

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 20-744

PHARMACOLOGY REVIEW(S)



**ROUTE OF ADMINISTRATION :** Intratracheally via endotracheal tube: one half of dose in the right main bronchus and one half in the left main bronchus.

**CLINICAL INDICATION :** To treat \_\_\_\_\_ respiratory distress syndrome (RDS) in premature infants.

**DOSE :** The initial dose is 200 mg/kg (= 2.5 mL/kg) in 2 divided doses ; 100 mg/kg (= 1.25 mL/kg) may be repeated at 12 hours and 24 hours after the first dose of 200 mg/kg if the baby is still intubated. Therefore, a total maximal dose of 400 mg/kg or 5 mL/kg.

**DOSAGE FORM :** Suspension in normal saline at a phospholipid concentration of approximately \_\_\_\_\_ DPPC mg/mL and 1 mg/mL protein, supplied in a 5 mL, \_\_\_\_\_ glass vial containing either 1.5 mL (120 mg DPPC) or 3.0 mL (240 mg DPPC) of CUR.

**COMPOSITION :** Phosphatidylcholine\*

SP-B and SP-C proteins

\* Dipalmitoylphosphatidylcholine constitutes at least \_\_\_\_\_ of this fraction.

pH ranges

For detailed quantitative information, see Chemist's review.

**PRECLINICAL DATA SUBMITTED :**

New studies reviewed here are indicated with an asterisk (\*).

**A. PHARMACOLOGICAL STUDIES :**

**EFFECTS RELATED TO THE PROPOSED THERAPEUTIC INDICATION: Vol.**

**1.10.**

- \*1. STATIC PRESSURE-VOLUME RECORDINGS IN PREMATURE NEWBORN RABBITS :
- \*2. IN VIVO LUNG MECHANICS IN ARTIFICIALLY VENTILATED PREMATURE NEWBORN RABBITS.
- \*3. IN VIVO LUNG MECHANICS AND LUNG GAS VOLUME (FUNCTIONAL RESIDUE CAPACITY) IN PREMATURE NEWBORN RABBITS :
- \*4. IN VIVO LUNG MECHANICS AFTER ADMINISTRATION OF CUROSURF AT VARIOUS CONCENTRATIONS IN PREMATURE NEWBORN RABBITS :
- \*5. DOSE-RESPONSE CURVE OF CUROSURF ON IN VIVO LUNG MECHANICS IN PREMATURE NEWBORN RABBITS :
- \*6. EFFECTS OF DIFFERENT DOSES OF CUROSURF ON IN VIVO LUNG MECHANICS IN PREMATURE NEWBORN RABBITS :
- 7. COMPARISON BETWEEN CLINICAL DOSES OF CUROSURF AND EXOSURF IN PREMATURE NEWBORN RABBITS :
- 8. EVALUATION OF THE EFFECTS OF CUROSURF AND EXOSURF IN COMPARISON WITH NON-IONIC DETERGENT ON IN VIVO LUNG MECHANICS IN PREMATURE NEWBORN RABBITS :
- \*9. EFFICACY OF SURFACTANT REPLACEMENT IN IMMATURE AND NEAR-TERM NEWBORN RABBITS VENTILATED TO PHYSIOLOGICAL TIDAL VOLUME:
- \*10. PASSIVE EXPIRATORY FLOW-VOLUME RECORDINGS IN IMMATURE NEWBORN RABBITS.
- \*11. EFFECTS OF CUROSURF ON LUNG PERMEABILITY IN IMMATURE NEWBORN RABBITS:
- \*12. EFFECTS OF CUROSURF ON LUNG PERMEABILITY IN IMMATURE NEWBORN RABBITS :

**EFFECTS RELATED TO POSSIBLE ADVERSE REACTIONS : Vol. 1.11.**

- 13. HEMODYNAMICS AND REGIONAL BLOOD FLOW IN SURFACTANT DEPLETED NEWBORN PIGLETS :
- 14. EFFECT OF INSTILLATION TECHNIQUE ON PULMONARY DISTRIBUTION AND EFFICACY OF EXOGENOUS SURFACTANT IN LUNG LAVAGED RABBITS :
- 15. TRACHEAL SURFACTANT INFUSION COMPARED TO BOLUS INSTILLATION IN RABBITS :
- \*16. EVALUATION OF CUROSURF IN VITRO HEMOLYTIC ACTIVITY.
- 17. BIOLOGICAL EFFECTS OF CUROSURF ON THE CULTURED TYPE II PNEUMOCYTE :
- \*18. HEMOLYSIS TEST OF CUROSURF :PROTECTIVE EFFECTS OF CUROSURF VERSUS ALVEOLAR EPITHELIAL INJURY INDUCED BY LYSOPHOSPHOLIPIDS : Vol. 1.11.

**OTHER EFFECTS ON MAINLY ON ADULT ANIMALS : Vol. 1.11.**

- \*19. EFFECT OF CUROSURF IN MECONIUM ASPIRATION SYNDROME IN NEWBORN RABBITS:
- \*20. EFFECT OF CUROSURF IN MECONIUM ASPIRATION SYNDROME IN ADULT RATS:
- \*21. EFFECT OF CUROSURF IN GROUP B STREPTOCOCCAL INFECTION IN NEONATAL RABBITS :
- \*22. EFFECT OF CUROSURF AND ANTISERUM TO TYPE 1a GROUP B STREPTOCOCCI ON OXIDATIVE METABOLISM OF NEUTROPHILS :
- \*23. EFFECT OF CUROSURF IN EXPERIMENTAL *E. COLI* PNEUMONIA IN ADULT RATS :

**B. PHARMACOKINETIC/ADME STUDIES : Vol. 1.14.**

ALVEOLAR CLEARANCE OF CUROSURF IN DEVELOPING AND MATURE LUNGS :

**C. TOXICITY STUDIES : Vol. 1.11 through Vol. 1.14.**

1. ACUTE IP (intraperitoneal) TOXICITY STUDY IN MICE.
2. ACUTE IP TOXICITY STUDY IN MICE.
3. ACUTE IP TOXICITY STUDY IN RATS.
4. ACUTE IP TOXICITY STUDY IN RATS.
5. ACUTE IT (intratracheal) TOXICITY STUDY IN RATS.
6. ACUTE IT TOXICITY STUDY IN GUINEA PIGS.
7. ACUTE IT TOXICITY STUDY IN RABBITS.
8. ACUTE IT TOXICITY STUDY IN DOGS.
9. 2-WEEK IT TOXICITY STUDY IN RATS.
10. 2-WEEK IT TOXICITY STUDY IN RABBITS.
11. 2-WEEK IT TOXICITY STUDY IN DOGS.
12. 4-WEEK IP TOXICITY STUDY IN RATS FOLLOWED BY A 4 WEEK RECOVERY PERIOD.
13. REVERSE MUTATION .
14. GENE MUTATION IN CHINESE HAMSTER V79 CELLS.
15. CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS IN VITRO.
16. UNSCHEDULED DNA SYNTHESIS IN HELA S3 CELLS IN VITRO.
17. MICRONUCLEUS TEST.
- \*18. PRELIMINARY IT TOLERABILITY STUDY IN GUINEA PIGS.
19. ACTIVE ANAPHYLAXIS IN GUINEA PIGS (SENSITIZED IT AND CHALLENGED IT).
20. ACTIVE ANAPHYLAXIS IN GUINEA PIGS SENSITIZE IP AND CHALLENGE IV ( intravenous).
21. ACTIVE ANAPHYLAXIS IN GUINEA PIGS (SENSITIZE SC AND CHALLENGE

IV).

22. PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) IN GUINEA PIGS.
23. GUINEA PIG MAXIMIZATION TEST.

#### SUMMARY AND EVALUATION :

The subject of this NDA is Curosurf<sup>®</sup> (CUR), a natural lung surfactant extracted from pig lungs, for the treatment of respiratory distress syndrome (RDS) in premature infants or with low birth weight. CUR is manufactured by Chiesi Farmaceutici S.p.A. in Parma, Italy.

A deficiency of pulmonary surfactant results in RDS, also known as hyaline membrane disease, commonly associated with prematurity of newborns, characterized by poor lung expansion during inspiration, inadequate gas exchange, and a gradual collapse of the lungs that results in atelectasis, and often death.

Premature infants with RDS have been shown to be deficient in endogenous surfactant due to prematurity of Type II cells in the lungs; thus, CUR will supplement the deficiency in surfactant to help these premature infants breath and survive.

The initial clinical dose of CUR is 200 mg/kg (= 2.5 mL/kg) in 2 divided doses, and 100 mg/kg (= 1.25 mL/kg) may be repeated at 12 hours and 24 hours after the first dose of 200 mg/kg if the baby is still intubated. Therefore, a total maximal dose is 400 mg/kg or 5 mL/kg.

The total daily dose of CUR is similar to other approved products, that have similar composition of active ingredients (DPPC and proteins).

The applicant submitted a total of 23 preclinical toxicity studies, in addition to 24 pharmacological/pharmacodynamic efficacy studies and distribution/clearance studies in adult rabbits and preterm rabbits. A total of 22 toxicity studies and 7 pharmacological efficacy studies were previously submitted under IND. All new studies are marked with asterisk (\*) and they were reviewed under this NDA.

Most pharmacological/pharmacodynamic efficacy studies were performed at Chiesi Farmaceutici in Italy. Most preclinical toxicity studies were performed either at

All toxicity studies were conducted under GLPs, but 5 toxicity studies performed at were not conducted under US GLP Regulations, mostly due to their completion before the regulations were established.

The mechanism of action for pulmonary surfactants (decreased alveolar surface tension and stabilizing of the alveoli during expiration) in premature animals as well as humans has been

well established. CUR also has been shown to lower surface tension in in vitro as well as in in vivo models of RDS, and CUR improved tidal volume and lung compliance dramatically and survival of immature preterm rabbits (artificially delivered on day 27 gestation except one study on day 28).

Examples of efficacy studies are as follows. The results of in vivo static pressure-volume recording of preterm rabbits showed that intratracheal instillation of CUR significantly improved lung volume, lung compliance and stability of immature lungs, when compared to untreated controls.

A dose-response survival curve of a study with 6 doses from 12.5 to 400 mg/kg of intratracheal administration of CUR in 8 to 14 preterm rabbit fetuses/group was a bell shaped curve, with the maximum 100% survival at 100 and 200 mg/kg. CUR administration improved tidal volume, lung compliance and survival of these fetuses dose-dependently (up to 100% survival at 100 and 200 mg/kg). The survival in saline control group was 43%. Tidal volume improved gradually with time and with dose, with the maximum at 30 minutes of artificial ventilation at pressure of 25 cmH<sub>2</sub>O. Decreased survival (83%) that occurred at 400 mg/kg CUR despite markedly increased tidal volume over 200 mg/kg was attributed to lung overdistension. Abnormal EKGs were reported in 8/14 saline controls, none in other 4 groups, but in 1/13 of 50 mg/kg and 1/11 of 400 mg/kg groups. Pneumothorax was not reported in saline controls and 100 and 200 mg/kg groups, but 2 each in 12.5 and 25 mg/kg groups, 1/13 of 50 mg/kg group and 1/11 in 400 mg/kg groups.

In another study, intratracheal instillation of 1 and 2 mL/kg CUR (80 and 160 mg/kg) in preterm rabbit fetuses produced dose-dependent increases in tidal volume and lung compliance with corresponding increases in lung gas volume; thus presumably an increase in the functional residual capacity (from evaluation of histological sections of the lungs) when compared to untreated controls.

The results of a study with 3 different concentrations and 3 different volumes [(40 mg/mL in 4 mL/kg, 55 mg/mL in 3 mL/kg and 80 mg/mL in 2 mL/kg) of CUR in 21 to 25 preterm rabbits/group showed that the best improvement of lung mechanics (tidal volume and compliance) was produced by 80 mg/mL CUR. It was stated that 80 mg/mL solution of CUR minimizes the liquid volume load, and the viscosity of the CUR suspension is such that it "warrants for even distribution". But, the survival rate in CUR treated groups was inversely dose-related : 100% for 40 mg/mL group, 96% for 55 mg/mL group, and 88% for 80 mg/mL group (vs. 25% for untreated controls). Thus, improved tidal volume and compliance did not show a direct relationship to the survival of preterm animals (improved survival) in this study.

Another dose-response study also suggested a bell shaped survival curve : the higher doses did resulted in lower survival : Four different (50, 100, 200 and 300 mg/kg) intratracheal doses of an 80 mg/mL CUR solution were studied with regard to effects on lung mechanics of 21 to 27 preterm rabbit fetuses/group. All parameters of lung mechanics (tidal volume, compliance,

AUC from both parameters, etc.) increased with CUR, dose-related manner when compared to untreated controls except the survival rates. The survival rate was 100% for 50 and 100 mg/kg groups, 93 % for the 200 mg/kg group and 92% for the 300 mg/kg group, showing decreased survival in spite of increased lung tidal volume and lung mechanics. The untreated controls had 38% survival. As in the previous study, greater improvement in tidal volume, lung compliance, and AUC from both parameters was not directly related to an improved survival rate.

The results of the above two dose-response and dose/concentration-response studies in preterm rabbits, where the clinical choice of the higher dose of 200 mg/kg that has a lower survival rate than 50 or 100 mg/kg, and also the choice of the highest concentration of 80 mg/mL solution that has a lower survival rate than 40 or 55 mg/mL, should have been considered in selection of the clinical dose.

For pharmacokinetic studies, a single dose of 200 mg/kg CUR with <sup>14</sup>C-DPPC was injected directly into the lungs of 3 day old newborn (4/group) and 6 to 8 week old adult rabbits (5/group). It was reported that approximately 50% of administered dose of CUR was removed from the alveoli within the first 3 hours in both newborn and adults. Over the next 24 hours, approximately 45% of labeled DPPC was cleared from the lungs of adults, but only approximately 20% in newborns was cleared with a larger portion of DPPC being recycled in newborns when compared to adult animals. The half life of CUR in the lungs was estimated as about 25 hours in adults and 67 hours in newborn rabbits. Very little DPPC was found in alveolar macrophages or any major organs in either newborn or adult animals at 48 hours. Metabolic fate of the proteins has not been studied in animals nor in humans. Protein content, LDH activity, and number of macrophages in lung lavage fluid did not increase after CUR administration, suggesting no significant increase in inflammatory responses in the lungs after CUR treatment. Similar PK data were reported for other pulmonary surfactants.

A total of 8 acute toxicity studies were performed: 2 intraperitoneal (IP) studies each in mice and rats and 4 intratracheal (IT) studies in rats, guinea pigs, rabbits and dogs.

After a single IP dose of 500, 1000, or 2000 mg/kg CUR in a mouse study or a single IP dose of 2000 mg/kg CUR in mice and rats, the animals were observed for 14 days. For all 4 IT studies, a single dose of 200 mg/kg CUR dose was administered via implanted catheter into the trachea, along with a sham control with catheter. The rat study also had an untreated control group. Clinical signs, body weight, gross pathology and histopathology of injection sites were observed/examined.

