CENTER FOR DRUG EVALUATION AND RESEARCH

Approval Package for:

Application Number :   020458

Trade Name :   GALZIN CAPSULES

Generic Name: Zinc Acetate Capsules

Sponsor :   Lemon Company

Approval Date: January 28, 1997

NDA 20-458

Lemmon Company
Attention: Deborah Jaskot
650 Cathill Road
Sellersville, PA 18960

JAN 2 8 1997

Dear Ms. Jaskot:

Please refer to your June 21, 1994 new drug application submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Galzin (Zinc Acetate) Capsules, 25 and 50 mg.

We acknowledge receipt of your amendments dated September 6 and October 31, 1995, January 3 and 29, March 27, April 8, August 7, September 13 and 24, October 8 and 14, 1996.

This new drug application provides for Galzin at a dose of 25-50 mg three times daily as maintenance treatment for patients with Wilson's disease initially treated with a chelating agent.

We have completed the review of this application and have concluded that adequate information has been presented to demonstrate that the drug is safe and effective for use as recommended in the final printed labeling submitted on October 8, 1996. Accordingly, the application is approved effective on the date of this letter.

We remind you of your Phase 4 commitments specified in your submission dated August 29, 1995 and confirmed in our letter of January 27, 1997. These commitments, along with any completion dates agreed upon, are listed below.

Protocols, data, and final reports should be submitted to your IND for this product and a copy of the cover letter sent to this NDA. Should an IND not be required to meet your Phase 4 commitments, please submit protocol, data, and final reports to this NDA as correspondences. In addition, we request under 21 CFR 314.81(b)(2)(vii) that you include in your annual report to this application, a status summary of each commitment. The status summary should include the number of patients entered in each study, expected completion and submission dates, and any changes in plans since the last annual report. For administrative purposes, all

submissions, including labeling supplements, relating to these Phase 4 commitments must be clearly designated "Phase 4 Commitments."

At the next printing of the package insert, please make the revisions specified below and notify the Agency in the next annual report:

1.   Overall:

   a.   The trademark symbol, (™) should be added to each instance of the word Galzin.

   b.   The top of the insert should be revised from "GALZIN ™ (Zinc Acetate)" to "GALZIN ™ (Zinc Acetate) Capsules."

2.   CLINICAL TRIALS section: The statement at the end of the table should be revised from "Some patients had more than one balance studies..." to "Some patients had more than one balance study..."

3.   DOSAGE AND ADMINISTRATION section:

   a.   The first sentence should be revised from "The recommended adult dose is 50 mg of elemental zinc..." to "The recommended adult dose is 50 mg as zinc..."

Further, the following revisions should be made to the DESCRIPTION section to ensure continuity of the package insert:

   b.   The first sentence should be revised from "Zinc Acetate as the dihydrate is a salt of metallic zinc used to inhibit..." to "Zinc Acetate as the dihydrate is a salt of zinc used to inhibit..."

   c.   The first sentence of the third paragraph should be revised from "GALZIN (Zinc Acetate) Capsules contain the equivalent of 25 or 50 mg of elemental zinc..." to "GALZIN™ (Zinc Acetate) Capsules contain the equivalent of 25 or 50 mg of zinc..."

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

promotional material and the package insert directly to:

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

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

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

If you have any questions, please contact Melodi McNeil, Consumer Safety Officer, at (301) 443-0483.

cc:

Original NDA 20-458	Sincerely yours,
HFD-180/Div. files	
HFD-180/CSO/M.McNeil	
HFD-180/Duffy	
HFD-180/Chen	Stephen B. Fredd, M.D.
HFD-180/Choudary	Director
HFD-720/Huque	Division of Gastrointestinal and Coagulation
HFD-820/ONDC Division Director	Drug Products
DISTRICT OFFICE	Office of Drug Evaluation III
HF-2/Medwatch (with labeling)	Center for Drug Evaluation and Research
HFD-92/DDM-DIAB (with labeling)	
HFD-40/DDMAC (with labeling)	
HFD-613/OGD (with labeling)	

HFD-735/DPE (with labeling) - for all NDAs and supplements for adverse reaction changes.

  HFI-20/Press Office (with labeling)
  HFD-021/ACS (with labeling)

drafted: mm/December 12, 1996/c:\wpfiles\cso\20458612.ap
r/d Initials: KJohnson 1/22/97
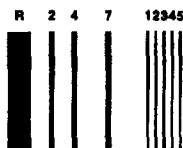   SFredd 1/27/97
final: January 28, 1997

APPROVAL (AP) [with Phase 4 Commitments]

GALZIN™ (ZINC ACETATE)
25 mg and 50 mg

PACKAGE INSERT LABELING

R 2 4 7 12345

215
208

GALZIN™

(Zinc Acetate)

DESCRIPTION

Zinc Acetate as the dihydrate is a salt of metallic zinc used to inhibit the absorption of copper in patients with Wilson's disease. Its chemical formula is $C_4H_6O_4Zn•2H_2O$; M.W. 219.51.

Zinc Acetate occurs as white crystals or granules, freely soluble in water and in boiling alcohol, and slightly soluble in alcohol.

GALZIN (Zinc Acetate) Capsules contain the equivalent of 25 or 50 mg of elemental zinc, in addition to corn starch and magnesium stearate in gelatin capsules. The 25 mg capsule shells contain titanium dioxide and the 50 mg capsule shells contain titanium dioxide, methyl paraben and propyl paraben. The 25 mg capsule shells contain FD&C Blue #1; the 50 mg capsule shells contain FD&C Red #40, D&C Red #28, and D&C Yellow #10.

CLINICAL PHARMACOLOGY
Introduction

Wilson's Disease (hepatolenticular degeneration) is an autosomal recessive metabolic defect in hepatic excretion of copper in the bile, resulting in accumulation of excess copper in the liver, and subsequently in other organs, including the brain, kidneys, eyes, bone, and muscles. In this disease, hepatocytes store excess copper, but when their capacity is exceeded copper is released into the blood and is taken up in extrahepatic sites, such as the brain, resulting in motor disorders (ataxia, tremors, speech difficulties) and psychiatric manifestations (irritability, depression, deterioration of work performance). Redistribution of excess copper in hepatocytes leads to hepatocellular injury, inflammation, necrosis, and eventual cirrhosis. Patients may present clinically with predominantly hepatic, neurologic, or psychiatric symptoms.

The disease has been treated by restricting copper in the diet, and the use of chelating agents to bind free copper to reduce its toxicity and facilitate its excretion. The purpose of initial treatment of symptomatic patients with a chelating agent is to detoxify copper. Once the patient's symptoms have stabilized clinically, maintenance treatment begins. Clinical measures are used to determine whether the patient remains stable (See **PRECAUTIONS: Monitoring Patients**).

The active moiety in Zinc Acetate is zinc cation. Regardless of the ligand, zinc blocks the intestinal absorption of copper from the diet and the reabsorption of endogenously secreted copper such as that from the saliva, gastric juice and bile. Zinc induces the production of metallothionein in the enterocyte, a protein that binds copper thereby preventing its serosal transfer into the blood. The bound copper is then lost in the stool following desquamation of the intestinal cells.

Pharmacokinetics

Because the proposed site of action of zinc is an effect on copper uptake at the level of the intestinal cell, pharmacokinetic evaluations based on blood levels of zinc do not provide useful information on zinc bioavailability at the site of action. Determinations of zinc content in the liver and the plasma zinc concentration after the oral administration of Zinc Acetate have yielded inconsistent results. However, foods and beverages have been shown to decrease the uptake of zinc thereby decreasing the levels of zinc in the plasma of healthy volunteers. For this reason, the oral dose of zinc should be separated from food and beverages, other than water, by at least one hour.

Pharmacodynamics

In pharmacodynamic studies, the methods used included net copper balance and radiolabeled copper uptake in Wilson's disease patients. These studies showed that a regimen of 50 mg t.i.d. of Zinc Acetate was effective in inducing a negative mean copper balance (-0.44 mg/day) and an adequate mean ^{64}Cu uptake (0.82% of the administered dose). A regimen of 25 mg t.i.d. of Zinc Acetate was also pharmacodynamically active but fewer patients have been treated with this regimen than 50 mg t.i.d.

CLINICAL TRIALS

In the single center United States trial, 60 patients with Wilson's disease (31 male, 29 female) who had adequate detoxification of copper after initial chelation therapy were entered into a copper balance study of various dose regimens of Zinc Acetate. Patients were hospitalized to carefully control food and liquid intake. Food, urine and feces were analyzed for copper content, and copper balance was defined as the difference between copper intake and copper elimination/excretion over a 10-day period. A patient was considered in adequate copper balance if the result was less than +0.25 mg copper/day. Results for the groups in each dose regimen tested and for adequacy of individual results are provided in the following table.

Dose Regimen (mg zinc x number of daily doses)	N*	Mean Copper Balance (mg/day)	Number of Patients Inadequately Controlled/Total number of patients studed
50 x 3	70	-0.36	6/70
50 x 2	5	-0.16	0/5
25 x 4	5	-0.21	0/5
25 x 3	11	-0.18	1/11
37.5 x 2	4	-0.02	1/4
75 x 1	8	0.16	2/8
25 x 2	4	0.15	1/4
25 x 1	10	-0.37	2/10
25 x 6	12	0.05	4/12
50 x 1	1	0.1	0/1
50 x 5	11	-0.3	1/11
0	6	0.52	—

*N = number of copper balance studies. Some patients had more than one balance studies, at different doses or at the same dose at widely separated intervals.

ing in motor disorders (ataxia, tremors, speech difficulties) and psychiatric manifestations (irritability, depression, deterioration of work performance). Redistribution of excess copper in hepatocytes leads to hepatocellular injury, inflammation, necrosis, and eventual cirrhosis. Patients may present clinically with predominantly hepatic, neurologic, or psychiatric symptoms.

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25 x 4	5	-0.21	0/5
25 x 3	11	-0.18	1/11
37.5 x 2	4	-0.02	1/4
75 x 1	8	0.16	2/8
25 x 2	4	0.15	1/4
25 x 1	10	-0.37	2/10
25 x 6	12	0.05	4/12
50 x 1	1	0.1	0/1
50 x 5	11	-0.3	1/11
0	6	0.52	—

*N = number of copper balance studies. Some patients had more than one balance studies, at different doses or at the same dose at widely separated intervals.

While all Zinc Acetate regimens appeared better than no therapy, there was little experience with doses other than 50 mg t.i.d. Once daily dosing did not appear to give satisfactory control in many cases, and would be inadequate in patients with poor compliance. Based on the limited data available 25 mg t.i.d. was also thought to be an adequate dose regimen, and not shown to be inferior to 50 mg t.i.d. Dose related toxicity was not found in this study.

Symptomatic Patients Initially Treated With a Chelating Drug
Clinical parameters such as neuropsychiatric status including evaluation of speech, and liver function tests were followed as the patients continued therapy on an adequate Zinc Acetate dose regimen. One hundred and thirty-three patients were followed for up to 14 years. There was no deterioration of neuropsychiatric function including speech and biochemical liver function tests, including bilirubin, transaminases, alkaline phosphatase and lactic dehydrogenase. The liver function tests remained either within normal range or slightly above the upper limit of normal for up to 9 years of treatment.

Pre-symptomatic Patients
In this study 30 pre-symptomatic patients were followed for up to 10 years. Diagnosis of the pre-symptomatic Wilson's disease was made on the basis of a liver copper value greater than 200 μg of copper per gram dry weight of tissue.

Non-ceruloplasmin copper levels, ^{64}Cu balance studies, and clinical parameters were assessed. No patient developed symptoms of Wilson's Disease in this cohort. Since the cloning and sequencing of the abnormal genes in Wilson's disease patients, many mutations have been identified that may affect the rate of disease progression. No matched historical control has been compared to this experience, nor has another center replicated this experience.

In a study in the Netherlands, using zinc sulfate, 27 patients were followed up to 29 years by mainly clinical parameters such as tremors, dysarthria, dystonia, ataxia and Kayser-Fleischer rings. No deterioration of the clinical status was observed. In some cases, Kayser-Fleischer rings disappeared and clinical signs and symptoms improved.

INDICATIONS AND USAGE
Zinc Acetate therapy is indicated for maintenance treatment of patients with Wilson's disease who have been initially treated with a chelating agent (See **PRECAUTIONS: Monitoring Patients**).

CONTRAINDICATIONS
Zinc Acetate Capsules are contraindicated in patients with known hypersensitivity to any of the components of the formulation.

PRECAUTIONS
General
Zinc Acetate is not recommended for the initial therapy of symptomatic patients because of the delay required for zinc-induced increase in enterocytic metallothionein and blockade of copper uptake. Symptomatic patients should be treated initially, using chelating agents. During initial therapy, neurological deterioration may occur as stores

GALZIN™ (ZINC ACETATE)
50 mg CAPSULES

LABEL

NDC 57844-208-52

GALZIN™ 50 mg
(zinc acetate) capsules

Each orange capsule contains: Zinc Acetate equivalent to 50mg elemental Zinc.

CAUTION: Federal law prohibits dispensing without prescription.

GATE **250 CAPSULES**

USUAL DOSAGE: See package insert for full prescribing information.
Store between 15°–30°C (59°–86°F).
Dispense in a tight, light-resistant container as defined in the USP/NF, with a child-resistant closure.
KEEP THIS AND ALL MEDICATIONS OUT OF THE REACH OF CHILDREN.

Manufactured for: GATE PHARMACEUTICALS
division of Lemmon Company, Sellersville, PA 18960
Manufactured by: LEMMON COMPANY, Sellersville, PA 18960

L18584

PG Iss. 7/96

57844-208-52

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NDA 20-458

GALZIN™ (ZINC ACETATE)
25 mg CAPSULES

LABEL

NDC 57844-215-52

GALZIN™ 25 mg
(zinc acetate)   capsules

Each aqua blue capsule contains: Zinc Acetate equivalent to 25mg elemental Zinc.

CAUTION: Federal law prohibits dispensing without prescription.

GATE

250 CAPSULES

USUAL DOSAGE: See package insert for full prescribing Information.

Store between 15°–30°C (59°–86°F).

Dispense in a tight, light-resistant container as defined in the USP/NF, with a child-resistant closure.

KEEP THIS AND ALL MEDICATIONS OUT OF THE REACH OF CHILDREN.

Manufactured for: GATE PHARMACEUTICALS
division of Lemmon Company, Sellersville, PA 18960
Manufactured by: LEMMON COMPANY, Sellersville, PA 18960

L18582

PG Iss. 7/96

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NDA 20-458

Lemmon Company   AUG 2 4 1995
Attention: Stanley Scheindlin, D.Sc.
1510 Delp Drive
Kulpsville, PA 19443

Dear Dr. Scheindlin:

Please refer to your June 21, 1994 new drug application submitted
under section 505(b) of the Federal Food, Drug, and Cosmetic Act
for Zinc Acetate, USP, Capsules.

We acknowledge receipt of your amendments dated July 29,
August 16, September 30, October 20, November 9, 11, and 16, 1994
and January 11, March 6, 7, 28, and 29, May 3 and August 3, 1995.

We have completed the review of this application as submitted
with draft labeling, and it is approvable. Before the
application may be approved, however, it will be necessary for
you to submit the following information:

   1.   A complete clinical report of the new patients studied
        by Dr. George Brewer who were cited at the
        Gastrointestinal Drugs Advisory Committee meeting on
        Wednesday, July 12, 1995. The Guidelines for the
        Format and Content of the Clinical and Statistical
        Section of New Drug Applications (1988) can be
        consulted for the content and format expected in such
        clinical reports of safety and efficacy. We would like
        the report to include case report tabulations and
        listings for each of the new patients. This report
        should also contain available data on dose, dose
        duration, copper balance, 24 hour urine copper,
        non-ceruloplasmin serum copper, ^{64}Cu studies, clinical
        evaluations including neuropsychiatric evaluations,
        liver function and histologic examinations, and adverse
        reactions, if any. Summary analysis, including means
        and standard deviations, where appropriate, should be
        provided. Results of these additional patients should
        be compared both to the results from the patients
        already provided in the NDA and to an historical
        control (with graphical comparisons, if possible) to
        demonstrate that Zinc Acetate maintained clinical
        control in the Wilson's disease patients studied to
        date.

2. An adequate response to the May 5 and 24, and June 14, 1995 chemistry information request letters. In addition, during recent GMP inspections of the manufacturing facilities for your NDA, a number of deficiencies were noted and conveyed to you or your suppliers by the inspector. Satisfactory inspections will be required before this application may be approved.

3. A report of the study protocol entitled "A bioavailability and dose proportionality study of Zinc Acetate (25 and 50 mg capsules) in healthy volunteers" upon completion for our review.

In addition, we request that you submit final printed labeling (FPL). The package insert should be identical in content to the enclosed draft labeling, with additional information added where indicated. Further, the storage statement on the immediate container labels should be revised to: "Store between 15^0-30^0 (59^0-86^0F)".

If additional information relating to the safety or effectiveness of this drug becomes available, revision of that FPL may be required.

Please submit sixteen copies of the printed labeling, ten of which are individually mounted on heavy weight paper or similar material.

Please note that, in the attached labeling, the indication for use in pre-symptomatic patients has not been accepted at this time since the single investigator trial submitted in the NDA has not been replicated. In order to pursue this indication, a randomized comparison study of patients who are pre-symptomatic would need to be conducted and submitted. This can be done in a supplemental application after approval of this NDA.

We also remind you of our request for post approval commitments made in an August 10, 1995 telephone conversation between yourself and Karen Oliver, CSO, of this Agency, and detailed in our August 17, 1995 letter. We await your written response to our request.

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

problems that may be identified. We also expect that drug samples for methods validation will be provided at our request at no cost to the Agency, even if that request occurs post-approval.

Within 10 days after the date of this letter, you are required to amend the application, notify us of your intent to file an amendment, or follow one of your other options under 21 CFR 314.110. In the absence of such action FDA may take action to withdraw the application.

The drug may not be legally marketed until you have been notified in writing that the application is approved.

Should you have any questions, please contact:

> Karen Oliver
> Consumer Safety Officer
> Telephone: (301) 443-0487

                    Sincerely yours,

                    Stephen B. Fredd, M.D.
                    Director
                    Division of Gastrointestinal
                        and Coagulation Drug Products
                    Office of Drug Evaluation I
                    Center for Drug Evaluation and
                        Research

Enclosure

EXCLUSIVITY SUMMARY for NDA #_20-458_____ SUPPL #_____

Trade Name _Galzin_____ Generic Name _Zinc Acetate_____
Applicant Name _Lemmon Company_____ HFD-180__

Approval Date _____

PART I IS AN EXCLUSIVITY DETERMINATION NEEDED?

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

    a) Is it an original NDA?

                     YES   /_X_/   NO /___/

    b) Is it an effectiveness supplement?

                                         YES /___/ NO /_X_/

        If yes, what type? (SE1, SE2, etc.)   _N/A___

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

                                           YES /_X_/ NO /___/

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

    _____

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

    _____

    _____

d) Did the applicant request exclusivity?

YES /___/   NO /_X_/

If the answer to (d) is "yes," how many years of exclusivity did the applicant request?

_____

IF YOU HAVE ANSWERED "NO" TO ALL OF THE ABOVE QUESTIONS, GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2.   Has a product with the same active ingredient(s), dosage form, strength, route of administration, and dosing schedule previously been approved by FDA for the same use?

YES /___/   NO /_X_/

If yes, NDA # _____   Drug Name_____

IF THE ANSWER TO QUESTION 2 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

3. Is this drug product or indication a DESI upgrade?

YES /___/   NO /_X_/

IF THE ANSWER TO QUESTION 3 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8 (even if a study was required for the upgrade).

PART II FIVE-YEAR EXCLUSIVITY FOR NEW CHEMICAL ENTITIES
(Answer either #1 or #2, as appropriate)

1.   Single active ingredient product.

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

<div align="center">YES /_X_/   NO /___/</div>

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

NDA # 18-959_____   _Zinc Chloride Inj.__

NDA # _(see attached)__   _____

NDA # _____   _____

2.   Combination product.

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

<div align="center">YES /___/   NO /_X_/</div>

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

NDA # _____   _____

NDA # _____   _____

NDA # _____   _____

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

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

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

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

<div align="center">YES / X / NO /___/</div>

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

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

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

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

<div align="center">YES / X /   NO /___/</div>

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

_____

_____

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

<div align="center">YES / _ / NO / X /</div>

Note: Although the firm DID include a list of published studies and Study #2 is from the literature, the application did not include a statement that publicly available data would not independently support approval.

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

<div align="center">YES / _ /   NO / _ /</div>

If yes, explain: _____

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

<div align="center">YES / _ /   NO / X /</div>

If yes, explain: _____

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

Investigation #1, Study #: "The Efficacy and Safety of Zinc Acetate as Maintenance Therapy of Wilson's Disease" (Conducted by Dr. George Brewer)

Investigation #2, Study #   "Management of Wilson's Disease with Zinc Sulphate: Experience in a Series of 27 Patients" (Hoogenraad: Journal of the Neurological Sciences. 1987, 77:137-146)

Investigation #3, Study # _____

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

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

Investigation #1   YES /___/   NO /_X_/

Investigation #2   YES /___/   NO /_X_/

Investigation #3   YES /___/   NO /___/

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

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

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

Investigation #1   YES /___/   NO /_X_/

Investigation #2   YES /___/   NO /_X_/

Investigation #3   YES /___/   NO /___/

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

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

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

Investigation #1, Study #:   "The Efficacy and Safety of Zinc Acetate as Maintenance Therapy of Wilson's Disease" (Conducted by Dr. George Brewer)

Investigation #2, Study #   "Management of Wilson's Disease with Zinc Sulphate: Experience in a Series of 27 Patients" (Hoogenraad: Journal of the Neurological Sciences. 1987, 77:137-146)

Investigation #_, Study # _____

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

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

Investigation #1

IND #   YES /_X__/ NO /___/ Explain: Note: Dr. Brewer is the sponsor of this IND; it is incorporated by reference into NDA 20-458

Investigation #2

IND # ____ YES /___/   NO /_X_/ Explain: This study is from the literature; it is incorporated by reference into NDA 20-458.

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

Investigation #1

YES /___/ Explain _____   NO /_X_/ Explain _____

_____   _____

_____   _____

Investigation #2
YES /___/ Explain _____   NO /_X_/ Explain _____

_____   _____

_____   _____

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

                           YES /___/   NO /_X_/

      If yes, explain: _____

      _____

      _____

      _____

_____ January 22, 1997
Signature   Date

Title:____CSO_____

_____ 1/20/97
Signature of Division Director   Date

cc: Original NDA   Division File   FD-85 Mary Ann Holovac

CERTIFICATION MADE PURSUANT TO THE
GENERIC DRUG ENFORCEMENT ACT OF 1992

On behalf of Lemmon Company, the applicant, I hereby certify, pursuant to Section 2(k) of the Generic Drug Enforcement Act of 1992, 21 U.S.C.§ 335a(k), that applicant has not used, is not using and will not in the future use in any capacity the services of any person who has been debarred pursuant to Section 2(a) and/or Section 2(b) of the Generic Drug Enforcement Act of 1992, 21 U.S.C.§§ 335a(a) and/or (b), in connection with this application.

Applicant further certifies that there have been no convictions of applicant for any of the types of crimes set forth in Section 2(a) and Section 2(b) of the Generic Drug Enforcement Act of 1992, 21 U.S.C. §§335a(a) and (b), within the five years prior to the date of this Certification, nor has any person affiliated with applicant, who is responsible in whole or in part for the development or submission of this application, been convicted of any crime of the types listed in Section 2(a) and Section 2(b) of the Generic Drug Enforcement Act of 1992, 21 U.S.C.§§ 335a(a) and (b), within the five years prior to the date of this Certification.

Dated: _January 11, 1995_

Stanley Scheindlin
Stanley Scheindlin, D.Sc.
Senior Director, Regulatory Projects

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NDA 20-458

ZINC ACETATE CAPSULES

PATENT INFORMATION

LEMMON Company declares that there are no patents which claim the drug or the drug product covered by this New Drug Application, or which claim a method of using the drug product, and with respect to which a claim of patent infringement could reasonably be asserted if a person not licensed by the owner of the patent engaged in the manufacture, use or sale of the drug product.

Stanley Scheindlin   _Jan. 11, 1995_

Stanley Scheindlin, D. Sc.   Date
Senior Director, Regulatory Projects

LEMMON

ZINC ACETATE CAPSULES

CERTIFICATION STATEMENT

In accord with 21 CFR 314.50, as revised in the Federal Register of September 8, 1993, LEMMON Company certifies that a field copy of the Technical Section for Chemistry has been provided to the applicant's home FDA district office, the Philadelphia district office.

Stanley Scheindlin

Stanley Scheindlin, D. Sc.
Senior Director, Regulatory Projects

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

NDA # 20-458   Trade (generic) names Zinc Acetate Capsules

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

_____ 1. A proposed claim in the draft labeling is directed toward a specific pediatric illness. The application contains adequate and well-controlled studies in pediatric patients to support that claim.

_____ 2. The draft labeling includes pediatric dosing information that is not based on adequate and well-controlled studies in children. The application contains a request under 21 CFR 210.58 or 314.126(c) for waiver of the requirement at 21 CFR 201.57(f) for A&WC studies in children.

  _____ a. The application contains data showing that the course of the disease and the effects of the drug are sufficiently similar in adults and children to permit extrapolation of the data from adults to children. The waiver request should be granted and a statement to that effect is included in the action letter.

  _____ b. The information included in the application does not adequately support the waiver request. The request should not be granted and a statement to that effect is included in the action letter. (Complete #3 or #4 below as appropriate.)

✓ 3. Pediatric studies (e.g., dose-finding, pharmacokinetic, adverse reaction, adequate and well-controlled for safety and efficacy) should be done after approval. The drug product has some potential for use in children, but there is no reason to expect early widespread pediatric use (because, for example, alternative drugs are available or the condition is uncommon in children).

  _____ a. The applicant has committed to doing such studies as will be required.

      _____ (1) Studies are ongoing.
      _____ (2) Protocols have been submitted and approved.
      _____ (3) Protocols have been submitted and are under review.
      _____ (4) If no protocol has been submitted, on the next page explain the status of discussions.

  _____ b. If the sponsor is not willing to do pediatric studies, attach copies of FDA's written request that such studies be done and of the sponsor's written response to that request.

_____ 4. Pediatric studies do not need to be encouraged because the drug product has little potential for use in children.

____ 5. If none of the above apply, explain.

Explain, as necessary, the foregoing items: _____

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_____   _____
Signature of Preparer   Date 8/17/90

cc: Orig NDA
HFD-180/Div File
NDA Action Package

MEDICAL OFFICER'S REVIEW

NDA:	20-458-BM (Minor Medical Amendment)
Date:	August 7, 1996
Sponsor:	Lemmon Company, PA
Drug:	GALZIN™ (Zinc Acetate 25 and 50 mg hard gelatin capsules)
Route of Administration:	Oral
Pharmacological Category:	Inducer of Copper Malabsorption
Draft to Supervisor:	October 4, 1996
Date Review Completed:	October 30, 1996
Material Reviewed:	a) Report entitled "Continuation Study of Zinc Acetate in the Treatment of Wilson's Disease" by Dr. George J. Brewer (submitted 08/07/96)
	b) Information in support of the approval of the use of Zinc Acetate in pre-symptomatic patients (submitted 09/24/96)
Reviewer:	Hugo E. Gallo-Torres, M.D., Ph.D.

I. BACKGROUND/INTRODUCTION

The sponsor of this NDA, Lemmon Company, is seeking approval of zinc acetate for   indications: a) the maintenance treatment of patients with Wilson's disease who have been initially treated with a chelating agent
                          Data in the initial NDA, submitted on June 21, 1994 were reviewed by the author of the present review (Hugo E. Gallo-Torres, M.D., Ph.D., MOReview of January 27, 1995). The NDA material also was the subject of discussions at the Gastrointestinal Drugs Advisory Committee meeting on Wednesday, July 12, 1995.

In action letter of August 24, 1995, the sponsor was informed that the application was approvable for the first indication. They were told that before the application may be approved it was necessary for Lemmon Co. to submit a complete clinical report of the new patients studied by Dr. Brewer who were cited at the July 12, 1995 G.I. Advisory Committee meeting. It was also noted that, in the labeling attached to the action letter, the indication

In response to the action letter of August 24, 1995 the sponsor has submitted

the following:

  a) Report entitled "Continuation study of zinc acetate in the treatment of Wilson's disease" by Dr. George J. Brewer.

The aim of the present review is to evaluate the information submitted in the above-listed documents.

II. <u>REPORT BY DR. GEORGE J. BREWER</u>

  1. <u>Objective</u>

To demonstrate the efficacy and safety of zinc acetate (ZnAC) for L-T therapy (maintenance therapy) in the additional 62 patients studied by Dr. Brewer post-NDA submission.

As per NDA portion of trial, the specific efficacy objectives were to maintain body Cu at subtoxic levels and prevent progressive target organ damage after adequately decoppering with chelating or other agents in patients who were symptomatic at the start of therapy.

  2. <u>Design/Plan</u>

This maintenance trial consisted of open-label evaluations where each patient served as his/her own control (clinical status and Cu variables prior to the start of maintenance therapy)[1].

In general, the period of initial Tx with chelating agents et al. was a minimum of two months. Patients returned for medical evaluations at intervals of ca. 12 mo. Additional visits were scheduled as indicated. Urine samples were collected at 6 month intervals and sent through the mail for Cu and Zn evaluation. As in the previous study, the duration of ZnAC treatment was indefinite. Lifetime Tx was anticipated unless withdrawal was clinically indicated or requested by the patient. Other aspects of the design/plan were as described for the previous NDA data.

  3. <u>Efficacy Variables</u>

These were the same as described for the previous NDA data.

  4. <u>Procedures</u>

The procedures at entry, pre-maintenance, maintenance, safety evaluations, assessment of concomitant medication/diet, were as described for the previous

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[1] As in the previous study, maintenance therapy was defined as that period of ZnAC therapy when the therapeutic objective is to prevent the accumulation or reaccumulation of Cu and to prevent the appearance of the symptoms of copper toxicity.

NDA data. At entry, the patients were categorized as having either the neurological form (N) or the hepatic form (H) of Wilson's disease. The N category included patients with the typical neurological manifestations of the movement disorder, such as dysarthria, dysphagia, tremor, dystonia, etc., or definite emotional depression, fall off in work or school performance, bizarre behaviors, etc. The H category included patients with a presentation of hepatic failure, with a hepatitis picture, or with a picture of cirrhosis.

5. Data Presentation

As previously, the data presentation consisted primarily of descriptive statistics. A formal prospective test of the hypothesis of clinical efficacy using a statistical model was not planned, primarily because the number of patients per group is small or too small.

The following efficacy parameters were evaluated and were presented in table form (sponsor's Appendix II), followed by summary statistics (means, standard deviations and number of data points):

	Sponsor's Table #
24-Hour Urine Copper	2
Non-ceruloplasmin Plasma Copper	3
24-Hour Urine Zinc	4
Speech Data	5
Neurological Data	6

For ^{64}Cu 1-2 hour peak, only 3 values were available, and for ^{64}Cu 24 hour/peak ratio only 1 value was available. These were shown in sponsor's Tables 7 and 8 respectively, but without summary statistics as such statistics would be meaningless.

The results of these additional patients were compared graphically with the results from the patients already provided in the NDA (sponsor's Appendix III). Each graph cited the NDA table from which the comparative data were plotted. Information on historical controls of untreated Wilson's disease patients was included in the original NDA.

The following safety parameters were presented in table form, followed by summary statistics:

	Sponsor's Table #
Bilirubin	9
Serum Albumin	10
ALT	11
AST	12
LDH	13
Alkaline Phosphatase	14
Serum Amylase	15
Serum Lipase	16

The safety tables were included in sponsor's Appendix IV and graphical comparisons with the NDA patients were given in sponsor's Appendix V.

Full data listings for patients 95-156 (the new patients) were enclosed in sponsor's Appendix VI, separated into efficacy parameters (sponsor's Table 17) and safety parameters (sponsor's Table 18). In the individual tables (sponsor's Appendices II and IV) patients 100 and 127 were not listed because no data were available for these patients while on zinc therapy alone.

6. Results

a. Generalities

Dr. Brewer is continuing his study essentially as described in the original NDA (Patients 1 through 94). Since the NDA cut-off (January 1, 1991) he has continued to enroll patients in the trial. The present cohort (Patients 95 to 156) has undergone treatment with ZnAC for anywhere from 1 to 5 years at this point in time. These patients were enrolled between March 1991 and October 1994. These patients, excluding those who left the study for reasons explained below, had at least one full year of follow-up while on zinc therapy.

b. Demographic Characteristics (Table 1)

As summarized in this Table, roughly half of the patients were females, mostly white, primarily symptomatic (most type N). Of the 62 patients, 5 were children (≤17y of age at the start of ZnAC therapy) and all except three, received ZnAC at the oral dose of 50 mg x 3 per day. The other three were children who were given 25 mg x 3 per day.

TABLE 1
The Brewer's Study
Post-NDA Continuation

Demographic Characteristics

Gender [n=62]	Race [n=62]	Disease Form [n=62]	Adults/Children [n=62]	Dose [n=62]	Withdrawals [n=9]
M = 35 F = 27[a]	W = 52 B = 3 A = 7	Symptomatic [n=54] N = 42 H = 12 Pre-symptomatic [n=8]	Adults 18 to 50y of age [n=57] Children ≤17y of age [n=5]	50 mg x 3 [n=59] 25 mg x 3 [n=3]	Deaths [n=2][a] Discharged by Investigator [n=4][c] Dropped Out [n=2][d] Liver Transplant [n=1][e]

a) A F patient, #130, became pregnant but lost her baby because of uterine rupture due to a previous C-section.
b) Patients #121 and 122.
c) Patients #98, #100, #119 and #127.
d) Patients #112 and #127.
e) Patient #106.

c. <u>Withdrawals (Table 2)</u>

● As noted in Table 1, two patients died, four were discharged by the
  investigator, two dropped out and one underwent liver transplant.
  Summary information on these nine discontinuations is given in Table 2.

● None of the two deaths was due to progression of Wilson's disease. One
  patient, with a hepatic presentation syndrome, presented with
  obstructive liver disease, the other patient expired after a flu
  epidemic swept through the nursing home where he had been placed.

● The four patients discharged by the investigator, one with hepatic the
  other three with neurological presentation of symptoms, were
  uncommunicative and uncooperative during therapy.

● The patient that underwent liver transplantation presented with a
  neurological form of disease but probably had autoimmune chronic active
  hepatitis rather than Wilson's disease.

<div align="center">

<u>TABLE 2</u>
The Brewer's Study
Post-NDA Continuation

Patient Discontinuations

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Pt. Identification	Prior Hx	ZnAC Treatment	Copper	Zinc	Comments
A. Deaths [n=2]					
#121 21y WF H	Trien	50 mg x 3 (12/10/92 to 01/25/95)	<u>Balance</u> = N/D ⁶⁴Cu = N/D <u>Urine</u> 0.251 to 0.048 <u>N-CER-PL</u> 0 <u>Liver</u> 174 ppm	<u>Urine</u> 7.42 to 1.55 <u>Plasma</u> 170 to 59	● Presented with liver Dz and chronic hepatitis. ● No K=F rings; diagnostic tests were inconclusive. ● A 2-y therapeutic trial failed to eliminate the active hepatitis + edema. ● Had mild hypersplenism with thrombocytopenia. ● There was some ↓ in serum trans-aminases, but they remained substantially ↑. ● Pt. died of liver failure. ● Pt. did not have W's Dz but presented with obstructive liver Dz.
#122 34y WM N	P, TM	50 mg x 3 (03/16/93 to 12/28/93)	<u>Balance</u> = -2.62 ⁶⁴Cu = N/D <u>Urine</u> 0.126 <u>N-CER-PL</u> N/D <u>Liver</u> N/D	<u>Urine</u> 5.35 mg <u>Plasma</u> N/D	● After an 8 week course of TM Tx the pt. showed moderate regression in his neurological symptoms. ● At home while on Zn Tx the pt. continued to regress, was placed in a Nursing Home. The continued regression may have been the results of depression. ● Expired after a flu epidemic swept through the nursing home. Cause of death was "Bilateral bronchopneumonia. Congestive splenomegaly".

B. Discharged by Investigator [n=4]					
#98 29y WF N	P	50 mg x 3 (04/26/91 to 04/04/94)			• Was pre-symptomatic at the time of diagnosis and subsequently developed mild dysarthria, dystonia and psychological problems. Improved later. • Discharged from the study for lack of cooperation and for noncompliance.
#100 30y WM H	T	50 mg x 3 (05/31/91 to 06/13/91)			• Alcoholic, extremely uncooperative. • Discharged for lack of cooperation.
#119 30y WM N	P	50 mg x 3 (09/16/92 to 10/24/94)			• Continued crack cocaine during therapy. He was discharged for lack of communication and cooperation during therapy.
#127 39y WM N	P, Z	50 mg x 3 (06/04/93 to 03/25/94)			• Failed to respond to the investigator's communications and requests and was discharged for lack of communication and cooperation during therapy.
C. Dropouts [n=2]					
#112 38y WM N	P	50 mg x 3 (07/15/92 to 01/05/93)			• His local doctor convinced him that zinc was only experimental and would not be in his best interest.
#148 24h BF N	P, Zn Gluconate	50 mg x 3 (09/08/94 to 12/01/94) <u>Also</u> (09/20/95 to 12/01/95)			• Had improved with penicillamine therapy. • In and out of therapy for W's Dz. • Was very difficult to reach and uncommunicative. She was not responsive to calls or written requests.
D. Liver Transplantation [n=1]					
#106 20y BF H	T	50 mg x 3 50 mg x 5 during Trien therapy			• Diagnostic tests for W's Dz were inconclusive. Did not have K-F rings. • Presented with jaundice + fatigue which continued unchanged during ZnAC therapy. • ALB was ↓ and remained unchanged throughout therapy. BIL was substantially ↑ and the serum transaminase moderately ↑ without improvement with therapy. • She probably had autoimmune chronic active hepatitis, instead of W/s Dz. • Underwent a liver transplant.

d.   Pharmacodynamic Effects (Copper values)

● As shown in Table 3, upper panel, all 24-h urine copper values (mg/day) after treatment initiation, were lower than at baseline. The high mean baseline value for post-NDA patients was due largely to four patients who had values greater than 0.5 mg/day [#109=0.852; #116=1.530; #133=1.440 and #137=0.582]. The mean values subsequent to baseline were 120 µg/24h or lower. This indicates adequate zinc therapy. Except for the noted difference at baseline, the curves for 24-h urine copper from the new patients was superimposable to that for the original NDA patients (Fig. 1).

Mean 24-Hour Urine Copper (mg/day)
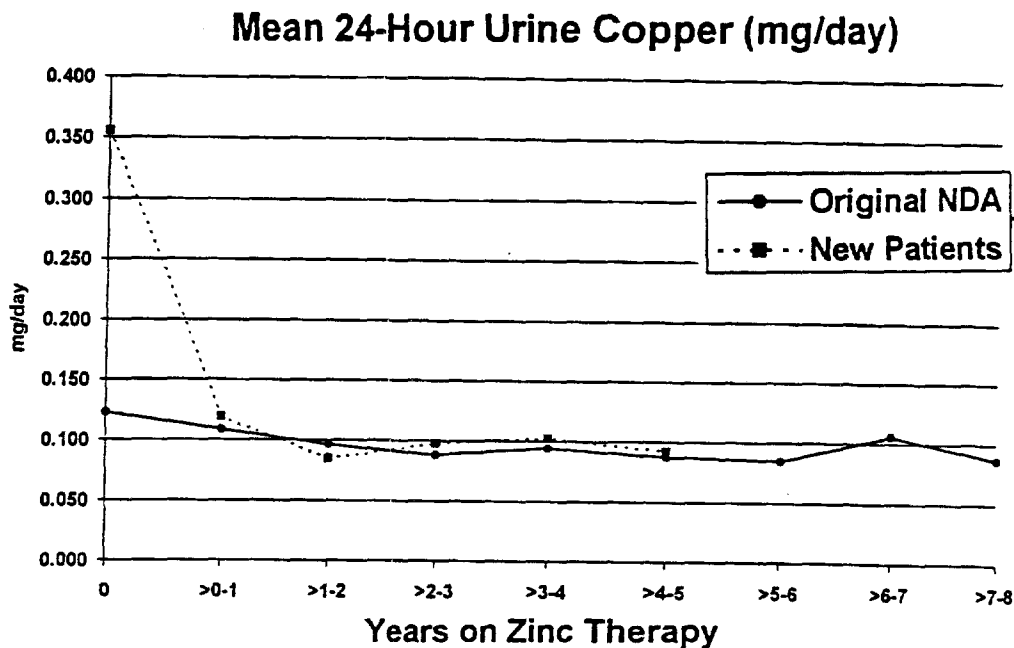
Fig. 1. - Post-NDA Continuation of the Brewer's Study: Mean 24-h Urine Cu (mg/dy). Original NDA data plotted from NDA Table D.9. New Patients data plotted from sponsor's Table 2 of the 08/07/96 Report.

● As seen in Table 3, lower panel, the mean non-ceruloplasmin plasma copper values were all below 20 µg/dl, as were the mean values for the NDA patients (Fig. 2).
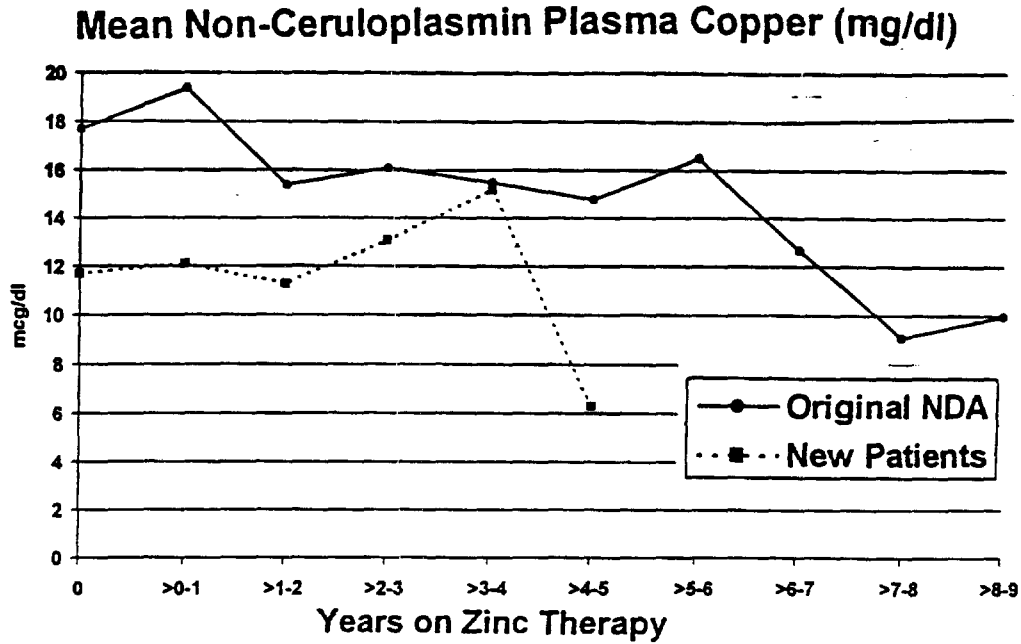
Mean Non-Ceruloplasmin Plasma Copper (mg/dl)

Years on Zinc Therapy

Fig. 2. - *Post-NDA Continuation of the Brewer's Study:*
Mean Non-ceruloplasmin Plasma Cu (μg/dl). Original
NDA plotted from NDA Table D.20. New Patients data
plotted from sponsor's Table 3 of the 08/07/96 Report.

TABLE 3
The Brewer's Study
Post-NDA Continuation

Pharmacodynamic Effects

BL	YEARS ON ZnAC THERAPY				
	> 0 to 1	> 1 to 2	> 2 to 3	> 3 to 4	> 4 to 5
A. MEAN 24-h URINE Cu (mg/day)					
0.355 [n=23]	0.120 [n=59]	0.085 [n=51]	0.098 [n=30]	0.103 [n=16]	0.093 [n=7]
B. MEAN NON-CERULOPLASMIN PLASMA Cu (μg/dl)					
11.7 [n=17]	12.1 [n=37]	11.3 [n=42]	13.1 [n=18]	15.2 [n=10]	6.3 [n=5]

BL = Baseline value (Pre ZnAC Therapy).

e. <u>Mean 24-h Urine Zinc (Fig. 3)</u>

As depicted below, during ZnAC therapy, the mean 24-h urine zinc values showed the expected enrichment in urinary zinc. The increases above baseline were very similar to those seen with the NDA patients (Fig. 3).

Mean 24-h urine zinc (mg/day)

BL	YEARS ON ZnAC THERAPY				
	> 0 to 1	> 1 to 2	> 2 to 3	> 3 to 4	> 4 to 5
1.280 [n=23]	4.377 [n=59]	4.601 [n=51]	5.040 [n=30]	4.728 [n=16]	3.930 [n=7]

Mean Urine Zinc (mg/day)
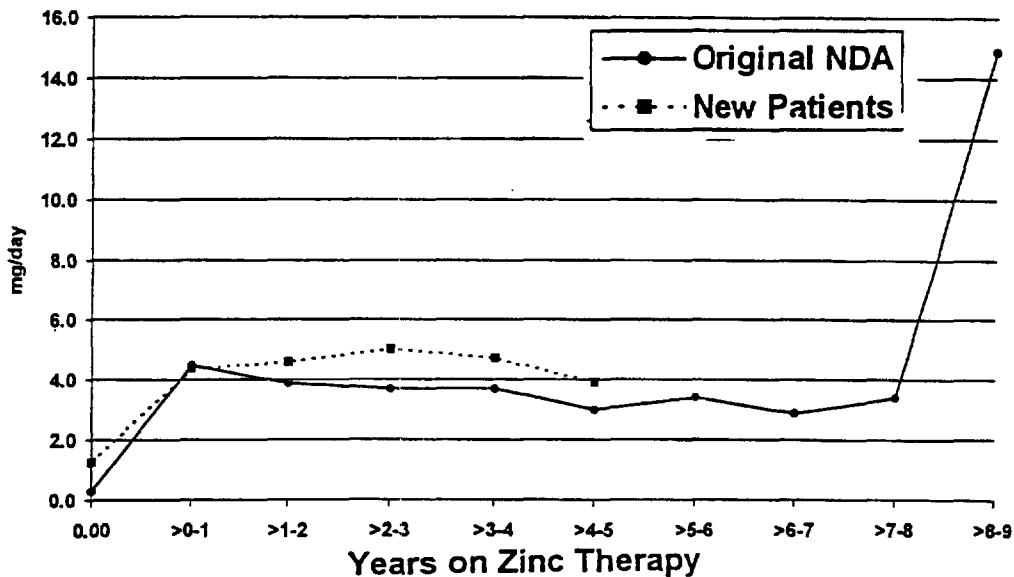
Fig. 3. - *Post-NDA Continuation of the Brewer's Study:*
Mean Urine Zinc (mg/day). Original NDA plotted from
NDA Table D.38. New Patients data plotted from
sponsor's Table 4 of the 08/07/96 Report.

f. <u>Clinical Efficacy Data</u>

● As shown in the upper panel of Table 4 and Fig. 4, in general, speech was less severely affected (all lower scores) in the post-NDA patients. But both lines seem to indicate response to Tx (maintenance).

TABLE 4
The Brewer's Study
Post-NDA Continuation

Clinical Response

	YEARS ON ZnAC THERAPY				
	> 0 to 1	> 1 to 2	> 2 to 3	> 3 ti 4	> 4 ti 5
A. SPEECH DATA (0=Normal; 7=Severe)					
2.2 [n=6]	2.8 [n=17]	2.1 [n=14]	1.6 [n=9]	1.1 [n=6]	2.0 [n=3]
B. NEUROLOGICAL DATA (0=Normal; 38=Severe)					
6.3 [n=7]	7.8 [n=15]	5.2 [n=13]	1.2 [n=7]	3.2 [n=5]	11.0 [n=1]

Mean Speech Rating Scale Results
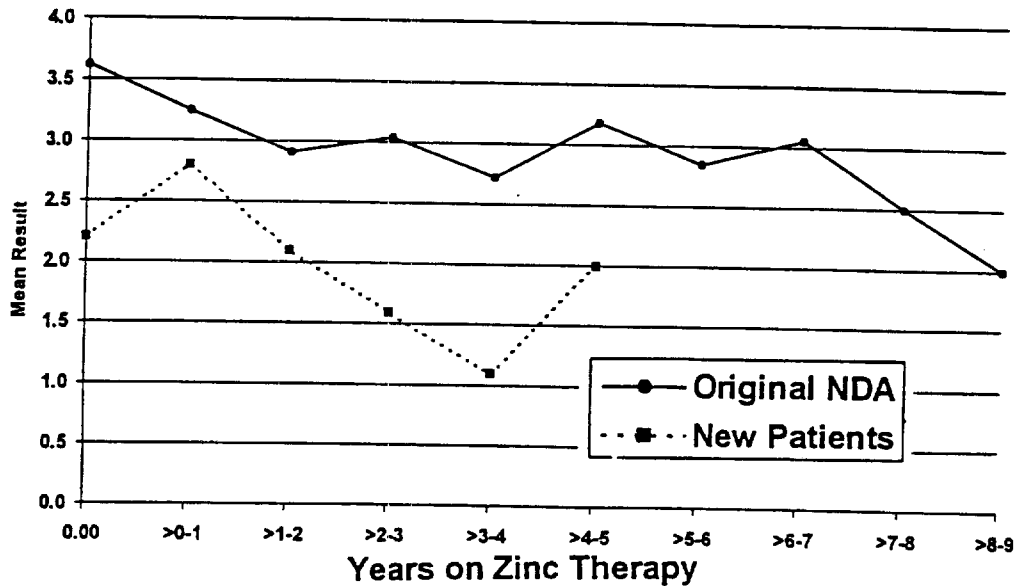
Fig. 4. - Post-NDA Continuation of the Brewer's Study:
Mean Speech Rating Scale Results. Original NDA
data plotted from NDA Table D.51. New Patients
data plotted from Table 5 of the 08/07/95 Report

• Similarly, response to Tx appears to be suggested by the neurological
data (Table 4, lower panel), with the post-NDA patients, in general,
having lower scores than the NDA patients (Fig. 5).

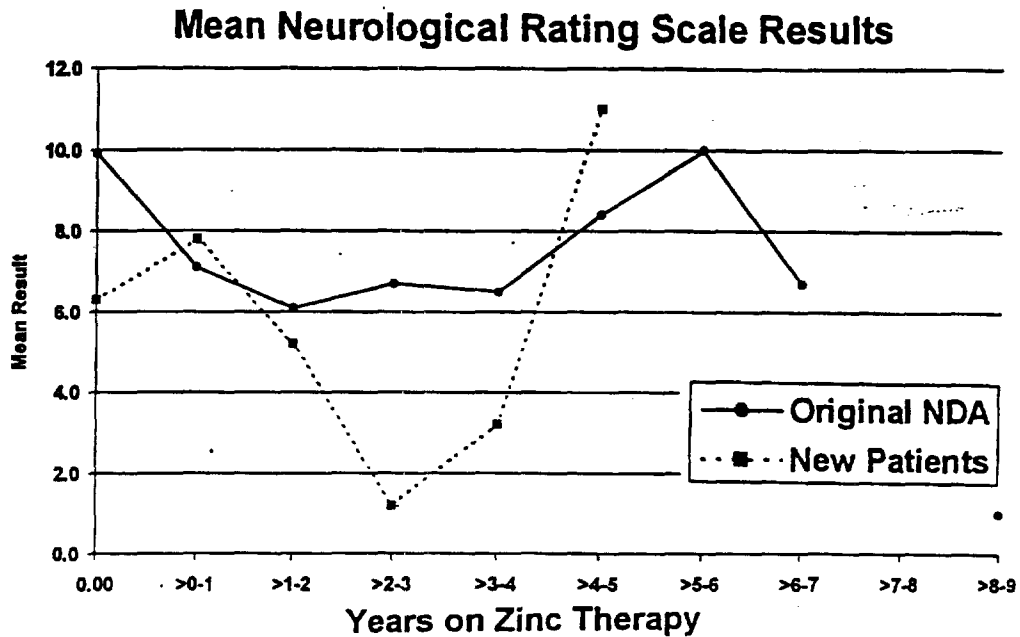## Mean Neurological Rating Scale Results

Fig. 5. - Post-NDA Continuation of the Brewer's Study:
    Mean Neurological Rating Scale Results. Original NDA
    data plotted from NDA Table D.58. New Patients data
    plotted from sponsor's Table 6 of the 08/07/96 Report.

g. Biochemical Markers of Liver Function (Table 5)

● As seen in this Table, all values for total serum bilirubin were lower
during treatment with ZnAC than at baseline. As shown, graphically, in
Fig. 6, the mean BIL values at baseline and at >0 to 1y were much higher
among the post-NDA than in the NDA patients as a group. This difference
was due to the inclusion of data from Pt. #106 who at baseline had
19.7 mg/dl and at the >0 to 1y interval had 15.5 mg/dl. This patient
was later found to have autoimmune chronic active hepatitis. Excluding
data from patient #106, the baseline mean value would be 1.32 and the
value at >0 to 1y would be 0.95 mg/dl. Thereafter, the two lines are
parallel and very similar (see Fig. 6).
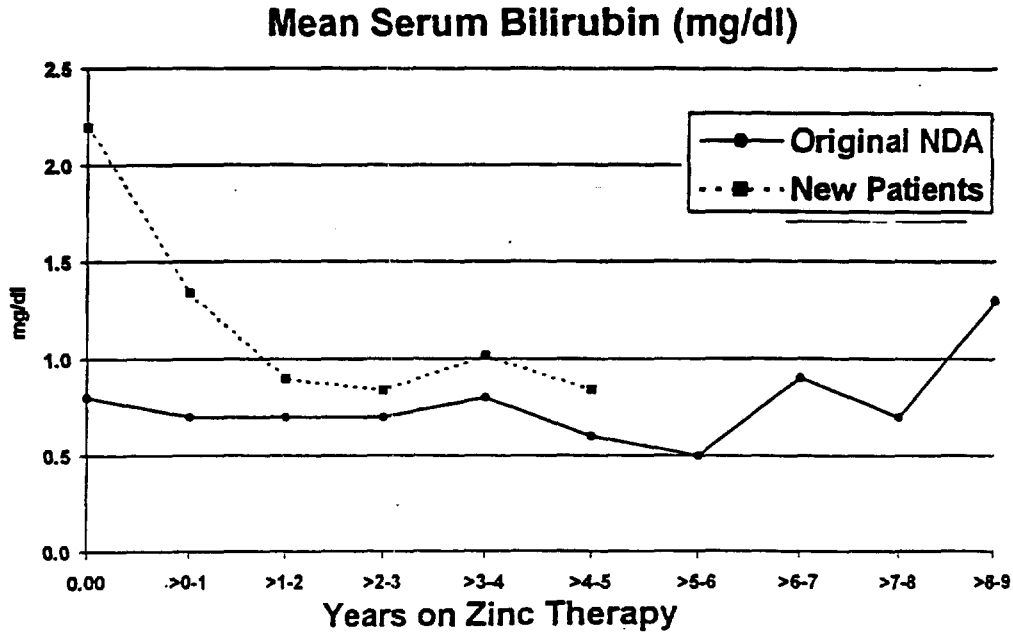
Mean Serum Bilirubin (mg/dl)

Fig. 6. - Post-NDA Continuation of the Brewer's Study:
Mean Serum Bilirubin (mg/dl). Original NDA data
plotted from NDA Table D.97. New Patients data
plotted from sponsor's Table 9 of the 08/07/96 Report.

● As shown in Table 5 and Fig. 7, for serum albumin, there were
essentially no differences between post-NDA and NDA patients.

TABLE 5
The Brewer's Study
Post-NDA Continuation

Biochemical Markers of Liver Function

	BL	YEARS ON ZnAC THERAPY				
		> 0 to 1	> 1 to 2	> 2 to 3	> 3 to 4	> 4 to 5
A. MEAN TOTAL SERUM BILIRUBIN (mg/dl)						
	2.2 [n=21]	1.3 [n=39]	0.9 [n=40]	0.3 [n=21]	1.0 [n=11]	0.8 [n=5]
B. MEAN SERUM ALBUMIN (g/dl)						
	3.8 [n=20]	3.7 [n=39]	3.9 [n=40]	4.1 [n=21]	4.1 [n=10]	4.2 [n=5]

C. MEAN SERUM ALT (IU/1)					
87 [n=20]	64 [n=39]	46 [n=39]	52 [n=21]	50 [n=11]	59 [n=5]
D. MEAN SERUM AST (IU/1)					
97 [n=21]	58 [n=39]	40 [n=40]	38 [n=21]	38 [n=11]	33 [n=5]
E. MEAN SERUM LDH (IU/1)					
173 [n=19]	167 [n=39]	165 [n=39]	164 [n=21]	173 [n=11]	145 [n=5]
F. MEAN SERUM AP (IU/1)					
118 [n=20]	137 [n=39]	130 [n=41]	129 [n=21]	102 [n=11]	108 [n=5]

● As shown in Table 5 and Fig. 7, for serum albumin, there were
essentially no differences between post-NDA and NDA patients.
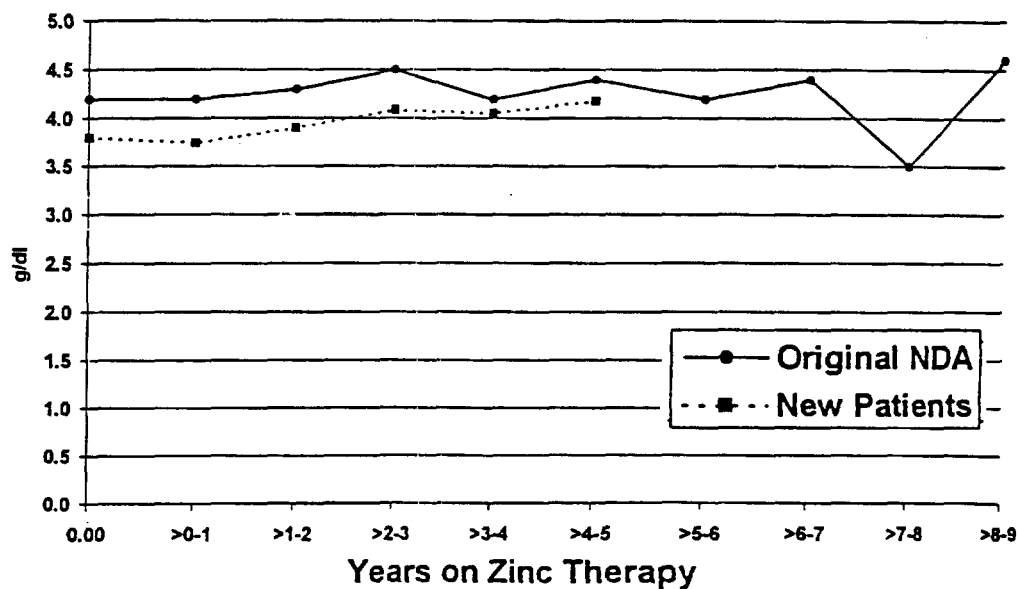
Mean Serum Albumin (g/dl)

Fig. 7. - Post-NDA Continuation of the Brewer's Study:
Mean Serum Albumin (g/dl). Original NDA data
plotted from NDA Table D.73. New Patients data
plotted from sponsor's Table 10 of the 08/07/96
Report.

• Also depicted in Table 5 are data on serum transaminases. For both, ALT
and AST, all values during treatment with ZnAC were lower than at
baseline. Except for the two initial points in the respective curves
(Figs. 8 and 9), the transaminase values during treatment with ZnAC to
the post-NDA patients were similar to those for the NDA patients.
Specifically, the following patients exhibited high ALT readings: #97
(176); #106 (155); #114 (213) and #121 (545 IU/L); but all of these
decreased sharply during treatment. Similarly, in the following
patients the AST readings were high at baseline: #97 (107); #106 (288)
and #121 (809). There were no F/U values for Pt. #106 (who was later
found to have autoimmune chronic active hepatitis) but for the other
two, the AST values decreased precipitously with treatment (observations
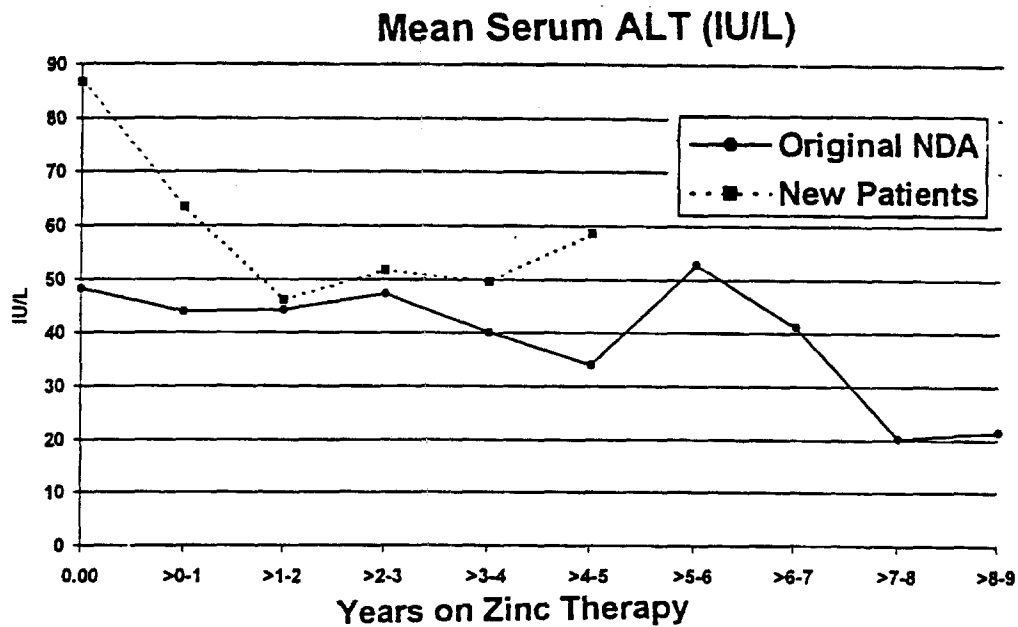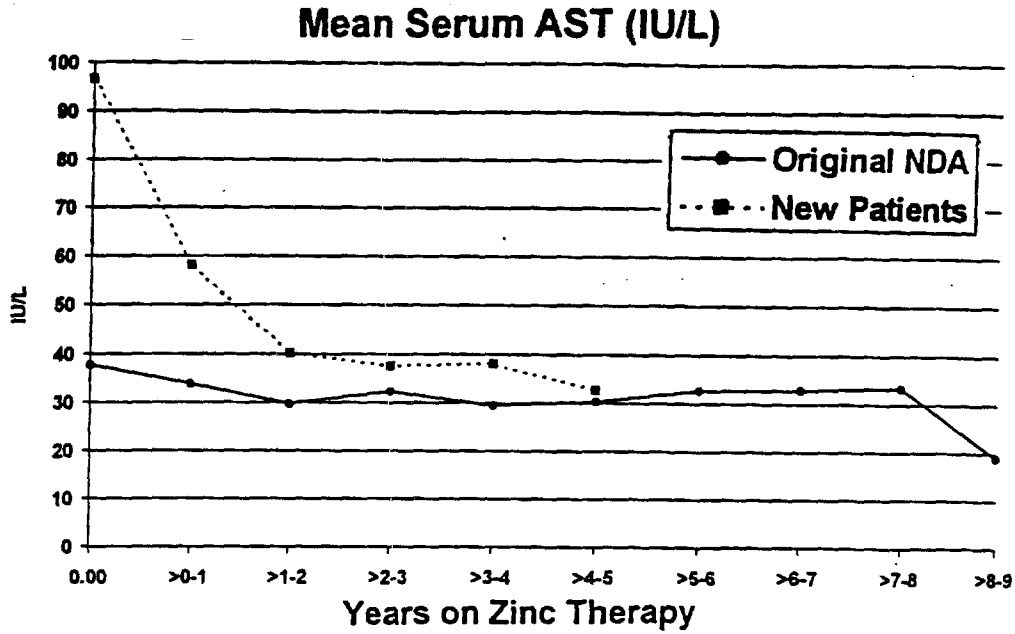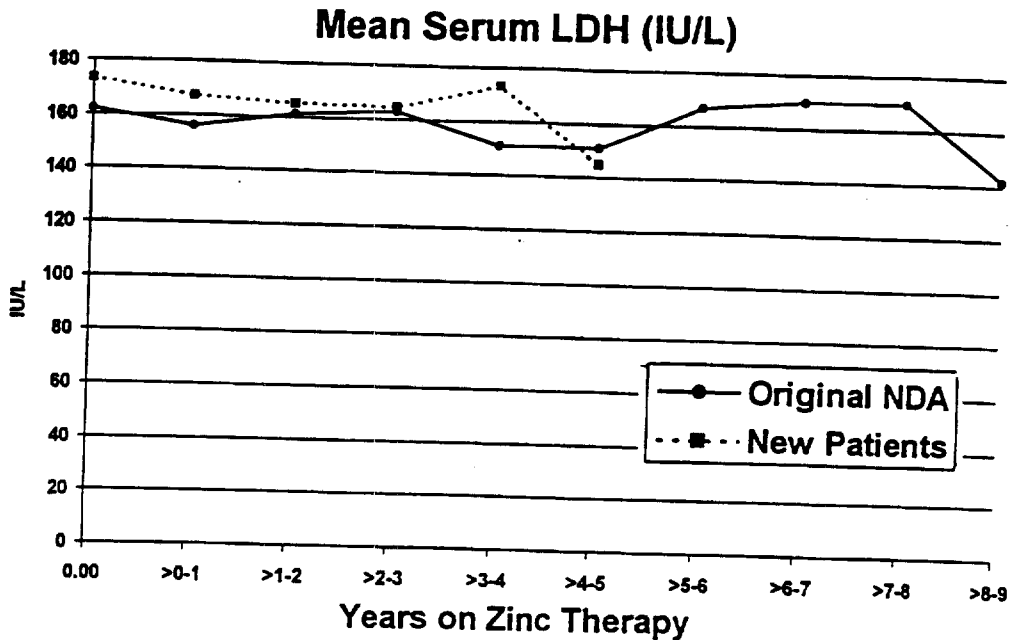at >1 to 2y).

Mean Serum ALT (IU/L)

Years on Zinc Therapy

Fig. 8. - _Post-NDA Continuation of the Brewer's Study:_
Mean Serum ALT (IU/l). Original NDA data plotted
from NDA Table D.81. New Patients data plotted
from sponsor's Table 11 of the 08/07/96 Report.

Mean Serum AST (IU/L)

Fig. 9. - Post-NDA Continuation of the Brewer's Study:
Mean Serum AST (IU/1). Original NDA data plotted
from NDA Table D.89. New Patients data plotted
from sponsor's Table 12 of the 08/07/96 Report.

● As shown in Table 5 and Fig. 10, for serum LDH there were essentially no differences between post-NDA and NDA patients. All means were below 200 IU/L.

Mean Serum LDH (IU/L)

Fig. 10. - Post-NDA Continuation of the Brewer's Study:
Mean Serum LDH (IU/L). Original NDA data plotted
from NDA Table D.105. New Patients data plotted
from sponsor's Table 13 of the 08/07/96 Report.

- For serum alkaline phosphatase (Table 5 and Fig. 11), mean values at >1
to 2y and >2 to 3y were somewhat higher for the new than for the NDA
patients. But means at all other times (Fig. 11) were similar in both
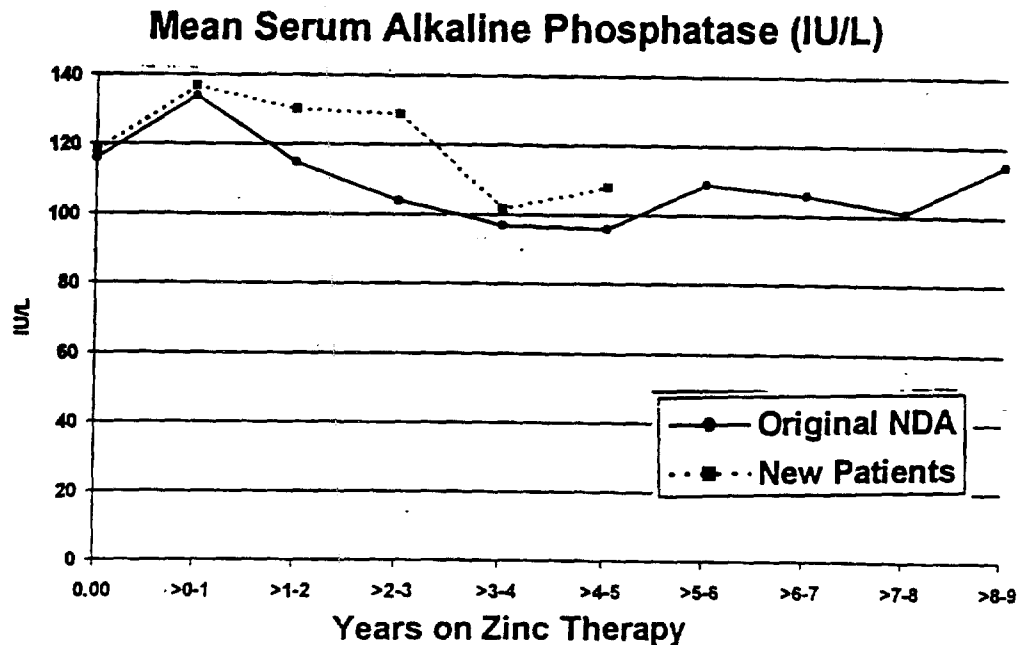groups of patients.

Mean Serum Alkaline Phosphatase (IU/L)

Years on Zinc Therapy

Fig. 11. - *Post-NDA Continuation of the Brewer's Study:
Mean Serum Alkaline Phosphatase (IU/L). Original
NDA data plotted from NDA Table D.113. New Patients
data plotted from sponsor's Table 14 of this Report.*

h. Results of Safety Evaluations

1) AEs Thought to be Related to ZnAC

The sponsor compiled the following list of 6 patients who had gastric
irritation with test medication. This occurred primarily with the first
morning dose. Each patient was told to take the medication in a different way
or time. This approach brought the problem under control.

Patients	Change that brought the problem under control
#101	Takes first dose mid-morning.
#109	Takes first dose mid-morning.
#111	Takes 25 mg 1 h after breakfast, 25 mg later in the morning, and then two 50 mg doses at regular times.
#118	Takes first dose mid-morning.
#124	Takes doses with Jello.
#143	Besides some intolerance of zinc this patient appeared to be not very interested in complying with various requirements of the program. She elected to discontinue zinc therapy.

2) **AEs Deemed Not Related to ZnAC Therapy**

The sponsor compiled the following list of 8 patients who experienced AEs that were deemed not related to therapy with ZnAC.

ADVERSE EVENTS DEEMED NOT RELATED TO ZnAC THERAPY

Patient	Adverse Event
#101	• Had an episode of variceal bleeding for which she was hospitalized for two weeks. This was due to her underlying cirrhosis and portal hypertension.
#124	• Fainting episodes over a period of a few months. These were later attributed to "stress".
#138, #141	• Both these patients (a brother and sister) were diagnosed as having multiple sclerosis in addition to having Wilson's disease. These diagnoses were made while the patients were on zinc therapy and they continue on zinc therapy. • They have had a 15 and 10 pound weight loss, respectively.
#140	• Has had an unintended weight loss of 15 pounds.
#143	• Has had an unintended weight loss of 30 pounds. • This patient is severely incapacitated because of his Wilson's disease.
#144	• Has been diagnosed as having HTLV-II virus infection. This appears to be asymptomatic.
#149	• This patient, who had been on penicillamine for many years, developed a mild tremor a few months after starting zinc. • There was no explanation for this event other than the possible coincident initiation of some other tremor-causing disease (such as Parkinsonism). • No diagnosis of another disorder has been made as yet.

Source: Sponsor's Addendums, p. 07 of 08/07/96 submission.

3) Changes in Serum Amylase and Lipase (Table 6)

In a fashion similar to the NDA patients, mean serum amylase values increased slightly after baseline (Table 6, Fig. 12). For serum lipase (Table 6) the post-NDA patients showed higher values at >0 to 1, >1 to 2 and >3 to 4 years (Fig. 13). But, as before, these relatively minor alterations of pancreatic function were not accompanied by clinical signs and/or symptoms of pancreatitis.
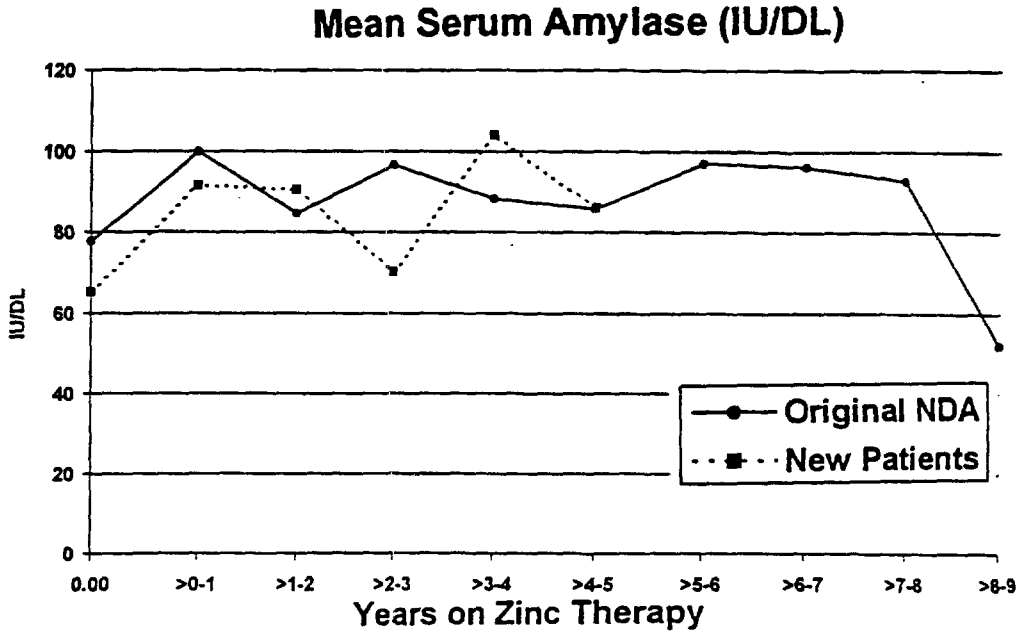
Mean Serum Amylase (IU/DL)

Fig. 12. - Post-NDA Continuation of the Brewer's Study: Mean Serum Amylase (IU/DL). Original NDA data plotted from NDA Table D.145. New Patients data plotted from sponsor's Table 15 of the 08/08/96 Report.

