20622

AF Letter

NDA 20-622

TEVA Pharmaceuticals USA
Attention: Dr. Stanley Scheindlin
1510 Delp Drive
Kulpsville, PA 19443

Dear Dr. Scheindlin:

Please refer to your June 13, 1995 new drug application (and your resubmission dated October 10, 1995) submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Copaxone (copolymer-1) injection.

We acknowledge receipt of your amendments dated:

November 30, 1995	December 12, 1995	January 10, 1996
January 12, 1996	January 19, 1996	February 1, 1996
February 29, 1996	March 29, 1996	April 22, 1996.

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

Labeling:

Attached to this letter is draft labeling. This labeling consists of text and requests for information and/or proposals for additional text (these proposals are embedded in the text and are bolded). Where specific wording is indicated, please adopt that wording. If you believe such wording is unacceptable in places, please propose alternative language, and include detailed support for these proposed changes. In those places where we have requested that you propose new language and/or tables, please do so, and, again, supply any information (or references to information already submitted) necessary to support your proposals.

We have concluded, based on the information submitted, that Copolymer 1 is effective in reducing the frequency of relapses in patients with relapsing remitting MS. The information submitted fails to demonstrate a beneficial effect of Cop-1 on the progression of MS, however. This view of the evidence conforms to that of the PCNS Advisory Committee.

The indication section of the draft labeling provided has been revised accordingly.

Gender Analyses

The Agency requires sponsors to perform analyses that examine the safety and effectiveness of treatments separately for men and women. We have been unable to locate such analyses in your application, and request that you perform and submit the results of these analyses in your response to this letter.

Chemistry:

Please provide the requested information communicated to you in the August 7, 1996 letter.

Phase 4 Requirements

Final approval of this application will be contingent upon your commitment to complete the following Phase 4 requirements. You should be aware, however, that this list may not be exhaustive, and the Approval letter may include additional items not anticipated at this time.

When a sensitive assay method becomes available for analyzing copolymer-1 in biological fluids, examination of the pharmacokinetics of Cop-I in humans should be performed.

Under 21 CFR 314.50(d)(5)(vi)(*b*), we request that you update your NDA by submitting all safety information you now have regarding Copolymer-1. Please provide updated information as listed below:

1.   Retabulate all safety data including results of trials that were still ongoing at the time of NDA submission. The tabulation can take the same form as in your initial submission. Tables comparing adverse reactions at the time the NDA was submitted vs now will certainly facilitate review.

2.   Retabulate drop-outs with new drop-outs identified. Discuss, if appropriate.

3.   Provide details of any significant changes or findings, if any.

4.   World Literature Update

        Prior to the approval we require an updated report on the world's archival literature pertaining to the safety of copolymer-1. This report should cover all published papers including clinical or preclinical data that were not submitted with the original NDA. We need your warrant that you have reviewed this literature systematically, and in detail, and that you have discovered no finding that would adversely affect conclusions about the safety of copolymer-1. The report should also detail how the literature

search was conducted, by whom (their credentials) and whether it relied on abstracts or full texts (including translations) of articles. The report should emphasize clinical data, but new findings in preclinical reports of potential significance should also be described. Should any report or finding be judged important, a copy (translated as required) should be submitted for our review.

5.   Foreign Regulatory Update/Labeling

We require a review of the status of all copolymer-1 actions taken or pending before foreign regulatory authorities. Approval actions can be noted, but we ask that you describe in detail any and all actions taken that have been negative, supplying a full explanation of the views of all parties and the resolution of the matter. In addition, we ask that you provide us any current foreign labeling for copolymer-1, if appropriate, along with English translations when needed.

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

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

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:

> Teresa Wheelous, R.Ph.
> Regulatory Management Officer
> Telephone: (301) 594-2777

Sincerely yours,

Robert Temple 10/4/96

Robert Temple, M.D.
Director
Office of Drug Evaluation 1
Center for Drug Evaluation and Research

20622

Copaxone

Food and Drug Administration
Rockville MD 20857

NDA 20-622

Teva Pharmaceuticals USA
Attention: Deborah Jaskot
1510 Delp Drive
Kulpsville, PA 19443

DEC 20 1996

Dear Ms. Jaskot:

Please refer to your June 15, 1995 new drug application and your resubmission dated October 11, 1995 submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Copaxone (glatiramer acetate) injection.

We also refer to an Agency Approvable letter dated October 4, 1996, and we acknowledge receipt of your response amendments dated:

October 2, 1996	October 21, 1996	October 31, 1996	November 6, 1996
November 11, 1996	November 27, 1996		

This new drug application provides for the indication of reduction of relapses in patients with relapsing-remitting multiple sclerosis.

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

Accompanying this letter (ATTACHMENT) is the labeling that should be used for marketing this drug product. These revisions are terms of the NDA approval. Marketing the product before making the agreed upon revisions in the product's labeling may render the product misbranded and an unapproved new drug.

We have the following additional comments:

Chemistry:

We remind you of the following specifications agreed upon in a December 3, 1996 telecon between Dr. Paul Leber, Dr. Russell Katz, Dr. Stanley Blum, Dr. Martha Heimann, and Ms. Teresa Wheelous of the Division and Dr. Carol Ben-Maimon and Debbie Jaskot of your firm:

RRT at peak maximum:

RRT at -2SD:

RRT at -1SD:

RRT at +1SD:

The approximate molecular weight range of   is acceptable for use in the
drug product labeling.

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.

Phase 4 Commitments

We remind you of the Phase 4 commitments specified in the October 4, 1996 approvable
letter. Protocols, data, and final reports should be submitted to your IND for this product
and a copy of the cover letter sent to this NDA. Should an IND not be required to meet your
Phase 4 commitments, please submit protocol, data, and final reports to this NDA as
correspondences. For administrative purposes, all submissions, including labeling
supplements, relating to these Phase 4 commitments must be clearly designated "Phase 4
Commitments."

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

Please submit sixteen copies of the FPL as soon as it is available, in no case more than 30 days
after it is printed. Please individually mount ten of the copies on heavy weight paper or
similar material. For administrative purposes this submission should be designated "FINAL
PRINTED LABELING" for approved NDA 20-622. Approval of this submission by FDA is
not required before the labeling is used.

Additionally, 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 the Division of Neuropharmacological Drug Products and two copies of both the promotional material and the package insert directly to:

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

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:

Teresa Wheelous, R.Ph.
Regulatory Management Officer
(301) 594-2777

Sincerely yours,

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

ENCLOSURE

FINAL PRINTED LABELING HAS NOT BEEN SUBMITTED TO THE FDA.

DRAFT LABELING IS NO LONGER BEING SUPPLIED SO AS TO ENSURE ONLY CORRECT AND CURRENT INFORMATION IS DISSEMINATED TO THE PUBLIC.

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

DATE: December 18, 1996

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

SUBJECT: NDA 20-622, Copaxone® [glatiramer acetate, formerly identified as copolymer-1]

TO: File NDA 20-622
&
Robert Temple, M.D.
Director, Office of New Drug Evaluation 1

This memorandum conveys the Division's recommendation that NDA 20-622 for the use of Copaxone® [glatiramer acetate, formerly identified as copolymer-1] in the management of patients with relapsing remitting MS be approved.

The sponsor has complied with the conditions of approval enumerated in the approvable action letter of October 4, 1996.   Follow receipt of the agency's letter, the firm initially sought extensive revisions to the text of product labeling proposed in the approvable action letter.   However, following discussions between its representatives and Division staff, the firm agreed that to accept, without substantive modification, the labeling that had been proposed by the agency.

Our Program Management staff have reviewed the latest draft of labeling, and find that, with the exception of a change in official generic name[1], it conforms in all but a few minor, and in my view ignorable, details to that conveyed in the agency's approvable action letter.   The sponsor has seen all but our last revision of the final draft; again, I believe the labeling under which Copaxone will be approved for marketing of Copaxone differs in only minor details from that the firm last reviewed.