There were no deaths in any studies, and body weight showed no treatment effect in any of the 8 studies. Toxic clinical signs in IP studies were hypoactivity (mice), piloerection (mice and rats), ptosis (mice), soft feces and hair loss (both for rats).

IT studies reported wheezing, red crust around nostrils, foamy fluid with/without red color

around nostrils, dyspnea, and gasping, all in rats and guinea pigs. These signs tended to be slightly greater in numbers in the CUR group than Sham group in both rats and guinea pigs. A single dose of IT 200 mg/kg CUR did not produce deaths in rats, guinea pigs, rabbits or beagle dogs, or any significant effects on body weight. Only transient respiratory insufficiency was reported in rats, guinea pigs and dogs, but this symptom seemed the most severe in dogs.

Four multidose toxicity studies were submitted : 2 week IT studies in rats, rabbits and dogs; and a 4 week IP study in rats.

In a 2 week IT study, rats were treated with 2.5 mL/kg (200 mg/kg) of CUR intratracheally via chronically implanted catheters for 14 days, along with a group of sham (air) treated control and an untreated control group.

Deaths occurred in 4 sham and 5 CUR groups, and the deaths in the sham control group were attributed to pneumonia; 5 deaths in CUR were during administration and attributed to suffocation caused by a "large volume" of CUR. Many of the findings were attributed to the presence of chronically implanted catheters in both sham and CUR treated groups. Significant depression of body weight gain was reported for both groups, but the difference between the two groups became significant from day 4 to the end of the study on day 15: at necropsy the CUR group had 13% greater depression. In addition, several catheter attributed findings were reported in the study. However, almost all of these changes showed a slight trend of marginal increases in the CUR group over the sham control group, although none of the changes were statistically significant.

In a 2 week IT toxicity study, a group of New Zealand white rabbits was treated with 1.25 mL/kg (100 mg/kg) CUR intratracheally via enterotracheal tube under anesthesia, once daily for 14 days. A second group was "sham" dosed (administration of anesthetic and insertion of intratracheal tube) and served as a control group.

100 mg/kg/day CUR for 14 days produced 3 deaths during the study, along with one control death. Two of 3 deaths in CUR treated group were attributed to CUR administration and lung changes, although anesthesia might have attributed to some degree. Sneezing and abnormal lung sounds were noted in the CUR treated animals, as well as in some control animals. Significantly increased lung weights were noted in CUR group. Gross pathology of CUR group included the lungs that failed to collapse upon opening the thorax. The histological changes were inflammatory reactions in the lungs of both groups, but slightly increased incidence and degree of alveolar macrophage aggregation, alveolar edema, and pneumonia were noted in CUR treated M. No drug-related changes were reported for body weight, food consumption, ophthalmology and blood chemistry except for some minor changes for WBC, lymphocytes, and neutrophils.

In a 2 week IT toxicity study in dogs, the first dog receiving 2.5 mL/kg on day 1 had to be killed after only 16 mL out of an intended 22 mL (=145 mg/kg), after severe convulsions that

were attributed to suffocation. The second dog after receiving only 1.25 mL/kg (100 mg/kg) also showed severe clinical signs of suffocation, thus the dose had to be decreased to 0.625 mL/kg (50 mg/kg). A control group was anesthetized and intubated but not treated. Daily intratracheal administration of 50 mg/kg/day CUR for 14 days in beagle dogs did not produce any significant increases in toxic effects on usual toxicity parameters including histopathology of the lungs, although some dogs had tachypnea and coughing, and lungs showed interstitial pneumonia, aggregations of alveolar macrophages and perivascular lymphocytes. Some findings in the lungs (such as fibrosis and congestion, reported in 2 dogs each) were limited to the CUR treated group, with slightly increased heart weights (RW) in M dogs. Therefore, 50 mg/kg/day or 0.625 mL/kg of CUR was considered the maximal practical dose for the dog, and 1.25 mL/kg (or 100 mg/kg) and 2.5 mL/kg (or 200 mg/kg) caused respiratory distress, mainly due to suffocation by physical means.

The following general observations also should be pointed out : 1). Almost all findings reported in 2 week IT studies are attributed to presence of catheters, that caused inflammation in the areas around the catheters. However, CUR had shown only a slightly greater tendency for incidence or degree of each finding over the vehicle control group, but this indicated that CUR probably brought about an increase in irritation/inflammation in animals. 2). In all three 2 week IT toxicity studies in rats, rabbits and dogs, only one single dose of CUR per day was tested, and the highest dose used was 200 mg/kg/day in rats, but only 100 mg/kg/day in rabbits and 50 mg/kg/day in dogs (only one dose group for each study, along with a control group), while the initial clinical dose is 200 mg/kg. 3). Dogs were the most sensitive species tested (rats, rabbits, lambs, ferrets, cynomolgus monkeys), even though they were young adults instead of newborn or premature pups that require artificial ventilation. Therefore, there was no clear cut NOAEL in any of these studies, although 50 mg/kg was considered the NOAEL in dogs by the applicant. A single dose of 200 mg/kg CUR was the NOAEL in acute IT toxicity studies in rabbits and dogs.

In a 4 week IP toxicity study in rats, the various groups were treated with saline, or 200, 350, or 600 mg/kg CUR, once daily by IP injection for 4 consecutive weeks. Additional rats from the 600 mg/kg and the control groups were kept for an additional 4 weeks as recovery groups.

CUR produced significant depression of body weight gain from the second week on, especially in M, although food consumption was not affected. M also had dose-related decreases in Hb with dose-related increases in WBCs, along with increased neutrophils. The Hb and WBC changes in 600 mg/kg M were also highly significant from the control values. F in 600 mg/kg also had increased WBCs, with significantly increased neutrophils and decreased lymphocytes. Changes in WBCs were attributed to infection at injection sites. Both coagulation times (PT and APPT) in 600 mg/kg M were significantly increased in spite of platelet increases for M and F which were marginally increased (dose-related). Serum proteins and some globulins, especially in 600 mg/kg M, were increased while albumin decreased, that led to decreased A/G ratio. These also were attributed to inflammation at the injection sites. Some other significant changes were noted, but the actual differences were either small (<10%) or limited to only

one sex, thus they may have no toxicological significance, but decreased potassium [6% ↓ (p < 0.05)] reported in F treated groups needs to be noted since this occurred in the recovery group (15% ↓ for potassium) as well. Among the organ weight changes, liver and spleen weight changes were most prominent: the increases were dose-related for AW and RW and statistically significant for M (for both organs) and F (liver), especially for the 600 mg/kg group, and the changes persisted until the end of a 4 week recovery period for the spleen. Spleen weight changes were also attributed to inflammation. Treatment related gross changes were found at the injection sites of treated groups but none in the control group. Adverse histopathology findings were limited to injection sites and the liver. Dose-related acute and/or chronic suppurative inflammation and fibrosis at the injection sites and dose-related reduction of margination of the cytoplasm in the liver of treated groups were reported at the end of the treatment as well as at the end of recovery period. Minimal to moderate centrilobular hepatocyte vacuolation was also reported mainly in 600 mg/kg M (9/15) at the end of treatment as well as after the recovery period. Therefore, some changes in the liver were persistent. Thus, the liver would be considered a target organ of toxicity in this study.

The hydrolysis of phospholipids normally occurs during the shelf life of surfactants. The resulting lyso-derivatives, because of their strong amphiphilic properties, can produce deleterious effects on cells by becoming inserted into and perturbing membrane stability. It has been speculated that the presence of lyso-phospholipids in the surfactants may have an impact on the safety of this product. Therefore, the toxicity of \_\_\_\_\_ was studied in premature rabbit fetuses, artificially delivered on day 28 of gestation, then treated with 5 different solutions [saline, CUR with low (standard) and high \_\_\_\_\_ low and high \_\_\_\_\_] just before being mechanically ventilated for 60 minutes at a constant volume of about 10 mL/kg. The degree of alveolar epithelial injury was assessed by means of LDH, hemoglobin and protein content in bronchoalveolar fluid (BAL), along with dynamic compliance. \_\_\_\_\_ dissolved in saline at 6.4 and 16 mg/mL produced dose-dependent tissue damage based on increased LDH, protein and Hb content in BAL and increased the incidence of pneumothorax (3/13 in high \_\_\_\_\_ group vs zero in CUR groups) as well as producing abnormal EKGs (6/13 in high \_\_\_\_\_ group). \_\_\_\_\_ produced significant increases in mortality when compared to 2 CUR treated animals: Survival rate was 100% in saline controls and 2 CUR groups but 92% for the low \_\_\_\_\_ and 31% (p < 0.001) in the high \_\_\_\_\_ group. Hb was not detected in saline treated rabbits, but it was detected in 1/25 CUR treated rabbits and 21/22 \_\_\_\_\_ treated rabbits. Therefore, the results indicated that up to 8% LPS in CUR did not affect the efficacy or survival rate of CUR. It was unusual that the survival rate of saline alone group was also 100% in this study, without abnormal EKGs or pneumothorax in 13 preterm rabbits.

In another in vitro hemolysis test, 2% and 8% \_\_\_\_\_ containing CUR produced much less hemolysis of human blood than 8% \_\_\_\_\_ solution in saline. This suggests that CUR may have some protective effect of \_\_\_\_\_.

Mutagenic potential of CUR has been tested in 5 genotoxicity studies. CUR was negative in all 5 genotoxicity studies (Ames Assays, In Vitro Gene Mutation Assays in Chinese Hamster V79

Cells, In Vitro Chromosomal Aberration Assays In Chinese Hamster Ovary Cells, Unscheduled DNA Synthesis, all with/without S-9, and In Vivo Micronucleus Tests for Chromosome Damage in CD-1 Mice), with/without metabolic activation, under the test conditions. All the positive controls produced statistically significant dose-related positive reactions as expected under the test conditions.

A preliminary tolerability study was conducted in M Dunkin-Hartley guinea pigs with IT doses of CUR at 25, 50, or 100 mg/kg or saline on days 1, 3, and 5, in order to establish effects that might be interpreted as an anaphylactic reaction. It was determined from the results that only 25 mg/kg (1/8th of clinical dose) was the maximum dose that would not lead to misinterpreted signs of anaphylaxis.

Among 5 antigenicity tests, CUR was negative in 3 active anaphylaxis tests and positive in 2 tests [passive cutaneous anaphylaxis test (PCA) and maximization test], all in guinea pigs under the test conditions, while the positive control produced marked anaphylaxis.

CUR produced measurable antibodies in a PCA in guinea pigs, although IgG antibodies were not detected under the conditions used. This is not surprising since CUR is an extract of natural pig lung surfactant, and contained minute amounts of (less than 2%) SP-B and SP-C in the final product.

The results of guinea pig dermal maximization tests were reported as "not useful or conclusive" for determining dermal sensitization potential, due to high positive reaction in the control group : 5 of 20 from undiluted CUR along with 2 controls and 1 of 20 from 50% CUR along with one control showed Grade 1 (weak) cutaneous reaction at 24 hours after dermal challenge. Forty eight hours after challenge, 2 of undiluted CUR still showed Grade 1 reaction along with one control. These positive cutaneous reactions were attributed to irritation. The applicant concluded that "it was not possible to assess the sensitization potential of the material using the results obtained". However, these results may indicate that CUR has a greater antigenic potential due to a high concentration of active ingredients.

The concentration of phospholipids (PL) as well as surfactant associated specific proteins (SP-B and SP-C) in CUR possibly could play important roles in its safety and efficacy as noted in the following paragraph.

CUR contains 80 mg PL/mL, with less than 2% of surfactant associated specific proteins (SP-B and SP-C) from pig lungs. Survanta contains 25 mg PL/mL, with less than 2% of surfactant associated specific proteins from bovine lung. But, Exosurf (synthetic surfactant) contains only 13.5 mg PL/mL without any foreign proteins. Therefore, CUR contains almost a 6-fold greater amount of the active ingredient, PL, than Exosurf, and thus, CUR may have an advantage over these other two surfactants due to the higher concentration of PL. This results in requiring a lesser volume being administered into the infant's lung.

However, the higher concentration of PL in CUR conceivably could be a disadvantage : It was not as effective at a lower concentration of PL on the survival of preterm rabbits in a previous dose/concentration study. Also, the higher concentration of PL in CUR may result in a higher viscosity than other surfactants administered to the lungs of premature infants which then may result in a hindrance of gaseous exchange when compared to surfactants with a lesser viscosity.

Due to its intended population and short term usage, reproductive studies and carcinogenicity studies are not required for an NDA approval.

CUR has been marketed and distributed in 22 countries, mainly in Europe. CUR has been used and studied extensively in European clinics, and at the end of 1995, it was estimated from the sale of the product that over 20,000 patients have been treated without notable problems to date; therefore, there is adequate human evidence to support relative safety in addition to preclinical data.

**RECOMMENDATIONS :**

1. This NDA is approvable from the preclinical standpoint.
2. Preclinical part of the proposed labeling on Animal Metabolism (the first paragraph, page 97, Vol. 1.1) and the statement on the mutagenic studies (the last sentence, page 104) need appropriate modifications as indicated previously.

**/S/**

Young S. Choi, Ph.D.  
Pharmacologist

**/S/**

Jan 3, 1997

cc:

Original (NDA 20-744)  
 HFD-570/Division file  
 HFD-570/MO/Pina  
 HFD-570/Sun  
 HFD-570/Choi  
 HFD-570/CSO/Kuzmik  
 R/D by Y. S. Choi/12/6/96  
 Revised by Y. S. Choi/1/3/97  
 Init. by J. Sun/ / /97  
 F/T by Y. S. Choi/ / /97, WP 0828F-1-  
 N:\NDA\20744\PHARM\96-7-3\REV

Attachment

[Redacted]

original review addendum of 3/10/95 CJSun 010397

**LETTER TO THE APPLICANT :**

We have reviewed your NDA submission dated 7/3/96 and have the following comments on the preclinical section :

The preclinical part of labeling on Animal Metabolism (the first paragraph, page 97, Vol. 1.1) and on Carcinogenesis, Mutagenesis, and Impairment of Fertility (the last sentence, page 104) needs modification.

For Animal Metabolism, the first paragraph should be deleted. For Carcinogenesis, Mutagenesis, and Impairment of Fertility, the following statement should be added : Studies to assess the potential carcinogenic and reproductive effects of CUROSURF have not been conducted

For Mutagenic studies, the names of studies should be added (Ames Assays, Gene Mutation Assays in Chinese Hamster V79 Cells, Chromosomal Aberration Assays In Chinese Hamster Ovary Cells, Unscheduled DNA Synthesis in HELA S3 Cells, all with/without metabolic activation, and In Vivo Mouse Micronucleus Tests.

**APPEARS THIS WAY  
ON ORIGINAL**

**NEW PRECLINICAL STUDIES REVIEWED :**

All new studies indicated by an asterisk (\*) beside the titles were reviewed under this NDA, and all other preclinical studies were reviewed previously under \_\_\_\_\_ except toxicity studies numbers 7 and 8 (comparison between CUR and Exosurf). See Addendum to the Original Review by this reviewer filed on March 10, 1995.

**PHARMACOLOGICAL STUDIES :****EFFECTS RELATED TO THE PROPOSED THERAPEUTIC INDICATION:**

COMMENTS : Studies were performed at Chiesi in Italy unless otherwise indicated.