TABLE 6
The Brewer's Study
Post-NDA Continuation

Biochemical Markers of Pancreatic Function

BL	YEARS ON ZnAC THERAPY				
	> 0 to 1	> 1 to 2	> 2 to 3	> 3 to 4	> 4 to 5
A. MEAN SERUM AMYLASE (IU/dl)					
65 [n=20]	92 [n=33]	91 [n=39]	70 [n=18]	104 [n=10]	86 [n=4]
B. MEAN SERUM LIPASE (IU/dl)					
16 [n=20]	34 [n=33]	30 [n=39]	17 [n=18]	32 [n=10]	21 [n=4]

Mean Serum Lipase (IU/DL)
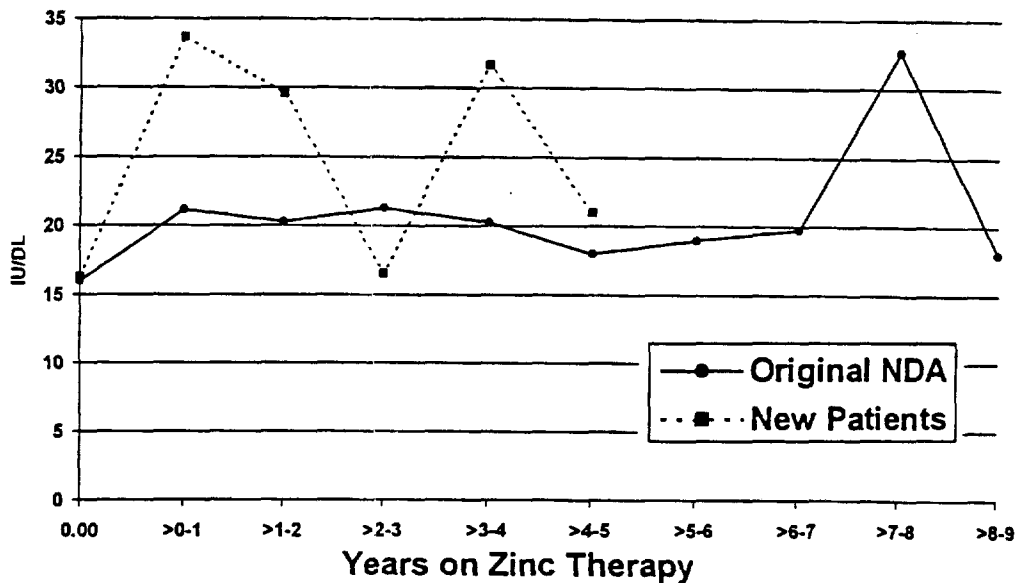

Fig. 13. - Post-NDA Continuation of the Brewer's Study:
Mean Serum Lipase (IU/DL). Original NDA data
plotted from NDA Table D.146. New Patients data
plotted from sponsor's Table 16 of the 08/07/96
Report.

7.   Reviewer's Conclusion

A review of the evidence presented by the sponsor in the post-NDA study by Dr.
Brewer (62 additional patients) demonstrates that ZnAC, at the orally

administered dose of 50 mg three times a day, is effective and safe for the maintenance of symptomatic Wilson's disease patients that have initially been treated with a chelating agent.

2 pages
PURGED

IV. RECOMMENDATIONS FOR REGULATORY ACTION

On the basis of the overall evidence at hand, the MO reiterates his
recommendations, as stipulated in the MOReview of January 27, 1995:

1. Approval of zinc acetate for the maintenance treatment of symptomatic
   Wilson's disease patients that have initially been treated with a
   chelating agent.

3. The recommended dose for 1. and 2. above is 50 mg three times a day.

4. The labeling should indicate that zinc acetate is effective and safe in
   the treatment of Wilson's disease women that become pregnant during the
   course of the disease. — *Checked w/ MO. Firm's FPL adequately addresses
   this concern. M. Merkul 1-27-97*

   NOTE: In order to facilitate a regulatory action on recommendation 1.
   above, the present review is being finalized.

                                                        November 1, 1996

                                                   Hugo E. Gallo-Torres, M.D., Ph.D.

CC:
NDA 20-458-BM
HFD-180
HFD-180/SFredd
HFD-180/HGallo-Torres
HFD-181/CSO
HFD-180/JChoudary

JAN 27 1995   , 7 |...

DIVISION OF GASTROINTESTINAL AND COAGULATION DRUG PRODUCTS

MEDICAL OFFICER'S REVIEW

NDA: 20-458

Date Submitted: June 21, 1994

Sponsor: Lemmon Company, PA

Drug: Zinc Acetate 50 and 25 mg hard gelatin
                                 capsules

Route of Administration: Oral

Pharmacological Category: Inducer of Copper Malabsorption

Proposed Indications: 1. Maintenance treatment of patients with
                                     Wilson's disease.

First Draft to Supervisor: 11/21/94

Date Review Completed: 1/25/95

Material Reviewed: **a) Initial Submission   Volume No.**
(Relevant to MOR)   1. Index to Application   1
                              2. Summary   1
                              5. Nonclinical Pharmacology/Toxicology   3
                              6. Human PKs and Bioavailability   4
                              8. Clinical Data   5-7
                             10. Statistical Section   8-10
                             11. Case Report Tabulations (Database) 11-12
                             12. CRFs (Appended to 8.)

                                 b) Submission dated October 24, 1994
                                      This contained tables of all
                                      parameters with data for symptomatic

Reviewer: '   Hugo E. Gallo-Torres, M.D., Ph.D.

REVIEW OF NDA 20-458
Table of Contents

I. BACKGROUND

Wilson's Dz is a recessively inherited metabolic disorder in which failure of copper excretion by way of the bile causes the metal to accumulate first in the liver and then in the brain, corneas, and kidneys, probably in that order. Excess metal in these tissues leads to chronic or occasionally more acute degenerative changes associated with the anticipated/predictable clinical signs and symptoms. The clinical picture is multiform. The disease may present as cirrhosis, subacute or chronic aggressive hepatitis, a predominantly hemolytic illness, or rarely, as acute hepatic failure with massive hemolysis (aptly named "fulminant Wilson's Dz"). Alternatively, the entire course of the illness may be one of progressive disintegration of the motor centers of the brain with or without personality changes. Rarely, the patient may present with metabolic bone disease or degenerative joint changes. What may be considered as the classic picture of a mixed hepatic-neurologic syndrome is, in practice, rather uncommon.

The Dz is probably not all that uncommon in the highly selective group seen by the pediatric hepatologist or by the neurologist handling patients between the ages of 10 and 30 years. The gene frequency may not be as rare as was previously believed; in East Germany an occurrence rate of 29 patients per million was reported, giving a gene frequency of 1 in 93. Prevalence data for Japan and the United States may be similar, and the frequency of the disease is highly unlikely to be lower elsewhere [I.H. Scheinberg, Lancet $\underline{1}$:1469 (1982)]. If these calculations are correct, there may be as many as 4000 cases in the U.S. alone [Scheinberg (1982) (locus cited)]. Moreover, if the majority remain undiagnosed, as seems likely, the resultant mortality approaches a major medical disaster and an untold well of human suffering.

Clinically, no case is a typical one of Wilson's Dz; all are different, although they may be variations on a theme. This applies as much to the hepatic cases as to the neurologic ones. The illness may be indistinguishable from acute hepatitis, subacute or chronic aggressive hepatitis, or symptomless cirrhosis diagnosed by finding hepatic and/or splenic enlargement on routine examination. With a mixed picture of hepatic disease and hemolysis, the diagnosis becomes highly probable in the absence of a history of poisoning. Hemolysis, which is Coombs' test-negative, may be low grade and chronic. More commonly it occurs as a series of crises at approximately monthly intervals, the hemoglobin seldom falling below 8 g/dl. Occasionally, hemolysis is associated with acute hepatic failure, giving the picture of "fulminant" Wilson's disease; such an illness can be rapidly fatal [S.J. Roche and T.P. Benhamou, Ann. Intern. Med. $\underline{86}$:301-303 (1977)]. Because of the pleomorphic nature of the liver lesion, the presenting symptoms are equally varied. The classic physical sign of this disease is the Kayser-Fleischer corneal ring[1]. This is always present when the nervous system is involved and usually, but not invariably, is found in the hepatic stage of the illness. It may or may not be present in pre-symptomatic patients. Copper deposition in the cornea is also said to occur in the syndromes of chronic familial cholestasis. But this has not been well documented; nor are there supportive slit lamp

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[1] The ring consists of a granular deposit of copper (probably a copper proteinate) in Descemet's membrane of the cornea. It usually is brown but occasionally is gray or perhaps green. The initial location is in the top crescent, from 11 to 1 o'clock, and then in the inferior crescent, from 5 to 7 o'clock. At first a faint brown smudge, the ring slowly spreads both laterally and centrally until it becomes a complete ring measuring up to 5 mm broad and eventually becoming heavily pigmented [J.E. Cairns et al., Trans. Ophthal. Soc. U.K. $\underline{90}$:187-190 (1971)]. A complete ring can be easily seen over a blue iris with the naked eye, and some patients can see their own rings in the mirror. Over a brown iris, the brown pigment may be detected without the aid of a slit lamp only by an experienced observer. The absence of copper deposition in the cornea of a patient who has only liver damage does not rule out the diagnosis of Wilson's disease.

photographs and postmortem histologic studies [C.R. Fleming et al., Ann. Intern. Med. 86:285-288 (1977)]. The rings seen in these cases are very attenuated and can only be seen with a slit lamp.

Hepatic Pathology

The earliest histologic changes found in the liver of a patient with Wilson's Dz are fatty droplets in the cytoplasm of the hepatocytes, inflammatory infiltration in the portal tracts, and prominent "ballooned" glycogen-containing nuclei [F. Schaffner et al., Amer. J. Pathol. 41:315-327 (1962)] although the significance of the last finding is disputed. At this stage the excess copper is diffusely distributed in the cytosol of the hepatocytes, never in the Kupffer cells. As the lesion progresses, fat becomes more prominent and the inflammatory cells spread into the lobules, usually leading to a slowly progressive cirrhosis. On occasion, the inflammatory exudate becomes prominent and is associated with PMN; the picture can become typical of chronic aggressive hepatitis. More often, the inflammatory reaction becomes quiescent, progresses slowly to cirrhosis, and eventually produces portal hypertension with all its various complications. Fulminant Wilson's disease with hepatitis and hemolysis that progresses rapidly to death is rare.

As the liver lesion evolves, copper is removed from the cytosol and becomes concentrated in electron-dense lysosomes [S. Goldfischer and I. Sternlieb, Amer. T. Pathol. 53:883-907 (1968)]. At this stage it is much easier to demonstrate the copper by cytochemical techniques[2]. The classic final picture of the liver in the more chronic forms of neurologic and mixed Wilson's disease is post-necrotic cirrhosis. Occasionally, the liver shows only relatively minor abnormalities.

Laboratory Findings

These are based on the concentration of ceruloplasmin in serum and the levels of copper in serum, urine and liver.

Ceruloplasmin is a metalloprotein of fixed, non-exchangeable copper content [I. Sternlieb et al., J. Clin. Invest. 40:1834-1840 (1961)] of approximately 0.3% [S.H. Laurie and E.S. Mohammed, Coordn. Chem. Rev. 33:279-314 (1980)]. In addition, there normally is a small percentage (<10%) of non-ceruloplasmin copper ("free copper") so that serum with a concentration of 30 mg of ceruloplasmin/dl must contain not less than 90 μg of copper, allowing for "free copper" of up to 10 μg, bringing the total to 100 μg/dl. However, much of the copper in the serum in patients with untreated Wilson's Dz is "free", and total copper is far in excess of the expected ceruloplasmin copper. This situation is corrected once the patient has had the excess metal removed by Tx; total serum copper then should closely match the ceruloplasmin copper. In patients with only low initial ceruloplasmin concentrations, the values will probably fall to zero and the copper level to a correspondingly low figure, 10 μg or less.

One problem relating to the copper/ceruloplasmin determinations is the wide range of variation that is found; concentrations of ceruloplasmin in normal persons overlap those in heterozygotes, and these, in turn, overlap those in patients with Wilson's Dz (Fig. 1). Thus, while the statistical difference between normal persons and patients is highly significant, the small degree of

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[2] Orcein staining for copper-associated protein [M. Salaspuro and P. Sipponen, Gut 17:787-790 (1976)] is not a prominent feature of the liver in Wilson's disease and, when present, is a late phenomenon. The orcein staining in primary biliary cirrhosis is always periportal in distribution, whereas in Wilson's disease it may be found more widely distributed around the lobule.

overlap between the 2 groups still leaves problems for the individual case.
J.M. Walshe has published guideline figures for patients, heterozygotes, and
normal persons (Table 1).
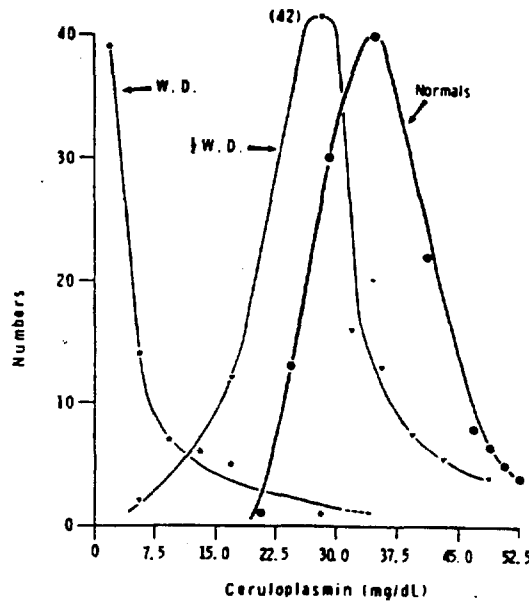
Fig. 1. - *A comparison of the concentrations of*
ceruloplasmin found in the sera of patients
with untreated Wilson's Dz, obligate
heterozygotes, and normal controls. Note
the marked range of overlap among the 3 groups.

TABLE 1

Approximate Guidelines for Copper Values in Controls,
Heterozygotes, and Patients with Wilson's Dz Before Tx

	Controls	Heterozygotes	Patients With Wilson's Dz
Serum copper (µg/dl)	90-150	60-150	10-100
Serum ceruloplasmin (mg/dl)	24-45*	5-45	0-30
Urine copper (µg/24 h)	<30	<50	80-800
Liver copper (µg/g wet Wt)	<10	<50	>50

a) M: 33 mg/dl ± 6 (SD);   F: 36 mg/dl ± 9 (SD)

Imaging studies with radionuclides, CT tomography or MR contribute relatively
little to the diagnosis of Wilson's Dz, and none can substitute for the
indices in Table 2 (E. Schiff).

TABLE 2

Minimal Pair of Criteria Sufficient to Confirm a Diagnosis of Wilson's Dz in Untreated Patients

	Serum Ceruloplasmin <20 mg/dl	Hepatic Copper >250 µg/g dry Wt	Kayser-Fleischer Rings	Radiocopper Incorporation
Asymptomatic	+	+	-	-
	+	-	+	-
	-	+	-	+
Hepatic	+	+	-	-
	+	-	+	-
	-	+	-	+
Neurologic	+	-	+	-
	-	-	+	+
Psychiatric	+	-	+	-
	-	-	+	+

[E. Schiff (1993)]

Ranges of Copper Concentrations in Three Tissues of Untreated Patients

Tissue	Wilson's Dz (µg/g dry Wt)	Normal (µg/g dry Wt)
Liver	94-1828	9-47
Brain	189- 362	13-39
Kidney	46- 166	5-36

As discussed in more detail below, knowing the duration of the patient's illness and what therapy, if any, he/she has received are essential in interpreting results of copper values in blood, urine, and liver. Without such information, drawing valid conclusions is impossible [K. Gibbs et al., Q.J. Med. 47:349-364 (1978)].

Interpreting ceruloplasmin results in patients with liver Dz must take into account that liver Fx of any etiology in its terminal stages may be associated with low concentrations of ceruloplasmin resulting from a Fx of the synthetic processes in the hepatocytes [J.M. Walshe and J. Briggs, Lancet 2:263-265 (1962)]. Under these circumstances, a discrepancy between the ceruloplasmin level and the serum concentration of copper is unlikely, as is abnormal urinary copper excretion. Another difficult diagnostic problem can arise in determining whether an individual is in the pre-symptomatic stages of Wilson's Dz or is heterozygous but with a very low concentration of serum ceruloplasmin. According to Walshe, to rely on the response to a provocative dose of penicillamine, as is often done, does not necessarily clarify the issue; some pre-symptomatic patients have only a very modest cupriuresis if the tissues have not reached the saturation point with the metal. The most reliable single test in this situation is an estimate of the copper concentration in the liver. The finding of abnormal liver function is a valuable pointer, but again these test results may be normal or only mildly disturbed early in the evolution of the illness. Studies using radioactive copper can be helpful but require much experience; such tests are best carried out by a practiced investigator.

Liver tests used routinely both for Dx and F/U purposes are equally applicable
in the management of Wilson's Dz.

In summary, Dx of Wilson's Dz, suspected on the basis of family or individual
Hx, P.E. or a low serum concentration of ceruloplasmin, is confirmed by the
demonstration of Kayser-Fleischer rings or, particularly in the asymptomatic
pt., by the quantitative demonstration of a liver Bx specimen of a
concentration of copper in excess of 250 μg/g dry Wt.

Treatment

[Part of this section was taken from Vol. 1, Seventh Edition (1993) Diseases
of the Liver book by the Schiffs.]

The goal of Tx is to remove the excess copper from the liver and from other
organs, prevent its reaccumulation and achieve a return to as normal hepatic
and cerebral function as possible. Two effective metal chelating agents –
penicillamine (CUPRIMINE®) and triethylene tetramine (trientine HCl, SYPRINE,
formerly CUPRID) – serve the purpose of achieving and maintaining a negative
copper balance. The specific wording in the Indications Section of the
penicillamine labeling is reproduced below:

"Tx has two objectives:

    (1) to minimize dietary intake and absorption of copper
    (2) to promote excretion of copper deposited in tissues.

"The first objective is attained by a daily diet that contains no more than
one or two milligrams of copper. Such a diet should exclude, most
importantly, chocolate, nuts, shellfish, mushrooms, liver, molasses, broccoli,
and cereals enriched with copper, and be composed to as great an extent as
possible of foods with a low copper content. Distilled or demineralized water
should be used if the patient's drinking water contains more than 0.1 mg of
copper per liter.

"For the second objective, a copper chelating agent is used. In symptomatic
patients this treatment usually produces marked neurologic improvement, fading
of Kayser-Fleischer rings, and gradual amelioration of hepatic dysfunction and
physic disturbances.

"Clinical experience to date suggests that life is prolonged with the above
regimen.

"Noticeable improvement may not occur for one to three months. Occasionally,
neurologic symptoms become worse during initiation of therapy with CUPRIMINE.
Despite this, the drug should not be discontinued permanently, although
temporary interruption may result in clinical improvement of the neurological
symptoms but it carries an increased risk of developing a sensitivity reaction
upon resumption of therapy.

"Treatment of symptomatic patients has been carried out for over ten years.
Symptoms and signs of the disease appear to be prevented indefinitely if daily
treatment with CUPRIMINE can be continued."

The wording in the Indications Section of the trientine HCl labeling reads:

    "SYPRINE is indicated in the Treatment of patients who are
    intolerant to penicillamine..."

ADRs with penicillamine include: significant proteinuria, nephrotic syndrome, thrombocytopenia, granulocytopenia, pemphigus, myasthenia, lupus, Goodpasture's syndrome, or severe arthralgias. Those with trientine HCl include: iron deficiency (anemia), and SLE in Wilson's Dz pts. [and many others in PBC pts.]. In addition, both penicillamine and trientine HCl are teratogenic in animals. Penicillamine was shown to be teratogenic in rats which given in doses 6 times higher than the highest dose recommended for human use. Skeletal defects, cleft palates and fetal toxicity (resorption) have been reported. There are no controlled studies on the use of penicillamine in pregnant women. Trientine HCl was teratogenic in rats at doses similar to the human dose. The frequencies of both resorptions and fetal abnormalities, including hemorrhage and edema, increased while fetal copper levels decreased when trientine HCl was given in the maternal diets of rats. There are no adequate and well-controlled studies on the use of trientine HCl in pregnant women. CUPRIMINE® and SYPRINE should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

II. RATIONALE

In Wilson's Dz, the biochemical lesion is related to an abnormal handling of copper by the body.

Copper is a trace element essential for life. It is present in the diet in amounts varying from 2 to 5 mg daily [I.H. Scheinberg and I. Sternlieb, Pharmacol. Rev. 12:355-381 (1960)]. Inorganic copper is rapidly absorbed from the stomach or upper small intestine and carried by way of the portal blood to the liver. This organ has a very efficient trap for the metal; the copper is rapidly removed from the circulation with about 70% of an absorbed dose being trapped within 12 h. Once in the liver, the metal may follow one of 3 courses: (1) it may remain in the liver bound to a metallothionein as storage copper; (2) it may be incorporated into the serum copper protein, ceruloplasmin; or (3) it may be excreted in the bile, the main excretory route for the metal. A small percentage of copper is carried in the plasma bound to albumin and amino acids; this is probably the exchange copper that enters the tissues for incorporation into cytochrome and other copper enzymes such as tyrosinase, monoamine oxidase, superoxide dismutase, and cerebrocuprein.

According to most investigators [I. Sternlieb et al., Gastroenterology 64:99-104 (1973)], deposition of excessive amounts of copper in several organs and tissues is the generally accepted cause of the pathologic changes which characterize Wilson's Dz. The pathogenetic mechanism which leads to the retention of the metal, although not known, is likely to involve one of the following: (1) increased absorption of dietary copper, (2) abnormally elevated tissue-protein binding affinity for copper, (3) diminished excretion of ceruloplasmin-bound copper associated with diminished ceruloplasmin synthesis, or (4) diminished biliary excretion of copper. Higher than normal association constants for copper of intracellular proteins have never been convincingly demonstrated, although the greater tissue concentrations of the metal result in greater concentrations of bound copper. Normally, each day the liver synthesizes ceruloplasmin containing 0.5 mg of copper, an amount roughly equal to the copper absorbed from the g.i. tract. Thus, a complete block in

ceruloplasmin synthesis[3] could result in retention of this much copper daily,
but such a mechanism cannot be the sole cause of copper retention in Wilson's
Dz because of the occurrence both of hypoceruloplasminemia, occasionally even
aceruloplasminemia, in 10% of heterozygotes without elevated or toxic amounts
of tissue copper; and of normal ceruloplasmin concentrations in rare patients
with Wilson's Dz. Bile appears to be the major physiologic route of excretion
of copper from the liver. Aside from the genetic disorder, Wilson's Dz, that
the above-mentioned study by Sternlieb et al. investigated, only an
obstructive process of long duration, such as biliary cirrhosis or atresia,
has been associated with significant hepatic retention of copper. Data
obtained in three different laboratories suggest that the genetic defect of
Wilson's Dz acts by diminishing biliary copper excretion and may be
responsible for the excess body copper in this illness. How the abnormal
copper-binding protein in the liver [that is supposed to be the primary
metabolic defect], leads to the toxic accumulation of the metal in other
tissues once the protein has become saturated is not clear unless there is
also an excretory defect.

As previously mentioned, copper, in the majority of patients with Wilson's Dz,
is not incorporated into ceruloplasmin, and this protein is deficient or
absent in the serum. The significance of this is not obvious, as the normal
physiologic role of ceruloplasmin has not been defined. Ceruloplasmin does
not appear to be essential for life, since many patients with well-treated
Wilson's Dz but with zero levels of this protein can live normally and without
apparent disability, provided they continue to take a chelating agent. What
is probably more germane to the pathogenesis of the disease is that the
biliary excretion of copper is also defective, so that not only is excretion
markedly reduced compared with normal, but the pattern of excretion also is
different [K. Gibbs and J.M. Walshe, Lancet 2:538-539 (1980)]. Thus, even if
the reduced rate of excretion of labeled copper in the bile were due to
dilution in an expanded body pool of the metal, a secondary rise (as seen in

---

[3] Deficiency of the plasma copper protein ceruloplasmin, caused by impaired synthesis of the protein,
is exhibited by 96% of Wilson's Dz gene homozygotes, as illustrated in the following Table:

Concentration of Ceruloplasmin in Serum of
Symptomatic Patients with Wilson's Dz at Diagnosis

Ceruloplasmin[a] (mg/dl)	Patients
0-0.9	121 (27%)
1-4.9	114 (25%)
5-9.9	107 (24%)
10-14.9	57 (13%)
15-19.9	30 (7%)
20-29.3	20 (4%)
TOTAL	449 (100%)

a) Normal 20-40 mg/dl

and by ca. 20% of heterozygous carriers of one Wilson's Dz gene, none of whom ever exhibits any clinical
stigmata of the disease. Ceruloplasmin is not the primary product of the Wilson's Dz gene since the gene for
ceruloplasmin is on chromosome 3.

At diagnosis, about 4% of patients with indubitable Wilson's Dz have ceruloplasmin concentrations greater than
20 mg/dl (WNR), although they are pathologically and clinically indistinguishable from the 96% of patients with
ceruloplasmin concentrations less than 20 mg/dl. In some patients, pregnancy, inflammation and certain
medications may account for a transient ↑ of the ceruloplasmin concentration into the low normal range of 20
to 30 mg/dl.

the normal person) is absent. This suggests that the primary genetic defect, the missing enzyme, would seem to occur somewhere between uptake of copper by the liver and its excretion from the hepatocyte into the biliary canaliculi. Patients with PBC whose livers may contain as much or more copper than patients with Wilson's Dz never develop neurologic symptoms from deposition of the metal in the brain. Moreover, even though their livers are approaching saturation with copper, the labeled metal can be shown to be incorporated into ceruloplasmin at a rate not much slower than normal [Ibid, Clin. Sci. 41:189-202 (1971)]. Presumably, therefore, the process of incorporating copper into ceruloplasmin in some way protects the tissues against copper toxicity; perhaps a common step is involved both in ceruloplasmin synthesis and in processing the metal for excretion by way of the bile.

The sponsor of the present NDA is seeking approval of zinc acetate to prevent [in patients with Wilson's Dz] the absorption of copper from the diet as well as the reabsorption of endogenously secreted copper such as that from the saliva and gastric juice. It is to be noted that the proposed site of action of Zn Acetate is in the enterocyte. In the intestinal cell the metal is expected to induce the production of metallothionein (MT), a protein that binds copper thereby preventing its serosal transfer into the blood. According to this approach, which is different from the metal-chelating properties of penicillamine and trientine HCl, the bound copper is then lost in the stool when the intestinal cell is sloughed. It is important to point out that Zn acetate is not proposed as a first line drug for symptomatic individuals. In other words, the Wilson's Dz patients must be initially treated with a chelating agent and then maintained with the salt of the metal.

Subject matters related to the scientific principles on which the use of Zn acetate is founded include bioavailability of copper, bioavailability of Zinc, and the many factors which may influence bioavailability of this metal.

One reason for the detailed review of abnormalities of copper metabolism in Wilson's Dz is two-fold. Although a decrease in copper absorption by Zn acetate may be demonstrated, it is not universally accepted that Wilson's Dz patients absorb any greater proportion of orally administered radiocopper than the control subjects. Dietary Cu, in general, is not expected to influence Cu levels in tissues. This is because, under normal circumstances, the amount of Cu in the diet is minimal, especially since, as mentioned above, these patients are already advised to be on a low copper diet. What this reviewer is saying is that maintenance [of the Wilson's Dz patient that is off chelating medication] cannot be judged on the basis of effects on copper absorption alone. Clinical data, demonstrating that the patients' signs and symptoms are not getting worse [after several years on Zinc] are as or more important than biochemical data on copper.

III. ZINC

Zinc is a nutritional trace element, necessary in the body (and hence in the diet). It forms an essential part of many enzymes (e.g., carbonic anhydrase, important in carbon dioxide metabolism) and plays an important role in protein synthesis and in cell division. Deficiency of Zn is associated with anemia, short stature, hypogonadism, impaired wound healing, and geophagia. Many Zn salts are used in medicine[4]. Five salts of Zn (chloride, gluconate, oxide,

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[4]
ex. acetate=styptic, astringent, emetic; caprylate, propionate=fungicide; chloride=astringent; citrate=used in toothpaste and mouthwash; insulin=antidiabetic; iodide=topical antiseptic, astringent; oxide=astringent, topical protectant; permanganate, tannate, stearate, salicylate, peroxide=antiseptic, astringent; sulfate=ophthalmic astringent, Zn supplement (ex. in TPN).

stearate and sulfate) (but not acetate) are listed in the CFR as GRAS nutrients. In comparison with other trace elements such as lead, cadmium, arsenic, and antimony, zinc is relatively nontoxic. Zinc is noncumulative and the proportion absorbed is thought to be inversely related to the amount ingested. Ingestion of high amounts of Zn sulfate (i.e. 12 g x 2 days) was characterized by drowsiness, lethargy, and increased serum concentrations of lipase and amylase. In pts. with renal failure following hemodialysis, N&V, fever and severe anemja have been observed. In addition to N&V, symptoms of Zn toxicity in humans[5] include dehydration, electrolyte imbalance, abdominal pain, nausea, lethargy, dizziness, and muscular incoordination.

IV. NONCLINICAL PHARMACOLOGY AND TOXICOLOGY

At this point in time there is no Pharmacology Review available. The sponsor's Summary is not very helpful because it is mostly qualitative. Few data in support of statements are provided. Reference is made to Zn "salts" as if all of these were equivalent. This may be so for some Zn salts, but no concerted approach to demonstrate equivalence has been submitted. Some of the findings are being attributed to zinc-induced copper deficiency but no evidence of how was deficiency demonstrated is presented. Reference is made to findings with several zinc salts (carbonate, chloride, oxide, sulfate). But the acetate, the salt of interest, does not appear to have been studied much. According to the Merck Index, for Zn acetate, the LD_{50} orally in rats is 2.46 g/Kg. Carcinogenicity studies (one species of animals only=mice) with Zn chloride and Zn sulfate have yielded controversial results. In rats given 2 to 6.3 mg/day Zn acetate in the diet for 29 weeks, no significant effects on fertility or health of parents or offspring were noted when this salt of Zn was given prior to mating and during gestation and lactation.

V. PHARMACOKINETICS/PHARMACODYNAMICS

In a fashion similar to section IV. above, there is no Biopharm. review available. Some of the material that follows was taken from the sponsor's summary of the NDA application. The reviewer emphasis is on factors that may interfere with the bioavailability of zinc acetate, data on equivalence between this salt and zinc sulfate, the effects of zinc acetate on oral ^{64}Cu absorption and the effects of zinc acetate on copper balance. Once again a number of the studies cited by the sponsor used several salts of zinc, not necessarily the acetate. But except for Study 1, all other data refer to Zn acetate.

A. Generalities: Zn as Micronutrient

The following was taken from Harrison's Textbook of Medicine. It is known that Zn absorption in the small intestine is <u>decreased</u> by fibers, phytate, phosphate, Ca. and Cu. In contrast, amino acids, peptides, iodoquinol and other chelating agents <u>increase</u> Zn absorption. Excretion of Zn occurs principally through secretions of the pancreas and intestine. Nearly 99 percent of total-body Zn is inside cells, the remainder is in plasma and extracellular fluids. Serum Zn, approximately 80% of which is loosely bound to albumin and other proteins, is the source of metal for cellular needs.

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[5] Acute renal failure caused by Zn chloride poisoning has been reported. The symptoms occurred within hours after large quantities of zinc were ingested. Death has occurred after the ingestion of 45 g of Zn sulfate. This dose is very massive in view of the fact that the daily requirement of Zn for man is considered to be in the range of 15 to 30 mg per day. In patients with sickle cell disease, 660 mg of Zn sulfate has been administered orally for nearly 1 year without any adverse effects.

Serum Zn content does not normally vary, but it decreases when intake or absorption is reduced (e.g., in regional enteritis) or when urinary losses are increased (e.g., in nephrotic syndrome; in cirrhosis of the liver or other hypoalbuminemic states; during the administration of penicillamine or other chelating agents; in high catabolic states as after trauma, burns or surgery; and in hemolytic anemias and sickle cell disease)[6]. Tissues with a high cellular turnover, including skin, gastrointestinal mucosa, chondrocytes, spermatogonia and thymocytes are characteristically affected in cases of Zn deficiency. The dermatologic abnormalities (hyperkeratosis, parakeratosis, acrodermatitis and alopecia) call attention to the possibility of Zn deficiency.

B. Absorption of Zinc (Study 1)

The sponsor states that the appearance of Zn in the peripheral blood has been used as a measure of the absorption of zinc, and is referred to as the zinc tolerance test. Study 1 is mentioned but no details about the protocol and study execution of this study are given. It is claimed that plasma levels of Zn after single oral doses in normal subjects demonstrated the similarity of Zn acetate and Zn sulfate and the "lesser absorption" of Zn carbonate. These data are reproduced in Fig. 2.
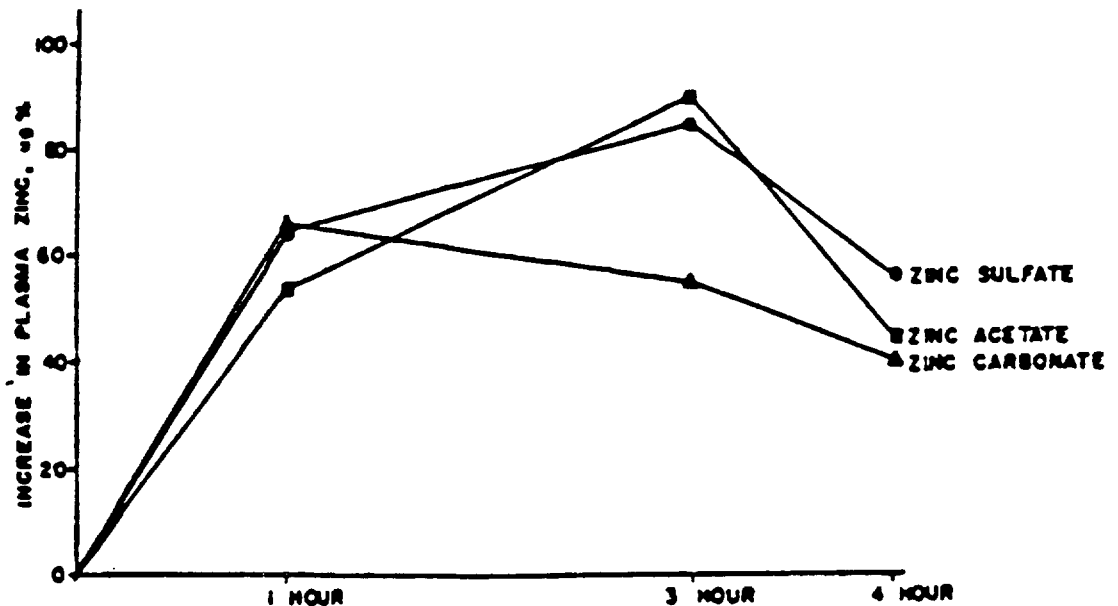
Fig. 2 - Study 1a: Zinc tolerance curves for three zinc salts, administered at the oral dose of 25 mg elemental zinc.

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[6] Plasma Zn also decreases in the acute phase of myocardial infarction, infections, malignancies, and hepatitis and other diseases. The decreases may be due to redistribution from plasma to tissues and are probably mediated by ACTH, cortisol, and/or a leukocyte protein (leukocyte endogenous mediator). Clinical deficiency may follow these decreases in serum content. The Zn requirement of the developing fetus, pregnant woman, and growing child or adolescent is higher than that of adult men or nonpregnant women. Therefore, the former groups are more susceptible to Zn depletion. Zn deficiency in pregnant animals can lead to fetal Zn deficiency, which results in high mortality rates or congenital malformations of nearly all organ systems. Zinc deficiency has not been described in pregnant women, but has been reported in adolescents who eat dirt, in patients who receive TPN without supplemental Zn, and in patients with the autosomal recessive defect acrodermatitis enteropathica. In the latter disease, deficiency in plasma Zn may be the consequence of a defect in Zn absorption. The onset of symptoms often occurs when an affected infant is weaned from human to cow's milk. Zn may also play a role in the maintenance of normal taste and in wound healing.

Comparison of AUCs at 3h

Zn Salt (No. of Subjects)	AUC[a] (%)	3-h Plasma Zn Concentration[b] (µg % ± S.D.)	p-value[c]
Sulfate (N=11)	100	186 ± 25	
Acetate (N=8)	92	196 ± 25	NS, p<0.05
Carbonate (N=5)	79	148 ± 35	p<0.01

a) AUC expressed as a percent of the area under the Sulfate curve. These areas reflect the increase in plasma Zn above BL values.
b) These are the actual values, not the increase over BL values (which averaged 98 µg % in these subjects.
c) Student's t-statistics (paired).

NOTE: This is important information because one of the studies used Zn acetate. In the other, the sulfate salt was used. The sponsor claims that the observations summarized in Fig. 1 support the premise that the acetate and sulfate salts of Zn are equivalent in their ability to provide adequate amounts of Zn after oral administration. Although this seems to be so on the basis of the clinical data, it is worth noting that the comparisons of AUCs give only a rudimentary picture regarding bioavailability of the acetate vs the sulfate salts (T_{max}, V_{max}, $T_{1/2}$, etc., etc. were not calculated). Equally important, it is not known if blood levels are an adequate approach to determine Zn bioavailability. Finally, since the proposed mechanism of action is an effect on copper uptake by the intestinal cell it would seem that important data should be either [intracellular] levels of Zn at the level of the enterocyte or intracellular levels of the induced metallothionein (intestinal cell MT), see below.

C. Factors Influencing Absorption of Zinc Salts (Study 1b)

In their Fig. C.2., the sponsor presented a diagrammatic representation (bars) of the effect of a number of substances on relative plasma zinc concentrations at 1, 2 vs 3h after oral ingestion of Zn sulfate (25 mg elemental Zn). Breads, vegetables, fruits and eggs and beverages such as milk and coffee appeared to interfere with the absorption of the metal.

NOTE: It is not known if these substances have the same effect on the absorption of zinc from Zn acetate. Details of the experimental conditions are not given. The sponsor states that because of these effects the oral doses of zinc in the clinical trials were always separated from food and beverages, other than water, for at least one hour.

D. Distribution, Metabolism and Excretion

Although Zn in the plasma is bound mainly to albumin and ceruloplasmin, other proteins such as α-macroglobulin, transferrin, haptoglobin, and γ globulin also bind small amounts of Zn. Liver, kidney, bone, retina, prostate, and muscle appear to be rich in zinc [A.S. Prasad, Ann. Rev. Pharmacol. Toxicol. 20:393-426 (1979)]. The liver is the main storage organ for Zn. Results of an attempt to characterize the pattern of Zn storage by the liver are summarized in Table 3. Depicted are the mean liver Zn concentrations for up

to 9 years in one of the L-T trials. After a period of initial elevation to
levels a little over 2-fold BL during the first 2 years, hepatic zinc
concentrations plateau and tended to come down in later years. This pattern
was also observed when the data were displayed for the subset of 5 patients
who have been studied at all times during the first three years (Fig. 3).

<div align="center">

TABLE 3
(Study 3)

Liver Zinc Data* vs Years of Zinc Acetate Therapy

</div>

	YEARS OF ZINC THERAPY								
BL (N=35)	0-1 (N=22)	1-2 (N=14)	2-3 (N=6)	3-4 (N=5)	4-5 (N=4)	5-6 (N=4)	6-7 (N=3)	7-8 (N=0)	8-9 (N=1)
431	1323	1202	1150	782	428	822	452	---	988
(180)	(719)	(523)	(407)	(585)	(174)	(220)	(221)	---	---

a) Depicted are the mean liver Zn concentration (± S.D. mean) values in μg zinc/g dry weight of tissue

E.   Zn Balance During L-T Zn Acetate Maintenance Therapy (Study 4)

Data on Zn balance during L-T therapy with Zn acetate in Wilson's Dz patients,
are summarized in Table 4. Zn balance tended to be positive during the first
year. A likely explanation for this finding is that many of the patients were
deficient in Zn due to prior Tx with penicillamine. After the first year, the
Zn balance stabilized at about +10 to +15 mg/day. It is important to
introduce a correction factor.

Undetectable losses, primarily from the skin surface, are estimated at about
10 to 15 mg/day in Wilson's Dz patients undergoing Zn therapy. According to
the data in Table 4, Zn balance become neutral after the first year and
remains so during L-T Tx. This pattern was consistent with the Zn levels in
the liver over time. According to the sponsor, these observations are
evidence that progressive Zn storage does not occur with chronic
administration of Zn to Wilson's Dz patients.
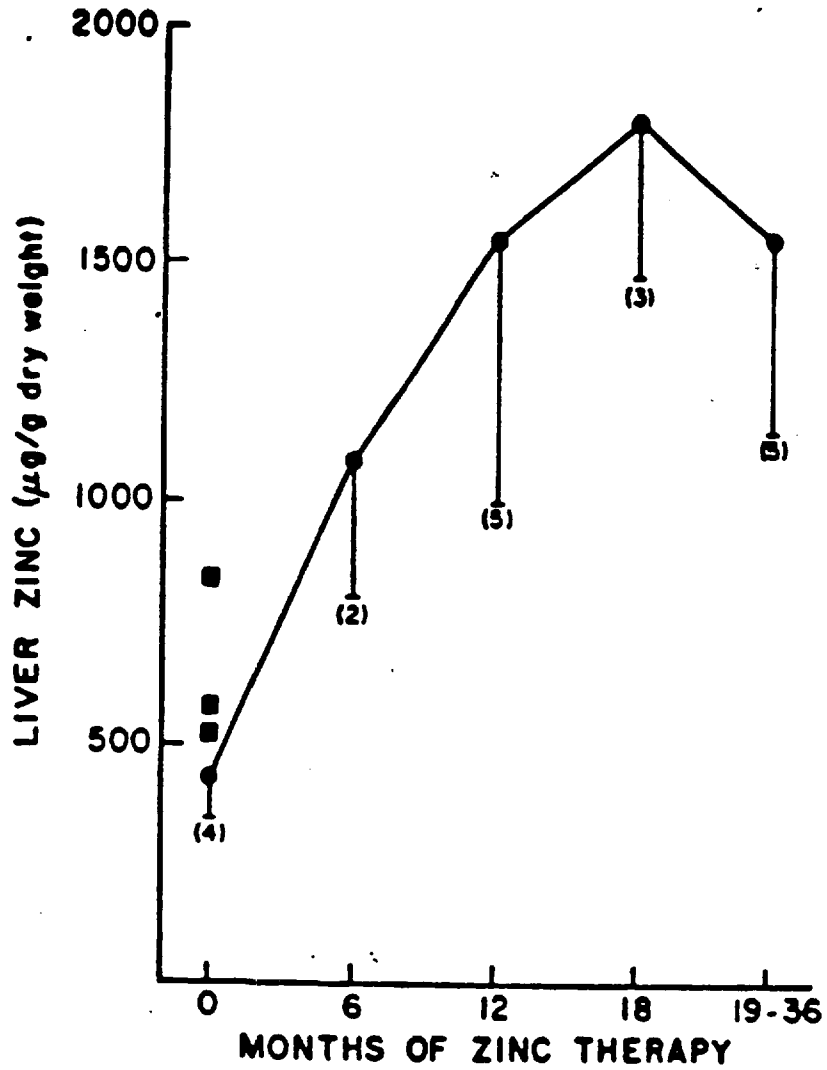
Fig. 3 - Study 3: Hepatic Zn concentrations from liver Bx specimens
of patients with Wilson's Dz (●) as a function of months of
Tx and three control values (■). ( ) = N for each period.

TABLE 4
Study 4

Zinc Balance (mg/day) During Zinc Acetate
Maintenance Therapy

YEARS OF THERAPY									
0 (N=15)	0-1 ((N=37)	1-2 (N=15)	2-3 (N=12)	3-4 (N=10)	4-5 (N=7)	5-6 (N=4)	6-7 (N=4)	7-8 (N=0)	8-9 (N=1)
-7.2	12.4	-1.5	6.0	37.0	20.0	25.9	-13.7	---	-8.3
(23.0)	(31.1)	(12.6)	(18.4)	(88.9)	(12.4)	(3.9)	(41.9)	---	---

( ) = S.D.

F. Pharmacodynamics

1. Effects on ^{64}Cu Uptake and Copper Balance (Study 6)

- The 50 patients with Wilson's Dz (23 M and 27 F) included in this study were well decoppered and in the maintenance phase of therapy with Zn.

- In all cases the patient was on the study therapy for at least 6 weeks to allow adequate time for the washout of the effect of the previous dose.

- The measurements included a 10 day Cu balance, ^{64}Cu uptake as often as possible, and usually 24h urine zinc and plasma Zn.

- The criteria for effectiveness of a Zn regimen were

   a) a copper balance of less than +0.25 mg copper/day[7] and
   b) ^{64}Cu uptake of 1.2% or less of the administered dose.

   Values not more than 25% above these values were considered "marginal".

- According to the publication by G.M. Hill et al. [Amer. J. Med. Sci. 292:344-349 (1986)] in the case of the ^{64}Cu test, a value of 1.2% or less is found in patients who are in neutral or negative Cu balance.

- Results from these evaluations are summarized in Table 5. Eleven (11) different regimens of Zn acetate were studied. Except for the 50 mg t.i.d. regimen (N=31) observations with the other regimens included few patients. For some the observations were too few (e.g., N=4 or less) to allow meaningful conclusions.

   - Pharmacodynamically, the 25 mg t.i.d., 25 mg q.i.d., 37.5 mg b.i.d. and 50 mg b.i.d. and 50 mg t.i.d. regimens all appeared to be effective.

   - From this Table, a pronounced pharmacodynamic effect was shown with Zn acetate. In the six patients that were not given Zn Ac, a

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[7] The rationale for the choice of +0.25 mg copper/day as the upper limit of the effective range for copper balance is that an estimated 0.34 mg copper/day is lost from the skin surface [R.A. Jacob et al., AJCN 34:1379-1383 (1981)] and not measured in balance studies. Thus, a completely neutral "apparent" balance would give a value of +0.34 on average.

positive mean copper balance (+0.52 mg/day) and the highest mean
^{64}Cu uptake (9.3% of the dose) were seen. Not unexpectedly, this
group of patients had the lowest plasma Zn (143 μg/dl) together
with the lowest urinary Zn (0.44 mg/day). By contrast, The
highest plasma Zn (274 μg/dl) and urinary Zn (6.7 mg/day) were
seen with the 50 mg x 5 regimen.

<u>TABLE 5</u>
Study 6

Summary of the Relevant Dose Response Data
in Wilson's Dz

Patients Administered Various Regimens of Zn Acetate

Dose Regimen	Mean Copper Balance (mg/day)	Mean ^{64}Cu Uptake (% of dose)	Rate of Adequately Controlled/ Total # of Patients	Mean	
				Plasma Zinc (μg/dl)	Urine Zinc (mg/day)
50 x 3	-0.44	0.82	28/31	226	4.65
50 x 2	-0.19	0.49	4/4	205	3.77
25 x 4	-0.21	0.64	4/4	255	6.01
25 x 3	-0.18	0.59	11/11	234	3.14
37.5 x 2	-0.02	0.73	4/4	155	2.85
75 x 1	0.16	2.10	4/8	156	2.10
25 x 2	0.15	2.09	0/4	237	1.32
25 x 1	0.16	2.05	2/4	183	2.20
25 x 6	-0.14	2.70	7/8	215	4.28
50 x 1	0.10	0.9	1/1	---	2.70
50 x 5	-0.36	0.37	8/9	274	6.70
0	0.52	9.30	6	143	0.44

● For illustration purposes, the results in the 31 individual patients
administered Zn acetate at the dose of 50 mg t.i.d. are displayed in
Table 6.

    - With this dose regimen, ^{64}Cu studies in pts. 21, 25 and 26 were
    >1.2%. The sponsor attributes this lack of efficacy to poor
    compliance, particularly in pts. 21 and 26. In support of this
    explanation are the low levels of plasma zinc in these two
    patients. However, urinary zinc levels were not necessarily low
    in these three patients (in comparison to pts. #5 (second test),
    6, 9 and 38. In addition, patient #26 showed the highest urinary
    excretion of zinc in this series (first test = 11.5 mg/day).

● The data depicted in detail in Table 6 demonstrate that the 50 mg x 3 Zn
Acetate regimen is pharmacodynamically effective since, on the average,
it induces a negative mean copper balance of -0.44 mg/day and a mean ^{64}Cu
uptake of 0.82% of the administered dose.

TABLE 6
Study 6

PD Effects in 31 Wilson's Dz Patients Given Zn Acetate
at the Oral Dose of 50 mg t.i.d.

Patient #	Copper Balance (mg/day)		⁶⁴Cu Uptake (% of dose)		Plasma Zinc µg/dl		Urine Zinc (mg/day)	
3								
4								
5								
6								
7								
9								
10								
12								
16								
17								
18								
21*								
24								
25*								
26*								
27								
31								
32								
38								
41								
44								
48								
49								
53								
60								
61								
63								
66								
69								
80								
81								
MEAN	-0.44		0.82		226		4.65	

The regimen was considered effective in all pts. except those 3 identified by an *.
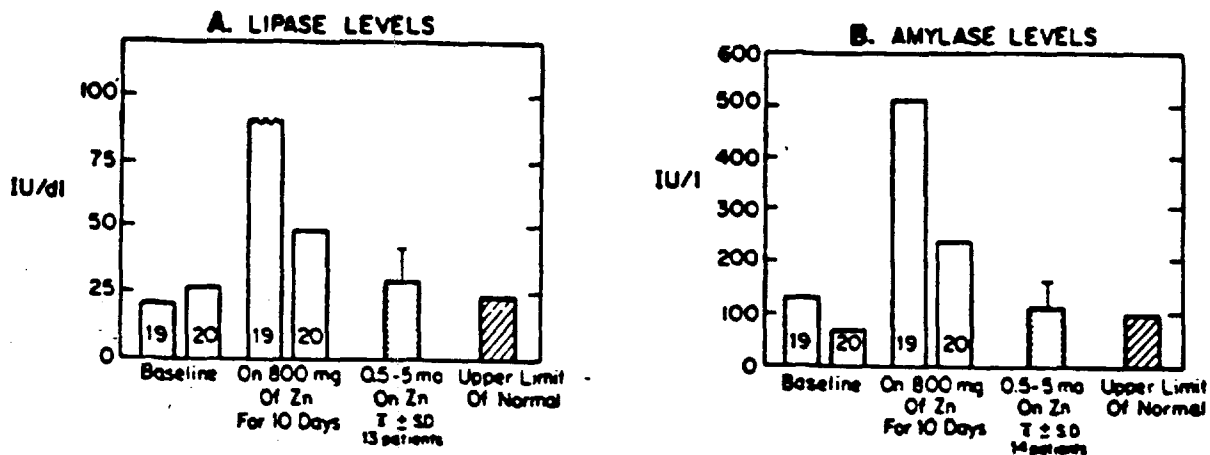
Fig. 4. - *Study 5: Elevations in plasma lipase and amylase concentrations in Pts. No. 19 and 20 after the oral administration of zinc acetate (800 mg/day).*

- Plasma Zn concentrations increased to 356 and 263 μg/dl, respectively.

  At the end of the 10 days of high dose Tx, both patients were discharged on their maintenance doses. Both remained asymptomatic. Amylase and lipase levels returned to their BL values on F/U about 6 months later in Patient 20. In Patient 19, serum amylase remained more elevated than usual for about two years and lipase about one year. Copper balance was measured in Patient 19 and did not show "unusual values for zinc therapy".

In conclusion, high doses of Zn acetate do not appear to be more effective than the recommended dose of 50 mg t.i.d. (150 mg/day). Although clinical tolerance was acceptable in these two patients, both developed biochemical but not clinical pancreatitis.

4. Nutrient-Drug and Drug-Drug Interactions (Study 2)

The methodology used involved blockade of oral ⁶⁴Cu absorption by zinc acetate and the effects of this drug on Cu balance in Wilson's Dz patients. The effects of vitamin C (1 g daily) and penicillamine (1 g daily), trientine (1 g daily) or ammonium tetrathiomolybdate (270 mg daily) (ATTM) were investigated.

As summarized in Table 7, the results showed that neither this nutrient nor the two approved drugs for Wilson's Dz significantly interfered with the effect of zinc acetate. In addition, based on observations on fewer patients, ATTM did not have an influence on the Zn acetate effect on copper balance/absorption (lower panel of Table 7).

5. Effects of Organ Diseases on Zn Acetate Absorption/Efficacy

Using the indirect methods (⁶⁴copper absorption and copper balance) evidence was presented that even severe liver Dz does not reduce Zn absorption or efficacy.

- 9 patients in the maintenance study had severe liver disease as evidenced by a presentation of hepatic failure; they were treated successfully with the usual dose of Zn acetate for up to 8 years.

TABLE 7
Studies 2a, 2b and 2c

Lack of significant influence of concomitant administration of
Vitamin C, penicillamine or trientine on the effects of
Zn acetate (50 mg t.i.d.) on Cu absorption in
Wilson's Dz patients

Dietary Intake	Copper (mg/day) Urinary Loss	Fecal Loss	Net Balance	64Cu Test (% of Dose)	Dietary Intake	Copper (mg/day) Urinary Loss	Fecal Loss	Net Balance	64Cu Test (% of dose)
Zn acetate (50 mg x 3) alone [N=6]					Zn acetate + Vitamin C (1000 mg) [N=6]				
0.89	0.117	1.43	-0.66	0.35	0.80	0.125	0.98	-0.30	0.57
Zn acetate (50 mg x 3) alone [N=5]					Zn acetate + Penicillamine (250 mg x 4) [N=5]				
0.98	0.076	1.08	-0.17	0.39	0.94	0.461	0.73	-0.25	0.26
Zn acetate (150 mg x 3) alone [N=5]					Zn acetate + Trientine (250 mg x 4) [N=5]				
1.33	0.128	1.48	-0.21	0.77	0.87	0.175	0.97	-0.25	0.61

NOTE: Displayed above are the means for the specified number of patients. For clarity of presentation, S.D. of the mean, t-test statistics and p-values have been omitted.

Study 2d

The study design to demonstrate lack of interaction between ATTM and Zn acetate was different. Results of this comparison are summarized below.

ATTM (270 mg/day) Alone Urine Cu (µg/24h)	Cu Balance (mg/day)	64Cu Uptake (% of Dose)	ATTM + Zn Acetate (50 mg x 3) Urine Cu (µg/24h)	Cu Balance (mg/day)	64Cu Uptake (% of Dose)
207a ± 62 [N=4]	-0.62 [N=3]	1.07a [N=1]	238 ± 86 [N=6]	-0.75 [N=4]	0.26a [N=2]

a) BL values
b, c) BL values = 5.98 mg/day

- The absorption of zinc in Wilson's disease patients with other concomitant organ impairment has not been studied specifically.

- Demographic characteristics such as ethnic background, age and sex appear to have little influence on zinc absorption and efficacy. These data are discussed along with the results of L-T trials.

6. Related Principal PD Effect (Study 7)

The aim of this study was to show that the induction of intestinal cell metallothionein (ICMT) by Zn acetate correlates with the suppression of oral 64Cu absorption.

NOTE: ICMT is a 61-amino-acid polypeptide with a high content (35%) of cysteine [J. Nutr. 111:1353-1361 (1981); Physiol. Rev. 65:238-309 (1985)]. The availability of a sensitive ELISA for human ICMT [J. Lab. Clin. Med.

113:221-228 (1989)] has allowed Brewer and his collaborators [J. Lab. Clin.
Med. 120:380-386 (1992)] to measure this cytosolic protein in endoscopic
small bowel Bx specimens from Wilson's Dz patients during Zn Ac Tx.

- Ten (10) patients (5M, 5F between the ages of 25 to 58y) with Wilson's Dz,
  were studied. There were three experimental groups.

    - Group I, consisting of two patients who had never taken Zn, was
      studied to show the effect of zinc on the levels of MT and ^{64}copper
      uptake by measurements taken before and after 5 or 6 days of zinc
      administration.

    - Group II, consisting of three other patients, was evaluated to show
      the consequences of suspending Zn therapy for up to 21 days by
      measurement of copper uptake and intestinal Bx.

    - Group III was similar to Group II, but the influence of zinc
      withdrawal was monitored only by ^{64}copper uptake in these five
      patients.

- The patients in Group II and III were clinically stable and in the
  maintenance phase of oral therapy with zinc acetate.

- In the five patients who were biopsied (Group I and Group II), the
  intestinal MT levels were greater on Zn Tx as compared to "off zinc"
  values. As shown in Table 8, the median zinc content was 2366 μg/g dry
  weight with zinc and 428 μg/g "off zinc"; this difference was highly
  statistically significant (p<0.001).

TABLE 8
Study 7

ICMT Concentrations in Wilson's Dz Patients
of Different Zinc Status

	ZINC STATUS			
	LOW		HIGH	
Pt. No.	ICMT (µg/g dry weight)	Length of Time Off Zn Acetate	ICMT (µg/g dry weight)	Length of Time On Zn Acetate
64				
52				
53				
20				
19				
Median	428		2366	

The null hypothesis that log (ICMT$_{High Zn}$ - ICMT$_{low Zn}$) = 0 in each patient was
tested using a one sample t-test; p <0.00001, t=23, df=4

- The relationship between increased ICMT and decreased ^{64}Cu uptake in 2
  patients (Nos. 52 and 64) is illustrated in Fig. 5. In these 2 patients
  there was a 2 to 4-fold increase in ICMT levels within 5 to 6 days after

the start of Zn acetate. This increase in ICMT levels was accompanied
by a reduction in ^{64}Cu uptake of 4.5% and 11.5% to 1.4% (nearly
effective) and 1.2% (effective), respectively.
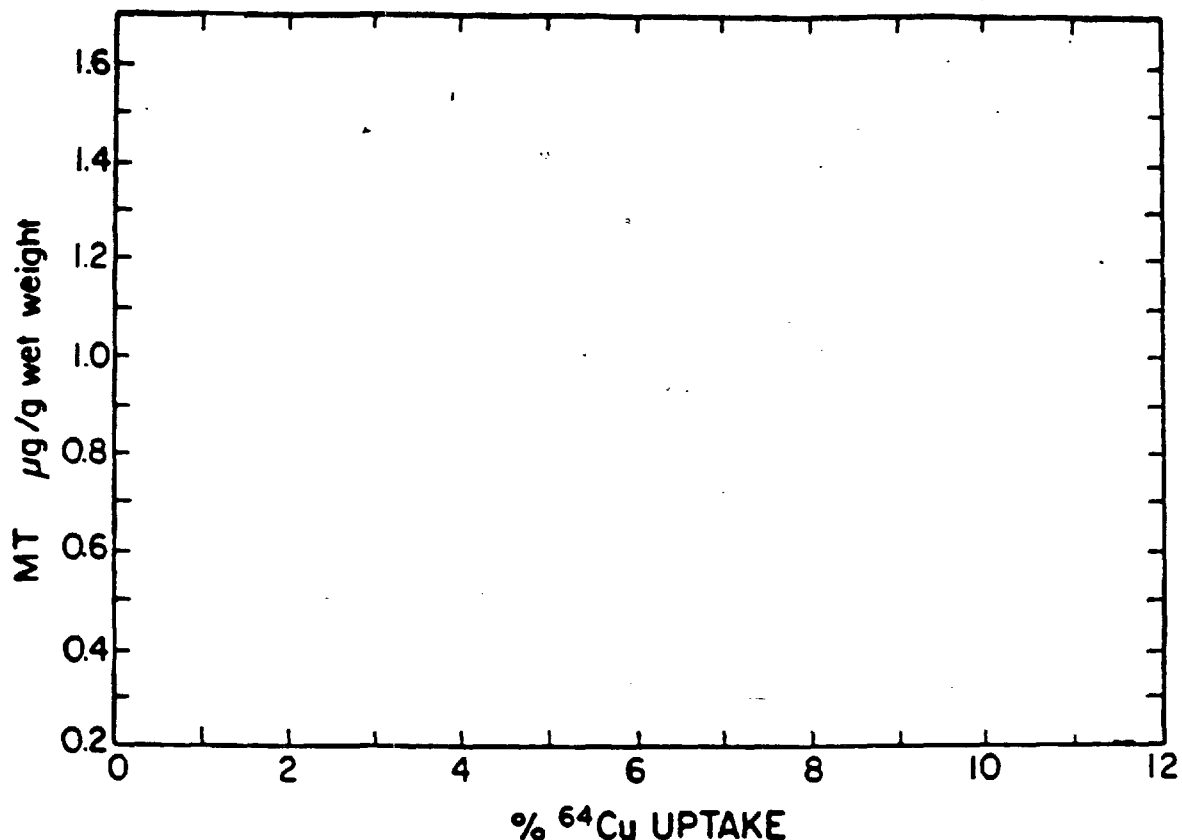
Fig. 5. - Study 7: Induction of intestinal ICMT and suppression of
^{64}Cu uptake following the initiation of Zn acetate therapy
in two Wilson's Dz patients. Numbers in parentheses indicate
days of Zn Tx.

• As shown in Fig. 6, the concentration of ICMT progressively decreased
and ^{64}Cu uptake increased in the three patients (#19, 20 and 53)
comprising Group II. This Fig. depicts ICMT results expressed either as
mg/g wet tissue or µg/g protein. The calculated half-time for loss of
^{64}Cu uptake suppression was 11, 10 and 15 for each of the above-mentioned
three patients.
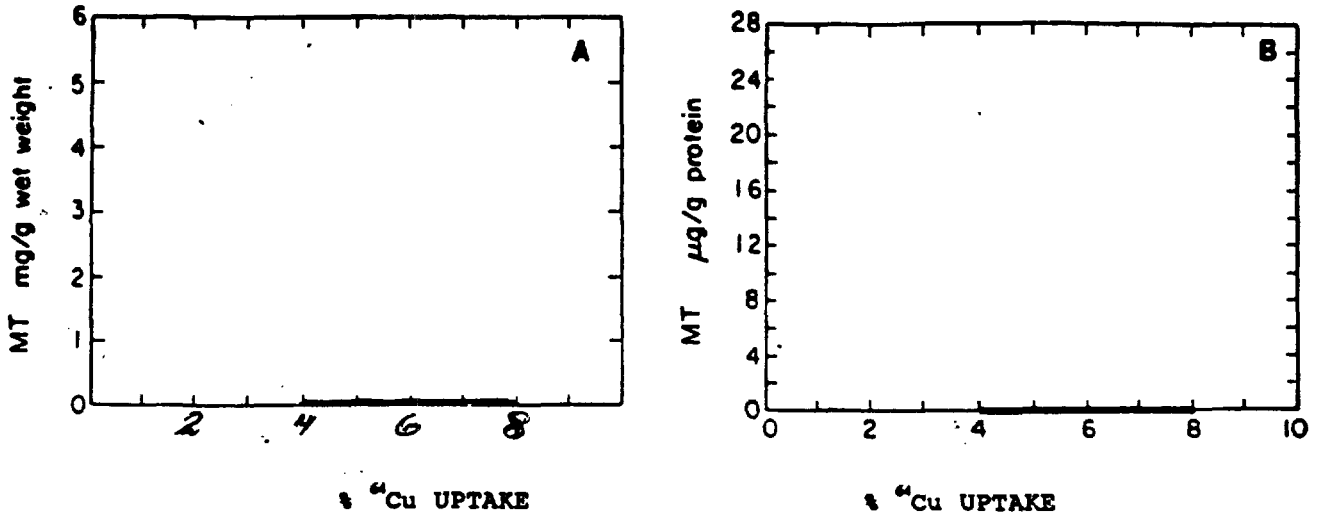
Fig. 6. - *Study 7: Serial data on the loss of* ^{64}*Cu uptake suppression concomitantly with decrease in the level of intestinal ICMT upon discontinuation of Zn acetate therapy in three Wilson's Dz patients. Numbers in parentheses indicate the number of days after the D/C of Zn acetate. The shaded area on the x-axis shows the mean and standard deviation of* ^{64}*Cu uptake levels in Wilson's Dz patients on no treatment.*

- Refer to Fig. 7. After discontinuation of zinc, the decline in urinary zinc excretion and ICMT levels is accompanied by increases in copper absorption. Linear regression analysis indicates a significant negative linear correlation between the number of days after stopping Zn acetate and urinary excretion of zinc and a positive linear correlation between intestinal ICMT levels and urinary excretion of zinc. The sponsor states that the ^{64}Cu uptake and urinary Zn levels suggest a hyperbolic relationsuip. But a formal statistical analysis is not warranted because the data are too sparse.

- Indirect support for the proposed mechanism of action had come from animal studies in which increased intraluminal Zn levels led to diminished radiocopper absorption [J. Nutr. 88:125-130 (1966)] and studies of patients with Wilson's Dz in which Zn treatment led to increased fecal Cu excretion [Ann. Intern. Med. 99:314-320 (1983)].
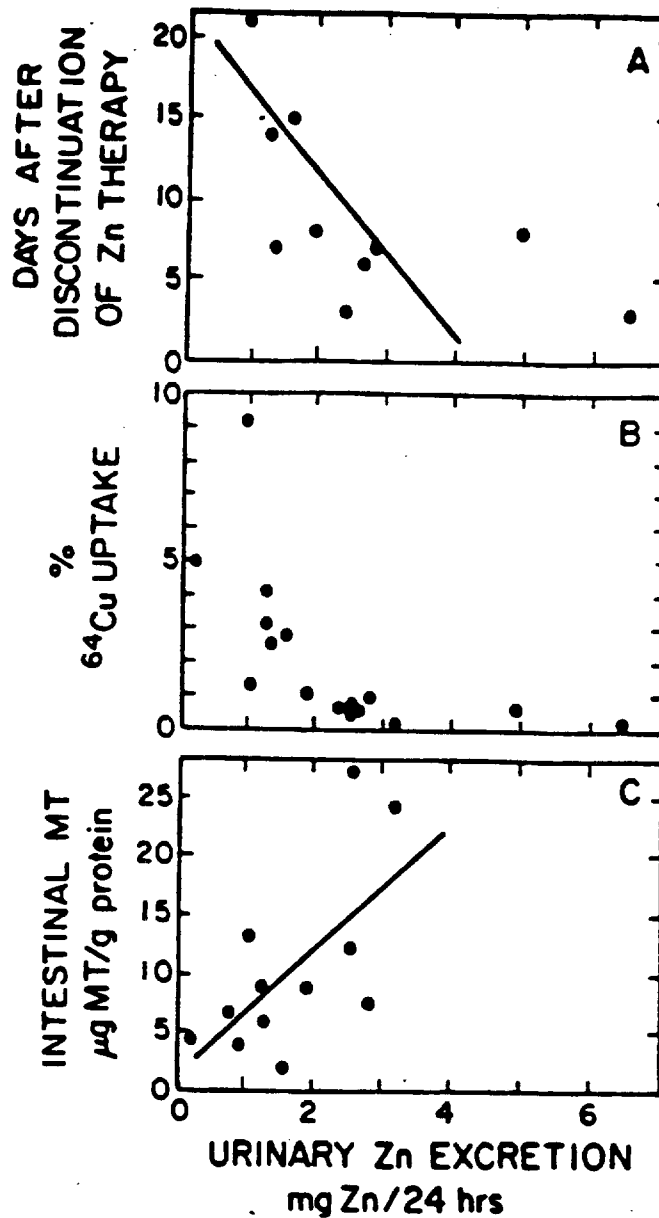
Fig. 7. - _(Study 7)_ __Panel A:__ _Decline of urinary zinc levels as a linear function of time after discontinuation of Zn acetate therapy._ __Panel B:__ _Inverse relationship of ⁶⁴Cu uptake to urinary zinc levels._ __Panel C:__ _Direct linear correlation of intestinal MT levels with urinary zinc excretion. The lines in Panel A and C reflect the regression equations._

VI. **CRITICAL TRIALS FOR THE INDICATION MAINTENANCE OF PATIENTS WITH WILSON'S DZ**

The sponsor has presented results of two clinical trials from here on identified as the Brewer study and the Hoogenraad study. In the two clinical reports, data are presented for all requested indications. From the Brewer's Report, it is not always possible to separate data for one from the other indications. Also, data from 50 mg t.i.d. Zn AC are mixed with other doses and regimens. In Table 9, the reviewer presents the summary of the study population and the main features of the Brewer's and Hoogenraad's trials as they apply to each of the   indications proposed by the sponsor. The reader is referred to the REMARKS column for an assessment of the adequacy of the trials for the maintenance of symptoms indication.

NOTE: Treatment of Wilson's Dz patients during pregnancy would not be a separate indication. If adequate, these data would be included in the labeling.

A. **The Brewer's Study**

The overall objectives of this study were to demonstrate the efficacy and safety of Zn acetate (Zn AC) for L-T therapy (maintenance therapy) in patients with Wilson's Dz.

1. Specific Efficacy Objectives

These were to show that Zn AC can:

- maintain body Cu at subtoxic levels and prevent progressive target organ damage after adequate decoppering with chelating agents, Zn (salts), or other agents in patients who were symptomatic at the start of therapy.

2. Materials and Methods

a. Overall Design and Plan

This maintenance trial consisted of open-label evaluations of the effect of Zn AC administered orally to patients with Wilson's Dz. Each patient served as his own control. The controls, or baselines (BLs), for the assessments of efficacy, were the patient's clinical status and Cu values prior to the start of maintenance therapy.

Definition

Maintenance therapy was defined as that period of Zn AC therapy when the therapeutic objective is to prevent the accumulation or reaccumulation of Cu and to prevent the appearance or reappearance of the symptoms of copper toxicity.

TABLE 9

Summary of the Study Population, Main features of the trial and an initial assessment of the adequacy of the controlled clinical trials presented by the sponsor in support of the efficacy of Zn acetate for the two indications in patients with Wilson's disease

	Study Population	Main Features of the Trial	Remarks
		I. Maintenance Tx of symptomatic patients that had been initially treated with another anticopper agent(s)	
Brewer	63 patients (35F; 28M; age range 16 to 55y) that were initially treated with penicillamine, trien or TTM (This includes 3 pts. that were treated with Zn acetate from the start)	Pts. treated with 75 to 150 mg Zn acetate, in 2 or 3 equally divided doses separated from meals by an hour. Pts. served as their own control. Clinical variables, such as speech and neurological status were measured. In addition, copper variables were measured as pharmacodynamic indicators of efficacy. Years followed: 3.8 (range=0.2 to 9.5y).	Adequate design to demonstrate maintenance. Pts. can serve as their own control because historical information indicates that the disease invariably progresses to a fatal outcome if untreated. Although survival data are presented, these data are weak because it is not known if those patients that died after D/C penicillamine are comparable to Brewer's patients. Cu variables, which can be measured reliably with fine precision, are good indicators of efficacy because Cu status is a good predictor of therapeutic efficacy. However, as clearly discussed in the PD section of this review, Cu variables (blood, urinary, hepatic) cannot be used as surrogate indicators of efficacy. L-T follow-up of clinical (and laboratory) variables, as done in this trial, is needed to demonstrate maintenance.
Hoogenraad	22 patients (11F; 11M; age range 11 to 38y). Of these: 7 were intolerant to penicillamine; 8 were not intolerant to penicillamine; 7 were treated with Zn SULF from the start	Pts. treated with (mg of Zn sulfate per day) 300 (N=1); 600 (N=9); 900 (N=11) or 1200 (N=1) in 3 divided doses 30 min. before meals. Pts. served as their own control. Parameters of evaluation as above. Years followed: 3.0 (range=0.1 to 6.8y).	Also a good design to demonstrate maintenance. But this study used a different kind of salt of Zn (sulfate) that the salt for which approval is being requested (acetate). Under these circumstances it may be necessary to demonstrate that these two salts give an equal level of Zn, the active principle (see Recommendations for Regulatory Action). Rest as above. Both trials give some information on the efficacy of Zn treatment in children with Wilson's Dz.

- According to the sponsor, the maintenance period followed a period of "initial" therapy during which time the Cu levels were reduced to sub-toxic amounts. In general, the period of initial Tx was a minimum of 2 months in duration for patients with Wilson's Dz who presented with elevated Cu values.

- Each patient returned for medical evaluations at interval of ca. 12 mo. Additional visits were scheduled as indicated. Urine samples were collected at 6 monthly intervals and sent through the mail for Cu and Zn evaluation. The duration of Zn AC Tx was indefinite. Lifetime Tx was anticipated unless withdrawal was clinically indicated or requested by the patient.

- The study was carried out according to the Declaration of Helsinki. IC was obtained from each patient. The study was conducted with the annual approval of the University of Michigan Committee on the Use of Humans as Research Subjects.

b. Assignment of Patients to Tx/Dose Selection

- All qualified/enrolled patients were maintained with Zn AC, usually at the oral dose of 50 mg t.i.d.

- The sponsor states that after 25 mg t.i.d. and 50 mg b.i.d. were shown to be effective in S-T balance studies, a few patients were placed on these maintenance doses to gain experience. Many of these patients were carefully studied in transition from chelating agents to Zn AC or in dose-ranging studies before beginning maintenance therapy with Zn AC.

- The sponsor further explains that the early indications were that 50 mg of elemental Zn 3 times daily was effective. Doses of 25 mg 4 times, 25 mg 3 times, and 50 mg 2 times daily were shown to be effective also. The sponsor reasons that these smaller doses have a lower margin of confidence, especially if the patient is not 100% compliant, because 25 mg 2 times and 75 mg 1 time daily were not adequately effective [G.J. Brewer et al., Ann. Intern. Med. 99:314-320 (1983)]. On the basis of these observations the preferred dose was 50 mg three times daily.

c. Test Materials/Dosing Instructions

- Initially, the investigational drug was available as Zn AC tablets containing 10 mg or 25 mg of elemental Zn. The tablets were prepared by the University Hospital Pharmacy at the University of Michigan in Ann Arbor, Michigan[9]. The chemical specifications, the finished product specifications, a sample label and the master formulary cards were given in Vol. 07, p. 001-008 (sponsor's Appendix III).

