 7 days post-dosing. No macroscopic abnormalities were detected in any of the mice examined. The LD_{50} in male rats was 2,555 mg/kb (1161 mg/lb) and 2,050 mg/kg (932 mg/lb) for female rats. Mottled kidneys were noted upon autopsy except at the lower dose levels. The mouse study was directed by Dr. A. S. Kelvin, the rat study by Dr. T. L. Hardy at the Beecham Pharmaceuticals Research Division, Harlow, Essex, England.

The toxicity of the clavulanate potassium in 60 male and 60 female mice by the intravenous route was evaluated by Dr. T. L. Hardy, Beecham Pharmaceuticals Research Division, Harlow, Essex, England. Potassium chloride was also investigated for its intravenous toxicity. Clavulanate potassium was administered at the following dosages: 118 (54), 168 (76), 240 (109), 343 (156) and 490 (223) mg/kg (mg/lb) and KCl was administered at 44 (20), 62 (28), 89 (40), 127 (58) and 181 (82) mg/kg (mg/lb). The mice were observed for 14 days. The LD_{50} of clavulanate potassium was 213 mg/kg (96.8 mg/lb) in males and 240 mg/kg (109 mg/lb) in females. These values are equivalent to the potassium contained in doses of 237-339 mg/kg (108-154 mg/lb).

The acute oral toxicity of clavulanate potassium was examined in sixty (30 male and 30 female) 4 day old rats by A. K. Palmer and P. A. Allen of

Huntingdon Research, Huntingdon, England. Dosages used were 0, 125 (57), 250 (114), 500 (227), 1,000 (455) or 2,000 (909) mg/kg (mg/lb). All animals were observed for 14 days. The LD_{50} was 1,359 mg/kg (618 mg/lb).

The acute oral toxicity of clavulanic acid alone and formulated with amoxicillin was evaluated in a total of 72 mice (36 male and 36 female). Twenty-four mice were treated with clavulanic acid, 24 with sodium clavulanate/amoxicillin (1:1) and 24 with sodium clavulanate/amoxicillin (1:10). All animals were observed for 14 days. Dosages used in all three groups were 2,500 (1136) and 5,000 (2273) mg/kg (mg/lb). No adverse clinical signs were observed after dosing in any animal. The LD_{50} of the two compounds at 1:1 or 1:10 were all in excess of 5,000 mg/kg (2273 mg/lb). This toxicity study was conducted under the supervision of of T. L. Hardy, Beecham Pharmaceuticals Research Division, Harlow, Essex, England.

Single acute oral toxicity studies were conducted with clavulanate potassium, amoxicillin and clavulanate potassium/amoxicillin (1:2) in 120 mice (60 male and 60 female) and 120 rats (60 male and 60 female). The animals were observed for 14 days. Dosages were 2,000 mg/kg (909 mg/lb) and 5,000 mg/kg (2273 mg/lb). The LD_{50} of clavulanate potassium formulated with amoxicillin was found to be in excess of 5,000 mg/kg (2273 mg/lb). The LD_{50} of the agents alone was also in excess of 5,000 mg/kg (2273 mg/lb). Single dosages of 5,000 mg/kg (2273 mg/lb) of clavulanate potassium/amoxicillin were well tolerated orally in mice and rats. Both studies were under the direction of Dr. T. L. Hardy, Beecham Pharmaceuticals Research Division, Harlow, Essex, England.

3. Sub-Acute Toxicity Studies

Clavulanic acid was administered orally to 112 mice (56 male and 56 female) at doses of 0, 100 (45) and 500 (227) mg/kg/day (mg/lb/day) at the highest level. Clavulanic acid was well tolerated by the mouse, with occurring changes being mild in nature. Histological changes in the kidney and liver showed evidence of regression although SGPT elevation was still evident after the regression period. This study was conducted by Dr. Hardy, Beecham Pharmaceuticals Research Division, Harlow, Essex, England.