**1. STATIC PRESSURE-VOLUME RECORDINGS IN PREMATURE NEWBORN RABBITS : Report No. DF/90/097 :**

Methods : Premature rabbits (HY/CR) were delivered on by uterine incision on day 27 of gestation, and were tracheotomized under anesthesia and connected to a system of parallel glass tubes that were connected to a reservoir bottle of stained water (see diagram on page 108, Vol. 1.10). The movement of stained water for each rabbit from several litter mates was recorded simultaneously up to 12 rabbits in a chamber. 77 fetuses were treated with CUR at 2 mL/kg (160 mg/kg) and 48 fetuses were not treated. Pressure was maintained and lung volume was obtained from the measurement of stained water movement multiplied by the tube capacity (6.83  $\mu$ l/mm) and referred to as the relative fetus body weight; and the static lung compliance was obtained from lung volume divided by the applied pressure level.

RESULTS : Control lungs required a transpulmonary pressure of 25 cm H<sub>2</sub>O to expand with the average volume at 30 cm H<sub>2</sub>O being 15.22  $\pm$  1.42 mL/kg. Lungs treated with CUR had an opening pressure of 15 cm H<sub>2</sub>O and the lung volume was 64.95  $\pm$  1.20 mL/kg. The treated lungs showed stability during deflation compared to non-treated control lungs.

**2. IN VIVO LUNG MECHANICS IN ARTIFICIALLY VENTILATED PREMATURE NEWBORN RABBITS : Report No. DF/90/061:**

Methods : Premature rabbits (HY/CR) were delivered on day 27 of gestation, and were tracheotomized under anesthesia, and then artificially ventilated. Lung mechanics were investigated in 80 fetuses after treating with 2

mL/kg (160 mg/kg) of CUR intratracheally before the onset of ventilation, along with 32 controls untreated.

**RESULTS :** CUR treated fetuses showed highly significant improvement in lung compliance over the untreated controls : Tidal volumes of the controls ranged between  $2.684 \pm 0.449$  and  $2.162 \pm 0.224$  mL/kg at 25 cm H<sub>2</sub>O in the first 15 minutes, while the tidal volume in CUR treated was significantly increased ( $p < 0.001$ ) :  $22.295 \pm 0.922$  to  $20.323 \pm 0.914$  mL/kg. At the lowest insufflation pressure of 15 cm H<sub>2</sub>O, the tidal volume of CUR treated was  $5.241 \pm 0.519$  mL/kg, but the same value in controls was only  $1.043 \pm 0.042$  mL/kg. Survival rate of fetuses was 37% for controls while it was 96% for CUR treated fetuses.

**3. IN VIVO LUNG MECHANICS AND LUNG GAS VOLUME (FUNCTIONAL RESIDUE CAPACITY) IN PREMATURE NEWBORN RABBITS : Report No. DF/90/151**

**Methods :** Premature rabbits (HY/CR) were delivered on day 27 of gestation, and were tracheotomized under anesthesia, and then artificially ventilated with 100% O<sub>2</sub> at 40 breaths/min. Lung mechanics, compliance, EKGs were investigated in fetuses after treating with 1 mL/kg (80 mg/kg, n=23) or 2 mL/kg (160 mg/kg, n=29) of CUR intratracheally before the onset of ventilation, along with 18 untreated controls. At the end of ventilation, animals were sacrificed, and lungs were collected, weighed, and lung volume was measured by water displacement and functional residual capacity (lung gas volume) was defined as the difference between lung volume and lung weight (assuming that the density of the lung tissue is approximately that of water). The lungs were fixed, stained and examined by light microscope for alveolar expansion, and the volume density (V<sub>v</sub>) was determined in 25 random microscopic fields.

**RESULTS :**

- a. Survival rate was 11% for controls, 95% for 1 mL/kg CUR treated group and 93% for the 2 mL/kg CUR treated group.
- b. Average lung weight was similar in all 3 groups (36.3 to 37 g).
- c. CUR produced dose-related increases in lung compliance : increases in average values for lung volume, lung gas volume, alveolar volume density, and tidal volume. Tidal volume in CUR treated fetuses was  $17.732 \pm 2.10$  mL/kg after 1 mL/kg CUR and  $21.287 \pm 1.411$  mL/kg after 2 mL/kg CUR when the control value was only  $2.315 \pm 0.383$  mL/kg. Compliance (mL/cmH<sub>2</sub>O) at 30 minutes in CUR treated fetuses was  $0.709 \pm 0.084$  after 1 mL/kg CUR and  $0.851 \pm 0.056$  after 2 mL/kg CUR while the control value was only was 0.093

±0.015.

- d. V<sub>v</sub> values decreased (dose-related) after CUR treatment (mean values of 0.29 and 0.25 vs. 0.31 in controls).

**4. IN VIVO LUNG MECHANICS AFTER ADMINISTRATION OF CUROSURF AT VARIOUS CONCENTRATIONS IN PREMATURE NEWBORN RABBITS : Report No. DF/90/099 :**

Each litter of preterm rabbit (HY/CR) fetuses was randomly treated with a fixed dose of 160 mg/kg CUR (batch 9002058) at 3 different concentrations : 80, 55 and 40 mg/mL via tracheal cannula in volumes of 2, 3 and 4 mL/kg, respectively, before the initiation of ventilation and at least one fetus was kept as control.

CUR produced inversely related concentration dependent effects on survival; tidal volume and lung compliance were directly related to concentration as shown below:

Group:	Control:	80 mg/mL:	55 mg/mL:	40 mg/mL:
mL/kg	0	2	3	4
n:	12	25	23	21
Body weight (g):	32.9	35.2	34.8	36.8
Survival (%):	25	88	96	100
Tidal volume (mL/kg):	2.06	23.7	19.2	17.1
at 5 minutes				
Compliance at 5 minutes (mL/cmH <sub>2</sub> O/kg):	0.082	0.95	0.77	0.69

**5. DOSE-RESPONSE CURVE OF CUROSURF ON IN VIVO LUNG MECHANICS IN PREMATURE NEWBORN RABBITS : Report No. DF/91/037 :**

Dose-response of intratracheal administration of CUR (batch 9007033) was studied after 7 doses from 12.5 to 400 mg/kg in saline (2.5, 5, 10, 20, 40 and 80 mg/mL, respectively) in randomly allocated preterm rabbits (HY/CR), along with one control fetus receiving saline treatment.

Administration of CUR improved tidal volume, lung mechanics and survival of these fetuses dose-dependently as shown below. The survival was best at 100 and 200 mg/kg. Tidal volume improved gradually with time at each dose with the maximum at 30 minutes at pressure of 25 cmH<sub>2</sub>O. Decreased survival that occurred at 400 mg/kg despite markedly increased tidal volume was attributed to lung overdistension, since

one pneumothorax was noted. Survival rate of 43% in saline was reported as an unexpected finding, and it was attributed to the presence of more mature babies.

Group: (mg/kg):	Saline:	12.5:	25 :	50:	100:	200:	400
Concentration:(mg/mL)	2.5	5	10	20	40	80	
n:	14	10	8	13	11	12	11
Body weight (g):	34.1	33.2	34.3	32.7	34.3	33.4	33.8
Survival (%):	43	83	80	86	100*	100*	83
Tidal volume (mL/kg):	3.6	6.6	8.96	12.2	15.1	18.6	27.2
at 30 minutes							
Compliance at 30 minutes							
(mL/cmH <sub>2</sub> O/kg):	0.14	0.26	0.36	0.49	0.61	0.74	1.09
Pneumothorax: n	0	2	2	1	0	0	1
Abnormal EKG: n	8	0	0	1	0	0	1

\*statistically significantly different from controls (Fisher's exact 2 tailed test).

#### 6. EFFECTS OF DIFFERENT DOSES OF CUROSURF ON IN VIVO LUNG MECHANICS IN PREMATURE NEWBORN RABBITS : Report No. DF/93/002 :

Effects of 4 doses of 80 mg/mL CUR (batch 9111011) on in vivo lung mechanics were studied in preterm rabbits (HY/CR), along with untreated controls.

Tidal volume, lung compliance, lung volume, AUC of lung (by trapezoidal rule) showed dose-related increases from untreated controls, but the survival was best at 50 and 100 mg/kg without any pneumothorax as shown below :

Group (mg/kg CUR):	Untreated :	50:	100:	200:	300:
n:	21	24	23	27	25
Body weight (g):	30.8	31.6	31.3	32.3	31.3
Survival (%):	38	100*	100*	93*	92*
Tidal volume (mL/kg):	4.6	15.9	18.2	21.7	26.9
at 30 minutes					
Compliance at 30 minutes					
(mL/cmH <sub>2</sub> O/kg):	0.18	0.63	0.7	0.9	1.1
Lung weight(g):	1.17	1.28	1.26	1.38	1.36
Lung volume (mL) :	1.17	1.36	1.41	1.64	1.66
AUC					
from tidal volume :	63.8	-	-215.1*	264.5*	296.0*
AUC from compliance:	2.8	8.9*	11.0*	12.3*	13.97*
Pneumothorax: n	0	0	0	1	1

\*significantly different from controls (Fisher's exact 2 tailed test).

**7. COMPARISON BETWEEN CLINICAL DOSES OF CUROSURF AND EXOSURF IN PREMATURE NEWBORN RABBITS :**

COMMENTS : Studies 7 through 12 were not reviewed, since identical studies were submitted previously under the IND or this NDA, as a whole study or a part of a study. Only the title will be given for each study.

**8. EVALUATION OF THE EFFECTS OF CUROSURF AND EXOSURF IN COMPARISON WITH NON-IONIC DETERGENT IN ON IN VIVO LUNG MECHANICS IN PREMATURE NEWBORN RABBITS :**

**9. EFFICACY OF SURFACTANT REPLACEMENT IN IMMATURE AND NEAR-TERM NEWBORN RABBITS VENTILATED TO PHYSIOLOGICAL TIDAL VOLUME:**

COMMENTS : A copy of a publication entitled "Lung Gas Volumes and Expiratory Time Constant in Immature Newborn Rabbits Treated with Natural or Synthetic Surfactant or Detergents" was submitted.

**10. PASSIVE EXPIRATORY FLOW-VOLUME RECORDINGS IN IMMATURE NEWBORN RABBITS :**

COMMENTS : A copy of a publication entitled "Application of a New Ventilator-multi-plethysmograph System for Testing Efficacy of Surfactant Replacement in Newborn Rabbits" was submitted.

**11. EFFECTS OF CUROSURF ON LUNG PERMEABILITY IN IMMATURE NEWBORN RABBITS:**

COMMENTS : A copy of a publication entitled "Passive Expiratory Flow-Volume Recordings in Immature Newborn Rabbits" was submitted, but not reviewed.

**12. EFFECTS OF CUROSURF ON LUNG PERMEABILITY IN IMMATURE NEWBORN RABBITS :**

COMMENTS : A copy of an abstract for publication was submitted without detailed information.

**13. REPORT No. 166/S : EVALUATION OF CUROSURF IN VITRO HEMOLYTIC ACTIVITY :**

times, and they were pooled and frozen for assays.

## RESULTS :

### 1). Summary of mortality, abnormal EKG and pneumothorax :

Treatment at birth	n	body weight (g)	Abnormal EKG (n)	Pneumothorax (n)	Survival (%)	
Saline	13	35.2±1.9	0	0	13	100
Saline + LPC 6.4 mg/mL	12	36.8±2.6	1	0	11	92
Saline + LPC 16 mg/mL	13	33.5±1.7	6	3	4	31 (p<0.001)
1% LPC CUR (0.6 mg/mL)	14	35.2±1.6	0	0	14	100
8% LPC CUR (10.2 mg/mL)	11	34.4±2.2	0	0	11	100

- 2). **Lung Mechanics** : Standard CUR (with low LPC) improved lung compliance of premature rabbits, followed by CUR with high LPC, that showed a slight improvement over the saline controls. Two LPC treated animals produced the worst lung compliance among 5 groups, although tidal volumes were similar in all groups (due to artificial ventilation).
- 3). **Alveolar Epithelial Damages** : Quantitatively, the released LDH in BAL was lowest for CUR with low LPC < CUR with high LPC ≤ saline < low LPC < high LPC. The released protein and hemoglobin (Hb) in BAL also followed a similar order. Hb was not detected in two CUR treated 25 rabbits except one while two LPC treated groups produced dose-dependent significant increases in Hb. Therefore, CUR protected epithelial lining, while increased LPC produced dose-dependent damage to alveolar epithelium.

COMMENT : Hb values for saline group were not submitted. But, \_\_\_\_\_ informed us on 12/6/96 by phone that although the data were not submitted in the NDA, the mean Hb value in the saline group was lower than the detection limit.

## 16. ALVEOLAR CLEARANCE OF CUROSURF IN DEVELOPING AND MATURE LUNGS:

Lab Performing the Study : \_\_\_\_\_

COMMENT : Identical study was submitted previously under IND.

**17. EFFECT OF CUROSURF IN MECONIUM ASPIRATION SYNDROME IN NEWBORN RABBITS:**

COMMENTS : A copy of a publication entitled "Surfactant Improves Lung Function and Morphology in Newborn Rabbits with Meconium Aspiration" was submitted.

Administration of 6 mL/kg via tracheal cannula of a human meconium suspension in 65 or 130 mg/mL in saline solutions in artificially ventilated nearly mature newborn rabbits (gestational age 29.5 days) produced statistically significant reduction in lung thorax compliance, elevated  $PCO_2$  in heart blood and reduced alveolar volume density ( $V_v$ ) in histological sections. Administration of 200 mg/kg CUR significantly improved all of these parameters when compared to saline controls. However, the CUR administration did not improve the survival rates at all: The survival rate in the saline controls were 92%, 79% in 65 mg/mL meconium alone group and 92% in 130 mg/mL meconium alone group (the same as the saline controls). After CUR treatment, the survival rates were still 79% in 65 mg/mL meconium group but 75% in 130 mg/mL meconium group. Thus, after CUR, the survival rate stayed the same in one group and decreased in the high dose meconium group, despite of improvement of other parameters in newborns. Pneumothorax was reported for one animal each in all groups. Abnormal EKGs were reported in one saline control group and 2 each in low dose meconium groups with/without CUR, and 3 occurred in the high dose meconium group with CUR but none in the high dose meconium alone group. Therefore, CUR did not have any protective effect on the heart.

**18. EFFECT OF CUROSURF IN MECONIUM ASPIRATION SYNDROME IN ADULT RATS:**

COMMENTS : A copy of an abstract entitled "SURFACTANT IMPROVES LUNG FUNCTION IN VENTILATED RATS WITH MECONIUM ASPIRATION" was submitted.

Administration of 6 mL/kg via tracheal cannula of a human meconium suspension in saline (25 mg/mL) in artificially ventilated adult rats produced statistically significant reduction in lung thorax compliances when compared to saline (4 to 6 mL/kg). Administration of 200 mg/kg CUR (80 mg/mL, 2.5 mg/kg) significantly improved lung compliance at 30 minutes and sustained to 180 minutes.