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[1] necessitated by USAN's ruling that the original generic name, copolymer-1, was unacceptable.

Accordingly, the other requirements of approval having been satisfied, the application may be approved.

Paul Leber, M.D.
December 18, 1996

REQUEST FOR TRADEMARK REVIEW

TO: Labeling and Nomenclature Committee
Attention: Daniel Boring, Chair, (HFD-530) MPN II, (827-2333)

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

From: Teresa Wheelous, Regulatory Management Officer (594-5535)
Division of Neuropharmacological Drug Products, HFD-120

Date: December 19, 1995

Subject: Request for Assessment of a Trademark for a Proposed Drug Product

Proposed Trademark: COPAXONE   **NDA#:** 20-622   *USAN NAME*

Established name, including form: Copolymer-1 for injection (IND)

Other trademarks by the same firm for companion products: None

Indications for Use (may be a summary if proposed statement is lengthy):
Slowing progression of disability and reducing frequency of relapses in patients with relapsing-remitting multiple sclerosis.

Initial comments from the submitter: (concerns, observations, etc.)

None.

cc:
NDA 20-622
HFD-120/division file
HFD-120/Leber
HFD-120/Katz/Rouzer-Kammeyer
HFD-120/SBlum/MHeimman
HFD-120/Wheelous

m:dos\wpfiles\nda\nomen.con
final: Dec 19, 1995

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

NDA # _X0-622_   Trade (generic) names _COPAXONE (Copolymer-1)_

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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_____   7/3/96   9/27/
Signature of Preparer   Date

cc: Orig NDA
HFD-___/Div File
NDA Action Package
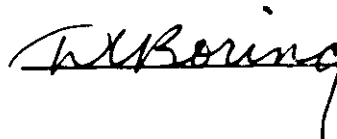
Consult #532 (HFD-120)

COPAXONE   copolymer-1 for injection

A review revealed no names which sound like or look like the proposed name. However, the Committee was uncertain about the USAN name since it does not appear in the current USAN handbook nor does it seem to comply with the usual USAN nomenclature conventions.

The Committee has no reason to find the proposed name unacceptable, but would suggest that the sponsor contact USAN regarding the use of the proposed USAN name.

CDER Labeling and Nomenclature Committee

_T.X. Boring_____, Chair

NDA 20-622

Teva Pharmaceuticals, USA
Attention: Stanley Scheindlin, D.Sc.
1510 Delp Drive
Kulpsville, Pennsylvania 19443

Dear Dr. Scheindlin:

Please refer to your New Drug Application (NDA) submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Copaxone® (Copolymer-1) Injection.

On the basis of our initial review of your new drug application referred to above, received on June 13. 1995, and acknowledged on July 5, 1995, we have determined that the application is not acceptable for filing under 21 CFR 314.101(d)(3) in that it is incomplete because it does not contain information required under 21 CFR 314.50(d)(1)(i and ii).   The critical deficiency resides solely in the chemistry, manufacturing, and control section. The deficiency is as follows:

The application fails to contain information necessary to evaluate the identity, quality, purity, and strength of the new drug substance/drug product (21 CFR 314.50(d)(1)(i & ii). Specifically, you have not submitted information describing the preparation and characterization of critical reference standards required for review of your application.

The materials described as Copolymer-1 markers and Copolymer-1 controls are critical primary reference standards for molecular weight determination in the methods listed below. No information about these materials, other than a brief paragraph (e.g. volume 1.3 pg. 038), has been provided.

Method No.

We are unable to evaluate the validity of these methods in the absence of information establishing the identity of the reference materials, i.e. the Copolymer-1 markers and controls. The following information is required for each reference sample.

     a.   A detailed description of the synthesis and purification of the marker.

b.   Spectroscopic and physical data to establish the chemical structure and any pertinent conformational properties, e.g. $\propto$-helical structure of the marker. This should include copies of actual spectra (e.g. NMR, IR, MS) for each marker.

c.   All analytical data and relevant calculations used to determine the molecular weight distribution of the markers.

Although not reasons for this Refuse to File Action, we have completed a preliminary review of your application and have identified the following deficiencies:

Chemistry and Manufacturing:

2.   We note that your application contains no evidence supporting your ability to scale up production of the drug substance. Should your application become approvable you will be limited to batches no larger than the pilot scale presently described until validated process scaleup information and detailed analytical data from production size batches of drug substance have been provided and reviewed.

3.   Please submit a table linking drug substance lot numbers, drug product lot numbers, and study description and number for all lots of drug substance / product used in both preclinical and clinical studies. Additionally, we request full analytical data for all of these lots including copies of the reverse phase

4.   The following applications and DMFs may not be referenced in support of the NDA until the IND and DMF holders submit Letters of Authorization (LoA) allowing the Agency to access their files on your behalf:

a.   IND   .   Currently TEVA is authorized by to access these applications only for the treatment protocol submitted under IND

b. DMF   Currently TEVA is authorized by Ben Venue to access this file for the treatment protocol IND   only.

c. DMF   submitted in the NDA is for Ben Venue, not TEVA. This LoA is not transferrable.

5. Please submit available analytical data tables for the drug substance and drug product lots on a 3.5 inch diskette in a spreadsheet format (i.e. Lotus or Excel).

6. In Section 3.2.6 Drug Substance Stability, please provide the following:

a. Supportive stability data referenced in this section.

b. Representative ⊦   for non-stressed samples and for samples exposed to each stress condition.

c. Moisture content, acetate content, and amino acid analysis at the end of the proposed 6 month retest period or any longer period proposed as an expiration date.

7. In Section 3.3.7.4, for manufacture of the drug product at Ben Venue Laboratories, a Master Formula should be provided and the Formula Card should indicate amounts of drug substance, excipients, and batch scale.

Pharmacology:

We request that you submit any data you might have addressing the issue of whether or not the antibodies produced as the result of administration of Copolymer-1 are neutralizing antibodies with respect to drug activity.

Within 30 days of the date of this letter, you may request in writing an informal conference about our refusal to file this application. To file this application over FDA's protest, you must avail yourself of this informal conference. We encourage you to avail yourself of a meeting with the Agency to discuss your resubmission. If you have any questions please call:
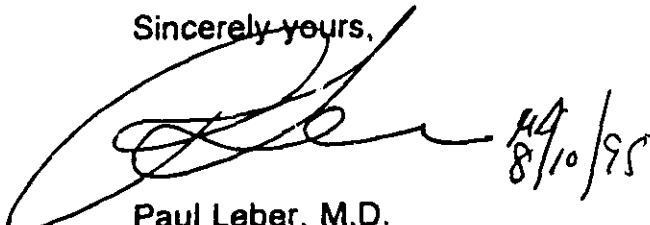
Teresa Wheeious, R.Ph.
Regulatory Management Officer
(301) 594-2777

If after the informal conference, you still do not agree with our conclusions, you may make a written request to file this application over protest, as authorized by 21 CFR

314.101(a)(3). The filing date will be 60 days after the date you requested the informal conference.

Under the Prescription Drug User Fee Act of 1992, FDA will refund one-half of the fee submitted with this application (25% of the total fee due). If you decide to file this application over protest, the filing of this application over protest will be regarded by the Agency as a new original application for user fee purposes, and you will be assessed a user fee applicable to new submission.