- In August 1985, the Lemmon Company supplied the drug as capsules containing 25 mg or 50 mg of Zn[10]. The Lemmon Company has been the source of the investigational drug from that time to the present. The specifications for zinc acetate, the inert ingredients and the finished product are given in the Chemistry, Manufacturing and Controls Section of the NDA.

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[9] Fine chemical grade zinc acetate was purchased commercially from   and used to make tablets.

[10] Each capsule contained 84.2 or 168.4 mg of Zn AC.

- The capsules were packaged in commercially available glass/plastic containers that contained 250 capsules. The capsules were dispensed to the patient in these containers.

- The patients were instructed to take each dose one hour before or one hour after meals or beverages. Patients experiencing gastric discomfort with the first morning dose were asked to take that dose one hour after breakfast rather than before. If this approach was not completely successful, the dose could be taken with a piece of lunch meat.

d. Efficacy Variables

1) Entry Procedures

Prospective patients for Zn AC therapy were admitted to the University Hospital for an initial evaluation to confirm the validity of the Dx and to establish their initial copper and clinical status. These procedures were performed during a hospitalization of about 4 to 7 days.

2) Pre-maintenance Therapy/Procedures

These depended on patient types.

- Patients on prior chelation therapy that were considered to be in the maintenance phase were switched to Zn AC for maintenance therapy.

- Symptomatic patients not previously treated were either initiated on Zn AC, or treated with ATTM for 8 weeks, and then switched to Zn AC. Listed below is the general categorization for the various types of pre-maintenance procedures. The therapeutic goal during the initial therapy period (pre-maintenance) was to bring patients' copper toxicity under control.

Initial Patient Categorization Relative to
Pre-Zn AC Therapy

Type of Pre-Zn AC Tx   # of Pts.

I. Pts. With Neurologic/Psychiatric Symptoms

Penicillamine	36
Penicillamine + Trien	5
Zn AC from the Beginning	3
ATTM	6
Subtotal	50 (N)[a]

[27F; 23M; Age Range 16 to 55y]

II. Pts. With Hepatic Symptoms

Penicillamine	10
Trien	2
Penicillamine + Trien	1
Subtotal	13 (N)[b]

[7F; 6M; Age Range 17 to 45y]
Total # of Symptomatic Pts   63

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a) The N category included patients with the typical neurological manifestations of the movement disorder, such as dysarthria, dysphagia, tremor, dystonia, etc., or definite emotional disorders of relatively recent (0 to 3y) onset, such as depression, fall off in work or school performance, bizarre behaviors, etc.
b) The N category included patients with a presentation of hepatic failure, with a hepatitis picture, or with a picture of cirrhosis.

3) Maintenance Procedures

- During maintenance therapy with Zn AC, the patients returned to the investigator's hospital about every 12 mo. for an assessment of their clinical condition and copper status (evaluations of the efficacy of Zn AC therapy).

- Speech was evaluated by the same speech pathologist throughout, using a quantitative scoring system ranging from 7 (severe) to 0 (normal).

  - 5 functional variables were scored in this test.

- Neurological function was evaluated by the same neurologist throughout using a quantitative scoring system ranging from 38 (severe) to 0 (normal).

  - 10 functional variables were scored in this test to end up with the final result.

- Brain MRI was evaluated by the same two radiologists throughout using a blinded quantitative scoring system running from 30 (severe) to 0 (normal).

  - 10 variables were scored in this test. The scores from the two radiologists were averaged to yield a final result.

- Each of the three clinical/radiological tests described above was done without specific knowledge of prior therapy and without reference to prior scores.

- Zinc concentrations in the liver and Zn balance were also measured to assess the Zn status over a period of prolonged administration of Zn.

  - A urine sample was obtained through the mail every 6 mo. to evaluate urine Cu and Zn.

e. Safety Evaluations

- These were adequate. ADRs were monitored by recording any unexpected or unwanted signs or symptoms whether observed by the investigator or reported by the patient. Also performed were hematology, a clinical chemistry panel, urinalysis and other selected blood parameters[11]. Chest X-rays and EKGs were done on most admissions.

- More frequent visits were scheduled with the investigator as clinically indicated. Patients who resided at a considerable distance from the investigator's location were followed by the referring physician.

f. Concomitant Medication/Diet

Routine therapy with penicillamine, trien, and Vitamin C in doses in excess of 100 mg was prohibited. Patients were not allowed to take vitamin/mineral supplements containing Cu. There were no restrictions on medications other

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[11] These included special studies such as lymphocyte function, carried out at the Pediatrics Dept. at Wayne State Univ. School of Med. by Dr. Joseph Kaplan, a qualified immunologist. These evaluations were controlled by comparison to normal controls, and by comparing data from the same patients before and after Zn AC administration.

than the agents just noted. Shellfish and liver were restricted to no more
than 1 time per week during maintenance therapy and not allowed at all during
initial therapy.

g. Data Quality Assurance

The following literature references were used for chemical analyses involving
Cu and Zn.

- [G.J. Brewer et al., Ann. Intern. Med. 99:314-320 (1983); G.M. Hall et
  al., Hepatology 7:522-529 (1987); G.J. Brewer et al., Zinc Therapy of
  Wilson's Disease: VIII. Dose response studies. Trace Elem. in Exp. Med.
  3:227-234 (1990)] for Cu balance. These were 10 days balances carried
  out in the Clin. Res. Center of the Univ. of Michigan Hosp.

- [G.M. Hill et al., Amer. J. Med. Sci. 292:344-349 (1980)] for ^{64}Cu
  absorption.

- [G.J. Brewer et al., PSEBM 184:446-455 (1987)] for urine Cu and Zn and
  for plasma Cu and Zn.

- [G.J. Brewer et al., J. Lab. Clin. Med. 109:526-531 (1987)] for the
  handling of liver tissue and the hepatic assay of Cu and Zn. These
  studies were done on percutaneous needle Bx material. The Bxs were
  performed by personnel of the Gastroenterology Div. of Univ. of Michigan
  Hosp.

h. Validity of the Database

The sponsor notes (Vol. 06, p. 014):

"The data entry person checks the validity of the entry by comparison of
the data being entered with the entry as it appears on the word-
processor screen.

"We have used a 'H' and 'L' flagging system to indicate data out of the
normal range. These data are evaluated by the staff, including Dr.
Brewer, for their validity.

"Suspicious values, if they involve copper or zinc assays, are
rechecked, since we save all samples. If they involve measurements from
the Hospital labs, we check against the values in the Hospital chart for
validity."

i. Data Presentation

- The entire set of efficacy and safety data are contained in the Data
  Listings.

- The tables in sponsor's Appendix II provided all of the values for each
  patient with an abnormal hepatic, hematologic, renal or other clinical
  laboratory test.

- For the Summary Tables (D.1 through D.151), the results were usually
  presented over the duration of maintenance therapy in yearly intervals
  and the value given was the mean when there was more than one value
  during an interval.

NOTE: Herein lies one of the difficulties when analyzing results from this trial. The average from too few observations (i.e. 2 or 3 patients) is not very meaningful.

j. Statistical Analyses

• A formal prospective test of the hypothesis of clinical efficacy using a statistical model was not planned.

• In some cases, particularly for copper variables and urine zinc, a series of desirable endpoints were determined, and the data collected were evaluated against those endpoints.

• In the case of almost all variables, the lack of an unfavorable trend over time of therapy in individual patients and in mean values, or the presence of a favorable trend in individual patients and in mean values, was used as being indicative of acceptable efficacy or lack of toxicity.

NOTE: This approach appears adequate. Although 63 patients is a large sample for a rare disease, the few cells under the various strata do not allow formal statistical approaches. For every addressed subcategory the reviewer provides clinical summaries of patients with "unusual" responses ("outliers" with respect to the mean).

• Descriptive statistics were used for the efficacy and safety variables where indicated.

3. Removal of Subjects From the Trial

The procedures to remove patients from the study were adequate. In their Table 1.2, the sponsor listed the 13 pts. who were no longer in the study. The reasons for these withdrawals are clearly specified in three Tables where the reviewer has listed Zn, clinical and Cu variables, results of LFTs and data on pancreatic function. This mode of presentation exemplifies the reviewer's approach during evaluation of the evidence presented in the present NDA: assessment of efficacy and safety data in <u>each</u> individual patient.

For each pt., a Comments column is given specifying the cause of death [(N=4) (Table 10)], reason for dropping out [(N=3) (Table 11)] or discharging patients from the trial [(N=6) (Table 12)]. The reason for the detailed information in these Tables is to determine the clinical/biochemical status of the patients at the time of their dismissal from the trial.

• All 4 deaths (2 accidents) were unrelated to test med.

• One of these (#08) appeared to be doing well in terms of control of Cu metabolism and liver function. Based on urine and plasma Zn levels, he seemed complaint. The information on clinical variables indicates that this pt. had severe speech problems (Grade 7) that did not change with time.

• The three additional patients that died appeared to be complying with test med. But the information on the clinical/biochemical/liver and pancreatic safety parameters is too incomplete to draw meaningful conclusions. We do not know if these patients were being properly maintained.

• The three dropouts (table 11) were also apparently compliant on the basis of urine and plasma Zn.

- Pt. #01 had a borderline hepatic failure from the start of the trial, with (primarily) moderately increased serum BIL. This patient was treated with Zn AC for a total of 8½y and clinical/biochemical status may have been maintained (but the information is incomplete). The pt. eventually elected to have a liver transplant.

  This appears to be an instance of Tx F.

- Pt. #28 experienced a very pronounced fall in liver Cu concentration; plasma Cu also decreased to normal values. Although this pt. was categorized as being of type H his LFTs did not seem to lend strong support to this categorization.

- The insufficient information for Pt. #40 does not allow the conclusion as to whether this pt. was being adequately or inadequately maintained.

• Except perhaps for Pts. 46 and #05 for all other four patients that were discharged from the trial [by the investigator(s)] the information is inadequate to draw efficacy/safety conclusions with certainty.

In summary, in the majority of patients that withdrew or were withdrawn from the trial, the available data are insufficient to determine drug effects on clinical/biochemical parameters. This is an important observation because the sponsor claims 100% efficacy (see below), that is, all pts. responded to or were properly maintained with Zn AC. The reviewer concludes that in a certain percentage of cases drug response cannot be evaluated due to the lack of important information. There is no convincing evidence that, as claimed by the sponsor [G.T. Brewer, Gastroenterology 104:1568 (1993)], the clinical/biochemical effectiveness of Zn AC is 100%.

4. Study Population and Data Sets Analyzed

• Of the 94 patients screened, the following 8 did not enter the maintenance trial for the reasons specified below.

Pt. Identif.	Reason	No. of Pts.
#23	Did not show up after being assigned a number	1
#13, #35	Did not have Wilson's Dz	2
#2, #11	Participated in S-T studies, but refused maintenance therapy	2
#15, #29	Uncooperative after initial discharge, and no maintenance data collected	2
#82	Died of bleeding varices prior to maintenance phase after TTM and Zn AC 50 mg t.i.d. for 5 weeks and then Zn AC t.i.d. for about one week	1
Total		8

TABLE 10
The Brewer's Study

DEATHS

Pt. Identif.	Zn Variables [Urine, Plasma]	Clinical Variables [Speech, Neuro/Psychiatr, MMR]	Cu Variables [Urine, Plasma, Liver]	LFTs [BIL, Transam, AP, LDH]	Pancreatic function [Amylase, Lipase]	COMMENTS
#08, WM, 27y old, M	Urine BL 5.1 / W/D 7.6; Plasma 181 / 248	Speech BL 7 / W/D 7; Neuro/Psychiatr N/D / 14; MMR N/D / N/D	Urine BL 0.051 / W/D 0.070; Plasma 11 / 4; Liver 307 / 147	BIL BL 0.5 / W/D 0.4; ALT N/D / N/D; AST 28 / 31; AP 109 / 142; LDH N/D / 187	AMYL BL N/D / W/D N/D; LIPASE N/D / N/D	• **Cause of death** = chronic pulmonary aspiration due to dysphagia (chronic pulmonary Dz). Refused gastrostomy. • Had severe, permanent neurological residual + severe dysphagia since enrollment into trial. • Was doing well in terms of control of Cu metabolism. • ^{64}Cu tests=0.6, 0.4% of adm. dose Cu balance=+0.24, +0.36, -0.13 mg/day. The +0.36 result was a home balance study while the pt. was also on vitamin C. • AP was WNL at BL but mildly elevated at the 1 to 2 year evaluation.
#33, WM, 22y old, M	Urine N/D 4.7; Plasma N/D 184	Speech N/D N/D; Neuro/Psychiatr N/D N/D; MMR N/D N/D	Urine 0.069 0.051; Plasma N/D 15; Liver N/D 310	BIL 1.4 1.5; ALT N/D N/D; AST 31 34; AP 117 145; LDH 201 191	AMYL N/D N/D; LIPASE N/D N/D	• **Cause of death** = Automobile accident. • This pt. entered the study with clinical symptoms of cirrhosis and acute hepatic encephalopathy after ca. 10 mos. of penicillamine therapy. It was determined that the hepatic encephalopathy was secondary to severe cirrhosis coupled with excessive dietary protein intake. • The pt. was treated with lactulose and penicillamine continued for a one mo. period, after which Zn AC was initiated and the penicillamine discontinued. • The pt. improved quite dramatically with only some minor difficulties remaining associated primarily with running and involuntary movements. • His K-F rings resolved over the course of Tx. • Laboratory BL safety values, including a mildly ↑ total BIL remained unchanged. • AP, which had been WNL at BL, was mildly ↑ at the 1 to 2 year evaluation.

#51, WM, 23y old, N

Zn		
Urine	0.5	4.7 [3-4y]
Plasma	N/D	184

Speech	5	N/D
Neuro/Psychiatr	9	N/D
MMR	N/D	N/D

Cu		
Urine	0.084	0.077
Plasma	N/D	N/D
Liver	98	N/D

BIL	0.7	N/D
ALT	40	N/D
AST	31	N/D
AP	60	N/D
LDH	124	N/D

AMYL	75	N/D
LIPASE	18	N/D

- **Cause of death** = drowning accident
- This pt. had mild neurological residual after being Tx with penicillamine for 1 yr.
- It is not known how was the pt. doing at the time of his death because the majority of BL observations were not repeated prior to patient's death.
- Based on urine Zn he was apparently compliant.
- Urine Cu did not change during the 5 mos. of Zn AC therapy.

#54, WM, 25y old, N

Zn		
Urine	N/D	6.0 [3-4y]
Plasma	N/D	N/D

Speech	6	N/D
Neuro/Psychiatr	N/D	N/D
MMR	N/D	N/D

Cu		
Urine	0.101	0.100 [3-4y]
Plasma	N/D	N/D
Liver	N/D	136 [0-1y] ?

BIL	0.8	N/D
ALT	70	N/D
AST	61	N/D
AP	211	N/D
LDH	202	N/D

AMYL	53	N/D
LIPASE	17	N/D

- **Cause of death** = bronchopneumonia probably due to aspiration.
- This pt. was seen at the investigator's institution on 5/87. He had severe permanent neurological residual and a psychiatric problem related to sex. He chronically exposed himself to the nurses during his admission to the Hosp. After returning home he was arrested for rape and eventually institutionalized in a Canadian mental Hosp. The pt. remained on Zn AC therapy until his death in 5/91.
- Urine samples showed appropriate levels of Zn.
- There was no information on clinical parameters.
- On the Cu variables, urine Cu remained higher than normal after 3-4y of Tx with Zn AC. Plasma Cu was not determined. Liver Cu was abnormally high. It is not known whether the cited value corresponded to BL (as suggested by the Summary CRF) or to the [0-1y] observation period (as suggested by sponsor's Table D.28, Vol. 06, page 115).
- At BL, serum BIL was WNL and LDH 2 fu/l above the upper limit of normal.
- Both transaminases and also AP were mildly elevated at BL. But there were no F/U values for any of the LFTs.
- Both tests of pancreatic function were WNL at BL but F/U observations on these safety parameters were not available.
- The safety parameters also included chronic thrombocytopenia and leukopenia due to hypersplenism from underlying cirrhosis.

W/D = Value at the time closest to the patient's death.
N/D = Not determined [parameter not evaluated or assay not done].

TABLE 11
The Brewer's Study

DROPOUTS

Pt. Identif.	Zn Variables [Urine, Plasma]	Clinical Variables [Speech, Neuro/ Psychiatr, NMR]	Cu Variables [Urine, Plasma, Liver]	LFTs [BIL, Transam, AP, LDH]	Pancreatic Function [Amylase, Lipase]	COMMENTS
#01, WM, 34y old M	Urine BL N/D — Y/O 5.9 [7-8y] ; Plasma N/D — 136 [3-4y]	Speech BL N/D — Y/O N/D ; Neuro/Psychiatr N/D — Y/O N/D ; NMR N/D — N/D	Urine BL N/D — Y/O 0.056 [7-8y] ; Plasma 12 — 7 [3-4y] ; Liver N/D — N/D	BIL BL 3.2 — Y/O 2.3 [3-4y] ; ALT N/D — N/D ; AST 54 — 30 [3-4y] ; AP 183 — 196 [3-4y] ; LDH N/D — N/D	AMYL BL N/D — Y/O N/D ; LIPASE N/D — N/D	• **Reasn:** After 3y in the trial, the pt. moved to Canada, but remained in the study for an additional 5 years. He eventually elected to have liver transplant (Tx Failure). • The pt. initially presented with hepatic symptoms and subsequently was placed on penicillamine several years prior to his admission. • He entered the Zn AC study because of continuing problems with fatigue, edema, incoordination and mild encephalopathy. In Oct. 1980 he underwent Zn and Cu balance studies and thereafter continued on penicillamine. • Cu balance: -0.42, -0.23, -0.03, -0.64, -0.145 mg day. 64Cu test was not done. • In July, 1981 the pt. was placed on zinc alone. The dosage was 25 mg x 4 daily. During the course of Tx with zinc therapy there were no changes in the patient's clinical symptoms. K-F rings were present at the beginning of therapy and disappeared with therapy. • Of the Cu variables, urine Cu was not done at BL but it was slightly elevated at the 7-8y sample. Plasma Cu remained WNLs. Liver Cu was not determined at any time. • This pt. had a borderline hepatic failure during the entire course. He required protein restriction to prevent encephalopathy. Serum BIL, AST and AP remained moderately elevated. ALT and LDH were not determined. • Of note, speech, neuro/psychiatr, or NMR evaluations were not done at any time.

| #28, WM, 19y old M | Urine N/D Plasma N/D | 7.4 [2-3y] 193 [1-2y] | Speech N/D Neuro/Psychiatr N/D MMR N/D | N/D N/D | Urine N/D Plasma 30 Liver 2468 | 0.090 [2-3y] 8 [1-2y] 265 [0-1y] | BIL 0.8 ALT N/D AST 56 AP 217 LDH N/D | 1.1 [2-3y] 45 [2-3y] 40 [2-3y] 112 [2-3y] 187 [2-3y] | AMYL N/D LIPASE N/D | 67 [2-3y] 20 [2-3y] | • **Reason:** The Mayo Clinic Drs. switched the pt. back to penicillamine because they thought he was failing Zn AC (high liver Cu levels) [Tx failure]. • This pt. had been on penicillamine for 6 mos. and was having "terrible" clinical problems with liver failure, pleural effusion, and poor healing of a thoracotomy incision. He was switched to Zn AC. The investigator claims that the pt. made a remarkable recovery to a normal life. But clinical evaluations of speech, neuro/psychiatr. and MMR were not done at any time. • Of the Cu variables, urine Cu was not determined at BL and remained elevated after 2-3y Tx. Plasma Cu became normal within 1-2y but these values were not determined thereafter. Although there was a dramatic ↓ in liver Cu, this parameter was still high at the 0-1y assay. • LFTs were slightly elevated/WNL. The pt. had leukopenia and marked thrombocytopenia from hypersplenism due to cirrhosis. His ALB normalized. There were no BL determinations of AMYL and LIPASE. At the 2-3y evaluation, both were WNL. |

#40, LM 34y old									
N	Urine 0.1	4.1 [0-1y]	Speech 4.5	4.5 [0-1y]	Urine 0.140	0.075 [0-1y]	BL 0.5	N/D	AMYL 42	71 [0-1y]
	Plasma 58	222 [0-1y]	Neuro/Psychiatr 15.0	10.3 [0-1y]	Plasma N/D	N/D	ALT 33	N/D	LIPASE 17	26 [0-1y]
			MMR N/D	N/D	Liver 433	N/D	AST 21	N/D		
							AP 84	N/D		
							LDH 105	N/D		

- **Reason:** Left hospital (end the study) after becoming fearful of impending neurosurgery.

- This formerly institutionalized patient entered the Zn AC study with stable, moderate neurological residual after 12y of penicillamine therapy. She presented with marked dysarthria and movement disorder. The sponsor claims that while on Zn AC and artane, this pt.'s neurological symptoms improved over a 1½ y period. Although numerical neurological exam values indicated a 33% improvement, this represents a decrease in the Rating Scale of 15 at BL to 10.3 at the 0-1y evaluation. The speech rating score (4.5 at BL) did not change. MMR evaluations were not done.

- The pt. continued to have many personal problems, including a divorce and the possible removal of her 4 children by the state. She was evaluated at the Univ. of Michigan for a stereotactically placed lesion in the ventral lateral nucleus of the thalamus. The night before this surgery, which the pt. had sought and agreed to, she became frightened and checked herself out of the hospital.

- There is insufficient information to determine the degree of Cu control in his pt. Urine Cu was still high at the 0-1y observation. Plasma Cu (Vol. 06, p. 101) was not determined. Liver Cu was very high at 433 at BL but there was no F/U value.

- There is also inadequate information about liver function. Although all LFTs were WNL at BL, there were no F/U determinations for any of these tests.

- Serum AMYL remained WNL; the increase in serum lipase (2 iu/dl) over the upper limit of normal is clinically insignificant.

W/D = Value at the time closest to the patient's death.
N/D = Not determined (or parameter not evaluated or assay not done).

TABLE 12
The Brewer's Study

DISCHARGED

Pt. Identif.	Zn Variables [Urine, Plasma]	Clinical Variables [Speech, Neuro/Psychiatr, NMR]	Cu Variables [Urine, Plasma, Liver]	LFTs [BIL, Transam, AP, LDH]	Pancreatic function [Amylase, Lipase]	COMMENTS
#04, UF, 41y old N	Urine BL N/D — W/D 3.8 [1-2y]; Plasma N/D 364 [0-1y]	Speech BL N/D — W/D 2.0 [0-1y]; Neuro/Psychiatr N/D N/D; NMR N/D N/D	Urine BL N/D — W/D 0.060 [1-2y]; Plasma 12 19 [0-1y]; Liver N/D N/D	BIL BL N/D — W/D 0.5 [1-2y]; ALT N/D N/D; AST N/D 20 [1-2y]; AP N/D 266 [1-2y]; LDH N/D N/D	AMYL BL N/D — W/D N/D; LIPASE N/D N/D	• **Reason**: Became abusive to nursing personnel; uncooperative. This pt. was very temperamental and a difficult management problem, particularly in the hospital. She was a sexual exhibitionist and had numerous tantrums. • Pt. had stable moderate neurological residual after several y of penicillamine when switched to Zn AC Tx. It is not documented if there was no change in these findings during her 13 m. course of Zn AC. K-F rings, present at the beginning of therapy, had disappeared by the time pt. was discharged. • There were insufficient data to evaluate response of clinical or Cu variables. Urine Cu was high (1-2y) but there was no BL value to compare with. Plasma Cu was WNL at the 0-1y evaluation, but this value could not be compared to BL. Liver Cu was not determined. Cu balance was -0.16, -0.55, -0.19 and -0.06; ^{64}Cu test was not done. • There was also insufficient information to assess liver function (no BL evaluations). At the 1-2y F/U evaluation BIL and AST were WNL and AP >2 time the ULN.
#05, UF, 32y old N	Urine N/D 1.8 [6-7y]; Plasma N/D 279 [6-7y]	Speech N/D 3.0 [6-7y]; Neuro/Psychiatr N/D 8.5 [6-7y]; NMR N/D 4.0 [6-7y]	Urine N/D 0.098 [6-7y]; Plasma N/D 16 [6-7y]; Liver N/D 426 [6-7y]	BIL N/D 0.1 [6-7y]; ALT N/D 27 [5-6y]; AST N/D 16 [6-7y]; AP N/D 75 [6-7y]; LDH N/D 166 [6-7y]	AMYL N/D 87 [6-7y]; LIPASE N/D 14 [6-7y]	• **Reason**: Uncooperative, unreliable. • The pt. had moderate neurological (and psychiatric residual). Abnormalities in speech, neuro/psychiatr. and NMR cannot be assessed with respect to BL. • Cu variables were not determined at BL; at the 6-7 evaluation, plasma Cu was WNL but both urine and liver Cu were ↑. Cu balance was -0.03, -0.36, 0.43, -0.24, -0.40, -2.03 mg/day. The ^{64}Cu test was 1.1 to 0.2% of the administered dose. • LFTs during several years allow the conclusion that these were normal and remained WNLs during the 7y period of Tx. • There were no BL observations of pancreatic ENZs. At 6-7y, both ENZ were WNL.

#46, WF, 16y old
N

Urine		Speech		Urine		BIL		AMYL		Reason
N/D	4.8 [1-2y]	1.5	1.5	0.322	0.107 [1-2y]	1.2	0.9 [1-2y]	80	73 [1-2y]	• **Reason:** Uncooperative. She was institutionalized prior to Dx for behavioral problems and attempted suicide. During the investigator's experience with her she continued to have behavioral difficulties and was uncooperative. For these reasons she was discharged from the study.
Plasma		Neuro/Psychiatr		Plasma		ALT		LIPASE		• The pt. entered the Zn AC study with mild neurological symptoms, after short period of penicillamine therapy. These included mild dysarthria, behavior problems and mood swings. The investigator claims that the pt. improved considerable during therapy. However, according to the available data, speech was 1.5 both at BL and F/U. The neuro/psychiatr. and MMR gave normal evaluation at BL (what is there to improve?) and there were no F/Us of these clinical variables. K-F rings were present at the beginning of therapy, but were resolving with therapy.
N/D	204 [1-2y]	0.0	N/D	15	25 [1-2y]	207	N/D	19	38 [1-2y]	• There was some improvement in urine cu, but plasma Cu became abnormal after 1-2y of Tx. Liver Cu was high at BL with no F/U determination to compare with. Cu balance was not done. ⁶⁴Cu test was 0.57 of administered dose.
		MMR		Liver		AST				• Of the LFTs, BIL (very slightly elevated) and AST (>2 time the ULN) normalized by 1-2y. ALT was x7 higher than normal and LDH WNL at BL but there was no F/U determination for these ENZs. AP was high at BL and still remained slightly high at the 1-2y observation time.
		0.0	N/D	983	N/D	111	27 [1-2y]			
						AP				
						255	149 [1-2y]			
						LDH				
						187	N/D			

#64, WF, 55y old
N

Urine		Speech		Urine		BIL		AMYL		Reason
0.2	N/D	4.5	N/D	0.059	N/D	1.1	N/D	153	N/D	• **Reason:** Lack of communication and cooperation.
Plasma		Neuro/Psychiatr		Plasma		ALT		LIPASE		• This pt. had moderate neurological symptoms after 35y of intermittent therapy with penicillamine and BAL. These included moderate dysarthria and an intention tremor. K-F rings were present.
70	N/D	N/D	N/D	14	N/D	15	N/D	34	N/D	• The available data are very insufficient to evaluate the clinical/biochemical response to Zn AC therapy in this pt.
		MMR		Liver		AST				
		5.0	N/D	N/D	N/D	26	N/D			
						AP				
						102	N/D			
						LDH				
						163	N/D			

Pt.						Comments
#67, WF, 37y old **N**	Urine N/D 5.0 [0-1y] Plasma N/D N/D	Speech N/D N/D Neuro/Psychiatr N/D N/D MMR N/D N/D	Urine 0.088 0.109 [0-1y] Plasma N/D N/D Liver N/D N/D	BIL N/D 0.8 [0-1y] ALT N/D 23 [0-1y] AST N/D 31 [0-1y] AP N/D 67 [0-1y] LDH N/D 105 [0-1y]	AMYL N/D 81 [0-1y] LIPASE N/D 16 [0-1y]	• **Reason:** Uncooperative. • This pt. had been Dx with Wilson's Dz 21y previously and treated with penicillamine and BAL. She had presented with neurological disease, dysarthria, tremor, dysphagia. She had been switched to zinc elsewhere 2 y previously, because of skin wrinkling from penicillamine. When seen by the investigator she had mild dysarthria and mild dystonia (by Hx) of one arm. • The available data are too insufficient to determine effects of Tx on clinical/biochemical parameters. • All liver and pancreatic function tests were WNL at the 0-1y evaluation but there were no BL evaluations to compare with.
#71, WM, 25y old **H**	Urine N/D 2.2 [0-1y] Plasma N/D N/D	Speech N/D N/D Neuro/Psychiatr N/D N/D MMR N/D N/D	Urine N/D 0.026 [0-1y] Plasma N/D N/D Liver N/D N/D	BIL 1.2 N/D ALT 80 N/D AST 48 N/D AP 90 N/D LDH 188 N/D	AMYL N/D N/D LIPASE N/D N/D	• **Reason:** Lack of communication and cooperation. • According to the Clinical Report this pt. presented with the hepatic form of Wilson's Dz. He was clinically asymptomatic when he entered this trial. K-F rings were negative at time of Dx. The available data are too insufficient to evaluate clinical/biochemical response to Zn AC maintenance therapy in this pt. The laboratory parameters of safety were not repeated prior to discharging pt. from the trial.

W/D = Value at the time closest to the patient's death.
N/D = Not determined [or parameter not evaluated or assay not done].

• The principal demographic characteristics of the 63 patients being reviewed for the maintenance therapy category were:

Category	Age Range	F/M	No. of Pts. Enrolled
■	16 to 55	27/23	50
■	17 to 45	7/7	13
			Total 63ª

a) Of these, 62 were W; one (#26) was Mexican-American
   The pts. were enrolled between October 1, 1980 and
   December 31, 1990.

• Length of Tx with Zn AC ranged from <3 mo. (Pt. #94) to 9.5y (Pt. #3). The distribution of patients as a function of dose duration was:

Years of Tx	No. of Pts
0 to 1	11ª
1 to 3	19
3 to 5	11
5 to 7	12
7 to 9	9
> 9	1
Total	63

a) 8 of these pts. [#83, 84, 85, 86, 88,
   90, 92 and 94] did not have ample time
   for F/U because they were Tx with Zn AC
   for <1 year.

5. Data Excluded from Analysis

The analysis of efficacy and safety data included all 63 pts. who were entered into the Zn AC maintenance therapy trial. Some data points were excluded but this applied to the evaluations of Cu-related variables (see 6. C., Biochemical Response, below).

6. Efficacy Results

a. Survival in Comparison to a Historical Control (Fig. 8)

[NOTE: These data are reviewed first because survival or
       significant prolongation of life is an important clinical
       parameter.]

The sponsor notes that a literature search turned up 16 pts.[12] that had D/C penicillamine therapy for a variety of reasons.

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[12] Pregnant pts. are not included in this analysis.

Survival Curve with No Treatment
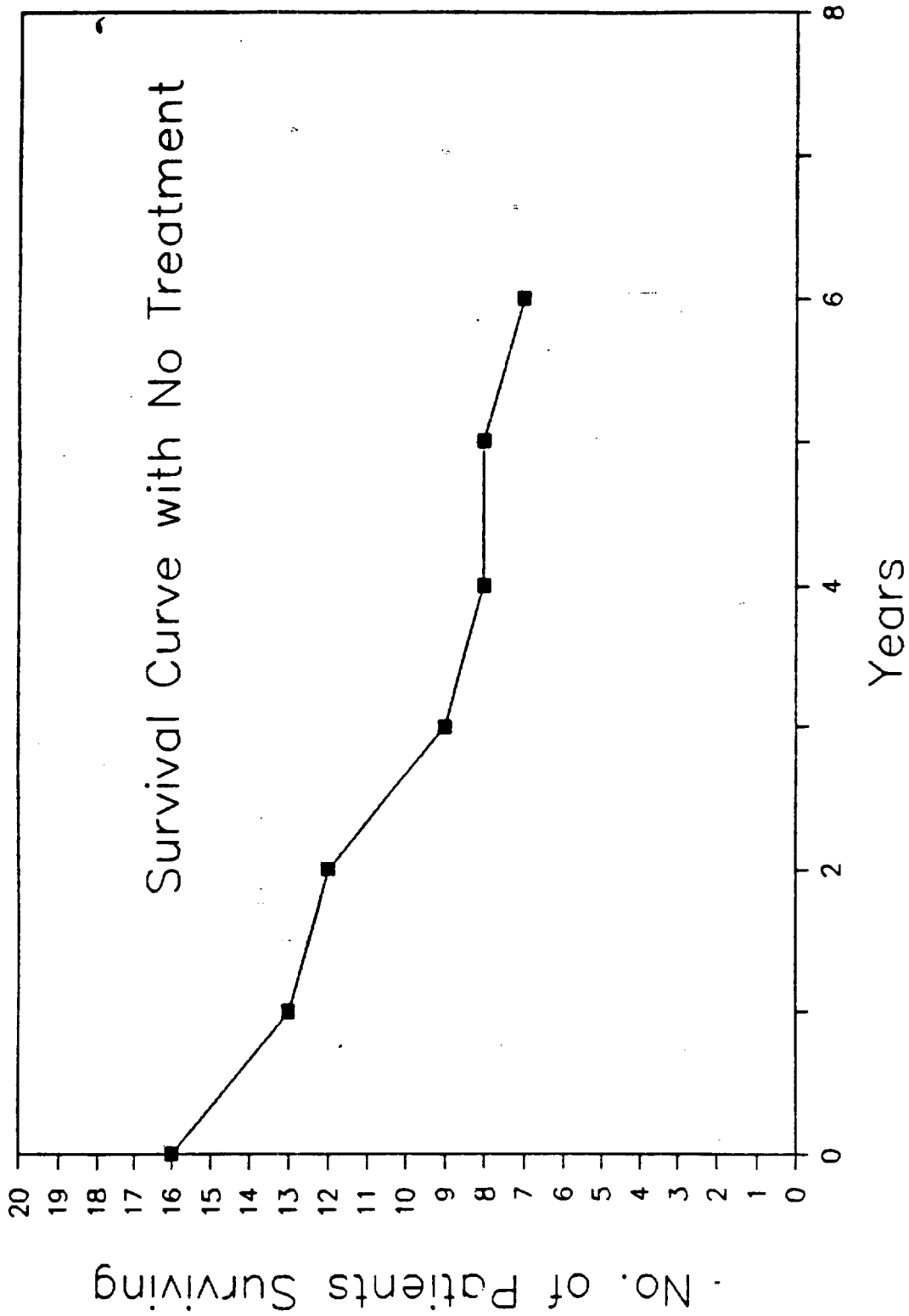
No. of Patients Surviving

Years

Fig. 8. – Death: Survival in patients in whom penicillamine has been discontinued.

- As shown in Fig. 8, half of these 16 pts. were dead in 4y.

- As summarized in Fig. 9, either death or major clinical deterioration [either neurological or hepatic] had occurred in all of these patients within 6 y.

- 7 of the 16 pts. (ca. half of the total number) had either died or suffered a major clinical deterioration by the end of the first year.

   [NOTE: Here, we are taking this important information at face value because the sponsor has not submitted the data (published?) in support of these evaluations/conclusions.]

- The sponsor presented a Fig. (p. 32c) depicting the cumulative summary of the number of years of Zn AC Tx of the 86 patients presented in the report as of January 1, 1991. Although the sponsor's Fig. is reproduced because it is illustrative (Fig. 10), the reviewer's computation below refers only to the 63 symptomatic pts. in the maintenance therapy indication. Of these,

   - 23 pts. have been Tx for 4+ years
   - 22 pts. have been Tx for 5+ years
   - 15 pts. have been Tx for 6+ years
   - 52 pts. have been Tx for >1+ years

   According to the Clinical Report, in no case has there been neurological or hepatic deterioration, or death due to progression of Wilson's Dz. It is further stated that there have been 4 deaths among these 63 pts. but none related to progression of damage from Cu toxicity. The reviewer agrees that these 4 patients did not die from progression of damage from Cu toxicity. But according to the very detailed assessment, in only one of the three pts. that died (#08) there was adequate information to suggest that he appeared to be stabilized at the time of his death. Although the other three appeared to be complaining with test medication, the information on the clinical/biochemical/hepatic/ pancreatic parameters is too incomplete to render an opinion as to whether there was any progression of damage from Cu toxicity or not.

COMMENT

Although survival data are reviewed first, the information provided by the sponsor is incomplete to allow the reviewer to come to an independent conclusion. The sponsor needs to provide information such as the reason for the patients to discontinue penicillamine and arguments in support of the statement that these patients are comparable to the Brewer's patients. Did the patients die of Wilson's Dz or its complications? If the validity of such comparison is established and the conclusion can be reached that this historical control group is reliable, then using survival alone as a parameter of efficacy, the reviewer agrees with the sponsor's conclusion that Zn AC therapy appears effective because no pt. treated for 4+ years has died from progression of Wilson's Dz. This is to be contrasted with the 50% mortality among the 16 pts. on the historical control (D/C of penicillamine).
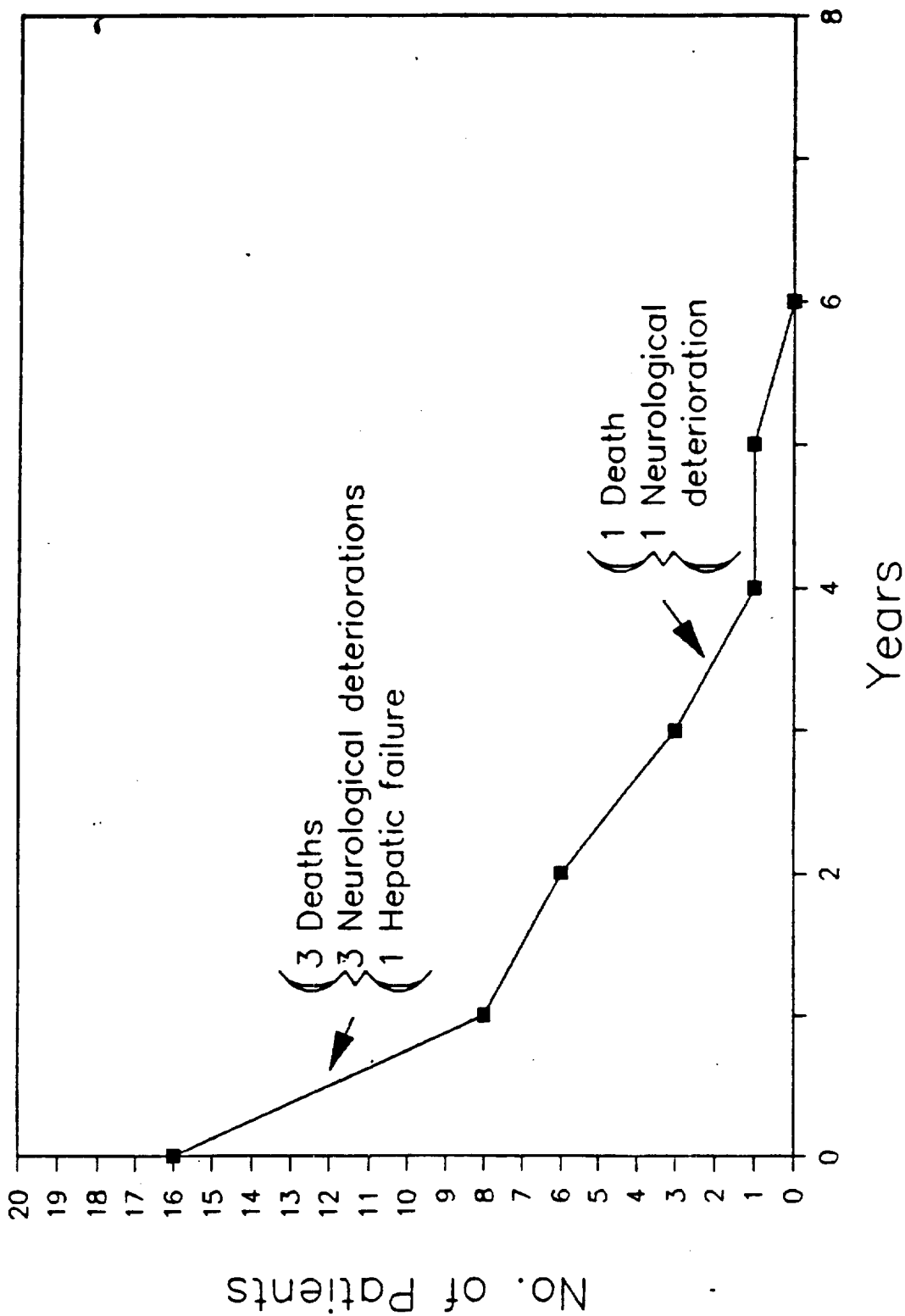
Fig. 9. — _Survival or Major Incident Curve with no treatment (patients off penicillamine)._

- An additional evaluation indicates that, in contrast to the 50% in the historical control, none of the 52 patients Tx with Zn AC for >1+ year (after having completed at least 1 year of therapy) died or deteriorated due to progression of Wilson's Dz.

- The initial conclusion from the above is that, all in all, Zn AC appears to be clinically effective in preventing the progression of Wilson's Dz. But, as already pointed out, for some pts., the information provided is too incomplete to evaluate the degree of effectiveness (as maintenance therapy) of the drug. It does not appear to be 100%, as the sponsor states.

b. Clinical Response

1) Speech Data

The sponsor presented the following Tables:

D.51: All 86 pts. on 3 effective doses of Zn AC
D.52: Pts. with Neurologic/Psychiatric presentation

- As shown in Summary Table 13, there were no clinically significant changes in speech during observations of up to 5+ years of Tx with Zn AC (50 mg x 3 per day). On the average, the pts. had speech alterations that can be categorized as mild to moderately low and did not change with time.

2) Neurologic/Psychiatric Data

These data were presented in sponsor's Tables   . D.59, D.60, D.61, D.62, D.63 and D.64. Of these, only D.60 is pertinent (but to the second indication) and this will be evaluated during the review of data for the pre-symptomatic indication. The following evaluation is from the computations in the October 24, 1994 submission.

- Refer to Table 13. On the average the pts. had abnormalities that can be characterized as mild to moderately low. These did not change with time. There were few observations (5 or less per time period) from 3+ years onwards.

3) NMR Data

These data were presented in sponsor's Tables D.65, D.66, D.67, D.68, D.69, D.70, D.71 and D.72. Of these, only D.68 is pertinent (but to the second indication) and this will be evaluated during the review of information for the pre-symptomatic indication. Computations in the October 24, 1994 submission are addressed here.
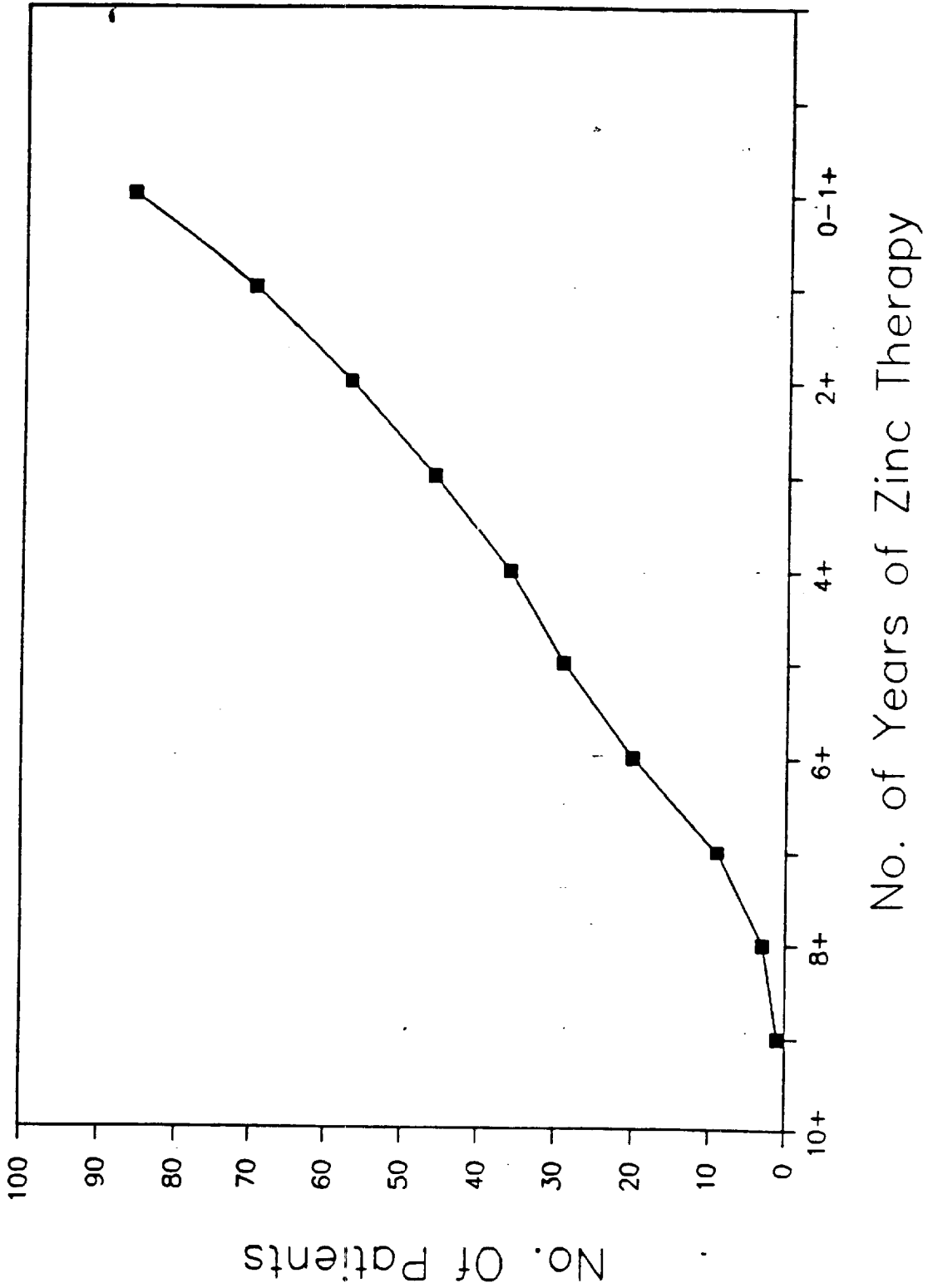
Fig. 10. - _Brewer Study: Cumulative summary of years of treatment. Zinc Acetate treated patients (all patients for both indications)._

TABLE 13
Brewer's Study

Comparison of Means in Clinical Parameters, LFTs and Tests of Pancreatic Function in Symptomatic Wilson's Dz Pt. Administered Zn AC at the Oral Dose of 50 mg x 3

Years of Zn AC Therapy									
0	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	Remarks
I. SPEECH									
N=23 / 2.6	25 / 3.3	16 / 3.0	12 / 3.0	10 / 2.7	10 / 3.3	8 / 2.8	5 / 2.9	2 / 2.5	• Few observations from 6+ years onwards. • The small changes are towards improvement [Scale, severe=7; normal=0].
II. NEURO/PSYCHIATR.									
N=13 / 9.8	16 / 7.7	10 / 5.1	10 / 6.4	5 / 9.6	4 / 7.0	3 / 10.0	1 / 8.5		• Few observations from 3+ years onwards. • No clinically meaningful changes. [Scale, severe=38; normal=0].
III. NMR									
N=9 / 4.3	14 / 7.2	10 / 4.4	7 / 3.0	8 / 3.6	7 / 3.2	5 / 3.8	5 / 2.6		• Few observations from 2+ years onwards. • No significant changes. The apparent ↑ at the 0-1y period in comparison to the BL value can be accounted for by 6 pts. with value ranging from 9 (higher than the highest value at BL) to 12.8 and, especially, 1 pt. (#85) with value of 17 at the 0-1y period and no evaluation at BL. [Scale, severe=20; normal=0].
IV. SERUM ALBUMIN									
N=30 / 4.1	28 / 4.0	24 / 4.2	16 / 4.1	12 / 4.3	8 / 4.3	8 / 4.2	5 / 4.1	2 / 3.5	• Few observations from 6+ years onwards. • Stable values throughout and WNL (Normal values=3.0 to 4.9 g/dl).

V. SERUM BIL

N=32 / 1.0	32 / 0.8	25 / 1.0	16 / 1.0	13 / 1.0	7 / 1.0	8 / 1.0	5 / 1.0	2 / 0.9

- Probably the best indicator that pts. are being properly maintained, from the bio-chemical view point. Most values are very slightly over the ULN.

(Normal=0.1 to 0.9 mg/dl).

VI. SERUM ALT

N=28 / 46.6	22 / 43.7	17 / 39.6	11 / 62.2	10 / 49.8	6 / 43.2	7 / 38.4	4 / 39.0	2 / 25.0

- Few observations from 4+ years onwards.
- No clinically meaningful changes.
- The ↑ at the 2-3y period was due to Pt. #55 in whom ALT was 228 (BL=39; 1-2y=42 IU/L). No F/U value available.

Normal values: 2-35 IU/L.

VII. SERUM AST

N=33 / 40.5	33 / 34.2	24 / 32.4	16 / 38.1	13 / 31.5	8 / 27.3	8 / 29.9	5 / 27.4	2 / 35.0

- Few observations from 6+ years onwards.
- All values are WNL.

Normal = 0-45 IU/L.

VIII. SERUM AP

N=33 / 127.2	33 / 138.7	25 / 130.1	16 / 118.8	13 / 106.5	8 / 109.4	8 / 123.5	5 / 106.6	2 / 195.0

- Few observations from 6+ years onwards.
- Only the 0-1y period value was very slightly above ULN. All others were WNL.

(Normal = 30-130 IU/L).

IX. SERUM LDH

N=27 / 162.9	24 / 160.6	17 / 162.9	11 / 165.2	7 / 162.3	6 / 148.2	7 / 162.4	5 / 162.8	2 / 170.5

- Few observations from 4+ years onwards.
- All values are WNLs.

(Normal = 60 to 200 IU/L).

In this Table, S.D. of the Mean is not shown, for clarity of presentation. The average for serum amylase and lipase are rounded figures.

- On the average, the pts. had NMR alterations of the mild to moderately low type and these did not significantly change with time, although from 2+ y onwards, the number of observations per period included only 8 patients or less.

4) Hepatic Function

For each of the five variables: BIL (Tables D.97 through D.104), ALT (Tables D.81 through D.88), AST (Tables D.89 through D.96), AP (Tables D.113 through D.120) and LDH (Tables D.105 through D.112) information mixed up data from symptomatic and asymptomatic patients. The latter information (Tables D.100=BIL; D.84=ALT; D.92=AST; D.118=AP and D.108=LDH) is pertinent to the pre-symptomatic indication. To those data in the October 24, 1994 submission, evaluations of changes in serum albumin have been added and discussed below.

- Serum albumin concentrations were WNL pre-drug and remained WNL during all periods of observation, up to 7-8 years, although the number of patients per period was gradually decreasing (Table 13).

- Serum BIL concentration was very slightly high (↑ of 0.1 mg/dl) at BL (Table 13). This parameter remained virtually unchanged for up to 7+ years of Zn AC maintenance therapy. BIL is probably the most stable biochemical parameter, with little if any normal changes throughout the years. This is a good indicator that these pts. are being properly maintained in regards to liver function.

- As shown in Table 13, ALT was, on the average, higher than the ULN by ca. 11 iU/L. The observed changes were not clinically important because they never amounted to even x2 the BL value, except in Pt. #55. In this pt., the ALT values were 39 and 41 iU/L at BL and the 1-2y observation periods, respectively. The ALT value ↑ to an abnormal 228 iU/L (> x 4 the BL value) but there were no follow-up observations in this pt. This change in ALT in this pt. should be considered together with changes in other parameters (see below).

- As shown in Table 13, AST was, on the average WNL at BL and remained WNL throughout the duration of the trial (up to 7+ years). For Pt. #55, changes in AST and other parameters of liver function are displayed below (after the LDH subsection).

- On the average, serum AP was WNL at BL and remained so throughout the trial (Table 13). The increase in 12 iU/L from the BL to the 0-1y period is not clinically significant but the changes in Pt. #55 are discussed below.

- LDH values were, on the average, WNL at the start and remained WNL throughout the trial (Table 13).

- Results of evaluations of parameters of liver function in Pt. #55 are summarized as follows.

1 page
PURGED

Individual Raw
Data

c. **Biochemical Response**

The sponsor presented data on Cu variables in urine, non-ceruloplasmin plasma
and liver. A large portion of these data were reviewed under V.
Pharmacokinetics/Pharmacodynamics. This demonstrated a L-T pharmacodynamic
effect by Zn AC, together with a S-T pharmacodynamic effect on ^{64}Cu absorption
and Cu balance. Although data from pre-symptomatic patients were presented
separately, the Tables for strata according to dose, mode of clinical
presentation (N or H) and gender included the data from pre-symptomatic
patients. These Tables need to be corrected so as to include symptomatic data
only. This should be requested of the sponsor.

In addition, the data in sponsor's Tables D.1 to D.19 did not include data
from a number of patients due to a number of reasons (for some pts. more than
one reason was listed). These reasons are valid not to include the
observations on the particular time points (day) for the particular Cu
parameter because the important PD effects are shown L-T and these exclusions
would not influence results (see Table 13a).

TABLE 13a
Brewer's Study

No. Pre-Tx With Zn	Cu Related Variables Data Excluded From Analyses and Reasons				
	Vit. C Adm.	Penicillamine Adm.	ATTM Adm.	TRIEN Adm.	Other
#3 [07-01-81]	#3 [10-17-89]	#3 [09-01-90]	#38 [08-05-88]	#12 [07-05-89]	#5 [01-01-83]ᵃ
#6 [12-01-82]	#5 [10-21-83]	#6 [05-13-83]	[08-12-88]	#44 [08-21-86]	#5 [05-25-86]ᵇ
#7 [03-01-83]	#38 [07-14-90]	#34 [11-06-84]	#56 [07-31-87]	[11-03-86]	#8 [10-15-83]ᵇ
#9 [05-22-83]	#41 [07-05-89]	#69 [11-09-88]	[08-11-87]	#50 [09-22-87]	#9 [01-31-84]ᵃ
	#44 [01-05-90]	#80 [08-05-89]	[08-20-87]	#53 [05-25-87]	#9 [06-25-86]ᵇ
	#48 [04-29-90]	#81 [08-05-89]	[09-11-87]	[07-08-87]	#12 [11-05-84]ᵇ
	#53 [01-05-90]		#61 [03-04-88]	#80 [06-13-80]	#16 [03-01-84]ᵇ
			[03-11-88]	#81 [06-03-80]	
			[04-09-88]		
			#63 [03-26-88]		
			[04-02-88]		
			#68 [10-19-88]		
			#79 [09-20-89]		
			[11-01-89]		
			#84 [05-29-90]		
			#85 [08-16-90]		

a) Pt. had been non-compliant prior to admission
b) Special study of high Cu diet
c) Technical problems with Cu balance

- Due to technical problems with the analysis, liver Zn data (sponsor's data D.150) did not include the Bx data for the following 4 pts. (on the dates indicated): #46 [05-05-86], #47 [05-16-86], #49 [08-21-86] and #55 [08-19-87].

    7. **Zn Variables as a Tool to Monitor Compliance**

        a. **Urine Zn (Table 14)**

Summarized in this Table are urine Zn data over time from all patients treated and results according to dose, clinical presentation (50 mg x 3 dose) or gender (also 50 mg x 3 dose).

TABLE 14
Brewer's Study

Urine Zn data (Mean mg/day) as a function of years of Tx
and according to patient subpopulations

YEARS ON ZN THERAPY									
0*	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9
I. OVERALL DATA[b] (ALL DOSE REGIMENS)									
N = 23 0.3	75 4.5	63 3.9	51 3.7	41 3.7	33 3.0	27 3.4	18 2.9	6 3.4	1 14.9
II. PTS. TREATED WITH 50 mg X 3									
N = 19 0.3	56 4.3	44 3.9	33 3.9	23 4.0	19 3.4	16 3.7	11 3.3	4 3.2	--- ---
III. PTS. TREATED WITH 50 mg X 2									
N = 2 0.3	6 6.4	6 4.7	6 4.6	6 4.2	5 2.9	4 4.0	3 3.2	1 5.5	1 14.9
IV. PTS. TREATED WITH 25 mg X 2									
N = 2 0.4	13 4.6	13 3.6	12 2.8	12 2.9	9 2.4	7 2.4	4 1.5	1 2.3	--- ---
V. Data from N pts. (50 mg X 3)									
N = 10 0.3	31 4.3	23 3.9	15 4.1	12 4.0	11 3.5	8 3.3	5 2.6	2 2.6	--- ---
VI. DATA FROM H PTS. (50 mg x 3)									
N = 0 ---	10 4.7	7 4.0	7 4.7	3 5.6	1 9.0	2 6.0	2 4.6	2 3.7	--- ---

VII. DATA FROM FEMALE PTS. (50 mg x 3)									
N = 12 0.3	29 4.3	23 3.5	18 3.7	14 3.3	13 3.2	11 3.1	8 2.3	4 2.2	--- ---

VIII. DATA FROM MALE PTS. (50 mg x 3)									
N = 9 0.3	29 4.3	23 4.3	17 4.1	11 4.9	8 3.7	7 4.7	5 5.2	2 5.9	--- ---

a) BL values ranged from 0.1 to 0.5 mg/day.
b) This and the other substrata include data from symptomatic and non-symptomatic pts. Values in individual pts. ranged from as low as 0.3 to as high as 14.9. Three pts., #s 18, 36 and 85, with no BL determinations, had very high values at the 0-1y interval: 13.1, 11 and 10.5. In the first, values came down with time. Values in the second showed marked fluctuation but also came somewhat down. There were no F/U data for the third pt.

- There is some suggestion of dose response. Although there were no marked differences between 50 mg x 3 and 50 mg x 2, in general, after the first year, Zn urine values after 25 mg x 2 were lower than with the other two regimens.

- Neither clinical presentation (N vs H) nor gender (F vs M) appeared to markedly influence urinary excretion of Zn. Some numerical differences noted in Table 14 are the result of too few observations per cell.

b. Plasma Zn (Table 15)

- Although the number of observations in some cells is too small, with a large degree of variability, it does not appear to be a dose effect.

- Neither the mode of clinical presentation (N vs H) nor gender (F vs M) appear to have an effect on plasma Zn concentration.

c. Liver Bx Zn

Normal values are given as 100 to 500 (ppm) μg/g day weight. The sponsor presented results of this evaluation in their Table D.150 (all pts. included).

- 14 of the 35 pts. that had liver Zn concentrations determined at BL showed values higher than the UNL (>500 ppm). The observed changes in this parameter are summarized below. The mean Zn concentrations tended to increase for up to 3 years of Tx. The values thereafter appear to be decreasing but the number of observations from 2+ y onwards is small.

Brewer's Study
Liver Bx Zn data (mg/g dry Wt) in patients
being treated L-T with Zn AC
(All patients included)

YEARS OF ZN AC THERAPY[a]									
0	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9
N = 35 431	22 1323	14 1202	6 1150	5 782	4 428	4 822	3 452	0 ---	1 988

a) Depicted are mean values as a function of time. S.D. mean are not depicted for clarity of presentation.

TABLE 15
Brewer's Study

Plasma Zn data (mean μg/dl) as a function of years of Tx
and according to patient subpopulations.

YEARS OF ZN THERAPY									
0*	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9
I. OVERALL DATA[b] (ALL)									
N = 18 / 89	59 / 218	47 / 223	33 / 226	28 / 199	21 / 214	15 / 208	10 / 183	3 / 177	1 / 321
II. PTS. TREATED WITH 50 mg X 3									
N = 15 / 84	41 / 200	33 / 212	18 / 220	13 / 213	11 / 222	10 / 217	5 / 221	2 / 140	--- / ---
III. PTS. TREATED WITH 50 mg X 2									
N = 2 / 126	6 / 234	4 / 215	5 / 229	6 / 167	3 / 197	2 / 174	2 / 143	0 / ---	1 / 321
IV. PTS. TREATED WITH 25 mg X 3									
N = 1 / 94	12 / 270	10 / 260	10 / 236	9 / 202	7 / 209	3 / 202	3 / 144	1 / 252	--- / ---
V. DATA FROM N PTS. (50 mg X 3)									
N = 20 / 90	23 / 229	16 / 224	10 / 242	7 / 221	8 / 234	6 / 211	4 / 224	1 / 208	--- / ---
VI. DATA FROM H PTS. (50 mg X 3)									
N = 8 / 80	8 / 146	5 / 210	4 / 201	3 / 199	0 / ---	0 / ---	0 / ---	1 / 72	0 / ---
VII. DATA FROM FEMALE PTS. (50 mg X 3)									
N = 11 / 90.3	31 / 233	24 / 214	20 / 228	14 / 198	13 / 241	9 / 200	7 / 205	3 / 177	0 / ---
VIII. DATA FROM MALE PTS. (50 mg X 3)									
N = 7 / 87	28 / 202	23 / 232	13 / 223	14 / 201	8 / 170	6 / 221	3 / 131	0 / ---	1 / 321

a) BL values ranged from 8.0 to 126 μg/dl
b) This and the other substrata include data from symptomatic and pre-symptomatic pts.

8. **Results of Safety Evaluations**

   a. **Deaths (N=4), Dropouts (N=3) and Discharges (N=6)**

• The reviewer gave a very detailed account of the events related to each of these 13 individual patients (Tables 10, 11 and 12).

   According to the sponsor, the progression of Cu toxicity, or the development of Zn toxicity was not considered contributory to the cause of death in any of the four patients (2 were accidental deaths). Although the reviewer does not entirely disagree with this assessment, the question of how well were these patients being maintained has been raised. The information is incomplete to answer this question with certainty.

• Similarly the sponsor states that none of the 3 pts. who dropped out did so because of side effects or intolerance of Zn, or progression of Cu toxicity. But Pt. #1 elected to have liver transplant and Pt. #28 was advised by Mayo Clinic doctors to go back to penicillamine therapy because of hepatic copper values. These appear to be instances of Tx F.

• Pts. # 4, 5, 46, 64, 67 and 71 were discharged from the trial primarily due to lack of adequate cooperation. None of these was discharged because of side effects or intolerance to Zn or because of a progression of Cu toxicity. But, in at least 4 of these patients (#s 5, 64, 67 and 71) the available information is too inadequate to draw conclusions on drug effects (beneficial or untoward) on clinical/biochemical parameters.

   b. **Other Adverse Events**

• "Gastric intolerance" to Zn AC was exhibited by 4 patients (#s 37, 39, 77 and 92), mostly during the first morning dose. In three cases this was corrected by having the pt. take the first morning dose at midmorning rather than prior to breakfast.

   The nature of this "gastric intolerance" has not been characterized. Endoscopy was not performed.

• One pt. developed pneumonia. This was Dx at a different institution as due to pseudomonas.

   c. **Results of Evaluation of Laboratory Parameters**

• As previously mentioned, the overall data on mean serum albumin (g/dl) showed little if any change as a function of time.

   - Of the 54 patients with observations before the start of Zn AC therapy, 8 have values of 3.5 or < g/dl and 7 had values 4.9 g/dl or higher.

   - As shown in Table 16, in H patients, the number of observations per cell is too few. Firm conclusions cannot be drawn but there appeared to be no marked differences between N and H patients.

   - Neither observations according to dose nor according to gender gave significant effects of those factors on serum albumin.

TABLE 16
Brewer's Study

Serum albumin concentrations (g/dl) as a
function of time

YEARS OF ZN AC THERAPY									
0	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9
I. ALL PATIENTS INCLUDED									
N = 54 4.2	51 4.2	50 4.3	32 4.5	30 4.2	24 4.4	15 4.2	11 4.4	3 3.5	1 4.6
II. DATA FROM N PTS. (50 mg x 3)									
N = 30 4.2	31 4.3	27 4.4	20 4.6	21 4.3	17 4.4	11 4.2	10 4.4	2 3.9	1 4.6
III. DATA FROM H PTS. (50 mg x 3)[a]									
N = 9 3.6	8 3.6	7 4.0	6 4.1	3 4.0	0 ---	0 ---	0 ---	1 2.8	0 ---

Depicted are mean values; S.D. mean have been omitted for clarity of presentation.
a) Included are data from Pts. #26 (compliance problems, according to the sponsor) and #69 (severe cirrhosis that may necessitate liver transplantation).

- The hematologic parameters either remained within the normal range or were stable over the years, with no consistent trend to change over time. Occasional low Hb values were seen (Pts. #16, 50, 62, 81 and 92), some changes in MCV, MCH and MCHC (again Pt. #62)[13]. Some female pts. may carry a somewhat low Hb. Some low WBC and platelet count values are expected from patients with hypersplenism accompanying the underlying condition. Wilson's Dz patients also have a tendency to show elevated reticulocyte counts at BL. High reticulocyte counts in Pts. #s 53 (6.9%) and 90 (4.5%) can be attributed to liver Dz. But in most pts. F/U observations were too few to draw well founded conclusions on the reticulocyte evolution as a function of L-T Tx.

    - Values on serum iron and transferrin tended to be normal and stable over time.

---

[13] Pt. #62 became iron deficient due to heavy menstrual periods. Her hematologic parameters changed accordingly:

Pertinent Hematologic Parameters in Pt. #62
Years on Zn AC Therapy
0   0-1   1-2

- Most serum ferritin values were stable over time. BL values in pts. #48, 53, 68, 77 and 79 were high but, with time, they eventually decreased to WNL values. The reason for this change and the decrease in serum ferritin (all changes WNLs) in 21 pts. is not known.

• The renal variables (creatinine, BUN, 24h Uric Acid) were, for the most part, stable over time, specially if the data for the up to 6-7 years time period are considered. Numerical variations, especially at the 7-8 and 8-9 time periods, are most likely due to the small number of observations. The range of values was always narrower if one computed data from the first 7 rather than the entire 9 years of observation.

- Only a few pts. showed any sporadic proteinuria.

• Some changes in lipid variables (Total CHOL, HDL-CHOL, LDL-CHOL, TGs) were noted. But, due to many reasons, these are of doubtful clinical relevance. The observations did not control for intake of dietary fat, or other factors influencing lipid metabolism. The "beneficial" findings reported by the sponsor were neither very dramatic nor always consistent. The CHDRF[14] did not change in either sex.

• Some changes in parameters of pancreatic function (amylase, lipase) are worth noting. The sponsor states that some Wilson's Dz patients have elevated serum levels of these enzymes. This was indeed seen in some pts. but it was not well documented because only ca. half of the pts. had BL determinations of pancreatic enzymes.

- When considering all the Zn AC-treated patients, 7 of the 44 pts. who had BL amylase determinations showed values >UNL (>100 iu/dl). In two of these 7 pts. [#58   and #64   the lipase values were simultaneously elevated (>24 iu/dl). An additional pt. (#19=   · also had simultaneous ↑s of serum amylase and serum lipase concentrations.

- In a number of pts. the amylase values often increased to slightly elevated values as Zn AC therapy was initiated. The average changes for those patients treated with 50 mg x 3 per day Zn AC are illustrated in Table 17.

- In pts. # 5, 7, 38 and 50 the increases in serum amylase concentrations occurred simultaneously to increases in serum lipase concentrations.

- Also listed in Table 17 are the changes occurring in Pt. #19.

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[14] Coronary Heart Disease Risk Factor

1 page
PURGED

INDIVIDUAL RAW
DATA

* Changes in routine laboratory tests including electrolytes, calcium, phosphorus, glucose and also other parameters such as EKG and chest X-ray were followed as a function of time. These were not necessarily part of the database. Results of these evaluations showed usually normal values at BL and little if any changes in those abnormal at BL.

9. Conclusions (Sponsor)

"From this study it is concluded that:

■ Zinc acetate in doses of 50 mg (elemental zinc) tid, 50 mg bid, and 25 mg tid is fully efficacious in the maintenance therapy of Wilson's disease.

■ The recommended dose for adults should be 50 mg tid, because this dose provides a safety factor, keeping in mind the lifelong nature of the treatment. The recommended dose for children is 25 mg tid.

■ The efficacy of zinc acetate is documented by comprehensive studies, involving:

- Copper studies, including copper balance, "copper uptake, urine copper, and non-ceruloplasmin plasma copper.

- Liver function studies

- Clinical studies including quantitative speech, neurological, and brain MRI exams.

■ The efficacy of zinc acetate is not affected by duration of use.

■ The efficacy of zinc acetate is 100% in this study. None of the [63] patients had progression of their disease from copper toxicity during the period of observation.

■ Zinc acetate in recommended doses has very little toxicity in Wilson's disease:

-The only observed side effect in the 86 patients studied was mild gastric symptoms with the first morning dose in five patients. This was rather easily mitigated by giving that dose in midmorning or having the patient take the dose with meat or seltzer water.

-Hematologic, chemical, and urine variables are all stable over a period of many years of therapy."

10. Comments

Data from the Brewer Study was submitted by the sponsor as one of the two trials in support of the approval of zinc acetate, as a second line drug, for the maintenance treatment of symptomatic Wilson's Dz patients that had initially been treated with a chelating agent. The trial was well-designed. Although information - sometimes critical - is missing for some patients, the study was, all in all, well executed. The open-label design used in the Brewer Study is adequate. The use of a concurrent PL control is ethically inappropriate because of the progressive and invariably fatal nature of the disease. The use of a concurrent active comparator (penicillamine, trientine HCl) would not be adequate either, for several reasons. These include: a) the patient population of interest is that where penicillamine has been

discontinued, not one where the patients continue taking the chelating drug;
b) to demonstrate superiority of the test medication over penicillamine is
probably an impossibility because this chelating agent is very effective.
(>90%); c) demonstration of clinical/biochemical equivalence is possible, but
again, this would be necessary if the sponsor were requesting the approval of
Zn AC as a first line drug; most important, d) both penicillamine and
trientine HCl (the other possible comparator) have been shown to be associated
with certain risks.

Under these circumstances, one is left with the use of a historical control.
If such historical control is certain then the use of each patient as his or
her own control, as in the Brewer Study, is a good approach. But for this
approach to be successful, an important requirement, is precisely, the
availability of baseline clinical and biochemical observations. If such pre-
drug evaluations are lacking then it become impossible to demonstrate the
effects of the test medication. One needs to know what clinical
manifestations of the disease are being maintained and to what extent.
However, in the absence of BL observations, it is possible to demonstrate that
clinical and biochemical parameters are being maintained if there are serial
observations of these parameters while the patient is taking the drug (several
years). The BL data to be used for comparison is moved forward.

The aim of the trial was to maintain body Cu at subtoxic levels and prevent
progressive target organ damage. This assessment was facilitated by the
availability of methods for the precise measurement of urinary, plasma and
hepatic copper. The study population consisted of patients that had undergone
adequate decoppering with chelating agents, Zn salts or other agents and were
symptomatic at the start of Zn AC therapy. It is important to note that
maintenance therapy was defined as that period of Zn AC therapy when the
therapeutic objective was to prevent the accumulation or reaccumulation of Cu
and to prevent the reappearance or the worsening of the symptoms of Cu
toxicity. The maintenance period was preceded by a 2-month period of
"initial" therapy. During this period, the Cu levels were to be reduced to
subtoxic amounts. Although this was not always accomplished or was not always
properly documented, this plan is sound. This is not getting a high Cu level
under control but rather keeping a low level low. Evidence in this and the
other trial (Zn SO4) indicates that this approach controls disease.

Depending on predominant clinical presentation, the pts. were classified
either as N if they had predominately neurologic/psychiatric symptoms (50
pts., 36 of which had been previously treated with penicillamine) or H if they
had predominantly hepatic symptoms (13 pts., 10 of which had been previously
treated with penicillamine). Although 63 pts. is a large sample for a rare
disease, the few cells under the various strata did not allow formal
statistical approaches. Efficacy and safety variables were evaluated on the
basis of descriptive statistics. A total of 13 pts. are no longer on the Zn
AC maintenance trial. Deaths (N=4) were unrelated to test medication. Two of
three dropouts appeared to be Tx Fs. Most discharges (total N=6) were due to
lack of cooperation. The reviewer attempted to determine whether these
patients were being adequately maintained during the period prior to their
dismissal from the trial. But in the majority of these pts. that withdrew or
were withdrawn from the trial the clinico/biochemical and safety data were
insufficient to determine drug effects. The point is made that in at least 10
of these patients drug response could not be evaluated due to lack of critical
information. These data do not seem to support the sponsor's claim of 100%
efficacy.

The trial included long-term evaluations. Of the 63 pts. enrolled, 52 were treated for one year or more (1 to 3y, N=19; 3 to 5y, N=11; 5 to 7y, N=12; 7 to 9y, N=9; >9y, N=1). Eight of the remaining 11 did not have ample time for F/U because they were treated for < 1 year.

NOTE ON THE RELIABILITY OF HISTORICAL DATA

The following information was taken from a memorandum, dated February 20, 1985, from Dr. William H. Bachrach to Dr. Raymond J. Lipicky (both of HFD-110). The subject matter is TRIEN (trientine HCl).

Summary of Outcome off Penicillamine (mean years)

	n	To Relapse[a]	n	To Death
All Cases	12	2.67	9	2.59

a) Symptoms, LFTs and free serum copper reverted to
   abnormal pre-penicillamine stating in an average of 2.7y
   (range=3 months to 9y; 3y or less in 9/12).

In the Brewer trial, Zn AC, 50 mg t.i.d. was shown to be efficacious. In no case has there been overt neurological or hepatic deterioration or death due to progression of Wilson's Dz in these patients treated continuously for 4+ years. This is to be contrasted with the 50% mortality among the 16 pts. that discontinued penicillamine and that served as a historical control. Moreover, none of the 52 pts. treated with Zn AC for > 1+ year (after having completed at least 1 year of therapy) died or deteriorated due to progression of Wilson's Dz. This is in contraposition to the 50% mortality among the 16 pts. in whom penicillamine had been discontinued.