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**19. EFFECT OF CUROSURF IN GROUP B STREPTOCOCCAL INFECTION IN NEONATAL RABBITS :**

COMMENTS : A copy of a one page abstract publication entitled "INFLAMMATORY RESPONSE IN EXPERIMENTAL GROUP B STREPTOCOCCAL (GBS) INFECTION IN NEONATAL RABBITS" was submitted.

Groups of near term (29.5 day gestational age) newborn rabbits after 15 minutes of artificial ventilation were administered IT with a 5 mL/kg ( $10^9$  colony forming units/mL) GBS strain 090 Ia LD (3 groups) or saline (one). One group of GBS treated (the control) was killed one minute later. Thirty minutes later, one group was treated with saline; and one group, with 2.5 mL/kg CUR IT. At the end of the experiment, lungs were aseptically removed, weighed, and homogenized. Free elastase activity was determined in the supernatant, and the inflammatory cells, edema, degree of bacterial proliferation were judged by light microscopy, with 4 degrees of a scale system for the severity of pneumonia.

Pneumonia did not occur in newborns after an IT inoculation of GBS in saline (controls, but killed one minute later) and the newborns treated with saline only (except one of 11 in saline group was reported to have less 10% pneumonia), but all 12 newborns in the group treated with GBS in saline had severe pneumonia; 7/12 had > 30% pneumonia. All but one of 11 newborns in the group treated with GBS in saline plus CUR resulted in pneumonia but only one had severe pneumonia. Elastase activity of neutrophils was increased significantly in the GBS treated group without CUR, but it decreased to almost less than half in the group with GBS plus CUR. Thus CUR showed some protective effect.

**20. EFFECT OF CUROSURF AND ANTISERUM TO TYPE 1a GROUP B STREPTOCOCCI ON OXIDATIVE METABOLISM OF NEUTROPHILS:**

COMMENTS : A copy of a one page abstract publication entitled "EFFECT OF SURFACTANT AND SURFACTANT ANTISERUM TO TYPE 1a GROUP B STREPTOCOCCAL (GSB) ON OXIDATIVE METABOLISM OF NEUTROPHILS" was submitted, but it was not reviewed.

**21. EFFECT OF CUROSURF IN EXPERIMENTAL *E. COLI* [PNEUMONIA IN ADULT RATS (abstract only).**

Methods : A total of 116 rats were treated with 0.2 mL/kg standard suspension of *E. coli* ( $4 \times 10^8$  bacteria/mL). After 2 to 3 days, 31 of the infected

animals showed symptoms of respiratory failure. They were intubated, artificially ventilated, and divided into 3 groups : one non treated, one treated with 2 mL/kg of normal saline, and one treated with 2 mL/kg CUR (160 mg/kg in 80 mg/mL CUR). Ten healthy animals served as the controls.

#### RESULTS :

- a. PaCO<sub>2</sub> and pH values of the blood samples were similar in all groups.
- b. Average PO<sub>2</sub> at 30 minutes was increased 76% ( $p < 0.01$ ) in CUR treated group over the pretreatment value, but it was not improved in other groups.
- c. The left lung weight/body weight ratio increased 3 fold in infected animals when compared to normal controls.
- d. The protein content of lung lavage fluid also increased 3 fold in infected rats when compared to normal controls, but the values of the total phospholipids and PC content were unchanged in animals not receiving CUR.
- e. Histopathology showed a wide-spread nonspecific pneumonia in infected animals, but alveolar expansion showed no difference between CUR treated and no treated animals.

#### SUMMARY OF NEW STUDIES NOT INCLUDED IN THE EVALUATION :

Administration of 200 mg/kg CUR did not improve the survival rate of near-term newborn rabbits with meconium aspiration, and it actually decreased the survival in high dose meconium aspirated newborn, in spite of the fact that CUR improved lung thorax compliance statistically significantly in newborn rabbits and adult rats. Also, CUR administration did not have any protective effect on the heart since abnormal EKGs were reported in 3 of 16 rabbits. Therefore, CUR did not improve the survival in meconium aspiration.

In a study with near term newborn rabbits inoculated with GBS with/without CUR, CUR was shown to produce significant decreases in incidence and severity of pneumonia and release of elastase and inflammatory cells (PMN) in the lung.

In an another study, 2 mL/kg CUR (160 mg/kg in 80 mg/mL CUR) significantly improved oxygenation of E. coli infected adult rats, but it did not improve lung thorax compliance nor alveolar expansion.

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*K. Choi*

MAR 10 1995

DIVISION OF ONCOLOGY AND PULMONARY DRUG PRODUCTS  
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY  
Addendum to the Original Review

IND : \_\_\_\_\_ Reviewer : Young S. Choi, Ph.D.

SUBMISSION : Date Originated : 5/4/93  
Date FDA Received : 5/5/93  
Date Assignment Received : 5/12/93  
Date Review Completed : 4/18/94

INFORMATION TO BE CONVEYED TO SPONSOR : Yes ( ), No (x).

SPONSOR : Chiesi Pharmaceuticals, Inc.  
150 Danbury Road  
Ridgefield, CT 06877

For preclinical information, call Dr. Andrew Snoddy at (201) 777-2800.

MANUFACTURER : Chiesi Farmaceutici S.p.A. in Parma, Italy  
U.S. agent : Dr. Hubert Loncin, M.D.

NAME OF DRUG : CUROSURF<sup>(R)</sup> (CUR)

CATEGORY : Pulmonary surfactant from pig lung.

ROUTE OF ADMINISTRATION : Intratracheally via endotracheal tube : one half of dose in the right main bronchus and one half in the left main bronchus.

CLINICAL INDICATION : To treat respiratory distress syndrome (RDS) in premature babies.

DOSE : CUR : 200 mg/kg (= 2.5 mL/kg) in 2 divided doses : 100 mg/kg (= 1.25 mL/kg) may be repeated at 12 hours and 24 hours after the first dose of 200 mg/kg if the baby is still intubated. Therefore, a total maximal dose of 400 mg/kg or 5 mL/kg.

Survanta: 100 mg/kg (= 4.0 mL/kg) in 4 divided doses, and the same dose of 100 mg/kg may be repeated every 6 hours up to a total of 4 doses. Therefore, a total maximal dose of 400 mg/kg or 16 mL/kg-in-24 hour period.

DOSAGE FORM : Suspension in normal saline at a phospholipids (PL) concentration of 80 mg/mL, supplied in a 5 mL, Type I glass vial containing either 1.5 mL (= 120 mg of PL) or 3.0 mL (= 240 mg PL) of CUR.

**CLINICAL STUDIES : Phase 3 studies :**

**"A DOUBLE-BLIND, MULTICENTER COMPARISON OF CUROSURF AND SURVANTA IN THE TREATMENT OF NEONATAL RESPIRATORY DISTRESS SYNDROME "**

Multicenter, randomized, double-blind, Phase 3 clinical studies : the study is a 28 day study to compare the safety and efficacy of two natural pulmonary surfactants, Curosurf (CUR) and Survanta (SUR).

CUR will be given at an initial dose of **200 mg/kg (= 2.5 mL/kg)** in 2 divided doses, each portion administered in each main bronchus, no later than 2 hours after entry into the study, then **100 mg/kg (= 1.25 mL/kg)** may be repeated at 12 and 24 hours after the first dose if the baby is still intubated and meets specified criteria. Therefore, a total maximal dose is **400 mg/kg** or **5 mL/kg** per infant.

SUR will be given according to marketing instructions : **100 mg/kg (= 4.0 mL/kg)** in 4 divided doses, administered into the lower trachea, and the same dose of 100 mg/kg may be repeated every 6 hours up to a total of 4 doses, if needed and meets certain criteria. Therefore, a total maximal dose is **400 mg/kg** or **16 mL/kg** per infant in 24 hour period.

**PRECLINICAL DATA SUBMITTED/REVIEWED :**

Sponsor submitted the following preclinical studies, in addition to Pharmacological and Pharmacokinetic studies :

1. ACUTE INTRAPERITONEAL TOXICITY STUDY IN MICE :
2. ACUTE INTRAPERITONEAL TOXICITY STUDY IN MICE :
3. ACUTE INTRAPERITONEAL TOXICITY STUDY IN RATS:
4. ACUTE INTRAPERITONEAL TOXICITY STUDY IN RATS:
5. ACUTE INTRATRACHEAL TOXICITY STUDY IN RATS :
6. ACUTE INTRATRACHEAL TOXICITY STUDY IN GUINEA PIGS :

7. ACUTE INTRATRACHEAL TOXICITY STUDY IN RABBITS :
8. ACUTE INTRATRACHEAL TOXICITY STUDY IN DOGS:
9. 2-WEEK INTRATRACHEAL TOXICITY STUDY IN RATS :
10. 2-WEEK INTRATRACHEAL TOXICITY STUDY IN RABBITS :
11. 2-WEEK INTRATRACHEAL TOXICITY STUDY IN DOGS :
12. 4-WEEK INTRAPERITONEAL TOXICITY STUDIES IN RATS FOLLOWED BY A 4 WEEK RECOVERY PERIOD :
13. REVERSE MUTATION
14. GENE MUTATION IN CHINESE HAMSTER V79 CELLS :
15. CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS IN VITRO :
16. UNSCHEDULED DNA SYNTHESIS IN HELA S3 CELLS IN VITRO :
17. MICRONUCLEUS TEST :
18. ACTIVE ANAPHYLAXIS IN THE GUINEA PIGS (SENSITIZE AND CHALLENGE INTRATRACHEALLY):
19. ACTIVE ANAPHYLAXIS IN THE GUINEA PIGS (SENSITIZE INTRAPERITONEALLY AND CHALLENGE INTRAVENOUSLY):
20. ACTIVE ANAPHYLAXIS IN THE GUINEA PIGS (SENSITIZE SUBCUTANEOUSLY AND CHALLENGE INTRAVENOUSLY):
21. PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) IN THE GUINEA PIGS :
22. SENSITIZATION TEST IN THE GUINEA PIG:

**REVIEW OF PRECLINICAL DATA :**

**GLP STATEMENTS :** Yes, submitted on page 028, Vol. 1.4 as well as with each study including each mutagenic studies.

**FOREIGN ANIMAL TOXICITY STUDIES :** Yes, most studies were performed either at .....  
 ..... Some acute

toxicity studies and a 2 week study were done at \_\_\_\_\_

**NOTE :** This submission was so poorly organized that the studies were rearranged in an attempt to facilitate evaluation.

**I. PHYSIOLOGICAL/PHARMACOLOGICAL/PHARMACODYNAMIC EFFECTS (EFFICACY STUDIES) OF CUROSURF:**

1. In- Vitro Pulsating Bubble Test and Wilhelmy Balance System : DPPC concentrations of Curosurf (CUR) greater than 5 mg/mL were adsorbed instantaneously (< 1 second) to an air-liquid interface, generating a surface film that reduced surface tension during compression.
2. Static Pressure-Volume (P/V) Recording in Premature Rabbit Pups (day 27 gestation): The changes in P/V in non-ventilated rabbits showed dramatic enhancement of lung expansion and good stability during deflation of the lungs.
3. Premature Newborn Rabbits : 12.5 to 400 mg/kg CUR administered intratracheally (IT) in rabbit fetuses with artificial ventilation improved tidal volumes in a dose-related manner over the saline control as well as survival rate of these fetuses.
4. Premature Rabbit Neonates : Improvement of lung mechanics was dependent on the concentrations of phospholipid (40-80 mg/mL); the higher the more effective.
5. Premature Rabbit Neonates : Dramatic improvement of lung mechanics was also produced under conditions of standardized insufflation pressure ventilation and resulted in significant improvement of the survival rate over untreated pups (96% vs 37%). Comparison of tidal volume changes by CUR and Exosurf (EXO).
6. Under conditions of standardized tidal volume (10 mL/kg) ventilation, high values for lung-thorax compliance were associated with an improvement of alveolar gas exchange, and the histological examination of the lungs from CUR treated animals showed a nearly uniform pattern with well aerated terminal air spaces and well preserved epithelium in conducting airways :
7. Experimental Model of Adult Respiratory Distress Syndrome (ARDS), guinea pig depleted of endogenous surfactant: CUR restored pulmonary gas exchange, preventing intra-alveolar edema and respiratory epithelial damage.

**II. PHARMACOKINETIC STUDIES :**

**Treatment:** A single dose of 200 mg/kg <sup>14</sup>C-DPPC intratracheally (IT) in Newborn

remain immobile, starting at 10 and 20 minutes post dosing, with "palpebral" ptosis. Slight piloerection was noted in 2,000 mg/kg group. Mice in 1,000 mg/kg group were fully recovered at 60-120 minutes post dosing, and 2,000 mg/kg group recovered at 180-360 minutes post dosing.

**Body Weight :** No significant differences between groups for weekly body weight were reported.

**Necropsy and Histopathology of Lungs and Tracheas :** No abnormality was reported.

2. **ACUTE IP TOXICITY STUDY IN MICE :** Study No. 047-051A :  
Report No. 047-051A/T/042/89 :

**Lab. Performing the Study :**

**Methods :** 5 M + 5 F of Swiss mice [CD-1(ICR)BR, mean body weight of 28 g for M and 23 g for F, no age was given] were administered a single dose of 2,000 mg/kg CUR (Lot no. P812050), after 18 hours fasting, then they were observed for 14 days. After necropsy, histopathology was performed on the injection sites of all mice. For details, see pages 154-156, Vol. 1.4.

**RESULTS :**

**Mortality :** None.

**Clinical Observations :** All mice showed hypoactivity and piloerection on the day of dosing, and they returned to normal by the 3rd day.

**Body Weight :** All mice gained weight during 14 day observation period.

**Necropsy Findings :** Urinary bladder of one M (No.18) had pale firm material and right ovary of one F (No.13) had cyst filled with red fluid.

**Histopathology of Injection Sites :** The injection sites in the skin of 2 M (#12 and 14) had marked cystic dilatation of preputial glands with minimal hyperplasia, and No.12 also had a keratin cyst rated minimal.

**Mortality :** None.

**Clinical Signs :** Signs of toxicity were **soft feces and piloerection** noted in all 10 rats within one hour after dosing, but they were normal the next day. **Hair loss** was reported in all 5 F beginning on day 7.

**Body Weight :** Weight gain was similar for 2 week period.

**Necropsy :** Two F (11 and 13) had minimal hair loss on the neck and head, and one F (17) had a dorsal scab.

**Histopathology :** Four of 5 M had minimal **hyperplasia of the mammary glands at the injection site**. This was **not** considered to be unusual pathology for M rats of this age. The other M had a minimally **hyperplastic preputial gland** with secretory activity, but was considered normal. The injection sites of F were normal, but there was one foci of epidermal hyperplasia. The hair loss or scab area of 2 F had minimal hyperkeratinization and epidermal hyperplasia.

## 5. ACUTE INTRATRACHEAL (IT) TOXICITY STUDY IN RATS :

### Lab. Performing Toxicity Studies:

**Methods :** 5 M + 5 F/group of Sprague-Dawley rats (95-130 g, 4-5 weeks old) were administered 2.5 mL/kg of 37°C CUR (200 mg/kg, no Lot no. was given) intratracheally by means of a chronically (3 days ahead) implanted catheter, over 5-8 minutes by infusion pump, along with a sham dosed control with catheter and a normal control group without catheter.