Sincerely yours,

Paul Leber, M.D.
Director
Division of Neuropharmacological
   Drug Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

NDA 20-622

OCT 20 1995

Teva Pharmaceuticals USA
Attention: Dr. Stanley Scheindlin
1510 Delp Drive
Kulpsville, PA 19443

Dear Dr. Scheindlin:

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

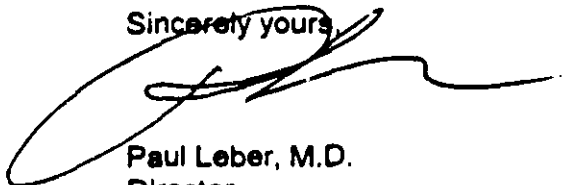|	
Name of Drug Product:	Copaxone® (Copolymer-1 for Injection)
Therapeutic Classification:	Standard
Date of resubmitted Application:	October 10, 1995
Date of Receipt:	October 11, 1995
Our Reference Number:	NDA 20-622

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

Should you have any questions, please contact:

Teresa Wheelous
Regulatory Management Officer
Telephone: (301) 594-2777

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

Sincerely yours,

Paul Leber, M.D.
Director
Division of Neuropharmacological
 Drug Products
Office of Drug Evaluation I
Center for Drug Evaluation and
 Research

Wheelous

NDA 20-622

TEVA Pharmaceuticals USA   AUG - 7 1996
Attention: Stanley Scheindlin, Ph.D.
1510 Delp Drive
Kulpsville, PA 19443

Dear Dr. Scheindlin:

Please refer to your pending October 10,1995 new drug application resubmitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Copaxone (copolymer-1) injection.

We also refer to your amendments dated January 10, 1996 and March 29, 1996.

We have completed our review of the microbiology and chemistry sections of your submission and have identified the following deficiencies:

Microbiology:

A.   Provide the following information about the drug product manufactured at Teva Pharmaceutical Industries:

1.   The bulk drug product prior to filtration is a peptide solution and does not contain a preservative.
a) Indicate whether the bulk solution supports microbial growth.
b) Submit information regarding the total bioburden load and volume of a batch of unfiltered bulk drug solution.
c) Indicate the kinds of microorganisms that can be recovered from the bulk solution.
d) Indicated the rationale for the   limit number in the unfiltered bulk solution. We note that specification of   for the bulk drug substance.
e) Indicate the alert and action limits for the bulk solution at the Teva facility.

2.   The sterilizing filters should ideally be validated with product inoculated with the challenge microorganism. Recirculation of drug product solution followed by a microbial filter challenge does not demonstrate the capabilities of the filter to sterilize the drug product solution. Please submit evidence that indicated that the sterilizing filters are capable of sterilizing the bulk solution. Indicted the actual CFU of P. diminuta used and recovered for assessing the microbial retentivity of the sterilizing filters.

3.   Filtration conditions are not specified in the submission. Describe conditions including bulk solution volume and filtration time, temperature, pressure, and

the set-up used during the filtration process. Indicated whether one or two
sterilizing filters are used to filter the bulk solution. In the event of a filter
failure, what actions would be taken?

4.   Indicated storage temperature and conditions during the holding periods for the
bulk product. Describe the sterilization validation of the holding tanks and vent
filters.

5.   A description of the   was omitted from
the application. Please describe the
includ

6.   Describe the autoclave loading patterns, the placement of the thermocouples and
biological indicators during the sterilization validation of the closures,
equipment, containers and components. Identify the commercial source, the
stability of the biological indicators used. Corroborate the microbial counts and
resistance and provide performance characteristics.

7.   Include a description of the bacterial endotoxin tests used for the product. The
description should include qualification of the laboratory, inhibition and
enhancement testing and results, determinations of noninhibitory concentration
and maximum valid dilution.

8.   Submit information on the sterilization validation of the freeze-drier.

B.   Provide additional information regarding the manufacturing process at the Ben
Venue Laboratories facility in Bedford, Ohio.

1.   The validation of the sterilizing filters as conducted at the Teva manufacturing
facility is inadequate. The sterilizing filters should ideally be validated with
product inoculated with the challenge microorganism. Recirculation of drug
product solution followed by a microbial filter challenge does not demonstrate
the capabilities of. the filter to sterilize the drug product solution. Please
submit evidence that indicates that the sterilizing filters are capable of sterilizing
the bulk solution or that organisms cannot be tested by direct inoculation into
the product. Indicate the actual CFU of P. diminuta used and recovered for
assessing the microbial retentivity of the sterilizing filters.

2.   Submit information on the sterilization of the freeze-drier.

3.   Provide data on the sterilization of the sterilizing and vent filters.

4. Specify what are actions #AN-S-3087-1 (p.039 237,241), #AN-S-086 (p. 039 240), and #AN-S-3-077 (p. 139 250, 252,253).

5. Include a description of the bacterial endotoxin tests used for the product. The description should include qualification of the laboratory, inhibition and enhancement testing and results, determinations of noninhibitory concentration and maximum valid dilution.

Chemistry:

1. Please provide additional information about the synthesis of copolymer-1 drug substance:

6 Pages
Purged

f.   Shelf-life instructions in patient insert (Vol. 3.14, p. 29)

The shelf-life of COPAXONE° as packaged for sale is 18 months when stored in a freezer (-20°C to -10°C). This product contains no preservatives and should be used immediately after reconstitution or discarded. Protect COPAXONE° from light.

We would appreciate your prompt written response so we can continue our evaluation of your NDA.

If you have any questions, please contact:

Teresa Wheelous, R.Ph.
Regulatory Management Officer
(301) 594-2777

Sincerely yours,

Paul Leber, M.D.
Director
Division of Neuropharmacological Drug Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

REVIEW AND EVALUATION OF CLINICAL DATA: SAFETY

Application Information

NDA # 20-622

Sponsor: Teva Pharmaceuticals

Clock Date   January 30, 1996

Drug Name

Generic Name: Copolymer 1

Proposed Trade Name: Copaxone

Drug Characterization

Pharmacological Category: Immunomodulator

Proposed Indication: Treatment of Multiple Sclerosis

NDA Classification:

Dosage Forms, Strengths, and Routes of Administration:
Subcutaneous injection, 20 milligram strengths.

Reviewer Information

Safety Reviewer: John Dikran Balian, M.D.

Review Completion Date: 3/14/96   Revised: 7/8/96

TABLE OF CONTENTS

1. Introduction

Multiple Sclerosis (MS) is a chronic inflammatory disease affecting the central nervous system (CNS). Myelin basic protein (MBP), the protective sheath that surrounds the axons of the CNS is the target for demyelination in MS. The animal model for MS, experimental allergic encephalomyelitis (EAE) is an autoimmune neurological disease induced by injections of MBP. The immunological processes in EAE are similar to those seen in human MS patients.

Copolymer-1 (Cop-1) is a synthetic copolymer of 4 amino acids (L-alanine, L-lysine, L-glutamic acid and L-tyrosine) in specific ratios but random order. These same 4 amino acids form the basic composition of the MBP. Cop-1 has been shown to be effective against EAE, possibly via interference with the immunological processes presumed to induce MS.

It is hypothesized that the basis of the efficacy of Cop-1 lies in its cross reactivity with MBP. The pre-clinical study results indicated binding of Cop-1 to the MHC class II molecules on antigen presenting cells. This in turn produces two specific effects that ameliorate the pathogenesis of MS: 1)Cop-1 induces specific suppressor T-cells and 2) inhibits specific effector T-cells.

Cop-1 is thought to initiate its immunomodulatory action at the site of the injection. Therapeutic effects are then mediated by systemic distribution of locally activated T-cells. In vitro and in vivo animal studies provided evidence that the drug is rapidly degraded at the site of injection and components reaching the circulation most likely are inactive. Exposure of non-immune systems (heart, lung, liver, kidneys, etc.) to the parent compound appears unlikely. The relevant effects of any systemic distribution of the drug itself or its degraded components are unknown.

Extrapolating from animal studies, serum concentrations of the drug in humans should be low or not detectable following subcutaneous administration of 20 mg once-daily. Therefore, even if detectable, blood levels of Cop-1 or its metabolites would not be expected to predict therapeutic effect.

Following the above findings, the sponsor decided to develop this drug as treatment for MS. In the 70s, studies in humans were begun and after initial encouraging results the sponsor expanded the trials from small open label studies to a small pilot controlled trial. The sponsor reported a trend toward protection from increasing neurologic disability. A trial in chronic progressive (CP) MS patients failed to demonstrate a statistically significant slowing of progression, hence the trials were focused upon the relapsing-remitting (RR) MS patient

group. The RR-MS patient group was studied in a series of open labelled and uncontrolled trials, one small controlled trial (BR-1) and one larger controlled study (01-9001). Study 01-9001 is designated pivotal by the sponsor, because it represents close to 90% of the overall exposure in the placebo-controlled trials of the RR-MS patient group. Except for study BR-2, the placebo-controlled trial in CP-MS, all the trials were performed using a single dose (20 mg once daily).