In the Brewer Study, the patients had speech, neurologic/psychiatric and NMR evaluations suggesting that - on the average - they had mild to moderate conditions. Proof of efficacy was that their clinical status did not significantly change with time. Except for a few outliers (for ex. Pt. #55 whose LFTs appeared to begin to deteriorate but whose serum bilirubin concentration was stable) most patients maintained parameters of liver function that did not change much with time. As a rule, there were little changes in albumin, transaminases (both OT and PT), AP, LDH and especially, bilirubin. The stability of the latter parameter is one of the best demonstrations that - from the hepatic function viewpoint- these patients were adequately maintained.

On the average, the oral dose of Zn AC of 50 mg x 3 per day produced an at least ten-fold enrichment in urinary zinc (BL=0.3; post-drug=3.2 to 4.3 mg/24h). Plasma zinc concentration showed a less dramatic although apparently consistent increase (BL=84, post-drug=140 to 222 μg/dl). Although Zn AC, at the dose of 50 mg x 3 per day produced - roughly - a 3-fold ↑ in liver Zn during the first three years of maintenance treatment, thereafter, the values varied considerably. It is not known whether these marked variations in plasma Zn were due to the fewer number of observations per cell, interference with Zn AC absorption, lack of compliance or combinations of these factors influencing Zn bioavailability.

In conclusion, Zn AC, at the oral dose of 50 mg t.i.d., is efficacious as a maintenance therapy in symptomatic Wilson's Dz patients that had been previously decoppered by administration of chelating agents. This trial also showed this dose and dose regimen of Zn AC to be well tolerated.

B. Hoogenraad's Studies

The sponsor submitted five (5) publications from Dr. Hoogenraad's work[15]. Of
these, the most recent [J. Neurolog. Sci. 77:137-146 (1987)] describes the
management of Wilson's Dz with Zn SULF in 27 patients. These data are
reviewed in detail below.

> NOTE: The 27 pts. in this study include five that were pre-symptomatic.
> Data from these five are reviewed under the second indication.
> The material that follows is considered pertinent to the first
> indication.

1. Introduction

The authors state that, in the Netherlands, Zn SULF has been used for many
years in the Tx of Wilson's Dz, the first report dating to 1961 when clinical
amelioration was shown in 2 pts. that were Tx with Zn SULF [G. Schouwink,
Thesis, with a Summary in English][16]. The authors believe that the
effectiveness of Zn SULF has been proven by these uncontrolled studies because
the disorder is invariably fatal without effective therapy [H.R. Wulff,
Rational Dx and Tx, An Introduction of Clinical Decision-Making. Blackwell
Sci. Publ., Oxford]. Hoogenraad maintains that the advantage of Zn SULF
therapy in comparison with penicillamine is that the toxicity of Zn SULF is
very low. Three references are mentioned in support of this statement
[M.W. Graeves and A.W. Skilen, who in 1981 published a paper on the effects of

L-T ingestion of Zn SULF in patients with venous leg ulceration; Floersheim
and Kull, Schweiz. Med. Wischr. 110:1250-1254 (1980) and Tschumi and
Floersheim, Ibid 111:1573-1577 (1981)]. Since 1977, most of Hoogenraad's
patients have been treated with Zn SULF.

2. Objective

The goal of therapy was to improve the clinical condition of the symptomatic
patients and to prevent the occurrence of symptoms and signs in the
asymptomatic pts. The study was conducted in accordance with the Helsinki
Agreement of 1975.

3. Study Population

- 25 pts. were referred to be treated for Wilson's Dz either at the time
  of Dx or after a period of Tx with penicillamine.

- 2 pts. (#1 and 2) who had been instituted on Zn SULF Tx since 1958 were
  also included in this study.

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[15] The other four publications are only briefly summarized for PK/PD, efficacy and safety but not
reviewed in detail. The first [Br. Med. J. 289:273-276 (1984)] consists of 2 case reports. The second [Trace
Ele. in Med. 1:84-87 (1984) describes ^{64}Cu loading tests for monitoring Zn therapy in Wilson's Dz. The other
two [Acta Neurol. Scand. 67:356-364 (1983) and Eur. Neurol. 18:205-211 (1979)] provide information on 3-y of
continuous oral Zn therapy in 4 pts. and on oral Zn SULF as L-T Tx in Wilson's Dz.

[16] This author also showed that Zn SULF induces the excretion of Cu via the stools.

- Dx was confirmed by criteria described by Scheinberg and Sternlieb [Wilson's Disease. Major Problems in Internal Medicine. Vol. 23. Saunders, Philadelphia (1984)].

- All pts. had very low incorporation of ^{64}Cu into ceruloplasmin.

- The following 3 groups of Pts. can be distinguished:

Group I: Consisted of 7 pts. (#11, 12, 13, 14, 15, 16 and 17) in whom intolerance to penicillamine had developed. These pts. had either systemic side effects while on penicillamine or had developed a paradoxical and serious increase of neurological signs after that chelating agent.

Group II: Consisted of 8 pts. (#18, 19, 20, 22, 23, 24, 25 and 27) that were switched over to Zn SULF after initial Tx with penicillamine but without development of intolerance.

Group III: Consisted of 7 symptomatic pts. (# 1, 2, 3, 4, 5, 6 and 9) who were Tx from the start with oral Zn SULF.

4. Materials

- Zn SULF (Zn $SO_4 \cdot 7H_2O$) was administered in capsules, 30 min. before meals.

- In adults the initial dose was 600 mg/day, in three divided doses.

- In children, the dose was 300 mg/day in three divided doses.

- All pts. were placed on a diet containing ca. 1.2 mg Cu/day.

5. Methods

- Assessment of the results of therapy was performed by interval Hx and physical, neurologic and corneal examination.

- Every 6 weeks CBC, platelet count, urinalysis, measurement of transaminase levels and analysis of plasma for Cu, Zn and ceruloplasmin was performed.

- Liver Bx were performed before and after Tx with Zn in 13 patients, with intervals varying from 1 to 8y.

- Plasma Cu and Zn were determined by atomic absorption spectrometry, ceruloplasmin by quantitative radial immunodiffusion and Cu and Zn concentrations in the liver by neutron activation analysis [J.L. Nooijen et al., Clin. Chim. Acta 113:335-338 (1981)].

6. Endpoints of Efficacy

- Primary: To improve the clinical condition of the symptomatic pt. and to prevent the occurrence of symptoms and signs in the asymptomatic pts.

- Secondary: A freely diffusible fraction of plasma Cu concentration. In effectively treated pts. the free plasma Cu concentration[17] should be <0.1 mg/l.

---

[17] Free Cu is the difference between total plasma Cu and ceruloplasmin-bound Cu.

[Scheinberg and Sternlieb (locus cited) (1984)].

7. Results

a. Group I (Table 18)

- 3 pts. in this group were treated with 200 mg x 3 and 4 pts. with 300 mg x 3. The number of pts. per dose is small. No dose-response was apparent.

- Pronounced enrichment of the liver Zn pool was shown in one pt. in each group. These two pts. (#17 and 16) also showed the highest plasma Zn values.

- 5 of the 7 pts. listed in Table 18 showed decreases in free Cu in the plasma; in 3 of these (Pts. #13, 12 and 16), free Cu had been above normal during Tx with penicillamine but the levels normalized after the start of Zn SULF therapy. In the two additional pts., no changes in this parameter were observed.

- There were no pronounced changes in the liver Cu concentration (3 pts.).

- Although evaluations were not done in a quantitative fashion (no scales or scores were used), this reviewer agrees with the sponsor that - clinically - all 7 pts. in this group reacted favorably to oral Zn SULF therapy (compare columns labeled symptoms and signs at the start vs end of Zn SULF therapy).

- Attention is called to the clinical evaluation of Pts. #15 and 16, also reported in detail previously [Hoogenraad et al., Ned. T. Geneesk, 129:529-532 (1985)]. These two pts. had developed a severe worsening of the neurological Dz during penicillamine, were bedridden, in great dismay and total desperation. A dramatic clinical recovery started in the first weeks after D/C of penicillamine and institution of Zn SULF.

b. Group II (Table 19)

- Of the 8 pts. listed in this Table, 3 received Zn SULF at the oral dose of 600 mg/day; 5 received 900 mg/day. Plasma Zn levels were higher (1.9 to 2.5 mg/l) in the latter than the former group (0.5 to 1.9 mg/l). The higher dose also gave higher liver Zn levels but there was only one determination of this parameter in the 600 mg/dl group.

- Liver Cu levels increased in 3 pts. (#22, 20 and 24) and decreased in one (Pt. #23; 900 mg/day) from x 3 WNL to slightly higher than UNL.

- Four pts. (#27, 19, 20 and 24) were asymptomatic at the start of Zn SULF Tx and remained asymptomatic after 3 to 4 years of Tx.

- The cirrhosis in Pt. #18 (treated for 2 years) did not change.

- The clinical signs/symptoms in Pts. #22 and 23 improved after 6.8 and 5.5y of Zn SULF Tx (600 and 900 mg/day, respectively). Nonetheless, Pt. #22 asked to be switched back to penicillamine.

TABLE 18
Hoogenraad's Study

Response to Orally Administered Zn SULF After Intolerance to Penicillimine

Pt. Identif.	Symptoms and Signs at the Start of Zn SULF Therapy	Length of Tx	Plasma Zn (mg/L)	Liver Zn BEF AFT	Free Cu (mg/l) BEF AFT	Liver Cu BEF AFT	Symptoms and Signs at the End of Zn SULF Therapy (Response to Change)
	I. PTS. TX WITH 600 mg/day (N=3)						
#11, F36							
#13, F37							
#17, F11							
	II. PTS. TX WITH 900 mg/day (N=4)						
#12, M37							
#14, F27							
#15, F23							
#16, M21							

• **Pt. #25, in the 600 mg/day Zn SULF subgroup, was a Tx F.**

At the start of Tx, the pt. had cirrhosis, esophageal varices and thrombocytopenia and remained in stable condition during the first 2y of continuous Zn SULF therapy. After an emergency operation because of torsio testis the pt.'s hepatic failure and ascites increased. He preferred to be switched back to penicillamine, the Tx on which he had been in stable condition for many years.

  – 8 mo. later, this pt. died of a meningococcal meningitis.

  – When pt. #25 was switched back to penicillamine, his brother (Pt. #26) also preferred to do the same.

• **Free Cu either did not change (5 pts.) or decreased with Tx (3 pts.).**

  c. <u>Group III (Table 20)</u>

  • The number of pts. per dose regimen was too small; no firm conclusions about dose response can be drawn.

  • Only in pts. #3 and 6 (900 mg/day) the liver Zn levels increased with respect to BL to values >UNL. In 3 other patients (#s 1, 2 and 4, receiving 600, 600 and 1200 mg/day, respectively) the liver Zn levels were WNL after Tx but there were no BL determinations to compare with.

  • Pts. #3 (900 mg/day) and #4 (1200 mg/day) that had markedly elevated liver Cu at BL (1100 ppm each) showed considerable decrease in this parameter after 3.2 and 7.5y of Tx with Zn SULF, respectively. In 2 additional patients (#1 and #6) Cu levels after Zn SULF therapy were <2 times higher than the UNL.

  • In six pts. (#s 1, 2, 5, 3, 6 and 4) free plasma Cu concentration normalized (0.1 to <0.1) after Tx with Zn SULF; in 5 of these, Pre-Tx free plasma Cu concentration ranged from 0.3 to 0.5 mg/l.

  • In this group, favorable response to oral Zn SULF therapy was noted in 6 pts. (#1, 2, 5, 3, 6 and 4).

  • This subgroup includes 2 pts. (#1 and #2) that have been Tx with Zn SULF the longest (600 mg/day for nearly 27y).

  • The investigator explains that when they first saw Pt. #1 in 1986 (25y after the institution of Zn SULF therapy (Schouwink 1961), she was found to be in a good condition: tremor had vanished and dysarthria had decreased. No Kayser-Fleischer rings could be seen although these had been present at the time of the initial diagnosis. The concentration of copper in the liver was only slightly above normal levels: 84 ppm dry weight (normal value: lower than 50 ppm dry weight), the concentration of Zn was 350 ppm dry weight (normal value 200-400 mg dry weight).

TABLE 19
Hoogenraad's Study

Response to Orally Administered Zn SULF After Penicillamine Without Intolerance

Pt. Identif.	Symptoms and Signs at the Start of Zn SULF Therapy	Length of Tx	Plasma Zn (mg/l)	Liver Zn BEF AFT	Free Cu (mg/l) BEF AFT	Liver Cu BEF AFT	Symptoms and Signs at the End of Zn SULF Therapy (Response to Change)
I. PTS. TX WITH 600 mg/day (N=3)							
#22, M26							
#25, M19							
#27, F18							
II. PTS. TX WITH 900 mg/day (N=5)							
#18, F34							
#19, M38							
#20, M32							
#23, M21							
#24, M22							

- In patient #2 all symptoms and signs, including Kayser-Fleischer rings, had disappeared after 25y of oral Zn SULF therapy. The liver copper concentration was 381 ppm dry weight, the Zn concentration 417 ppm dry weight.

- In patient #3 (900 mg/day) the first improvement of the neurological symptoms was seen a few weeks after the institution of therapy.

- In pts. #5 (600 mg/day), #6 (900 mg/day) and #4 (1200 mg/day) improvement of the neurological symptoms was seen after a few months.

- Pt. #9 was a Tx Failure. She was desperately ill when therapy was started. The condition seemed beyond hope because of severe hepatic insufficiency, hemolytic anemia and ascites. For the first 3 weeks after the institution of Zn SULF therapy her clinical condition seemed to improve and free plasma copper concentration and transaminases decreased (values not shown in Table 20). However, she died in hepatic coma 4 weeks after the start of Zn SULF therapy. Postmortem examination of the liver showed extreme cirrhosis with only small islands of liver tissue left. It is to be noted that this pt. was given the lowest dose of Zn SULF (300 mg/day) for a very short period of time (1 month).

8. Conclusions (From Hoogenraad's publication)

"The over-all results with zinc sulphate in the three groups of patients were good. At first glance, the results look even better than those usually obtained with penicillamine: (1) patients who did not tolerate penicillamine did well on zinc sulphate, (2) worsening of neurological signs in the first weeks of treatment was not observed, (3) there were no major adverse effects of treatment, (4) free plasma copper concentration normalized in all patients, even in those patients who had elevated levels during treatment with penicillamine.

"Clinical goals are often difficult to assess objectively and in these circumstances it is necessary to set an objective intermediate goal (Melman et al. 1980). For Wilson's disease a low free plasma copper concentration has been demonstrated to be the best intermediate endpoint because it is correlated best to the ultimate clinical benefit (Scheinberg and Sternlieb 1984). In this series we found that the free plasma copper concentration normalized in all patients during zinc therapy, also in the patients in whom this level had been elevated during treatment with penicillamine. Our results give no indication that zinc therapy needs to be restricted to patients who do not tolerate penicillamine (Brewer et al. 1983; Van Caillie-Bertrant et al. 1985).

TABLE 20
Hoogenraad's Study

Response to Oral Zn SULF Therapy Administered From the Start

Pt. Identif.	Symptoms and Signs at the Start of Zn SULF Therapy	Length of Tx	Plasma Zn (mg/l)	Liver Zn BEF	AFT	Free Cu [mg/l] BEF	AFT	Liver Cu BEF	AFT	Symptoms and Signs at the End of Zn SULF Therapy (Response to Change)
		I. PTS. TX WITH 300 mg/day (N=1)								
#9, F11										
		II. PTS. TX WITH 600 mg/day (N=3)								
#1, F34										
#2, M26										
#5, F18										
		III. PTS. TX WITH 900 mg/day (N=2)								
#3, F26										
#6, M16										
		IV. PTS. TX WITH 1200 mg/day (N=1)								
#4, M16										

"Dosage: In adults we usually started therapy with a dosage of 200 mg thrice daily. Judging the clinical results and the free plasma copper level we adjusted the dose. We found that for the majority of the patients the dosage had to be increased.

"Although there are only few investigations on the effects of long-term treatment on changes in the liver of patients treated with penicillamine, we have the impression that liver biopsies may be of help. We aim at a decrease of the copper zinc ratio (Nooyen et al 1981). We decided to increase the dosage of zinc sulphate when the copper zinc ratio did not decrease. One should beware of the conclusion that absence of a decrease of the liver copper indicates ineffectivity of therapy; Scheinberg and Sternlieb (1984) have described that in 3 out of 6 patients treated with penicillamine the liver copper did not significantly decrease after effective therapy during 3 years. Even an increase of liver copper may occur in a previously decoppered patient during effective therapy; we studied liver copper in 2 patients during effective maintenance therapy with penicillamine; in both patients the liver copper increased.

"Altogether it is difficult to assess the safety of a new strategy on a series of consecutive cases. However, a favorable situation arises when the new drug has dramatically fewer side-effects than the previously available one (Lasagna 1982; Moses 1984). These circumstances were present in this study. Zinc therapy seems to be very safe and this is the major reason why, in our evaluation, zinc sulphate comes out as a better choice than penicillamine.

"Patient compliance was no major problem in our series. Patient 21 had severe increase of liver copper after 2 years of oral zinc therapy. It came out that she had taken less than half of the prescribed dose of 600 mg/day and that a copper-containing intrauterine anticonceptive device had been used during a period of one year.

"Zinc sulphate is easily available in most countries. The costs of the drug are very low. In some Third World countries penicillamine is not available (Longe et al. 1982) and zinc therapy may be the only choice.

"We conclude from this study that zinc sulphate should be considered as initial treatment in Wilson's disease and certainly for patients who do not tolerate penicillamine. A controlled comparative study would be needed to measure the merits of zinc sulphate for the management of patients who do well on penicillamine."

9. Comments

This is the second study submitted by the sponsor in support of the approval of the marketing of Zn AC for the maintenance of Wilson Dz patients. The reviewer is basing his evaluations on the publications. No raw data or computations preceding the publication are available. Based on literature evidence the author and his colleagues have treated Wilson's Dz patients, some of them for a number of years and successfully in most. The salt of Zn used has been the SULFATE and this is different from the ACETATE, the subject of the present NDA. Provided that clinical/biochemical data are adequate to show efficacy and safety, bioavailability of the salts is not important. Both products liberate elemental Zn^{++} and this is the active moiety and the effect is in the gut.

In the present study, Hoogenraad and his co-workers carried out this Netherlands trial with the aim of improving the clinical condition of the symptomatic patient and to prevent the recurrence of symptoms and signs in those that were asymptomatic. So, from the viewpoint of their clinical

condition, there were two pt. populations: those who had no symptoms upon
entry (ex. Pts. #2, 25, 19, 20 and 24) and those that, upon entry, had signs
and/or symptoms of disease (all other patients). To demonstrate efficacy in
the first group, one needs to demonstrate that, after several years of Tx with
Zn SULF the patients remained asymptomatic. Proof of efficacy in the second
group requires the demonstration that the patient's clinical condition is not
changing [his/her signs and symptoms of disease, are not getting worse].
These signs and symptoms could even improve but, knowing the progressive
nature of the disease, improvement should not be a requirement to claim
maintenance.

Using both definitions of maintenance, this reviewer believes that efficacy of
Zn SULF has been demonstrated. Emphasis is put on clinical data. There were
three groups of patients analyzed. Results from these three groups are
reviewed separately and the data were further stratified on the bases of Zn
SULF dose. This approach resulted in too few patients per stratum which
precludes drawing firm conclusions about dose response.

Group I consisted of 7 pts., 6 of whom were symptomatic. These pts. had
either systemic side effects while on penicillamine or had developed a
paradoxical or serious ↑ of neurological signs after taking penicillamine.
All of these patients were maintained. This statement is based on clinical
findings at the end of Zn SULF therapy, including decrease in neuropsychiatric
signs, disappearance or recovery of dermatopathy, fading of K-F rings (3 of
the 4 cases that had K-F rings at the start of Zn SULF Tx), disappearance of
urticaria, normalization of hair growth, improvement of dysarthria, and
improvement of ataxia. In the only asymptomatic patient (#12) there was
regression of biochemical signs and still no signs of Wilson's Dz after 44 mo.
of Tx with Zn SULF. Both dose levels of compound [600 mg/day, N=3;
900 mg/day, N=4] were effective.

Group II consisted of 8 patients who were switched over to Zn SULF after an
initial Tx with penicillamine. These pts. had not developed intolerance to
penicillamine. Five of these patients were asymptomatic at the start of Zn
SULF therapy, although they may have had an underlying condition, such as
liver cirrhosis. Six of these eight pts. were adequately maintained. Four of
these six, who were asymptomatic at BL, remained asymptomatic after 37 to 50
months of Zn SULF therapy. The cirrhosis did not change in one of the other
two and in the other, there was ameliorating of dysarthria but his dystonia
did not change after 66 months of Tx. After being treated with Zn SULF for 29
months, one pt. (#25) developed ascites after emergency operation; he was
changed back to penicillamine. An additional pt. (#22) who appeared to be
properly maintained (actually his signs/symptoms had improved after 6.8 years
of Tx with Zn SULF), asked to be switched back to penicillamine.

In this trial, an additional demonstration of adequate maintenance were the
clinical results in patients in Group III. This consisted of 7 patients who
were treated with oral Zn SULF from the start. All of them were symptomatic
at the beginning of Zn SULF therapy. The doses of Zn SULF shown to be
effective varied from 600 mg/day (N=3) to 900 mg/day (N=2) to 1200 mg/day
(N=1). Actually, this group included the pts. that have been treated
(maintained) the longest with 600 mg/day Zn SULF, pt. #1 (323 months) and pt.
#2 (also 323 months) and Pt. #4 (90 months). These 6 patients responded to
maintenance therapy because their clinical condition either did not change or
actually improved. Demonstration of improvement were complete disappearance
of K-F rings in 3 and fading of these signs in 2, improvement of ataxia and
arthralgia (Pt. #1), disappearance of rigidity (Pt. #5), disappearance of
tremors, rigidity, dysarthria (Pt. #3), improvement of rigidity, dysarthria
(Pt. #6) and improvement of dysarthria (Pt. #4).

Pt. #9 had cirrhosis, hemolytic crisis and K-F rings at the start of Zn SULF therapy. This pt. received the lowest dose (300 mg/day) for the shortest period of time (1 month). After this, she developed severe hepatic failure and died. This appears to be a case of therapeutic insufficiency. The low dose of compound was not given enough time to act. Since higher doses appear to be effective, there is no reason to start the pt. with such a low dose. Alternatively, symptomatic pts. may need to be treated with more rapidly acting agents such as ATTM before being switched to Zn SULF.

A computation of the doses tested indicate that 300 mg/day (N=1) may be too low and that 1200 mg/day (N=1) may not be necessary. But the data in support of this note are too insufficient. The total number of patients treated with Zn SULF, 600 mg/day was 9; those treated with 900 mg/day amounted to 11 patients. Both dose levels are efficacious and there is no evidence of dose response. Also, both dose levels are well tolerated, although in terms of length of administration there is more experience with the 600 mg/day dose.

It is of interest to reiterate that the 600 mg/day dose of Zn SULF has been given for very prolonged periods of time: virtually 27 years to patients #1 and #2.

VII. CLINICAL DATA SUBMITTED IN SUPPORT OF THE INDICATION THERAPY FOR PRE-SYMPTOMATIC AFFECTED SIBLINGS

A. The Brewer's Study

A separate section for this indication was not presented by the sponsor. What follows is material extracted from the numerous tables included in the Clinical Report.

In the Clinical Report, these patients are identified as belonging to the P (pre-symptomatic) category. Included were 23 patients without signs and symptoms of Wilson's Dz at the start of Zn AC therapy. Most of these patients were diagnosed during the screening of asymptomatic siblings of a newly diagnosed patient. The main objective of the study was to show that oral Zn AC can maintain body Cu at subtoxic levels in patients who were asymptomatic at the start of therapy. Aside of the difference in study population all other aspects of the trial were as described under IV. A. above.

6. Results

- Of the 23[18] pts., 10 were F and 13 M, age ranged from 11 to 42y.

- Their initial categorization relative to pre-Zn AC therapy was:

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[18]The identification #s for these 23 pts. were:

#10	#31	#57	#73	#89
#20	#32	#58	#74	#91
#21	#42	#59	#75	#93
#25	#43	#62	#76	
#27	#45	#72	#87	

Type of Pre-Zn AC Tx	No. of Pts.
Penicillamine	9
Zn AC from the beginning	14
Total	23 (P)

- The data are presented primarily in a descriptive fashion because information on **each** patient was evaluated. The sponsor presented mean ± SDM for the many parameters of efficacy and safety. These are duly reproduced by the reviewer but, as documented in the pertinent Tables, in many instances, the number of observations from which such a mean was derived was too small.

- There were neither deaths nor discharges among the 23 pts. in the group: Pt. #10 (see below) dropped out of the trial.

- Pt. #10 is a 33y old WF. She was the sister of one of the symptomatic pts., who joined the program at the urging of her affected sibling. The patient received Tx (50 mg x 3) for 3.67y (6/16/83 to 02/16/87). Her biochemical/clinical response (and status) at the time of dropping out is summarized in Table 21.

- The info. displayed in Table 21 is not adequate/sufficient to determine the biochemical/clinical status of this pt. close to the time she left the trial. The pt. lived in Florida and did not like the annual visit normally required of the pts. entering the trial. Also, there was an incident of a disappearing telephone from her hospital room when she was discharged, and she resented being queried about it. She made the decision to resume prophylactic penicillamine therapy with her Florida physician.

- The distribution of pts. according to length of Tx was:

Years of Tx	No. of Pts.
0 to 1	4
1 to 2	5
2 to 3	1
3 to 4	4
4 to 5	1
5 to 6	2
6 to 7	6
Total	23

- Excluded from analysis were Cu-related variables for Pt. #20 on 08-10-90 (only). TRIEN was adm. on that day. [This pt. was treated with Zn AC between 01/28/84 and 01/01/91 = nearly 7 years.]

- Results of clinical and hepatic biochemical evaluations are summarized in Table 22. It is important to note that in many instances the number of observations per cell is very small (1 to 3 patients). But, all in all, it appears that these patients remained asymptomatic throughout observation periods of 5+ years. No noticeable changes in clinical variables were recorded. During 6+ years, serum albumin did not change, BIL and liver enzymes remained stable during this period.

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Individual Raw
Data

- In Pt. #27 BIL was 0.8 mg/dl for the 0-1 and 1-2 years of observation (BL BIL was not done). These values were slightly increased at 1.2 (2-3y) and 1.5 (4-5y). It will be of interest to further F/U serum BIL concentrations in this pt. who also had mild increases (± x 2 over UNL) in serum ALT concentrations.

- In Pt. #57, BL BIL was 1.2 and remained as such for ca. 3 years. At the 3-4y period of observation BIL decreased to 0.4 mg/dl.

- Serum BIL in Pt. #58 was grossly abnormal at 3.1 mg/dl at BL. Throughout the 4 years of Zn AC Tx, this value changed little.

- Two pts. (#21 and 27) experienced mild increases of serum ALT, above the UNL, but in these pts.. no BL determinations were available. Pt. #32 had no BL determinations. The first available values was at 1-2y at 44 iu/l (very slightly above UNL). But in the next 2 years the serum ALT increased to ca. x 4 UNL and remained > x 2 UNL by the 4+y observation time. F/U evaluations are needed for this pt.

- Sporadic, mild changes in serum AST concentration were noted in Pt. #32 (no BL values available).

- In pt. #75, the AP seen at BL (380 iu/l) was still elevated at the 0-1y evaluation time (=431 iu/l). F/U on this pt. is needed.

• Also noted in Table 22 are some increases in serum amylase and serum lipase. Such mild increases in these enzymes were seen especially during the firs 2y of Tx for amylase but throughout the L-T period for lipase. As discussed elsewhere, these findings may be characterized as mild biochemical pancreatitis without overt clinical pancreatitis.

• Cu balance was done at the 0-1y period (no F/U) in 7 of the P patients; in 6, the balances were negative, ranging from -0.24 to -1.31.

- Cu balance was + in pt. #25=0.13 but this assessment was not repeated.

- The mean Cu balance in the 7 pts. was -0.60.

• ^{64}Cu uptake was done in 5 pts. only. In three (#s 27, 31 and 32 with 0.5-0.4, 0.6 and 0.1% of the administered dose, respectively) the test show that Zn AC was "effective". But, using this parameter, the Tx with Zn AC, 50 mg x 3 per day, was judged "ineffective" for pts. #21 (^{64}Cu=3.2% of the given dose) and #25 (^{64}Cu=1.9% of the administered dose).

- In spite of these findings on ^{64}Cu uptake both patients appeared to be well maintained when clinical and biochemical (LFTs) parameters were analyzed.

TABLE 22
Brewer's Study

Comparison of Means in Clinical Parameters, LFTs and Tests of Pancreatic Function in Pre-symptomatic Wilson's Dz Pts. Administered Zn AC at the Oral Dose of 50 mg x 3

	Years of Zn AC Therapy									Remarks
	0	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	
I. SPEECH										
N = 3 / 3.0	1 / 2.0	3 / 2.8	1 / 2.0	2 / 3.0	2 / 2.0	1 / 2.0	1 / 2.0	1 / 2.0		• Too few observations per cell. • Pt. #20 had a BL rating scale of 2 and this did not change (yearly observations for 8 years).
II. NEURO/PSYCHIATR.										
N = 2 / 6.0	1 / 5.5	2 / 7.5	1 / 3.0	2 / 4.3	0 / ---	0 / ---	0 / ---	0 / ---		• Too few observations per cell. • Practically worthless data.
III. NMR										
N = 5 / 1.8	6 / 0.7	2 / 2.5	3 / 0.7	2 / 1.3	2 / 2.3	4 / 1.0	1 / 4.0	0 / ---		• Few observations per cell. • In Pts. #21, 27 and 31 the rating scale was 0 (Normal) at BL or the 0-1y time period. This was also 0 at the 5-6y period of observation.
IV. SERUM ALBUMIN										
N = 15 / 4.4	12 / 4.4	16 / 4.3	6 / 4.3	6 / 4.1	7 / 4.3	4 / 4.3	1 / 4.4	0 / ---		• This parameter remained remarkably stable for 6+ years.
V. SERUM BIL										
N = 18 / 0.7	14 / 0.8	16 / 0.7	7 / 0.7	6 / 1.0	7 / 0.6	2 / 0.7	1 / 0.6	0 / ---		• This parameter was stable for 6+ years, even in pt. #58 in whom BIL was 3.1 mg/dl at BL.

VI. SERUM ALT

N = 12	9	13	5	7	5	4	1	0	
62.2	57.9	57.2	52.7	50.9	36.0	68.3	89.5	---	• The serum ALT in pts. #75 and 76 were high at x 3 and x 7 UNL, respectively. At the 0-1y of Tx these values 1 to x 2 and x 4 UNL, respectively.

VII. SERUM AST

N = 18	14	16	7	7	7	4	1	0	
41.0	45.6	31.7	37.2	39.5	37.3	35.1	42.5	---	• In Pt. #25, BL values was x 3 UNL, but normalized at the 0-1 and 1-2y times of observation

VIII. SERUM AP

N = 18	14	16	7	6	7	4	1	0	
108	138	95	87	104	100	89	142	---	• Mild increases to < x 2 UNL were seen in Pt. #20 (BL=normal)

IX. SERUM LDH

N = 11	11	13	5	4	6	4	1	0	
159	152	162	165	125	168	172	161	---	• Very mild increases noted in Pt. #75 (BL=220, 0-1y=292 iu/l) suggest the need for F/U in this pt.

X. SERUM AMYLASE

N = 14	8	15	7	5	5	4	1	0	
74	90	80	75	75	68	72	90	---	• BL value in Pt. #58 was slightly above UNL and remained so for up to 3-4 yrs, which was the total length of Tx in this pt.

XI. SERUM LIPASE

N = 16	7	15	7	5	4	4	1	0	
15	19	20	19	23	19	21	33	---	• Very minor changes were noted in some pts. but there elevations were never > x 2 UNL.

In this Table, S.D. of the mean is not presented, for clarity of presentation. The averages for serum amylase and (lipase are rounded figures.

● Of more interest are observations on Cu variables after L-T administration of Zn AC and these are summarized in Table 23. Mean data are presented; only minor inconsistent changes are noted. It would appear that, in the main, these parameters of Cu metabolism (24-h urine Cu, nonceruloplasmin Cu and hepatic Cu) did not consistently change even after 6-7y of Tx with Zn AC.

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TABLE 23
Brewer's Study

Mean Values of Cu Variables in P Patients
Treated L-T With Zn AC (50 mg x 3 per day)

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YEARS ON ZN AC THERAPY								
0	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8
I. 24-h URINE Cu								
N = 17 0.137	18 0.144	18 0.111	14 0.102	11 0.107	8 0.114	6 0.107	4 0.136	0 ---
II. NONCERULOPLASMIN PLASMA Cu								
N = 17 17.9	14 22.2	13 18.8	6 16.3	4 12.2	4 15.2	4 13.3	1 24.0	0 ---
III. HEPATIC Cu								
N = 17 601	6 585	5 551	2 1317	2 370	2 1004	1 450	1 365	0 ---

In this Table, S.E. Mean has been omitted for clarity of presentation.

- Of the 17 24-h urine Cu values averaged in this Table, 16 had abnormal values at BL. In one pt. (#10) the BL 24-h urine Cu was WNL. In 8 of the patients (#s 21, 25, 32, 42, 57, 62, 75 and 76), the BL values were x 3 or >UNL and in one (#76), this BL value was as high as 350 $\mu g/24h$ (Normal=20 to 50 $\mu g/24h$). In 6 of these 8 patients (including #76) 24-h urine Cu values decreased with Zn AC Tx although not to normal values. In pt. #32 decreases seen at 0-1, 1-2 and 2-3y of therapy, were followed by slight increases at 4-5 and 5-6y of observation.

  Only 1 pt. (#10) had normal 24-h urine Cu at BL (10 $\mu g/24h$). This increased momentarily to 73 at the 2-3y and was practically in the upper limit of range at 3-4y of Zn AC Tx.

● 5 of the 17 pts. listed in Table 23 with nonceruloplasmin plasma Cu levels had BL increases of up to x 2 the upper limit of normal but no higher. In 3 of these 5 pts. (#s 25, 57 and 62) the free Cu values decreased to WNL or nearly so after Tx with Zn AC. In 5 pts. (#72, 73, 74, 75 and 76) the BL free Cu values were reported as being lower than the lowest normal value; F/U in 3 of these 5 pts. was WNL or very slightly higher (Pt. #76).

- **Evidence of biochemical abnormality in Cu metabolism in these pre-symptomatic pts. is given by the fact that 16 of the 17 pts. listed in Table 23 had _very high_ concentrations of Cu in the liver. These increases ranged up to X 25 the UNL of hepatic Cu and were never < x 5 the UNL. The only pt. with hepatic Cu WNL at BL was #43.**

  - The F/U information is quite incomplete in that 11 of the 17 pts. listed in Table 23 as having BL values of hepatic Cu did not have any evaluation after BL. From the little data at hand, hepatic Cu levels appeared to be maintained as a function of time.

  - The exception was Pt. #43 where the WNL value of 38 $\mu g/g$ dry wt. at BL increased to a very high of 833 at the 4-5y observation time. However, in spite of these changes in liver Cu, this patient was being properly maintained. That is, clinical (speech, neuro/psychiatr, NMR) and biochemical (LFTs, etc) parameters were stable for up to 4-6 years (this patient's dose duration). These findings appear to suggest that, in some pts., liver Cu concentration may not be a reliable parameter of Dz activity/progression.

- **Results of evaluations of urine, plasma and liver Zn are summarized in Table 24.**

- **All 9 pts. listed in Table 24 as having pre-Zn AC determinations, had urine Zn WNL. On the average, these values increased at least 10 times and remained so during L-T Tx with Zn AC.**

- **For plasma Zn, values during Zn AC therapy were higher than those at BL (Table 24); these increments were roughly, up to x 2 the upper limit of normal at BL.**

- **Little F/U data on liver Zn were available. Indeed, of the 16 pts. listed in Table 24 as having liver Zn concentration determinations pre-Zn AC treatment, _only three_ (#20, 21 and 25) had F/U data.**

7. Comments

This trial's purpose, part of the larger study by Brewer, was to demonstrate the efficacy and safety of Zn AC, 50 mg t.i.d., for maintenance therapy in siblings of Wilson's disease patients. These siblings do not yet show signs/symptoms of disease, although nearly all of them showed abnormalities in Cu metabolism. It is not known what is the most reliable parameter of Cu status. At BL, 16/17 pts. had abnormal (high) values of urinary Cu, 5/17 had increases of up to x 2 the upper limit of normal in nonceruloplasmin plasma Cu concentrations and 16/17 had very high concentrations of Cu in the liver. Aside of these indications of deranged Cu metabolism, all of these patients were pre-symptomatic. Fourteen (14) of the pts. had apparently never been treated for their subclinical Wilson's disease and Zn AC was their first treatment for their condition. Nine (9) other patients, that were being treated orally with penicillamine, were switched to Zn AC. The aim of the trial was to maintain body Cu at subtoxic levels and prevent the appearance of signs and symptoms of the disease.

TABLE 24
Brewer's Study

Mean Values of Zinc Variables in P Pts. Treated L-T with Zn AC (50 mg x 3 per day)

YEARS OF ZN AC THERAPY								
0	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8
I. URINE ZN								
N = 9	15	14	11	8	7	6	4	0
0.3	4.0	3.8	3.1	3.4	2.4	3.5	3.6	---
II. PLASMA ZN								
N = 14	10	12	4	3	3	4	1	0
109	178	197	183	209	189	227	210	---
III. LIVER ZN								
N = 16	6	5	2	0	2	1	0	0
426	1501	870	1391	---	410	937	---	---

The S.D. Mean is not included for clarity of presentation. The means for liver Zn are rounded figures.

This is a somewhat different situation to the first indication. These patients do not have disease but they do have increased Cu levels. Efficacy is shown by the ability to control. There were neither deaths nor discharges and only one patient (#10) dropped out from the trial after receiving test medication for 3.7 years. In this patient, clinico/biochemical information was inadequate to determine her status at the time she left the trial. She lived in Florida and made the decision to be switched to penicillamine therapy there.

There were not too many pts. per cell per the stratified L-T periods (1-2y, N=5; 2-3y, N=1; 3-4y, N=4; 4-5y, N=1; 5-6y, N=2 and 6-7y, N=6) but these numbers are not necessarily low if one considers the total number of subjects with this condition.

All in all, Zn AC, at the oral dose of 50 mg t.i.d. was effective in preventing the appearance of signs and symptoms of Wilson's disease. In no case has there been neurologic, hepatic or other morbid manifestations of Wilson's disease in these pts. treated continuously for 6+ years. Although the information was not always complete, these pts. had speech, neuro/psychiatric and NMR evaluations suggesting that their clinical status did not significantly change with time. Similarly, except for isolated, inconsistent, relatively mild abnormalities, biochemical parameters of liver function, including albumin, bilirubin, ALT, AST, AP and LDH remained nearly WNL and stable throughout the trial. As in the case of symptomatic patients with Wilson's disease, the patients in the present trial had little changes in their serum bilirubin and albumin concentration for the 6+y of observation.

In a fashion similar to that for the symptomatic patient, in these pre-symptomatic individuals, the oral dose of Zn AC of 50 mg x 3 per day produced an at least ten-fold enrichment in urinary zinc (BL=0.3, post-drug=2.4 to 4.0 mg/24h). An increase in plasma Zn concentration was also noted (BL=109; post-drug=178 to 227 µg/dl). At the given dose, Zn AC produced a 2- to 4-fold increase in liver Zn concentration (BL=426; post-drug=410 to 1501 µg/g dry Wt.) but the values varied considerably. One of the factors contributing to this variability is the fewer number of observations per cell after the 0-1y interval.

In conclusion, Zn AC, at the oral dose of 50 mg t.i.d. is efficacious in preventing a) the appearance of clinical signs and symptoms and b) alteration of biochemical parameters of liver function in pre-symptomatic Wilson's disease subjects. This trial also showed that this dose and dose regimen of Zn AC is well tolerated.

> ### B.   The Hoogenraad's Study (Table 25)
>
> * Data from the 5 pre-symptomatic pts. treated with Zn SULF (anywhere from 300 to 1200 mg per day) are summarized in the Table.
>
> * 2 of the 5 pts. were children.
>
> * After varying length of Tx lasting from 1 to 49 months, all pts. remained asymptomatic. However, Pt. #26, switched back to penicillamine. This was not necessarily a Tx failure. As noted above, this pt. asked to be switched to penicillamine because his brother had preferred to be switched back to penicillamine after his hepatic failure and ascites increased due to an emergency operation because of torsio testis.
>
> * Biochemically, free Cu remained at the low level of <0.1 mg/l. Liver Cu concentrations were WNL and increased dramatically to 1200 µg/g wet Wt in Pt. #21. The BL liver Cu value in Pt. #8 was high at 863 µg/g wet Wt. and decreased to 377 after Tx with low dose Zn SULF (300 mg per day for 49 months). In spite of these marked changes in liver Cu, going in opposite directions, both patients remained pre-symptomatic. This information suggests that liver Cu concentration is not always a reliable indicator of the patient's Cu status.
>
> * In 2 of the 5 pts. (#s 21 and 7) liver Zn increased x 3 from BL and in a third pt. (#8) the increment was not so pronounced. But in all 3 pts. the BL values of hepatic Zn were WNL.

Reviewer's Comments

These data with Zn SULF, administered in doses of up to 1200 mg/day for up to 49 months, show that this compound is efficacious in preventing the appearance of clinical signs and symptoms in pre-symptomatic Wilson's disease patients. Of the 5 patients studied, 1 was intolerant to penicillamine, 2 did not have intolerance to penicillamine and 2 received Zn SULF from the start. All 5 patients remained pre-symptomatic, although one (#26) asked to be switched back to penicillamine because his brother had asked to be switched back to the chelating agent.

TABLE 25
Hoogenraad's Study

Response to Orally Administered Zn SULF in Pre-symptomatic
Wilson's Dz Patients

Pt. Identif.	Length of Tx	Plasma Zn (mg/l)	Liver Zn		Free Cu (mg/l)		Liver Cu		Remarks
			BEF	AFT	BEF	AFT	BEF	AFT	
#10, F38									
#21, F30									
#26, F18									
#7, M14									
#8, F10									

These doses of Zn SULF, given for the specified lengths of time were also well tolerated.

VIII. **SPONSOR'S REQUEST TO INCLUDE IN THE LABELING INFORMATION ON CLINICAL DATA IN SUPPORT OF THE TREATMENT OF WILSON'S DZ PATIENTS DURING PREGNANCY**

   A. **Brewer's Study**

   - In this study, the following 5 pts. became pregnant and delivered normal babies while on Zn AC therapy:

Pt. No.	No. of Pregnancies
32	2
34	1
45	2
49	1
52	2
70	1
Total	9

   Patients #45, 49 and 52 were on a maintenance dose of 25 mg x 3. In the other patients the 50 mg X 3 dose was reduced to 25 mg x 3 or 25 mg X 2 as soon as pregnancy was discovered, and this dose was maintained until lactation was ended.

   - Available data on Cu metabolism L-T in these pts. is summarized in Table 26.

      - All 4 pts. in whom BL values were available had elevated (x 2-4 upper limit of normal) 24-h urine Cu levels. There were no pronounced changes in this parameter in this 4 pts. and an additional 2 in whom BL values were not determined. Two patients (#32 and 34) were treated with Zn AC for 5+ years.

      - There was little F/U information for pts. with regards to nonceruloplasmin plasma or liver Cu.

      - In Pt. #34, Cu balance was 0.05 and the ^{64}Cu uptake was marginal at 1.4% of the dose. In this Pt., urine Zn was 2.1 mg/day and plasma Zn was 240 μg/dl.

      - Cu balance in Pt. #49 was either -0.20 (sponsor's Table D.7, Vol. 06, p. 081) or 0.20 (sponsor's Table D.4, Vol. 06, p. 078).

   - There were no adverse effects of Tx noted in the babies. This was established by data forms filled out by the mother for each pregnancy, and by attending obstetricians and pediatricians.

TABLE 26
Brewer's Study

Cu Metabolism Variables as a Function of Time After L-T
Administration of Oral Zn AC to Six Women That
Became Pregnant During the Trial

Pt. Identif.	YEARS ON ZN AC THERAPY							
	0	0-1	1-2	2-3	3-4	4-5	5-6	6-7
I. 24-h URINE CU								
#32 #34 #45 #49 #52 #70								
II. NONCERULOPLASMIN PLASMA CU								
#32 #34 #45 #49 #52 #70								
III. LIVER CU								
#32 #34 #45 #49 #52 #70								

B. Hoogenraad's Study

Two women in this study delivered healthy children. A brief summary of their evolution is given below.

1. Pt. #21

She was 30y old and pre-symptomatic when she entered the trial. She was listed as having been on penicillamine without intolerance. The pt. received 900 mg/day Zn SULF for 39 months. Her plasma Zn concentration was 1.9 mg/l, free Cu <0.1 and <0.1 before and after Zn SULF therapy, respectively. Her liver Cu was 34 and 1200 μg/g dry Wt. before and after administration of Zn SULF. Her liver Zn was 168 and 627 μg/g dry Wt. before and after Zn SULF therapy.

- This pt. remained pre-symptomatic and delivered a healthy child.

2. Pt. #5

She was 18y old and at entry had rigidity and K-F rings. She was listed as
having received Zn SULF from the start. The pt. took medication at the oral
dose of 600 mg per day, for 45 months. Before Tx, her free Cu was 0.4 mg/l
and this decreased to 0.1 after Tx with Zn SULF. Liver Cu evaluations were
not done. Her plasma Zn was 1.7 mg/l. Liver Zn was not done.

- This pt. was not only maintained but her clinical status improved after
  Zn SULF since her signs and symptoms disappeared. In addition, the pt.
  delivered a healthy child.

C. Reviewer's Comments

These studies, one with Zn AC, the other with Zn SULF show that women of
child-bearing age who become pregnant while taking these medications can be
safely treated with either salt of Zn without untowards effects to the patient
or the babies. The dose level of Zn AC tested (25 mg x 3 per day) was half of
that recommended for the two requested indications (50 mg x 3 per day). Two
of these patients were treated with Zn AC for 5+ years. The dose levels of Zn
SULF were the same as those demonstrated to be effective for the two
indications being seeked (600 to 900 mg per day). One patient was treated for
a total of 39 months, the other, for 45 months. In addition to being safe
from the pregnancy viewpoint and effective in maintaining/treating the
clinical condition, both salts of Zn proved to be well tolerated in this
patient population.

IX. OVERALL CONCLUSIONS/RECOMMENDATIONS FOR REGULATORY ACTION

1. A review of the evidence in the Brewer's study demonstrates that Zn AC,
   at the orally administered dose of 50 mg three times a day is effective
   and safe for the two indications requested by the sponsor (maintenance
   in symptomatic Wilson's disease patients and initial therapy for pre-
   symptomatic affected siblings of Wilson's disease patients).

2. A review of the evidence in the Hoogenraad's trial shows that another
   salt of zinc, Zn SULF, at the oral dose of 600 or 900 mg per day is also
   effective for the two indications proposed by the sponsor (maintenance
   in symptomatic patients and initial therapy for pre-symptomatic
   siblings).

3. According to 1. and 2. above, each compound is safe and effective in its
   own right. Since a) both compounds liberate Zn^{++}; b) elemental Zinc is
   the active moiety; and c) the effect is at the level of the enterocyte,
   there is no need to compare the bioavailability of one salt in
   comparison to the other. Blood levels and PK parameters of Zinc are
   unrelated to the site of action (the gut). It follows that the results
   of the Hoogenraad's trial are replicative of those in the Brewer's
   study. In conjunction, both trials give substantial evidence of
   effectiveness and safety for the two indications requested by the
   sponsor. The following is therefore recommended:

   a) Approval of zinc acetate, as a second line drug, for the
      maintenance treatment of symptomatic Wilson's disease patients
      that have initially been treated with a chelating agent.

b) Approval of zinc acetate as a first line drug for the maintenance treatment of pre-symptomatic affected siblings of Wilson's disease patients.

c) The labeling should indicate that zinc acetate is effective and safe in the treatment of Wilson's disease women that become pregnant during the course of the disease.

4. The recommended dose for a) and b) above is 50 mg three times a day.

5. Both trials included children. The labeling should contain a section (to be prepared by the sponsor) on efficacy and safety in children.

6. From the review of the evidence it is also concluded that, although of some utility in predicting therapeutic outcome, copper variables (urinary, plasma, but especially liver) are unreliable. The main reason for this unreliability may be the unpredictability of tissue saturation. There were many instances where, in the individual patient, urinary Cu, nonceruloplasmin Cu and liver Cu did not correlate among themselves and/or with the evolution of the clinical condition. In addition, measurements of Cu in these two fluids and liver is incomplete because an important route of Cu excretion (fecal) is ignored. Furthermore, parameters such as Cu balance or ^{64}Cu absorption are good indicators of short- but not long-term copper metabolism. It therefore follows that copper variables are not recommended as useful surrogate indicators of efficacy. These data ("copper variables") cannot serve as the basis for approval.

January 26, 1995

Hugo E. Gallo-Torres, M.D., Ph.D.

cc:
NDA 20-458
HFD-180
HFD-180/SFredd
HFD-180/HGallo-Torres
HFD-181/CSO
HFD-180/JChoudary
HFD-180/JGibbs
r/d 11/21/94 jgw
f/t 1/26/95 jgw
MED\N\20458410.OHG

1/27/95
Concur that the drug is effective for maint. therapy. Not sure why second line is noted for MD indication.
For preasymp. siblings, copper control would need to be accepted as endpoint acceptable for clinical benefit.
Experience with pregnancy valuable for labeling, but not an indication section.

STATISTICAL REVIEW AND EVALUATION

NDA #: 20-458

Drug: Zinc Acetate Capsules

Applicant: Lemmon Company

Indication: Maintenance Treatment of Wilson's Disease

NDA Drug Classification: 2S

Clinical Reviewer: The issues addressed in this review have been discussed with the medical reviewer, Gallo-Tores, M.D.

Volumes Reviewed: 1.8-1.10 June 24, 1994

Date Transferred to this Reviewer: August 1, 1994

Draft Date December 20, 1994

Introduction

Zinc acetate USP is a copper antagonist for removal of excess copper from the body. It is available as capsules for oral administration containing the equivalent of 25 mg or 50 mg of elemental zinc. Wilson's disease (hepatolenticular degeneration) is a defect in copper metabolism that is inherited as an autosomal recessive trait. Individuals with this disease are able to absorb copper from the diet normally but are unable to excrete it in a normal way, so copper accumulates in the body. Excess copper in the body is toxic especially to the liver and the nervous system. If untreated, the disease invariably progresses to a fatal outcome. Zinc is an efficient inhibitor of copper absorption. A therapeutic goal is to reduce the copper level in the system by administering agents such as penicillamine or trien which increases copper excretion in the urine through copper chelation. Even though effective, these agents are known to be very toxic. The rationale for using zinc acetate to treat Wilson's disease is that zinc reduces the absorption of copper from the diet and the reabsorption of endogenously secreted copper (in the gastrointestinal tract).

The sponsor has submitted two open, non-randomized, no controlled maintenance studies in support of the effectiveness claim for zinc acetate. Both studies are described as a well patient-controlled studies (i.e., patients serve as their own control). This review addresses the efficacy of 50 mg tid of zinc acetate from the Brewer study.

1

I U. S. STUDY (Investigator, George Brewer, MD)

1.1 STUDY DESIGN

This was an open, no concurrent control groups, maintenance study of zinc acetate administered orally to all patients with Wilson's disease who entered the study; patients served as their own control. The sponsor indicated that ethical and toxicity considerations, in addition to the fact that active agents such as penicillamine are known to be 100% effective, were the main reasons for not using concurrent placebo or active control group. The patient's copper values and clinical status prior to the start of the therapy served as control for the assessment of efficacy and safety.

The objectives of this study were to demonstrate that oral zinc can <u>maintain body copper at subtoxic levels and prevent progressive target organ damage</u> in patients who were: i) symptomatic but adequately detoxified prior to the maintenance therapy, ii) asymptomatic at the start of zinc therapy, and iii) pregnant.

Maintenance therapy is defined as that period of zinc therapy when the therapeutic objective is to prevent the accumulation or reaccumulation of copper, and to prevent the appearance or reappearance of the symptoms of copper toxicity. The maintenance period of this study was preceded by a pre-maintenance therapy period of minimum two months duration. In the pre-maintenance therapy period, patients with Wilson's disease who presented with elevated copper values were treated to reduce their copper levels to sub-toxic levels. The duration of the (zinc acetate) maintenance therapy period ranged from less than 3 months to 9.5 years.

Visit Schedule

<u>1. Initial Workup.</u>

During a hospitalization period of 4 to 7 days, prospective patients for zinc therapy were (initially) evaluated to confirm the validity of the diagnosis and establish their initial copper and clinical status.

<u>2. Pre-maintenance Therapy.</u>

 Patients who qualified for the pre-maintenance phase were placed in one of three groups:

i)   symptomatic patients on prior chelation therapy were switched to zinc therapy (N=63),
ii) symptomatic patients not previously treated were either initiated with zinc, or treated with ammonium tetrathiomolybdate for 8 weeks and then switched to zinc,
iii) presymptomatic patients were initiated on zinc from the beginning (N=23).

3. Maintenance Phase.

During the maintenance period with zinc, patients returned to the investigator's hospital annually for an assessment of their copper and clinical status for efficacy evaluation of zinc therapy.

Dosing Regimen

Patients who qualified for the maintenance phase were treated with zinc acetate. Patients were first administered with either 25 mg tid, or 50 mg bid, 50 mg tid dose of zinc acetate in short term balance studies. After these doses were shown to be effective, a few patient were continued on the 25 mg tid and 50 mg bid maintenance doses to gain further experience with these doses, while the rest were placed on the preferred 50 mg tid dose regimen. Patients were instructed to take each dose one hour before or after meals or beverages to avoid interaction with food. Patients who experienced gastric discomfort with the first morning dose were instructed to take that (dose) one hour after breakfast. Otherwise the dose was to be taken with a piece of meat.

Study Population

This is an all comers patient population study. Ninety-four patients were screened and 86 (39 males and 47 females) of these entered the maintenance phase of the study. The ages of these 86 (4 Mexican-American and 82 Caucasians) patients ranged from 11 to 55 years at the beginning of the study.

The table below summarizes patient disposition among the three clinical groups: 1) Neurological/ Psychiatric (N), 2) Hepatic (H), and 3) Presymptomatic (P).

Clinical Group	All Patients	Males/ Females	Age Range
Neurological	50	23/27	16-55
Hepatic	13	6/7	17-45
Presymptomatic	23	10/13	11-42
All Patients: Total	86	39/47	11-55

1.2 ENDPOINTS AND METHODS OF ASSESSMENT

The endpoints studied in this trial can be put into three (3) categories:

1. Efficacy Endpoints (Parameters)

This category includes three clinical and a number of biochemical parameters.

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A. Clinical

i) Speech

Using quantitative scoring system running from 7 (severe) to 0 (normal), the same speech pathologist evaluated speech (on 5 functional variables) throughout the maintenance therapy phase. Each test was to be done without specific knowledge of prior therapy and without reference to prior scores.

ii) Neurological function

Using quantitative scoring system running from 38 (severe) to 0 (normal), neurological function was evaluated by the same neurologist (on 10 functional variables to end up with a final results) throughout the maintenance therapy phase. Each test was to be done without specific knowledge of prior therapy and without reference to prior scores.

iii) Brain Magnetic Resonance Imaging (MRI)

Using blinded quantitative scoring system running from 30 (severe) to 0 (normal), the same two radiologists evaluated brain MRI (on 10 functional variables to end up with a final result obtained by averaging the scores from the two radiologists) throughout the maintenance therapy phase. Each test was to be done without specific knowledge of prior therapy and without reference to prior scores.

B. Biochemical Endpoints (Parameters)

The biochemical parameters are serum bilirubin, serum AST, serum ALT, serum alkaline phosphate, gamma GT and LDH.

2. Pharmacodynamic Endpoints (Parameters)

Copper Balance and Absorption (surrogate endpoint): Short Term Assessment of Efficacy

ii) Copper balance is an early measure of the effectiveness of zinc. It has, therefore, been identified as a useful surrogate endpoint in Wilson's disease for short term assessment but not for long term assessment. Normal people lose approximately 0.34 mg of copper per day from the skin surface. According to the sponsor, the level of copper in the perspiration of a Wilson's disease patient is at least equal to that of a normal person. The categorization of copper balance is as follows:

	Category	Range
1.	Positive Balance	> +0.25 mg/day
	Marginal	+0.26 mg/day to +0.31 mg/day
2.	Neutral Balance	-0.15 mg/day to +0.25 mg/day
3.	Negative Balance	< -0.15 mg/day

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A reversal of positive copper balance to negative or neutral copper balance is considered a therapeutic success.

ii) The effect of zinc is to block intestinal copper absorption, and this can be evaluated by a test dose of ^{64}copper, and measuring the amount of radioactivity absorbed into the blood. The peak occurs at 1-2 hours after oral ingestion. The effect of zinc is induction of intestinal cell metallothionein. According to document submitted by the sponsor, copper test evaluates the efficacy of zinc and compliance to the program over the 1-2 week period before the study.

The average blood peak of radioactivity in the absence of zinc therapy is 6% (with a range 3% to 12%). Effective blockade of copper absorption is represented by a peak of 1.2% or less; values between 1.3% and 1.5% are considered marginal.

3. Compliance Endpoints (Parameters)

Liver Zinc Levels and Zinc Balance (long Term Assessment of Efficacy)

i) These were measured to assess the zinc status over a period of prolonged zinc administration. Urine samples were obtained (by mail) every 6 months to evaluate urine copper and zinc status.

Safety Endpoint (Parameter)

Safety was monitored by recording any unexpected or unwanted symptoms observed by the investigator or reported by the patient. Hematology, clinical chemistry, urinalysis and other blood analysis were done.

Method of Efficacy Assessment

There was no concurrent control group; patients served as their own control. There was no prospectively planned statistical method of analysis for the assessment of clinical and statistical effectiveness of the therapy, and no formal statistical analysis was carried out. The lack of an unfavorable trend over the time of the therapy, or the presence of a favorable trend in the individual patients, or the mean values, were used as indicative of acceptable efficacy or lack of toxicity.

1.3 EFFICACY RESULTS/ REVIEWER'S COMMENTS

The sponsor 's documentation indicated that all 86 patients entered into the maintenance therapy phase were included in the efficacy and safety assessments. However, sponsor's database indicate majority of patients had missing data. For some of the endpoints the sparseness of the data was too high to render any statistical analyses meaningless. Therefore, patients with fewer than 4 of the possible 9 data points (baseline, year 1 through year 8 visits) are excluded from this

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reviewer's data summary below. The following endpoints are, therefore, not included in the statistical summary below: neurological clinical endpoint, serum ALT, LDH chemical endpoint, and hepatic copper. Furthermore, only the data for the symptomatic indication is considered.

CHANGES IN LEVELS FROM YEAR ONE FOR SYMPTOMATIC PATIENTS ON ZINC WITH ≤3 MISSING DATA: 50 MG TID

ENDPOINTS	Time on Zinc	N	Mean	Std Error	2-Sided P-Value
CLINICAL	Baseline	5	3.2000		
SPEECH RATINGS	YEAR1	12	3.0833	0.3580	
	YEAR2	12	2.8417	0.3329	
	YEAR3	12	2.7917	0.3560	
	YEAR4	12	2.7917	0.3452	
	YEAR5	10	2.9000	0.4460	
	YEAR6	4	3.6250	0.7181	

DIFFERENCE(=Mean Yeari - Mean Year1, i=2,...,6)					
DIFF1		12	-0.2417	0.1873	0.2233
DIFF2		12	-0.2917	0.2784	0.3172
DIFF3		12	-0.2917	0.2981	0.3489
DIFF4		10	-0.0500	0.2522	0.8473
DIFF5		4	0.1250	0.4732	0.8088

NMR

	Time on Zinc	N	Mean	Std Error	2-Sided P-Value
	Y1	9	37.1111	4.3699	
	Y2	9	40.6667	4.2850	
	Y3	9	43.1111	5.6579	
	Y4	8	53.1250	11.7434	
	Y5	3	60.0000	21.8251	
	Y6	1	51.0000	.	

DIFFERENCE(=Mean Yeari - Mean Year1, i=2,...,6)					
DIFF1		9	3.5556	4.8250	0.4822
DIFF2		9	6.0000	4.7346	0.2407
DIFF3		8	16.5000	9.7009	0.1328
DIFF4		3	25.0000	22.9129	0.3892
DIFF5		1	18.0000	.	.

BIOCHEMICAL

SERUM BILIRUBIN	Baseline	4	0.6000		
	Y1	15	0.6933	0.1475	
	Y2	15	0.7267	0.1999	
	Y3	15	0.6667	0.1440	
	Y4	14	0.6143	0.1456	
	Y5	9	0.5889	0.1207	
	Y6	2	0.3500	0.0500	

DIFFERENCE(=Mean Yeari - Mean Year1, i=2,...,5)					
DIFF1		15	0.0333	0.0747	0.6625
DIFF2		15	-0.0267	0.0746	_0.7262
DIFF3		14	-0.0929	0.0539	0.1088
DIFF4		9	0.0333	0.0782	0.6811
DIFF5		2	0.0000	0.0000	.

SERUM AST	Baseline	4	25.7500		
	Y1	15	30.4667	4.3882	
	Y2	15	29.2667	2.5643	
	Y3	15	28.2000	2.3688	
	Y4	14	30.2143	2.5831	
	Y5	9	27.4444	3.4283	
	Y6	3	36.3333	6.3333	

DIFFERENCE(=Mean Yeari - Mean Year1, i=2,...,6)					
DIFF1		15	-1.2000	2.9588	0.6912
DIFF2		15	-2.2667	4.1271	0.5915
DIFF3		14	0.7857	4.2880	0.8574
DIFF4		9	0.6667	7.0985	0.9275
DIFF5		3	15.3333	4.6667	0.0815

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ENDPOINTS	Time on Zinc	N	Mean	Std Error	2-Sided P-Value

BIOCHEMICAL (CONT'ED)

	Time on Zinc	N	Mean	Std Error	2-Sided P-Value
SERUM ALKALINE PHOSPHATE	Baseline	3	120.0000		
	Y1	17	136.5294	14.9615	
	Y2	17	122.7647	12.9051	
	Y3	15	116.2000	10.6745	
	Y4	14	116.5000	14.5564	
	Y5	9	107.3333	10.3521	
	Y6	3	108.6667	6.4893	

DIFFERENCE(=Mean Yeari - Mean Year1, i=2,...,5)

		N	Mean	Std Error	2-Sided P-Value
DIFF1		17	-13.7647	9.3803	0.1616
DIFF2		15	-15.3333	10.6693	0.1727
DIFF3		14	-11.1429	10.7299	0.3180
DIFF4		9	-4.2222	14.8153	0.7829
DIFF5		3	18.6667	6.3333	0.0984

COMPLIANCE

URINE ZINC LEVELS

	Time on Zinc	N	Mean	Std Error	2-Sided P-Value
	Y1	16	3.8750	0.4106	
	Y2	14	3.6929	0.4282	
	Y3	15	4.0733	0.5316	
	Y4	16	4.5063	0.6117	
	Y5	13	3.8308	0.6113	
	Y6	11	3.8727	0.6556	
	Y7	8	3.8125	1.0914	

DIFFERENCE(=Mean Yeari - Mean Year1, i=2,...,6)

		N	Mean	Std Error	2-Sided P-Value
DIFF1		14	-0.3071	0.3997	0.4559
DIFF2		15	0.1933	0.5614	0.7357
DIFF3		16	0.6313	0.5400	0.2607
DIFF4		13	-0.2615	0.4317	0.5559
DIFF5		11	-0.2636	0.3248	0.4359
DIFF6		8	-0.6500	0.8783	0.4833

PLASMA ZINC LEVELS

	Time on Zinc	N	Mean	Std Error	2-Sided P-Value
	Y1	13	236.5385	21.9386	
	Y2	11	221.4545	17.4212	
	Y3	9	257.3333	22.0914	
	Y4	10	216.5000	19.4098	
	Y5	8	234.5000	24.3083	
	Y6	7	206.1429	10.3220	
	Y7	4	227.2500	25.4996	

DIFFERENCE(=Mean Yeari - Mean Year1, i=2,...,6)

		N	Mean	Std Error	2-Sided P-Value
DIFF1		11	-17.1818	21.4522	0.4418
DIFF2		9	39.4444	32.3626	0.2576
DIFF3		10	-22.0000	35.8150	0.5542
DIFF4		8	-32.3750	30.0915	0.3177
DIFF5		7	-39.0000	23.6603	0.1504
DIFF6		4	-33.5000	43.3945	0.4964

PHARMACODYNAMIC (PD)

URINE COPPER LEVELS

	Time on Zinc	N	Mean	Std Error	2-Sided P-Value
	Y1	17	0.1258	0.0333	
	Y2	15	0.1242	0.0260	
	Y3	16	0.1039	0.0168	
	Y4	17	0.1094	0.0158	
	Y5	13	0.0954	0.0203	
	Y6	12	0.0969	0.0222	
	Y7	8	0.1386	0.0501	

DIFFERENCE(=Mean Yeari - Mean Year1, i=2,...,6)

		N	Mean	Std Error	2-Sided P-Value
DIFF1		15	-0.0117	0.0185	0.5359
DIFF2		16	-0.0248	0.0225	0.2878
DIFF3		17	-0.0164	0.0291	0.5808
DIFF4		13	-0.0423	0.0349	0.2483
DIFF5		12	-0.0106	0.0380	0.7857
DIFF6		8	0.0639	0.0536	0.2720

Comments/ Conclusions

Note that the data summary above includes patients with less than 2 consecutive missing visit data points per endpoint only. The summary statistics are the mean statistics for baseline (year 0) and subsequent annual visits (year 0-1 thru year 5-6). Since most of the patients have missing baseline visit data, a measure of maintenance or improvement for the efficacy endpoints (clinical and biochemical parameters) is indicated by a lack of increase in mean-year 2 through mean-year 6 reading compared with mean-year 1 (which is used here as reference reading). Therefore, a non-positive difference between year 1 and subsequent annual visits would indicate a non-degenerating condition. Moreover, mean annual readings that are within the accepted normal mean reading ranges for each parameter (see **Attachment I**) would indicate therapeutic benefit.

For the assessment of compliance, PD and biochemical endpoints, baseline (year 0) reference summary is also given where possible; because for these parameters it is of more interest to compare patients progress from baseline.

From the summary results on pages 6 and 7, the following observations can be made:

A. Clinical Endpoints

For the speech efficacy data, the mean annual ratings for the first 5 years (year1 - year4) are 3 or less. Compared to first year on therapy, the data seem to indicate an improvement trend.

For the NMR efficacy data, there is no indication of an improvement trend. The mean annual ratings based on a scale of 38 (worst) to 0 (normal) are at least 37 for year1 through year4.

B. Biochemical Endpoints

For both serum bilirubin and serum AST, the mean annual readings are well within normal ranges (.1 - .9 mg/dl) for bilirubin and (0 - 45 iu/l) for AST.

Except for year1, the mean annual readings for serum alkaline phosphate data are all within ranges (30 - 130 iu/l).

Thus for biochemical endpoints, the efficacy data seem to indicating a therapeutic benefit.

C. Pharmacodynamic Endpoint

For urine copper levels, the mean annual readings are all less than .125 mg/day, an indication of copper control.

8
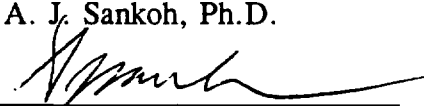
D. Compliance Endpoints

For urine zinc levels, the mean annual readings are all within accepted normal readings. For plasma zinc levels, the efficacy data do not indicate zinc control as all mean annual readings are above the upper normal limit of 125 μg/dl.

OVERALL CONCLUSION
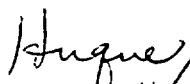
Two-sided p-values (based on simple t-tests) have been included among the summary statistics in the table on pages 6 and 7 for completeness of summary only. No meaningful statistical interpretation can be made from these regarding the statistical effectiveness of zinc therapy because of the sparseness of the data (see **Attachment II**). However, this reviewer's perusal observation of the summary data seems to suggest some therapeutic benefit.

A. J. Sankoh, Ph.D.
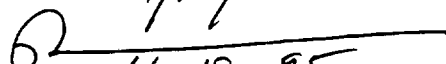
_____
Mathematical Statistician

Concur:

Dr. Huque

Dr. Dubey

cc: Orig. NDA 19-594
  HFD - 180
  HFD - 180/Dr. Fredd
  HFD - 180/Dr. Gallo-Tores
  HFD - 180/Ms. Oliver
  HFD - 713/Dr. Dubey [File: DRU 1.3.2 NDA]
  HFD - 713/Dr. Huque
  HFD - 713/Dr. Sankoh
  Chron.
  Sankoh/x4594/AJS/04-13-95

Table of normal values

Urine copper	20-50 ug/24 hours
Urine zinc	200-500 ug/24 hours
Nonceruloplasmin plasma copper	10-20 ug/dl
Liver copper	20-50 (ppm) ug/g dry weight
Plasma zinc	75-125 ug/dl
Serum albumin	3.5-4.9 g/dl
Serum ALT	2-35 iu/l
Serum AST	0-45 iu/l
Serum bilirubin	0.1-0.9 mg/dl
Serum lactic dehydrogenase	60-200 iu/l
Serum alkaline phosphatase	30-130 iu/l
Blood hemoglobin - men	13.5-18 g/dl
Blood hemoglobin - women	12-16 g/dl
Red blood cell MCV	80-100.0 fl
Red blood cell MCH	27.0-31.0 pg
Red blood cell MCHC	32.0-36.0 %
White blood cell counts	4.0-10.0 k/mm^3
Platelet counts	200-400 k/mm^3
Reticulocyte counts	0.5-1.5 %
Serum creatinine	0.6-1.0 mg/dl
Serum BUN	8-20 mg/dl
Serum uric acid	2.2-6.0 mg/dl
Urine protein	0
Serum total cholesterol	110-240 mg/dl
Serum HDL-cholesterol	35-72 mg/dl
Serum LDL-cholesterol	75-160 mg/dl
Serum triglycerides	30-170 mg/dl
Serum amylase	30-100 iu/dl
Serum lipase	4-24 iu/dl
Serum iron	33-150 ug/dl
Serum transferrin	210-400 ug/dl
Serum ferritin	5.0-202.0 ng/ml
Hepatic zinc	100-500 (ppm) ug/g dry weight

2 pages
PURGED

Individual RAW
DATA

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA: 20-458 Amendment

REC'D
MAY 3 0 1996
HFD-180

SUBMISSION DATE: Jan. 29, 1996
Mar. 27, 1996

Zinc Acetate, 25 mg & 50 mg Capsules
Lemmon Company
1510 Delp Drive
Kulpsville, PA 19443
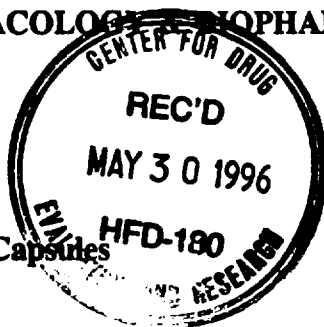
REVIEWER: Hae-Ryun Choi, Ph.D.

REVIEW OF A BIOAVAILABILITY STUDY

SYNOPSIS: The firm submitted a bio study entitled "A bioavailability and dose proportionality study of zinc acetate (25 mg and 50 mg capsules) in healthy volunteers." in response to the Agency's request dated 08/23/94. It provides a single dose PK, multiple dose PK, and dose proportionality results on zinc acetate in both male and females, and includes data on baseline/diurnal variations for endogenous zinc in normal subjects. As per the Agency's recommendations dated 04/11/95, the protocol was amended to included an additional blood samples at 30 minute time point post-dose and equal numbers of male and female subjects in the study.

Since the firm only provided the pharmacokinetic parameters determined from the baseline corrected serum zinc concentration data, the pharmacokinetic parameters uncorrected for baseline were recalculated by noncompartmental methods by this reviewer (see attached).

A review of the study is as follows:

TITLE: A bioavailability and dose proportionality study of zinc acetate (25 mg and 50 mg capsules) in healthy volunteers.

OBJECTIVES: 1) To evaluate the pharmacokinetics and dose proportionality of zinc acetate following 25 mg and 50 mg single oral doses and 2) to determine the relative bioavailability of multiple versus single doses of 50 mg zinc acetate capsules.

INVESTIGATOR AND STUDY SITE:

FORMULATIONS:

- Zinc Acetate Capsules 25 mg, lot no.:3862, lot size: . mfg. site: Lemmon Co., Sellersville, PA.
- Zinc Acetate Capsules 50 mg, lot no.:5060, lot size: mfg. site: Lemmon Co., Sellersville, PA.

STUDY DESIGN: This was a fasting single, stepped-dose challenge and profile and multiple dose challenge and profile. Sixteen healthy adults (8 males and 8 females) participated and completed the study. Subjects were confined in the study unit approximately 62.5 hours prior to Dose 1 and continued for 128 hours after Dose 1. Standardized meals were served at specified times throughout confinement. The study consisted of following three steps:

Step I of the study involved Days -1 to 3 and commenced with establishing a zinc baseline diet on Days -1, 1, and 2. Study day 2 involved sampling baseline serum zinc concentrations. On Study day 3, each subject was administered a single 25 mg oral zinc acetate capsule followed by a serum zinc profile.

Step II involved a washout on Day 4 with dosing on Day 5. On Day 5, a single 50 mg oral zinc acetate capsule was administered followed by a serum zinc profile.

Step III involved multiple daily dosing with 50 mg zinc acetate capsules on Days 6, 7, and 8 with a serum profile on Day 8. On Days 6 and 7 subjects were administered a 50 mg zinc acetate capsules every eight hours. On Day 8 a single dose, zinc acetate 50 mg capsule, was administered followed by a serum zinc profile.

All subjects fasted from 8 hours prior to Doses 1, 2, 3, and 6 and at least 2 hours prior to Doses 4, 5, 7 and 8. Two hours prior to Dose 9, an apple was served. No food was allowed for 4 hours after the dose.