24 hours later, the catheter was removed under anesthesia and sterile conditions, and animals were observed for 14 days and necropsied after CO<sub>2</sub>. Histopathology was done only for lungs and tracheas of all animals. For detailed methodology, see pages 96-100, Vol. 1.4.

### RESULTS :

**Mortality :** None in all 3 groups. .

**Clinical Signs :** 3/10 in sham control group had debility, wheezing, and red crusts around the nostrils, and these disappeared on day 3.

10/10 in CUR group had red crust around the nostrils, still present in 7/10 on day 2 and disappeared on day 3.

8/10 in CUR group also had sneezing during the administration of CUR and emitted "very modest quantity" of foamy liquid, sometimes reddish, from the nostrils. 2 more in this group showed symptoms of suffocation (dyspnea, wheezing, agitation, gasping) during administration and decreased within 15-20 minutes.

**Body Weight :** No significant differences between groups.

**Gross Pathology/Histopathology (Lungs and Tracheas) :** No differences between groups.

## 6. ACUTE INTRATRACHEAL TOXICITY STUDY IN THE GUINEA PIGS :

### Lab. Performing Toxicity Studies:

**Methods :** 5 M + 5 F/group of Dunkin-Hartley guinea pigs (210-270 g, no age was given) were treated with 2.5 mL/kg of 37°C CUR (200 mg/kg, no Lot no. was given) intratracheally by means of a chronically (4 days ahead) implanted catheter, over 5-8 minutes with infusion pump, along with a sham dosed control with catheter.

24 hours later, the catheter was removed under anesthesia and sterile conditions, and animals were observed for 14 days and necropsied following CO<sub>2</sub>. Histopathology was performed only for lungs and trachea of all animals. For detailed methodology, see pages 108-111, Vol. 1.4.

### RESULTS :

**Mortality :** None in either group.

**Clinical Signs :** During CUR administration, all animals emitted a small quantity of foamy liquid from the nostrils and exhibited transitory suffocation (dyspnea, agitation, shaking, urination), and 3 fell on one side and gasped during the final phase of administration; they returned to normal 30-60 minutes later.

Animals in sham control became agitated in the final phase of administration. Animals in both CUR and sham control groups showed debility, wheezing, and red crusts around nose, which were attributed to the presence of catheter.

**Body Weight :** No difference between groups.

**Gross Pathology/Histopathology :** No difference between groups.

7. **ACUTE INTRATRACHEAL TOXICITY STUDY IN RABBITS:** Study  
**No. 047-053 : Report No. 047-053/T/048/89 :**

**Lab. Performing the Study :** .....

**GLP Statements :** Yes, submitted on page 064, Vol. 4.

**Methods :** 5 M + 5 F/group of New Zealand white rabbits (no age or body weight was given) were injected with a single dose of 2.5 mL/kg CUR (=200 mg/kg, Lot No.p812050, 80 mg/mL) over 10 seconds into the lungs via endotracheal tube, followed by 5 cc of air, along with a sham dosed control group, and the animals were observed for 14 days before necropsy. Histopathology of the respiratory tract was performed. For details, see pages 070-072, Vol.1.4.

**RESULTS :**

**Mortality :** None.

**Clinical Observations :** No drug-related abnormality was reported.

**Body Weight:** Group mean values on days 0, 7 and 14 showed no significant differences between the 2 groups.

**Gross Pathology :** No treatment related findings were reported.

**Histopathology :** No treatment related changes were reported.

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8. ACUTE INTRATRACHEAL TOXICITY STUDY IN DOGS: Study  
No. 047-054 : Report No. 047-054/T/055/89 :

Lab. Performing the Study :

GLP Statements : Yes, submitted on page 033, Vol. 4.

Methods : 3 M + 3 F/group of Beagle dogs (16 weeks old and 5-5.9 kg) received a single dose of 2.5 mL/kg CUR (=200 mg/kg, Lot no. 051233/P812050, 80 mg/mL) over 4-5 minutes into the lungs via endotracheal tube, along with a sham dosed control group, and the dogs were observed for 14 days before necropsy. Histopathology of the respiratory tract was performed. For details, see pages 039-042, Vol.1.4.

RESULTS :

Clinical Observations : No abnormality was reported. See Table 3, on pages 47-48.

Food Consumption: Weekly group mean value showed no toxic drug effects.

Body Weight: Group mean values on days 0, 3, 7, 10 and 14 showed no significant differences between groups.

Physical Examination : Veterinary examination on days minus 1 and plus 13 were unremarkable.

Gross Pathology : No treatment related findings were reported.

Histopathology : No drug-related changes were reported. Minimal to moderate pneumonitis and lymphocytic infiltration were reported for most of the dogs in both groups.

Summary of All Acute Toxicity Studies :

Total of 8 acute toxicity studies were performed on 5 M + 5 F animals per dose : 2 intraperitoneal (IP) studies each in mice and rats and 4 intratracheal (IT) studies in rats, guinea pigs, rabbits and dogs.

After a single IP dose of 500, 1000, or 2000 mg/kg CUR in a mouse study or a single 2000 mg/kg CUR in mice and rats, the animals were observed for 14 days. Only clinical signs, body weight, gross pathology and histopathology of injection sites were observed/examined. For all 4 IT studies, a single dose of 200 mg/kg CUR dose was administered via implanted catheter into trachea, along with a sham control

with catheter. Rat study also had an untreated control group.

There were no deaths in any studies, and body weight showed no treatment effect in all 8 studies. Toxic clinical signs in IP studies were hypoactivity (mice), piloerection (mice and rats), ptosis (mice), soft feces and hair loss (both for rats). IT studies reported wheezing, red crust around nostrils, foamy fluid with/without red color around nostrils, dyspnea, gasping, all reported in rats and guineas; urination (guinea pigs only). These signs tended to be slightly greater in numbers in CUR group than Sham group in both rats and guinea pigs.

Some histopathology findings were reported in IP studies in mice and rats, but they were considered "normal" for the age. Focal tracheitis and some subacute inflammation reported from lung histopathology in IT studies in rats and guinea pigs were attributed to the presence of chronically (3 days) implanted endotracheal catheter.

A single dose of IT 200 mg/kg CUR did not produce any significant toxic signs or effects on body weight nor any gross or histopathology in the lungs or tracheas in rabbit or dog study. Therefore, 200 mg/kg was the NOEL in both species under the conditions studied.

## 9. 2-WEEK INTRATRACHEAL TOXICITY STUDIES IN RATS :

### Lab. Performing Toxicity Studies:

**Methods :** 8 M + 8 F/group of Sprague-Dawley rats (approximately 88-106 g, 4-5 weeks old) were administered CUR into trachea via chronically implanted endotracheal catheter at 200 mg/kg (2.5 mL/kg, Batch No. December 86, 80 mg/mL, for composition, see page 026, Vol. 1.5) over 5-8 minutes daily for 14 days, along with a untreated control group and a sham control group with the insertion of the catheter and 2.5 mL/kg air administration. For detailed methodology, see pages 005-013, Vol. 1.5.

### Measurements and Observations :

Clinical Signs : daily.

Body Weight and Food consumption : daily.

Blood Collection : from aorta after overnight fasting and after sacrifice.

Urinalysis : 16 hour period prior to sacrifice.

Necropsy and organ weights : days 15 and 16.

Study Duration : June 15 through 28, 1987.

### RESULTS :

**Mortality :** Total of 9 died : 2 M + 2 F in sham control group and 2 M + 3 F in the CUR group.

All deaths in sham group and one in CUR group were due to pneumonia, and rest in CUR group were due to suffocation as result of relatively large volume of moderately viscous liquid in the lungs.

**Clinical Observations :** In 30-40% of rats, the presence of implanted catheter in the trachea of sham control group produced clinical abnormalities : slight debility, ataxia and signs of "pneumonia like" labored breathing, red crusts around nostrils, and signs of stress as chromodacryorrea.

CUR induced transient **dyspnea** immediately after the treatment in 20-40% of rats, with gasping, agitation, and asphyxia like movements during the treatment. There were "limited cases" of asphyxia like convulsions that quickly led to **death** or induced **prostration, dyspnea and ataxia** during 2 hours after the treatment. Primarily at the end of treatment, 10-60% of rats discharged a small amount of **foamy liquid**, sometimes reddish, from nostrils.

**Body Weight :** Chronic implanted catheterization itself in sham control produced moderate but significant **decreases** in weight **gain** on day 1 compared to untreated control group. The difference in total body weight gain between sham and CUR groups was increased with time, and became statistically significant ( $p < 0.01$ ) on day 4. The group mean body weight (Table 22 on page 051) of sham treated M at the **necropsy** was **14%** less than control M and CUR group M was **27%** less than control M.

**COMMENT :** Due to technical difficulty, **sham** treated group was designated as 1 mg/kg in all of tables.

**Food Consumption :** Chronic catheterization itself produced moderate but some significant decreases in food consumption in sham and CUR groups compared to untreated control, but the decreases were not well correlated with depression of body weight gain.

**Hematology :** Chronic implanted catheterization itself, despite the precautions taken, produced **inflammation** in a number of rats, that resulted in moderate but significant **leukocytosis** in sham as well as CUR group when compared to untreated control group. The **increases** of WBCs were CUR > Sham > untreated, especially in F. The increases of **neutrophils** were also CUR > sham > untreated in M; but in F, sham was slightly

>CUR, although both values were significantly greater than untreated control values. Group mean lymphocyte values in sham and CUR groups were smaller than untreated control values, significantly ( $p < 0.05$ ) smaller in F (sham > CUR), although S.D.s were relatively large.

Coagulation Time : Not done.

Clinical Chemistry : No CUR-induced changes were reported.

Urinalysis : No treatment induced changes were reported.

Organ Weight : Due to decreased body weight in 2 treated groups over the untreated control group at necropsy, many organs showed decreased absolute weights (AW), but significant treatment-related changes were reported for the lungs and thymus, and possibly spleen for F, but all AW only.

	SHAM		CUR	
	M:	F:	M:	F:
Body weight	↓14%	↓19%	↓27%	↓25%
Lungs (AW)	↑27%**	↑23%**	↑43%**	↑42%**
(RW)	↑47%	↑52%	↑96%	↑91%
Spleen (AW)		↓19%*		↓22%*
(RW)				
Thymus (AW)	↓24%**	↓19%**	↓29%**	↓30%**
(RW)	↓11%	≈	↓3%	↓7%

\*\* :  $p < 0.01$ , \* :  $p < 0.05$ .

Some significant values were reported for other organs, but changes for AW and RW were opposite directions.

COMMENT : The changes in all thymus weights and nearly all lung weights were attributed to stress (thymus atrophy) and pneumonia (lungs) caused by the presence of catheter. Varying degrees of severity of pneumonia resulted in focal consolidation of the parenchyma that was responsible for organ weight increase, and slightly more severe in CUR group than sham group.

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Page 15

**Gross Pathology :** Findings in the respiratory tract were attributed to the presence of a catheter. Findings in CUR group were slightly more than sham control group suggesting CUR may have added effects, although none of the values were S.S as shown in Table below :

Findings	Group	Sham	CUR
Salivary glands :	congestion and abscess in the area adjacent to the catheter	33%	27%
Lungs:			
	Moderate multifocal consolidation:	42%	54%
	Fibrinoid mucus in bronchial tracts:	58%	73%
Trachea :	Fibrinoid mucus :	50%	73%
	Inflammation radiated from area of catheter:	58%	73%

*None of these is significant*  
3/1/95  
/S/

**Histopathology :** Histopathological examination confirmed the necropsy findings. Essentially inflammatory reactions were noted in the lungs of both sham and CUR groups. CUR had a slight but not significantly increased incidence of alveolar histiocytosis. Findings in the pulmonary system are summarized in a table below :

DESCRIPTION	PERCENTAGE OF SURVIVING ANIMALS AFFECTED		
	NORMAL CONTROL	SHAM CONTROL	CURSORF
Alveolar histiocytosis	19	42	63
Atelectasis	6	67	63
Interstitial subacute inflammation	0	25	36
Perivascular lymphoid hyperplasia	13	100	100
Congestion	6	100	100
Peribronchial lymphocytic aggregates	13	92	100
Granulomatous inflammation - focal and multifocal	19	42	55
Empyema	0	17	18

*None of these appear to be drug related*  
3/1/95  
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Additional findings reported as foreign body reaction are as follows :

Findings	Groups	Sham	CUR
Subacute tracheitis		58%	72%
Cystic dilation of the submucosal glands		67%	81%

COMMENT: All these findings were attributed to the presence of catheter. But the above data suggest a trend of CUR producing slightly greater reactions than the sham control group, but none of the values were S.S.

*None of these are related to CUR*  
*15/11/90*

## Summary of 2 Week Intratracheal Toxicity Study in Rats:

Group of 8 M + 8 F/group rats were treated with 2.5 mL/kg (200 mg/kg) of CUR intratracheally via chronically implanted catheter for 14 days, along with a group of sham (air) treated control and an untreated control group.

Deaths resulted in 4 of sham (50% mortality) and 5 in CUR (63% mortality) group, and the deaths were attributed to pneumonia by the sponsor. 4/5 deaths in CUR were during administration and attributed to suffocation caused by large volume of CUR.

Many of findings were attributed to the presence of chronically implanted catheters in both sham and CUR treated groups. Significant depression of body weight gain was reported for both groups, but the difference between the two groups became significant ( $p < 0.05$ ) from day 4 to the end of the study on day 15 at necropsy ( $\downarrow 14\%$  vs  $\downarrow 27\%$  in CUR). In addition to  $\downarrow$  body weight gain, catheter attributed findings were  $\downarrow$  food consumption,  $\uparrow$  WBC,  $\uparrow$  neutrophils with  $\downarrow$  lymphocytes, organ weight changes ( $\uparrow$  lung and  $\downarrow$  thymus and spleen in F, all 3 for AW only), and inflammatory gross pathological changes as well as corresponding histopathological changes in the lungs at the sites of catheter or surrounding areas, as shown on previous 2 pages. Marginal increase in alveolar histiocytosis in the lungs was reported for CUR.

However, almost of all of these changes showed a slight trend of increases in CUR group than in the sham control group, although none of the changes were S.S.

## 10. 2-WEEK INTRATRACHEAL TOXICITY STUDIES IN RABBITS : Report Reference No. 9/90 : - -

Lab. Performing the Study :

*not body weight!*  
*subjective criteria*  
*what was*  
*about*  
*15/11/90*

**Methods :** A group of 5 M + 5 F New Zealand white rabbits (1.6-1.9 kg but exact age was not given) were treated with 1.25 mL/kg (100 mg/kg) CUR (warmed to 35°C, Batch No. 907018, 80 mg/mL) intratracheally via enterotracheal tube under anesthesia by IV Propofol (10 mg/mL, 1 mL/kg), once daily for 14 days. The second group was "sham" dosed (administration of anesthetic and insertion of intratracheal tube) and served as a control. Usual toxicity parameters were obtained before killing with iv pentobarbitone overdose. For detailed methodology, see pages 323-, Vol. 1.4.