The main adverse events reported, across all trials consisted of injection site reactions and transient reactions during which patients noted flushing, sweating, palpitations, a feeling of tightness in the chest, dyspnea and associated anxiety (these series of concurrent symptoms were later coined as "systemic reaction").

The local and "systemic reactions" seen in the early clinical trials prompted pre-clinical investigations designed to test the effect of Cop-1 on the various organ systems. No significant abnormalities were reported in the non-immune systems (cardiovascular, respiratory, etc.) of the animals studied. However, immune complex deposition in the glomeruli of kidneys from chronically dosed rats (6 mos) and monkeys (1 year) were noted.

A brief mention of pertinent positive findings in animal studies may be of use here, (for thorough evaluation of this area please refer to the pharmacology review). During the multidose toxicity studies of subcutaneous administration of Cop-1, the main adverse event noted was local lesions at injection sites. These appeared to be dose related. At doses of 30 mg/Kg the injection site reactions were poorly tolerated by rats. The other notable finding was in the area of immunotoxicity. Studies performed in rats, monkeys, guinea pigs and mice confirmed the antigenic properties of the study drug. All studies confirmed the formation of IgG after repeated administration of Cop-1.

In rats and monkeys, following chronic exposure of 30 mg/Kg for 1 year, evidence of immune complex deposition in the glomeruli of kidneys could be found as both drug and complement were found in the glomeruli of the kidneys. No pathological effects of immune complex deposition were reported. However, in support of immune complex disease, there were reports of fibroid arterial lesions with immunohistochemical evidence of Cop-1 and complement deposits in the glomeruli in monkeys and anti-DNA and anti-histone antibodies in both rats and monkeys. Other animal toxicity data revealed some transient effects such as arrythmic changes and hemodynamic changes in 2 dogs.

In the latest version of the annotated labeling (submitted 3/26/96), Copolymer-1 is described as an "immunomodulator that blocks myelin-specific autoimmune responses" with a mechanism of

action of ameliorating the pathogenesis of MS by binding to the MHC class II molecules on antigen presenting cells with two specific effects: 1)induction of specific suppressor T-cells, and 2) inhibition of specific effector T-cells. It is indicated for "slowing the progression of disability and reducing the frequency of relapses in patients with RR-MS". In the adverse events section of the labeling, there is special mention of injection site reactions and a "transient, self-limited reaction immediately following subcutaneous injection". A brief explanation of this "transient, self-limited reaction," without mention of the symptoms is also included in the labeling.

2 S⌐   ⌐or the Review

The   JDA integrated safety summary (ISS), individual study summaries and reports, the data listings, Case Report Forms (CRFs), Patient Narratives (PNs), reports of deaths, premature terminations, common and serious adverse effects, overdose reports, and reports of treatment emergent changes in vital signs, clinical laboratory values, and ECGs were the sources used to review the safety aspects of this drug.

3. Methods of Review

For the safety review the entire database was evaluated for all adverse events, dropouts, uncommon and serious adverse events, suicides and deaths. Where appropriate, the overall data is mentioned in the review, but most tables presented in the review reflect data obtained from the placebo-controlled trials. Data from uncontrolled trials would not be useful to draw any comparisons with placebo. Also, a specific review of the most commonly reported adverse events (occurrence of >5% and 2 times placebo) noted in the placebo-controlled trials were reviewed specifically. The above results are discussed section by section below.

a. Quality of Submission

A critical review of the NDA and the data collection methods for the safety review was performed and the following can be reported:
                                            •

a.1 Completeness of Submission

Overall, the submission meets the criteria noted in the 45 day refuse to file report of the DNDP for filing and review of the NDA. The Integrated Safety Summary (ISS) submitted is complete, but it is not a document that can be relied upon, because of its inadequate information contents and at least at one point contradictory data (inconsistent figures are given for patient exposure data). Because the ISS is not a reliable source for the review, I concentrated on the individual study reports, which are complete and adequate. The sposnsor was frequently contacted for clarification, confirmation, or reanalysis of specific areas and the sponsor was tremendously helpful.

The tables generally requested by the agency, such as 1% adverse events table and premature terminations table were properly presented by the sponsor. Line Listings of patients of special interest are listed, but not indexed properly for cross referencing. Patient Narrative Summaries cf only premature terminations, deaths and hospitalizations are provided. All PNs provided were reviewed and the narratives were found to be sketchy and not comprehensive. PNs are not indexed properly for

cross referencing to locate the same individual in the data listings. The case report forms (CRFs) of deaths and dropouts are also provided. All CRFs of deaths and 20 dropouts (randomly selected) were reviewed. Most useful aspect of CRFs is the listing of reported adverse events, but to formulate a history or a "patient discharge summary" is not possible. The reported adverse events in the CRFs are not indexed to locate and verify the transferred information in the data listings.

There is a lack of information and follow-up regarding deaths. In three of the cases, it is not possible to draw clear conclusions regarding cause of death due to the lack of information in the CRFs and the FNs. Repeated requests made to the sponsor did not materialize in uncovering new information to clarify the histories of these deaths.

b. Quality of Coding

Investigator and patient descriptions for adverse events were categorized by the sponsor using the COSTART II dictionary. Data collection and tabulations of adverse events for the uncontrolled trials and the pivotal controlled trial 01-9001, were recorded directly from the CRFs (reported event, date of onset, duration, severity and outcome). For the other two controlled trials Br-1 and BR-2, information was gathered from CRFs designed to record adverse experiences through a set of symptom checklists. Adverse experiences data for BR-3 and the clinical pharmacology trials, were derived from clinical evaluation of source documents, publications or a letter from the investigator. All of these data were assigned preferred terms using COSTART terminology. Overall, it appears that the sponsor's coding approach was neither too conservative nor too inclusive.

c. Review of Study Design Adherence

The investigators and sponsor seem to have adhered to the protocol designs of all trials, and there is no evidence to the contrary.

There is a well devised plan in place to capture adverse events and to follow patients post termination (two follow-up visits, one 6 months and the second 12 months after termination are in the design of the studies) in the phase II-III trials. Patients who withdrew prematurely from any trial due to adverse experiences were characterized as those who either gave adverse experiences as their principal reason for withdrawal or who had data from the CRF indicating an adverse experience at the time of the withdrawal. Other categories for premature termination were (i)investigator decision based upon investigator's judgement that continued treatment was not in the best interest of the patient, (ii)pregnancy, (iii)poor compliance, (iv)progressive disease, (v)loss to follow-up, and (vi)patient decision (under this fall

patient's decision to discontinue for any reason other than adverse events).

Early phase II-III studies revealed no significant laboratory abnormalities, hence the investigators decided to perform laboratory testing at three to six month month intervals. However, due to the reported adverse events of local skin reactions and "systemic reaction"s in phase I studies and early phase II-III studies, the investigators made special note of capturing these adverse events in subsequent studies.

d. Review of Specific Definitions

Treatment emergent adverse events were interpreted properly by the sponsor: all adverse events, whether considered drug related or not were reported.