TYPE of MEALS: Subjects were served standardized meals and beverages. On Study days -1 through 7, subjects were served a 3-6 oz. portion of lean beef in the evening to provide a nutritional baseline source of zinc. Meals were the same in content and quantity on study Days 3, 5, and 8 (serum zinc profile days). The daily meals on Days 1, 2, 4, 6, and 7 were similar in content but not identical.

SPECIMENS: Forty-eight blood samples per subject were collected over the course of the study for determination of serum zinc concentration. Blood samples for diurnal variation were collected at -24, -18, -12 hours and immediately prior to Dose 1 on Day 3. Blood samples for zinc bioavailability were collected at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, and 12 hours after Dose 1 on Day 3. Blood samples were collected immediately prior to Dose 2 on Day 5 and after dosing at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, and 12 hours. Multiple dosing commenced on Day 6 (Dose 3) and continue to Dose 9. Morning blood sampling occurred immediately prior to Dose 3, Dose 6, and Dose 9. Blood samples were collected immediately prior to Dose 9 (Day 8) and after dosing at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, and 8 hours.

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ASSAY METHOD:

Zinc serum levels were analyzed using

Overall assay is satisfactory.

DATA ANALYSIS: Cmax, Tmax and AUC(0-t) were determined for zinc from the baseline uncorrected serum zinc concentration data by noncompartmental methods.

RESULTS: The serum zinc levels for each subject are attached. The mean pharmacokinetic parameters (± SD) for zinc uncorrected for baseline are listed in Table 1. The mean serum zinc concentration time profile is given in Figure 1.

Table 1. Mean Zinc Pharmacokinetic Parameters (± SD)

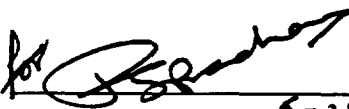| Dose (mg)	Cmax (μg/mL)	Tmax (hours)	AUC(0-t*) (μg.h/mL)
25 mg single dose	2.64 (± 0.42)	2.44 (± 0.60)	13.11 (± 2.72)
50 mg single dose	3.20 (± 0.46)	2.28 (± 0.66)	16.10 (± 2.80)
50 mg multiple dose	2.93 (± 0.33)	2.44 (± 0.57)	15.09 (± 1.78)

* t denotes 12 hours for single dose; 8 hours for multiple dose.

3

Figure 1.

ZINC ACETATE MEAN DATA
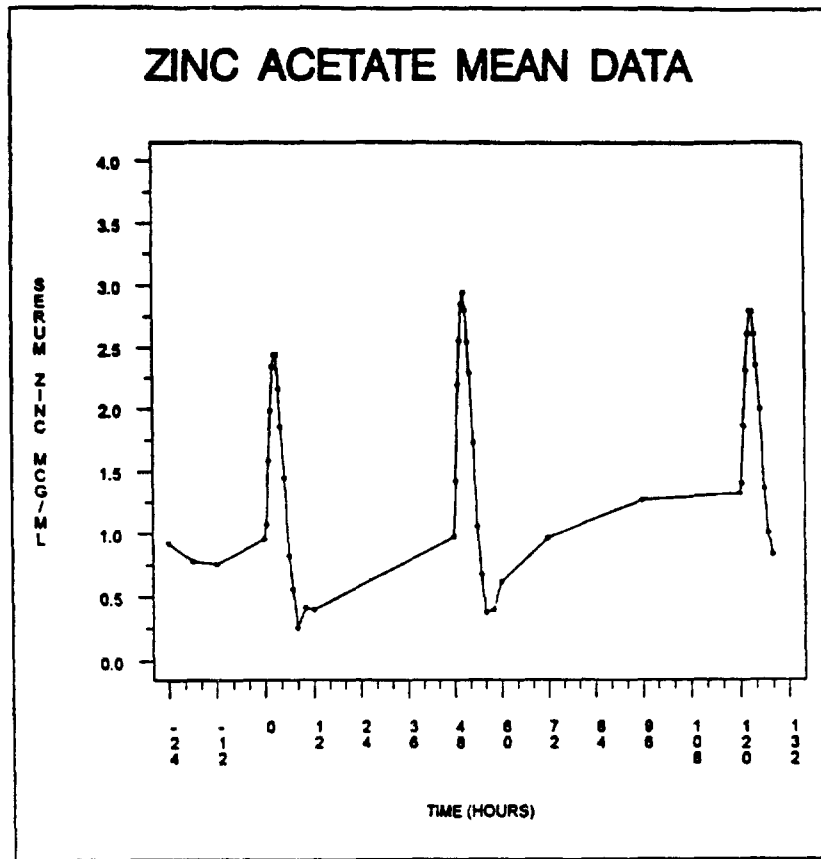
CONCLUSIONS:

A comparison of the mean pharmacokinetic parameters uncorrected for baseline showed that the AUC after the 25 mg single dose was approximately 81% of the AUC after the 50 mg single dose. The mean Cmax values uncorrected for baseline following a 25 mg single dose was 82% of those following a 50 mg single dose.

The AUC following multiple dosing of the 50 mg capsules was approximately the same as the AUC measured from dosing to return to baseline following a single 50 mg dose. The Cmax after multiple dosing appeared to be lower than that after a single dose.

Blood samples for diurnal variation were collected at -24, -18, -12 hours and immediately prior to Dose 1. The mean baseline serum zinc concentration established in this study by a zinc rich diet was 0.855 µg/ml.

4

RECOMMENDATIONS:

The Division of Pharmaceutical Evaluation II has reviewed the submissions dated on 01/29/96 and 03/27/96 and found acceptable.

The sponsor is recommended that the following information be incorporated in the labeling:

"A single, stepped-dose and multiple dose study was performed in eight healthy male subjects and eight healthy female subjects to compare the bioavailability and dose proportionality of zinc acetate (25 mg and 50 mg capsules) capsules under fasting conditions. In addition the relative bioavailability of multiple doses versus a single dose of zinc acetate (50 mg) capsules was compared. Standardized meals were served at specified times throughout confinement and subjects received at least one serving of lean beef to provide a nutritional baseline source of zinc. The mean baseline serum zinc concentration established in this study by a zinc rich diet was 0.855 μg/ml.

A comparison of the mean zinc pharmacokinetic parameters uncorrected for baseline showed that the AUC after the 25 mg single dose was approximately 81% of the AUC after the 50 mg single dose. The mean Cmax values following a 25 mg single dose was 82% of those following a 50 mg single dose. The AUC following multiple dosing of the 50 mg capsules was approximately the same as the AUC measured from dosing to return to baseline following a single 50 mg dose. The Cmax after multiple dosing appeared to be lower than that after a single dose."

It is suggested that the sponsors tabulate the data as follows:

Table 1. Mean Zinc Pharmacokinetic Parameters ($\pm$ SD)

Dose (mg)	Cmax (μg/mL)	Tmax (hours)	AUC(0-t*) (μg.h/mL)
25 mg single dose	2.64 ($\pm$ 0.42)	2.44 ($\pm$ 0.60)	13.11 ($\pm$ 2.72)
50 mg single dose	3.20 ($\pm$ 0.46)	2.28 ($\pm$ 0.66)	16.10 ($\pm$ 2.80)
50 mg multiple dose	2.93 ($\pm$ 0.33)	2.44 ($\pm$ 0.57)	15.09 ($\pm$ 1.78)

* t denotes 12 hours for single dose data; 8 hours for multiple dose data.

Please convey the above recommendation to the firm.

5

Hae-Ryun Choi 05/28/96

Hae-Ryun Choi, Ph.D.
Division of Pharmaceutical Evaluation II

FT initialed by Lydia Kaus, Ph.D.

R.S. PRADHAN 5-28-96

cc: NDA 20-458, HFD-180, HFD-850 (Lesko), HFD-860 (Malinowski), HFD-870 (M.Chen, Kaus, Choi), HFD-880 (Fleischer), HFD-340 (Viswanathan), HFD-870 (Chron, Drug, Reviewer), HFD-205 (FOI).

NDA: 20-458

SUBMISSION DATE: Jun. 21, 1994
Mar. 06, 1995
Mar. 07, 1995

Zinc Acetate, 25 & 50 mg Capsules
Lemmon Company
1510 Delp Drive
Kulpsville, PA 19443

REVIEWER: Hae-Ryun Choi, Ph.D.

TYPE OF SUBMISSION: Original NDA　　　**PRIORITY:** 2 S
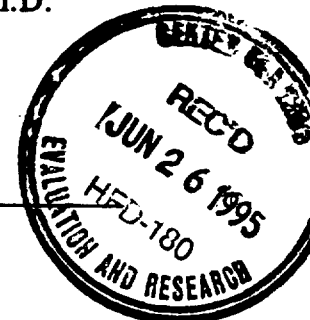
SYNOPSIS:

NDA 20-458 for Zinc Acetate Capsules, 25 mg and 50 mg, was submitted (dated: 06/21/94) by Lemmon Company for the following indications: 1) maintenance treatment of patients with Wilson's disease who have been initially treated with the chelating agents penicillamine, trientine, or ammonium tetrathiomolybdate;

On 11/07/85, zinc acetate received designation as an orphan drug for the treatment of Wilson's disease.

The Human Pharmacokinetics and Bioavailability Section of this NDA contains limited information. The firm has submitted the zinc plasma levels and urinary excretion of zinc which were measured yearly for up to 9.5 years in 86 Wilson's disease patients during the oral administration of zinc acetate over many years of treatment.

The sponsor has submitted several clinical pharmacology studies, which included some information regarding the drug interaction between zinc and penicillamine, trientine, and ammonium tetrathiomolybdate, the effect of Vitamin C on zinc efficacy, and foods or beverages effect on zinc absorption. The sponsor also submitted a literature report (1977) of a bioequivalency study which compared the zinc plasma levels after single oral doses of three different salts of zinc (i.e., zinc acetate, zinc sulfate, and zinc carbonate) in normal subjects. However, the details of protocol, assay validation, and study execution for these studies are not provided.

After the 45-day filing meeting, the firm was requested by the Agency to conduct an additional pharmacokinetic study. On 03/06/95, the sponsor submitted a protocol entitled "A bioavailability and dose proportionality study of zinc acetate (25 and 50 mg capsules) in healthy volunteers". The study will be a 16-subject trial which will include single dose PK (50 mg capsules), multiple dose PK (50 mg capsules), and single dose PK (25 mg capsules) to assess dose proportionality. The protocol has been reviewed (letter date: 04/07/95) by the Div. of Biopharmaceutics and the summary is attached in Appendix.

The formulation composition information has been provided for market formulations and investigational formulations used in clinical trials. Zinc Acetate 25 mg capsules are not compositionally proportional to Zinc Acetate 50 mg capsules. The sponsor provided adequate

in-vitro dissolution information on the capsule formulations used in clinical trials and proposed drug products.

RECOMMENDATION:

NDA 20-458 for Zinc Acetate Capsules, submitted on 06/21/94, 03/06/95, and 03/07/95, has been reviewed by the Division of Biopharmaceutics. The sponsor's NDA 20-458 appears to be deficient for meeting the biopharmaceutics requirements. Deficiencies #1 - #2 and Comment #3 on page 14 should be conveyed to the sponsor. Comments #1, #2 and #4 should be forwarded to the medical officer in HFD-180.

TABLE OF CONTENTS:

BACKGROUND:

Wilson's disease (hepatoenticular degeneration) is a defect in copper metabolism which is inherited as an autosomal recessive trait. Individuals with Wilson's disease are able to absorb copper from the diet normally but are unable to excrete the copper by way of the bile in a normal fashion, so copper accumulates in the body. The excess free copper is toxic especially to the liver and the nervous system. If the disease is not treated properly, it is ultimately fatal. The rationale for using zinc acetate for the treatment of Wilson's disease is that zinc prevents the absorption of copper from the diet and the reabsorption of endogenously secreted copper such as that from the saliva, gastric juice and other GI secretions.

The proposed site of action of zinc is in the intestinal cell where zinc induces the production of metallothionein, a protein that complexes copper and the bound copper is then excreted when the intestinal cell is shed in the stool.

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Zinc acetate is white crystals or granules, having a slight acetous odor and an astringent taste. It has a molecular weight of 219.50 and a chemical formula of $(CH_3COO^-)_2Zn.2H_2O$. Zinc acetate is slightly efflorescent. It is freely soluble in water (ca. 0.43 g/mL) and in boiling alcohol (ca. 1 g/mL), and slightly soluble in alcohol (0.033 g/mL).

Zinc acetate, the drug substance, is official in USP XXIII, but the dosage form, the hard gelatin capsules containing zinc acetate, is not compendial.

Clinical evaluation of zinc acetate was initiated by George J. Brewer, M.D., Department of Human Genetics, University of Michigan, who was the principal investigator for all clinical studies in the United States.

The sponsor has submitted two controlled (patients serve as their own control) clinical studies to support the efficacy claims for this NDA. One study was conducted by George J. Brewer at the University of Michigan who treated 86 Wilson's disease patients with zinc acetate for up to 9.5 years. The second study was by Hoogenraad in the Netherlands who treated 27 Wilson's disease patients with zinc sulfate for up to 27 years.

It was stated that the sponsor is unaware of any foreign marketing experience with zinc acetate for the treatment of Wilson's disease. Zinc sulfate has been studied extensively outside the United States for Wilson's disease, however, the sponsor is unaware of any regulatory approvals of the safety and efficacy of zinc sulfate for this purpose..

Several zinc salts (e.g., zinc sulfate, zinc gluconate, complex zinc carbonates) are available for OTC's as a zinc dietary supplement to treat or prevent zinc deficiencies (average adult dose is 25 to 50 mg zinc daily). The zinc salts are poorly absorbed from GI tract; 20% to 30% of dietary zinc is absorbed. The main excretion route is through the intestine. Only minor amounts are lost in the urine ($\approx 2\%$) (Facts and Comparisons, pp 41, 1993).

The recommended adult treatment dose for Wilson's disease is 50 mg of zinc TID (not with meals) and that for children is 25 mg of zinc TID until age 15, or until the body weight is 50 kg. The dosage for pregnant patient is 25 mg of zinc TID.

SUMMARY OF BIOAVAILABILITY/PHARMACOKINETICS/PHARMACODYNAMICS:

I. BIOEQUIVALENCE STUDY:

The firm submitted this study, published by Oelshlegel and Brewer in 1977 (Zinc metabolism: current aspects in health and disease. Alan R. Liss, Inc., New York, NY, pp. 299-311), on the absorption of zinc from three different salts of zinc. The sponsor claimed that the raw data are not available as the study was conducted approximately twenty years ago.

In this study, eleven subjects received 25 mg of zinc as zinc sulfate, eight of the same subjects

received 25 mg of zinc as zinc acetate, and five of the same subjects received 25 mg of zinc as zinc carbonate. Zinc levels at 3 hours after zinc acetate and zinc sulfate were not significantly different from each other, however, the difference between zinc carbonate and zinc sulfate is significant at the p<0.05 level.

**THREE HOUR PLASMA ZINC VALUES AFTER INGESTION OF EQUIVALENT AMOUNTS OF VARIOUS ZINC SALTS**

Zinc Salt	Dosage	Number of Subjects	Area under Curve[1]	Three hour plasma zinc ± S.D.[2]	p value for difference[3]
Zinc sulfate	25 mg Zn	11	100%	186 ± 25 ug%	NS ⎤
Zinc acetate	25 mg Zn	8	92%	196 ± 25 ug%	⎦ p<0.05
Zinc carbonate	25 mg Zn	5	79%	148 ± 32 ug%	p<0.01

[1]Areas under curves from Figure C.1 expressed as a percent of the area under the sulfate curve. Note that these areas reflect the increase in plasma zinc above baseline values.

[2]The three hour plasma zinc values here are the actual values, not the increase over baseline values, (which averaged 98 ug% in these subjects).

[3]T-statistic (Paired). NS = not significant.

The mean increase in plasma zinc over baseline values after administration of three different zinc salts are shown below.
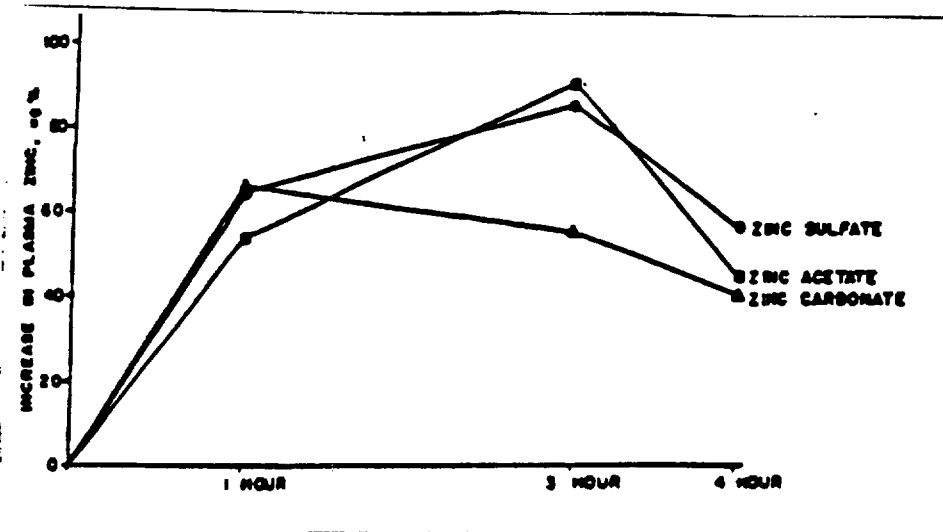
II. PHARMACOKINETICS:

Eighty-six patients with Wilson's disease were treated with zinc acetate up to 9.5 years by George J. Brewer, M.D. The 86 patients included 39 males and 47 females ranging in age from 11 to 55 years at the beginning of the study. The patients were Caucasian except for four who were of Mexican-American ancestry. During this maintenance study, the zinc plasma levels and urinary excretion of zinc were measured yearly during oral administration of zinc acetate over many years of treatment. Zinc as 50 mg TID or 25 mg TID usually was administered to patients, the latter being given in special circumstances such as pregnancy. However, the information regarding the sampling time for each measurement is not provided.

A. Plasma Zinc

a. Overall Data: The overall data for plasma zinc during this maintenance study are shown below.

Years on Zinc Therapy

Year	0[d]	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9
mean[a]	89	218	223	226	199	214	208	183	177	321
sd[b]	22	74	55	65	63	61	44	60	77	0
n[c]	18	59	47	33	28	21	15	10	3	1

[a]: mean zinc level in plasma (mcg/dl)   [b]: standard deviation
[c]: number of patients   [d]: years of treatment

The mean plasma zinc level was 218 mcg/dl with a standard deviation of 74 mcg/dl in 59 Wilson's disease patients during the first year of therapy (normal baseline range: 75-125 mcg/dl). The mean baseline zinc level was 89 mcg/dl and individual levels were within the normal range in 17 of 18 Wilson's disease patients. The mean plasma levels were then sustained above the normal level throughout the study. The individual patient data and demographics are provided in Appendix.

b. Dose: No apparent dose effect on plasma zinc is shown. Tables 1 to 3 present the mean plasma zinc data over time in patients with 50 mg X 3, 50 mg X 2, and 25 mg X 3, respectively.

Table 1. 50 mg X 3

Year	0	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9

	0	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9
mean[a]	84	200	212	220	213	222	217	221	140	
sd	20	68	46	65	56	62	47	41	68	
n	15	41	33	18	13	11	10	5	2	

[a]: mean zinc level in plasma (mcg/dl)

Table 2.   50 mg X 2

Year	0	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9
mean[a]	126	234	215	229	167	197	174	143		321
sd	8	64	63	71	49	31	32	14		0
n	2	6	4	5	6	3	2	2	0	1

[a]: mean zinc level in plasma (mcg/dl)

Table 3.   25 mg X 3

Year	0	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9
mean[a]	94	270	260	236	202	209	202	144	252	
sd	0	72	62	61	73	67	24	64	0	
n	1	12	10	10	9	7	3	3	1	

[a]: mean zinc level in plasma (mcg/dl)

c. Gender Effect: No effect of gender is apparent. The mean plasma zinc data on patients treated with 50 mg X 3 according to gender are shown below:

Female

Year	0	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9
mean[a]	90.3	233	214	228	198	241	200	205	177	
sd	21.5	73	57	70	79	49	34	43	77	
n	11	31	24	20	14	13	9	7	3	

[a]: mean zinc level in plasma (mcg/dl)

Male

Year	0	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9
mean[a]	87	202	232	223	201	170	221	131		321
sd	24	72	52	57	43	51	53	64		0
n	7	28	23	13	14	8	6	3	0	1

[a]: mean zinc level in plasma (mcg/dl)

B. Urine Zinc

The overall urinary excretion data during this maintenance study are shown below.

Years on Zinc Therapy

year	0[d]	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9
mean[a]	0.3	4.5	3.9	3.7	3.7	3.0	3.4	2.9	3.4	14.9
sd[b]	0.1	2.3	1.9	1.9	2.2	1.7	2.0	2.4	1.7	0.0
n[c]	23	75	63	51	41	33	27	18	6	1

[a]: mean urinary excretion of zinc for 24 hours (mg)   [b]: standard deviation
[c]: number of patients   [d]: years of treatment

The mean baseline urinary excretion was 0.3 mg/24 hours in 23 patients with Wilson's disease and individual levels were all within the normal range of 0.2 to 0.5 mg/24 hours. The daily urinary excretion of zinc was 4.5 mg/day during the first year of treatment. Over the years of maintenance therapy, the levels of urinary excretion were clearly higher than normal levels throughout the study. The individual patient urinary data are provided in Appendix.

III. FOOD EFFECT:

The sponsor presented a diagrammatic representation of the effect of several substances on relative plasma zinc concentrations after oral administration of zinc sulfate (25 mg elemental zinc). Comparisons were made between the plasma zinc value obtained when zinc was taken along with the foodstuff under study and the corresponding value obtained in the same individual when the zinc was taken without the foodstuff. The numbers within the columns

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indicate the time point of measure, generally 1 or 3 hours after zinc ingestion. Breads, vegetables, fruits, eggs and beverages such as milk and coffee appeared to reduce the zinc levels after oral administration of zinc sulfate (Oelshlegel and Brewer, Zinc metabolism: current aspects in health and disease. Alan R. Liss, Inc., New York, NY pp. 299-311, 1977).
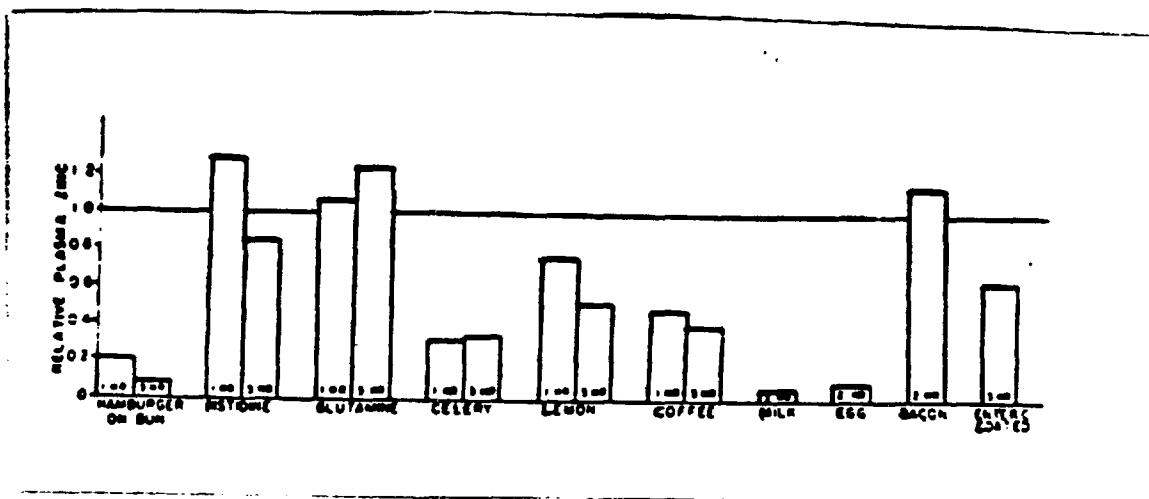
IV. SPECIAL POPULATIONS:

No pharmacokinetic studies were conducted in special populations (e.g., patients with hepatic or renal diseases, etc.).

V. DRUG INTERACTIONS:

A. Vitamin C: It has been shown that there is no significant interference with the effect of zinc by Vitamin C at 1.0 mg daily in patients with Wilson's disease during 8 weeks of study. It is based upon equivalent ^{64}Cu uptake and copper balance results when zinc is given alone, or given with Vitamin C.

Zinc Acetate (50 mg X 3) (N=6)					Zinc Acetate (50 mg X 3) + Vitamin C (1000 mg) (N=6)				
Copper (mg/day)					Copper (mg/day)				
Dietary Intake	Urinary Loss	Fecal Loss	Net Balance	^{64}Cu Test (% of Dose)	Dietary Intake	Urinary Loss	Fecal Loss	Net Balance	^{64}Cu Test (% of Dose)
0.89 ± 0.28[a]	0.117± 0.08	1.43± 0.76	-0.66± 0.91	0.35±0.17	0.80 ± 0.24	0.125± 0.088	0.98± 0.27	-0.30± 0.238	0.57±0.342
	NS[b]	NS	NS	NS					

[a]: standard deviation   [b]: not significant by paired t-test (p>0.05)

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B. Penicillamine and Trientine: Penicillamine 250 mg QID or trientine 250 mg QID, the chelating agents which are available for the treatment of Wilson's disease, does not interfere with the efficacy of zinc in patients with Wilson's disease during 2 weeks of study. This is based upon equivalent ^{64}Cu and copper balance results when zinc is given alone, or given with either of these drugs.

Zinc Acetate (50 mg X 3) (N=5)					Zinc Acetate (50 mg X 3) + Penicillamine (250 mg X 4) (N=5)				
Copper (mg/day)				^{64}Cu Test (% of Dose)	Copper (mg/day)				^{64}Cu Test (% of Dose)
Dietary Intake	Urinary Loss	Fecal Loss	Net Balance		Dietary Intake	Urinary Loss	Fecal Loss	Net Balance	
0.98 ± 0.27[a]	0.076± 0.035	1.08± 0.42	-0.17± 0.30	0.39±0.27	0.94 ± 0.31	0.461± 0.20	0.73± 0.37	-0.25± 0.25	0.26±0.21
	0.02	0.05	NS[b]	NS					

[a]: standard deviation   [b]: not significant by paired t-test (p>0.05)

Zinc Acetate (50 mg X 3) (N=5)					Zinc Acetate (50 mg X 3) + Trientine (250 mg X 4) (N=5)				
Copper (mg/day)				^{64}Cu Test (% of Dose)	Copper (mg/day)				^{64}Cu Test (% of Dose)
Dietary Intake	Urinary Loss	Fecal Loss	Net Balance		Dietary Intake	Urinary Loss	Fecal Loss	Net Balance	
1.33 ± 0.56[a]	0.128± 0.06	1.48± 0.72	-021 ± 0.36	0.77±0.85	0.87 ± 0.25	0.175± 0.07	0.97± 0.40	-0.25± 0.30	0.61±0.32
	0.03	NS[b]	NS	NS					

[a]: standard deviation   [b]: not significant by paired t-test (p>0.05)

C. Ammonium Tetrathiomolybdate (ATTM):   ATTM at 270 mg daily, an investigational drug, did not interfere with the effect of zinc on copper balance or absorption in patients with Wilson's disease during 2 weeks of study.

ATTM (270 mg/Day)			ATTM + Zinc Acetate (50 mg X 3)		
Urine Cu (mcg/24h)	Cu Balance (mg/day)	^{64}Cu Uptake[a] (% of Dose)	Urine Cu (mcg/24h)	Cu Balance (mg/day)	^{64}Cu Uptake[a] (% of Dose)
207±72 (n=4)	-0.62 (N=3)	1.07 (N=1)	238± 86 (N=6)	-0.75 (N=4)	0.26 (N=2)

a: baseline values = 5.98 mg/day

VI. PHARMACOKINETIC/PHARMACODYNAMIC RELATIONSHIPS:

A study was conducted to investigate the changes in the level of intestinal metallothionein

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(MT) in patients with Wilson's disease as a function of zinc treatment and concomitantly with copper absorption (Yuzbasiyan-Gurkan et al., J. Lab. Clin. Med. 120:380-386, 1992).

Five male and five female patients with Wilson's disease (between ages of 25 and 45 years) participated in this study. There were three experimental groups.

Group 1, consisting of two patients who had not taken zinc for 2 weeks, was studied to show the effect of zinc on the levels of MT and ^{64}Cu uptake by measurements taken before and after 5 or 6 days of zinc administration (50 mg zinc TID).

Group II, consisting of three other patients, was studied to show the consequences of suspending zinc therapy for up to 21 days by measurement of copper uptake and intestinal biopsy.

Group III was similar in purpose in Group II; but the influence of zinc withdrawal was monitored only by ^{64}Cu uptake in these five patients.

The results showed that there was a 2- to 4-fold increase in MT levels within 5 or 6 days after zinc treatment in the two patients in Group I. This increase in MT was accompanied by a reduction in ^{64}Cu uptake of 4.5% and 11.5% to 1.4% and 1.2%, respectively. This relationship is shown in Figure 1.
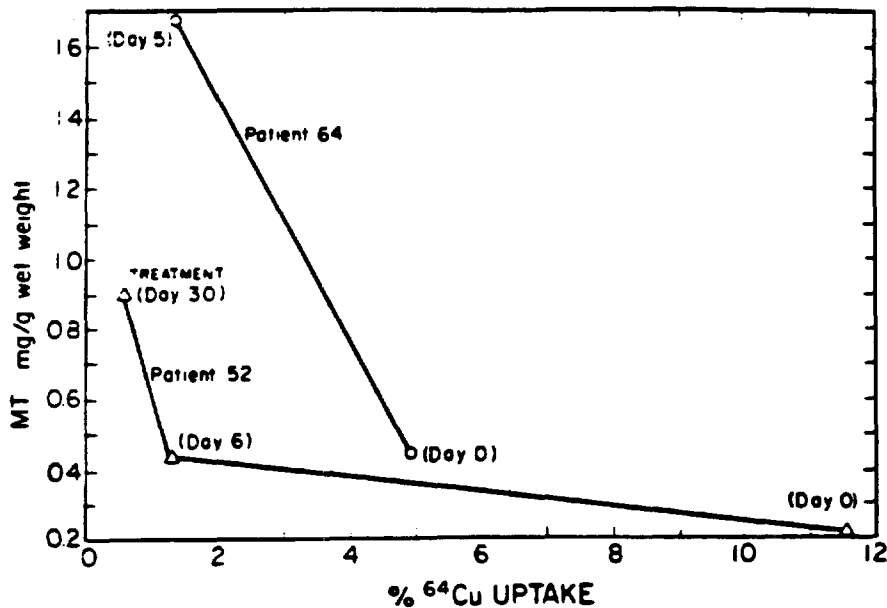
Fig. 1. Induction of intestinal metallothionein (MT) and suppression of ^{64}Cu uptake after initiation of zinc therapy in two patients with WD. *Numbers in parentheses* indicate days of zinc treatment.

Figure 2 shows that MT concentration progressively decreases with increasing ^{64}Cu uptake in each of the three patients in Group II. The suppression of ^{64}Cu uptake is short-lived after cessation of oral zinc treatment with about one-half of the effect lost within 10-13 days.
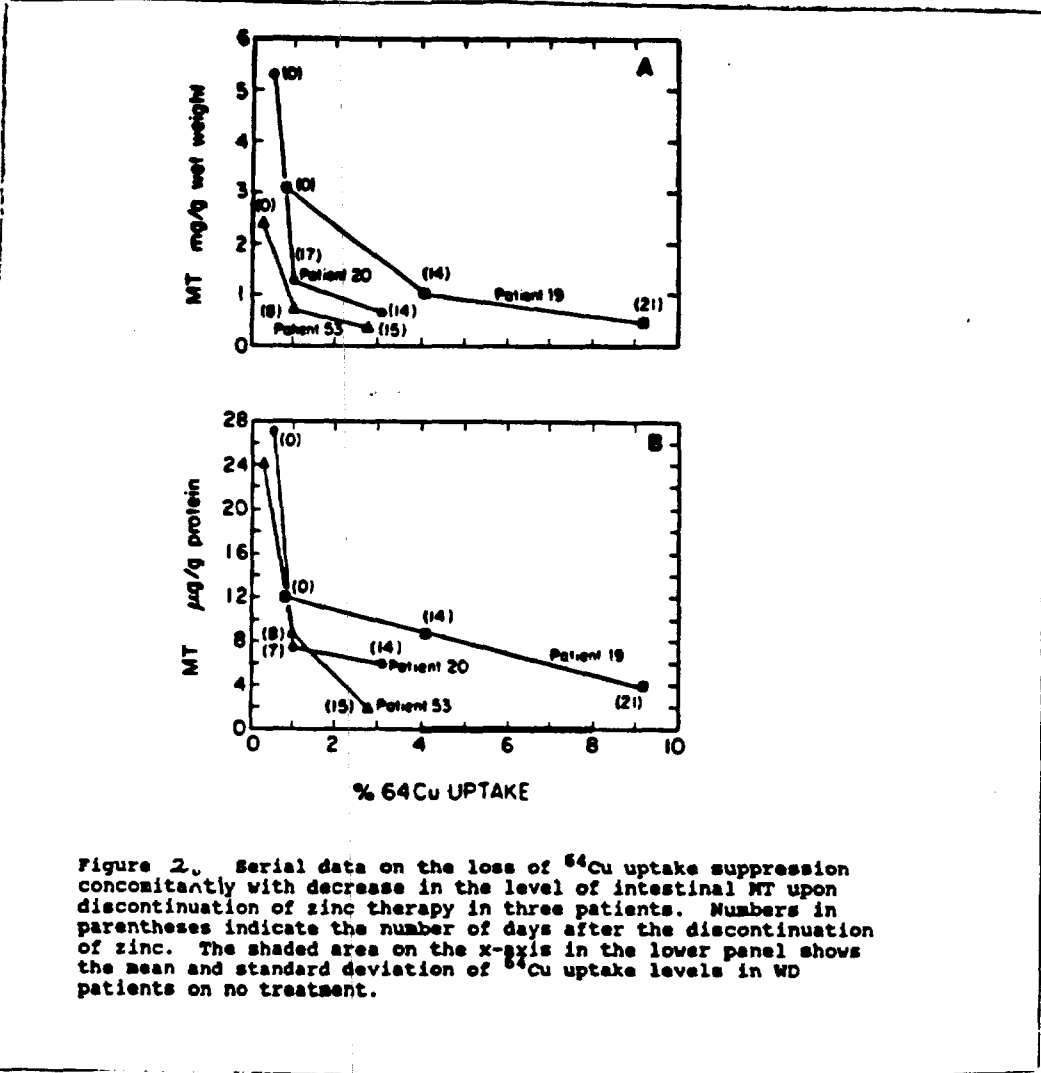
Figure 2. Serial data on the loss of ^{64}Cu uptake suppression concomitantly with decrease in the level of intestinal MT upon discontinuation of zinc therapy in three patients. Numbers in parentheses indicate the number of days after the discontinuation of zinc. The shaded area on the x-axis in the lower panel shows the mean and standard deviation of ^{64}Cu uptake levels in WD patients on no treatment.

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Figure 3 shows that after discontinuation of zinc, the decline in urinary zinc excretion and MT levels is accompanied by the increases in copper absorption. Linear regression analysis indicates that a significant negative linear correlation between the number of days after stopping zinc and urinary excretion of zinc and a positive linear correlation between intestinal MT levels and urinary excretion of zinc.
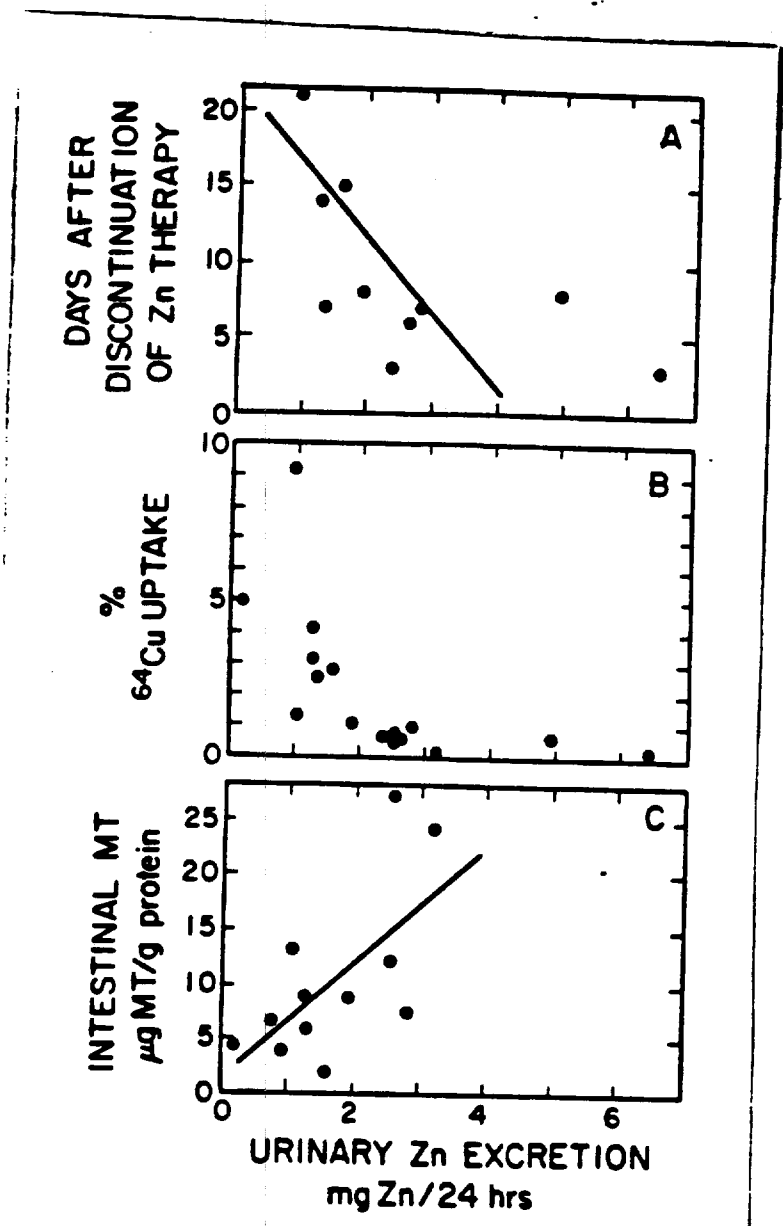
Fig. 3. A. Decline of urinary zinc levels as a linear function of time after discontinuation of zinc therapy. B. Inverse relationship of ^{64}Cu uptake and urinary zinc levels. C. Direct linear correlation of intestinal metallothionein levels with urinary zinc excretion. Lines in A and C reflect regression equations.

VII. FORMULATIONS:

The proposed drug product, Zinc Acetate Capsule, is available in two strengths, capsules containing the equivalent of 25 mg and 50 mg of zinc, respectively. The quantitative composition of each capsule is as follows:

Zinc Acetate Capsules, 50 mg
> Zinc Acetate USP   167.84 mg
> Corn Starch NF
> Magnesium Stearate NF

Zinc Acetate Capsules, 25 mg
> Zinc Acetate USP   83.29 mg
> Corn Starch NF
> Magnesium Stearate NF

The dosage form is conventional immediate release hard gelatin capsule.

Five lots of Zinc Acetate Capsules 50 mg and five lots of Zinc Acetate Capsules 25 mg were provided to George J. Brewer, M.D., for clinical study by the sponsor. The quantitative composition and lot number of each dosage form used in George Brewer's study are provided in Appendix. The summary of changes/differences in formulations during clinical development is also attached. Capsule formulations used in clinical study were the same as the capsule formulations to be marketed except for the minor change in the content of starch in the first clinical lot provided by the sponsor. The amount of starch was reduced by 10 mg (3% change) to improve the encapsulation process in the second clinical lot and this change remains thereafter. Initially, zinc acetate tablets containing 10 mg or 25 mg of zinc were available for clinical study before the sponsor supplied the drug as capsules containing 25 mg or 50 mg of zinc in August 1985.

VIII. DISSOLUTION:

Dissolution test results for lots used in clinical trials and the proposed drug products are located in Appendix. The sponsor proposed the following dissolution method and specification:

The comparative in-vitro dissolution profiles for the proposed market formulations in water, simulated gastric fluid without enzyme, and simulated intestinal fluid without enzyme, have been provided (see Appendix). The dissolution was rapid and almost complete at 30 minutes

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in simulated gastric fluid (without enzyme). The Division of Biopharmaceutics recommends a specification of Q not less than ⎯ at 30 minutes in 0.1 N HCL medium.

IX. ASSAY:

Assay validation for zinc acetate is not provided. The sponsor plans to submit the data as part of the future study report.

COMMENTS:

1. The sponsor submitted the clinical studies in Holland by Hoogenraad as the second study to support the efficacy claim. In this study, zinc sulfate was used instead of zinc acetate. The Agency requested the firm to submit the bioequivalence data comparing zinc sulfate and zinc acetate for the NDA. Consequently, the firm provided a literature report (1977) comparing the zinc plasma levels at 3 hour point after single oral doses of 3 different zinc salts as 25 mg of elemental zinc. The formulation information and the raw data were not provided. Although fasting zinc plasma levels at 3 hours after zinc acetate and zinc sulfate were shown to be similar, an adequate bioequivalency data haven't been provided to link the clinically tested zinc sulfate formulations and Zinc Acetate Capsules to be marketed.

2. It was suggested by Dr. Gallo-Torres (Medical Officer, HFD-180) that since Zinc Acetate Capsules are not intended to be administered to patients concomitantly with other chelating agents (e.g., penicillamine, trientine, ammonium tetrathiomolybdate), new drug interaction studies might not be necessary.

3. The Division of Biopharmaceutics recommends a dissolution specification of not less than ⎯ at 30 minutes. The dissolution condition is as follows:

4. Labeling comments will be made after the study report (protocol submission date: 03/06/95) is submitted to the Division of Biopharmaceutics.

DEFICIENCIES:

1. No definitive pharmacokinetic information regarding Zinc Acetate Capsules has been provided in this NDA. No assay validation for Zinc Acetate in biological fluids has been provided.

2. The sponsor has proposed to conduct an additional pharmacokinetic study. The sponsor submitted a protocol on 03/06/95 to the Agency. However, the study report has not been

submitted.

Hae-Ryun Choi 06/26/95
Hae-Ryun Choi, Ph.D.
Pharmacokinetic Review Branch II

FT initialed by Mei-Ling Chen, Ph.D. _Mei-Ling Chen_ 6/23/95

Biopharm Day on 06/19/95 (Drs. Ludden, M. Chen, Fleischer, Gillespie, Hussain, and Choi).

cc: NDA 20-458, HFD-180, HFD-427 (M.Chen, Choi), HFD-426 (Fleischer), HFD-340 (Viswanathan), HFD-420 (Chron, Drug, Reviewer), HFD-19 (FOI).

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MEMORANDUM ◆r   DEPARTMENT OF HEALTH & HUMAN SERVICES
                                          PUBLIC HEALTH SERViCE
                                    FOOD AND DRUG ADMINISTRATION
                                CENTER FOR DRUG EVALUATION AND RESEARCH DRUG

DATE: April 6, 1995

FROM: Hae-Ryun Choi, Ph.D., Reviewer
           Division of Biopharmaceutics, HFD-427

TO: NDA 20-458, Zinc Acetate Capsules

THROUGH: Mei-Ling Chen, Ph.D., Branch Chief   4/5/95
           Division of Biopharmaceutics, HFD-427

SUBJECT: Protocol for Zinc Acetate Capsules
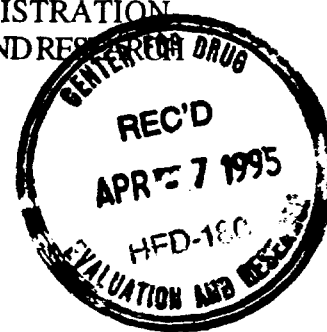
BACKGROUND:

NDA 20-458 for Zinc Acetate Capsules was submitted (dated 06/21/94) by Lemmon Company
for the following indications: 1) the maintenance treatment of patients with Wilson's disease who
have been initially treated with the chelating agents penicillamine, trientine, or ammonium
tetrathiomolybdate; 2) the initial therapy for asymptomatic affected siblings of Wilson's disease
patients; and 3) the treatment of Wilson's disease patients during pregnancy. Zinc Acetate has
been designated as Orphan Drug for these indications.

The Human Pharmacokinetics and Bioavailability Section of the NDA contains limited information.
The firm submitted only two studies. In the first study, zinc plasma levels were collected yearly
for 9.5 years. The second study is a literature report (1977) of a bioequivalency study which
compared zinc acetate and zinc sulfate using zinc plasma levels.

On March 6, 1995, in response to the Agency's letter (dated 08/23/94) requesting biopharmaceutic
information, the firm submitted a protocol entitled "A bioavailability and dose proportionality
study of zinc acetate (25 and 50 mg capsules) in healthy volunteers".

The following is the biopharmaceutic review of the protocol:

PROTOCOL SUMMARY:

Title: A bioavailability and dose proportionality study of zinc acetate (25 mg and 50 mg capsules)
in healthy volunteers.

Objectives: 1) To evaluate the absorption, disposition and clearance as well as dose proportionality
of zinc acetate from a 25 and 50 mg oral capsule following a single dose and

2) to determine the relative bioavailability of multiple versus single doses of zinc acetate.

<u>Investigator and Study Site:</u>

<u>Formulations:</u> 25 mg and 50 mg Zinc Acetate Capsules by Lemmon Company.

<u>Study Design:</u> Fasting, stepped single dose followed by multiple doses.

<u>Subjects:</u> 16 healthy adult male volunteers. Dismissals and dropouts will not be replaced over the course of the study.

<u>Drug Administration:</u> Subjects will be admitted to the study unit on Day -1 for a controlled evening meal. Standardized meals will be given at specified times throughout confinement with each evening meal including lean beef. <u>Step I</u> of the study will involve Days -1 to 3 and will commence with establishing a zinc baseline diet on Days -1, 1, and 2. Study Day 2 will involve sampling baseline serum zinc concentrations. On study Day 3, each subject will be administered a single 25 mg oral zinc acetate capsule followed by a serum profile for zinc. <u>Step II</u> will involve a washout on Day 4 with dosing on Day 5. On Day 5, a single 50 mg oral zinc acetate capsule will be administered followed by a serum zinc profile. <u>Step III</u> will involve multiple daily dosing with 50 mg zinc acetate capsules on Days 6, 7, and 8 with a serum profile on Day 8. Days 6 and 7 will involve three separate 50 mg zinc acetate capsule doses each day and on Day 8 a single dose will be provided in the morning followed by the serum profile.

All subjects will fast from 8 hours prior to doses 1, 2, 3, 6, and 9 and at least 2 hours prior to doses 4, 5, 7 and 8. No food will be allowed within 2 hours prior to or after each dose. During each blood profile, no food will be allowed for 2 hours prior or 4 hours after the dose.

<u>Specimens:</u> Blood sampling to determine zinc concentration will total 40 samples over the course of the study. Blood samples at -24, -18, -12 hours and immediately prior to dose 1 (Day 3) will be collected to determine the diurnal variation of zinc concentrations. Blood samples at 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, and 12 hours after dose 1 (Day 3). Blood samples will be collected immediately prior to dose 2 (Day 5) and after dosing at 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, and 12 hours. Multiple dosing will commence on Day 6 (dose 3) and continue to dose 9. Morning blood sampling will occur immediately prior to dose 3, dose 6, and dose 9. Blood samples will be collected immediately prior to dose 9 (Day 8) and after dosing at 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, and 8 hours.

<u>Analytical Methodology:</u> The analytical method, assay validation and analytical report will be submitted in the final report. In addition to the full validation of the assay for zinc acetate in serum, the analytical report will include a statement regarding the stability of the frozen samples, limit of quantitation, recovery, and a summary of the standard curves.

<u>Pharmacokinetic and Statistical analysis:</u> The statistical analysis will describe and compare the 25 and 50 mg products and determine dose proportionality. In addition, the analysis will evaluate and compare the 50 mg single and multiple dose data and describe the steady state data.

The descriptive analysis will include the serum concentration-time data for zinc; [SINGLE DOSE DATA] AUC(0-t), AUC(0-infinity), Cmax, Tmax, elimination rate, and half-life; [MULTIPLE DOSE DATA] AUC(0-8), Cmax and Tmax, etc. The data will be adjusted for baseline zinc values as needed.

<u>Sample Shipment:</u> Serum samples for zinc acetate analysis are to be shipped frozen to

GENERAL COMMENT (Need not be sent to the firm):

1. The firm plans to submit the drug interaction information obtained from the literature. This reviewer discussed with Dr. Gallo-Torres (Medical Officer, HFD-180). It was suggested that since Zinc Acetate Capsules are not intended to be administered to patients concomitantly with other chelating agents (e.g., penicillamine, trientine, ammonium tetrathiomolybdate), new drug interaction studies might not be necessary.

SPECIFIC COMMENTS (To be sent to the firm):

1. The sponsor has proposed to collect serum samples up to 12 hours post-dose. It is assumed that the length of the proposed sampling time represents at least three half-lives of zinc acetate. If there is an uncertainty about the length of half-life, it is recommended that a pilot study be conducted prior to the proposed study in order to obtain this information. In addition, a sufficient washout period should be allowed between 25 mg single dose and 50 mg single dose.

2. The sample collection schedule should also be amended to include a 30-minute time point post-dose.

3. Sixteen subjects might be enough for the proposed study, however, it is recommended that both male and female subjects (preferably equal numbers) be included in the study.

3

RECOMMENDATION:

The Division of Biopharmaceutics has reviewed the protocol submitted on 03/06/95. Specific Comments #1-#3 should be forwarded to the firm.

Hae-Ryun Choi, Ph.D.
Division of Biopharmaceutics

cc: NDA 20-458, HFD-180, HFD-427 (M.Chen, Choi), HFD-426 (Fleischer), HFD- 340 (Viswanathan), HFD-420 (Chron, Drug, Reviewer)

NDA # 20,458   Review # 1

Sponsor & Address: Lemmon Company
                        Kulpsville, PA 19443   JUN - 6 1995

Reviewer: Shannon P. Williams, Ph.D.
               Pharmacologist

Date of Submission: Original Submission: June 21, 1994;
                         Amendment of September 30, 1994

Date of HFD-180 Receipt: Original Submission: June 24, 1994
                              Amendment of 9/30/94 received 10/4/94

Date of Review: April 10, 1995

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original Summary

Drug: Zinc Acetate USP, Capsules

Molecular Weight: 219.5

Chemical Name: Acetic Acid, Zinc Salt, Dihydrate

Chemical Structure: $(CH3COO-)_2Zn \cdot H_2O$

Molecular Weight: 219.50

Drug Formulation: Zinc Acetate will be available in 25 and 50 mg
gelatin capsules. The 25 mg capsule will contain: Zinc Acetate
USP, 83.92 mg [Equivalent to 25 mg Elemental Zinc]; Corn Starch
NF,   and Magnesium Stearate NF   The 50 mg/kg
capsule will contain Zinc Acetate USP, 167.84 mg [Equivalent to
50 mg Zinc]; Corn Starch NF,   and Magnesium Stearate
NF,   Capsule shells contain FD&C RED #40, D&C Red # 28 and
D&C Yellow # 10.

Drug Category: Copper antagonist

Proposed Marketing Indication: Zinc acetate is indicated for
1) the maintenance treatment of patients with Wilson's disease
(hepatolinticular degeneration) who have been initially treated
with a chelating agent-penicillamine, trientine or ammonium,
tetra-thiomolybdate

Related INDs/NDAs: IND

PRECLINICAL STUDIES AND TESTING LABORATORIES:
PHARMACOLOGY:

Absorption, Distribution, and Excretion:
1.   **Absorption**
     Zinc Chloride in Rats, Mice, Dogs, and Humans
     Effects of Dietary Factors on Zinc Availability in Rats and Chicks.

2.   **Distribution**
     Zinc Acetate, Oxide, Citrate or Maleate in Rats
     $Zn^{65}Cl_2$ in Rats .
     Zinc Oxide in Dogs and Cats

3.   **Excretion**
     Zinc Acetate, Oxide, Citrate or Maleate in Rats
     $Zn^{65}Cl_2$ in Rats
     Zinc Oxide in Dogs and Cats

TOXICOLOGY:

I.   **Toxicology studies specific for Zinc Acetate:**

     Acute Toxicology:

        Rat: Acute Oral Toxicity of Zinc Acetate In Rats.

     Subacute/Subchronic/Chronic Toxicology:

        Rat: 35- to 53-Week Oral Toxicity Study

     Reproductive Toxicology:

        Rat: Effects of Oral Zinc (Oxide, **Acetate**, Maleate, and
                Citrate) on Fertility, Reproduction and Growth

     Special Toxicology Studies:

        Rat: 2-Month Oral Pancreatic Toxicity Study

II. **Toxicology Studies Specific for Zinc Oxide or Other Zinc Salts:**

     Subacute/Subchronic/Chronic Toxicology:

        Rat: 1) 5- to 6-Week Oral Toxicity Study with Zinc
                     Carbonate
                 2) 6-Week Oral Toxicity Study with Zinc Oxide

3) 14-Week Oral Toxicity Study with Zinc Sulphate

4) 6- to 15-Month Oral Toxicity Study with Zinc Chloride

5) 21-Month Oral Toxicity Study with Zinc Sulphate

6) Three Generation Oral toxicology of Zinc Oxide and Various Zinc Salts (Chloride, Carbonate and Sulphate)

Mice: One Year Oral Toxicity Study with Zinc Sulphate

Dog: 1) 3- to 19-Week Oral Toxicity Study with Zinc Oxide

2) 70-Week Oral Toxicity Study with Zinc Sulphate

Cat: 10- to 53-Week Oral Toxicity Study with Zinc Oxide

Swine: 1) 6-Week Oral Toxicity of Zinc Carbonate in Weanling Pigs

2) 27-Week Oral Toxicity Study with Zinc Sulphate in Swine.(**Note** part of 4-Week Oral Toxicity Study with Zinc Sulphate in Rats)

Ovine: 6- to 10-Week Oral Toxicity of Zinc Oxide in Lambs

Chicken: 4- to 10-Week Oral Toxicity of Zinc Oxide, Sulphate or Carbonate

Reproductive Toxicology:

Rat: 1) Effects of Dietary Zinc Carbonate on Growth, Reproduction and Blood Changes.

2) Oral Segment II Teratology Study with Zinc Sulphate[1]

Rabbit: Oral Segment II Teratology Study with Zinc Sulphate[2].

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[1]Studies were conducted by

[2]Studies were conducted by

Mice: 1)   Oral Segment II Teratology Study with Zinc Sulphate[1]

       2)   Effects of Excess Dietary Zinc Carbonate on Fetal Development and Growth

Hamsters: Oral Segment II Teratology Study with Zinc Sulphate[1].

Ovine: 1)   Toxicity of Zinc (form not indicated) in Pregnant Sheep

       2)   Effects of Zinc Sulphate on Pregnant Sheep and Fetal Development.

Mutagenicity:

1.   Mutagenicity of Zinc Oxide in the Ames Assay[3].

2.   Mutagenicity of Zinc Stearate in the Ames Assay[3]

3.   In Vivo Host Mediated Assay in Mice and Direct In Vitro Mutagenicity Assay With Zinc Sulphate

4.   Rat Bone Marrow Chromosomal Aberration and In Vitro Chromosomal Aberration Assay in Human Embryonic Lung Cultures with Zinc Sulphate

5.   Dominate Lethal Assay with Zinc Sulphate in Rats

Special Toxicity Studies:

1.   Induction of Pantothenic Acid Deficiency by Chronic Administration of Zinc Chloride in Rats

2.   The formation of DNA-Zinc Products following Oral Administration of Zinc in Rats.

Carcinogenicity:

Mice: One Year Oral Carcinogenicity Study with Zinc (Oleate and Sulphate)

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[3]Studies were conducted by

PHARMACOLOGY

Zinc Acetate is a soluble zinc salt which provides elemental Zinc for absorption following oral administration. Once absorbed in the intestinal cell, zinc induces the production of metallothionein (MT), a protein which binds copper and prevents its serosal transfer into the blood. The bound copper is then lost in the stool when the intestinal cell is sloughed. As such, zinc prevents the absorption of copper from the diet and the reabsorption of endogenously secreted copper such as that from the saliva and gastrointestinal juices. Thus, orally administered Zinc acetate has potential therapeutic use in the treatment of patients with Wilson's Disease, an autosomal recessive disease characterized by increased copper absorption and a deficiency of the α_2-Globulin fraction of the plasma proteins to which circulating copper is normally tightly bound.

I. Primary Pharmacology:

Effects on Copper Concentrations and Intestinal Metallothionein Levels:

In male weanling Long Evans rats, administration of dietary zinc oxide at doses of 0.6% of the diet for 3 weeks ($\sim$ 1.8 g/kg), 0.5% for 4 weeks ($\sim$ 1.5 g/kg), and 0.4 % for 8 weeks ($\sim$ 1.2 to 0.24 g/kg), produced increased tissue zinc levels in liver (2 to 5.9 fold) and kidney (2.7 fold) relative to control levels (See table 1). Reductions in body weight gains (21 to 53.6%) were observed in all zinc-treated groups, whereas reductions in tissue copper levels, in liver (35-60%) and plasma (73%), were only observed after 8 weeks of treatment at the 0.4% dose (See Table 1, on the following page).

Table 1.   **Effects of Excessive Dietary Zinc on Weight gains, and Zinc and
Copper Levels in Liver, Kidney, and/or Plasma**

Group (weeks)	Wt. gain (g)	Zinc Content (µg/g dry Wt.)		Copper Content (µg/g dry Wt.)		
		Liver	Kidney	Liver	Kidney	Plasma
Control 0.6% Zinc Oxide (3)	69±6 32±7	--- ---	--- ---	7.5±2 8.0±2	24±10 25±10	--- ---
Control 0.5% Zinc Oxide (4)	87±13 59±10	291±39 573±142	295±83 801±19	9±2 9±2	17±4 14±3	--- ---
Control[1] 0.4% Zinc Oxide(8)	165±40 129±35	107±20 627±172	--- ---	10±3 4±1	--- ---	81.0 21.9
Control[2] 0.4% Zinc Oxide (8)	165±34 129±31	98±9 530±199	--- ---	8±2 5±1	--- ---	--- ---

1 n=10 rats/group
2 n=10 rats/group
--- No data available for these values

A second study showed that treatment of male and female (Wistar
and Sprague Dawley) rats with dietary zinc (oxide, carbonate or
chloride) at levels of 0.75 and 1.0% (~0.45 g/kg and 0.6 g/kg)
for periods of 5 weeks each produced the following: 1) reductions
in body weight gains (17 to 48%, relative to control gains of 145
± 8 g); 2) large increases in liver zinc content (10 to 14 fold
increases, relative to control values of 37.21 ± 3.26 µg/g dry
wt.); and 3) reduced liver copper concentrations (59 to 74%,
relative to control levels of 15.69 ± 0.57 µg/g dry wt.)

In another study, in weanling rats, repeated (5-weeks) oral
administration of zinc sulphate at a high dose of 120 mg zinc/kg
diet (~36 mg/kg/day) produced increased serum zinc concentrations
(165 µg/dl, relative to values of 134 and 150 µg/dl in rats
treated with low, 7.5 mg, ~2.25 mg/kg/day and recommended, 30 mg,
~9.0 mg/kg/day, doses of zinc). In addition, the high dose zinc
animals had reduced serum copper levels (approximately 42%,
relative copper levels of ~97 µg/dl at the low and recommended
doses (Fisher, PWF, et al., J. Nutr. 1983; 113:462-469).

In a final study, administration of excess dietary zinc oxide
(0.4%; ~245-308 mg/kg) for a period of 22 days in nulliparous
female rats resulted in: 1) reduced fetal body weights (-20%,
relative to control weights of 546.3 ± 13.6 mg); 2) increased

maternal (M) and fetal (F) tissue concentrations of zinc in fetal
body (F, from basal levels of 41.6 ppm up to 48.6 ppm); liver (M,
from basal levels of 62.5 ppm up to 431.6 ppm and for F, from
54.5 ppm up to 277.1 ppm), kidney (M, from basal levels of 61.2
ppm up to 146.7 ppm) and brain (M, from basal levels of 34.1 ppm
up to 39.8 ppm); and 3) reduced maternal and fetal tissue copper
levels for whole body (F, from basal levels of 10.3 ppm down to
3.4 ppm) and liver (M, from basal levels of 9.2 ppm down to 5.5
ppm and for F, from basal levels of 11.0 ppm down to 2.3 ppm).

In regard to Zinc's proposed mechanism of action (i.e. the
induction of metallothionein synthesis), studies in rats showed
that oral administration of a single dose of zinc sulphate
(0.5 mg; 2.86-3.33 mg/kg body weight or <0.004 mg; <0.0229-
0.0267 mg/kg body weight [controls]) increased serum zinc
concentrations (up to 320 μg/dl at 3 hours after feeding versus
50 μg/dl in control rats) and increased metallothionein synthesis
(from basal values of approximately 60 cpm/mg cytosol protein to
maximal levels of 220 cpm/mg cytosol protein at 9 hours after
dosing; [measured via incorporation of ^{35}S-cystine into
metallothionein]). Increased translatable poly (A)[+]
metallothionein mRNA, relative to total mRNA (5-fold increase,
compared to control rats)[4] was also observed. The increased
mucosal metallothioein levels were associated with increased
mucosal zinc concentrations (from basal levels of 87 ug/g to
maximal levels of 150 ug/g at 6 hours post dosing) due to
increased zinc binding to newly synthesized mucosal
metallothionein (from basal levels of 75 ng zinc/mg cytosol
protein to 170 ng/mg by 9 hours after dosing). Finally, pre-
exposure to zinc sulphate (0.5 mg; ~2.86-3.33 mg/kg) reduced the
absorption of a subsequently administered oral dose of zinc (50%,
relative to that absorbed in control rats which were initially
fed <0.004 mg zinc).

The kinetics of serosal [67]Cu transfer and the distribution of
copper between metallothionein (MT) and a high molecular weight
protein fraction (HMWPF), within mucosal cells were studied in
segments of duodenum from weanling rats treated with low
(7.5 mg/kg diet, ~2.25 mg/kg/day), recommended (30 mg/kg diet,
~9.0 mg/kg/day), and high doses of zinc sulphate (120 mg zinc/kg
diet, ~36 mg/kg/day) for a period of 5 weeks. Administration of
zinc sulphate at the low, recommended, or high doses had no
effect on either the V_{max} or K_m for serosal transfer of copper,
but did effect differences in the in vitro binding of copper to
MT and HMWPF (See Figure 1, on the following page).

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[4] Metallothionein mRNA was measured indirectly by measuring
metallothionein synthesis in a wheat-germ cell free in vitro
translation system using 6.4 μg mRNA.

Figure 1. <u>The binding of ^{67}Cu to the high-molecular-weight</u>
<u>fraction and to metallothionein following a 45 min</u>
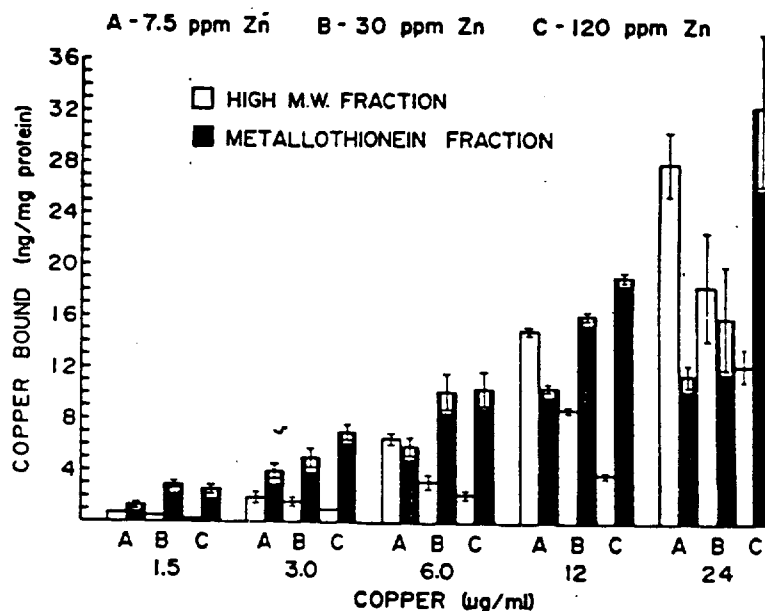<u>incubation period</u>. (Taken from Fisher et al., 1983)

Figure 1 shows that, all segments (regardless of zinc status)
incubated in a low copper media had a the majority of copper
bound to MT.   However, in a high copper media; rats fed the high
dose of zinc sulphate had the majority of the bound copper
associated with MT, whereas rats fed the low zinc diet had the
majority of the bound copper associated with the HMWPF.   Such
increased binding of copper to metallothionein relative to HMWPF
induced by zinc sulphate would reduce the absorption of copper,
since copper bound to metallothionein, unlike that bound to
HMWPF, is not available for serosal transfer.

In another study in rats, the site(s) at which zinc interferes
with intestinal absorption of ^{64}Cu was investigated.   This latter
study showed intraduodenally and intraperitoneally administered
zinc nitrate (1 mg Zinc in 0.4 ml i.e. ~5 to 2.8 mg/kg for rats
weighing 200 to 350 g) produced comparable increases in zinc
levels in plasma and liver (9.6 μg/ml and 146 μg/g dry wt. versus
8.4 μg/ml and 161 μg/g, respectively).   Concurrent intraduodenal
administration of zinc was shown to reduced the absorption of ^{64}Cu
(-50%, relative to control values of 22.6% [expressed as a % ^{64}Cu
recovered in sampled tissues]) and the % disappearance of ^{64}Cu
from the ligated segment (-38%, relative to control values of
50.6%).   In contrast, IP administration of zinc, either
concurrently or at 2 and 18 hours prior to ^{64}Cu administration had

no effect on the % ^{64}Cu recovered or on the % disappearance of ^{64}Cu from the intestinal segment. These data suggest that the zinc-induced depression of copper absorption was mediated either in, or on the intestine.

Studies in rats have also shown effects of zinc on the utilization and excretion of copper. In one study, administration of dietary zinc (oxide, carbonate or chloride at levels of 0.75 and 1.0%; ~0.45 g/kg and 0.6 g/kg, for a period of 5 weeks) had no significant effects on the percent of recovered radioactivity in the gastrointestinal tracts and feces of the control and zinc-treated rats (98% and 96%, respectively) following a single p.o. dose (100 μCi) of ^{64}Cu. However, zinc treatment did result in significant reductions in the % of radioactivity distributed to liver (37.8 ± 7.2% versus 57.5 ± 1.6% in zinc treated versus controls, respectively) and increased the percent found in the urine (45.5 ± 7.2% versus 21.7 ± 0.7% in controls). Thus, the latter results suggest that zinc treatment affects copper metabolism by reduced utilization and increased urinary excretion of the metal.

In conclusion, studies in rats showed that repeated administration of various zinc oxide or other zinc salts results in reduced serum and/or tissue copper levels. Possible mechanisms for this effect included: 1) induction of intestinal metallothionein synthesis which in turn binds copper and prevents it's absorption and 2) decreased utilization and increased urinary copper excretion.

II.   Secondary Pharmacology:

A.   Effects on Iron and Iron Storage Proteins:

A study in male weanling Long Evans rats showed that administration of zinc oxide at doses ranging from 240 mg/kg for 8 weeks to 1800 mg/kg for 3 weeks resulted in reduced tissue iron levels (52 to 78% in liver and 31% in kidney; see Table 2, on the following page).

Table 2. **Effects Zinc on Iron Levels following Dietary Administration of at levels of 0.6% for 3 Weeks or at 0.4% for 8 Weeks.**

Treatment Regimen Group (Duration)	Dose (mg/kg)	Iron (μg/g dry Wt)	
		Liver	Kidney
Control[1] (3 weeks) 0.6% Zinc Oxide	~ 1.8 g/kg	378±74 179±52	230±55 159±45
Control[2] (4 weeks) 0.5% Zinc Oxide	~ 1.5 g/kg	362±79 227±60	105±10 73±12
Control[2] (8 weeks) 0.4% Zinc Oxide	~ 1.2 to 0.24 g/kg	520±186 209±40	--- ---
Control[2] (8 weeks) 0.4% Zinc Oxide	~ 1.2 to 0.24 g/kg	564±230 124±30	--- ---

1 n=12 rats/group
2 n=10 rats/group

In a second study, administration of zinc (oxide, carbonate or chloride) to male and female Wistar and Sprague Dawley rats at doses of ~450 mg/kg and 600 mg/kg for periods of 5 weeks each also resulted in reduced liver iron levels (25% and 62%, relative to control liver iron levels of 425.72 ± 43.91 μg/g dry wt.). This latter study also indicated that the effects of zinc on iron levels were independent of its effects on copper levels, since copper supplementation reversed the zinc induced reductions in copper (see primary pharmacology section), but failed to increase the concentration of liver iron.

In yet another study in male rats, administration of high levels of dietary zinc oxide (0.75%, ~839 mg/kg) for a period of 5 weeks produced blood zinc levels which were 2.6 fold greater than control values of 60 μg/ml and reduced blood iron levels (-44% relative to average control values of 1479.2 μg/ml). Adjunct experiments which investigated the effects of zinc treatment on the pattern of iron excretion also showed that zinc treatment results in increased fecal excretion of injected ^{59}Fe, beginning around 10 to 12 days after the injection.(Figure 2, on the following page).

Figure 2 <u>**Excretion of 59Fe in control and High Zinc Fed Rats**</u>
<u>**(each point represents the mean of 3 rats)**</u>. (reproduced
from Sponsor's Figure 4, Amendment dated 9/30/94,
Vol. 1.3 pp 077).
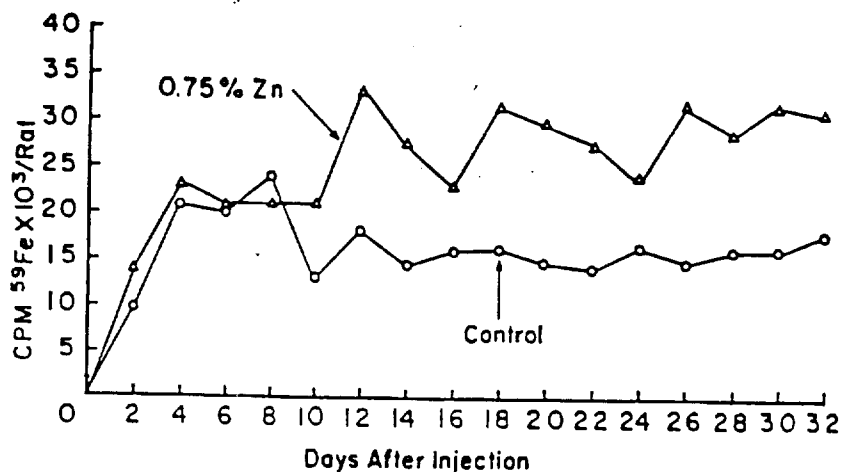
The 10 to 12 day delay period corresponded to a shortened life
span for red blood cells (approximately 1/4 to 1/5 of the life
span in untreated controls) in the zinc-treated rats. Thus, the
current study, suggests that the reductions in blood iron
concentrations result from increased fecal excretion of iron due
to shorten life span and increased turnover of red blood cells.

In still another study in rats, administration of zinc oxide
(0.4%; ~240 mg/kg) for a period of 8 weeks produced reduced total
liver iron by 74%, relative to basal control levels of 178.9 ±
29.4 μg/g of wet weight of tissue. In addition, iron bound to
ferritin, hemosiderin, and hemoglobin were reduced by 80.7%,
66.6%, and 32.8%, relative to basal control values of 126.7 ±
21.4, 38.9 ± 11.4, and 13.4 ± 4.0 μg/g of wet weight of tissue,
respectively. The study further showed that the loss of ferritin
contributed greatest (77.2%) to the total amount of iron loss
compared to that contributed by hemosiderin (19.6%) and
hemoglobin (3.3%) reductions.

In contrast to the aforementioned reports, a study conducted in
nulliparous female rats showed no effects on maternal tissue iron
levels following treatment with zinc oxide (245-308 mg/kg for a
period of 22 days during gestation). However, analysis of fetal
iron levels in this same study showed reductions in whole body
iron concentrations (from 161.8 ppm down to 39.4 ppm), but no
effects on fetal liver iron.

Thus, the majority of the available studies suggest that excessive dietary zinc (at levels shown to affect copper metabolism) can also reduce blood and tissue iron levels via increased fecal excretion of iron due to shorten life span and increased turnover of red blood cells and through the loss of ferritin, a more labile iron binding protein compared to hemosiderin.

In Vitro Effects on Ion Transport: In a series of in vitro studies using isolated bovine heart mitochondria, Zinc (150 μM) produced an increased (~2 fold, relative to control values of 75 to 108 μmoles Mg^{2+}/mg mitochondria) in the energy linked accumulation of mitochondrial Mg++ in the absence of associated increased ATPase activity. In other in vitro studies, zinc acetate (67 to 100 μM) also stimulated the mitochondrial transport of K+ (see Figure 2A below). The effects of zinc on the transport of Mg++ and K+ was purportedly due to direct effects on the permeability of the membrane to both ions.

Figure 2A. Effects of varying concentration of zinc on the beef heart mitochondrial K^+ accumulation (measured as K^+-dependent changes in mitochondrial swelling; monitored at 540 mμ mal-cm cuvett; reproduced from Sponsor's Figure 2, Amendment dated 9/30/94, Vol. 1.3 pp 121).
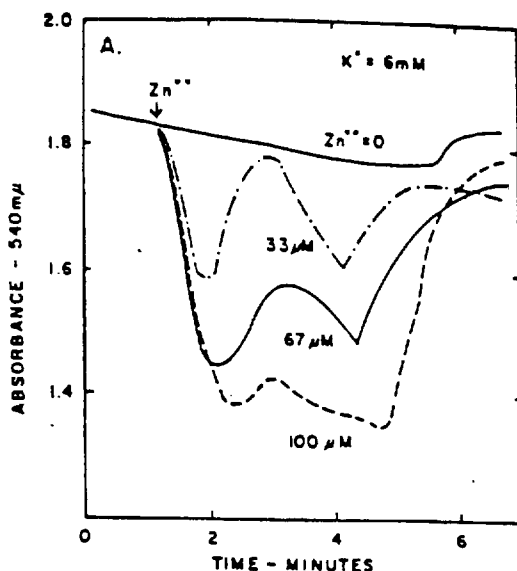
Ex Vivo Effects of Zinc on Mineral Levels: In addition to zinc's effects on copper and iron levels. Other reports also showed that treatment with zinc affected a number of other minerals. A tabulated summary of the reported effects of zinc on minerals/elements other that copper and iron is presented in Table 3 (on the following page).