A 14 day repeat dose study with clavulanic acid in 16 Beagle dogs (8 male and 8 female) was carried out by Beecham Research at Harlow, England, under the direction of Dr. Hardy. Clavulanic acid was administered orally (2 male and 2 female) and intramuscularly (3 male and 3 female) at 100 mg/kg/day (45.5 mg/lb/day). Two males and 2 females served as controls. Dosing was followed by a 14 day regression period. The oral dose produced only minimal renal effects and the compound appeared to exert low toxicity at this dose level. The intramuscular dosage of 100 mg/kg (45 mg/lb) did result in some histological renal and hepatic changes that were still apparent at the end of the regression period. These changes included hepatocyte hypertrophy in the liver and degenerative changes in the proximal tubules of the renal cortex.

Oral and subcutaneous 14 day repeat dose studies were conducted in 12 Rhesus monkeys (6 male and 6 female). Dosages were 0, 100 (45) and 500 (227) mg/kg/day (mg/lb/day) both orally and subcutaneously. Toxicity was seen in the subcutaneously administered 500 mg/kg/day (227 mg/lb) group.

The oral 100 mg/kg (45 mg/lb) group showed no significant lesions. The subcutaneous 100 mg/kg (45 mg/lb) group showed minimal change in the kidney on histological examination. Dr. Rivett of Huntingdon Research, Huntingdon, England, directed this study.

A comparison of the potential toxicity of the sodium and potassium salts of clavulanic acid in 64 male and 64 female rats was conducted by Dr. T. L. Hardy, Beecham Pharmaceuticals Research Division, Harlow, Essex, England. The dosages were 0, 100 (45), 200 (91) and 400 (182) mg/kg (mg/lb) once a day by gavage for 14 days. No significant gross or histological changes or variations from normal were observed. No target organ was demonstrated for either salt of clavulanic acid.

A 14 day repeat subcutaneous comparative toxicity study of the sodium and potassium salts of clavulanic acid was conducted in 128 rats (64 male and 64 female). The dosage level for each drug was 0, 100 (45), 200 (91) and 400 (132) mg/kg (mg/lb) per day. Both salts were tolerated. The toxic effects were dose related and included anemia, marked reduction in serum albumin and increase in spleen, kidney and liverweights. Generally, the effects seen with the potassium salt were slightly less marked than those seen with the sodium salt. The no-effect level for both salts was considered to be less than 100 mg/kg (45 mg/lb). This study was conducted under the supervision of Dr. T. L. Hardy, Beecham Pharmaceuticals Research Division, Harlow, Essex, England.

A comparison of the sodium and potassium salts of clavulanic acid in 24 Beagle dogs (12 male and 12 female) following oral dosages of 0, 25 (11), 50 (23) and 100 (45) mg/kg/day (mg/lb/day) for 14 days was conducted by Dr. T. L. Hardy, Beecham Pharmaceuticals Research Division, Harlow, Essex, England. At oral dose levels of 25 (11) and 50 (23) mg/kg/day (mg/lb/day), there were no clear-cut differences in the toxicological response of dogs treated with either salt. At 100 mg/kg/day (45 mg/lb/day), there were more marked effects with the potassium salt. Microscopic changes in the kidneys were observed.

Clavulanic acid/amoxicillin trihydrate was administered to 200 mice (100 male and 100 female) and 160 rats (80 male and 80 female) for 4 weeks at the following dosages: 0, 50/50 (23/23), 5/50 (2.3/23) and 50/500 (23/227) mg/kg/day (mg/lb/day). The mice were dosed by oral gavage once a day for 7 days a week. The only reactions noted in mice were production of softer feces and cecal enlargement. No histological changes or variations from normal was seen that could be attributed to the drug. The study was conducted by Dr. K. F. Rivett, Huntingdon Research Center in Huntingdon, England. Cecum enlargement was also noted in the rats. Histological evaluation of the tissue of the rats revealed no changes or variations from normal that were drug related.