The term "systemic reaction" is an underlying theme throughout the ISS. This is a term or rather a case definition that the sponsor uses in an attempt to classify a confusing event, which has defied clinical description. This "systemic reaction" groups a series of adverse events that are "transient, self-limited reactions immediately following subcutaneous injection" of the drug. The issue of this "reaction" came to light in 1987, when Dr. Bornstein coined it as a "vasomotor response." Later, upon the suggestion of this division, clinical consultants devised a case definition for these concurrently occurring adverse events and the term "systemic reaction" was utilized as an umbrella for these events. The adverse events that characterize the case definition of "systemic reaction" are "vasodilatation or chest pain with palpitations, anxiety, and/or dyspnea". Hence any patient with a reported adverse event of vasodilatation or chest pain and an additional concomitant report of palpitations, anxiety, and/or dyspnea would be classified as a patient that experienced "systemic reaction". In this reviewer's opinion, the sponsor's arbitrary case definition for "systemic reaction" is restrictive. for example, the symptoms of "vasodilatation", chest pain, palpitations, anxiety, angioedema, flushing, urticaria, constriction of the throat and dyspnea might be all relevant. There appears to be a clear event that triggers the simultaneous appearance of some of these adverse events. A discussion with the sponsor to reach an appropriate case definition with a broader grouping of adverse events under this umbrella may be needed. This may facilitate future surveillance and reporting of the "systemic reaction".

Vasodilatation is a COSTART term that the sponsor has used as a blanket term to describe a multitude of reported events, such as "blood rushing to head, diffuse flush, face redness, flushed and warm skin" and many other symptoms that impart the idea of flushing, redness and warmth.

Angioedema was not listed as a COSTART term by the sponsor in the dictionary of adverse events of this submission. Additionally, "angioedema" was not among the patient or investigator reported adverse events, however there were symptoms listed under "vasodilatation" and "facial edema" that may be consistent with angioedema.

e. Findings From the Audit

An audit of CRFs and Patient Narratives (PNs) was performed, as mentioned above. A random sample was reviewed and there were no contradictions or misreporting.

Due to the lack of indexing and cross referencing, it is not possible to perform an audit to validate the proper transfer of the adverse events from the CRFs to the data listings.

4. Quality of Adverse Events Surveillance in the Development Program

A review of the CRFs revealed a rather thorough surveillance of the spontaneous reporting of the adverse events at every visit. But, it was not possible to certify the transfer of these reports to the data listings or verify their coding due to absence of cross-referencing and indexing. Aside from the spontaneous reporting system, surveillance or searches for specific adverse events were lacking. Another major weakness of the submission (this is common to almost all NDAs) is the total absence of clinical descriptions of the adverse events in the CRFs. Issues of co-morbidity, previous history, workup, follow-up, clinical characterization of a symptom, special testing, special treatment and start and stop dates of a symptom are usually not addressed in the CRFs. Occasionally, PNs may shed some light on these issues, but most PNs are very scanty and when not reflective of the contents of the CRF a reviewer can not determine their reliability. When the above were requested, the sponsor made a genuine attempt to be as comprehensive as possible and submitted a data listing of the adverse events that attempted to characterize them. But these were tables of the reported events, which revealed when and how often they occurred and whether the investigator considered them drug related or not. Although helpful, by no means these tables are explanatory when it comes to specific adverse events that need further investigation.

5. Study Population and Demographics

There are three adequate and well-controlled trials (01-9001 with its extension 9001E, BR-1 and BR-2) in this submission. The safety data presentations of this review will concentrate on these controlled studies, without disregarding the other studies and the entire safety database.

Study 01-9001, the largest of the controlled studies is a two-year, placebo-controlled, randomized, parallel-group, double-blind study involving 251 patients (Cop-1 125 and placebo 126). Patients 18-45 years of age, who met the protocol criteria of RR-MS were enrolled. Aside from the various efficacy outcome measures, the sponsor's safety analysis included looking at relapse episodes, hospitalizations, antibody levels, and clinically significant effects on vital signs, ECG or laboratory abnormalities. At the end of the two years of assigned treatment, the patients had the option of continuing on the same treatment under blinded conditions. 80% of Cop-1 patients and 83% of placebo patients from the original enrollment groups decided to extend their treatment for 35 months.

a. Extent of Exposure

The number of unique normal subjects and patients receiving Cop-1 worldwide is as follows:

Phase I (Clinical Pharmacology)

Drug	Number of Patients
Cop-1	49

Phase II-III (Clinical Trials)

Drug	Number of Patients
Cop-1	852
Placebo	206

The total clinical program (excluding the clinical pharmacology trials) consists of 11 clinical trials in which a total of 852 patients with MS have been exposed. Of 779 patients with RR-MS exposed to Cop-1, 670 were exposed for at least 6 months; 490 received the drug for at least 12 months, 290 for at least 2 years, 87 for at least 3 years, 15 for at least 5 years, and 4 for at least 10 years. With the exception of 63 patients in one trial in which the drug was administered at a dose of 20 mg every other day, all the rest were administered a single daily dose of 20 mg.

A total of 73 patients (BR-2 and BR-3) with CP-MS were exposed to Cop-1. In trial BR-2 the dose was 15 mg twice daily and in trial

BR-3, 20 mg once daily.

Due to missing data, precise information on patient years of exposure for the entire database is difficult to assess. Table 5.a.1 displays the exposure for the studies with reliable data:

Table 5.a.1
Duration of Patient Exposure in Patient Years

Type of Trial		COP-1	Placebo
Controlled Trials (9001/9001E, BR-1)	N Patient Years	150 338.7	151 356.2
Uncontrolled Trials (9002,1110-1,1110-2)	N Patient Years	586 753.7	0
Total	N Patient Years	736 1092.4	151 356.2

b. Extent of Exposure by Dose

Appendices 5.b.1 and 5.b.2 show the number of patients with RR-MS and CP-MS exposed to Cop-1. For all practical purposes, this NDA is a single dose exposure development (20 mg subcutaneous injections once daily).

c. Extent of Exposure by Disease Type

Relatively few patients with CP-MS were enrolled into the studies.

d. Demographics

Appendix 5.d.1 shows the demographics of all RR-MS studies, 5.d.2 the RR-MS controlled trials and 5.d.3 the CP-MS controlled trial. The RR-MS patients receiving the drug in these trials are representative in terms of demographic and disease characteristic of those likely to receive the drug after it is marketed. Each of the trials had more females than males, consistent with the overall MS population. The ages ranged from 18-68, with an average age of 30 years.

6. Review of Deaths

In the Cop-1 NDA, a total of 7 patient deaths were reported across all the clinical studies. These 7 deaths are summarized in Appendix 6.1. Two of the deaths were in RR-MS patients and the remaining five were from the CP-MS cohort.

There is no duration of exposure data from studies BR-3 and BR-2 (CP-MS trials), where 5 deaths occurred, hence it was not feasible to assess a crude rate of mortality and the mortality adjusted for time of exposure to drug. The 2 other deaths occurred in study 1110-1, an uncontrolled open label study. There were no deaths reported in the placebo group.

The patient narratives (PNs) and the CRFs on these patients are not very revealing. For all practical purposes, there is no information provided on one patient (#2039, study BR-3). For the rest, I relied upon sketchy PNs. Most had no post mortems performed. Patient #8501 from study 1110-1 may have had a post mortem (there are conflicting reports about whether there was a post mortem or not), in any case there is no appended report and the PN simply states that nothing significant was noted. The sponsor could not provide any further information on these deaths.

Two deaths are noteworthy for their possible association with a group of adverse events falling under the case definition of "systemic reaction" (discussed above and in greater detail below in section 10). Patient 01-2038 from study BR-3, a 46 year old male expired after approximately 3 years of treatment with Cop-1. 2 years into treatment, the patient started experiencing symptoms consistent with the description of "systemic reaction". The patient started reporting these symptoms two weeks prior to lapsing into an unexplained "coma". While hospitalized he continued receiving injections of Cop-1 and the family reported recurrences of the same symptoms (chest tightness, dyspnea with constriction of the throat and anxiety). The patient expired in the process of changing of his tracheostomy tube.

Patient 01-2039 from study BR-3, a 48 year old female expired after approximately 1.5 years of treatment with Cop-1. The case report form covers the treatment period up to two weeks prior to termination of study and a month prior to death. During this time, the patient reported symptoms consistent with the description of "systemic reaction" including constriction of the throat. There are no further details.

It is difficult to draw any conclusion regarding the causal relationship of the deaths to "systemic reaction", and hence to study medication.