Table 3. **Tabulated Summary of the Reported Effects of Dietary Zinc on Tissue Mineral Content.**

Mineral	Species (Tissue)	Zinc Form	Dose (mg/kg)	Duration	Basal Levels	(+)increase (-)decrease
Calcium	Rat (femur)	ZnO	778 and 1638	15 days	176.2(1)	(-)4.8 and 29%
Phosphorus	Rat (femur)	ZnO	778 and 1638	15 days	104.1(1)	(-)1.5 and 4.1%
Calcium	Rat (femur)	$ZnCO_2$	707-2123	4 weeks	146 ± 8(1)	(-)1.7 to 26.6%
Phosphorus	Rat (femur)	$ZnCO_2$	707-2123	4 weeks	71.6 ± 2.4(1)	(-)2.9 to 14.4%
Maternal Calcium	Rat† (heart)	ZnO	245-308	22 days	0.0(2)	9.9 ± 1.4(2)
Calcium	Rat† (brain)	ZnO	245-308	22 days	57.5 ± 3.2(2)	(+)51%
Calcium	Rat† (kidney)	ZnO	245-308	22 days	87.1 ± 6.3(2)	(-)36%
Magnesium	Rat† (spleen)	ZnO	245-308	22 days	3535.4 ± 251(2)	(-)58%
Magnesium	Rat† (kidney)	ZnO	245-308	22 days	1861.5 ± 273 (2)	(-)5.8%
Fetal Calcium	Rat‡ (liver)	ZnO	245-308	22 days	5.1 ± 1.2 (2)	(+)2.55 fold
Calcium	Rat‡ (body)	ZnO	245-308	22 days	6832.6 ± 217.1 (2)	(-)7.1%
Magnesium	Rat‡ (liver)	ZnO	245-308	22 days	632.0 ± 96.4 (2)	(+)79%
Magnesium	Rat‡ (body)	ZnO	245-308	22 days	1594.3 ± 42.3 (2)	(+)22%

†Nulliparous females Day 22 of gestation.
‡Fetal tissue obtained at day 22 from Zinc treated females
(1) mg/g dry weight
(2) parts per million

Table 3 shows that in rats, oral treatment with ZnO (778 and 1638 mg/kg for 15 days) or with zinc carbonate (~707 to 2123 mg/kg for 4 weeks) reduced bone (femur) calcium and phosphorus contents.

In the 15 day study, reductions in calcium and phosphorus were associated with reduced body weight gains (16 and 69%, relative to gains of 42 g in controls); reduced femur dry weights (10 and 23%, relative to control values of 0.3875 ± 0.009 g/femur); and reduced total ash (22 and 50%) and percent ash content (7 and 26%), relative to control values of 0.1990 ± 0.0065 g ash/femur and 51.4 ± 0.94% of femur dry weight, respectively.

In the 4-week study, depicted in Table 3, reductions in calcium and phosphorus were accompanied by increased femur zinc content (6 to 26 fold, relative to control levels of 0.09 ± 0.01 mg/g dry weight) and suppressed weight gains (14.4 and 39.4%, relative to control gains of 188 ± 8 g). However, no effect on the deposition of magnesium in bone was detected. Separate experiments in the 4-week study also showed that dietary supplementation with a combination of either 0.8% calcium and phosphorus or 1.2% calcium and phosphorus reversed the increased deposition of zinc in bone, the zinc-induced suppression of body weight gains, and the zinc-induced reductions in bone calcium and phosphorus levels.

Finally, Table 3 shows that administration of zinc oxide (245-308 mg/kg) to nulliparous female rats during days 1-22 of gestation affected both calcium and magnesium concentrations as follows: 1) In the fetus: calcium concentrations were increased in liver (2.54 fold), but decreased in the whole body (-7%), whereas magnesium concentrations were increased in the liver and body (78.5% and 22%, respectively); and 2) In the maternal tissues: calcium concentrations were increased in heart and brain (from 0.0 ppm up to 9.9 ppm and ~51%, respectively), but reduced in kidneys (-36%), whereas magnesium concentrations were lower in spleen and kidney (-58% and -6%, respectively).

Other studies in rats have which examined the effects of zinc (administered as the oxide at 0.5 and 1.0% of the diet; ~300 and 600 mg/kg) for 15 days on the metabolism of nitrogen (N), phosphorus (P) and sulphur (S), indicated that administration of zinc reduced the retention of N (17.6 and 32.3%), P (54.5 and 89.6%), and S (62.08 and 127.7%), relative to values of 2646.63 mg of N; 526.44 mg of P and -53.15 mg of S which were retained in control animals over the 15 day period. Further analysis of the relative loss of each element via urine or feces indicated that for nitrogen, the said reductions were associated with increased fecal (22 and 26% from control of 892.76 mg) and urinary (58% at the high dose, from control of 338.71 mg) excretion of N. In contrast, increased fecal excretion of P (3.32 and 3.98 X control values of 143.79 mg) and S (1.48 fold and 1.79 fold greater than control values of 86.90 mg) were primarily responsible for the reduced retention of these elements, since urinary excretion of P and S were both reduced (~ 89% and 30%, relative to control values of 41.37 and 23.94 mg).

In general, the aforementioned studies suggest that outside of its effects on copper and iron metabolism, administration of dietary zinc (oxide and carbonate) at doses $\geq$ ~300 mg/kg can also produced mild to moderate effects on the metabolism of other elements such as inhibition the deposition of calcium and phosphorus in bone and the assimilation of nitrogen, phosphorus and sulphur.

Zinc-Induced Anemic Effects:

One of the more prominent preclinical effects, reported following
administration of excess dietary zinc is the development of a
microcytic hypochromic anemia. In this regard, studies in rats
showed that dietary administration of zinc oxide to male weanling
Long Evans rats, at doses ranging from 240 mg/kg for 8 weeks to
1800 mg/kg for 3 weeks produced reduced hemoglobin (32-52%) and
hematocrit (26-49%; See Table 4 below).

Table 4. **Effects Zinc on Hemoglobin and Hematocrit following
Dietary Administration of at levels of 0.6% for 3 Weeks
or at 0.4% for 8 Weeks**.

Group (Duration)	Dose (g/kg)	Hb gm %	Hct %
Control[1] (3 weeks) 0.6% Zinc Oxide	~ 1.8	12.7±6 8.7±0.5	33±6 24±1
Control[2] (8 weeks) 0.4% Zinc Oxide	~ 1.2 to 0.24	14.5±0.7 6.9±1.2	39±2 20±5
Control[2] (8 weeks) 0.4% Zinc Oxide	~ 1.2 to 0.24	14.9±1.2 9.1±2.0	38±3 28±6

1 n=12 rats/group
2 n=10 rats/group

The onset of the zinc-induced anemic effects in the latter study
was correlated with reductions in liver iron levels which were
evident in animals at the ~ 1.8 g/kg dose after 3 weeks, but not
with reductions in liver copper levels which occurred only after 8
weeks of zinc administration at the 0.4% dose level.

Other studies which reported reduced hemoglobin values following
administration of excess dietary zinc included: 1) a study in male
and female Wistar and Sprague Dawley rats in which hemoglobin
values were reduced (9 to 26%, relative to control values of 13.58
± 0.46 g/100 ml blood), following dietary administration of zinc,
as the oxide, carbonate or chloride at doses of ~450 mg/kg and
600 mg/kg for periods of 5 weeks each; 2) a study in male Wistar
rats in which hemoglobin was reduced by 36 and 59% (control values
not indicated), following administration of zinc carbonate at doses

of 300 and 420 mg/kg for a period of 4 to 5 weeks; 3) a study in
male and female Sprague Dawley rats in which suppressed weight
gains (32 and 46%, relative to control gains of 149 g) and reduced
hemoglobin concentrations (60 and 68%, relative to control
concentrations of 14.1 g/dl) were observed following dietary
administration of Zinc (form not indicated) at doses of 1.0 and
1.5% of the diet; (~600 and 900 mg/kg zinc, respectively) for a
period of 5 weeks (Note: The latter study also showed that
supplementation of the diet with copper (0.03%) partially reversed
zinc's inhibitory effects on growth and hemoglobin); and finally,
4) a study in nulliparous female rats in which slight reductions in
maternal serum hemoglobin (-17%, relative to control values of
144 ± 2.5 g%) were reported following treatment with zinc oxide at
levels of 245 to 308 mg/kg for a period of 22 days.

Morphological effects on red blood cells were also reported in
another study in male rats, following dietary administration of
high levels zinc oxide (0.75%, ~839 mg/kg) for a period of 5 weeks.
Observed changes in red blood cells included: 1) irregularly
shaped, microcytic and hypochromic red blood cells (RBC); 2)
increased RBC osmotic fragility (i.e. hemolysis occurred at 0.32%
NaCl versus 0.4% in controls) and 3) reduced RBC life span
(utilization of glycine-2 ^{14}C) to 1/3 of that seen in control
groups. However, the incorporation of ^{55}Ir into RBC was not
affected by zinc treatment.

Collectively, the aforementioned studies in rats show that repeated
administration of excess dietary zinc at doses comparable to those
which reduce copper levels (i.e. ≥ ~245 mg/kg), result in the
development of a hypochromic, microcytic anemia in rats.

Effects of Zinc on Enzyme Activity (In Vitro Studies):

In vitro studies using rat liver homogenates reported that zinc
(crystalline zinc insulin or zinc sulphate) at levels of 2 x 10-5 M
zinc ion inhibited succinoxidase (~38%, relative to control levels
of ~83.9 μl O$_2$ per 10 min). However, under conditions which favored
oxidative phosphorylation, zinc sulphate (2 x 10^{-5}M) had a triphasic
effect, first producing almost complete inhibition of oxidative
phosphorylation for 20 min, followed by recovery to a level greater
than that seen in controls and finally returning to a level below
that of control (See Figure 3, below). The latter fall in the rate
of oxidation was attributable to a zinc-induced inhibition of the
oxidation of Krebs Cycle intermediates, particularly,
α-ketoglutarate and citrate.

Figure 3. <u>Transitory inhibition of the oxidation of succinate by</u>
<u>zinc sulfate in an isotonic KCl liver homogenate system</u>.
(Reproduced from Sponsor's Figure 2, Amendment dated
9/30/94, Vol. 1.3 pp 030).
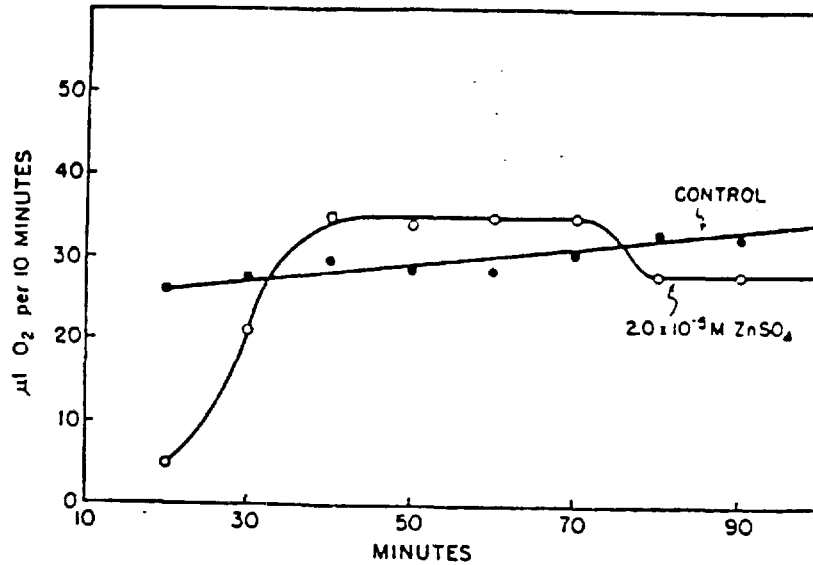
Comparison of zinc's inhibitory effects on succinic dehydrogenase
activity with the inhibitory effects of a large number of other
diverse compounds including: quinonoid structures, sulphydryl
reagents and compounds, copper, selenite and arsenite suggested
that the ability to react with sulfhydryl groups was common to all
agents studied. Thus, the inhibitory effects of zinc on succinic
dehydrogenase activity was attributed to an interaction with
sulfhydryl groups on the enzyme.

<u>In vitro</u> studies have also demonstrated effects of zinc on Myosin-
Linked ATPase Activity. In one study, high concentrations of zinc
chloride (1 mg/2.16 ml of reaction buffer;~ 3.4 x 10-3 M)
completely inhibited the myosin linked enzymatic hydrolysis of ATP.
However, other studies indicated that Zn^{++} (form not indicated) had
a biphasic effect, with, activation of the myosin ATPase at
concentrations up to 1.5×10^{-5} M, but inhibition at higher
concentrations. In the latter studies, the effects of Zn^{++} on the
myosin ATPase activity were also purported to occur via
interactions with SH groups of myosin.

Several reports were also available on the in vitro effects zinc on
the activity of alkaline phosphatase obtained from rabbits, rats
and humans. In one study, optimal enzyme activity of alkaline β-
glycerophosphatase derived from rabbit small intestine required a
zinc sulphate concentration of 0.3-1 m-equiv. zinc/l, whereas
concentrations beyond this level had strong inhibitory effects.

In other in vitro studies using rat bone, kidney, and intestinal preparations, zinc (administered as either the chloride, sulphate or nitrate salt, at concentrations of 4×10^{-6} M to 70×10^{-6} M) produced dose-dependent inhibition of alkaline phosphatase activity in bone and kidney preparations, but increased activity by 40 to 100% in preparations from intestine. No differences between the three zinc salts tested were reported. Following dialysis of the intestinal preparation, the stimulatory effects were reversed to inhibition. The dialyzable zinc co-activator in the intestinal preparation was shown to be a product of mucosal autolysis, with properties similar to those of α-amino acids such as glycine.

Finally, in vitro studies using purified alkaline phosphatase from humans showed that Zinc ions (form not indicated) inhibits phosphatase activity, when the concentration of zinc is greater than that of the substrate, (pyrophosphate). The inhibitory effects of zinc were suggested to be due to the formation of a Zinc complex with the substrate.

In other in vitro studies effects of zinc on the activity of phosphoglucomutase, plasmin, trypsin and thrombin have also been demonstrated.

At concentrations of 5×10^{-5} M and 1×10^{-4} M, zinc insulin was shown to inhibit the activity of phosphoglucomutase isolated from yeast by 53 and 91%, respectively (basal levels not provided). This study also showed that addition of the metal binding agents, cysteine (0.02 M) and 8-hydroxyquinoline (0.003 M) reversed the inhibitory effects of zinc (5×10^{-5} M).

In vitro studies on the effects of Zinc on human and/or bovine plasmin, trypsin and thrombin showed that at a concentration of 1×10^{-3} M, Zinc (form not indicated) inhibited human plasmin and bovine trypsin by 81% each and inhibited bovine plasmin and bovine thrombin by 59% and 29%, respectively.

Finally, zinc was reported to have strong inhibitory effects on prolidase, the intestinal peptidase which splits glycyl-1-proline and to a lesser extent glycyl-1-alanine, in in vitro preparations from hog duodenum. However, neither the extent of inhibition (other than "strongly") nor the form or concentration of zinc used were indicated.

Effects of Zinc on Enzyme Activity (Ex Vivo Studies):

A number of literature reports in which ex vivo effects of zinc on various enzymes were also submitted. A tabulated summary of the reported ex vivo effects of Zinc on the activity of various enzymes is provided in Tables 5A and 5B (succeeding pages).

Table 5A.   <u>Tabulated Summary of the Reported Effects of Dietary Zinc on the Ex Vivo Activity of Various Enzymes.</u>

Enzyme	Species	Zinc Form	Dose (mg/kg)	Duration	Basal Levels	%(+)increase %(-)decrease
Alkaline Phosphatase	Rat (liver)	$ZnCO_2$	300 and 420	4-5 wk	0.54-0.69 (1)	no effect and (+)2.6x
Alkaline Phosphatase	Rat (bone)	$ZnSO_4$	300	4 wk	31% (2)	(+)61%
Alkaline Phosphatase	Rat (Gut)	$ZnSO_4$	300	4 wk	21% (2)	(+)34%
Alkaline Phosphatase	Rat (Gut)	ZnO	1875 to 2500	15 days	0.62 ± 0.04†	(-)19%
Alkaline Phosphatase	Rat (liver)	ZnO	1875 to 2500	15 days	0.07 ± 0.005†	(+)68%
Alkaline Phosphatase	Rat (bone)	ZnO	1875 to 2500	15 days	0.10 ± 0.007†	(+)29%
Alkaline Phosphatase	Rat kidney	ZnO	1875 to 2500	15 days	0.25 ± 0.01†	(+)89%
Catalase	Rat (liver)	$ZnCO_2$	300 and 420	4-5 wk	5.65 ± 0.2(2)	(-)50%
Ferroxidase	Rat‡	ZnO	245-308	22 days	47± 2.5*	None

(1) μmol of phosphate liberated/mg protein
(2) % hydrolysis of glycerophosphate in 21 hours
*   International units
†   mg phosphate liberated
‡   Nulliparous females.

Table 5B.   Tabulated Summary of the Reported Effects of Dietary Zinc on the Ex
Vivo Activity of Various Enzymes.

Enzyme	Species	Zinc Form	Dose (mg/kg)	Duration	Basal Levels	%(+)increase %(-)decrease
Cytochrome Oxidase	Rat (liver)	ZnCO₂	300 and 420	4-5 wk	1.89 ± 0.2(1)	(-)67%
Cytochrome Oxidase	Rat (heart)	ZnCO₂ ZnCl₂ ZnO	450 and 600	5 wk	10.9 ± 1.4(2)	(-)28 to 50%
Cytochrome Oxidase	Rat (heart)	N.S.	600 and 900	5 wk	1699 (3)	(-)71 to 84%
Cytochrome Oxidase	Rat† (liver)	ZnO	245-308	16 days	270 ± 2.6(4)	(-)30%
Cytochrome Oxidase	Rat† (heart)	ZnO	245-308	16 days	461 ± 1.4(4)	(-)28%
Cytochrome Oxidase	Rat‡ (liver)	ZnO	245-308	16 days	448 ± 28.7(4)	(-)31%
Xanthine Oxidase	Rat† (heart)	ZnO	245-308	22 days	440 ± 48.8(5)	(-)47%
Xanthine Oxidase	Rat† (liver)	ZnO	245-308	22 days	708 ± 48.8(5)	(-)11% n.s.
Xanthine Oxidase	Rat (liver)	ZnO	240	4-14 days	8.4- 12.3(6)	(-)50 to 70%

N.S. = Zinc form not stated; n.s. = not significant
†Nulliparous females.
‡Fetal tissue obtained at day 16 Zinc treated females
(1) ΔOD/mg protein
(2) ΔOD/min/mg protein
(3) $QO_2 = \mu l$ of O_2 uptake/hr/ng organic matter
(4) μl of O_2/mg dry weight tissue/hour
(5) μl of O_2/g dry weight tissue/hour
(6) μmol of xanthine disappearance/hour/g wet weight of tissues

Other data from the 22 day study in nulliparous females indicated
that treatment with Zinc (245 to 308 mg/kg) had no effects on
maternal or fetal succinic dehydrogenase activity in liver or heart
tissue. These latter findings are in contrast to the reported
inhibitory effects of zinc in in vitro studies.

As is indicated in Tables 5A and 5B, administration of excess
dietary zinc to rats results in altered activity of a number of
enzymes including alkaline phosphatase, catalase, ferroxidase,
cytochrome oxidase and xanthine oxidase. In general, ex vivo
effects of zinc were observed following administration of zinc in
the diet at doses ≥ 245 mg/kg/day for periods ≥ 2 weeks. In the
majority of cases zinc-treatment had inhibitory effects on enzyme
activity, except for alkaline phosphatase activity, where zinc
treatment almost always resulted in augmented activity.

Gastrointestinal Effects of Zinc: In dogs, administration of a zinc sulfate solution (75 mg/kg dose of a 0.75 to 1.5% solution) was followed by three distinct response periods. In the first period, salivation, retching and vomiting was observed within 10-20 min of zinc administration and persisted for 15 to 20 min. In the second period (45-60 min following dosing) no effects on gastric acid secretion (volume or concentration) were observed relative to control values of 0.9 ml/hr and 0 meq/60 min, respectively). Finally in the third period (60-180 min), zinc treated animals showed increased the gastric volume (~ 75% up to levels of 3.5 to 3.8 ml/hr) and acid concentrations (up to levels of 0.32 mEq/hr, relative to none in controls). A similar sequence of events was also observed following administration of $CuSO_4$ and Varioloid at emetic doses. These latter results suggest that the gastrointestinal effects of zinc were related to the emetic event, rather than zinc treatment per se.

Effects of Zinc (Zinc Insulin versus Zinc Free Insulin) on the Hypoglycemic Response to Insulin in Rabbits: In rabbits, subcutaneous administration of insulin (0.5 unit/kg) containing zinc at levels of 2 and 4 mg/1000 units of insulin, prolonged the hypoglycemic response to insulin, whereas no effects were seen at a lower dose of (1 mg/1000 units of insulin; 0.5 unit/kg). Adjunct in vitro studies further showed that if zinc is present in sufficient amounts the insulin protein is completely adsorbed on the insoluble zinc salts at pH 7.0 or thereabout. These latter findings suggested that the prolongation of insulin's hypoglycemic effect induced by zinc could be due to the adsorption of insulin protein on insoluble zinc salts.

Effects of Zinc on Pancreatic Function: Studies in cats showed that administration of dietary zinc oxide (71-86 mg/kg for 12 to 16 weeks) in cats resulted in an average weight loss of 900 g/cat. In addition, pancreatic and liver zinc levels were increased by 7 and 15 times, relative to control values of 0.072 and 0.058 mg zinc/g tissue, respectively). Zinc-fed animals also showed reductions in weights for pancreas (~ 50%, relative to control weights of 4.22 g), but no difference in liver weights. Finally, zinc-fed animals showed marked fibrotic changes and increased insulin content per gram of pancreas (~ 52%, relative to control content of 1.68 units of insulin/gram of pancreas).

In other experiments conducted in chicks, dietary administration of zinc oxide at levels of 1060 mg/kg diet (~127 mg/kg body weight) for a period of 9 days resulted in zinc oxide treatment produced decreased body weight gains (18%) and feed intake (-13%), relative to control gains of 26.0 g/chick and feed intakes of and 57.4 g/chick over the 9 day test period. The 127 mg/kg doses also

reduced pancreatic enzymes including: amylase, lipase, trypsinogen and chymotrypsinogen (20 to 30 % of control activities for all enzymes). Zinc associated reductions pancreatic enzyme activities were associated with reduced digestibility of dietary starch and reduced α-tocopherol concentrations (35 to 48% at the 76 mg Zn/kg and 59 to 70% at the 127 mg Zn/kg dose). Zinc treatment did not however affect plasma glucose concentrations. These findings suggest that the pancreas may be a target organ of zinc toxicity in the chick

Effects of Zinc Deficiency: Several reports from studies in rats also examined the effects of zinc deficiency (i.e. administration of diets containing zinc at levels far below the recommended daily intake of 30 ppm (~30 mg/kg diet or ~1.8 mg/kg for a 250 mg/kg rat which consumes an average of 15 g of diet/day).

In one study, rats administered diets deficient in zinc (as a single daily meal or ad lib.), showed the following: 1) reduced weight gains (-0.1 g/day versus 1.8 g/day in controls; and 0.7 g/day versus 5.5 g/day in controls, for rats fed a single meal and ad lib., respectively); 2) reduced incorporation of [^{14}C]glucose into fatty acids of epididymal fat pads (70%, relative to control incorporation of 12.4 μmol [^{14}C]-glucose incorporated/g tissue in the meal fed rats), but only slight reductions in incorporation into liver fatty acids. In contrast, zinc deficiency in the ad lib. fed rats had no effect on [^{14}C]glucose incorporation into fatty acids of epidermal pads and produced only a slight increase in [^{14}C]glucose incorporation into liver fatty acids. These data suggest that zinc deficiency can result in alterations in glucose metabolism in the rat, and the single meal-fed rat model may be most useful for delineating these effects.

In another study, rats fed diets deficient in zinc for a period of 4 days had reduced serum zinc levels (-78%, relative to control values of 16.3 $\pm$ 0.2 μmol/l) and reduced activities of serum angiotensin converting enzyme (-28%, relative to control values of 543 $\pm$ 13 nmol/ml per min). Finally, zinc deficiency in rats was reported to result in reduced intestinal phosphatase activity (-40%, relative to control values of 45.3% hydrolysis of glycerophosphate over a 21 hour period). Thus, zinc appears to be

In other experiments conducted in chicks, dietary administration of zinc oxide at levels of 1060 mg/kg diet (~127 mg/kg body weight) for a period of 9 days resulted in zinc oxide treatment produced decreased body weight gains (18%) and feed intake (-13%), relative to control gains of 26.0 g/chick and feed intakes of and 57.4 g/chick over the 9 day test period. The 127 mg/kg doses also

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION: (ADME)

Absorption of Zinc[65] Chloride from Ligated Segments of the Rat Gastrointestinal Tract (Van Campen, DR and EA Mitchell, J. Nutr. 1965; 86:120-124)

Methods: Portions of rat intestinal tract were ligated to provide the following isolated in vivo segments: stomach, duodenum, midsection or ileum. Radioactive Zinc[65] chloride (0.15 ml of a 0.3 μM/ml solution) was then injected into one of the ligated segments. Rats were sacrificed 3 hours later and tissues were taken for direct gamma counting in order to determine the rate of absorption in the various segments.

Results: Analysis of the sums of tissue radioactivity (blood, heart, kidneys, liver) showed that highest tissue Zn^{65} levels were detected following injection into the duodenum, compared to other segments. The rank order for uptake in the other segments was ileum > mid-section > stomach.

Absorption of $Zn^{65}Cl_2$ in Mice, Rats, Dogs, and Humans Subjects and Tissue Distribution in Rats.

Methods: The absorption and retention (as a function of time) of a single oral dose of $Zn^{65}Cl_2$ were determined in mice (0.5 μCi), rats (1.0 μCi), dogs (2.0 μCi), and 4 human subjects (0.6 to 1.0 μCi) at 30 min and periodically thereafter using a 4π whole body liquid scintillation counter. The time variation of Zn^{65} distribution in rats was also determined following single oral administration of 1.2 μCi, followed by determination of radioactivity in the liver GI tract, testes, pelt, carcass (remains after removal of visceral organs and pelt) and remains (accessory sex organs, blood fat and visceral organs).

Results: Figure 4, below shows the average retention values expressed as a % of the administered dose as a function of time after ingestion of Zn^{65} in humans, dogs, rats and mice.

Figure 4. <u>Average retention values expressed as a % of the
administered dose as a function of time after ingestion
of Zn^{65} in humans, dogs, rats and mice</u>. (Reproduced from
Sponsor's Figure 1, Amendment dated 9/30/94, Vol. 1.2
pp 210)
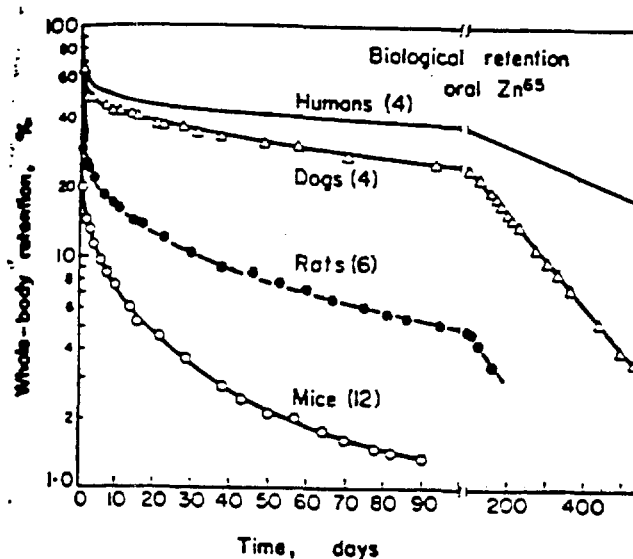
Briefly, the plots in Figure 4 show that the elimination of Zn^{65} in
humans, dogs, rats, and mice was multiphasic and could be
accurately represented by multiple rate equations consisting of
three exponential terms. The relative absorption of orally
administered Zn65 in mice, rats, dogs, and humans was 13.2%, 23%,
48.4%, and 54.8%, respectively (calculated as the administered dose
minus the intercept of the first component of the retention
function). In addition, there was a positive correlation between
the amount of zinc retained and body weights of the various
species. In all species, the majority of the administered dose of
zinc was excreted in the feces. Distribution studies in rats
showed that peak tissue concentrations of zinc were attained within
5 days of oral administration. Figure 5 on the following page
shows the average Zn^{65} content in rat tissues as a function of time.

Figure 5. **Average Zn[65] Content in Rat Tissues as a Function of Time.** (Reproduced from Sponsor's Figure 2, Amendment dated 9/30/94, Vol. 1.2 pp213)
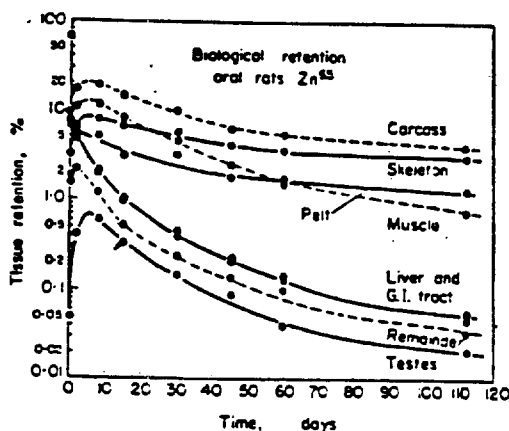
As is shown in Figure 5 above, the rates of elimination were similar in most tissues, with the exceptions of bone and pelt which showed delayed elimination. The average effective half life of absorbed zinc in mice, rats, dogs, and humans, was 91 days, 75 days, 94 days and 154 days.

In another study in rats, the absorption of Zinc[65]Chloride was estimated following dietary and gavage administration (based on comparison of the retention curves of zinc following oral dosing with that observed following intramuscular injection). This latter study indicated that approximately 31% of the dose was absorbed when administered in the diet versus 36% when administered by gavage. This latter study, like the previous one, indicated that the elimination of zinc was triphasic following oral dosing and biphasic following i.m. administration. Elimination half life values for zinc in the first, second, and terminal phases of elimination were 8.8 and 8.4 hours, 316 and 338 hours (13-14 days), and 721 and 713 hours (~30 days each), following dietary and gavage administration, respectively.

Absorption, Deposition and Placental Transfer of Zn[65]Cl$_2$ in the Rat
(Feaster et al. Am. J. Physiol. 1955, 181:287-290)

Methods: Forty-four nulliparous female rats (200-325 g) at definite stages of pregnancy, ranging from the 9th to the 21st day were administered single tracer doses of zinc[65] chloride (actual doses not indicated) orally or i.p. Urine and feces samples were then collected and analyzed for total and radio-zinc content. At death, following balance periods of 0.5 to 96 hours, fetuses were removed, weighed and measured. Total zinc and radio-zinc content in fetuses, maternal blood, whole body, were also assessed.

Results: Only about 5% of an oral tracer dose of radio-zinc was retained following oral dosing in the adult rat. Approximately 68% of the oral dose was eliminated in the feces within 24 hours and 86% was eliminated within 48 hours after dosing. Urinary excretion of orally administered Zn^{65} was insignificant (only about 0.2% of the dose). Zn^{65} which was absorbed was distributed primarily to the kidney, liver and pancreas, with relatively little isotope noted in muscle, hide, hair, or bones. Assessments of placental transfer of zinc showed that for a given stage of fetal development, the amount of isotope transferred to a litter was almost directly proportional to the number and age of fetuses present. Relatively little zinc was transferred prior to gestation day 18 (0.1 to 0.3% per fetus). However, a sharp increase in placental transfer was observed from days 18 through 22 (up to 9% per fetus of the retained dose on gestation day 22).

Separate studies on the quantity and rate of transfer of radio-zinc in milk from the maternal rat showed that by 96 hours after an i.p. injection of $zinc^{65}$, 57% of the retained dose, was passed via the milk to the suckling pup.

Distribution and Elimination of Zinc Oxide and Various Zinc Salts Including Zinc Acetate in Rats following Repeated Dosing.

Methods: Thirty-five white rats (Mus norvegicus albinus; males and females 5-22 weeks of age), including 13 control rats, were fed zinc as the oxide (2.7 to 32.4 mg/day; 10.8-136.8 mg zinc/kg in a gum acacia suspension) or as one of the following salt solutions: zinc acetate (1.9 to 6.3 mg/day; 7.6-25.2 mg zinc/kg), zinc citrate (9.7 mg/day; 38.8 mg zinc/kg), or zinc maleate (11.1 to 16.5 mg/day; 44.4-66 mg zinc/kg) for periods of time ranging from 35 to 53 weeks. One group of rats whose members received one of the three zinc salt solutions were mated after 29 weeks of dosing and then continued on zinc but some on different salts for another 6 to 18 weeks. Urine and fecal samples were collected periodically throughout the treatment period for urinalysis and determination of zinc content. At the conclusion of each experiment, rats were sacrificed and tissue zinc levels were determined. (**Note:** Review of the toxicological assessments made during the current study are included in the Toxicology section of the review.)

Results: Analysis of excreta from control and zinc-treated groups indicated that fecal excretion of zinc was the main route of elimination in rats. In this regard, zinc excretion in the urine and feces of control rats averaged 0.10 ± 0.01 mg/day and 6.3 ± 0.81 mg/day, respectively. In comparison ingestion of zinc as the oxide over 15 and 19 week periods at average daily doses of 1.14,

3.32, 5.74, and 23.48 mg/day resulted in average daily urinary excretions of zinc of 0.009, 0.011, 0.012, and 0.017 mg/ day and average excretion in the feces of 1.106, 3.32, 5.83, and 23.3 mg/day, respectively. These data clearly show that the GI tract was the major route for zinc excretion in the rat and that increasing dose produced only minimal increases in urinary excretion.

Analysis of Zinc concentrations in the various organs including blood, hide, muscle, carcass, G.I. tract, liver, kidneys, brain, pancreas, spleen, showed that in general ingestion of zinc oxide or organic zinc salts, over long periods of time, and even in large amounts, did not significantly increased the zinc concentration in the tissues and organs of the rat. Two exceptions included: 1) the blood of rats ingesting the organic zinc salts, which was increased to an average of 0.015 mg zinc/g blood compared to 0.007 mg/g for control rats and 0.005 for the Zinc oxide group; and 2) the kidneys which showed only mild increased zinc concentrations, which would be expected in an organ involved in the excretion of zinc.

In conclusion, repeated oral dosing with zinc (administered as the oxide or an organic salt) in rat showed no evidence of accumulation or tissue specific storage, with fecal excretion being the major route of elimination for all forms of zinc tested in the rat.

Effects of Various Dietary Factors on Zinc Availability:

Studies in male weanling rats showed that the absorption of zinc from soy protein diets was less compared to that in casein diets (availability = 44% versus 84%, respectively). In a study conducted in chicks, addition of phytic acid (15%) to a casein diet reduced chick growth to about the same extent as that observed with a soy protein diet of the same zinc content (-55% versus -64%; relative to control weights of 460 g at 4 weeks). Thus, phytic acid was identified as the component of soy protein responsible for the reduced absorption of zinc.

Effects (although not always consistent) of calcium and/or phosphorus on the absorption and/or effects of zinc have also been reported. In this regard, a study in male albino rats (4-6 weeks of age) showed that increased dietary calcium (0.6% and 1.76%) in a soy protein diet consistently reduced the absorption of oral zinc[65]chloride and decreased its turnover in the body. A second study in male albino rats (22-28 days old), also showed that dietary administration of calcium (1.2%) or phosphorus (1.2%) independently resulted in reduced body weight gains in rats fed diets containing low (18 ppm) levels of zinc oxide, whereas, increasing the levels of Zinc oxide to (42 ppm) abated the effects of calcium and phosphorus on body weights. In contrast, a third

study in male weanling rats showed that while calcium supplementation (up to 1.6% of the diet) of casein or soy protein diets containing 18-54 ppm of zinc for 42 days produced depressed weight gains (-45%, compared to average weight gains of 186.7 g), no apparent effects on either the absorption or excretion of zinc administered in either diet.

The Excretion and Storage of Zinc in Cats and Dogs Following Long-Term Dietary Administration of Zinc Oxide.

Methods: The excretion and storage of zinc in cats and dogs were studied following long-term dietary administration of zinc oxide at doses ranging from 33 to 268 mg/kg for periods of 10 to 53 weeks (in cats) and from 36-77 mg/kg for periods of 3 to 19 weeks (in dogs). Urine and fecal samples were collected during the treatment period for examination zinc content. At the end of the study period all animals were sacrificed and underwent complete autopsy with determinations of zinc content in the skin, bone, and all organs. Zinc content in samples and organs were determined following ashing in an electric muffle furnace using either a ferrocyanide turbidimetric method or a ferrocyanide titration method.

Results: Normal urinary excretion of zinc in cats and dogs averaged 0.18 mg/24 hr and 0.35 mg/24 hr, respectively. In comparison, average urinary excretion of zinc more than doubled in four cats given daily doses of zinc oxide (200-300 mg/day) and in 3 dogs, (one given 1 g zinc/day and the other two given 0.5 g zinc/day during the zinc feeding period. However, in both cats and dogs the majority of zinc was excreted in the feces. Normal fecal excretion of zinc, measured in one 24 hr cat specimen was 4.4 mg/24 hr and in dogs ranged from 5.0 to 25 mg/24 hr. The available information regarding the relationship between fecal excretion and dose levels only indicated that comparison of the daily zinc input with the daily zinc output over a long period of time was in approximate equilibrium, with no information on either absorption or biliary excretion of zinc oxide following oral administration.

Figures 6 and 7 on the following page show the tissue distribution of zinc in cats and dogs following oral administration at various levels.

Figure 6. (Reproduced from Sponsor's Figure 6, Amendment dated 9/30/94, Vol. 1.1 pp 224).
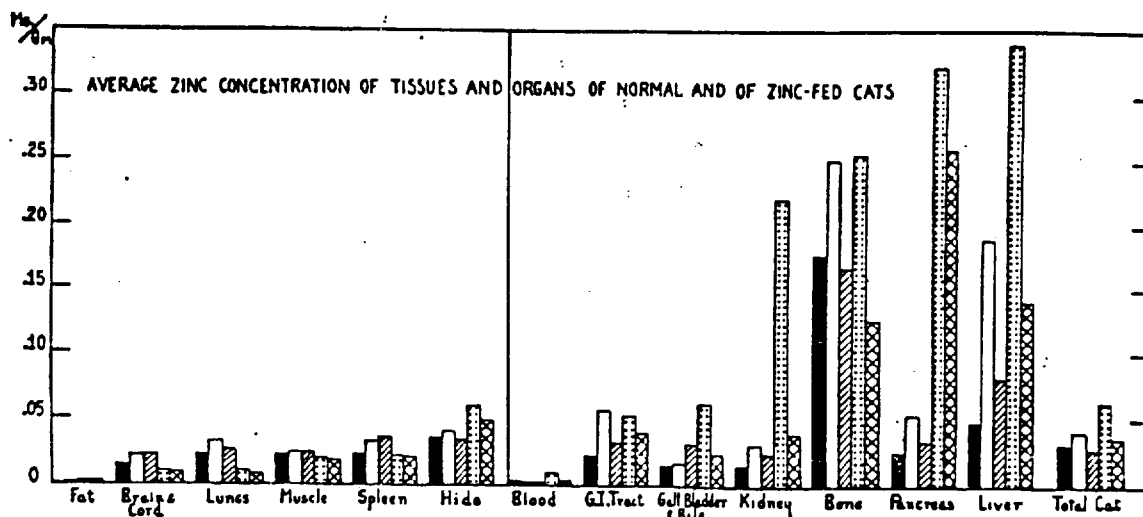

Fig. 6. Average zinc concentration of tissues and organs of normal cats (black columns); of cats receiving 200 to 354 mgm. zinc oxide daily and killed at height of zinc administration (white columns); of cats receiving similar doses for similar periods of time but not killed until two weeks after discontinuance of zinc dosing (single cross-hatched columns); of cats receiving excessively large doses of zinc oxide (681 to 1000 mgm.) and killed during zinc administration (dotted columns); and of one cat receiving a similar dose for a similar period of time but not killed until two weeks after discontinuance of zinc (double cross-hatched columns). See text for comment on noteworthy points.

Figure 7. (Reproduced from Sponsor's Figure 7, Amendment dated 9/30/94, Vol. 1.1, pp 225).
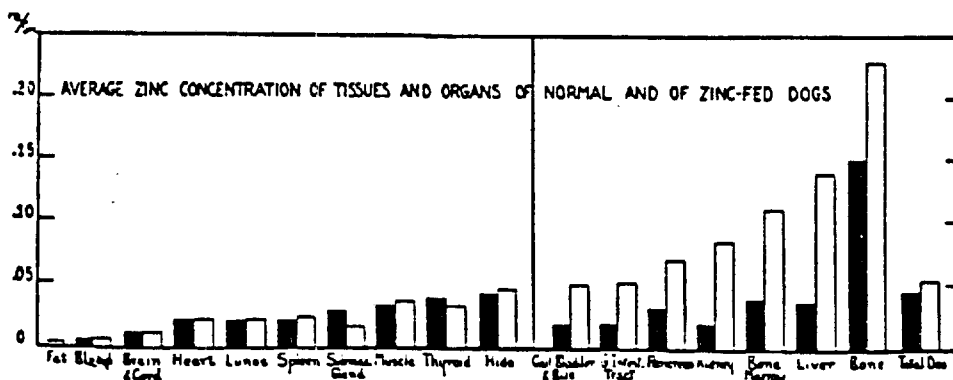
Fig. 7. Average zinc concentrations of tissues and organs of normal dogs (black columns) plotted against average zinc concentrations of tissues and organs of zinc-fed dogs (white columns). See text for comment on significant points.

Briefly, Figure 6 shows that increasing oral doses of zinc in cats resulted in dose proportional increases in tissue zinc concentrations, mainly in organs involved in the excretion of zinc (i.e. liver, gall bladder with its content of bile, gastrointestinal tract, and kidney), but also in bone and pancreas. Figure 7 also shows that the distribution of zinc in dogs was similar to that seen in cats. Finally, Figure 6 shows that zinc levels in all of the aforementioned organs quickly return to normal levels (by 2-weeks) when zinc dosing is discontinued.

Summary of the ADME For Zinc

ADME studies in rats showed that zinc is primarily absorbed from the duodenum. The relative absorption of orally administered Zn65 chloride in mice, rats, dogs, and humans was approximately 13.2%, 23%, 48.4%, and 54.8%, respectively. Studies in rats and chicks showed that the absorption of zinc was reduced, when administered in combination with phytic acid (a soy protein component). Although, some reports in rats showed that calcium and phosphorus also reduced the absorption of oral zinc, others showed no effects. In rats, peak tissue concentrations of Zn^{65} were reached within 5 days after oral dosing. Distribution studies in rats, cats and dogs showed that highest levels of zinc were attained in liver, gall bladder, gastrointestinal tract, kidney, bone, bone marrow, and pancreas following oral administration. In cats, tissue levels in all of the aforementioned organs quickly return to normal levels (by 2-weeks) when zinc dosing was discontinued, whereas in rats, elimination from bone and pelt was delayed. However, no evidence of tissue accumulation was observed in rats following repeated dosing. The elimination of Zn^{65} in humans, dogs, rats, and mice was triphasic, with half life values for the terminal phase of 91 days, 75 days, 94 days and 154 days for mice, rats, dogs, and humans, respectively. In all of the said species, the majority of excretion occurred via the feces, with only a small fraction excreted in the urine.

TOXICOLOGY

I. Toxicology Studies Specific for Zinc Acetate:

Acute Toxicology:

Rat: In acute oral toxicity studies with Zinc Acetate (200 mg/ml in lard) in rats, single oral LD_{50} values 2.46 g/kg (range = 1.6 to 3.78 g/kg) were reported, with no additional information available.

Subacute/Subchronic/Chronic Toxicology:

35- to 53- Week Oral Toxicity Study of Zinc Oxide and Various Zinc Salts Including Zinc Acetate in Rats.

Methods: Briefly, a total of 35 white rats (Mus norvegicus albinus; males and females, 5-22 weeks of age), including 13 control rats, were fed zinc as the oxide, acetate, citrate, or maleate in gum acacia suspensions for periods of 35 to 53 weeks. The rats were divided into three groups as follows: Group I: a) eight male rats on varying doses of zinc oxide suspended in 3.5 per cent gum acacia (0.5 to 22.8 mg. of zinc daily [~2 to 91.2 mg/kg]) for 36 to 43 weeks; b) two male rats on zinc acetate solution (1.9 and 3.6 mg [~7.6 and 14.4 mg/kg] of zinc daily) for 53 and 48 weeks, respectively; and c) two control male rats, one on 3.5 per cent gum acacia solution and one on distilled water, both for 34 weeks. Four of the zinc oxide rats served also as controls for the first 6 weeks of the experiment, receiving during this time distilled water instead of the zinc oxide suspension. Group II: a) three male rats caged together and ingesting average daily doses of 34.4 mg [~137.6 mg/kg] of zinc in the form of zinc oxide suspended in 3.5 per cent gum acacia solution for periods of 35 to 36 weeks and b) five male control rats which were caged together and received as their only liquid for 34 to 35 weeks 3.5 per cent gum acacia solution. Group III: a) three males on zinc malate solution (12.4 mg zinc/rat/day [~49.6 mg/kg]); b) three females on zinc acetate solution (4.4 mg/day [~17.6 mg/kg]); c) three females on zinc citrate solution (9.7 mg/day [~38.8 mg/kg]); and d) six control rats, three male and three female, on distilled water (all for a period of 29 weeks). At the end of this period, group III rats were mated and continued on zinc, but some not on their original solutions, for another 6 to 18 weeks. Controls were also mated and continued on distilled water. Rats in all groups were observed to food and water consumption, and body weights were determined weekly or biweekly. Urine and fecal samples were collected periodically throughout the treatment period for urinalysis and determinations of zinc content. Blood samples were obtained just before autopsy via the rat tail vein for determination of red and white cell counts and hemoglobin determinations. At autopsy, with gross and microscopic examinations were performed and tissue zinc levels were determined. Results from pharmacokinetic assessments conducted in the current study were reported in the ADME section of the review.

Results: Treatment with zinc oxide or any of the other zinc salts including zinc acetate produced no evidence of toxicity, no treatment-related effects on body weight gain or food consumption. Two rats, one treated with zinc oxide (34.4 mg zinc/day) and the second treated with zinc acetate (4.4 mg/day) died during the study as a result of a pneumonia infection which swept through both the control and treated groups during the summer. Reduced water consumption was noted as a result of the unpalatability of the higher doses of zinc solution.

There were no treatment-related changes in red cells, white cells, or percent hemoglobin in any group. Results from urinalysis showed one zinc treated rat #7 and one control rat #12 which always presented with a heavy precipitate of albumin in the urine. However, no clinical signs ascribable to renal alterations were observed and the kidneys of both animals appeared normal at autopsy.

Gross pathological findings were limited to the following: 1) small consolidated areas and pus sacs in the lungs of 7 zinc treated rats and 3 control rats (mainly confined to the upper lobes but occurring in patches throughout the lungs in 4 of the aforementioned treated animals and 2 of the aforementioned control animals); 2) a pus pocket found in the bladder of one animals treated with zinc acetate (1.9 mg/day) and 3) parasitic cysts found in the livers of three treated rats (one with zinc oxide (13.7 and 34.4 mg zinc/day) and the other 2 with zinc acetate (1.9 mg/day). Histological correlates of bronchopneumonia or ·its sequelae (i.e. patches of leukocytic infiltration, atelectatic cavities and dilated bronchi filled with exudate, or encapsulated chronic abscesses, apparently originating from pneumonic foci) were seen to varying degrees in all animals which showed gross pulmonary lesions. Finally, 2 treated animals (one treated with zinc oxide (34.4 mg/day) and a second treated with the zinc maleate salt (12.4 to 16.5 mg/day) showed some cloudy swelling of the liver (attributed to pneumonic infection). None of the aforementioned findings appeared related to treatment with zinc.

In conclusion, the aforementioned studies in rats indicated that daily oral doses of zinc (0.5 to 34.4 mg zinc; ~2 to 137.6 mg zinc/kg body weight), derived from zinc oxide, zinc acetate, zinc citrate, and zinc maleate for periods of 35 to 53, weeks produced no evidence of toxicity at any level tested. However, the usefulness of the study is limited in that only 1-3 animals/dose level were tested and in that the doses tested were not high enough to elicit toxicological effects.

Reprodutive Toxicology:

Effects of Oral Zinc (Acetate, Citrate and Maleate) on Fertility, Reproduction and Growth in the Albino Rat.

Methods: Male (n=5) and female (n=5) albino rats 6 weeks of age were orally administered 1 of 3 organic Zinc salt solutions (Zinc Acetate, Zinc maleate or Zinc Citrate), daily at doses of Zinc ranging from 4.4 to 12.4 mg (17.6 to 49.6 mg/kg) for a period of 29 weeks. A separate group of 3 males (26 weeks of age) had received heavy suspensions of Zinc oxide (34.4 mg; ~137.6 mg/kg) in Gum acacia for a period of 29 weeks prior to mating. However, after serving as sires in three matings, one of which proved sterile, the latter males were removed from the study due to infection with "rat pneumonia". The removed males were replaced with one of the zinc maleate males and a second male which had received zinc acetate (2 mg/day zinc) for a period of 46 weeks (nearly 2x the age of the female mate). Finally, three rats/sex, of the same age, maintained in the same surroundings and administered the same diet, but given water only, served as controls. Males which received treatment with a particular salt were mated successively (2 or 3 times) with females which received a different zinc salt solution. After the initial mating, paired animals were kept together throughout the remainder of the experiment and continued to receive one of the zinc salt solutions or the zinc oxide solution at average daily doses (calculated as zinc) ranging from 1.75 to 38 mg (7 to 152 mg/kg). At birth, two of the pups from each litter were weighed and analyzed for zinc content, while the remaining pups were allowed to remain with the mother for 23 days. ·These pups were then distributed to cages and received the same regimen as the parents had received during the lactation period (i.e. litters from water parents were put on water, litters from zinc maleate parents were put on Zinc maleate, etc.), with one exception. In the exception, one of the second litters from a zinc maleate group was put on zinc oxide after weaning due to a lack of zinc oxide offspring. All pups were allowed to grow to at least 60 days of age in order to determine the effect of zinc on their early growth curves.

Results: Pairing schedules for mating along with zinc doses, the number of litters, total numbers of offspring, and the number of pups which died (from all causes) during the period they were with their parents are presented in Table 6 on the following page.

Table 6. <u>Mating schedule and litter data for rats treated with Zinc oxide or zinc salts prior to mating and throughout gestation and lactation.</u> (Reproduced from Sponsor's Table 1, Amendment dated 9/30/94, Vol. 1.1 pp 143)

Results of fertility experiments with zinced rats and their controls

	PARENTS*			OFFSPRING		REMARKS
SERIES	Average daily dose of each pair of rats after mating (calculated as zinc)	Average daily dose of each rat before mating (calculated as zinc)†		September 28, 1925-January 2, 1926		
		Male	Female	Total number per female in number of litters	Deaths	
	mgm.	mgm.	mgm.			
D	Zinc acetate, 6.3	Zinc malate, 12.4	Zinc acetate, 4.4	6 in 2 litters	0	
E	Zinc malate, 16.5	Zinc malate, 12.4	Zinc acetate, 4.4	18 in 2 litters	0	8 embryos found at autopsy (1 litter)
P	Zinc malate, 11.1	Zinc malate, 12.4	Zinc citrate, 9.7	11 in 2 litters	1	
H	Zinc citrate, 10.2	Zinc oxide, 34.4	Zinc citrate, 9.7	15 in 3 litters	3	
L	Zinc acetate, 1.75	Zinc acetate, 2.0				
I	Zinc oxide, 6.5	Zinc oxide A, 34.4	Zinc citrate, 9.7	20 in 2 litters	19	Series I was sterile
J	Zinc oxide, 22.2	Zinc oxide B, 34.4				
M	Zinc oxide, 38.0	Zinc malate, 12.4				
Totals for five zinced females............				70 in 11 litters	23	
Average number per liter................				6.4		

			Controls			
A	Water	Water	Water	14 in 3 litters	14	
B	Water	Water	Water	23 in 3 litters	12	
C	Water	Water	Water	19 in 2 litters	2	4 embryos found at autopsy (1 litter)
Totals for three control females............				56 in 8 litters	28	
Average number per litter............				7.0		

* All rats approximately 250 days old when mated, except the males in series H, L, I and J, which were approximately 410 days old.

† All rats were on zinc for 29 weeks previous to mating, except the male in series L (46 weeks).

Briefly the Data in Table 6 show that during the 96 day mating period, 70 offspring were born to the five females, with eight unborn embryos also found in one female at autopsy. A total of 12 litters (one embryonic) from 15 matings were recorded for an average of 15.6 offspring/female and 5.2 offspring/mating (including embryos). Table 6 also shows that 32.9% of the pups died or were killed by their parents during the lactation period. No pups, control or treated, died after weaning.

The aforementioned findings were comparable to values in the 3 control rats, to which 56 offspring (also 4 embryos found) were born during the same time period (20.0 offspring/dam or 6.6 offspring/mating; including embryos). Fifty percent of the young born to control animals died or were killed by their parents during the lactation period.

Examination of post weaning weight gains in F1 offspring showed no treatment-related differences in mean body weight gains, compared to the weight gains in F1 offspring from control groups or compared to growth curves for normal male and female albino rats (numerical values not reported).

In summary, the aforementioned study though limited in it's scope suggested that feeding of organic Zinc salts, or Zinc oxide to rats, at doses from 2 to 38 mg (8 to 152 mg/kg) of zinc daily for many weeks prior to mating, during pregnancy and lactation and no significant effects on the health of the parents, fertility, nor upon the health and early growth of the F1 offspring. Finally, treatment of F1 offspring with organic zinc salts or Zinc oxide (post delivery Day 23 throughout Day 60) had no apparent effects on their survival growth or maturation.

Special Toxiology Studies:

2-Month Oral Pancreatic Toxicity Study with Zinc Acetate in Rats.

Methods: The potential for Zinc to produce pancreatic toxicity was investigated in rats. Briefly, groups of male Sprague Dawley (9-10/group) rats were orally administered Zinc Acetate (aqueous solution), by gavage at total daily doses of 5.7, 28.5, and 57.1 mg/kg, (administered as 2 divided doses each in a total volume of 0.5 ml) for a period of 2 months. Two additional groups of rats, groups A and B (6 rats/group), served as controls. All animals except Control Group A received a diet supplemented with copper (100 ppm; ~6 mg/kg) to insure that any observed effects were due to Zinc treatment and not a zinc-induced copper deficiency. At the end of the two month treatment period animals were sacrificed and the pancreas was removed fixed in 10% buffered formalin solution and examined histologically.

Results: Administration of Zinc Acetate to rats for 2 months at doses up to 57.1 mg/kg produced no clinical signs of toxicity and no treatment-related histological effects on the pancreas.

II. Toxicology Studies Specific for Zinc Oxide and Other Zinc Salts:

Subacute/Subchronic/Chronic Toxicology:

Rats:

5- to 6-week Oral Toxicity of Zinc Carbonate in Rats

Methods: In preliminary studies, Sprague Dawley rats (4 to 6 weeks of age; ~ 50 g in weight; numbers not indicated) received dietary zinc carbonate over a dose range of 0.4% to 1.0% (~1200 to 3000 mg/kg) for 6 weeks to assess its effects on survival and hemoglobin levels. The possible mechanism(s) for zinc's induction of anemia and suppression of growth were examined in two additional studies. In the first study, groups of rats (both sexes; 5/group; 30-40 days old; and 36-71 g in weight) were administered zinc carbonate (0.7% of the diet; 2916 to 1479 mg/kg) for a period of 6 weeks; either alone or in combination with one or all of the following: iron (2 mg/day as ferric pyrophosphate); copper (0.2 mg/day as cupric sulphate); cobalt (0.2 mg as cobaltous chloride) and liver extract (1 g/day). In the second experiment, rats (5/sex/group; 26-29 days old; 42-82 g) were administered zinc at a dietary level of 1.0% (3571 to 1829 mg/kg) in combination with a liver extract and/or a mixture of iron copper and cobalt salts (at the same levels as for exp. #1) for a period of 5 weeks. Rats were monitored for effects on mortality, clinical signs of toxicity and changes in body weights and hematological parameters

Results: Qualitative results from preliminary experiments indicated the following: 1) at a level of 1.0 % (~3,000 mg/ kg) zinc carbonate produced severe anemia, complete inhibition of growth and approximately 75% mortality within 3-5 weeks (No. of rats tested not provided); 2) at levels ≥ 0.7% (~2100 mg/kg) in the diet, zinc carbonate produced marked anemia in 4 weeks, but no mortality; and finally, 3) at a level of 0.4% (1200 mg/kg), zinc carbonate had no effects on growth or hemoglobin levels. The first of the two main studies showed that zinc containing diets which were supplemented with copper or extracts from liver had increased hemoglobin concentrations (31% and 30%, respectively) relative to groups which received zinc (0.7%) alone. The combination of zinc (0.7%) and liver extract, was also reported to produce "better growth" (extent of difference not indicated) relative to those which received zinc (0.7%) alone. Results from the second main experiment showed that dietary zinc at the 1% level (~3000 mg/kg) for 5 weeks, suppressed body weight gains (49%, relative to gains of 112,2 g in control rats) and reduced hemoglobin (-48%); Hematocrit (-30%); Mean cell volume, (-37%); mean hemoglobin concentration (-26%), and mean cell hemoglobin levels (54%)

relative to respective control values of 16.04 g/dl; 50.4%; 59.3 cu microns; 31.8%; and 18 micrograms. The second study also showed that the anemic effects of zinc were partially reversed by the addition of the combination of copper, iron and cobalt salt mixture, but not by the liver extracts. In contrast, addition of the liver extracts did help to ameliorate the zinc induced suppression of body weights.

In conclusion, the current study, showed that repeated administration of zinc carbonate in the diet of rats, at levels of ≥ 0.7% (~2100 mg/kg) for 3-5 weeks, produced hypochromic anemic effects and suppression of body weight gains. In addition, the study showed that supplementation with copper and liver extracts ameliorated the effects of zinc on hemoglobin and body weights, respectively. However, the usefulness of the study was limited in regard to the following: 1) only single dose levels of 0.7% and 1.0% zinc carbonate were evaluated in the two main studies; 2) no untreated control groups were utilized in the first of the 2 main studies; and finally, 3) the toxicological focus of the study was limited to zinc's effects on mortality, growth and hematological parameters.

6-week Oral Toxicity of Zinc Oxide in Rats

Methods: Five groups (6/group) of Wistar hooded male rats (5 to 6 weeks of age) were assigned to the following dietary groups: Group A: Basal Diet; Group B: Basal Diet + Copper (0.4 mg/day); Group C: Basal diet + zinc oxide (0.5% zinc oxide; 647-938 mg/kg) Group D: Basal Diet + Copper (4 mg/day) + Zinc (0.5%; 556-961 mg/ kg); Group E: Basal Diet (food consumption restricted to that of group C) for a period of 6 weeks. Animals were weighed 3 x /week and hemoglobin determinations were made at the beginning, every 2 weeks and at the end of the study period. Food consumption was recorded daily and group E received the amount consumed by rats in group C on the previous day. Blood samples were also obtained via cardiac puncture at the end of the experiment for determination of hemoglobin concentrations. Animals were sacrificed at the end of the study period and organ dry weights for liver and kidneys were determined. Body copper and fat contents were determined.

Results: Zinc-fed rats showed suppressed body weight gains -69.2% and reduced food consumption (-37.9%) relative to weight gains of 117 g over 6 weeks and average food consumption of 7 g/week in control animals. Likewise diet matched rats (Group E) also showed 45% suppression of body weight gains. Zinc-fed rats also showed significant reductions in blood hemoglobin levels (-46.7%) relative to control values of 13.7 g/dL; with no effects on hemoglobin in the diet matched group. In addition, zinc-fed rats showed

significant reductions in copper levels for blood (-76%), liver
(-46%), kidneys (-54%) and body (-38%), relative to respective
values of 1.03, 12.5, 21.6 and 3.7 p.p.m. Cu on a dry basis in
control rats. Finally, zinc-fed rats had reduced values for mean
percentage body fat (46%), relative to control values of 38.6%.
Addition of Copper to the zinc diets produced a partial improvement
in growth, but no corresponding increase in food consumption; a
partial reversal of the reductions in hemoglobin; and reversal of
the reductions in tissue copper levels induced by Zinc treatment.
Collectively, the data suggest that reduced food consumption may be
the primary factor involved in the reduced growt, whereas the zinc-
induced reduction in tissue copper levels cannot fully account for
the zinc-induced anemia in rats.

In conclusion, the current study showed that in rats administration
of excess dietary zinc, at a level of 0.5% (~556 to 961 mg zinc/kg)
for 6 weeks, reduced food consumption, suppressed body weight gains
and reduced hemoglobin and body fat. Incorporation of diet-matched
and copper supplemented control groups also showed that the zinc-
induced reductions in body weight gain resulted from reductions in
food consumption, whereas reductions in hemoglobin were only
partially related to reduced copper levels. Although the current
dose tested (0.5%; ~556 to 961 mg zinc/kg) was appropriately high,
the usefulness of the current study was limited in regard to the
following: 1) only a single dose level of zinc was tested; and 2)
the toxicological focus of the study was narrow in scope (i.e. only
effects of zinc on growth, hemoglobin concentrations, and body fat
were examined.

14-Week Oral Toxicity of Zinc Sulfate in Rats.

Methods: In the current study Wistar weanling rats (3-5 rats) were
administered either zinc sulfate in the diet at levels 1000 p.p.m.
(~60 mg/kg) or basal diet for a period of 14 weeks, in order to
examine the effects on body weight gains and tissue copper levels.

Results: Zinc administration produced no signs of toxicity in
terms of effects on body weight gains. In addition, dietary zinc
(1000 p.p.m.; ~60 mg/kg) did not appear to have any appreciable
effects on either the concentrations of copper in liver, kidneys
and femurs of rats. However, zinc treatment was associated with a
lower uptake of ^{64}Cu in blood and red bone (extent of reduction not
provided). The study was of limited value in that only effects on
weight gain and tissue copper levels were evaluated and the single
dose of zinc sulphate which was tested was not high enough to
elicit effects on either parameter.

6- to 15-Month Oral Toxicity of Zinc Chloride in Wistar Rats

Methods: Groups of Wistar albino rats (25/group) were fed diets dampened with solutions zinc chloride (approximate daily doses of 0 [water control], 60, 120, and 600 mg/rat/day; ~0, 240, 480 and 2400 mg/kg) for a period of 6 months at the 2400 mg/kg dose and for a period of 15 months in the remaining groups. Animals were weighed every other day and rats which died, along with all rats which survived to the end of the treatment period underwent careful gross post mortem examinations.

Results: Rats treated with zinc chloride at the 240 and 480 mg/kg doses showed no treatment-related effects on body weights and no gross or histological lesions. However at 2400 mg/kg dose, Zinc Chloride produced a sudden reduction in weight (from an average of 210 g to one of 150 g, during the first two weeks) and clinical signs of toxicity which included: general malaise, staring coat, and huddled posture. Death was observed in 19 of 25 rats at the 2400 mg/kg dose group beginning at 14 days after the start of the treatment. The intestines were the only target organ of toxicity identified in the study with discoloration (black to grey and slimy character) observed grossly at the 2400 mg/kg. However, additional information was unavailable. The 120 mg/ rat/day (480 mg/kg) dose was the no effect dose for the study.

21-month Oral Toxicity of Zinc Sulfate in Rats.-

Methods: Four groups of Osborn Mendel weanling rats (4/sex/group) were administered diets containing Zinc sulphate at levels of 1000, 500, 100, and 0 p.p.m. (~60, 30 and 6 mg/kg/day) for 21 months. Body weights and food consumption in rats were determined weekly, with hematology determinations conducted at 8 intervals throughout the study and bone marrow smears conducted at the end of the treatment period. Gross post mortem examinations were apparently conducted on all rats, whereas complete histological evaluations were limited to 6 high dose rats and 6 control rats. Histological exams in animals of the other groups were limited to liver, kidneys and testes (if male).

Results: Treatment of rats with zinc sulfate at doses up to 1000 p.p.m. (~60 mg/kg) in the diet produced no significant effects on body weight gains or food consumption. Hematological changes, characterized by microcytosis, coupled with polychromasia in some cases and hyperchromia in others were noted at all doses in rats by the 16-17th months of feeding, but subsequently returned to normal size, despite continued feeding with zinc. Compared to values in

control groups, all zinc-treated groups showed slight decreases in total myeloid numbers (-14 to -21% vs 61% in control), slight increases in erythroid numbers (35 to 45% vs 30% in controls), and depression of the myeloid to erythroid ratios (37 to 46% vs a ratio of 2.4 in controls). Finally, gross and histological examinations of rats at the end of the study showed that males at the 30 and 60 mg/kg doses had kidneys which appeared larger (weights not determined) and more granular than in males in the control and 100 p.p.m. (~6 mg/kg) groups. Histologically, males in all groups including controls showed signs of spontaneous nephritis. There were 6 males with a moderate degree of nephritis and 5 males with severe nephritis, all the latter 5 animals were in the 30 and 60 mg/kg dose groups. Thus, zinc appeared to increase the severity of normally occurring nephritis or nephrosis in male rats. No tumorigenic effects were noted at any dose tested.

Three Generation Oral Toxicology of Zinc Oxide and Various Zinc Salts (Chloride, Carbonate, and Sulphate) in Rats

Methods: Randomly bred immature rats (2/sex/group) were orally administered zinc (0.25% ~163 mg zinc/kg) as the oxide, chloride, carbonate, and sulfate. Two additional groups of rats received a higher zinc dose of 0.5% (~326 mg zinc/kg) as either Zinc Oxide (2 males and 3 females) or chloride (2 males and 7 females). Upon reaching maturity, rats were mated and the F1 offspring were continued on similar rations through mating and delivery of the F2 generation. The number of treated F1 offspring ranged from 20 to 44 rats/group at the 0.25% (~163 mg zinc/kg) dose and 11 and 27 in the two additional 0.5% (~326 mg zinc/kg) ZnO and 0.5% (~326 mg zinc/kg) $ZnCl_2$ groups, respectively. Estimated periods of treatment for the F0 and F1 generations were ~6 to 12 weeks, excluding 3-4 weeks of nursing (based on approximate period required for normal sexual maturity in rats). All rats and their offspring were observed for effects on growth and clinical signs of toxicity. Matured animals underwent gross pathological examinations and zinc levels in heart, liver, kidneys, spleen lungs and testicles and in samples of feed, urine, and feces were determined.

Results: Administration of Zinc, as the oxide or various zinc salts (0.25% zinc ion in rats) had no effects on growth or reproduction and normal functions in the rat through three generations. A qualitative reduction in normal growth was reported for F1 offspring at the 0.5% ZnO dose ([+++], relative to normal growth [++++] for control animals). However, no actual data on the differences were provided. In addition, no gross lesions or pathological conditions were reported in zinc-treated rats. Further, analysis of zinc content in heart, liver, kidneys, spleen

lungs and testicles from zinc-treated rats revealed no perceptible increase in the zinc content in the treated versus control animals. Finally analysis of zinc content in the samples of urine and feces indicated that elimination of Zinc occurred primarily with the feces with relatively little excretion occurring via the urine.

In conclusion, administration of excess dietary zinc (administered as the oxide, chloride, carbonate and sulphate) over three generations in rats at a level of 0.25% (~163 mg zinc/kg) for (periods of ~6-12 weeks for the F0 or F1 generations) had no effects on growth, reproduction, or tissue zinc levels. However, zinc oxide at a dose of 0.5% (~326 mg zinc/kg) was reported to supress body weight gains in the F1 generation. The usefulness of the study was limited in that the doses tested were too low to elicit toxicity; the majority of the information provided was only qualitative in nature; and only effects on clinical signs, body weight gains, tissue zinc levels, and gross pathological observations were recorded.

Mice:

One Year Toxicity Study with Zinc Sulfate in Mice:

Methods: One hundred and fifty mice of the CH_3 strain (males and females, 6-weeks to 2-months of age) received Zinc sulphate in their drinking water at levels of 0.5 g/l (~100 mg/kg) for up to 14 months. Control mice (number not indicated) received untreated distilled water. Control and test animals were removed from the colonies in groups of 5, usually at monthly intervals for determination of the following: 1) zinc levels in plasma and tissue (skin, liver, and spleen) 2) glucose and insulin levels in plasma, 3) histological assessment of adrenal, pancreas, and hypophysis cerebri, 4) cholesterol content of adrenals, and 5) electron microscopic examination of the adrenal, pancreas, and adenohypophysis.

Results: All animals treated with zinc remained healthy, with no clinical signs of toxicity observed. Weights in control animals ranged from 21 to 30 grams, with weights in the Zinc-treated groups being about 1 g higher than controls. Mean plasma levels of zinc in the control population averaged 15 ± 1.35 μM (i.e. 1.02 ± 0.09 ug/ml) but were increased to a level of 31 μM (2.1 ug/ml) by day 3 in the zinc-treated groups. Tissue levels of zinc liver, spleen and skin were not altered in the zinc supplemented mice versus controls. In addition, zinc treatment had no effects on the levels of plasma insulin or glucose levels.

Only qualitative descriptions of histopathologic findings were available and no incidence data was provided. In this regard, the available information indicated that zinc treatment produced morphological changes in the adrenal cortex, the pancreatic islets, and in the cells of the anterior lobe of the pituitary gland.

Reported effects in the adrenal cortex from the zinc-treated mice included: 1) hypertrophy and vacuolization (lipid deposition) of the cells of the zona fasciculata; 2) contracted blood vessels and granular deposits in the blood vessels and sub-endothelial space of the zona fasciculata; 3) anastomosing cords of cells in the zona reticularis, separated by very wide vascular channels; and 4) strongly positive cholesterol reaction throughout all three cortical zones after 6 months of zinc treatment versus mainly cells of the zona glomerulosa in the untreated control groups.

In the pancreas, zinc treatment was reported to produce increased islet size, hypertrophy of the islet beta cells, and an apparent increase in beta cell granules. However, as was indicated previously, no effects on the levels of plasma insulin or glucose were observed.

Finally, zinc treatment was reported to result in hypertrophy of the anterior pituitary, with evidence of increased synthetic and secretory activity (increased granulation of the ACTH cells, which were also surrounded by somatotropin cells compared to agranular ACTH cells in control mice).

In conclusion, the information provided in the current study suggests that prolonged administration of zinc at a level of -100 mg/kg can result in morphological and possibly functional changes in endocrine tissues in the mouse. However, the biological significance of the aforementioned findings is unclear since no other toxicological effects or changes in insulin or plasma glucose levels were detected.

Dogs:

3 to 19-week Oral (Dietary) Toxicology Study with Zinc Oxide in Dogs

Methods: Three dogs (breed not indicated) were administered zinc oxide in the diet at dose of 36.1, 59.9, and 76.5 mg zinc/kg for periods of 19, 3, and 15 weeks, respectively. Animals were observed daily for clinical signs of toxicity and weighed weekly during the treatment periods. Urinalysis was performed on samples were collected periodically during the treatment period. Blood samples were obtained from a few animals in the early part of the experiment and from all animals just before sacrifice for

determination of hemoglobin and blood cell counts. At the end of each study period, dogs were sacrificed and underwent complete gross and histological examinations.

Results: The dog dosed at the 59.9 mg zinc/kg died of distemper pneumonia after three weeks of treatment with zinc and thus, was not considered treatment-related. The other two dogs at the 36.1 and 76.5 mg zinc/kg doses showed no overt clinical signs of toxicity, no effects on body weights, hematological parameters (RBC counts, WBC counts or hemoglobin levels) or urinalysis and no treatment related gross or histological lesions.

In conclusion, the current study showed no apparent treatment-related effects in dogs following up to 19 weeks of zinc ingestion at a dose up to 77 mg/kg. However, the usefulness of the current study was limited in that a total of only 2 dogs, one each at different levels of zinc were evaluated and that neither of the doses tested were high enough to elicit toxicity.

70-Week Oral Toxicity of Zinc Sulfate in Dogs.

Methods: Dogs (4 dalmatian puppies 10 weeks of age) were administered zinc sulfate (by capsule) for 70 weeks according to the following schedule: initially all four dogs were started on a dose of 200 mg/kg, which, as a result of emesis, was reduced to 100 mg/kg, after 7 weeks, with a further reduction to 50 mg/kg after 3 weeks at the 100 mg/kg dose in 1 dogs and after 32 weeks in the other 3 dogs. Complete blood counts were conducted periodically. Other methodological details of the dog study were not provided.

Results: In dogs, the initial 200 mg/kg dose produced severe emesis which necessitated dose reduction to 100 mg/kg, after 7 weeks of treatment. The 100 mg/kg dose also induced emesis which necessitated a further dose reduction to 50 mg/kg (after 3 weeks at the 100 mg/kg dose in 1 dog and after 32 weeks in the other 3 dogs). One dog which had exhibited weight loss and was in moribund condition, was sacrificed in extremis at one week after the final dose reduction to the 50 mg/kg level. The remaining, dogs showed normal appetite and growth until sacrifice at the end of the 70-week treatment period. Hematological analysis of the 4 treated dogs showed hypochromic anemia (decreased hemoglobin, -29%, but with normal red cell counts). In dogs, the only pathological change attributed to the administration of zinc occurred in the bone marrow where uniformed slight hyper-plasticity was observed, but with no apparent alterations in the myeloid to erythroid ratios observed. However marrow counts in dogs were not performed.