**Measurements and Observations :**

Mortality and morbidity : twice daily.  
Clinical Signs : during dosing and during recovery from anesthetic.  
Body Weight and Food consumption : weekly.  
Ophthalmology : pretest and during week 2.  
Blood Collection : pretest and week 2 after overnight fasting.  
Urinalysis : prior to sacrifice when possible from bladder.  
Necropsy and organ weights : days 14 and 15.  
Study Duration : November 14 through 28, 1989.

**RESULTS :**

**Mortality :** Excluding one M (10M) that died after CUR and replaced on day 1, one control and 3 CUR treated rabbits died during the study : control M on day 8, probably due to over dose of anesthetics, and 2 M (one replacement) and one F died during CUR dosing on days 3, 5 and 6, respectively.

No cause of death was identified for one control M and one F from CUR group, but "lung changes" were considered to be the cause of death for 2 M in CUR group (No.s 6 and 100). See page 358, Vol. 1.4 for pathologist's report.

**Clinical Observations :** All in the control group coughed during insertion of the cannula. All rabbits in CUR group coughed and sneezed during dosing, and 2 rabbits in this group also had abnormal respiratory noises.

**Body Weight :** Group mean total body weight gain in CUR group was same as the control group.

**Food Consumption :** No significant effect was noted, although the treated F rabbits ate less than the control F, it was reported that the control F ate an unusually large amount of food.

**Ophthalmology :** No drug-induced abnormalities were reported.

- Hematology :** Group mean values of **WBC** and **lymphocytes** in M and F from CUR group were slightly increased, but group mean values of **neutrophils** were decreased over the respective control values although S.D.s were large and no drug-related changes were reported by the sponsor.
- Coagulation Time :** Group mean PT values showed no difference between groups, but group mean APTT values in M and F from CUR group were larger than the respective control, although S.D.s were very large.
- Clinical Chemistry :** No drug-related or dose-related changes.
- Urinalysis :** Urine composition and cellularity showed no difference between groups.
- Organ Weight :** AW and RW of the **lungs** in M from CUR group were significantly ( $\uparrow > 80\%$ ,  $p < 0.05$ ) increased over the M control value, although S.D.s were large.
- Gross Pathology :** Treatment-related finding was the **occurrence of lungs which failed to collapse** on opening the thorax in 2 M (No. 6 and 100) that received 100 mg/kg CUR and died during the study.
- Histopathology :** CUR treated group had **increased incidence and degree** of alveolar macrophage aggregation and the occurrence of alveolar edema and pneumonia over the control group, although inflammatory changes were noted in both CUR and control groups.

**Summary of 2 Week Intratracheal Toxicity Study in Rabbits :**

100 mg/kg/day CUR for 14 days produced 3 deaths during the study, and two of 3 deaths were due to CUR administration : CUR caused sneezing as well as abnormal sounds in the lungs, histological changes of the lungs, and failure to collapse upon opening the thorax. The histological changes were increased incidence and degree of alveolar macrophage aggregation, alveolar edema, and pneumonia, along with significantly ( $p < 0.05$ ) increased lung (A+R) weights in treated M. No drug-related changes were reported for body weight, food consumption, ophthalmology and blood chemistry except some minor changes for WBC, lymphocytes, and neutrophils.

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11. 2-WEEK INTRATRACHEAL TOXICITY STUDIES IN DOGS : Toxicol Report  
Reference No. \_\_\_\_\_ /10/89 :

Lab. Performing the Study :

**Methods :** It was planned to treat a group of 4 M + 4 F pure bred beagle dogs (6.1-8.2 kg and 19-21 weeks of age) with 2.5 mL/kg (200 mg/kg) CUR warmed to 35°C (Batch No. 907018, 80 mg/mL) intratracheally via intratracheal tube under anesthesia by IV Propofol (10 mg/mL, 1 mL/kg).

The intention was to dose **once daily** for 14 days. However, the first dog receiving this dose on day 1 had to be killed in extremis, and the second dog after receiving only 1.25 mL/kg (100 mg/kg) showed **severe clinical signs**, thus the remaining dogs received only 0.625 mL/kg (50 mg/kg) on day 1 and then onward. The second group was "sham" dosed (administration of anesthetic and insertion of intratracheal tube) and served as a control. Usual toxicity parameters were obtained before sacrificing the dogs with iv pentobarbitone

For detailed methodology, see pages 198-205, Vol. 1.4.

**Measurements and Observations :**

Mortality and morbidity : twice daily.

Clinical Signs : during dosing and during recovery from anesthetic.

Body Weight : weekly.

Food consumption : daily, but reported weekly.

Ophthalmology : pretest and during week 2.

Blood Collection : pretest and week 2 after overnight fasting.

Urinalysis : overnight during the fasting.

Necropsy and organ weights : days 15 and 16.

Study Duration : August and September, 1989.

**RESULTS :**

**Mortality :** It was the intent to dose with 2.5 mL/kg. But when only 16 out of 22 mL were administered to the first dog (989M), an equivalent of 145 mg/kg/day, the dog showed severe signs of **respiratory distress** (the dogs were under anesthesia). **Opisthotonus and tetanic spasms of the forelimbs** were noted and this was followed by signs of cerebral anoxia, although there was an apparent recovery period. Therefore, the dog was killed in extremis. The signs of toxicity were attributed to excessive volume of CUR.

**Clinical Observations :** There were no clinical signs considered to have been caused by a toxic effect of CUR.

Due to the finding in the first dog, the second dog (991M) was given ½ the dose, or 1.25 mL/kg (9.4 mL total), and this also provoked signs of respiratory distress for 13 minutes immediately after dosing.

Beginning with the third dog, only 0.65 mL/kg (3.7-6 mL) was administered, but it still provoked signs of respiratory distress in most dogs.

**COMMENT :** This is the first dog study we have received for any of the surfactants, and dogs seem to be extremely sensitive to volume effects and/or viscosity.

**Body Weight :** No significant effect on weekly body weight gain.

**Food Consumption :** No significant effect on weekly food consumption (g/dog/week).

**Ophthalmology :** No treatment-related changes.

**Hematology :** No drug-induced significant changes.

**Coagulation Time :** No drug-related consistent changes for PT and APTT.

**Clinical Chemistry :** No treatment-related changes.

**Urinalysis :** Urine composition and cellularity showed no drug-related changes.

**Organ Weight :** No treatment-related changes were reported, although group mean heart weights (A:absolute; R:relative) of treated M were 10 and 11% larger than M control values, and the increase for RW in M (11%) was highly significantly ( $p < 0.001$ ) different from controls.

Group mean thyroid weights (AW and RW) in treated M and F were smaller than respective control values, although S.D.s were relatively large for both sexes, and the decrease for AW in F was significantly ( $p < 0.05$ ) different from the control value.

AW and RW of pituitary glands in M and of gonads for both M and F were somewhat larger than respective control values, although S.D.s were large.

**Gross Pathology :** No treatment-related changes in the lungs or other organs and tissues.

**Histopathology :** Although no treatment-related changes in the lungs or other organs and

tissues were reported by the pathologist, the following findings were reported **only** in CUR treated groups in Appendix 14 (pages 242-255, Vol.1.4).

Chronic inflammation of the heart : 1 dog.  
hemorrhage of the heart : 1 dog.  
congestion of the liver : 1 dog.  
hepatocyte vacuolation : 1 dog.  
Lung fibrosis : 2 dogs.  
congested lungs : 2 dogs.  
granuloma of the lungs : 1 dog  
bronchiolitis : 1 dog. See pages 243 and 244, Vol. 1.4.

#### Summary of 14 Day Intratracheal Toxicity Study in Beagle Dogs:

The first dog receiving 2.5 mL/kg on day 1 had to be killed after only 16 mL out of 22 (=145 mg/kg), due to severe convulsions. The second dog after receiving only 1.25 mL/kg (100 mg/kg) also showed severe clinical signs, thus the dose had to be decreased again, and only 0.625 mL/kg (50 mg/kg) was used.

Daily intratracheal administration of 50 mg/kg/day CUR for 14 days in beagle dogs did not produce any significant toxic effects on usual toxicity parameters including histopathology of the lungs, although some findings in the lungs and liver were limited to CUR treated group.

50 mg/kg/day or 0.625 mL/kg of CUR was considered the maximal practical dose for the dog, and 1.25 mL/kg or 100 mg/kg/day and up caused respiratory distress syndrome, mainly due to suffocation by physical means.

#### 12. 4 WEEK IP TOXICITY STUDY IN RATS FOLLOWED BY A 4 WEEK

RECOVERY PERIOD : Study No.: 244-047-063 : Report No.:  
244-047-063/T/051/90 :

Lab Performing the Study :

**Methods :** Three groups of 10 M + 10 F/group of Sprague-Dawley rats (160-177 g, 30-40 days old) were treated with 200, 350, or 600 mg/kg CUR (Batch No. 9001012) by IP injection once daily at 2 sites in rotation, along with a vehicle control receiving saline (same volume as HD) for 4 consecutive weeks. 600 mg/kg and the control had an additional 5 M + 5 F/group for 4 weeks of treatment, followed by a 4 week recovery period. Animals were necropsied after killing them with CO<sub>2</sub>, and selected organs with liver from all rats were included for

histopathology. For detailed information, see pages 130 through 139, Vol. 1.5.

**Measurements and Observations :**

- Mortality, Morbidity, and Clinical Signs : daily
- Body Weight : minus day 7 (day of allocation), then weekly and just before necropsy.
- Food consumption : weekly.
- Ophthalmology : minus day 7 (day of allocation) and during week 4.
- Hematology : during weeks 4 and 5, and week 4 for recovery group after overnight fasting.
- Clinical Chemistry: during weeks 4 and 5, and week 4 for recovery group after overnight fasting.
- Urine : during week 3.
- Necropsy, organ weights : after 4 week treatment and after 4 week recovery periods.
- Study Duration : Dosing started on 2/26/90 and necropsied on 4/26/90.

**RESULTS :**

**Mortality :** No treatment related deaths were reported. Two M in 600 mg/kg group died from accident during bleeding.

**Clinical Observations :** Dose-related **swelling** was reported at the site of injection, mainly after 2 weeks treatment through the end of treatment.

- COMMENTS:**
1. Nothing was mentioned about the injection sites after recovery.
  2. It was stated that Appendix 1 (starting on page 232) contained clinical signs, but it contained data for individual rat only, without any summary table.
  3. Exact page numbers were not given for each result section.

**Body Weight :** Mean body weight gain per day was **depressed** in M and F of 350 and 600 mg/kg groups on days **15 and 22**, significantly ( $p < 0.05$ ) in 350 mg/kg M on day 15 and 600 mg/kg M for both days (page 152) and 350 mg/kg F on day 22 (page 153).

For recovery group, mean body weight gain per day in M of 600 mg/kg group was also **significantly ( $p < 0.05$ ) depressed on day 36** (see page 154), although mean body weight of M and F in 600 mg/kg group was slightly larger than the control values at the end of recovery period, day **57** (pages 150 and 151).

**Food Consumption :** Group mean food consumption of treated groups was

Recovery 600 mg/kg group did not show any significant changes.

COMMENT : It was stated that "the toxicological significance of these findings is unclear." This reviewer agree with the sponsor's statement.

Clinical Chemistry : Mean glucose values in all 3 F treated groups and 600 mg/kg M were increased and the increases were significant at  $p < 0.05$  (13% ↑ in F of 200 mg/kg and 9% ↑ in M and 12% ↑ in F of 600 mg/kg groups), although S.D.s were large.

Mean total protein values were increased in treated M at the end of treatment as well as at the end of recovery period, and the 5% increase in 600 mg/kg group was highly significant ( $p < 0.01$ ) at the end of 4 week treatment.

Mean albumin values in all treated M were decreased significantly (6-9%), while  $\alpha, \beta$ , gamma globulins were increased (10-25%), significantly in most, and thus these resulted in significantly decreased albumin/globulin ratios, although none of changes showed clear dose-relationship. See page 169, Vol. 1.5.

Treated F showed similar direction of changes, although less significant: 600 mg/kg F had significantly decreased albumin (4% ↓) with increased  $\beta$ -globulins (9% ↑) and gamma globulin (11% ↑), and decreased A/G ratio (9% ↓), without dose-relationship.

COMMENT : These changes in serum proteins are reported as probably related to the route of administration and secondary to the inflammatory process noted at injection sites. They probably have no toxicological significance.

F in 600 mg/kg group had small but statistically significant decreases for chloride (3% ↓,  $p < 0.01$ ) and potassium (6% ↓,  $p < 0.05$ ) values. See page 173, Vol. 1.5.

Recovery group had several significant differences/values from the control values, but the only changes which carried over from those reported at the end of treatment are : 13% increased glucose in F, that is similar to that seen at the end of treatment, and 15% decreased potassium in F, that is a 3 fold increase over recovery period. See pages 175-179, vol. 1.5.

Urinalysis : No treatment related changes were reported for urine volume and specific gravity, but the urine volume in treated groups was slightly lower than the control group in both sexes. Hemoglobin in urine was reported in 2 M + 3 F of 600 mg/kg group at week 3. Recovery group showed no consistent changes.

Minimal to moderate **centrilobular hepatocyte vacuolation** was reported for 9/14 M in 600 mg/kg group at the end of treatment (page 227, attributed to metabolism of test material by liver), and also in all M (4/4) of 600 mg/kg group and 2/5 control M at the end of recovery period. See page 230, Vol. 1.5.

Dose-related acute and/or chronic suppurative **inflammation** and **fibrosis** were reported at the injection sites in the treated at the end of treatment.

"Evidence of vacuolar degeneration in the liver was observed in some of the control and in all high dose males sacrificed after recovery". See lines 9-10 from the bottom of page 142, Vol. 1.5. This may be a misstatement since no such finding was reported and only **centrilobular hepatocyte vacuolation** was listed. For report of unscheduled deaths, see page 231, Vol. 1.5.

COMMENT : Summary table (10A, page 226-231, Vol. 1.5) contains data from the control and 600 mg/kg groups, and the liver only for 2 mid dose groups and recovery groups.

#### Summary of 4 Week IP Toxicity Followed by a 4 Week Recovery Study in Rats :

Two groups of 15 M + 15 F/group of rats received 600 mg/kg CUR or saline, and 10 M + 10 F/group of rats received 200 or 350 mg/kg CUR, once daily by IP injection for 4 consecutive weeks, then 10 M + 10 F/group of rats were killed, and 5 M + 5 F/group from 600 mg/kg and control groups were kept for an additional 4 weeks as recovery groups.

CUR produced significant depression of body weight gain from the second week on, especially in M, although food consumption was not affected. M also had **dose-related decreases in Hb** with **dose-related increases in WBCs**, along with increased neutrophils. The Hb and WBC changes in M were also **highly ( $p < 0.01$ ) significant** from the control values. F also had increased WBCs, with significantly increased neutrophils and decreased lymphocytes. Changes in WBCs were due to infection at injection sites. Both **coagulation times (PT and APPT)** in 600 mg/kg M were significantly increased in spite of **platelet increases** for M and F which were marginally increased (dose-related). **Serum proteins and some globulins**, especially in 600 mg/kg M, were increased while albumin were decreased, that led to decreased A/G ratio. These are also due to inflammation at the injection sites. Some other significant changes were noted, but the actual differences were either small (<10%) or limited to only one sex, thus they may have no toxicological significance, but **decreased potassium and increased glucose**, both reported in F treated groups, need to be mentioned since they occurred in **recovery group** as well.