7. Review of Serious Events

The Code of Federal Regulations (CFR) defines serious adverse events as "...any experience that is fatal or life-threatening, is permanently disabling, requires inpatient hospitalization, or is a congenital anomaly, cancer, or overdose" (21 CFR § 312.32). Of note, there was an apriori arrangement between the sponsor and agency, where the sponsor was allowed to separate hospitalizations from serious adverse events. For example, if a patient suffered an MI and was subsequently hospitalized, the patient would be reported under the serious AEs for the MI. However, a patient hospitalized due to an accident would not be reported under the serious AEs but would be listed under hospitalizations. There is separate reporting for all hospitalized patients.

The overall incidence rates of serious adverse events were reported to be 6.5% (55/844) in the Cop-1 group and 6.8% (16/206) in the placebo group. There were no serious adverse events reported in study BR-1, while in the other two controlled trials the incidence was reported to be 28.6% (36/176) in the Cop-1 group and 12.7% (23/181) in the placebo group. The overall (including phase I) incidence rates of hospitalizations are reported to be 6.5% (58/893, of which 19 were secondary to aggravation of MS) in the Cop-1 group and 13.6% (28/206, of which 23 were secondary to MS) in the placebo group. In the controlled trials the incidence was reported to be 10.9% (22/201, of which 14 were secondary to MS) in the Cop-1 group and 13.6% (28/206, of which 23 were secondary to MS) in the placebo group.

Additionally, incidence rates of serious events (as defined by the CFR) are reported under specific headings (review of systems, etc.). It should be noted, once again that most information (CRFs and PNs) is very sketchy, when available, and to draw conclusions as to whether an event is drug related or not is very difficult. Nonetheless, an attempt was made to classify the events as drug related or not and lists prepared (if a case falls under the related category, it simply means that in this reviewer's clinical jugdement from reading the sketchy PNs, there is no strong evidence to rule out disassociation from the drug). Appendices 13.1 and 13.2 display a listing of drug unrelated serious adverse events and hospitalizations and appendix 13.3 displays a listing of serious adverse events that may possibly be drug related. These appendices closely resemble the information and tables provided by the sponsor. In the text, some cases of interest that are thought to be possibly causally related to treatment are discussed (e.g. the two death cases). The incidence rates are low and not sufficient to relate causality on a statistical basis.

8. Review of Dropouts

a Overall Pattern of Dropouts

When all studies are taken into consideration, both controlled and uncontrolled, approximately 23.7% (200/844) of Cop-1 assigned patients dropped out (this probably reflects longer duration of treatment in the uncontrolled studies) and 16.0% (33/206) for placebo. The highest dropout rate in the placebo group is due to patient decision (8.74%), while the highest rate of dropout in the Cop-1 group is for adverse reactions (7.5%). Over the entire database, with 49 patients treated in clinical pharmacology studies and 844 in phase II-III studies, a total of 72 (72/893=8.1%) patients terminated prematurely due to an adverse event.

Table 8.a summarizes the reasons for patients's premature terminations in the database for the RR-MS controlled trials of the phase 2-3 studies:

Table 8.a
Distribution of Patients (RR-MS) who Prematurely Terminated Treatment

Reason	9001/9001E		BR-1		Total	
	COP-1 N=125	Placebo N=126	COP-1 N=25	Placebo N=25	COP-1 N=150	Placebo N=151
Adverse Experience	17	4	2	0	19	4
Investigator Decision	0	0	0	2	0	2
Patient Decision	7	17	0	1	7	18
Protocol Violation	0	6			0	6
Disease Progression	1	0			1	0
Treatment Failure	1	0			1	0
Lost to Follow-up	2	2			2	2
Unspecified			1	1	1	1
Total	28(22%)	29(23%)	3(12%)	4(16%)	31(21%)	33(22%)

In the RR-MS controlled trials the treatment groups of Cop-1 and placebo are similar in the total number of dropouts. The main reason for dropouts in the Cop-1 arm is adverse experience, while in the placebo, patient decision and protocol violation. The sponsor's explanation of "patient decision" is discontinuation by patient for any reason other than adverse events.

Table 8.b summarizes the reasons for patients's premature terminations in the database for the CP-MS controlled trials of the phase 2-3 studies:

Table 8.b
Distribution of Patients (CP-MS) who Prematurely Terminated Treatment

Reason Discontinued	BR-2	
	COP-1 (N=51)	Placebo (N=55)
Adverse Experience	6	1
Investigator's Decision	0	0
Patient Decision	4	5
Protocol Violation	0	1
Disease Progression	7	13
Treatment Failure	0	0
Lost to Follow-up	0	1
Unspecified	0	0
Total	17(33%)	21(38%)

In the Cop-1 arm of trial BR-2, the main reason for dropout is disease progression and adverse experience, while in the placebo, disease progression and patient decision.

b. Dropout Secondary to Adverse Events

Appendices 7.b.1 and 7.b.2 display all patients who dropped out secondary to an adverse event occurrence in the placebo-controlled studies. The most common adverse event associated with dropout was injection site reaction (all injection site reactions combined: 13/201=6.5% for Cop-1 and 2/206=1% for placebo, in trials 01-9001, BR-1 and BR-2). "systemic reaction" is not listed as a separate adverse event, but based on the definition of

"systemic reaction" not more than four patients could have dropped out secondary to "systemic reaction" from all three studies, since only one patient dropped out secondary to chest pain and 3 secondary to vasodilatation.

9. Other Safety Findings

a. ADR Incidence Table And AE Lists

Appendices 9.a.1, 9.a.2 and 9.a.3 display the incidence of adverse events in the placebo-controlled studies 01-9001, BR-1 and BR-2, respectively. Because of the small sample size and to avoid inclusion of every reported adverse event, for study BR-1 and BR-2 the usual $\geq$ 1% table was replaced with a $\geq$ 2% table. Pertinent adverse events are discussed in section 10.a under the review of systems.

b. Dose Response For Common Adverse Events

It is not possible to draw any conclusion about dose response relationships in this NDA, since all but one (BR-2) trials were fixed dose (20 mg/day).

c. Common and Drug Related Adverse Events

Adverse events with an incidence of $\geq$ 5% and reported at least twice as frequently in the Copolymer-1 group as in the placebo group are displayed in tables 9.c.1, 9.c.2 and 9.c.3.

Table 9.c.1
Controlled Study 01-9001/01-9001E

Body System	Adverse Experience	Number of Patients (%)	
		COP-1 (N=125)	Placebo (N=126)
Body as a Whole	chest pain	33 (26.4)	13 (10.3)
	face edema	11 (8.8)	2 (1.6)
	injection site erythema	73 (58.4)	17 (13.5)
	injection site hemorrhage	9 (7.2)	4 (3.2)
	injection site induration	25 (20.0)	1 (0.8)
	injection site inflammation	35 (28.0)	9 (7.1)
	injection site mass	33 (26.4)	10 (7.9)
	injection site pruritus	48 (38.4)	5 (4.0)
	injection site urticaria	9 (7.2)	0 (0)
	injection site welt	19 (15.2)	5 (4.0)
Cardiovascular	palpitation	14 (11.2)	6 (4.8)
	syncope	8 (6.4)	4 (3.2)
	vasodilatation	34 (27.2)	14 (11.1)
Metabolic and Nutritional	peripheral edema	14 (11.2)	7 (5.6)
	weight gain	7 (5.6)	0 (0)
Nervous	tremor	14 (11.2)	7 (5.6)
Respiratory	dyspnea	23 (18.4)	8 (6.3)
Skin and Appendages	erythema	8 (6.4)	4 (3.2)
Special Senses	eye disorder	8 (6.4)	1 (0.8)

Table 9.c.2
Controlled Study BR-1

Adverse Experience	Number of Patients (%)	
	COP-1 (N=25)	Placebo (N=25)
fever	2 (8.0)	0
injection site inflammation	22 (88.0)	4 (16)
injection site pain	23 (92)	9 (36,
injection site pruritus	3 (12)	0
injection site reaction	2 (8)	0 (0)
vasodilatation	3 (12)	0
vomiting	2 (8)	1 (4)
hypesthesia	2 (8)	1 (4)
insomnus	2 (8)	0
dyspnea	3(12)	0
pruritus	18 (72)	7 (28)