Cats:

10 to 53-week Oral (Dietary) Toxicology Study with Zinc Oxide in Cats

Methods: Ten cats (breed not indicated) were administered zinc oxide in the diet at doses ranging from 34 to 268 mg zinc/kg for varying periods (10 to 53 weeks). In order to determine the rate at which potentially stored zinc was eliminated, eight of the 10 cats were divided into 4 groups of 2 cats/group and each group was maintained on the same dose of zinc for the same duration. One cat/pair was sacrificed at the end of treatment and the other after a 2 weeks recovery period. Differences in tissue zinc levels between the two cats in each pair were then used as an index of the rate of elimination of stored zinc. Cats were observed daily for clinical signs of toxicity and weighed weekly during the treatment periods. Urinalysis was performed on samples collected periodically during the treatment period. Blood samples were obtained from a few animals in the early part of the experiment and from all animals just before sacrifice for determination of hemoglobin and blood cell counts. At the end of each study period, cats were sacrificed and underwent complete gross and histological examinations.

Results: Addition of zinc oxide to the diet at doses up 268 mg/kg in cats produced no treatment related clinical signs of toxicity in cats. Three cats which received heavy concentrations of zinc oxide approximately 681-1000 mg/day (189-268 mg/kg) for 16, 21, and 21 weeks lost 0.4, 0.7 and 0.3 kg over the treatment period, with loss of appetite (due mainly to the unpalatable nature of the food) being the only noticeable symptom. Hematological examinations showed no treatment-related effects on RBC counts, WBC counts or hemoglobin concentrations. However, the three cats which received the extremely high doses of zinc oxide (189-268 mg/kg) presented with pancreases, which were harder than normal and covered with firm nodules. Two of these cats also had histological correlates of extensive proliferation of fibrous tissue in the pancreases. The third cat was not examined histologically. Otherwise, no treatment-related histological findings were evident in any of the other treated animals.

In conclusion, repeated dosing in cats with zinc at levels up to 156.2 mg/kg for periods up to 53 weeks were well tolerated in cats with no significant toxicity observed. However, higher doses (189-268 mg/kg) produced body weight loss and morphological changes in the pancreas (i.e. grossly hardened and covered with firm nodules, with histological correlates of extensive fibrous tissue proliferation). The major limitation of the study was that only 2 cats/dose lever were studied, and only one of these was examined at the end of the treatment period.

Swine:

6-Week Oral Toxicity of Zinc Carbonate in Weanling Pigs.

Methods: The oral toxicity of excess dietary zinc carbonate was assessed in a series of 3 experiments conducted in weanling pigs. In experiment #1, groups of weanling pigs (6/group) were administered Zinc Carbonate in the diet at levels of 0, 0.05, 0.1, 0.2, 0.4, and 0.8% (0 and ~60, 114, 213, 343, and 318 mg/kg, respectively) for a period of 42 days. In experiment #2, groups of weanling pigs (5/group) were administered Zinc Carbonate in the diet at levels of 0, 0.05, 0.1, 0.2, and 0.4% (0 and ~73, 131, 271, and 365 mg/kg, respectively) for a period of 35 days. Finally, in experiment # 3, two groups of weanling pigs (8/group) were administered Zinc Carbonate in the diet at levels of 0 (controls) and 0.4% (~290 mg/kg) for a period of 42 days. The effects of zinc on mortality, weight gains, food consumption, feed efficiency were determined and gross post mortum examinations were conducted.

Results: Zinc treatment had no effects at dose levels up to 0.1% of the diet (~ 131 mg/kg). However, at levels of 0.2, 0.4, and 0.8% of the diet (i.e. ~213, 343, 318 mg/kg) for 42 weeks, zinc produced dose-dependent suppression of body weight gains (21-83%, relative to control gains of 29.71 kg), reduced feed consumption (-7 to 67%, relative to control consumption of 82.85 kg) and reduced feed efficiency (14-50%, relative to control gain feed ratios of 0.36).

Treatment-related mortality in experiment #1 included: 2 of 6 pigs on days 15 and 31 at the 0.2% (~213 mg/kg) level; 3 of 6 pigs on days 15, 19, and 31 at the 0.4%, (343 mg/kg) and 1 of 6 pigs at the 0.8% (318 mg/kg) level on day 24, with the remaining pigs at the 0.8% level sacrificed in moribund condition on day 31. Similar incidences of mortality (3 of 4 and 3 of 8 pigs each at the 0.4% level) were observed in experiments 2 and 3.

Although incidence data was not available, the report did describe a set general toxic reactions which occurred at the 0.2 and 0.4% (~213 and 343 mg/kg) doses and were characteristic of zinc toxicity in the pig. These included: arthritis; extensive hemorrhages in the axillary spaces; marked gastritis with some ulceration, extensive hemorrhage and marked catarrhal enteritis of the intestines, congestion of the mesentery and hemorrhagic conditions in the ventricles of the brain, spleen, lymph nodes and viscera.

27-Week Oral Toxicity of Zinc Sulfate in Swine.

Methods: In the current study, swine were administered zinc sulfate at 1000 p.p.m. (~21 mg/kg/day) for periods of 27 weeks, in order to examine the effects of zinc on body weight gains and tissue copper levels.

Results: Zinc administration produced no signs of toxicity in terms of effects on body weight gains or degenerative changes in the spinal cord, leg joints, of muscle tissues. In addition, no appreciable effects on either the concentrations of copper in the liver, kidneys and femurs swine or uptake or metabolism of copper in swine were observed. Thus, the single dose level tested was not high enough to elicit toxicological effects.

Ovine:

6- to 10-Week Oral Toxicity of Zinc Oxide in Lambs

The oral toxicological effects of excess dietary zinc oxide, in terms of its effects on food and water consumption, body weight changes and mortality was investigated in a series of 3 experiments involving Wether lambs. Animals which died were also examined for gross and histological lesions. In the first experiment, lambs (6/group, weighing 22-29 kg) were administered zinc oxide at levels of 0, 0.5, 1.0, 2.0, and 4.0 g/kg of diet (~29.2, 50.8, 88.3, and 105.9 mg/kg), with 2 lambs/group slaughtered at 6 weeks and the remaining 4 slaughtered at 10 weeks. In the second experiment, 8 groups of lambs (10/group, mean weight: 38.6 kg) received zinc oxide in the diet at levels of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 g/kg of diet (~23.3, 45.3, 59.1, 74.6, 87.6, 104.1, and 101.6 mg/kg) for 10 weeks. Finally in the third experiment lambs (2/group; mean weight: 37.6 kg) received zinc sulphate in the diet at high doses of 0, 2.0, 4.0, or 6.0 g/kg of diet for 11 days (~76.59 at the 2.0 g/kg dose, but not calculated for the 4 and 6 g/kg doses due to severe reductions in food consumptions).

Results: Experiments 1 and 2 showed that zinc oxide produced dose-dependent suppression of body weight gains begining at doses of 1 to to 2 g/kg diet (~45.3 to 88.3 mg/kg); dose-dependent reductions in food consumption (-12.7 to -52.7% reductions) and decreased feed efficiency (38.2% or greater) at doses ≥ 1.5 g/kg (~59.1 mg/kg); body weight loss at levels ≥4.0 g/kg diet (~106 mg/kg); and increased mineral intake at all doses tested. Fiveof 10 and 2 of 10 lambs in the 3.0 g/kg and 3.5 g/kg diet (~104.1 and 101.6 mg/kg)

groups died during the 8th to 10th weeks of experiment No.2, respectively. Acute symptoms in animals which died included: extension of limbs, convulsions, and opisthotonos occurring 6 to 8 hours prior to death.

In Experiment #3, lambs which received 4.0 and 6.0 g of zinc/day showed immediate reductions in food and water consumption leading to dehydration and emaciation. The study was terminated after 11 days of dosing due to the death of one lamb in the 6.0 g of zinc/day group. Post mortem examinations of the lambs which died revealed a sporadic incidence of endocardial hemorrhages, lung congestions, pleuritis, enteritis and brain damage whose relationship to treatment was considered equivocal. The 0.5 g/kg dose level (23-29 mg/kg) was the no effect dose in lambs.

Chickens:

4- to 10-Week Oral Toxicity of Zinc Oxide, Sulphate, or Carbonate in Chickens:
The oral toxicity of zinc was examined in two 4-week toxicity studies in broiler chicks and in a 4-day study in adult broiler hens. In the 2 four week chick studies, excess zinc oxide was added to the diet at levels of 1000 (~66 mg/kg), 2000 (~129 mg/kg), 4000 (~494 mg/kg), or 6,000 (mg/kg dose not calculated due to excess food spillage at 6000 mg/kg level). Finally, in the 4 day study in hens zinc oxide was administered at doses of 10000 and/or 20000 mg/kg diet (~31 and 23 mg/kg, respectively). Results showed that in chicks, dietary zinc at levels of 2000, 4000, and 6000 mg/kg diet produced dose-dependent reductions in body weights (7-59%), and high mortality (2 and 9 of 22 chicks each at the 4000 and 6000 mg/kg doses in experiment # 1 and 9 of 20 chicks at the 4000 mg/kg level in experiment #2, respectively). A few of the birds which died at the 6000 mg/kg dose showed dissecting aneurism. In addition, administration of zinc at doses $\geq$ 1000 mg/kg diet (~65.62 mg/kg) was associated with lesions of the pancreas, whereas an increased incidence of gizzard erosions were observed at doses $\geq$ 2000 mg zinc/kg diet (~129.4 mg/kg). Histologic lesions of the gizzard included: excessive desquamation of epithelial cells, heterophils and erythrocytes into an abnormally spongiform koilin, to erosion of koilin, glands and pits and pancreatic lesions included: dilation of the acinar lumina, cytoplasmic vacuolation, cytoplasmic globule formation, and necrosis of the exocrine cells with intra-parenchymal fibrosis.

Similar lesions of the gizzard and pancreas were observed in hens administered 10000 and 20000 mg/kg diet (~31 and 23 mg/kg), zinc induced after only 4 days of treatment. The latter effects were not seen however in hens administered 10000 mg/kg diet for 4 days followed by normal zinc intake for 28 days.

In a 5-week study, 4 week old New Hampshire chicks were administered diets supplemented with zinc oxide, at levels of 57 to 823 p.p.m. (~4.7 to 69.3 mg/kg), for a period of 5 weeks. Examination of body weight gains and feed efficiency showed no observable effects on either parameter, to the age of 9 weeks in chicks.

In a series of oral dose tolerance studies in male White Leghorn Chicks, zinc (oxide, carbonate or sulphate) was administered in the feed at doses ranging from 200 to 3000 ppm (14.8 to 291.6 mg/kg) for the first 4 or 5 weeks after hatching. Results from these studies showed that zinc administered in either of the three forms was well tolerated at doses up to 1000 ppm (54-76 mg/kg) each. However, at a levels from 1500 ppm (~89 to 95 mg/kg) to 3000 ppm (211 to 292 mg/kg) for 4 weeks, all three of the dose forms (ZnO, $ZnSO_4$, and $ZnCO_2$) produced reduced body weight gains (-7 to -32% for ZnO; -18 to -45% for $ZnSO_4$ and -20 to -61% for $ZnCO_2$, relative to average control weight gains of 446 or 441 g) and reduced feed efficiency (i.e. increased feed to gain ratio (15 to 23% for ZnO; 22 to 32% for $ZnSO_4$ and 24 to 86% for $ZnCO_2$) respectively. Increased mortality was observed in one 4 week study with the carbonate form at a dose of 3000 ppm (~292 mg/kg), with death observed in 7 of 30 chicks tested. This data suggest that the oxide was best tolerated followed by the sulphate and finally the carbonate. The 1000 ppm (~54 to 76 mg/kg) dose was the no effect dose in chicks for each of the dose forms tested.

In 16-week oral studies in cross-bred broiler chicks zinc oxide was administered in the feed at doses ranging from 540 to 5000 ppm (~19.23 to 188.5 mg/kg) for the first 10 weeks after hatching, followed by a 6 week recovery period. Results from this experiment showed that zinc in the form of zinc oxide was well tolerated in cross-bred broiler chicks when administered in diets containing 1000 ppm (~35.1 mg/kg) and produced only slight suppression of growth at a level of 2000 ppm (71.51 mg/kg). However, levels $\geq$2391 ppm (88 mg/kg) produced suppressed body weight gain (~10% relative to control gains of 1635 g). Levels >4000 ppm (145.8 mg/kg) also resulted in reduced efficiency of food utilization. Effects on growth and food utilization were fully reversible. The 1000 ppm (35.1 mg/kg) level was the no effect level for the study.

Reproductive Toxicology:

Rats:

Effects of Dietary Zinc Carbonate on Growth, Reproduction and Blood Changes in Rats.

Methods: Young rats (40-50 g) were distributed in groups of 5 (3 females and 2 males/group) and administered zinc carbonate in the diet at concentrations of 0.10, 0.50, and 1.0% (~60, 300, 600 mg/ kg) of zinc for up to 39 weeks. Rats were monitored for effects on growth, reproduction and blood changes.

Results: Growth was reportedly normal for rats on the 0.1 and 0.5% (~60 and 300 mg/kg) Zinc diets. However at the 1.0% (~600 mg/kg) level all rats failed to grow normally, and death occurred in some of these animals within 4 weeks of dosing. Unlike growth, effects on reproduction were observed beginning at the 0.5% (~300 mg/kg) level, with extensive weight loss in the dams following parturition, (30 g in one female and 40 g in another). In addition, 2 of the three females at the 0.5% level had stillborn offspring (1 of 3 in one litter and 5 of 5 in the second). The live fetuses (3 from one litter and 2 from a second) were mothered satisfactorily and weighed almost 30 g at 21 days of age. The F0 females again mated when returned to their cages. However, all pups from the second mating (a total of 23) were still born. After 5 months on the rations, females at the 0.5% (~300 mg/kg) level ceased to become visibly pregnant and reproduction was completely absent at the 1.0% (~600 mg/kg) level. The 0.1% (60 mg/kg) level had no effects on reproduction.

Additional analysis of blood changes in the three groups showed that, neither hemoglobin (Hb) nor red blood cell counts (RBC) were affected at the 0.1% (~60 mg/kg) level. However, after 39 weeks on the 0.5% (~300 mg/kg) zinc diet, rats became anemic with average Hb of only 10.2 g per cent (-28.9% reduction). However, no change in the number of RBC were observed at the 0.5% (~300 mg/kg) level. Reductions in both Hb (-52.6%) and RBC (-28%) were observed at the 1.0% (~600 mg/kg) zinc level. Morphological analysis of the blood cell picture at the 1.0% (~600 mg/kg) level also revealed RBC which were irregular in shape and size, with an apparent excess of white and immature RBC in every case. Both Hb and reproduction became normal in the animals at the 0.5% zinc level (~300 mg/kg), when the zinc carbonate was removed from the diet. The 0.1% (~60 mg/kg) level was the no effect dose for the study.

In conclusion, the current study showed that zinc carbonate, at both the mid (~300 mg/kg) and the high doses (~600 mg/kg) was maternally toxic, producing anemic effects at both doses and reductions in weight gains and death at the high dose. Thus, the

effects of zinc on reproductive function at these doses are confounded by the induced maternal toxicity. However, at the level of 0.1% (~60 mg/kg), which was not maternally toxic, zinc carbonate had no effects on reproductive function.

Oral Segment II Teratology Study with Zinc Sulfate in Rats

Methods: Pregnant albino Wistar Rats (25/group) were orally administered (by gavage) zinc sulfate at doses of 0.0 (control) 0.4, 2.0, 9.1, and 42.5 mg/kg during day 6 through 15 of gestation. The basis of dose selection was not indicated. Dams were observed daily for appearance and behavior with particular attention to food consumption and weights. Body weights were recorded on days 0, 6, 11, 15, and 20 of gestation. On gestation day 20, all dams were subjected to Caesarean section, with determinations of the numbers of implantation sites, resorption sites and live and dead fetuses recorded. Body weights of live pups were also recorded. In addition, all fetuses underwent gross examinations for the presence of external congenital abnormalities, with 1/3 of the fetuses in each litter subjected to detailed visceral examinations, and the remaining 2/3 examined for skeletal defects.

Results: Table 7 on the following page provides a tabulated summary of maternal and fetal observations at the Cesarean Section. As can be seen, administration of zinc sulfate at doses up to 42.5 mg/kg (body weight) for 10 consecutive days during gestation, had no discernable effect on nidation or maternal or fetal survival in rats.

Table 7. <u>**Maternal and Fetal Parameters in Rats at Cesarean Section**</u>

	Sham Control	Zinc Sulfate (mg/kg)			
		0.4	2.0	9.1	42.5
No. of Litters	20	22	22	23	25
No. of Fetuses	225	220	219	66	78
Fetuses Viable1 Non Viable1	11.2 0.05	9.95 0.05	11.9 0.00	11.2 0.00	10.9 0.00
Total Implantations1	11.4	10.4	11.9	11.3	11.0
Total Resorptions1	0.1	0.41	0.05	0.09	0.04
Corpora Lutea1	11.7	11.3	12.3	11.8	12.4
Sex Ratio (M/F)	0.85	1.01	0.98	0.92	0.90
Fetal Wt.(g)	3.88	3.83	3.82	3.82	3.81

1 Values indicate the mean value per litter

The incidence of skeletal findings is summarized in Table 8 below. The data in Table 8 show that, administration of zinc had no effects on the incidence of abnormalities in skeletal tissues compared to the sham-treated control group. The detailed visceral examinations conducted on 1/3 of the F1 offspring revealed no soft tissue abnormalities in the Zinc-treated groups.

Fetal Evaluations:

Table 8. **Summary of the Incidence of Skeletal Abnormalities in Rats**

Dose groups (mg/kg)	Zinc Sulfate (mg/kg)				
	0	0.4	2.0	9.1	42.5
No. of litters	20	22	22	23	25
No. fetuses examined	159	169	167	180	166
Skeletal Variations:	**No. of Fetuses /No. of litters**				
-Sternebrae					
Incomplete Oss.	66/19	77/22	80/21	69/18	95/21
Bipartite	1/1	1/1	1/1	0/0	1/1
Missing	17/8	6/3	8/6	23/9	5/4
-Ribs					
Wavy	16/7	8/3	11/6	7/7	12/5
More than 13	1/1	0/0	1/1	6/3	1/1
-Vertebrae					
Incomplete Oss.	20/10	17/9	10/8	9/7	14/6
-Skull					
Incomplete closure	21/11	16/10	22/9	24/13	20/9
-Hyoid bone					
Missing	19/8	21/10	21/11	29/13	19/10
Reduced	3/3	14/7	17/9	17/10	12/6

In conclusion, administration of dietary zinc sulfate at doses up to 42.5 mg/kg during for 10 consecutive days during the period of organogenesis was not teratogenic in rats.

Rabbits:

Segment II Teratology Study with Zinc Sulfate in Rabbits

Methods: Pregnant Dutch-belted Rabbits (10 to 14/group) were orally administered (by gavage) zinc sulfate at doses of 0.0 (control) 0.6, 2.8, 13.0, and 60.0 mg/kg during day 6 through 18 of gestation. The basis of dose selection was not indicated. Dams were observed for observed daily for appearance and behavior, with particular attention to food consumption and weights. Body weights were recorded on days 0, 6, 12, 18 and 29 of gestation. On gestation day 29, all dams were subjected to Caesarean section, with determinations of the numbers of implantation sites, resorption sites and live and dead fetuses recorded. Body weights of live pups were also recorded. In addition, all fetuses underwent gross and visceral examinations for the presence of external congenital and visceral abnormalities, respectively. All fetuses also were examined for skeletal defects.

Results: Table 9 below provides a tabulated summary of maternal and fetal observations at the Cesarean Section. As can be seen administration of Zinc sulfate at doses up to 60 mg/kg (body weight) for 13 consecutive days during gestation, had no discernable effect on nidation or maternal or fetal survival in rabbits. Fetal weights in all the zinc-treated groups were slightly less (4-14%) than in the sham control rats. However, observed reductions were not dose dependent, and thus not considered treatment related.

Table 9. **Maternal and Fetal Observations in Rabbits at Cesarean Section**

	Sham Control	Zinc Sulfate (mg/kg)			
		0.6	2.8,	13.0	60.0
No. of Litters	11	9	10	10	10
No. of Fetuses	67	71	80	66	78

Fetuses
  Viable1
  Non Viable1
	6.09
	0.00
		6.50
		0.67
			6.58
			0.1
				6.50
				0.1
					7.09
					0.00

Total Implants1	7.0	8.5	8.67	7.4	8.27
Total Resorptions1	0.36	0.80	0.67	0.30	0.45
Corpora Lutea1	9.45	10.7	10.7	9.40	9.50
Sex Ratio (M/F)	0.72	1.10	1.47	1.41	1.05
Fetal Wt.(g)	42.3	40.5	3ℓ.3	40.3	38.4

1 Values indicate the mean value per litter

The incidence of skeletal findings is summarized in Table 10 below. The data in Table 10 show that, administration of zinc had no effects on the incidence of abnormalities in skeletal tissues compared to the sham-treated control group. The detailed visceral examinations only showed the following: 1) Umbilical hernia in one offspring at the 0.6 mg/kg dose, 2) Tripodal; enterohepatocele in a second F1 at the 0.6 and 3) Entero-hepatocele in one F1 offspring at the 13 mg/kg dose. The aforementioned soft tissue abnormalities were sporadic and unrelated to dose and thus, are not considered treatment-related.

**Table 10. Summary of the Incidence of Skeletal Abnormalities in F1
Rabbits**

Dose groups (mg/kg)	0	Zinc Sulfate (mg/kg) 0.6	2.8	13.0	60.0
No. of litters	11	9	10 -	10	10
No. fetuses examined	67	65	79	65	79

Skeletal Variations	No. of Fetuses /No. of litters				
-**Sternebrae**					
Incomplete Oss.	8/6	6/5	10/6	6/4	5/3
Bipartite	1/1	1/1	1/1	1/1	1/1
Fused	2/2	0/0	1/1	·0/0	1/1
Missing	7/4	10/4	5/3	5/2	6/2
-**Ribs**					
Fused/Split	1/1	2/2	1/1	1/1	0/0
Skeletal Malformations					
-**Vertebrae**					
Scrambled	1/1	0/0	1/1	0/0	0/0

In conclusion, administration of dietary zinc sulfate at doses up
to 60 mg/kg, for 13 consecutive days, during the period of
organogenesis was not teratogenic in rabbits.

Mice:

Segment II Teratology Study with Zinc Sulfate in Mice

Methods: Pregnant albino CD-1 out bred mice (21 to 23/group) were
orally administered (by gavage) zinc sulfate at doses of 0.0
(control), 0.3, 1.4, 6.5, and 30.0 mg/kg during day 6 through 15 of
gestation. The basis of dose selection was not indicated. Dams
were observed for observed daily for appearance and behavior with
particular attention to food consumption and weights. Body weights
were recorded on days 0, 6, 11, 15, and 17 of gestation. On
gestation day 17, all dams were subjected to Caesarean section,
with determinations of the numbers of implantation sites,
resorption sites and live and dead fetuses recorded. Body weights
of live pups were also recorded. In addition, all fetuses
underwent gross examinations for the presence of external
congenital abnormalities, with 1/3 of the fetuses in each litter
subjected to detailed visceral examinations, and the remaining 2/3
examined for skeletal defects.

Results: Table 11 below provides a tabulated summary of maternal and fetal observations at the Cesarean Section. As can be seen administration of Zinc sulfate at doses up to 30 mg/kg (body weight) for 10 consecutive days during gestation, had no discernable effect on nidation or maternal or fetal survival in mice.

Table 11. <u>Maternal and Fetal Observations in Mice at Cesarean Section</u>.

	Sham Control	Zinc Sulfate (mg/kg)			
		0.3	1.4	6.5	30.0
No. of Litters	21	22	21	22	22
No. of Fetuses	233	240	238	255	237
<u>Fetuses</u> Viable1 Non Viable1	10.9 0.19	10.4 0.05	11.3 0.00	11.6 0.05	10.8 0.00
Total Implantations1	11.5	11.3	11.5	12.3	11.1
Total Resorptions1	0.43	0.86	0.14	0.68	0.36
Corpora Lutea1	12.3	12.6	11.3	14.0	12.1
Sex Ratio (M/F)	1.04	1.10	1.03	0.90	0.81
Fetal Wt.(g)	0.87	0.89	0.92	0.92	0.87

1 Values indicate the mean values per litter

The incidence of skeletal findings is summarized in Table 12 below. The data in Table 12 show that administration of zinc had no effects on the incidence or quality of abnormalities in skeletal tissues compared to the sham-treated control group. The detailed visceral examinations conducted on 1/3 of the F1 offspring revealed no soft tissue abnormalities in any of the offspring from the groups treated with zinc.

Table 12. **Summary of the Incidence of Skeletal Abnormalities in Mice**

Dose groups (mg/kg)	Zinc Sulfate (mg/kg)				
	0	0.3	1.4	6.5	30.0
No. of litters	21	22	21	22	22
No. fetuses examined	158	169	167	180	166
Skeletal Variations	**No. of Fetuses /No. of litters**				
-**Sternebrae**					
Incomplete Oss.	95/20	76/20	67/19	72/19	94/20
Bipartite	3/3	4/4	3/3	2/2	4/4
Extra	0/0	1/1	9/3	0/0	0/0
Missing	20/7	6/4	16/6	16/8	19/10
-**Ribs**					
More than 13	18/9	43/15	63/15	30/15	29/10
-**Vertebrae**					
Incomplete Oss.	8/3	3/3	1/1	0/0	6/1
-**Skull**					
Incomplete Closure	2/1	1/1	0/0	0/0	5/1
-**Extremities**					
Incomplete Oss.	8/3	3/3	0/0	4/3	6/1
-**Hyoid**					
Missing	57/16	18/9	35/14	30/11	27/13
Reduced	23/13	23/11	18/12	15/13	35/16
-**Pelvic Bone**					
Incomplete	0/0	1/1	0/0	0/0	4/1

In conclusion, oral administration of excess zinc sulfate at doses up to 30 mg/kg for 10 consecutive during gestation was not teratogenic in mice.

Effects of Excess Dietary Zinc Carbonate on Fetal Development and Growth in the Mouse

Methods: The effects of excess dietary zinc during gestation/lactation/and post weaning development were studied in female weanling C57BL/6J mice (21 days old). Briefly, weanling mice were administered diets containing Zinc Carbonate at either 50 ppm (normal control amount, ~7.9 mg/kg) or 2000 ppm (~590 mg/ kg) until mating at 6 week of age. Mated dams and their litters were then distributed to 10 groups which were administered either 50 (normal dietary zinc content) or 2000 ppm zinc during gestation/ lactation/and the post weaning period according to the following: 1) 50/50/50 (control); 2) 50/50/2000; 3) 2000/50/50; 4) 2000/2000/50; 5) 2000/50/2000; 7) 50/2000/2000; 8) 2000/ 2000/2000; 9) 50/50/50 (pair fed to group 8); and 10) chow/ chow/chow. At

approximately 8 weeks of age, F1 offspring were sacrificed, with
determinations of the following parameters: body weights;
hematocrit %, and zinc concentrations in the tibia.

Results: Administration of zinc carbonate (2000/2000/2000;
~590 mg/kg; group 8) during gestation/lactation/and post weaning
development resulted in the following: 1) reduced mean body weights
(-48.24% and -43% in F1 male and female mice respectively compared
to respective control weights of 19.90 and 17.94); 2) increased
zinc content in the bone (from control values of 179.29 μg/g dry
bone up to 661.00 μg/g dry bone); 3) reduced plasma copper
concentrations (from control values of 137.00 μg/ml to 47.67 μg/
ml); and 4) reduced hematocrit (-40.5%). Group 8 F1 offspring also
developed alopecia at approximately 2-4 weeks of age.

By 5 weeks of age, the alopecia was most severe from all mice in
groups 7 and 8. In addition, the skin from the aforementioned mice
was notably thinner compared to control mice. By about 6 weeks of
age hair regrowth began and was complete, but lighter in color by 8
weeks of age. In all other groups, where alopecia was observed,
not all the pups in any one litter were affected and the alopecia
was more moderate. Achromotrichia, was also observed, but again
was most severe in groups 7 and 8.

Hamsters:

Segment II Teratology Study with Zinc Sulfate in the Hamster

Methods: Pregnant out bred golden hamsters (21 to 22/group) were
orally administered (by gavage) zinc sulfate at doses of 0.0
(control), 0.9, 4.1, 19.0, and 88.0 mg/kg during day 6 through 10
of gestation. The basis of dose selection was not indicated. Dams
were observed for observed daily for appearance and behavior, with
particular attention to food consumption and weights. Body weights
were recorded on days 0, 8, 10, and 14 of gestation. On gestation
day 14, all dams were subjected to Caesarean section, with
determinations of the numbers of implantation sites, resorption
sites and live and dead fetuses recorded. Body weights of live
pups were also recorded. In addition, all fetuses underwent gross
examinations for the presence of external congenital abnormalities,
with 1/3 of the fetuses in each litter subjected to detailed
visceral examinations, and the remaining 2/3 examined for skeletal
defects.

Results: Table 13 on the following page provides a tabulated
summary of maternal and fetal observations at the Cesarean Section.
As can be seen administration of Zinc sulfate at doses up to 88
mg/kg (body weight) for 10 consecutive days during gestation, had
no discernable effect on nidation or maternal or fetal survival in
hamsters.

Table 13. **Maternal and Fetal Observations in Hamsters at Cesarean Section.**

	Sham Control	Zinc Sulfate (mg/kg)			
		0.9	4.1	19.0	88.0
No. of Litters	24	22	21	21	21
No. of Fetuses	303	278	253	260	27
Fetuses Viable1 Non Viable1	12.6 0.00	12.4 0.27	12.0 0.5	12.3 0.05	12.9 0.14
Total Implantations1	13.1	13.1	12.2	12.7	13.2
Total Resorptions1	0.45	0.41	0.14	0.29	0.14
Corpora Lutea1	13.2	12.0	11.7	12.6	12.8
Sex Ratio (M/F)	0.64	0.75	0.85	1.06	0.84
Fetal Body Wt.(g)	1.70	1.67	1.71	1.78	1.75

1 Values indicate the mean value per litter

The incidence of skeletal findings is summarized in Table 14 on the following page. The data in Table 14 show that, administration of zinc had no effects on the incidence of abnormalities in skeletal tissues compared to the sham-treated control group. The detailed visceral examinations conducted on 1/3 of the F1 offspring revealed only a single incidence of anasarca in one F1 offspring at the 4.1 mg/kg dose. This latter finding was considered incidental.

Table 14. Summary of the Incidence of Skeletal Abnormalities in Hamsters

Dose groups (mg/kg)	0	Zinc Sulfate (mg/kg)			
		0.9	4.1	19.0	88.0
No. of litters	24	22	21	21	21
No. fetuses examined	211	191	176	178	186
Skeletal Variations	**No. of Fetuses /No. of litters**				
-**Sternebrae**					
Incomplete Oss.	76/21	77/22	61/18	26/14	51/16
Bipartite	39/14	26/13	31/13	19/11	16/13
Missing	44/16	36/17	35/14	13/9	19/11
-**Ribs**					
Fused/split	0/0	0/0	1/1	0/0	1/1
More than 13	43/17	36/13	32/11	31/12	32/14
-**Vertebrae**					
Incomplete Oss.	11/8	5/3	6/6	2/2	4/3
-**Extremities**					
Incomplete Oss.	62/20	81/16	49/17	14/8	30/13
-**Hyoid**					
Missing	4/4	2/2	7/7	1/1	3/2
Reduced	5/4	7/4	9/9	7/5	7/4

In conclusion, administration of dietary zinc sulfate at doses up to 88 mg/kg, for 5 consecutive days during the period of organogenesis was not teratogenic in hamsters.

Ovine:

The Toxicity of Zinc (Form Not Indicated) in Pregnant Sheep.

The toxicity of zinc (form not indicated) at daily doses of 30, 150, and 750 mg/kg diet (~1.0, 5.0 and 20 mg/kg) for 90 days was evaluated in 36 Cheviot sheep (0-6 weeks pregnant). (**Note:** The 30 mg/kg diet; ~1.0 mg/kg, was the control group for the experiment, since this level represents the normal daily intake of zinc in sheep). Results from the aforementioned experiments indicated that administration of zinc at the 20 mg/kg level resulted in increased plasma zinc levels of 6.71 mg/L versus 1.05 mg/L in control animals after 90 days of treatment. The 20 mg/kg doses also produced suppressed weight gains (-65%), food consumption (-23%) and efficiency of feed utilization (-72%), relative to daily gains of 0.26 kg, daily food consumption of 2.1 kg and a feed efficiency of 0.11 in control groups which received normal levels of dietary zinc

(~1.05 mg/kg). The 20 mg/kg dose also produced reduced plasma copper (-45%), ceruloplasmin (-89%) and amine oxidase (-42%) relative to values of 0.98 mg Cu/L; 10.1 U ceruloplasmin/L; and 55.9 U amine oxidase/L in animals which received normal levels of zinc.

The 20 mg/kg dose also resulted in severe impairment of reproductive performance, with numerous abortions and perinatal deaths resulting in 17 of 20 nonviable lambs (nonviable = any lamb which was aborted, still born or died for any reason within 7 days of birth). In comparison, only 3 of 14 and 4 of 23 non viable lambs were observed at the 1.05 (normal zinc levels) and 5.0 mg/kg doses, respectively. None of the deaths in the 1.05 and 5.0 mg/kg groups appeared treatment-related.

Compared to control levels, lambs which died in the 20 mg/kg group showed very high liver zinc concentrations (10 fold increase) and low liver copper concentrations (90% reduction), relative to values of 251.9 mg zinc/kg and 79.8 mg Cu/kg in control sheep. Liver zinc concentrations in lambs from the 20 mg/kg dose group were increased 3 fold relative to controls, with no significant differences in tissue (liver) copper levels. In addition, long bone radiographs from nonviable lambs in the 20 mg/kg diet group showed lines of arrested growth not seen in the 2 lower dose zinc groups. Finally, one nonviable lamb in the 20 mg/kg diet group showed severe renal damage (hyperemia, and extravasation of blood cells, almost complete ischemic necrosis of the cortex, and necrosis of the arcuate and interlobular arteries.

In a separate experiment, the diets of pregnant ewes administered 20 mg/kg zinc, were supplemented with copper (10 mg/kg diet) in an attempt to alleviate the effect of zinc on reproductive performance. Results from this latter experiment showed that copper supplementation prevented the reductions in tissue copper which were observed, but failed to prevent the deleterious effects on lamb viability, with 12 of 14 lambs being non viable in the groups supplemented with copper versus 12 of 12 in the unsupplemented group and only 2 of 11 lambs in a group administered zinc at 30 mg/kg with no copper supplement beyond the 2.5 mg contained in the diet.

The no effect dose, with the exception of increased tissues levels of zinc was 5.0 mg/kg diet.

The Effects of Zinc Sulphate on Pregnant Ewes and Fetal Development.

Zinc sulfate (5 mg/kg/body weight/day) was administered to 2 groups of pregnant ewes (2 ewes/group) either for the first 45 days of gestation (group 1) or throughout gestation (147 and 152 days, group 2). The reported results only indicated that zinc accumulated in the liver (from control values from 171 ppm up to 365 ppm) and possibly the bone (from control values of 69 ppm up to 94 ppm) of the fetal lamb. However, no other treatment-related effects on lambs or on the health of ewes were reported.

Mutagenicity:

Evaluation of Zinc Oxide USP in the Ames Mutagenicity Assay.

Testing Laboratories:

Study Conducted: February 12, 1976

GLP Requirements: No statement of compliance with the GLP regulations and quality assurance unit was included.

Methods: The mutagenic activity of zinc oxide USP at concentrations of 0.4, 0.8 and 1.6% (w/v) (i.e. 5000, 10000 and 20000 ug zinc oxide/plate), was tested using the AMES test; tester strains Salmonella typhimurium TA1537, TA1535, and TA1538 in both the presence and absence of an S-9 mix (metabolic activation system) and at concentrations of 1.25, 2.50, and 5.0% (w/v) (i.e. 15625, 31250 and 62500 ug/plate) in the yeast strain Saccharomyces cerevisiae D4. The highest test concentration used in the current assays was 5.0% (w/v) or the concentration which was previously determined to result in 50% survival, with the mid and low concentrations representing 1/2 and 1/4 of the high concentration. Tests for mutagenicity were conducted using both the plate overlay method and the suspension methods, with and without metabolic activation systems from rat mouse and monkeys. Assay validity was insured by using the following positive controls: Ethylmethane-sulfonate, 2-Nitrofluorene, and Quinacrine mustard (for the non activation assays) and Dimethylnitrosamine, 2-Acety-laminofluorene, 8-aminoquinoline, and 2-aminoanthracine (for the activation assays). Criteria for a positive mutagenic effect were not indicated. However, the "mutagenicity" of zinc oxide USP was assessed in terms of its ability to produce a dose-related increase in the total number of mutants and mutation frequencies in the absence of excess cytotoxic effects (i.e. <25% survival).

Results: In the AMES tests, concentrations of 1.6% (w/v) (20000 ug zinc oxide/plate) resulted 50% reductions in cell survival, whereas 50% reductions in yeast survival were reported at the 5.0 % (w/v) (62500 ug zinc oxide/plate) concentration. Results from the plate assay (overlay method) were negative in all cases, with no increases in the number of revertant colonies/plate observed in any of the salmonella strains with or without metabolic activation. In addition, zinc oxide produced no increase the mutation frequency in the non-activated suspension assays with either Salmonella typhimurium strains or the D4 yeast strain. Further, zinc oxide did not increase the mutation frequency in the activated suspension tests using the Salmonella TA1535 and TA1538 strains or in the D4 yeast strain. Although, zinc oxide did produce an increase in mutation frequency in suspension assays using the TA-1537 strain (primarily detects frame shift mutagens), activated with mouse liver and lung fractions compared to respective homogenate control groups, these latter findings were considered incidental, since activation by liver and lung fractions from both rats and monkeys failed to demonstrate similar effects in the TA1537 strain, respectively. Appropriate response to both positive and negative controls were observed insuring the validity of all assays. Thus, zinc oxide did not exhibit mutagenic activity in the Ames assays reviewed herein.

Evaluation of Zinc Stearate USP in the Ames Mutagenicity Assay.

Testing Laboratories:

Study Conducted: May 10, 1977

GLP Requirements: No statement of compliance with the GLP regulations and quality assurance unit was included.

Methods: The mutagenic activity of zinc stearate USP at concentrations of 0.02875, 0.0575 and 0.115 % (w/v) (i.e. 359, 719 and 1437 ug zinc stearate/plate), was tested using the AMES assay system; tester strains Salmonella typhimurium strains TA1535, TA1537, and TA1538, TA98 and TA100 in both the presence and absence of an S-9 mix (metabolic activation system) and at concentrations of 1.25, 2.50, and 5.0 % (w/v) (i.e. 15625, 31250 and 62500 ug zinc stearate/plate) in the yeast strain Saccharomyces cerevisiae D4. The highest test concentration used in the current assays was 5.0 % (w/v) (62500 ug zinc stearate/plate) or the concentration which was previously determined to result in 50% survival, with the mid and low concentrations representing 1/2 and 1/4 of the high concentration. Tests for mutagenicity were conducted using both the plate overlay and the suspension methods, with and without metabolic activation systems from rat mouse and monkeys. Assay validity was insured by using the following positive controls:

Methylnitrosoguanidine, 2-Nitrofluorene, and Quinacrine mustard (for the non activation assays) and Dimethylnitrosamine, 2-Acetylaminofluorene, 8-aminoquinoline, and 2-aminoanthracine (for the activation assays).

The criteria for a positive test are as follows: 1) for strains, TA1535, TA1537, and TA1538, a solvent control value which is within the normal range and a positive response over three concentrations, with the lowest value equal to twice the solvent control value; and 2) for strains TA98, TA100, and D4: a solvent control value which is within the normal range, a positive response over three concentrations, with the highest value equal to twice the solvent control value. In addition, results will be evaluated based on the pattern of mutagenicity observed (i.e. strains which were derived from the same parental strain; TA1535 and TA100 [both derived from G-46 parent strain] or TA1538 and TA98 [both derived from D3052 parent strain] should show similar responses to a particular mutagen. And finally, a positive response in one test must be reproducible.

Results: In the AMES tests, concentrations of 0.115 % (w/v) (1437 ug zinc stearate/plate), resulted 50% reductions in cell survival, whereas 50% reductions in yeast survival were reported at the 5.0 % (w/v) concentration. Results from the plate assay (overlay method) were negative in all cases, with no increases in the number of revertant colonies/plate observed in any of the salmonella strains with or without metabolic activation. In addition, zinc stearate did not increase the mutation frequency in all non-activated or activated suspension assays with either Salmonella typhimurium strains or the D4 yeast strain. Appropriate response to both positive and negative controls were observed insuring the validity of all assays. Thus, zinc stearate did not exhibit mutagenic activity in the Ames ascays reviewed herein.

In Vivo (Host Mediated Assay) and Direct In Vitro Evaluation of the Mutagenicity of Zinc Sulfate

Testing Laboratories:

Study Dates: Not provided

GLP Requirements: No statement of compliance with the GLP regulations and quality assurance unit was included.

Methods: In the _in vivo_ Host Mediated Assay, 10 mice/group were orally administered zinc sulfate at doses of 2.75, 27.5, and 275 mg/kg once in acute studies and daily for a period of 5 days in subacute studies. The 275 mg/kg dose (the oral LD_5 dose in rats)

was selected as the high dose based on the results of an in vivo (intubation) toxicity study in male rats in which an LD_{50} dose of 920 mg/kg was identified. Negative controls received saline and positive control groups received either Ethyl Methane Sulfonate (350 mg/kg) or Dimethyl Nitrosamine (100 mg/kg). Following dosing, all groups received i.p. injections (2 ml) of the indicator organism (6 x 10^8 cells for Salmonella strains G-46 and TA-1530 and 1 x 10^7 cells for the D-3 diploid strain of Saccharomyces crevisiae). Three hours later, the Salmonella bacteria were withdrawn, plated and examined for the induction of reverse mutations, with determination of the mutation frequency. Likewise, the D-3 diploid strain of Saccharomyces cerevisiae was withdrawn, plated and examined for mitotic recombination, with the recombinant frequency calculated. Animals in the subacute studies were inoculated with the test organism at 30 min after the last dosing and handled in the same fashion as those in the acute study.

The direct in vitro mutagenic effects of zinc sulphate in S. tryphimurium G-46 (concentration not indicated) and TA-1530 and 2.0 % w/v for Saccharomyces cerevisiae D-3) were also examined. In vitro, results from the assays with TA1530 and G-46 were reported only as +, -, or questionable for mutagenic effects, whereas in vitro results from the Saccharomyces cerevisiae D-3 experiments were reported as sample concentrations, percent survival, and recombinants/10^5 survivors.

Results: In the Host Mediated Assay, treatment with zinc sulfate produced no significant increases in mutant frequencies at the dose levels used in vitro or in vivo against either the TA-1530 or the G-46 Salmonella strains. However, in tests using the Saccharomyces D3 strain, zinc sulfate treatment resulted in a dose-related increase in recombinant frequencies in both the acute experiment (from mean recombinant frequencies of 4.06 x 10^{-5} to values of 8.23, 10.36, and 13.22 x 10^{-5} values at the low mid and high doses, respectively) and in the subacute experiment (from mean recombinant frequencies of 4.51 x 10^{-5} to values of 8.85, 12.56, and 117.16 x 10^{-5} values at the low mid and high doses, respectively). In comparison, the mean recombinant frequency in the positive control group from the acute studies was 38.37 x 10^{-5}. Finally, in the in vitro assay using the Saccharomyces D3, treatment with zinc sulfate (2.0% w/v) produced a small increase in the recombinant frequencies, from negative control values of 4 x 10^5 up to 14 x 10^5. In comparison, mean recombinant frequencies in the positive controls were increased to 347 x 10^5 in the D-3 strain.

In conclusion, zinc sulphate exhibited a positive mutagenic effects (increased mitotic recombination) when tested against the Saccharomyces D3 strain in both the in vivo Host Mediated Assay and in the direct in vitro challenge. However, zinc sulphate was not

mutagenic when tested against both the <u>Salmonella</u> TA-1530 and G-46 strains in both <u>in vivo</u> Host Mediated Assays and in direct in vitro challenges.

<u>In Vivo Rat Bone Marrow Chromosomal Aberration Assay and In Vitro Chromosomal Aberration Assay in Human Embryonic Lung Cultures with Zinc Sulphate</u>.

<u>Testing Laboratories</u>:

<u>Study Dates</u>: Not provided

<u>GLP Requirements</u>: No statement of compliance with the GLP regulations and quality assurance unit was included.

<u>Methods</u>: Both in vivo and in vitro chromosomal aberration studies were conducted. In the <u>in vivo</u> studies, 10-12 wk old male albino rats (5/dose group/sacrifice time point) were administered zinc sulfate by intragastric intubation at doses of 2.75, 27.5, and 275 mg/kg, once in acute studies and daily for a period of 5 days in subacute studies. Negative controls received saline and positive controls received Triethylene Melamine (0.3 mg/kg). Animals in the acute studies were sacrificed at 6, 24, and 48 hours after dosing, and at 6 hours after the last dose in the subacute study. Bone marrow cells (arrested in C-mitosis by Cholcemid injected i.p. 4 hours prior to sacrifice) were removed and diploid cells were analyzed for chromatid gaps, breaks, reunions, polyploidy, pulverization and other chromosomal aberrations.

In the <u>in vitro</u> chromosomal aberration studies, human embryonic lung cultures (WI-38) were exposed to zinc sulfate at concentrations of 0.01, 1.0, and 10.0 μg/ml for periods of 24, 48, and 72 hours. The high concentration tested (10 μg/ml was one dose below the cytopathic dose (i.e. 25 μg/ml determined in a dose ranging study). Cells from the in vitro studies were evaluated for mutagenic effects by observing cells in anaphase for chromosomal aberrations (bridges, pseudochiasmata, multipolar cells, acentric fragments etc.) which arise from chromatids which do not migrate to the daughter cells. Such aberrations are sensitive indicators of genetic damage.

<u>Results</u>: Results from the <u>in vivo</u> studies, showed no significant detectable aberrations of the bone marrow metaphase chromosomes in following single dose or 5-day repeated dose oral administration of zinc sulfate in the rat. Negative controls and positive controls produced the expected responses and the mitotic indices were within the normal limits in both the acute and subacute experiments. Examinations of WI-38 cells at anaphase in the <u>in vitro</u> studies, showed that only 1% of the cells treated with zinc sulfate at the mid and high concentrations (0.1 and 1.0 μg/ml, respectively)

levels had bridges. In comparison, 12 % of the cells treated with the positive control (triethylene melamine, 0.1 μg/ml), had bridges and 3% also had acentric fragments. The small incidence of bridges observed at the mid and high doses were not considered biologically significant and thus the zinc sulfate was considered negative for induction of chromosomal aberrations in both the _in vivo_ and _in vitro_ assays.

Evaluation of the Mutagenicity of Zinc Sulfate in the Dominant Lethal Assay in Rats

Testing Laboratories:

Study Dates: Not provided

GLP Requirements: No statement of compliance with the GLP regulations and quality assurance unit was included.

Methods: In the Dominant Lethal Assay, male random bred rats (10/group; 10-12 weeks of age) were orally administered zinc sulfate (by gastric intubation) at doses of 2.75, 27.5, and 275 mg/kg for one day (acute study or for 5 consecutive days (subacute study). The 275 mg/kg dose (the oral LD_5 dose in rats) was selected as the high dose based on the results of an in vivo (intubation) toxicity study in male rats in which an LD_{50} dose of 920 mg/kg was identified. Negative and positive control groups were concurrently administered saline and triethylene melamine (0.3 mg/kg), respectively. Following treatment males were sequentially mated with 2 virgin females/week for 8 weeks (acute study) and 7 weeks (subacute study). Females were killed 14 days after separation from the male and examined for, corpora lutea, early and late deaths, and total implantations.

Results: In the acute Dominant Lethal Assay, treatment of rats with zinc sulfate at the low dose (2.75 mg/kg) was associated with decreased average implantations (week #2) and corpora lutea (week 4), neither of the aforementioned effects were observed in the positive control groups. The positive control group did show a significant increase in the average resorptions (week 2) and in the proportion of females with one or more dead implants and in dead implants to total implants. In the subacute study, a few significant differences between negative controls and experimental groups were observed. However, no strong indications were observed. Positive controls were not included in the subacute assay. The lack of any dose relationship and the sporadic nature of the differences in the groups treated with zinc, indicate that zinc sulfate was non-mutagenic in the Dominant Lethal Assay in rats at doses up to 275 mg/kg.

In conclusion, the zinc acetate salt has not been tested for its mutagenic potential. However, the mutagenic potential of zinc oxide and or other zinc salts (as specified) have been tested in the following assay systems: 1) the Ames assay with and without metabolic activation (ZnO and Zn Stearate); 2) In vivo acute and subacute Host Mediated Assays and direct in vitro assays with Salmonella strains G-46 and TA-1530 and Saccharomyces cerevisiae D4 strain (zinc sulphate); 3) In vivo rat bone marrow chromosomal aberration assay in rats and in vitro chromosomal aberration assay in human embryonic lung cells (zinc sulphate); and 4) the Dominant lethal assay in rats (zinc sulphate). Zinc oxide and the indicated zinc salts tested negative for mutagenic activity in all but the Saccharomyces cerevisiae D4 strain in the acute and subacute Host Mediated and the direct in vitro assays. In all of the latter instances with the Saccharomyces D4 strain, zinc sulphate exhibited positive mutagenic effects (increased mitotic recombination). In regard to these positive findings another in vivo study in rats indicated that orally administered zinc chloride results in increased binding of zinc to DNA bases in various organs, an effect which could interfere with base pair binding producing a destabilization of the helix (See Special Toxicology Section below).

Special Toxicology Studies:

Induction of Clinical Manifestations of Pantothenic Acid Deficiency by Chronic Administration of Zinc Chloride.

Methods: Control female rats (Group 1; 21 to 24 days of age weighing around 40 g; no. of rats not indicated) were administered synthetic diets suboptimal only in pantothenic acid (Supplied by 100 mg of Labco Rice Polish factor II) for a period of 20 weeks. Three additional groups of rats (albino, white or black rats as specified) received the same diet as controls, but supplemented with the following as specified: Group 2 (eight albino rats) addition of 4 mg of zinc chloride dissolved in cod liver oil and administered orally; Group 3 (6 black and 8 white rats), addition of 5 to 6 mg of zinc chloride, dissolved in olive oil and administered orally; and finally Group 4 (6 groups of white rats, 4 rats/group) addition of a daily dose of synthetic calcium pantothenate 100 μg/day and varying amounts of Zinc chloride initially at a doses ranging from 4-15 mg, but increased to 75, 100, 125, 150, 200, and 250, mg/kg in the fourth week.

Results: Administration of the diet with suboptimal amounts of pantothenic acid in control animals resulted in weekly gains of around 12-15 g, which were some what less than those reported for

rats fed diets with optimal levels of pantothenic acid. In addition, Group 1 control rats showed some rusting of the fur which cleared up spontaneously, but no skin lesions (alopecia, crusting of the snout, with blood caked whiskers, ruffled and matted fur, redness of the skin hemorrhagic ears and crusted tails,) commonly associated with pantothenic acid deficiency.

At 3 to 5 weeks after the beginning of the experiment, Group 2 rats showed retardation of the growth and various skin manifestations (severe alopecia, severe rusting of the fur, ruffled appearance, crusting of the nose and chins and eyelids) and death in 1 of 8 rats at 5½ weeks. These signs are commonly associated with pantothenic acid deficiency. Addition of pantothenic acid to Group 2 rats (at the end of week 6) resulted in immediate weight gains (12 to 27 g for the first week) and reversal of all the signs of deficiency within 3 weeks.

At 4 to 5 weeks after the start of treatment, black rats in group 3 showed severe greying of the fur, while severe rusting of the fur was seen in the white rats along with manifestations (skin lesions) of pantothenic acid deficiency similar to those seen in Group 2 animals.

Administration of Zinc Chloride (4-15 mg/day) in Group 4 rats which received the pantothenic acid supplement showed weekly body weight gains of around 20 g and hardly any signs of toxicity during the first 3 weeks of treatment. Therefore, Zinc Chloride was increased in these animals to levels of 75, 100, 125, 150, 200, and 250 mg/kg during week 4. By week 6, the increased doses of zinc chloride caused weight curves to plateau or decline and produced rusting of the fur in most animals, but was not associated with skin lesions seen in other groups. However, the high doses of zinc chloride (dissolved in oil) did produce caustic and nauseating effects. Therefore, beginning at the end of week 6, the desired amount of the zinc chloride was incorporated into 5 g of the food mixture and fed to the animals daily. This latter change resulted in a prompt increase in weight gains over the next two weeks at which time the study was terminated.

In conclusion, the current study showed that administration of zinc chloride could initiate clinical manifestations of pantothenic acid deficiency in rats maintained on diets with sub optimal levels of pantothenic acid. Although, the exact nature of this interaction was not evident, a direct toxicological effect was not indicated, since dietary supplementation with additional pantothenic acid could reverse the observed effects.

The Formation of DNA-Zinc Products Following Oral Administration of Zinc Chloride in Rats

Methods: The in vivo interaction of Zinc with DNA from liver, kidney, ileum, colon and brain of rats were studied following repeated administration of zinc (23 ppm; ~3.5 mg/kg/day) to rats (n=4) in their drinking water for periods of 1, 3, or 7 days (**Note**: On average each rat drank 21 ml of water/day, corresponding to 7.4 μmol Zn/day). At the end of each test period rats were sacrificed and the DNA from liver, kidney, ileum, colon and brain was isolated, hydrolysed and the purine bases were separated using a Sephadex G-10 column. The DNA products of metalation were then eluted.

Results: Administration of Zinc (23 ppm) in the drinking water resulted in increased Zn in the DNA of liver, kidney, ileum, colon and brain. Analysis of the purine bases from various organs of rats yielded 3 distinct Zinc-containing peaks (B, C, and D), none of which were chemically identified. The major product (Peak D) eluted after adenine. Maximum levels of Zinc in peak D occurred on day 1 for colon and liver, and on day 3 for ileum, but were highest on day 7 in brain and kidney (suggestive of possible accumulation in these latter two organs). By Day 7, Zinc levels in DNA from colon and ileum had returned to control levels and was nearing control levels in DNA from liver, despite continued administration of Zinc. This study showed that administration of zinc in vivo resulted in increased binding of Zinc to DNA in various organs of rats. Such binding of Zinc to DNA bases could disrupt base pair bonding and lead to a destabilization of the helix in order to accommodate the zinc ion. This latter effect could in turn lead to a carcinogenic result. In regard to this latter possibility, no conventional carcinogenicity studies with either zinc acetate of other zinc salts have been performed. However, in one 45 to 53 week study in mice, dietary administration of zinc oleate at doses of ~624 mg zinc/kg for 3 months, reduced to 312 mg zinc/kg for the next 3 months and then to 156 mg zinc/kg for another 6 months or addition of zinc sulphate to the drinking water at doses of 81 and 405 mg/kg for periods of one year each produced no carcinogenic effects.

Carcinogenicity Studies

Mice

45 to 53-Week Oral Carcinogenicity Study with Zinc (Oleate and Sulphate) in Mice (Walters, M and FJC Roe, Food Cosmet. Toxic. 1965; 3:271-276)

Methods: Zinc oleate was added to the diets of lactating mothers of and subsequently to their weaned litters at initial doses of 50 g/kg for 3 months, which was reduced to 25 g/kg for 3 months, then to 12.5 g/kg for 6 months, (calculated zinc ion levels were ~5.2 g/kg, 2.6 g/kg and 1.3 g/kg of the diet or ~624, 312, and 156 mg zinc/kg/day). (**Note:** reductions in the dose were due to deaths from anemia). Other groups received Zinc sulphate (1000 and 5000 ppm; ~81 and 405 mg/kg) in their drinking water for periods of 45-53 weeks. Additional weanling mice were added to the control group (tap water and basic diet) and to the dietary zinc groups, due to death or sacrifice of numerous mice in these groups following an outbreak of ectromelia. Animals were examined weekly and weighed every 2 weeks. Mice which died or were killed after 45 to 53 weeks of treatment were subjected to a thorough post mortem, including histological examination of any possible neoplastic lesions and macroscopic and microscopic examination of the stomach for tumors, or other changes. No further information on the methodology was provided.

Results: Excessive mortality (30 deaths) was observed in the group receiving dietary zinc oleate. Most of the deaths which occurred within the first 8 weeks were due, directly or indirectly to an outbreak of ectromelia, while the development of marked anemia was responsible for 17 of the 30 deaths which occurred between week 4 and week 15 (i.e. blood sampled from the orbital sinus of 4 randomly selected zinc oleate mice had a hemoglobin content which was only 40% of the control level). The effects of zinc administration on the incidence and types of tumors in mice is presented in Table 15, on the following page.

Table 15. Incidence and Types of Tumors in Mice Surviving 45-53 Weeks of Zinc Treatment (adapted from Walters and Roe, 1965)

Dose of Zinc Ion (mg/kg)	# examined	No. of Mice; original or (weanling)* with		
		Hepatoma	Malignant lymphoma	Lung adenoma
0 (Control)	24	1 (2)	2 (1)	8 (2)
81[1]	28	3	4	9
405[2]	22	3	2	5
624 to 156[3]	23	1 (6)	2	9

* No. of tumors in originally treated or (added weanling mice). Control; n = 19 original and 5 weanling mice
[1] ZnSO4 in drinking water
[2] ZnSO4 in drinking water
[3] Zinc Oleate in diet; n = 11 original and 12 weanling mice

Feeding of zinc oleate in the diet at levels of 1250 to 5000 ppm (~156 to 624 mg/kg) failed to increase the incidence of malignant lymphoma or pulmonary adenoma above control levels. The incidence of hepatomas were increased in the group which received dietary zinc to 7 of the 23 total mice in this group versus 3 of 24 controls. However, the observed increase was not equally distributed among the original versus weanling groups (i.e. 1 versus 6, respectively) and no increase incidence of hepatomas was observed in the mice which received a comparable dose of zinc (~405 mg/kg) in their drinking water over the same duration. Thus the biological significance of this latter occurrence is doubtful.

In conclusion, no definitive evidence of a zinc induced carcinogenic effect was observed in mice following oral administration of zinc oleate (~156 to 624 mg/kg) in the diet or zinc sulphate (~81 to 405 mg/kg) in the drinking water for periods of 45-53 weeks. However, the usefulness of the current study is limited in that the duration of administration currently employed was only 45-53 weeks, which is well below the 1 ½ to 2 years duration employed in conventional carcinogenicity studies in mice.

LABELING:

The annotated labeling of Zinc Acetate generally conforms to the format specified under CFR 21, subpart B, 201.50 to 201.57, dated April 1, 1992. However, the following changes should be made.

1) The Sponsors heading Titled **"Carcinogenesis and Mutagenesis"** should be titled: "CARCINOGENESIS and MUTAGENESIS AND IMPAIRMENT OF FERTILITY". In addition, the Sponsor's statement under this section only states the following:

   " In-vivo or in-vitro studies in laboratory species have demonstrated no undue risk of carcinogenic or mutagenic effects from zinc."

   This statement should be changed to incorporate a complete picture of the carcinogenic and mutagenic effects which have been reported for zinc, along with available information regarding the reported effects of zinc on reproduction. Rewritten, this section should state:

   "No conventional oral carcinogenicity studies with zinc acetate, zinc oxide or other zinc salts have been conducted. However, an available one year oral toxicology study in mice showed no definitive evidence of a zinc induced carcinogenic effect following administration of zinc oleate (~156 to 624 mg/kg; 372 to 1872 mg/m^2) in the diet or zinc sulphate (~81 to 405 mg/kg; 243 to 1215 mg/m^2) in the drinking water for periods of 45-53 weeks. The aforementioned doses·are 2.2 to 17 fold greater than the proposed human dose of 150 mg/day (111 mg/m^2).

   The zinc acetate salt has not been tested for mutagenicity. However, studies on the mutagenicity of zinc oxide and/or other zinc salts (as specified) have ben conducted. These latter studies showed that zinc tested negative for mutagenic activity in the following assay systems: the Ames assay, with and without metabolic activation (ZnO and Zn Stearate); Host Mediated and In Vitro Assays with <u>Salmonella</u> strains G-46 and TA-1530 (zinc sulphate); Chromosomal Aberration Assay in rats and In Vitro chromosomal aberration assay in human embryonic lung cells (zinc sulphate); and finally in the Dominant lethal assay in rats. In contrast, zinc sulphate tested positive for mutagenicity against the <u>Saccharomyces</u> cerevisiae D4 strain in two Host Mediated Assays and in a direct in vitro assay, suggesting a propensity of zinc to increase mitotic recombination. In a related study in rats, orally administered zinc chloride was shown to bind to DNA bases in various organs. Such an effect could interfere with DNA base pair binding leading to destabilization of the DNA helix.

Studies on the effects of zinc on fertility and reproductive performance were conducted in rats and sheep. In one rat study, zinc administered orally as the oxide, acetate, citrate, and maleate, at doses up to 38 mg/day (~ 152 mg/kg) for up to 29 weeks prior to and continuing through mating, pregnancy, and lactation had no effects on fertility or reproductive performance (Thompson, PK et al., <u>Amer. J. Physiol.</u> 1927, 80:65-74). Similarly, a second study in rats showed no adverse effects on fertility following oral administration of zinc (0.25%, ~163 mg Zn/kg) as the oxide chloride, carbonate, and sulphate) in tne diets through several generations (Heller VG and AD Burk, <u>J. Biol. Chem.</u>, 1927, 74:85-93). Although, higher oral doses of zinc carbonate (0.5% and 0.1% of the diet,~300 and 600 mg/kg) have been reported to inhibit reproduction in rats, these doses were also associated with substantial maternal toxicity (Sutton, WR and VE Nelson <u>Proc. Iowa Acad. Sci.</u> 1937, 44:117-121). Finally a study in sheep indicated that doses as low as 20 to 10 mg/kg produced maternal toxicity and severe impairment of reproductive performance, whereas doses ~5 mg/kg or less had no effects on either parameter Cambell and Mills, <u>Environ. Res.</u> 1979; 20:1-13)".

2) Under TERATOGENIC EFFECTS: PREGNANCY CATEGORY C, The Sponsor States the following:

"Pregnancy Category B (No evidence of risk in humans). The pregnant patient should continue anticopper therapy since copper toxicosis can develop during pregnancy if anticopper therapy is stopped. Zinc therapy is a reasonable option to the chelating agents, penicillamine and trientine, because these drugs are teratogenic. Zinc has been shown to have no teratogenic effects in laboratory species. As a precaution, it may be wise to reduce the dose of zinc to a minimally effective dose of 25 mg three times daily as long as the patient is compliant with therapy."

The aforementioned statements are not in accordance with the prescribed format as outlined in CFR 21, subpart B, 201.57, dated April 1, 1994. Therefore, this section should be revised to read as follows:

"Reproduction studies have been carried out with the zinc sulphate salt in rats (at doses up to 42.5 mg/kg; 250.8 mg/m^2); mice (at doses up to 30 mg/kg; 90 mg/m^2); rabbits (at doses up to 60 mg/kg; 514.2 mg/m^2) and hamster (at doses up to 88 mg/kg; 470.1 mg/m^2) and have revealed no harm to the fetus due to zinc. Compared on a mg/m^2 basis the ratios of doses in rats, mice, rabbits and hamsters to the proposed daily human dose of 150 mg/day (i.e. 111 mg/m^2) are 2.3, 0.81, 4.6 and 4.24

respectively. There are however, no adequate and well
controlled studies in pregnant women. Because animal
reproduction studies are not always predictive of human
response, this drug should be used during pregnancy only if
clearly needed."

SUMMARY AND EVALUATION:

Zinc is an essential element in the nutrition of humans, animals,
and plants. It is an integral component of a number of
metalloenzymes including carbonic anhydrase, alcohol dehydrogenase,
carboxypeptidase, glutamic dehydrogenase, lactate dehydrogenase,
and alkaline dehydrogenase. The acetate salt of zinc is a soluble
zinc form which provides elemental zinc for absorption following
oral administration. In vivo studies in rats have shown that once
absorbed in the intestinal cell, elemental zinc induces the
production of metallothionein (MT), a protein which binds copper
and prevents its serosal transfer into the blood. The bound copper
is then lost in the stool when the intestinal cell is sloughed.
The aforementioned effects would prevent the absorption of copper
from the diet and the reabsorption of endogenously secreted copper
such as that from the saliva and gastrointestinal juices.

Thus, orally administered Zinc acetate has potential therapeutic
use in the treatment of patients with Wilson's Disease, an
autosomal recessive disease, associated with increased copper
absorption and a deficiency of the α_2-Globulin fraction of the
plasma proteins to which circulating copper is normally tightly
bound.

Two drugs already approved for the treatment of Wilson's Disease
include: penicillamine and trientine. However, penicillamine has
been associated with serious adverse hematologic and renal
reactions and trientine has been associated with iron deficiency
and systemic lupus erythematosus. In comparison, orally
administered zinc has been associated with relatively few side
effects, which would offer advantage over the currently approved
agents.

In the present New Drug Application, the Sponsor proposes to use
Zinc Acetate (25 and 50 mg gelatin capsules) for the following:
1) the maintenance treatment of patients with Wilson's disease
(hepatolinticular degeneration) who have been initially treated
with a chelating agent-penicillamine, trientine or ammonium, tetra-
thiomolybdate

The available preclinical information submitted in support of the application consisted almost entirely of reports from the literature and only 6 of the studies utilized the acetate salt form of zinc which is currently proposed for clinical use. Studies which used zinc acetate included: 1) An in vitro pharmacology study on the effects of zinc on ion transport by heart mitochondria; 2) distribution and excretion studies in rats; 3) an acute oral toxicity study in rats; 4) a 35 to 53 week oral toxicity study in rats; 5) a study on the effects of orally administered zinc acetate, maleate or citrate on fertility, reproduction, and growth in rats and 6) a special 2-month oral pancreatic toxicity study in rats.

Other studies currently submitted in support of the current application utilized various other forms of zinc (oxide or salts: carbonate, chloride, citrate, maleate, nitrate, oleate, stearate or sulphate) and included: (1) pharmacology, (2) ADME studies including: absorption studies with zinc chloride in rats, mice, dogs, and humans, with zinc carbonate in rats, and with zinc oxide, zinc carbonate, and zinc sulphate in chicks; distribution studies with zinc oxide and zinc chloride in rats; a metabolism study with zinc carbonate in rats; and excretion studies with zinc oxide, zinc citrate, and zinc chloride in rats and with zinc oxide in dogs; (3) toxicity studies in rats including: a 4-week oral study with zinc sulphate, a 5- to 6-week oral study with zinc carbonate; a 6-week oral toxicity study with zinc chloride, a 6- to 15-month oral toxicity study with zinc chloride, a 21-month oral toxicity study with zinc sulphate, and a 3 generation oral toxicity study with zinc oxide and organic zinc salts (4) a 1 year·oral toxicity study with zinc sulphate in mice; (5) toxicity studies in dogs including: a 3- to 19-week oral study with zinc oxide and a 70-week oral study with zinc sulphate; (6) a 10- to 53-week oral toxicity study in cats; (7) toxicity studies in swine including: a 6-week oral toxicity study with zinc carbonate in weanling pigs and a 27-week oral toxicity study with zinc sulphate; (8) a 6- to 10-week oral toxicity study with zinc oxide in lambs; (9) 4- to 10-week oral toxicity studies with either zinc oxide sulphate or carbonate in chickens; (10) effects of oral zinc carbonate on growth, reproduction and blood changes in rats (11) Oral Segment II teratology study in rats; (12) Oral Segment II Teratology Study with Zinc Sulphate in rabbits; (13) Oral Segment II Teratology Study with Zinc Sulphate in mice; (14) Effects of Excess Dietary Zinc Carbonate on Fetal Development and Growth in mice; (15) Oral Segment II Teratology Study with Zinc Sulphate in hamsters; (16) Toxicity of Zinc (form not indicated) in Pregnant Sheep; (17) Effects of Zinc Sulphate on Pregnant Sheep and Fetal Development; (18) Mutagenicity of Zinc Oxide in the Ames Assay; (19) Mutagenicity of Zinc Stearate in the Ames Assay; (20) In vivo bone marrow chromosomal aberration assay in rats and in vitro chromosomal aberration assay using human embryonic lung cultures; (21) Host Mediated Assay in Mice and direct in vitro mutagenicity

assay; (22) Dominate Lethal Assay in Rats; (23) Special toxicity studies including: induction of pantothenic acid deficiency by chronic administration of zinc chloride in rats and the formation of DNA-zinc products following oral administration of zinc in rats; and finally a 1-Year oral toxicity "Carcinogenicity" Study with Zinc (Oleate and Sulphate).

The majority of the available ADME information was obtained from studies which utilized zinc oxide or one of the other organic zinc salts. One such study in rats showed that zinc is primarily absorbed from the duodenum. The relative absorption of orally administered Zn^{65} chloride in mice, rats, dogs, and humans was approximately 13.2%, 23%, 48.4%, and 54.8%, respectively. Studies in rats and chicks showed that the absorption of zinc was reduced, when administered in combination with phytic acid (a soy protein component). Some reports in rats also showed that calcium and phosphorus reduced the absorption of oral zinc. However, others showed no such effects. In rats, peak tissue concentrations of Zn^{65} were reached within 5 days after oral dosing. Distribution studies in rats, cats, and dogs showed that highest levels of zinc were attained in liver, gall bladder, gastrointestinal tract, kidney, bone, bone marrow, and pancreas following oral administration. In cats, tissue levels in all of the aforementioned organs quickly return to normal levels (by 2-weeks) when zinc dosing was discontinued, whereas in rats, elimination from bone and pelt was delayed. However, no evidence of tissue accumulation was observed in rats following repeated dosing. The elimination of Zn^{65} in humans, dogs, rats, and mice was triphasic, with half life values for the terminal phase of 91 days, 75 days, 94 days and 154 days for mice, rats, dogs, and humans, respectively. In all of the said species, the majority of excretion occurred via the feces, with only a small fraction excreted in the urine.

The available information regarding the pharmacokinetic behavior of zinc from zinc acetate was limited to cne 35-53 week distribution and elimination study conducted in rats. The latter study only indicated that repeated oral dosing with zinc acetate (7.6 to 25.2 mg zinc/kg) for periods up to one year showed no signs of accumulation or tissue specific storage of zinc and that the feces was the major route of elimination following oral dosing. Although the same study reported similar findings in rats which were administered zinc oxide (10.8 to 136.8 mg zinc/kg), zinc citrate (38.8 mg zinc/kg), or zinc maleate (44.4 to 66 mg zinc/kg) for comparable periods of time, no data for direct comparisons between salts were provided. However, since zinc acetate is a soluble salt form of zinc, the ADME data for Zinc derived from the acetate would be expected to parallel that of zinc derived form other soluble forms of zinc (i.e. sulphate, oxide or carbonate and chloride). As

a corollary, the toxicological effects of zinc derived from other soluble forms of zinc should provide a reasonable estimate of the toxicological effects which would be expected for zinc derived from the acetate salt.

As was indicated, only four studies concerning the toxicological effects of zinc acetate were available. The information provided in these studies was insufficient to directly assess the toxicological profile of the zinc acetate salt.

For instance, information on the acute toxicity of zinc acetate only identified an oral LD_{50} value of 2.46 g zinc acetate/kg in rats, with no other information provided. In the other three zinc acetate studies (summarized below), either the doses tested were two low to elicit toxicological effects, two few animals were tested, or the focus of the study was two narrow.

In a 35- to 53- week toxicity oral study in rats, the effects of zinc acetate (~7.6 to ~17.6 mg/kg, p.o.), on body weights, food consumption, hematology (red and white cell counts and percent hemoglobin) and gross or microscopic changes were evaluated. No effects on any of the aforementioned parameters were observed. However, this was not a very useful study, since in most cases only 1-3 rats/dose level were tested and since the doses of zinc acetate tested (~7.6 to ~17.6 mg/kg) were not high enough to elicit toxic effects.

In an oral reproductive toxicity study in rats, the effects of zinc acetate on fertility, reproduction, and early growth of the F1 offspring were evaluated following oral administration at doses of 2 to 38 mg zinc/day (8 to 17.6 mg zinc/kg) for many weeks prior to mating, during pregnancy and lactation. No effects on any of the said parameters were observed. In addition, the study indicated that treatment of F1 offspring with zinc acetate at the same levels as the parents received (post delivery Day 23 through Day 60) had no apparent effects on their survival growth or maturation. This study like the previous one was of limited value in that only a couple of F0 rats/sex were tested and the doses tested were not high enough to elicit any toxicological signs.

Finally, in a special 2-month oral "pancreatic" toxicity study in rats, zinc acetate was administered at total daily doses of 5.7, 28.5 and 57.1 mg/kg (2 divided doses/day). The focus of the study was essentially limited to effects on the pancreas (gross and microscopic pathology) and no signs of pancreatic toxicity were observed. The study also indicated that rats continued to grow and no diarrhea was observed. However, no data regarding the latter

parameters were provided. Thus, the usefulness of the study in defining the toxicological profile of zinc acetate was limited, since essentially only effects on the pancreas were evaluated and in that at the doses tested no toxicity was observed.

The numerous other toxicology studies currently submitted used either zinc oxide or zinc salts, other than zinc acetate. In general, these studies were also flawed or limited in their design with regard the following: 1) only effects on a limited number of toxicological parameters were examined per study; 2) the doses tested were not high enough to elicit toxicological effects; and 3) the number of animals studied were insufficient for drawing firm conclusions. Brief summaries of these latter studies are provided below.