Among the organ weight changes, **liver and spleen** weight changes were most prominent, since the **increases** were dose-related for AW and RW and significant for M (for both organs) and F (liver), especially for 600 mg/kg group, and the changes persisted until end of

No significant cytotoxicity was produced in preliminary assays, with/without activation.

14. GENE MUTATION IN CHINESE HAMSTER V79 CELLS : Report  
No. 047031-M-06388 :

Lab. Performing the Study :

**Method :** 5 concentrations of CUR (Batch July 87) from 650 up to 10,100  $\mu\text{g}/\text{mL}$  were tested for induction of 6-thioguanine resistant mutants in two independent assays in Chinese hamster V79 cells after *in vitro* treatment along with appropriate vehicle (12.5% saline), and positive control groups (ethylmethanesulphonate, 7,12-dimethylbenz(a)anthracene), with/without metabolic activation, with liver S-9 fraction from rats pretreated with phenobarbitone and betanaphthoflavone, with 2-fold dilutions from 10,000  $\mu\text{g}/\text{mL}$  to 625  $\mu\text{g}/\text{mL}$ . Two independent tests were done, after a preliminary cytotoxicity test. For detailed methodology, See Appendix I and pages 009-010, Vol. 1.6.

**RESULTS :** The actual data showed the following :

- a. The positive control groups gave not only dose-related increases in mutant frequencies but also more than 26x increases in mutant frequencies over the respective control values on both days. See a summary Table 16, page 030, Vol. 1.6.
- b. Average mutant frequencies increased at 2 high doses after metabolic activation on both days in Experiment (EXP) II. The largest increase was approximately 2x for 5,000  $\mu\text{g}/\text{mL}$  and approximately 3.5x for 10,000  $\mu\text{g}/\text{mL}$  over the controls after metabolic activation on day 9.
- c. The data from individual plate counts (Table 13 on page 027) for day 6 showed no significant increases for any dose group.
- d. The data on day 9 after metabolic activation in EXP II showed that only one each out of 5 plates had increased mutation : 2x for 5000  $\mu\text{g}/\text{mL}$  and 3x for 10,000  $\mu\text{g}/\text{mL}$  over the controls, with relatively large S.D. If those 2 high values are excluded, the remaining data showed no differences from the control values.

Therefore, CUR did not induce reproducible S.S increases in numbers of mutants or mutation frequencies on days 6 and 9 over the control values at any dose level, with/without metabolic activation. Thus CUR was considered **negative** under the test

condition.

No significant cytotoxicity was produced in preliminary assays, with/without metabolic activation.

CUR did not change pH or osmolarity of test solutions. Slight decreases in survival of cells were produced at 10,000  $\mu\text{g}/\text{mL}$  with/without metabolic activation. See pages 018 and 019, Vol. 1.5.

15. **CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS  
IN VITRO :** Report No. 047030-M-06288 :

Lab. Performing the Study :

**Method :** 8 concentrations of CUR (Batch July 87) from 46.4 up to 10,000  $\mu\text{g}/\text{mL}$  were tested for induction of chromosomal damage in Chinese hamster ovary cells following *in vitro* treatment, along with appropriate vehicle (physiological saline), and positive control groups (mitomycin C, 0.1  $\mu\text{g}/\text{mL}$  and cyclophosphamide, 6.6  $\mu\text{g}/\text{mL}$  with S-9), with/without metabolic activation, with liver S-9 fraction from rats pretreated with phenobarbitone and betanaphthoflavone, with 2-fold dilutions from 10,000  $\mu\text{g}/\text{mL}$  to 625  $\mu\text{g}/\text{mL}$ . Two independent tests were done, after scoring chromosomal aberrations from a preliminary test. **One hundred metaphase spreads were scored for chromosomal aberrations from each culture at each dose.** Without S-9 activation, 1000, 2150, 4640  $\mu\text{g}/\text{mL}$  CUR were chosen, and the cells were treated for an entire cell cycle and were harvested after 24 hours. With S-9, 2150, 4640, and 10,000  $\mu\text{g}/\text{mL}$  CUR were chosen, and the cells were treated 3 hours but harvested at 12 and 24 hours. Air dried slides were prepared and stained with 3% Gimsa. Two cell cultures were prepared at each test points. For detailed methodology, See Appendix I and pages 070-071, Vol. 1.6.

**RESULTS :** CUR did not increase significantly the numbers of cells bearing chromosomal aberrations, with/without metabolic activation, while the positive controls produced significant increases in frequency of aberrant cells. For actual data, see a summary table (10, page 087) and pages 082-086, Vol. 1.6. For mitotic index, see Tables 1-3 on pages 077-079, Vol.1.6.

16. **UNSCHEDULED DNA SYNTHESIS IN HELA S3 CELLS IN VITRO :**  
Report No. 047032-M-06488 :

Methods : 8 groups 10 M Dunkin Hartley guinea pigs ( $496 \pm 62$  g) were treated IP with saline or CUR (Batch no.s: 904010 and 904009, groups 3 and 4) or ovalbumin, once daily, 3 times on alternate days, and then 21 days later, they were challenged IV as following table shows. Animals were observed at 10 minute intervals for one hour, then hourly intervals for 3 hours, then they were killed by cervical dislocation or overdose of sodium pentobarbitone. For detailed methodology, see pages 259-261 and 266, Vol. 1.6. Treatment started on 3/26/90 and ended on 4/21/90.

Group number	Animal numbers	Sensitization Treatment (i.p.)	Challenge Treatment (i.v.)
1	101 - 110	Saline	Saline
2	111 - 120	Saline	100mg/kg Curosurf
3	121 - 130	100mg/kg Curosurf	100mg/kg Curosurf
4	131 - 140	50mg/kg Curosurf	50mg/kg Curosurf
5	141 - 150	25mg/kg Curosurf	25mg/kg Curosurf
6	151 - 160	Ovalbumin (*) 1.1mg/kg	Ovalbumin (*) 1.1mg/kg
7	161 - 170	Ovalbumin (**) 0.55mg/kg	Ovalbumin (**) 0.55mg/kg
8	171 - 180	Ovalbumin (***) 0.275mg/kg	Ovalbumin (***) 0.275mg/kg

Curosurf batches 904009 and 904010 contain 1.1% proteins.

- \* amount per kg corresponding to protein content of 100mg/kg Curosurf
- \*\* amount per kg corresponding to protein content in 50mg/kg Curosurf
- \*\*\* amount per kg corresponding to protein content in 25mg/kg Curosurf

RESULTS : No anaphylaxis was produced in CUR treated groups, while the positive control group produced extreme anaphylaxis.

20. **ACTIVE ANAPHYLAXIS IN GUINEA PIGS (Sensitized by SC and Challenged by IV): Toxicological Report Reference : /21/PH :**

Lab Performing the Study :

Methods : 4 groups 10 M Dunkin Hartley guinea pigs (533-635 g) were treated SC with 2.5 mL/kg saline (groups 1 and 2) or 200 mg/kg CUR (Batch

nos: 904010 and 904009, group 3) or 2.2 mg/kg ovalbumin (group 4), once daily, for 7 consecutive days, in a volume of 2.5 mL/kg. 10 days later, they were challenged IV with 1.25 mL/kg saline for group 1, with 100 mg/kg CUR for groups 2 and 3, and with 1.1 mg/kg ovalbumin for group 4. Animals were observed for 30 minutes, then at 10 minute interval for one hour, then hourly interval for 3 hours. They were kept for further observation on the next day and subsequently killed by overdose of sodium pentobarbitone. The lungs and tracheas from all animals were inflated and fixed. For detailed methodology, see pages 283-284, Vol. 1.6. Treatment started on 6/11/90 and ended on 6/28/90.

**RESULTS :** No anaphylaxis was produced in saline or CUR treated groups (groups 1, 2 and 3), while the ovalbumin treated group produced varying degrees of anaphylaxis: 8/19 suffered extreme anaphylaxis and died within 9 minutes after challenge and two showed moderate anaphylaxis. But, histopathology of the lungs and tracheas from all animals treated with CUR reported no significant abnormalities, and although group 4 had an increased incidence and degree of focal alveolar hemorrhage, they were considered "agonal and unrelated to treatment". For pathologist's report, see pages 299-301, Vol. 1.6.

**21. PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) IN GUINEA PIGS :**  
**Toxicological Report Reference : /15/PH :**

Lab Performing the Study : \_\_\_\_\_

**Methods :** Preliminary study : 7 groups 2 M/group of Dunkin Hartley guinea pigs ( $472 \pm 17$  g) were treated with 2 SC injections, one week apart, with 2.5 mL/kg saline or 0.01, 0.1, 1, 10, or 100 mg/kg CUR (Batch P902030) or 1 mg/kg bovine serum albumin (BSA) in Freund's complete adjuvant (FCA). 2 weeks later, animals were challenged with 100 mg CUR + 20 mg Evans blue dye in saline by IV, since this dose did not give any reaction in nonsensitized animals. Animals were sacrificed after blood collection by cardiac puncture under anesthesia. Serum from each animal was isolated, and levels of anaphylactic antibodies were assayed. For detailed methodology, see page 348-352, Vol. 1.6.

**RESULTS :** A positive PCA response (diameter greater than 5 mm) was reported in one of 4 sensitized with BSA and challenged with BSA, at the 1/2 dilution. Complete dosing at challenge from animals given serum from BSA sensitized animals was not possible due to no available ear veins

(sponsor).

NOTE : Guinea pigs are often injected IV into the large vein of the penis.

No other PCA response was shown. No antibodies were detected from CUR treated groups.

Methods : **Principle Study** : 5 groups of 4 M/group of Dunkin-Hartley guinea pigs were treated with 2 SC injections, one week apart, with 2.5 mL/kg saline or 1, 10, or 100 mg/kg CUR (Batch P902030) or 1 mg/kg BSA in FCA. 2 weeks later, animals were challenged with 100 mg CUR + 20 mg Evans blue dye in 1.25 mL saline by IV. Animals were sacrificed after blood collection by cardiac puncture under anesthesia. Serum from each was isolated, and each serum sample was assayed for **total Ig and IgG levels** in 3 guinea pigs each: 0.05 mL of each serum dilution was injected **ID** in the shaved back of guinea pig, and serum from 2 study animals was tested in each guinea pig. Approximately 24 hours after ID injection, all received IV injection of 100 mg CUR + 20 mg Evans blue except the positive control groups (2 and 15) which received 10 mg BSA + 20 mg Evans Blue. 30 minute later, they were killed and the diameter of the blue area at injection sites were assessed from 2 measurements. For assay of anaphylactic antibodies, see page 351, Vol. 1.6.

RESULTS: One animal in BSA group died of anaphylactic shock immediately after challenge, and 3 other serum samples from this group gave positive PCA responses.

One serum sample each out of 3 sensitized with 1 mg/kg CUR and 10 mg/kg CUR also gave a positive reaction for **total Ig assay**, but none from 100 mg/kg CUR had a positive reaction (thus not dose-related), and no IgG was detected for CUR treated animals, while all in the positive control gave positive IgG reactions.

22. **SENSITIZATION TEST IN THE GUINEA PIG: MAGNUSSON AND KLIGMAN MAXIMIZATION METHOD** : Toxicol Report Number A/M/20240 :  
January 1990 : Test period : 9/6/89 to 10/12/89 :

Lab Performing the Study : .....

Methods : **Preliminary Test** : to determine the optimal concentration of CUR (maximum non-irritating concentration and a minimum irritating concentration), a dose-ranging study was performed with 4 previously

mg/kg CUR treated group. None of CUR treated animals showed IgG response. All BSA treated animals gave marked PCA reactions, and they gave positive reactions not only for total Ig but also IgG, and one in this group died of anaphylaxis immediately after challenge by accidental iv administration.

The Maximization Test in Guinea Pigs was NOT conclusive or useful, due to high positive response (15% at 24 hours and 10% at 48 hours) in the control group produced by "irritation". The sponsor concluded that "it was not possible to assess the sensitization potential of the material using the results obtained." This test is to determine if a material has delayed sensitization (type 4 immune reaction) potential.

#### IV. CLINICAL TRIALS :

A summary of European clinical studies were submitted on Table 2 on page 007, Vol. 1.1

#### SUMMARY AND EVALUATION :

This addendum contains an in depth review of all preclinical studies submitted to the original submission of this IND. Only a preliminary original review had been completed previously (7/1/93), as discussed in a PRE-IND meeting with the sponsor on 5/11/91: the available human data were concluded as sufficient to proceed with Phase 3 clinical trials. Division's IND meeting on 6/1/93 came to the same conclusion.

The subject of this IND is Curosurf<sup>®</sup> (CUR), a natural lung surfactant, extracted from pig lungs. CUR is manufactured by Chiesi Farmaceutici S.p.A. in Parma, Italy.

CUR will be used for treatment of neonatal respiratory distress syndrome (RDS) in premature infants.

Sponsor submitted the following 21 preclinical toxicity studies, in addition to Pharmacological and Pharmacodynamic/Pharmacokinetic studies :

#### Study Number:

- 1-3. ACUTE IP TOXICITY STUDIES (2 in mice and 2 in rats).
- 5-8. ACUTE IT TOXICITY STUDIES (rats, guinea pigs, rabbits, and dogs).
- 9-11. 2-WEEK IT TOXICITY STUDIES (rats, rabbits, and dogs).
- 12. 4-WEEK IP TOXICITY STUDY IN RATS FOLLOWED BY A 4 WEEK RECOVERY PERIOD.
- 13-17. MUTAGENICITY STUDIES : REVERSE MUTATION IN SALMONELLA TYPHIMURIUM, GENE MUTATION IN CHINESE HAMSTER V79 CELLS, CHROMOSOME ABERRATIONS IN CHINESE HAMSTER

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- OVARY CELLS IN VITRO, UNSCHEDULED DNA SYNTHESIS IN HELA S3 CELLS IN VITRO, and MICRONUCLEUS TEST.
- 18-20. ANAPHYLAXIS TESTS IN THE GUINEA PIGS : 3 ACTIVE ANAPHYLAXIS (SENSITIZED AND CHALLENGED IT, SENSITIZED IP AND CHALLENGED IV, SENSITIZE SC AND CHALLENGED IV),
21. PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) IN GUINEA PIGS.
22. GUINEA PIG MAXIMIZATION TEST.

Preclinical studies were performed either at \_\_\_\_\_ acute toxicity studies and a 2 week study were done at \_\_\_\_\_ GLP statements were submitted for the all studies including mutagenicity studies.

Efficacy of CUR as a pulmonary surfactant has been well established in in vitro and in vivo animal models such as premature rabbits as shown on pages 4 and 5. Pharmacokinetic data (half-life) of IT CUR with <sup>14</sup>C-DPPC in newborn and adult rabbits were similar to marketed products [Survanta (SUR) and Exosurf (EXO)]. The half life of CUR in the lungs was about 25 hours in adults and 67 hours in newborn rabbits (see page 5).