A clavulanic acid/amoxicillin trihydrate toxicity study was conducted in 24 dogs (12 male and 12 female). This was a repeat dosage study involving dosages of 0, 50/50 (23/23), 5/50 (2.3/23), 50/500 (23/227) mg/kg/day (mg/lb/day) by oral gavage 7 days a week for 4 weeks. No deaths were observed in this study. No abnormalities were seen on macroscopic or histological examination that were considered to be related to the test compound. All organ weights were within normal limits. The study was carried out under the direction of Dr. K. F. Rivett of Huntingdon Research Center, Huntingdon, England. Pathology

was evaluated by Dr. A. J. Newman, Lee Newman Research Associates, Barnstaple Devon, England.

A similar study utilizing the same dosage levels to the one just described in the dog was conducted by the same people in 24 Rhesus monkeys (12 male and 12 female). No abnormalities were observed in gross or histological reviews of the pathology.

Amoxicillin trihydrate/clavulanic acid was administered to 80 rats (40 male and 40 female) in an oral 28 day repeat dose study. This work was under the direction of Dr. T. L. Hardy, Beecham Pharmaceuticals Research Division, Harlow, Essex, England. The drug was administered at levels of 0, 30 (14), 90 (41) and 270 (123) mg/kg/d ay (mg/lb/day). No significant changes were observed and it was concluded that the drug was without apparent toxic effect in the rat at dose levels up to 270 mg/ kg/day (123 mg/lb/day) for 28 days.

Amoxicillin trihydrate/clavulanic acid was evaluated in an oral 28 day toxicity study in 16 Beagle dogs (8 male and 8 female) by Dr. T. L. Hardy, Beecham Pharmaceuticals Research Division, Harlow, Essex, England. The drug was dosed at 0, 30 (14), 90 (41) and 270 (123) mg/kg/day (mg/lb/day). Vomiting was noted in the high dose group 270 mg/kg/day or 123 mg/lb/day and to a lesser extent at the 90 mg/kg dose (41 mg/lb). There was some histological evidence indicative of intestinal irritation at the high dose level. There was no evidence of any other specific toxic effects.

4. Chronic Toxicity Studies

Clavulanic acid was evaluated in a 13 week oral toxicity study in 56 Beagle dogs (28 male and 28 female) with a 4 week regression period. This study was conducted by Dr. Rivett of Huntingdon Research, Huntingdon, England. Clavulanic acid was administered at 0, 5 (2.3), 50 (23) and 400 (182) mg/kg/day (mg/lb/day). The high dose group experienced a loss of appetite, loss of condition and a high incidence of vomiting. Vomiting continued in the high dose group until the drug was reduced to 50 mg/kg/b.i.d (23 mg/lb). No significant adverse reactions were noted.

A 13 week oral repeat dose study followed by a 4 week regression period was carried out in 56 Rhesus monkeys (28 male and 28 female). The dosages used were 0, 5 (2.3), 50 (23) and 400 (182) mg/kg/day (mg/lb/day). Vomiting and loose feces were seen in the 400 mg/kg/day (182 mg/lb/day) group. No toxicity was observed in the 5 mg/kg (2.3 mg/lb) group and minimal toxicity was noted in the 50 mg/kg (23 mg/lb) group. This study was conducted by Dr. Rivett, Huntingdon Research, Huntingdon, England.

Clavulanate potassium was orally administered to 170 rats (85 male and 85 female) for 26 weeks followed by a 4 week regression period. This study was under the direction of Dr. Rivett, Huntingdon Research, Huntingdon, England. Dosages used were 0, 10 (4.5), 30 (14), 90 (41) and 400 (182) mg/kg/day (mg/lb/day). Histological changes, including those in hepatocytes, were noted in the 400 mg/kg/day (182 mg/lb/day) group. The lower treatment groups were relatively clean with no changes in the 10 mg/kg/day (4.5 mg/lb/day) group. A second 26 week oral toxicity study with 170 rats (85 male and 85 female) was carried out by Dr. Rivett at Huntingdon Research utilizing 0, 10

(4.5), 20 (9), 50 (23) and 400 (182) mg/kg/day (mg/lb/day) with similar findings.