Table 9.c.3
Controlled Study BR-2

Adverse Experience	Number of Patients (%)	
	COP-1 (N=51)	Placebo (N=55)
Chills	3(6)	1(2)
infection	4 (8.0)	1(2)
injection site inflammation	41 (80.0)	9 (16)
injection site hemorrhage	3 (6)	1(2)
injection site pruritus	29 (57)	7(13)
injection site welt	3 (6)	0
injection site mess	19 (37)	9 (16)
vasodilatation	18(35)	7(13)
palpitation	14 (27)	6 (11)
pain	3 (6)	0

It is apparent that most of the adverse events reported, reflect the commonly experienced problems with injection site reactions and symptoms associated with "systemic reactions". The most commonly experienced adverse events such as injection site reactions, chest pain, eye disorder, etc. are discussed in section 10.a under the review of systems.

d. Adverse Event Incidence Over Phase 2-3 Integrated Primary Database

Appendix 9.d.1 includes all other adverse events reported from the clinical trials that are not reported in the incidence ≥ 1% table (Appendices 9.a.1, 9.a.2 and 9.a.3).

10. Review of Systems

In this section I will concentrate, system by system, on the commonly reported adverse events. However, aside from reporting incidence rates and occasional commentary, it is not possible to analyze specific AEs or cases. As mentioned in section 4, issues of co-morbidity, previous history, workup, follow-up, clinical characterization of a symptom, special testing, special treatment and start and stop dates of a symptom are not available. Aside from symptoms of injection site reactions and the "systemic reaction", 11 adverse events (eye disorder, weight gain, edema, facial edema, tremor, confusion, agitation, nystagmus, chest pain, syncope, and lymphadenopathy) were selected for specific analysis, because they were the most commonly reported adverse events in study 01-9001.

For an unknown reason, study 01-9001 had a higher reporting rate for all the commonly reported AEs, when compared to the other controlled trials or to the rest of the database. There was no specific analysis done by the sponsor to clarify the discrepancy in the reporting frequencies.

a.1 Neurology--Obviously, a thorough neurologic evaluation and reporting was performed at every visit to evaluate the effect of Cop-1 on the progression of MS. There were no seizures reported.

In study 01-9001, tremor (a COSTART term used by the sponsor that encompassed a series of reported events that included tremor, tremble, shaky feeling) was reported in 11.2% (14/125) of cop-1 patients and 5.6% (7/126) of placebo patients. In all controlled trials combined, tremor was reported in 7.5% (15/201) of cop-1 patients and 3.4% (7/206) of placebo patients. The incidence of tremor overall was reported to be 2.6% (22/844) of cop-1 patients and 3.4% (7/206) of placebo patients.

In study 01-9001, confusion (a COSTART term used by the sponsor that encompassed a series of reported events that included confusion, dazed, disorientation) was reported in 4% (5/125) of cop-1 patients and 0.8% (1/126) of placebo patients. In all controlled trials combined, confusion was reported in 3% (6/201) of cop-1 patients and 0.5% (1/206) of placebo patients. The incidence of confusion overall was reported to be 1.2% (10/844) of cop-1 patients and 0.5% (1/206) of placebo patients.

In study 01-9001, agitation (a COSTART term used by the sponsor that encompassed a series of reported events that included agitation, irritation, possible panic attacks, wired feeling) was reported in 5.6% (7/125) of cop-1 patients and 3.2% (4/126) of placebo patients. In all controlled trials combined, agitation was reported in 4.5% (9/201) of cop-1 patients and 1.9% (4/206) of placebo patients. The incidence of agitation overall was reported to be 1.4% (12/844) of cop-1 patients and 1.9% (4/206)

of placebo patients.

All three adverse events were COSTART terms for a series of symptoms reported. There were no specific tests done by the sponsor to study the three frequently reported neurological symptoms. In the overall database the incidence rate for serious AEs related to the nervous system was 1.7%(14/844) in the drug group and 2.9%(6/206) in the placebo group.

a.2 Opthalmology--Eye disorder was a COSTART term used by the sponsor that encompassed a series of reported events that included stye, eye irritation, eye contusion, "eye problems", etc.. In study 01-9001, eye disorder was reported in 6.4% (8/125) of cop-1 patients and 0.8% (1/126) of placebo patients. In all controlled trials combined, eye disorder was reported in 4.5% (9/201) of cop-1 patients and 0.5% (1/206) of placebo patients. The incidence of eye disorder overall was reported to be 1.1% (9/844) of cop-1 patients and 0.5% (1/206) of placebo patients.

Similarly with nystagmus. It was a COSTART term used by the sponsor that encompassed a series of reported events that included oscillocopsia, "eye problems", eye jerkiness, etc.. In study 01-9001, nystagmus was reported in 5% (4/125) of cop-1 patients and 1.6% (2/126) of placebo patients. In all controlled trials combined, nystagmus was reported in 2.5% (5/201) of cop-1 patients and 1.0% (21/206) of placebo patients. The incidence of nystagmus overall was reported to be 0.4% (5/844) of cop-1 patients and 1.0% (2/206) of placebo patients.

Both these AEs, almost exclusively, seem to be reported in study 01-9001. There were no specific tests done by the sponsor to study opthalmologic symptoms reported such as doing visual field studies. No serious AEs were reported for this system.

a.3 Psychiatry--There were no reported completed suicides in this NDA submission. One Cop-1 patient attempted suicide (overdose; patient #08-813 study 01-9001). The patient recovered without sequelae.

In a review of the patient narrative summaries, 3 more treatment emergent suicide attempts (overdoses using other drugs--patients 04-403 and 03-302 study 01-9001 and patient 01-106 study BR-2), and a patient (07-712, study 01-9001) with suicidal ideation were discovered. In the overall database the incidence rate for serious AEs related to psychiatry was 1.1%(9/844) in the drug group and 1.0%(2/206) in the placebo group.

a.4 Pulmonary--No specific tests done. Despite the frequently reported adverse event of dyspnea and/or "constriction of the throat" in association with "systemic reaction", there were no specific attempts made to do peak flows, spirometry or other studies to measure the presence and severity of bronchospasm. In

the overall database the incidence rate for serious AEs related
to pulmonary was 0.4%(3/844) in the drug group and 0% in the
placebo group.

a.4 Cardiovascular--As in pulmonary, symptoms associated with
"systemic reaction" included chest tightness, palpitation and
"vasodilation", but there was no cardiovascular testing beyond
the ECG at the termination of the study.

Chest pain was a COSTART term used by the sponsor that
encompassed chest pain and chest tightness. In study 01-9001,
chest pain was reported in 26.4% (33/125) of cop-1 patients and
10.3% (13/126) of placebo patients. In all controlled trials
combined, chest pain was reported in 22% (44/201) of cop-1
patients and 10.7% (22/206) of placebo patients. The incidence of
chest pain overall was reported to be 10.3% (87/844) of cop-1
patients and 10.7% (22/206) of placebo patients.

This time, studies 01-9001 (33/125=26.4%) and BR-2 (11/51=21.5%)
had a higher reporting rate of chest pain when compared to the
rest of the database (none were reported in BR-1). There was no
explanation regarding the discrepancy in the reporting
frequencies in the different studies.

In trial 9001/9001E, there were 33 cases of chest pain (or
tightness) in the cop-1 group. Included in these numbers are 6
cases that met the sponsor set criteria of "systemic reaction."
In other words, of the 19 cases from trial 9001/9001E that the
sponsor classified as experiencing ""systemic reaction"" 6 gave
chest pain as their primary symptom. In all cases the chest pain
was reported as a short episode (usually few minutes) not
requiring therapeutic intervention.