The results of animal toxicity studies submitted were summarized in the REVIEW section : page 11 for all 8 acute toxicity studies, page 16 for 2 week IT study in rats, page 18 for 2 week IT study in rabbits, page 21 for a 2 week IT study in dogs, page 26 for 4 week IP study in rats, page 30 for 5 mutagenic studies and page 35 for 5 antigenicity studies.

The results of IT toxicity studies showed **no unusual or unexpected** toxicity findings from CUR, including histopathology of the lungs. The adverse findings produced by CUR were similar to those reported for SUR, including the antigenic potential.

But, the results of IP toxicity studies have only limited value since almost all findings were attributed to **inflammatory reactions at the injection sites**, that made the evaluation of toxicity from CUR when given alone equivocal.

CUR was **negative** in 5 genotoxicity studies, with/without metabolic activation, although there were some sporadic increases in mutation; and at high concentrations, CUR was cytotoxic in some strains of bacteria, under the test conditions, while all the positive controls produced S.S dose-related positive reactions.

Among 5 antigenicity tests, CUR was **negative** in 3 active anaphylaxis tests in guinea pigs under the test conditions, while the positive control produced marked anaphylaxis.

CUR produced measurable **antibodies** in a passive cutaneous anaphylaxis test (PCA) in guinea pigs, although **IgG** antibodies were **not** detected under the conditions used. This is

*This does not make the findings equivalent to CUR when given alone. Value Toxicology studies were conducted.*

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not surprising since CUR is an extract of natural pig lung surfactant, and contained minute amounts of (less than 2%) Surfactant Specific Proteins B and C in the final product.

The results of guinea pig Magnusson and Kligman dermal **maximization test** were **not** useful or conclusive for determining **dermal sensitization potential**, due to high positive reaction in the control group : **5 of 20** from undiluted CUR along with **2 controls** and **1 of 20** from 50% CUR along with one control showed Grade 1 (weak) cutaneous reaction at **24 hours** after dermal challenge. **48 hours** after challenge, **2 of undiluted CUR** still showed Grade 1 reaction along with one control. These positive cutaneous reactions were probably due to irritation. The sponsor concluded that "it was not possible to assess the sensitization potential of the material using the results obtained". But this study does not need to be repeated, since CUR is antigenic as described in the previous paragraph.

It should be pointed out that all three **2 week IT** toxicity studies in rats, rabbits and dogs tested only a **single dose** of CUR per day, and the high dose was **200 mg/kg/day** in rats, but only **100 mg/kg/day** in rabbits and **50 mg/kg/day** in dogs (only one dose group for each study, along with a control group). Therefore, there was no clear cut NOEL in any of these studies, although 50 mg/kg was considered the NOEL in dogs by the sponsor. A single dose of **200 mg/kg** CUR was the NOEL in acute IT toxicity studies in rabbits and dogs.

**Proposed clinical starting dose is 200 mg/kg, and the maximum dose is up to 400 mg/kg within 24 hours.**

The IT toxicity study in the dogs is the first toxicity study submitted for any of the surfactant products in this species. Dogs could not tolerate even the clinical single dose of 200 mg/kg, or a half dose, 100 mg/kg. The first dog had to be killed in extremis after a 200 mg/kg. Dogs were the most sensitive species tested (rats, rabbits, lambs, ferrets, cynomolgus monkeys), even though they were young adults instead of newborn or premature pups that require artificial ventilation.

CUR contains **80 mg phospholipids/mL**, with less than 2% of surfactant specific proteins (B and C) from porcine lung. SUR contains **25 mg phospholipids/mL**, with less than 2.4% of surfactant specific proteins from bovine lung. But EXO contains only **13.5 mg phospholipids/mL** without any foreign proteins. Therefore, CUR contains almost 6-fold greater amount of active ingredient than EXO.

Therefore, CUR may have an **advantage** over these other two surfactants, due to the higher concentration of phospholipids in one mL, that results in requiring less volume being administered into the infant's lung, thus lessening/minimizing distress that has been observed in premature infants with SUR and EXO.

The following Table shows comparative composition, clinical single dose, and volume of single dose of 3 surfactants :

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Surfactant	PL conc.: mg/mL	PL : mg/kg	Vol.: mL/kg
Curosurf	80	200	2.5
Survanta	25	100	4.5
Exosurf	13.5	67.5	5.0

PL = phospholipids

But, the high concentration conceivably could be a disadvantage : A **2 Week IT Toxicity Study in Rats** resulted in relatively high death rates: 4 in sham (50% mortality) and 5 in CUR (63% mortality) group, although the deaths were attributed to pneumonia by the sponsor. **4/5 deaths in CUR was during administration procedure and attributed to suffocation from "large" volume of CUR.** CUR was administered at **2.5 mL/kg**, but the clinical volume of other surfactants is much **greater** than CUR: **4 mL/kg** for Survanta and **5 mL/kg** for Exosurf, and their mortality rates in the animal studies were not as high as in this study (less than 30-45% even in neonatal animals for the two approved surfactants). Vehicles for all 3 surfactants are basically similar, but CUR has the **greatest concentration of DPPC/mL**, making it the most viscous. Therefore, viscosity may play a role in this case, especially in **dogs**.

It should be also pointed out that almost all findings reported in 2 week IT studies are attributed to **presence of catheters**, that caused **inflammation** in the areas around the catheters. However, CUR had shown a slightly **greater** tendency for incidence or degree of each finding over the vehicle control group, especially in rats, indicating that CUR apparently had an **added burden** in animals (see Tables on pages 14-16 of this review).

**Clinically**, intratracheal CUR has been studied extensively over the past 8 years in over 2,900 babies for the treatment of RDS in Europe as shown in the table on page 36.

*I do not agree with this conclusion.*  
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**IS/**

Since this product has been used extensively in European clinics without notable problems to date, there are adequate human data to support relative safety.

CUR has been marketed and been distributed in Italy, France, Spain, Portugal and Brazil since January, 1993.

Since 1989, 2 lung surfactants, SUR and EXO have been marketed in U.S.A. for the treatment of RDS in premature infants. They are currently being studied in ARDS, including

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AIDS and CF.

## CONCLUSIONS :

The results of animal toxicity studies, especially intratracheal 2 week studies, are similar to those produced by SUR, including histopathology of the lungs and antigenic potential.

The preclinical data are relatively weak in support of safety, but none of the data presented would preclude proceeding with clinical trials.

*The preclinical data are adequate*

Since Curosurf has been marketed in Italy, France, Switzerland, Spain, Portugal, and Brazil and since the product has been used and/or studied extensively in European clinics, there are adequate human data to support relative safety.

Therefore, the proposed Phase 3 clinical studies may be initiated based on clinical experience with 2,943 premature babies, with either 600 mg/kg or 300 mg/kg, provided medical officer finds the clinical protocol acceptable.

## RECOMMENDATIONS :

The proposed Phase 3 clinical studies may be initiated with proposed doses of \_\_\_\_\_ primarily based on the available clinical data from Europe and marketing experience, provided medical officer finds the clinical protocol acceptable.

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Young S. Choi, Ph.D.  
Pharmacologist

cc:

Original (IND)  
HFD-150/Division File  
HFD-150/MO/Himmel  
HFD-150/DeGeorge  
HFD-150/Choi  
HFD-151/CSO  
HFD-340

R/D by Y. Choi/4/18/94

Revised by Y. Choi/3/7/95

R/D init. by J. DeGeorge/3/16/95

F/T by Y. Choi/ / /95, Wp #689T-1addendum

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I my supervising review of this review  
I have disagreed with several of the  
conclusions and statements made by  
the reviewer. These are indicated  
at the appropriate locations in the  
text. Where any statements are  
crossed out this is signed and initialed

The opinion of the supervising pharmacist?  
and are valid scientific disagreements on data interpretation  
between the reviewer and supervisor  
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3/10/95

Division of Pulmonary Drug Products  
Pharmacology Consult review

NDA 20744

Date of submission: July 3, 1996  
Date of consult request; March 4, 1997  
Date of review: April 9, 1997

Reviewer: C. Joseph Sun  
Applicant: Dey Laboratories  
Drug: Curosurf (poractant)

Review and evaluation:

This review is in response to a consult requested by Dr. Nashed, the review chemist for the NDA, to assess the technical aspect of the in vivo method and appropriateness of the chosen parameters.

The applicant proposed a method for evaluating in vivo biological activity of Curosurf as follow.

The in vivo activity is evaluated by measuring tidal volume ( $V_t$ ) and thorax-lung compliance (C) in Charles river Italy rabbit fetuses delivered prematurely on the 27th day of gestation. Each litter is divided for three treatments as follow: (1) Curosurf, (2) no treatment and (3) Internal reference standard of Curosurf (positive control). Every test batch would be tested on at least six fetuses delivered from at least two does. The volume administered is 2 ml/kg (80mg/ml or 160 mg/kg of total phospholipid). The ventilation pressure is gradually increased to 35  $H_2O$  for 1 minute and then decreased to 25 cm  $H_2O$  for 15 minutes, to 20 and 15 cm  $H_2O$  for 5 minutes each and increased to 25 cm  $H_2O$  for further 5 minutes. The respiratory volume is recorded every 5 minutes, while the ECG is taken before and at the end of the ventilation cycle.

At the end of each experiment, autopsies were performed to determine pneumothorax-in which case the fetus is rejected from the test. The maturity of a litter is checked at the beginning (5 minutes) of ventilation and is considered acceptable only if the mean value of control is less than 5.5 ml/kg and positive control is greater than 11 ml/kg.

Dose selection was based on the two submitted reports entitled " Effects of different doses of curosurf on in vivo lung mechanics in premature newborn rabbits" and "Dose-response curve of Curosurf on in vivo lung mechanics in premature new born rabbits."

The result from the first study showed that Curosurf improved survival, Vt and C at dose range of 50-300 mg/kg. No statistical differences were found between 100 mg/kg and 300 mg/kg in comparison to 200 mg/kg while the dose of 50 mg/kg was less effective. The 2nd study demonstrated that Curosurf at dose range between 25 and 400 mg/kg improved lung mechanics in a dose-dependent manner and the least effective single dose was 100 mg/kg. Thus, the chosen dose of 160 mg/kg for the proposed study is justified.

The batch acceptance criteria established by the applicant were based on data generated from a series of experiments. The study was carried out on 110 rabbits fetuses at gestation age of 27 days, 48 of which received no material and served as controls and 62 treated intratracheal with different batches (K5/87, K5/88, BS/812050, BS/812049, BS/902030). The Vt and C values at 25 cm H<sub>2</sub>O at the beginning (5 min) and at the end (30 min) of the cycle represent the most significant parameters. Furthermore, the positive controls (batched used in the pivotal clinical studies) showed no significant difference among the Vt values at the beginning (5 min) and at the end (30 min) of the ventilation cycle. Thus, the proposed time for specifications of Vt and C at 5 and 30 min. of Curosurf is appropriate.

Data of the study are shown below:

		Mean	Standard Deviation
Vt (ml/kg)	5 min	18.69	8.21
	30 min	21.02	9.03
C (ml/cm H <sub>2</sub> O.kg)	5 min	0.748	0.328
	30 min	0.841	0.361

Based on the above, the applicant proposed the specification (the lower 95% confidence limit) for in vivo biological activity as follow:

Tidal volume: 5 min, >12.8 ml/kg; 30 min, >14.6 ml/kg

Lung compliance: 5 min, > 0.51 ml/cm H<sub>2</sub>O.kg; 30 min, >0.58 ml/cm H<sub>2</sub>O.kg

Since the acceptance criteria and the proposed specification were based on the results from the 5 batches which were not used in the pivotal clinical trials, a linkage of these five batches to the clinical batches should be established.

Recommendation:

The proposed method of evaluating *in vivo* biological activity by monitoring tidal volume and lung compliance at 25 cm H<sub>2</sub>O at 5 min and 30 min of the ventilation cycle is scientifically sound. However, the batch acceptance criteria were based on the data generated from five batches which were not used in the pivotal clinical studies, an equivalent linkage between these five batches and the clinical batches should be established. Otherwise, the batch acceptance criteria and the proposed specification should be based on the data generated from the batches which have been used in the pivotal clinical studies. Furthermore, acceptance of the proposed specifications (the lower 95 % confidence limit) for these parameters will be pending on statistical evaluation.

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C. Joseph Sun Ph. D.  
Pharmacology Team Leader

Orig. NDA  
HFD-570/Division file  
HFD-570/Sun  
HFD-570/Pina  
HFD-570/Nashed

Apr: 19, 1997

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April 9, 1997

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ON ORIGINAL

Division of Pulmonary Drug Products  
Pharmacology Consult review

NDA 20744

Date of submission: July 3, 1996  
Date of consult request; March 4, 1997  
Date of review: April 9, 1997

Reviewer: C. Joseph Sun  
Applicant: Dey Laboratories  
Drug: Curosurf (poractant)

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The applicant proposed a method for evaluating in vivo biological activity of Curosurf as follow.

The in vivo activity is evaluated by measuring tidal volume ( $V_t$ ) and thorax-lung compliance (C) in Charles river Italy rabbit fetuses delivered prematurely on the 27th day of gestation. Each litter is divided for three treatments as follow: (1) Curosurf, (2) no treatment and (3) Internal reference standard of Curosurf (positive control). Every test batch would be tested on at least six fetuses delivered from at least two does. The volume administered is 2 ml/kg (80mg/ml or 160 mg/kg of total phospholipid). The ventilation pressure is gradually increased to 35  $H_2O$  for 1 minute and then decreased to 25 cm  $H_2O$  for 15 minutes, to 20 and 15 cm  $H_2O$  for 5 minutes each and increased to 25 cm  $H_2O$  for further 5 minutes. The respiratory volume is recorded every 5 minutes, while the ECG is taken before and at the end of the ventilation cycle.

At the end of each experiment, autopsies were performed to determine pneumothorax-in which case the fetus is rejected from the test. The maturity of a litter is checked at the beginning (5 minutes) of ventilation and is considered acceptable only if the mean value of control is less than 5.5 ml/kg and positive control is greater than 11 ml/kg.

Dose selection was based on the two submitted reports entitled " Effects of different doses of curosurf on in vivo lung mechanics in premature newborn rabbits" and "Dose-response curve of Curosurf on in vivo lung mechanics in premature new born rabbits."

Recommendation:

The proposed method of evaluating in vivo biological activity by monitoring tidal volume and lung compliance at 25 cm H<sub>2</sub>O at 5 min and 30 min of the ventilation cycle is scientifically sound. However, the batch acceptance criteria were based on the data generated from five batches which were not used in the pivotal clinical studies, an equivalent linkage between these five batches and the clinical batches should be established. Otherwise, the batch acceptance criteria and the proposed specification should be based on the data generated from the batches which have been used in the pivotal clinical studies. Furthermore, acceptance of the proposed specifications (the lower 95 % confidence limit) for these parameters will be pending on statistical evaluation.

ISI

C. Joseph Sun, Ph. D.  
Pharmacology Team Leader

April 9, 1997

Orig. NDA  
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