An oral 26 week toxicity study with a 4 week withdrawal period was conducted in 32 Beagle dogs (16 male and 16 female) by Dr. Rivett, Huntingdon Research, Huntingdon, England. Clavulanate potassium was administered at dosages of 0, 10 (4.5), 30 (14) and 90 (41) mg/kg/day (mg/lb/day). During the 23rd week, an improperly manufactured batch of drug (contaminated with an impurity) was used resulting in data difficult to interpret.

A 26 week oral repeat dosage study with a 4 week regression period was conducted in 130 rats (65 male and 65 female) by Dr. Hunter, Huntingdon Research, Huntingdon, England. The test drug was amoxicillin trihydrate/clavulanate potassium. The dosage levels were 0, 30 (14), 60 (27), 150 (68) and 1,200 (545) mg/kg/day (mg/lb/day). Minor changes occurred in most parameters in the high dose group. Histological changes were observed in the liver, non-glandular epithelium of the stomach and distention of the cecum. The lower treatment groups were unremarkable.

5. Other Special Studies

The effect of intravenous administration of clavulanic acid on the heart rate, blood pressure, respiration and electrocardiography was studied in two anesthetized Beagle dogs (one male and one female) by Dr. J. Hopkins, Toxicology and Drug Safety Unit, Beecham Pharmaceuticals, Harlow, Essex, England. Clavulanic acid was administered at dosages of 50 (23), 100 (45) and 250 (114) mg/kg (mg/lb). Blood pressure decreased in a dose related fashion. Slight and variable effects occurred in heart rate while respiration was increased in one dog following the highest dose. T-waves became flattened at 50 mg/kg (23 mg/lb) and inverted at 100-250 mg/kg (45-114 mg/lb).

A formulation of amoxicillin sodium and clavulanate potassium 30 (14), 150 (68) and 375 (170) mg/kg (mg/lb) was conducted in four (2 male and 2 female) anesthetized dogs to assess the safety (cardiovascular) of this compound following intravenous administration. Potassium chloride (47 mg/kg or 21.4 mg/l b) and sodium amoxicillin (125 mg/kg or 56.8 mg/lb) were also administered to assess the effect of potassium ion on the cardiovascular system. There was no evidence of any synergistic toxic effects on the cardiovascular system. With the exception of the well known adverse effects of potassium ion on cardiac conduction and consequential changes in blood pressure and heart rate evident after administration of clavulanate potassium but not after sodium amoxicillin and clavulanate potassium, these materials had no toxic effects on the cardiovascular system. The test compounds did not appear to have any specific antagonistic activity against acetylcholine, histamine, isoprenaline or noradrenalin and they did not appear to have any consistent effect on autonomic reflexes. This study was under the direction of Dr. A. Cockburn, Beecham Pharmaceuticals Research Division, Harlow, Essex, England.

VI. HUMAN SAFETY:

No special precautions needed.

VII. AGENCY CONCLUSIONS:

The data submitted in support of this NADA comply with the requirements of Section 512 of the Act and Section 514.111 of the implementing regulations. It demonstrates that SYNULOX™ when used under its approved conditions of use is safe and effective.

This product is Rx (prescription only) because the expertise of a veterinarian is necessary for diagnosis of the condition for which the product is indicated.

Section 514.1(b)(B)(v.) of 21 Code of Federal Regulations states the requirements for efficacy of drug combinations as:

> "Each ingredient designated as active in any new animal drug combination must make a contribution to the effect in the manner claimed or suggested in the labeling, and, if in the absence of express labeling claims of advantages for the combination such a product purports to be better than either component alone, it must be established that the new animal drug has that purported effectiveness."

Resistance of bacteria to the activity of antimicrobials has been recognized as a major problem in therapeutic regimens. The enzyme penicillinase which is elaborated by various bacteria, is a natural defense mechanism of these bacteria to the activity of penicillin and its synthetic analogs. Clavulanate potassium possesses a low index of antimicrobial activity; it has another characteristic of irreversibly tying up penicillinase. It is incorporated in the SYNULOX™ formulation because of this characteristic. This NADA contains both laboratory and clinical studies indicating the combined effect of the two drugs is greater than the effect of each individual active ingredient.