In a preliminary 6-week oral toxicity study in weanling rats, the effects of zinc carbonate (0.4% to 1.0% of the diet; ~1200 to 3000 mg/kg) on survival and hemoglobin levels were assessed. In two follow up studies of 6- and 5-weeks duration in weanling rats, zinc carbonate was administered at levels of 0.7%; ~2916 to 1479 mg/kg (6-week study) or 1.0% of the diet; 3571 to 1829 mg/kg (5-week study) either alone or in combination with iron, copper, cobalt and/or liver extract in order to study the mechanism(s) of zinc's induction of anemia and suppression of growth. In the preliminary study, at levels $\geq$ 0.7% of the diet (~2100 mg/kg), zinc carbonate produced hypochromic anemic effects and suppression of body weight gains after 3-5 weeks of dosing, while at the 1.0% dose (~3571 to 1829 mg/kg), zinc carbonate was associated with complete inhibition of growth and death in approximately 75% of the animals. No toxic effects were reported for the 0.4 % dose level (~1200 mg/kg). The 6- and 5-week studies showed that supplementation with copper and liver extracts ameliorated the effects of zinc on hemoglobin and body weights, respectively. However, the usefulness of the study was limited in regard to the following: 1) only doses of 0.7% and 1.0% zinc carbonate were evaluated in the two main studies; 2) no untreated control groups were utilized in the 6-week main study; and finally, 3) the toxicological focus of the study was limited to zinc's effects on mortality, growth and hemoglobin concentrations.

In a 6-week oral toxicity study in male rats, the effects of zinc oxide at a dose of 0.5% (~961 to 556 mg/kg) on body weights, food consumption, hemoglobin, organ weights for kidney and liver and tissue copper and fat content were assessed. Zinc-treatment resulted in reduced food consumption, suppressed body weight gains, reduced hemoglobin levels, reduced tissue copper levels and reduced body fat. Additional data suggested that the zinc-induced suppression of body weight gains occurred secondary to reduced food consumption and zinc-induced reductions in tissue copper levels, whereas reductions in hemoglobin levels could be partially reversed

by supplementing the diet with copper. Although, in general this study was well controlled, its usefulness was limited in that only one dose level of zinc was tested and only a limited number of parameters were examined.

In a 14-week oral toxicity study in rats the effects of zinc sulphate (1000 mg/kg diet; ~60 mg/kg) on body weights and tissue copper levels were examined. Zinc treatment had no effects on body weights or tissue copper levels, but was associated with a lower uptake of ^{64}Cu in blood and red bone. The study was of limited value in that only effects on weight gains and tissue copper levels were evaluated and the single dose of zinc was tested was not high enough to elicit effects on either parameter.

In a 6- to 15-month oral toxicity study in rats zinc chloride was administered at doses of 60 and 120 mg/rat/day (~240 and 480 mg/kg) for periods of 15 months or at a dose of 600 mg/rat/day (~2400 mg/kg) for a period of 6 months. Effects on clinical signs of toxicity, body weights, food consumption were monitored and gross examinations were conducted. The 240 and 480 mg/kg doses had no effects on any of the aforementioned parameters. However, at the 600 mg/day dose (~2400 mg/kg), zinc produced reduced body weights, general malaise, staring coat, huddled posture and death in 19 of 25 rats, beginning at 14 days after the start of the treatment. The intestines were identified as a target organ of toxicity showing gross lesions of discoloration (black to grey slimy character). The dose levels tested were sufficient to elicit toxicity and adequate numbers of animals were tested. However, most of the information provided was only qualitative in nature no incidence data and no information on effects on hematology, clinical chemistry or histopathology was available, thus limiting the usefulness of the study.

In a 21-month oral toxicity study in rats the effects of zinc sulphate (100, 500, and 1000 ppm; ~60, 30, and 6 mg/kg) for a period of 21-months, on body weights, food consumption, hematology, bone marrow smears, gross and microscopic pathological analysis. Zinc treatment induced transient hematological effects (microcytosis, polychromasia and/or hyperchromia) in all dose groups which spontaneously resolved, despite continued treatment. Treatment-related toxicological effects were limited to reduced myeloid to erythroid ratios (all dose levels) and increased severity of spontaneous nephritis or nephrosis in males at the 30 and 60 mg/kg doses. No tumorigenic effects were noted at any dose tested. In general the study appeared to be well conducted. However, the doses tested appeared too low to elicit frank toxicological effects, thus limiting the usefulness of the study.

In a three generation oral toxicology study F0 and F1 generations
of rats were administered excess dietary zinc (as the oxide,
chloride, carbonate, and sulphate) at levels of 0.25% (~163 mg
zinc/kg) or as the oxide and chloride 0.5% (~326 mg zinc/kg) for a
period of ~6-12 weeks, for determination of zinc's effects on
growth, clinical signs, gross pathology, and tissue zinc levels.
The only observed treatment related effects reported was a
reduction in body weights in the F1 offspring treated with zinc
oxide at the 0.5% (326 mg/kg) dose. The 0.25% dose (all zinc forms
tested) had no effects in either the F0 or F1 generations. The
usefulness of the current study was limited in regard to the
following: 1) the doses tested were not high enough to elicit
toxicity; 2) the majority of the information provided was
qualitative in nature; and 3) evaluations of the effects of zinc
were limited to effects on clinical signs, body weight gains, and
gross pathological observations.

In a one year oral toxicity study in mice, the effects of zinc
sulphate, at levels of 0.5 g/l (100 mg/kg) for up to 14 months on
zinc levels and endocrine function (i.e., plasma insulin and
glucose levels, histological and/or electron microscopic assessment
of adrenals, pancreas, and hypophysis cerebri and adrenal
cholesterol content). Zinc treatment produced no clinical signs of
toxicity, no adverse effects on body weights and no effects on
plasma insulin or glucose levels. Only qualitative descriptions of
the histological and/or electron microscopic findings were
provided, with no incidence data available. Reported, histological
effects included hypertrophy, vacuolization increased cholesterol
deposition in the adrenal cortex; hypertrophy and increased
granulation of the beta islet cells of the pancreas and
hypertrophy, increased synthetic and secretory activity
[granulation] of ACTH cells in the anterior lobe of the pituitary.
However, in the absence of corresponding effects on body weights or
plasma insulin levels the toxicological significance of the
aforementioned findings was not apparent. Thus the current study
suggested that long term administration of zinc can affect
endocrine function in the mouse. However, overall the study was of
limited use in that only a limited number of parameters were
evaluated and since the only dose tested was not high enough to
elicit toxicity.

In a 3- to 19-week oral toxicity study, three dogs received zinc
oxide in the diet at doses of 36.1, 59.9, and 76.5 mg zinc/kg for
periods of 19, 3, and 15 weeks, respectively. Effects of zinc on
clinical signs, body weights, urinalysis, hematology, tissue zinc
levels were determined and complete gross and histological
examinations were conducted. One dog died of distemper pneumonia
during week three and in the other two dogs, zinc carbonate had no
effects on body weights, hematological parameters (RBC counts, WBC
counts or hemoglobin levels), or urinalysis and produced no
treatment related gross or histological lesions. The usefulness of

the current study was limited in that a total of only 2 dogs, each at a different level of zinc were available for evaluation and in that neither of doses tested were not high enough to elicit toxicity.

In a 70-week oral toxicity study in four immature Dalmatian puppies, the effects of zinc sulphate on blood and bone marrow were examined. Zinc sulfate was administered by capsule at an initial dose of 200 mg/kg, which was reduced to 100 mg/kg after 7 weeks, and then further to 50 mg/kg after 3 or 32 weeks at the 100 mg/kg dose as a result of severe emesis. One moribund dog which had exhibited weight loss, was sacrificed in extremis at one week after reduction to the 50 mg/kg level. The remaining, dogs showed normal appetite and growth until sacrifice at the end of the 70-week treatment period. The hematopoietic system was the target organ of toxicity with hypochromic anemia (decreased hemoglobin, -29%, but with normal red cell counts) and a uniformed slight hyperplasticity of the bone marrow observed. The narrow focus of the study (bone marrow and blood) limited its overall usefulness.

In a 3-week to 53-week oral toxicity study in cats, zinc oxide was administered in the diet at doses ranging from 34 to 268 mg zinc/kg for periods of 10 to 53 weeks, with effects on clinical signs, body weights, urinalysis, hematology, tissue zinc levels determined and complete gross and histological examinations conducted. Zinc had no effects at doses up to 268 mg/kg, but produced severe reductions in food consumption (due to unpalatability of the diet) and associated losses in body weights at doses of 681 to 1000 mg/kg. The pancreas was the target organ of toxicity at the 681 to 1000 mg/kg doses, grossly being harder and covered with firm nodules, with histological correlates of extensive proliferation of fibrous tissue. The major limitation of the study was that only two cats/dose level were studied and only one of these was examined at the end of the treatment period.

In a series of three oral toxicity studies in weanling pigs, zinc carbonate was administered at doses ranging form 0.05% to 0.8% of the diet (~60 to 365 mg/kg) for periods of 35 to 42 days. The effects of zinc on mortality, weight gains, food consumption, feed efficiency were determined and gross post mortem examinations were conducted. Zinc treatment had no effects at dose levels up to 0.1% of the diet (~ 131.6 mg/kg). However, at levels of 0.2, 0.4, and 0.8% of the diet (i.e. ~213.2, 342.9, 318.34 mg/kg) for 42 weeks, zinc produced dose-dependent suppression of body weight gains (21 to 83%), reduced feed consumption (7 to 67%) reduced feed efficiency (14 to 50%) and treatment-related mortality (2 of 6 and 3 of 6 pigs at the ~213.2 mg/kg and 342.9 mg/kg doses, respectively, and all pigs at the 0.8%; 318.34 mg/kg level). At the 0.2 and 0.4 % doses (~213 and 343 mg/kg) common signs of zinc toxicity included: arthritis; extensive hemorrhages in the axillary

spaces; marked gastritis with some ulceration, extensive hemorrhage and marked catarrhal enteritis of the intestines, congestion of the mesentery and hemorrhagic conditions in the ventricles of the brain, spleen, lymph nodes and viscera. No incidence data for the aforementioned findings was provided.

In a 27-week oral toxicity study in swine, the effects of zinc sulfate (1000 ppm; ~21 mg/kg/day) on body weight gains and tissue copper levels, and copper uptake were determined. In addition, gross pathology of leg joints and muscle of the gluteal region and histopathology of the cerebrum, cerebellum, and spinal cord were performed. No treatment-related changes were observed in any of the aforementioned parameters. Thus, the study was of limited value in that only one dose of zinc was tested and that dose was not high enough to elicit toxicity in the pig.

In a series of 6- to 10-week oral toxicity studies in lambs, the effects of zinc oxide (0.5, 1.0, 2.0, and 4.0 g/kg of diet; ~29.2, 50.8, 88.3, and 105.9 mg/kg) on food and water consumption, body weight changes, and mortality were determined. Animals which died were also examined for gross and histological lesions. Zinc treatment produced dose-dependent reductions in body weight gains at doses $\geq$ 1 g/kg diet (~45.3 mg/kg); reduced food consumption at doses $\geq$ 1.5 mg/kg (~59.1 mg/kg); and body weight loss at doses $\geq$ 4 g/kg diet (~106 mg/kg) and increased mineral intake at all doses tested. Doses $\geq$ 3.0 g/kg diet (~104 mg/kg) were lethal in some animals. Animals which died showed clinical signs of extension of limbs, convulsions, and opisthotonos, but had no pathological findings which were definitively attributable to zinc treatment. The no effect dose in lambs was 0.5 g/kg diet (23 to 29 mg/kg).

Several oral toxicity studies in chickens using either zinc oxide, sulphate or carbonate for periods of 4 to 10-weeks were submitted. A 4-week study in chicks showed that at doses $\geq$ 2000 mg/kg diet (~129 mg/kg), zinc oxide, produced dose-dependent depression of body weights at doses $\geq$ 2000 mg/kg diet (~129 mg/kg) and treatment-related mortality at doses $\geq$ 4000 mg/kg (~494 mg/kg). Doses $\geq$ 1000 mg/kg (~66 mg/kg) resulted in histological lesions in the pancreas (dilation of the acinar lumina, cytoplasmic vacuolation, cytoplasmic globule formation, and necrosis of the exocrine cells with intra-parenchymal fibrosis), with lesions in the gizzard (desquamation of the epithelial cells, heterophiles and erythrocytes, erosion of the koilin glands and pits) observed at doses $\geq$ 2000 mg/kg (~129 mg/kg). An adjunct study conducted in hens reported similar lesions of the gizzard and pancreas after only 4 days of administration of zinc at doses of 10 g/kg diet and 20 g/kg diet (only ~31 and 23 mg/kg due to reduced food consumption).

In a 5-week oral toxicity study conducted in New Hampshire chicks, administration of zinc at doses up to 823 mg/kg diet (~69 mg/kg) for up to 9 weeks was without apparent toxicity.

In 5-week studies in White Leghorn Chicks, zinc (oxide carbonate or sulphate) were administered at dose ranging from 200 to 3000 ppm (~15 to 292 mg/kg). At a doses up to 1000 mg/kg diet (~54 to 76 mg/kg) was without apparent toxicity, whereas at doses $\geq$ 1500 ppm (~89 to 95 mg/kg) all three forms suppressed body weight gains -7 to -61%) and reduced feed efficiency (15 to 86%). In regard to induction of the said effects, the oxide was more potent than the sulphate which was more potent than the carbonate.

Finally, in 16-week studies in cross-bred broiler chicks, zinc oxide levels up to 1000 mg/kg diet (~35 mg/kg) was without apparent toxicity. However at higher levels zinc oxide induced dose-dependent inhibition of growth ($\geq$ 2000 mg/kg diet; ~72 mg/kg) and reduced feed efficiency (4000 mg/kg diet; ~146 mg/kg).

Three studies on the effects of zinc on reproductive function in rats were provided. One study, was previously summarized within the zinc acetate section of the summary and evaluation. The other two are summarized below.

In one of the two latter "reproductive toxicology" studies in rats, zinc carbonate was administered in the diet at doses of 0.1, 0.5, and 1% (~60, 300, and 600 mg/kg) for 39 weeks and effects on growth, reproduction, and blood changes were determined. Both the 0.5% (300 mg/kg) and the 1.0% (600 mg/kg) dose levels were reported to interfere with reproductive function in rats. However, both doses were maternally toxic (i.e. both doses produced anemic effects and reductions in weight gains and death were observed at the high dose). The 0.1% dose (~60 mg/kg) which was not maternally toxic also had no apparent effects on reproduction in rats. Thus, at non-maternally toxic doses, zinc carbonate, had no apparent effects reproductive function.

In the other "reproductive toxicology" study in rats, zinc was administered in the diet as the oxide, chloride, carbonate and sulphate, each at levels of 0.25% (~163 mg zinc/kg), or as the oxide or chloride at levels of 0.5% (~326 mg zinc/kg) over three generations in rats (i.e. periods of ~6-12 weeks for the F0 and F1 generations) and effects on growth, reproduction and tissue zinc levels were determined. At the 0.25% level (~163 mg zinc/kg), the oxide or other salts had no effects on any of the aforementioned parameters. However, at a dose of 0.5% (~326 mg zinc/kg), zinc oxide was reported to suppress body weight gains in the F1 generation. This study like the first, was of limited use in that the doses tested were two low to elicit toxicity. In addition, the

majority of the information provided was only qualitative in nature; and only effects on clinical signs, body weight gains, tissue zinc levels, and gross pathological observations were recorded.

In a Segment II teratology study, pregnant albino Wistar Rats were orally administered (by gavage) zinc sulfate at doses of 0.0 (control), 0.4, 2.0, 9.1, and 42.5 mg/kg during day 6 through 15 of gestation (period of organogenesis). Zinc sulphate had no discernable effects on nidation, or maternal or fetal survival in rats. Zinc sulphate was not teratogenic in rats at any doses tested in this study.

In a Segment II teratology study, pregnant Dutch-belted rabbits were orally administered (by gavage) zinc sulfate at doses of 0.0 (control), 0.6, 2.8, 13.0, and 60.0 mg/kg during day 6 through 18 of gestation (period of organogenesis). Zinc sulphate had no discernable effects on nidation, or maternal or fetal survival in rabbits. Zinc sulphate was not teratogenic in rabbits at any doses tested in this study.

In a Segment II teratology study, pregnant albino CD-1 out-bred mice were orally administered (by gavage) zinc sulfate at doses of 0.0 (control) 0.3, 1.4, 6.5, and 30.0 mg/kg during day 6 through 15 of gestation (period of organogenesis). Zinc sulphate had no discernable effects on nidation, or maternal or fetal survival in mice. Zinc sulphate was not teratogenic in mice at any doses tested in this study.

In a Segment II teratology study, pregnant out-bred golden hamsters were orally administered (by gavage) zinc sulfate at doses of 0.0 (control), 0.9, 4.1, 19.0, and 88.0 mg/kg during day 6 through 10 of gestation (period of organogenesis). Zinc sulphate had no discernable effects on nidation, or maternal or fetal survival in hamsters. Zinc sulphate was not teratogenic in hamsters at any doses tested in this study.

The effects of zinc carbonate on development, body weights, hematocrit and zinc concentrations in F1 offspring were investigated in an experiment of factorial design in mice in which doses of either 50 ppm (~7.9 mg/kg, normal daily intake) or 2000 ppm (~590 mg/kg) during the periods of F0 gestation, lactation, and during early F1 development (up to 8 weeks of age) were administered. All possible combinations of the 2 doses during the three periods were tested. Treatment with the high dose of zinc carbonate during all three time periods or during the last 2 time periods produced reduced body weights, increased bone zinc content, reduced plasma copper levels, reduced hematocrit and induced the development of alopecia in F1 offspring. Thus high levels of zinc can have deleterious effects on offspring development when

administered during lactation and early offspring development in mice. However, the study was limited in that only one dose which was ~74 times greater than the normal daily intake was tested and other than growth, no developmental parameters were monitored.

Two other reproductive toxicology studies were conducted in sheep. In the first pregnant ewes, were administered Zinc (form not indicated) orally, at doses of 30, 150, and 750 mg/kg diet (~1, 5, and 20 mg/kg). The 20 mg/kg dose was maternally toxic (i.e. reduced body weight gains and reduced feed consumption) and was associated with numerous abortions and perinatal deaths resulting in 17 of 20 nonviable lambs. Lambs which died showed very high tissue (liver) zinc concentrations (10 fold increase) and low tissue (liver) copper concentrations (90% reduction). Long bone radiographs from nonviable lambs in the 20 mg/kg diet group also showed lines of arrested growth and one lamb showed severe renal damage (hyperemia, and extravasation of blood cells, almost complete ischemic necrosis of the cortex, and necrosis of the arcuate and interlobular arteries). The 1 and 5 mg/kg doses resulted in increased liver zinc concentrations, but were without other signs of toxicity. Thus, effects on reproduction reported herein occurred at the 20 mg/kg dose, which was maternally toxic in sheep, where as no effects were observed at doses up to 5 mg/kg which were not maternally toxic.

In the second reproductive toxicology study, pregnant sheep were orally administered zinc sulphate at a dose of 5 mg/kg body weight/day throughout gestation. Zinc treatment resulted in increased fetal liver zinc levels, with no effects on lambs or the health of the ewes reported.

The zinc acetate salt was not tested for mutagenicity. However, studies on the mutagenicity of zinc oxide and/or other zinc salts (as specified) have ben conducted. These latter studies showed that zinc tested negative for mutagenic activity in the following assay systems: the Ames assay, with and without metabolic activation (ZnO and Zn Stearate); Host Mediated and In Vitro Assays with Salmonella strains G-46 and TA-1530 (zinc sulphate); Bone Marrow Chromosomal Aberration Assay in rats and In Vitro chromosomal aberration assay in human embryonic lung cells (zinc sulphate); and finally in the Dominant lethal assay in rats. However, zinc sulphate tested positive for mutagenicity against the Saccharomyces cerevisiae D4 strain in two Host Mediated Assays and in a direct in vitro assay, suggesting a propensity of zinc to increase mitotic recombination.

In a special toxicity study on the formation of DNA-zinc products following oral administration of zinc chloride in rats, addition of zinc (23 ppm; ~3.5 mg/kg/day) to the drinking water of rats for 1, 3, or 7 days resulted in increased binding of zinc to DNA isolated from liver, kidney, ileum, colon and brain. Such binding of Zinc

to DNA could disrupt base pair bonding and potentially lead to tumorigenic effects. This latter possibility, in the context of the available chronic toxicity studies in mice and rats is discussed below.

In another special toxicity study in rats the ability of chronically administered zinc chloride to induce pantothenic acid deficiency was investigated. This study showed that, administration of zinc chloride (250 mg/kg) to rats maintained on suboptimal concentrations of pantothenic acid induced the development of clinic manifestations consistent with pantothenic acid deficiency (i.e. inhibition of growth and rusting of the fur). These effects were reversible upon supplementation of the diet with additional pantothenic acid.

No conventional oral carcinogenicity studies with zinc acetate, zinc oxide or other zinc salts have been conducted. However, an available one year oral toxicology study in mice showed no definitive evidence of a zinc induced carcinogenic effect following administration of zinc oleate (~156 to 624 mg/kg; 372 to 1872 mg/m^2) in the diet or zinc sulphate (~81 to 405 mg/kg; 243 to 1215 mg/m^2) in the drinking water for periods of 45-53 weeks. In addition, no tumorigenic effects were reported in a 21-month oral toxicity study in rats which were administered zinc sulphate at daily doses of 6, 30, and 60 mg/kg. Thus although, zinc binding to DNA was demonstrated in an ex vivo study in rats, the aforementioned chronic toxicology studies in mice and rats showed no evidence of a tumorigenic potential. In addition, the preponderance of available studies on the mutagenicity/ clastogenicity of ·zinc (oxide, stearate, or sulphate) have been negative. Thus, overall the available data suggest that the risks of a tumorigenic effect for zinc are minimal.

In conclusion, almost all of the preclinical studies submitted in support of the current application were derived from the literature and were not conducted in accordance with GLP standards. In addition, only 6 studies which used the acetate salt of zinc (form intended for clinical use) were available, and none were of practical use in identifying either the toxicological or pharmacokinetic profiles for zinc acetate. It is note worthy however to indicate that the available toxicology studies which used zinc acetate (all rat studies) reported no effects on growth or pancreatic toxicity at doses up to 57.1 mg/kg given for 2 months, no effects on reproduction at doses up to 17.6 given many weeks prior to mating, during pregnancy and lactation, and finally no effects at doses up to 17.6 mg/kg given for 35 to 53 weeks.

The majority of other toxicology studies which used zinc oxide or a zinc salt other than zinc acetate also contained design flaws (i.e. insufficient numbers of animals or doses tested; inappropriate dose

selection, and/or limited numbers of parameters examined) which also limited their individual usefulness. However, collective examination of the latter studies did show that: 1) in general, doses of zinc (oxide, sulphate, and carbonate) up to: 150 mg/kg in rats, 76.5 mg/kg in dogs, 131 mg/kg in pigs, 88 mg/kg in lambs, and ~70 mg in chickens for periods ranging from 5 to 29 had no significant toxicological effects and 2) a pattern of effects associated with administration of excessive amounts of zinc, regardless of the zinc form used.

The most complete picture of this pattern of effects was provided from the numerous studies in rats which were submitted. In this regard, the available rat studies showed that oral administration of zinc (oxide, carbonate, or chloride) at doses $\geq$ 245 mg/kg for periods $\geq$ 2 to 5 weeks or longer produced: 1) reduced tissue copper, iron, calcium and/or phosphorus levels, 2) enzyme inhibition (i.e. cytochrome oxidase, catalase, ferrioxidase, and xanthine oxidase) or stimulation (i.e. alkaline phosphatase) and toxicological effects (i.e. suppression of body weight gains and induction of hypochromic microcytic anemia). In general the available studies in rats indicated that, regardless of the form used, zinc had no effects following repeated oral administration at doses up to ~150 mg/kg (~0.25% of the diet).

Toxicological effects similar to those observed in rats (i.e. suppression of body weight gains and anemic effects) were also reported in other species. For instance, zinc-induced suppression of body weight gains were reported in: 1) pigs given zinc carbonate at doses $\geq$ 213 mg/kg for 6 weeks; 2) lambs/sheep given zinc oxide at doses of $\geq$ 20 to 45 mg/kg for 6 to 12 weeks; and 3) chicks given zinc oxide, carbonate, or chloride at doses $\geq$ ~89 mg/kg for $\geq$ 4 weeks, whereas zinc-induced anemic effects were reported in: dogs given zinc sulphate at doses of 200 to 50 mg/kg for 70 weeks and in mice given zinc oleate at initial doses of 624 mg/kg which were reduced to 156 mg/kg (due to anemia) for one year.

Additional target organs of toxicity which were identified in at least two different species and with at least two different forms of zinc included the pancreas and the intestines. Pancreatic toxicity (islet cell hypertrophy, vacuolation, fibrosis and/or necrosis) was reported in: cats given zinc oxide either at doses of 71 to 86 mg/kg for 12 to 16 weeks or at doses of 189 to 256 mg/kg for 10 to 53 weeks; mice given zinc sulphate at a dose of 100 mg/kg for 14 months; and chicks given zinc oxide at 66 mg/kg for 4 weeks. However, a 2-month study in rats (designed specifically to assess pancreatic toxicity) showed that at doses up to 57.1 mg/kg, zinc acetate (form intended for clinical use), had no effects on the pancreas. Toxicological effects in the intestine/gut were seen in pigs given zinc carbonate given at doses $\geq$ 213 mg/kg for 6 weeks (gastritis, ulceration, marked catarrhal enteritis of the

intestines, congestion of the mesenteric) and in rats given lethal
doses of zinc chloride, ~600 mg/kg after 14 days of treatment
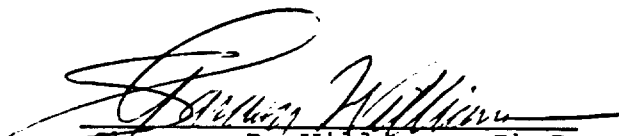(black to grey discoloration and slimy character).

The similar pattern of effects/toxicities reported in multiple
species and with for multiple forms of zinc, suggests that the zinc
ion (the common denominator in all zinc forms) was responsible for
the observed effects. In addition, since most of the
aforementioned effects were elicited using soluble forms of zinc
(i.e. zinc oxide, chloride, carbonate or sulphate), the observed
pattern of effects for the aforementioned salts should reflect the
profile of effects which would be expected for zinc derived from
the acetate form of zinc (also a soluble zinc salt).

Finally, zinc acetate is currently approved for use as a component
in (Vitaline) a multivitamin/mineral replacement supplement for
renal patients at a daily dose of 50 mg.

Therefore, sufficient preclinical information regarding the
expected ADME and toxicological effects of zinc acetate were
provided by available studies which utilized other soluable forms
of zinc. The available studies demonstrated a wide margin of
difference between the no effect doses of zinc (regardless of the
salt used) and the maximum total daily dose of 150 mg/day; ~3 mg/kg
for a 50 kg person, currently proposed for clinical use.
Therefore, from a preclinical stand point the application is
approvable.

RECOMMENDATIONS:

1.   From a preclinical standpoint the application is approvable.

2.   Sponsor should be asked to modify the label and suggested in
      the text of the review.

Shannon P. Williams   4/10/95
Shannon P. Williams, Ph.D.
Pharmacologist, HFD-180

cc:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd
HFD-180/Dr. Williams

SPW/hw/4/10/95
C:\WPFILES\PHARM\N\20458502.OSW

① Noted

② See the accompanying
Supervisory Pharmacologist's
Addendum.

5/21/95

SUPERVISORY PHARMACOLOGIST'S ADDENDUM
TO DR. S.P. WILLIAMS' PHARMACOLOGY REVIEW
OF APRIL 10, 1995

1. Noted

2. Adequate or acceptable toxicity studies of zinc acetate are
not available in the present submission.

(a) In the "35- to 53-week toxicity study in rats" (pages
32 and 33 of the review and pages 2 241 to 2 263, volume 3.2
of NDA), only 2 male rats and 1 female rat received
exclusive zinc acetate treatment. The doses employed were
approximately 7.6 mg/kg/day for 53 weeks in one male (rat #
3 of group I), 14.4 mg/kg/day for 48 weeks in one male (rat
2 of group I) and 17.6 mg/kg/day for the first 29 weeks
followed by 25.2 mg/kg/day for the last 13 weeks in the
female (rat # 27 of group III). It would be presumptuous to
assume that this study of 3 animals treated with
small/modest doses of zinc acetate can provide adequate
information about its toxicity or facilitate identification
of its safety margins. However, this study along with the
toxicity studies of other zinc salts in different species do
provide aggregate toxicity information for all zinc salts.

(b) In the oral toxicity study for effects on fertility,
reproduction and growth in the rat (pages 33, 34 and 35 of
the review and pages 1 141 to 1 150, volume 3.1 of NDA),
several salts of zinc, i.e. acetate, malate, citrate and
oxide were employed. None of the females or males in this
study received the same salt or the same dose of any salt
throughout the study and for that reason, ro conclusion can
be drawn on the exclusive effects of acetate salt. On the
other hand, collectively the results of the study indicated
that the aforementioned zinc salts in a dose range of 7 to
152 mg/kg/day did not exert an adverse effect on fertility
and reproductive performance of adult male and female rats
or growth of their offspring.

3. For aforementioned reasons (# 2), we are constrained to rely
on studies of other zinc salts (zinc chloride, zinc sulfate,
zinc carbonate, zinc citrate, zinc malate, zinc oleate, zinc
stearate, zinc oxide) for chronic toxicity: 1 year or longer in
rats, 1 year in mice, 70 weeks in dogs and 10 to 53 weeks in
cats; reproductive toxicity: fertility and general reproductive
activity study in rats, teratology studies in rats, rabbits, mice
and hamsters; and mutagenicity: Ames assays, in vivo rat bone
marrow cell chromosomal aberration, in vitro human embryonic lung
cell chromosomal aberration assay and in vivo rat dominant lethal
assay.

Although head to head comparison of the above salts of zinc and zinc acetate for absorption characteristics in animals was not undertaken, limited comparison in healthy clinical subjects do not disclose major differences between zinc acetate and zinc sulfate (Medical Officer's review dated January 27, 1995). The above listed studies of different salts of zinc do not reveal any particular overwhelming limiting toxicity.

4. Appropriate oral carcinogenicity studies of zinc salts were not submitted to the NDA. Two of the four reports provided by the sponsor are foreign language articles (pages 1 265 to 1 272 and 1 273 to 1 274 of volume 3.1 of NDA) and therefore not amenable for review. The English summary provided by the sponsor (page 03 044 of volume 1.3 of NDA) does not indicate that the studies are appropriately designed or valid. The third report (pages 1 275 to 1 276, volume 3.1 of NDA) describes intratesticular injection of zinc chloride solution (prepared with water and 1N hydrochloric acid) in hamsters and observations after 10 weeks. The fourth report (page 1 277 of volume 3.1 of NDA) gives a one year progress of an "ongoing study in mice". None of the above reports can be considered as suitable material to judge the carcinogenic potential of zinc. However, absence of carcinogenicity studies is somewhat alleviated by the following:

   (a) As per 21 CFR, part 182 (April 1, 1995) most of the zinc salts quoted in # 3 above are considered as "substances generally recognized as safe" for humans.

   (b) As per FDA/BF-77/103 (November 1973) report prepared by the select committee on GRAS substances, zinc salts in general are not considered carcinogenic.

   (c) Zinc is an essential trace element in the nutrition of man. It is an integral part of a number of cellular components in the body.

   (d) The following salts of zinc have been previously approved as OTC or prescription products. Some of them are available as part of multivitamin supplements:

   I.   Zinc sulfate injection (NDAs 19,553 and 19,229)
   II.   Zinc chloride injection (NDA 18,959)
   III. Zinc sulfate (Rx May-Vita Elixir-dose of zinc:
         15 mg/day, Vicon Forte Capsule-dose of zinc:
         50 mg/day)
   IV.   Zinc gluconate (OTC Mega™-dose of zinc: 25 mg/day,
         ZE CAPS-dose of zinc: 75 mg/day)
   V.   Zinc acetate (Rx and OTC Vitaline tablets-dose of
         zinc: 50 mg/day)

5. Unlike the previously approved drugs, Cuprimine® and Syprine® for the treatment of Wilson's disease, salts of zinc (particularly zinc sulfate) were not teratogenic in pregnant rats, rabbits, mice and hamsters. Eight women with Wilson's disease while on zinc acetate or zinc sulfate treatment conceived and delivered 11 healthy babies (Medical Officer's review dated January 27, 1995). Thus zinc acetate offers definite advantage in the treatment of Wilson's disease patients who are of child bearing potential or already pregnant.

6. RECOMMENDATIONS:

   a. From a preclinical standpoint, approval of this application is recommended.

   b. Sponsor should be asked to make the following changes in the labeling:

      I. Carcinogenesis, Mutagenesis, Impairment of Fertility

   Evaluation and Recommendation:

   No appropriate oral carcinogenicity studies of zinc acetate or other salts of zinc are available in this submission. Therefore the labeling under carcinogenesis simply should state that no long-term studies in animals have been performed to evaluate the carcinogenic potential of zinc acetate.

   Zinc acetate has not been tested for mutagenicity potential. It also has not been tested adequately for effect on fertility and reproductive performance in animals. However, information on the testing of other salts of zinc for mutagenic potential and effects on fertility and reproductive performance is available. Labeling should include the results of this testing.

   Suggested Version:

   "Carcinogenesis, Mutagenesis, Impairment of Fertility.

   Zinc acetate has not been tested for its carcinogenic potential in long-term animal studies, for its mutagenic potential or for its effect of fertility in animals.

   However, testing with other salts of zinc (zinc oxide, zinc stearate, zinc sulfate) did not reveal a mutagenicity potential in _in vitro_ Ames assays, human

embryonic lung cell chromosomal aberration assay and in
in vivo rat dominant lethal assay and rat bone marrow
cell chromosomal aberration assay.

Other salts of zinc (zinc oxide, zinc chloride, zinc
citrate, zinc maleate, zinc carbonate, zinc sulfate)
and pure zinc dust at oral doses up to 326 mg/kg/day
(18 times the recommended human dose based on body
surface area) were found to have no effect on fertility
and reproductive performance of male and female rats."

II. Pregnancy: Teratogenic Effects. Pregnancy
Category.

Evaluation and Recommendation:

As per the Medical Officer's review dated January 27,
1995, eight women with Wilson's disease while on
treatment with zinc acetate (1.5 to 3.0 mg/kg/day or
55.5 to 111.0 mg/m^2/ day) and zinc sulfate (12 to
18 mg/kg/day or 444 to 666 mg/ m^2/day) became pregnant
and delivered 11 healthy babies. These clinical
results merit a classification of Pregnancy Category A.
Oral zinc sulfate was also tested in pregnant rats at
doses up to 42.5 mg/kg/day (255 mg/m^2/day), mice at
doses up to 30 mg/kg/day (90 mg/m^2/day), rabbits at
doses up to 60 mg/kg/day (708 mg/m^2/day) and hamsters
at doses up to 88 mg/kg/day (528 mg/m^2/day) and found
to have no teratogenic potential. As per 21 CFR, part
201.57, both the clinical and preclinical information
should be incorporated.

Suggested Version:

"Pregnancy: Teratogenic Effects. Pregnancy Category A.

Studies in pregnant women have not shown that zinc
acetate or zinc sulfate increases the risk of fetal
abnormalities if administered during all trimesters of
pregnancy. If this drug is used during pregnancy, the
possibility of fetal harm appears remote. Because
studies cannot rule out the possibility of harm,
however, zinc acetate or zinc sulfate should be used
during pregnancy only if clearly needed. Oral
teratology studies have ben performed with zinc sulfate
in pregnant rats at doses up to 42.5 mg/kg/day (2 times
the recommended human dose based on body surface area),
mice at doses up to 30 mg/kg/day (1 time the

recommended human dose based on body surface area),
rabbits at doses up to 60 mg/ kg/day (6 times the
recommended human dose based on body surface area) and
hamsters at doses up to 88 mg/kg/day (5 times the
recommended human dose based on body surface area) and
have revealed no evidence of impaired fertility or harm
to the fetus due to zinc sulfate."

7. The following <u>corrections and/or additions to Dr. Williams'</u>
<u>review</u> should be taken into consideration. They are identified
by the titles and page numbers of his review.

(a) The citations (volume number and page numbers) for
those contents of review which are copied from the
Submission are inaccurate. The correct citations are
provided below.

Review Page	Incorrect Citation	Correct Citation
11	Vol. 1.3 pp 077	Vol. 3.3, p. 3 077
12	Vol. 1.3 pp 121	Vol. 3.3, p. 3 121
17	Vol. 1.3 pp 030	Vol. 3.3, p. 3 030
24	Vol. 1.2 pp 210	Vol. 3.2, p. 2 210
25	Vol. 1.2 pp 213	Vol. 3.2, p. 2 213
29	Vol. 1.1 pp 224	Vol. 3.1, p. 1 224
29	Vol. 1.1 pp 225	Vol. 3.1, p. 1 225
34	Vol. 1.1 pp 143	Vol. 3.1, p. 1 143

(b) **Page 37   6-Week Oral Toxicity of Zinc Oxide in Rats**

The quoted copper supplement (4 mg/day) for group D is
incorrect. The correct quantity is 0.4 mg/day (page 3 069,
volume 3.3 of NDA). The quoted food consumption of 7 g/week
for the control animals is also incorrect. The correct
quantity is 87 g/week (page 3 070, volume 3.3 of NDA).

(c) **Pages 50 - 52   Oral Segment II Teratology Study With**
Zinc Sulfate in Rats Table 7 (page 51):

The number of fetuses (line 2) for the zinc sulfate groups
of 2.0, 9.1 and 42.5 mg/kg are inaccurate. The correct
numbers as per Sponsor's Table on page 2 164, volume 3.2 of
NDA are as follows: 2.0 mg/kg group - 261 fetuses, 9.1
mg/kg group - 257 fetuses and 42.5 mg/kg group - 273
fetuses.

(d) **Pages 56-57** **"Effects of Excess Zinc Carbonate on Fetal Development and Growth in the Mouse":** Page 57

The quoted plasma copper concentrations in F_1 treated and control mice (137 μg/ml to 47.67 μg/ml) are in error. As per page 2 141, volume 3.2 of NDA, the values are for deciliter of plasma. The correct values are 137.00 ± 29.55 μg/100 ml in group 1 controls (50 ppm zinc in diet) and 47.67 ± 45.84 μg/100 ml in group 8 (2000 ppm zinc in diet).

(e) **Pages 57 - 59** **"Segment II Teratology Study with Zinc Sulfate in the Hamster** Table 13 (page 58):

The quoted number of fetuses (line 2) for the zinc sulfate group of 88 mg/kg (27) is incorrect. The correct number of fetuses for this group as per Sponsor's table on page 2 178, volume 3.2 of NDA is 274.

(f) **Pages 63 - 65** **"In Vivo (Host Mediated Assay) and Direct In Vitro Evaluation of the Mutagenicity of Zinc Sulfate"** Results (page 64):

The quoted recombinant frequency of 117.6 for the high dose zinc sulfate (275 mg/kg) group in the subacute groups with diploid strain (D-3) saccharomyces cerevisiae is incorrect. The correct recombinant frequency as per sponsor's table on page 2 018, volume 3.2 of NDA is 17.16.

(g) **Pages 70-71**

"Carcinogenicity Studies,

Mice,

45 to 53-Week Oral Carcinogenicity Study with Zinc (oleate and sulphate) in Mice (Walters, M and FJC Roe, **Food Cosmet. Toxic.** 1965, 3:271-276)"

Designation of this one year study as carcinogenicity study is clearly a misnomer. It is not a carcinogenicity study. Even as a chronic toxicity study, it had problems of intercurrent infections and consequent mortalities and the confounding drug toxicities at continuously varying doses. Neither the starting number of animals in each group was provided, nor the non-availability of such important information identified.

(h) Page 72-74 "LABELING:"

Carcinogenesis (page 72)

No reports of valid and appropriately designed carcinogenicity studies are available. The one year mouse toxicity study is not a carcinogenicity study. This portion of labeling should simply state that no long term studies in animals were performed to evaluate carcinogenic potential of Zinc Acetate.

Mutagenesis (page 72)

Reviewer's quote of the strain of saccharomyces cerevisiae (D4) in host mediated and in vitro assays is inaccurate. The correct strain is D3. The results of host mediated assays should be deleted since the assay is obsolete. In addition, the in vitro response was considered as negative (page 2 016, volume 3.2 of NDA).

The portion dealing with binding of unlabelled zinc to DNA in different rat organs should be deleted since no effort was made to identify the source of zinc in this study (pages 2 294-2 303, volume 3.2 of NDA). The study itself is preliminary in nature as indicated by the title of the article and no distinction was made between the different DNA-Zinc adducts formed in control and treated rat tissues and despite the continued administration of a small dose of zinc for 1-week the number of adducts formed decreased in different tissues.

Impairment of Fertility (page 73)

The portion dealing with the sheep study should be deleted since it does not deal with the fertility and reproductive performance of rams and ewes. The study deals with treatment of pregnant ewes. In addition, the study was also not well controlled since the unspecified salt of zinc was administered to pregnant ewes anytime between 0 to 6 weeks of pregnancy.

Teratogenic Effects: Pregnancy Category C (page 73)

Pregnancy Category C is inappropriate for zinc salts since studies in pregnant rats, mice, rabbits and hamsters did not demonstrate any teratogenic risk (harm to the fetus) or a adverse effect on pregnancy. For accurate classification see the above item # 6, b., II.

(1)   Pages 74-88   "SUMMARY AND EVALUATION:"

Page 75 Paragraph 2 (2)   ADME Studies

There are inaccuracies in the listing of the studies. This
review does not include the following: (1) "absorption zinc
carbonate in rats", (2) "absorption of zinc oxide, zinc
carbonate and zinc sulphate in chicks" and (3) "a metabolism
study with zinc carbonate in rats".

Page 75 Paragraph 2 (3) Toxicity Studies in Rats Including:

The following inaccuracy should be noted. There was no 4-
week rat toxicity study of zinc sulfate in either the
submission or the text of the review.

**Page 77 Paragraph 5 Oral Reproductive Toxicity Study in
Rats, the effects of zinc acetate on --------:**

The summary in this paragraph conveys the impression that
zinc acetate was tested for effects on fertility etc. of
adult male and female rats and the development of their
offspring. In this particular study as conveyed on page 33
of the review text and the submission on pages 1 141 -
1 150, vol. 3.1 of NDA, zinc acetate was not the only salt
tested. They included acetate, malate, citrate and oxide
salts of zinc. In no instance both sexes of the animals or
a single sex of animal were treated with the same salt or
the same dose throughout. The quoted dose ranges 8 to
17.6 mg/kg are inaccurate. The correct ranges are 7 to
152 mg/kg which had no adverse effect on fertility and
reproductive performance of adult female and male rats and
on growth of their offspring.

Page 79 Paragraph 4, 21-Month Toxicity Study in Rats:

The intent of the study was not to determine the tumorigenic
effect of zinc sulfate. Only a small number of animals
(n=4/sex/group) were employed in the study. Histopathology
examination was very limited. The authors of the
publication (E.C. Hagon et al, J. Am. Pharm. Assoc. 42:700-
702, 1953, volume 3.1, p. 1 262 to 1 264 of NDA) did not
claim any intent of studying the tumorigenic effects or the
lack of tumorigenic outcome.

Page 81 Paragraph 3, 3-Week to 53-Week Oral Toxicity Study in Cats:

The duration of the study quoted (3 to 53 weeks) is at variance with the duration quoted on page 44 of the review text (10 to 53 weeks). The correct duration of the study is 10 to 53 weeks (individual cats studied for 10, 11, 12, 13, 16, 21, 23, 31, 33 and 53 weeks). Each cat received a different dose. The quoted highest doses of 681 and 1000 mg/kg are incorrect. They represent doses per animal. The correct mg/kg doses are 188.5 for terminal 8 weeks of the 16 week period, 233.0 for terminal 13 weeks of the 21 week period and 267.6 for terminal 13 weeks of the 21 week period (pages 1 198-1 233, volume 3.1 of NDA). Histopathological examination of the pancreatic tissues (2 of 3 cats) showed excessive fibrous tissue infiltration and diminished number of well organized acini and islets of langerhans. These changes are correlated with the presence of excessive pancreatic zinc concentrations (0.41, 0.23 and 0.26 mg/g tissue) when compared to the concentrations in untreated cats (0.026 mg/g tissue).

Page 83 Paragraph 6 (last), In the other "Reproductive Toxicology" study in rats, -------:

This is essentially a repetition of paragraph 1 of page 80. They describe the results of the same study. Daily treatment with 163 mg/kg of zinc dust, zinc chloride, zinc carbonate or zinc sulfate or 326 mg/kg of zinc oxide or zinc chloride did not interfere with reproduction in male and female rats. The reviewer's comment that doses tested were too low to elicit toxicity is not relevant to a reproductive toxicity study since the high dose (~326 mg/kg) suppressed body weight gains of the treated F_1 animals in a qualitative sense. In addition, provoking overt toxicity in a reproductive toxicity study is undesirable.

Page 85 "Mutagenicity":

The quoted strain of saccharomyces cerevisiae (D4) for the host mediated assay is inaccurate. The correct strain is D3. The results of this obsolete test showed a weak positive response. The in vitro test results were interpreted as negative (pages 2 015 and 2 016, volume 3.2 of NDA).

Pages 85 and 86 "DNA-Zinc Products and Tumorigenic Effect of Zinc"

Reviewer's comments on the "DNA-Zinc products" in rats should be disregarded since the study itself is preliminary in nature as indicated by the title of the report (pages 2 294 - 2 303 of volume 3.2 of NDA) and no clear distinction was made between different DNA-Zinc adducts (peaks D, A, B and C) formed in control and treated rat tissues. In addition, despite the continued administration of the small dose of zinc for 1 week, there were decreases in the number of adducts formed in different tissues from day 1 to day 7. The number of adducts reported were erratic within each organ. Appropriate oral carcinogenicity studies of zinc salts were not submitted to the NDA. The studies cited by the reviewer, i.e. the 1-year oral toxicology study in mice and the 21-month oral toxicity study in rats were neither appropriate (see # 8 under comment for page 79) nor adequate for assessing the tumorigenic potential of zinc.

Page 87, Last paragraph, Additional Target Organs of Toxicity --:

The type of histopathology lesions observed in pancreatic tissues of cats, mice and chicken were not similar. As indicated below, the endocrine pancreas was affected only in mice. In cats and chickens the affected portion was the exocrine pancreas.
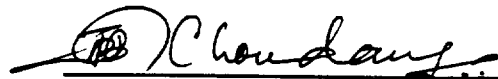
In mice, the lesions were in the endocrine portion of the pancreas: increase in the size of islets, hypertrophy of beta cells and increase in the granular contents of beta cells (pages 3 227 - 3 240, volume 3.3 of NDA).

There were no pancreatic lesions in the cats receiving doses less than 188.5 mg/kg/day. In the three cats receiving zinc oxide at 188.5, 223.0 and 267.6 mg/kg/day during the last 8 weeks of 16-weeks treatment period, 13 weeks of 21-week treatment period and 13 weeks of 21-week treatment period, respectively, the predominant lesion was heavy fibrotic invasion of the exocrine portion. Islet cell lesions were not noted (pages 1 198 - 1 233, volume 3.1 of NDA).

In the chickens, the pancreatic lesions were confined to the exocrine pancreas: dilation of the acinar lumina, cytoplasmic vacuolation, cytoplasmic globule formation and necrosis of the exocrine cells with interparenchymal fibrosis (pages 3 215 - 3 226, volume 3.3 of NDA).

Page 88, First paragraph:

The quoted lethal dose for rats is incorrect. The correct
dose is 2400 mg/kg.

_____ 6/6/95
Jasti B. Choudary, Ph.D., B.V.Sc.
Supervisory Pharmacologist
June 3, 1995

cc:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd
HFD-180/Dr. Williams
HFD-102/Assistant Director (Pharmacology)
HFD-345/Dr. James

JBC/hw/6/6/95
C:\WPFILES\PHARM\N\20458505.0JC

DIVISION OF GASTROINTESTINAL AND COAGULATION DRUG PRODUCTS

Review of Chemistry, Manufacturing, and Controls

NDA 20-458   **CHEM.REVIEW #: 1**   **REVIEW DATE: 21 NOV 1994**

SUBMISSION TYPE   **DATES**

	DOCUMENT	**CDER**	**ASSIGNED**	**REVIEW**	**NUM**	**LETTER**	**ST**
ORIGINAL	21Jun94	23Jun94	01Aug94	16Nov94			

NAME & ADDRESS OF APPLICANT:
  LEMMON COMPANY
  1510 DELP DRIVE
  Kulpsville, PA 19443

DRUG PRODUCT NAME
  Proprietary: Zinc Acetate Capsules
  Nonproprietary/USAN: Acetic acid, zinc salt, dihydrate
  Code Name/#:
  Chem.Type/Ther.Class: 2S

ANDA Suitability Petition/DESI/Patent Status: None

PHARMACOL.CATEGORY/INDICATION:
copper antagonist, metallothionein inducing agent

1. Maintenance treatment of patients with Wilson's disease.

DOSAGE FORM: Hard Gelatin Capsule

STRENGTHS: 25 mg Zinc
                     50 mg Zinc

ROUTE OF ADMINISTRATION: Oral

DISPENSED: __X__ Rx _____ OTC

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:
Zinc Acetate Dihydrate
$C_4H_6O_4Zn \cdot 2H_2O$
Mwt 219.51
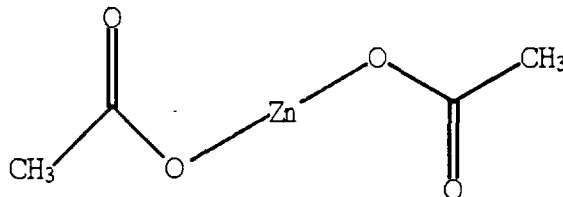
SUPPORTING DOCUMENTS:
Document/Contents   Holder/Proposed Supplier
Investigational IND
    IND

RELATED DOCUMENTS (if applicable): None

CONSULTS:

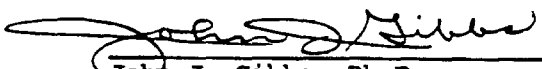| Type	Status
Environmental Assessment July 5, 1994 Dr. Phillip Vincent	Review #1 Completed in this review.
Stability October 3, 1994 Dr. Karl Lin	Pending

REMARKS/COMMENTS:
This is the review of the original submission.

CONCLUSIONS & RECOMMENDATIONS:
This application is not approvable. The sponsor should address the deficiencies noted in the information request letter of 23Aug94, and the deficiencies noted in this review.

George T. Chen 12/2/94
_____
George T. Chen, Ph.D.
Review Chemist, HFD-180

John J. Gibbs 12/9/94
_____
John J. Gibbs, Ph.D.
Supervisory Chemist, HFD-180

cc:
Orig. NDA 20-458
HFD-180/Division File
HFD-180/KOliver
HFD-180/GChen
R/D Init by:JGibbs/11-25-94
GC/dob DRAFT 11-29-94\F/T 12-2-94\WP: c:\wp51\chem\n\20458412.1GC

DIVISION OF GASTROINTESTINAL AND COAGULATION DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA 20-458   **CHEM REVIEW #2** **REVIEW DATE:** March 29, 1995   APR 25 1995

SUBMISSION TYPE   DATES

	DOCUMENT	CDER	ASSIGNED	REVIEW	NUM	LETTER	ST
ORIGINAL	21Jun94	23Jun94	01Aug94	21Nov94	1	30Dec94	AE
BC Amend	11Jan95	13Jan95	18Jan95	28Feb95	2		

NAME & ADDRESS OF APPLICANT :
LEMMON Company
1510 Delp Drive
Kulpsville, PA 19443

DRUG PRODUCT NAME:
Proprietary: Zinc Acetate Capsules
Nonproprietary/USAN:
Code Name/#:
Chem.Type/Ther.Class: 2S

PHARMACOLOGICAL CATEGORY: copper antagonist, metallothionein
inducing agent

INDICATIONS:
1. maintenance treatment of patients with Wilson's disease

DOSAGE FORM: gelatin capsule

STRENGTHS: 25 mg and 50 mg zinc

ROUTE OF ADMINISTRATION: oral

HOW DISPENSED: X Rx   OTC

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:
See Chemistry Review #1.

SUPPORTING DOCUMENTS:
See Chemistry Review #1.

RELATED DOCUMENTS (if applicable): None

CONSULTS:
Type	Status
Environmental Assessment	Review #1 Completed, see Chemistry
July 5, 1994	Review #1.
Dr. Phillip Vincent	

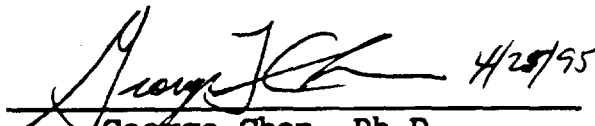Stability   Pending
October 3, 1994
Dr. Karl Lin

REMARKS/COMMENTS:
This review, Chemistry Review #2, is of LEMMON's response to the
information requested letter (IR) of 23Aug94 from the 45 day
filing meeting.

The deficiencies from Chemistry Review #1 issued in the letter of
30Dec94 remain outstanding.

CONCLUSIONS & RECOMMENDATIONS:
This application remains "not approvable". LEMMON must respond
to the deficiency letter of December 30, 1994 from Chemistry
Review #1. These additional deficiencies should be conveyed to
LEMMON.

_____ 4/25/95
George Chen, Ph.D.
Review Chemist, HFD-180

_____ 4/25/95
John J. Gibbs, Ph.D.
Supervisory Chemist, HFD-180

cc:
NDA 20-458
HFD-180/Division File
HFD-180/SFredd
HFD-181/CSO
HFD-180/GChen
R/D Init.:JGibbs/4-6-95
GC/dob DRAFT 4-13-95/F/T 4-24-95
WP: c:\wpfiles\chem\N\20458504.2GC

DIVISION OF GASTROINTESTINAL AND COAGULATION DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA 20-458   CHEM REVIEW #3 REVIEW DATE: May 17, 1995   JUN - 5 1995

SUBMISSION TYPE   DATES

	DOCUMENT	CDER	ASSIGNED	REVIEW	NUM	LETTER	ST
ORIGINAL	21Jun94	23Jun94	01Aug94	21Nov94	1	30Dec94	AE
AMENDMENT							
BC	11Jan95	13Jan95	18Jan95	28Feb95	2	05May95	AE
AC	30Mar95	30Mar95	04Apr95	15May95	3		

NAME & ADDRESS OF APPLICANT :
    LEMMON Company
    1510 Delp Drive
    Kulpsville, PA 19443

DRUG PRODUCT NAME:
    Proprietary: ZIRAC
    Nonproprietary/USAN: zinc acetate dihydrate
    Code Name/#:
    Chem.Type/Ther.Class: 2S

PHARMACOLOGICAL CATEGORY:
copper antagonist, metallothionein inducing agent

INDICATIONS:
1. maintenance treatment of patients with Wilson's disease,

DOSAGE FORM: gelatin capsule

STRENGTHS: 25 and 50 mg zinc

ROUTE OF ADMINISTRATION: oral

HOW DISPENSED: _X_Rx ___OTC

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:
See Chemistry Review #1

SUPPORTING DOCUMENTS:
See Chemistry Review #1

RELATED DOCUMENTS (if applicable):
None

CONSULTS:

Type	Division	Date/Status
Tradename	Labeling & Nomenclature Committee	13Apr95/ Pending
Stability (Exp Date)	Division of Biometrics HFD-715	15May95/ Pending

REMARKS/COMMENTS:
The 23Aug94 information request letter from the 45 day filing meeting resulted in the Amendment of 11Jan95, and was reviewed in Chemistry Review #2.

The 30Dec94 approvable letter issued from Chemistry Review #1 of the original submission. The 29 March 1995 BC response is reviewed here as Chemistry Review #3.

The deficiencies noted in Chemistry Review #2 and issued to Lemmon in the approvable letter of 05May95 remain outstanding.

CONCLUSIONS & RECOMMENDATIONS:
This application remains approvable "AE". Deficiencies from Chemistry Review #2 remain outstanding. The few remaining CMC and environmental assessment deficiencies should be forwarded to Lemmon.

George T. Chen 6/2/95

George T. Chen, Ph.D.
Review Chemist, HFD-180

John J. Gibbs 6/5/95

John J. Gibbs, Ph.D.
Supervisory Chemist, HFD-180

cc:
NDA 20-458
HFD-180/Division File
HFD-180/SFredd
HFD-181/CSO
HFD-180/GChen
R/D Init by: J Gibbs/5-26-95
GC/dob DRAFT 5-30-95\F/T 6-1-95
Wp:c:\wpfiles\chem\N\20458505.3GC

DIVISION OF GASTROINTESTINAL AND COAGULATION DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA 20-458   **CHEM.REVIEW # 4**   **REVIEW DATE: September 1, 1995**

SUBMISSION TYPE			DATES				
	DOCUMENT	CDER	ASSIGNED	REVIEWED	NUM	LETTER	ST
ORIG.	21Jun94	23Jun94	01Aug94	21Nov94	1	30Dec94	AE
AMEND.							
BC	11Jan95	13Jan95	18Jan95	28Feb95	2	05May95	AE
AC	30Mar95	30Mar95	04Apr95	15May95	3	14Jun95	AE
BC	03Aug95	04Aug95	08Aug95	01Sep95	4		

NAME & ADDRESS OF APPLICANT:
LEMMON Company
1510 Delp Drive
Kulpsville, PA. 19443

DRUG PRODUCT NAME
     Proprietary: Pending
(Note: Proposed tradename ZIRAC has been declined by the Labeling
Committee.)   Nonproprietary/USAN: Zinc Acetate
     Code Name/#:
     Chem.Type/Ther.Class: 2S

ANDA Suitability Petition/DESI/Patent Status: N/A

PHARMACOL.CATEGORY/INDICATION:
copper antagonist, metallothionein inducing agent/maintenance
treatment of patients with Wilson's disease

DOSAGE FORM: gelatin capsule
STRENGTHS: 25 and 50 mg zinc
ROUTE OF ADMINISTRATION: oral
DISPENSED: __X__ Rx _____ OTC

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:
See Chemistry Review #1

SUPPORTING DOCUMENTS:
See Chemistry Review #1

RELATED DOCUMENTS (if applicable):
None

CONSULTS:

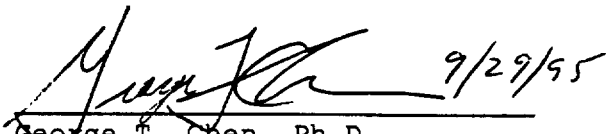| Type	Division	Date/Status
Tradename	Labeling & Nomenclature	13Apr95/Proposed Name, ZIRAC, **NA** (see Consult #431, 15May95)
Stability	Division of Biometrics	15May95/**NA**. See Consult of 14Jun95

REMARKS/COMMENTS:
Review History
1. IR Letter 23Aug94/Amendment 11Jan95/Chemistry Review #2
The 23Aug94 information request letter from the 45 day filing meeting resulted in the Amendment of 11Jan95, and was reviewed in Chemistry Review #2. Deficiencies were forwarded in the letter of 05May95.

2. AE Letter 30Dec94/Original Submission 23Jun94/Chemistry Review #1
Chemistry Review #1 of the original submission resulted in the approvable (AE) action letter of 30Dec94.

3. AE Letter 14Jun95/Amendment 29Mar95/Chemistry Review #3
Chemistry Review #3 is of the BC Amendment, 29Mar95, submitted in response to the AP letter 30Dec94 from Chemistry Review #1. Deficiencies have been forwarded in the letter 14Jun95.

4. AE Letter May 5, 1995/BC Amendment, 03Aug95/Chemistry Review #4
This review, Chemistry Review #4, is of the BC Amendment, 03Aug95, submitted in response to the AP letter 05May95 from Chemistry Review #2.

5. The deficiencies from Chemistry Review #3 issued in the letter of 14Jun95 remain outstanding.
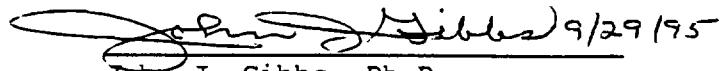
Problems:

2. The tradename ZIRAC has been recommended as "not approvable" by the labeling and trademark committee.

CONCLUSIONS & RECOMMENDATIONS:
This application remains approvable (AE). The deficiencies should be forwarded to the firm.

_____ 9/29/95
George T. Chen, Ph.D.
Review Chemist, HFD-180

_____ 9/29/95
John J. Gibbs, Ph.D.
Supervisory Chemist, HFD-180

cc:
Orig. NDA 20-458
HFD-180/Division File
HFD-180/GChen
HFD-180/KOlivier
R/D Init: JGibbs/9-27-95
GC/dob DRAFT 9-27-95**F/T 9-28-95**\WP: c:\wpfiles\chem\N\20458508.4gc

DIVISION OF GASTROINTESTINAL AND COAGULATION DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

<u>NDA:</u> **20-458**   <u>CHEM.REVIEW:</u> #5   <u>REVIEW DATE:</u> 04Jan96   JAN 16 1997

<u>SUBMISSION TYPE</u>		<u>DATES</u>					
	<u>DOCUMENT</u>	<u>CDER</u>	<u>ASSIGNED</u>	<u>REVIEWED</u>	<u>NM</u>	<u>LET</u>	<u>ST</u>
ORIG.	21Jun94	23Jun94	01Aug94	21Nov94	1	30Dec94	AE
AMEND.							
BC	11Jan95	13Jan95	18Jan95	28Feb95	2	05May95	AE
AC	30Mar95	30Mar95	04Apr95	15May95	3	14Jun95	AE
BC	03Aug95	04Aug95	08Aug95	01Sep95	4	12Oct95	AE
BC	06Sep95	07Sep95	12Sep95	29Feb96	5		
BC	03Jan96	04Jan96	18Jan96	29Feb96	5		
BC	08Apr96	09Apr96	12Apr96	02Jul96	5		

<u>NAME & ADDRESS OF APPLICANT:</u>
LEMMON Company
1510 Delp Drive
Kulpsville, PA 19443

<u>DRUG PRODUCT NAME</u>
<u>Proprietary:</u>   **PENDING.**
(Note, the originally proposed names, ZIRAC and ZINATE, have been found
unacceptable by the Labeling and Nomenclature Committee. The proposed
name **GALZIN** has been found **acceptable**, see Consult of 5Jan96.)
<u>Nonproprietary/USAN:</u>   Zinc Acetate
<u>Code Name/#:</u>
<u>Chem.Type/Ther.Class:</u>   2S

<u>ANDA Suitability Petition/DESI/Patent Status:</u>   N/A

<u>PHARMACOL.CATEGORY/INDICATION:</u>
copper antagonist, metallothionein inducing agent

maintenance treatment of patients with Wilson's disease

<u>DOSAGE FORM:</u>   gelatin capsule
<u>STRENGTHS:</u>   25 and 50 mg zinc
<u>ROUTE OF ADMINISTRATION:</u>   oral
<u>DISPENSED:</u>   __X__ Rx _____ OTC

<u>CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:</u>
See Chemistry Review #4.

<u>SUPPORTING DOCUMENTS:</u>
See Chemistry Review #4.

<u>RELATED DOCUMENTS (if applicable):</u>
None

CONSULTS:

Type	Division	Date/Status
Trade name	Labeling & Nomenclature	13Apr95/Proposed Name, ZIRAC, **NA** (see Consult #431, 15May95)
		05Jan96/Proposed Name, GALVACE or GALZIN, **AP** (see Consult #579, 30Apr96)
Stability	Division of Biometrics	15May95/**NA**. See Consult of 14Jun95
EER	Division of Manufacturing and Product Quality, HFD-320	29Feb96/FUR. **Acceptable. EER 9705 is dated 9/12/96**

REMARKS/COMMENTS:

This review covers three BC Amendments.
1. the BC Amendment of September 6, 1995, the response to the letter of June 14, 1995 from Chemistry Review #3,
2. the BC Amendment of January 3, 1996, the response to the letter of October 12, 1995 from Chemistry Review #4, and
3. the BC Amendment of April 8, 1996, revised MPR from validation run.

All responses to chemistry deficiencies have been submitted.

Complete review history is summarized in the REMARKS section of Chemistry Review #4.
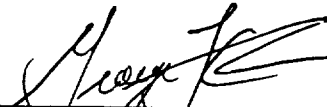
Outstanding Issues:

2. Trademark Review
The current proposed proprietary name, GALVACE or GALZEN (see Trademark Consult, 05Jan96), has been "approved" by the Trademark Review Committee (see Consult #579, 30Apr96). The recommended name GALZEN will be communicated to the sponsor as part of this action letter.

3. Environmental Assessment
A freedom of information environmental assessment must be submitted to support the FONSI.

CONCLUSIONS & RECOMMENDATIONS:

This application remains approvable. The remaining deficiencies should be forwarded to the sponsor.

1/16/97

GEORGE T. CHEN, Ph.D.
Review Chemist, HFD-180

1/16/97

ERIC P. DUFFY, Ph.D.
Chemistry Team Leader, HFD-180

cc:
Orig. NDA 20-458
HFD-180/Division File
HFD-180/GChen
HFD-180/KOliver
R/D Init by: EDuffy/1-10-97
GC/dob F/T 1-14-96\WP: c:\wpfiles\chem\N\20458510.5gc

DIVISION OF GASTROINTESTINAL AND COAGULATION DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

<u>NDA:</u> 20-458 <u>CHEM.REVIEW</u> : #6   <u>REVIEW DATE:</u> December 5, 1996

<u>SUBMISSION TYPE</u>

	DOCUMENT	CDER DATE	ASSIGNED	REVIEWED	NUM	LET	ST
ORIG.	21Jun94	23Jun94	01Aug94	21Nov94	1	30Dec94	AE
AMEND.							
BC	11Jan95	13Jan95	18Jan95	28Feb95	2	05May95	AE
AC	30Mar95	30Mar95	04Apr95	15May95	3	14Jun95	AE
BC	03Aug95	04Aug95	08Aug95	01Sep95	4	12Oct95	AE
BC	06Sep95	07Sep95	12Sep95	29Feb96	5		
BC	03Jan96	04Jan96	18Jan96	29Feb96	5		
BC	08Apr96	09Apr96	12Apr96	02Jul96	5		
AF	08Oct96	16Oct96	28Oct96	05Dec96	6		
BC	14Oct96	18Oct96	25Oct96	05Dec96	6		

<u>NAME & ADDRESS OF APPLICANT:</u>
LEMMON Company
1510 Delp Drive
Kulpsville, PA 19443

<u>DRUG PRODUCT NAME</u>
<u>Proprietary:</u>   GALZIN
<u>Nonproprietary/USAN:</u>   Zinc Acetate
<u>Code Name/#:</u>
<u>Chem.Type/Ther.Class:</u>   2S

<u>ANDA Suitability Petition/DESI/Patent Status:</u>   N/A

<u>PHARMACOL.CATEGORY/INDICATION:</u>
copper antagonist, metallothionein inducing agent

maintenance treatment of patients with Wilson's disease

<u>DOSAGE FORM:</u>   gelatin capsule
<u>STRENGTHS:</u>   25 and 50 mg zinc
<u>ROUTE OF ADMINISTRATION:</u>   oral
<u>DISPENSED:</u>   __X__ **Rx** _____ **OTC**

<u>CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:</u>
See Chemistry Review #4

<u>SUPPORTING DOCUMENTS:</u>
See Chemistry Review #4.

<u>RELATED DOCUMENTS (if applicable):</u>
None.

CONSULTS:

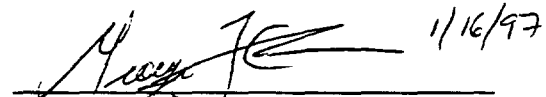| Type	Division	Date/Status
Trademark	Labeling & Nomenclature	05Jan96/GALZIN, AP
Stability	Division of Biometrics	14Jun95/NA
EER	Division of Manufacturing And Product Quality, HFD-320	12Sep96/AC

REMARKS/COMMENTS:

Chemistry Review #5 remains outstanding. The three (3) deficiencies in the action letter should be transmitted to the firm. Two require post-approval studies and the other requires an FOI copy of the environmental assessment.

In response to Karen Oliver, Project Manager, telephone request of September 24 and 25, 1996, LEMMON has submitted an FOI version of their environmental assessment.

This review is of the FOI environmental assessment and the remaining expiration dating issue.

CONCLUSIONS & RECOMMENDATIONS:

This application may be approved once LEMMON commits to the post-approval studies from Chemistry Review #5.

_____ 1/16/97

George T. Chen, Ph.D.
Review Chemist, HFD-180

_____ 1/16/97

Eric P. Duffy, Ph.D.
Chemistry Team Leader, HFD-180

cc:
Orig. NDA 20-458
HFD-180/Division File
HFD-180/CSO\MMcNeil
HFD-180/GChen
R/D Init:EDuffy/1-13-97
GC/dob F/T 1-14-97\WP: c:\wpfiles\chem\N\20458612.6GC

McNeil

NDA 20-458

OCT 2 8 1996

Lemmon Company
Attention: Deborah A. Jaskot
650 Cathill Road
Sellersville, PA 18960

Dear Ms. Jaskot:

We acknowledge receipt on October 16, 1996 of your October 8, 1996 amendment to your new drug application (NDA) for Zinc Acetate Capsules.

This amendment contains final printed labeling (FPL), submitted in response to our August 24, 1995 approvable letter.

We consider this a major amendment under 21 CFR 314.60 of the regulations and it constitutes a full response to our letter.

The due date under the Prescription Drug User Fee Act of 1992 (PDUFA) is April 16, 1997.

Should you have any questions, please contact Melodi McNeil, Consumer Safety Officer, at (301) 443-0483.

Sincerely yours, MM 10/25/96

Stephen B. Fredd, M.D.
Director
Division of Gastrointestinal and Coagulation Drug
    Products
Office of Drug Evaluation III
Center for Drug Evaluation and Research

cc:
  Original NDA 20-458
  HFD-180/Div. Files
  HFD-180/CSO/M.McNeil   SF 10/14/96
  HFD-180/EDuffy
  HFD-180/GChen
  HFD-180/HGallo-Torres
  DISTRICT OFFICE

drafted: mm/October 25, 1996/c:\wpfiles\cso\n\20458610.ack
RD init: KOliver 10/25/96
Final: October 25, 1996

ACKNOWLEDGEMENT (AC)

NDA 20-458

Lemmon Company
Attention: Stanley Scheindlin, D.Sc.
1510 Delp Drive
Kulpsville, PA 19443

Dear Dr. Scheindlin:

Please refer to your New Drug Application submitted
June 21, 1994 pursuant to section 505(b) of the Federal Food,
Drug, and Cosmetic Act for Zinc Acetate Capsules.

We have completed the review of your proposed tradename, Zinate,
and are concerned that the proposed tradename may be misleading
as defined in 21 CFR 201.10(c)(5), because of similarity in the
spelling or pronunciation that may cause confusion with the
proprietary name or the established name of a different drug or
ingredient, for example, Zincate and Zenate.

Please propose alternative tradenames, and send them to the
Agency for consideration.

If you have any questions, please contact:

> Karen Oliver
> Consumer Safety Officer
> (301) 443-0487

Sincerely yours,

> Stephen B. Fredd, M.D.
> Director
> Division of Gastrointestinal
>   and Coagulation Drug Products
> Office of Drug Evaluation III
> Center for Drug Evaluation and Research

REQUEST FOR TRADEMARK REVIEW
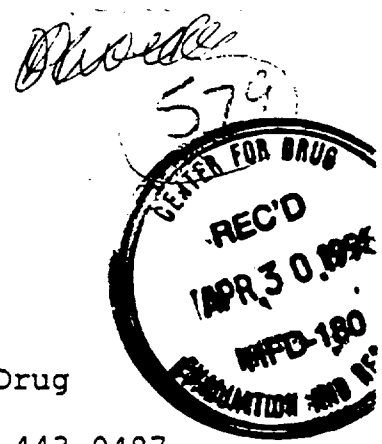
TO: CDER Labeling and Nomenclature Committee
Attention: Daniel L. Boring, Ph.D., Chair,
(301) 827-2333
HFD-530, Corporate Building, Room N461

From: Division of Gastrointestinal and Coagulation Drug
Products, HFD-180
Attention: Karen Oliver, CSO   Phone: (301) 443-0487

K.Oliver 1/05/96

Date: January 5, 1996

Subject: Request for Assessment of a Trademark for a Proposed Drug
Product

Proposed Trademark: GALVACE, or GALZIN as a possible alternate
NDA/ANDA#:   20-458

Established name, including form: Zinc Acetate Capsules

Other trademarks by the same firm for companion products: None

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

Lemmon Company submitted NDA 20-458 for Zinc Acetate Capsules for
the following indications: (1) the maintenance treatment of
patients with Wilson's disease who have initially been treated with
the chelating agents Penicillamine, trientine, or ammonium
tetrathiomolybdate;

An
approvable letter was issued August 24, 1995.

BACKGROUND:

An April 3, 1995, request for trademark review, with a
May 15, 1995 response from the committee revealed several problems
with the proposed name Zirac (review attached). In May 24, 1995
letter, the firm was notified of the Agency's concern that the
proposed name, Zirac, may be misleading, as defined in
21 CFR 201.10(c)(5). On August 16, 1995, the firm submitted a
proposed new tradename, ZINATE. The trademark review committee, in
response to an August 25, 1995 request for the trademark review,
revealed several names which sound or like or look like the
proposed name including Zincate and Zenate (review attached). The
committee believed there would be significant potential for
confusion involving both of the names and the proposed names, as
defined in 21 CFR 201.10(c)(5). The firm was notified, and
responded on January 3, 1996 with the proposed tradename GALVACE.

Initial comments from the submitter: (concerns, observations, etc.)

Note: Meetings of the Committee are scheduled for the 4th Tuesday of the month. Please submit this form at least one week ahead of the meeting. Responses will be as timely as possible.

ATTACHMENTS

cc: Original 20-458
     HFD-180/division file
     HFD-180/K.Oliver
     HFD-180/G.Chen
     HFD-180/J.Gibbs

KO/01/05/96/c:\wpwin\karenfil\misc\20458601.0ko

Rev Dec. 1990

Consult #579 (HFD-180)

GALVACE
GALZIN   zinc acetate capsules

      The LNC found no look alike/sound alike conflicts or misleading aspects in either name. However, the syllable "-ace" is often associated with angiotensin converting envyme inhibitors and is also a phonetic variant of "-ase", a USAN stem syllable for enzyme products. The LNC would recommend against the use of GALVACE for the above reasons but finds the proposed trademark GALZIN to be acceptable.

_____, Chair
CDER Labeling and Nomenclature Committee