As mentioned in section 4, there is total absence of clinical
descriptions of the adverse events in the CRFs. When specific
information regarding the chest pains were requested, the sponsor
made a genuine attempt to be as comprehensive as possible and
submitted a data listing of the adverse event that attempted to
characterize them, but these were tables of the reported events,
that revealed when and how often they occurred and whether the
investigator considered them drug related or not. Although
helpful, by no means these tables answer burning issues of
interest.

In most instances the AE chest pain occurred while as an
outpatient and the patient did not report the event until the
next visit. There are no ECGs done while the episode was in
progress and follow-up ECGs (when done at all, mostly done at
study termination) were not significant. From all cases and
reports reviewed, the indication is that the chest pain or
tightness reported does not lead to any lasting cardiac injury.
From the information provided, it is difficult to assess the

relationship of time of onset of chest pain to injection of the drug or placebo, although in some instances it is reported to occur immediately following injection, but for the vast majority this information is not provided. Most episodes appear to be brief, 2/3 of the cases are recurrent (on the average 3 episodes), very few cases discontinued secondary to this AE and few more had temporary interruption of treatment. Whenever available, the vast majority of follow-up ECGs are unchanged from baseline. There is also no evidence to support the hypotheses whether the drug may or may not cause transient ischemia from decreased perfusion of the cardiac muscles. Any thoughts regarding possible transient coronary vessel constriction (as may occur with cocaine or other drugs) can not be substantiated with the data provided. Further investigation of this issue is warranted.

In study 01-9001, syncope was reported in 6.4% (8/125) of cop-1 patients and 3.2% (4/126) of placebo patients. In all controlled trials combined, syncope was reported in 5% (10/201) of cop-1 patients and 2.4% (5/206) of placebo patients. The incidence of syncope overall was reported to be 1.3% (11/844) of cop-1 patients and 2.4% (5/206) of placebo patients. As is the case with chest pain, the causal relationship of syncopal events to cop-1 is difficult to assess.

In the overall database the incidence rate for serious AEs related to the cardiovascular system was 0.6%(5/844) in the drug group and 2.4%(5/206) in the placebo group. Chest pain itself was reported as a serious event in only 2 patients in study 01-9001/9001E.

a.5 Renal--There was no specific testing done, such as looking for immune complex disease on autopsy specimens.

a.6 Gastrointestinal--No specific focus in AE surveillance or conduct of specific testing. In the overall database the incidence rate for serious AEs related to this system was 1.4%(12/844) in the drug group and 1.0%(2/206) in the placebo group.

a.7 Musculoskeletal--No specific focus in AE surveillance or conduct of specific testing. In the overall database the incidence rate for serious AEs related to this system was 1.4%(12/844) in the drug group and 0% in the placebo group.

a.8 Hematologic--No specific focus in AE surveillance or conduct of specific testing (such as biopsy) despite the appearance of lymphadenopathy as a frequent AE.

Lymphadenopathy was a COSTART term used by the sponsor that encompassed a series of reported events that included swollen neck lymph glands, groin lymphadenopathy, lump in the groin, lump

in the left lower quadrant, submandibular swelling, etc.. In study 01-9001 lymphadenopathy was reported in 18.4% (23/125) of cop-1 patients and 9.5% (12/126) of placebo patients. All controlled trials combined, lymphadenopathy was reported in 12.4% (25/201) of cop-1 patients and 5.8% (12/206) of placebo patients. The incidence of lymphadenopathy overall was reported to be 4.3% (36/844) of cop-1 patients and 5.8% (12/206) of placebo patients. Again, the causal relationship of lymphadenopathy events to cop-1 is difficult to assess.

In the overall database the incidence rate for serious AEs related to the hematologic/lymphatic system was 0.2% (2/844) in the drug group and 0% in the placebo group. One of these cases is of interest: Patient 707, study 01-9001, was a 26 year old female that after 39 days of cop-1 treatment experienced enlarged lymph nodes that increased in size with continued treatment. Upon a temporary stoppage of treatmment due to an unrelated event, the lymph nodes decreased in size. Upon rechallenge, the lymph nodes once again were enlarged. An excision biopsy revealed "reactive nodes in the left groin and the remaining nodes were benign". Although, the PN mentions a pathology report, it was not attached and the sponsor states that there is no more information at hand.

a.9 Body as a Whole--No specific focus in AE surveillance or conduct of specific testing.

In study 01-9001, weight gain was reported in 5.6% (7/125) of cop-1 patients and 0% (0/126) of placebo patients. In all controlled trials combined, weight gain was reported in 3.5% (7/201) of cop-1 patients and 0 (0/206) of placebo patients. The incidence of weight gain overall was reported to be 1.4% (22/844) of cop-1 patients and 0% (0/206) of placebo patients.

In study 01-9001, edema was reported in 4% (5/125) of cop-1 patients and 0.8% (1/126) of placebo patients. In all controlled trials combined, edema was reported in 2.5% (5/201) of cop-1 patients and 0.5% (1/206) of placebo patients. The incidence of edema overall was reported to be 1.4% (12/844) of cop-1 patients and 0.5% (1/206) of placebo patients.

In study 01-9001, facial edema was reported in 8.8% (11/125) of cop-1 patients and 1.6% (2/126) of placebo patients. In all controlled trials combined, facial edema was reported in 6% (12/201) of cop-1 patients and 1.0% (2/206) of placebo patients. The incidence of facial edema overall was reported to be 1.8% (15/844) of cop-1 patients and 1.0% (2/206) of placebo patients. There were no cases of angioedema reported and angioedema was not listed under the AEs in the sponsor's dictionary.

All three adverse events were COSTART terms for a series of symptoms reported. Once again, study 01-9001 had a higher reporting rate when compared to the other controlled trials and

to the rest of the database. There were no specific tests done by the sponsor to either clarify the discrepancy in the reporting frequencies or to study the reported events.

In the overall database the incidence rate for serious AEs related to the body as a whole was 4.5%(38/844) in the drug group and 3.4%(7/206) in the placebo group.

a.10 Endocrine/Metabolic--No specific focus in AE surveillance or conduct of specific testing. In the overall database the incidence rate for serious AEs related to this system was 0.2%(2/844) in the drug group and 0% in the placebo group.

a.11 Immunology

Human allergic reactions are caused by immediate release of mediators from mast cells and basophils after interaction with an antigen. These mediators, such as histamine, induce the characteristic clinical signs and symptoms of the allergic response. Activation of the mediators can be both immunologic (IgE) and non-immunologic (direct activation by the agent without antibody involvement). For the immunologic process, prior exposure to the antigen is necessary (Anderson, JAMA 1992; Champion et al. Br J Dermatol 1969).

Considering the mechanism of action of Cop-1 (activation of T-cells), and the two most common adverse events ("systemic reaction" and injection site reaction), the critical issue becomes whether an immunologic process is responsible for these effects. A series of studies were performed by the sponsor in an attempt to discover an etiology for these reactions and thus an explanation whether the drug is immunogenic or not.

In one such study (placebo-controlled trial 01-9001), serum samples were monitored every 3 months for the development of Cop-1 reactive antibodies. Results revealed that, antibody levels reached maximum values within 3-6 months of exposure. 80% of the patients experienced increases of >150% over baseline levels. These levels declined subsequently to around 50% above baseline values in majority of the patients. Placebo treated patients did not experience a significant or consistent response. The peak antibody levels in the placebo group (in 80% of the patients were below 50% over baseline values) were not as high as in the Cop-1 group. Also the peaks in the placebo group were random and occurring at random timepoints. There is evidence (from animal and human data) that the Cop-1 reactive antibody is IgG and not IgE.

Another small study revealed that Cop-1 induced histamine release from basophils only at very high concentrations: concentrations much higher than would be expected from regular dosing of 20 mg/day.

Skin testing of intradermal injections of Cop-1 caused a positive reaction (a wheal of >5mm) in naive as well as in previously exposed patients; prior administration of an antihistaminic agent (terfenadine) greatly reduced the size of the skin wheal.

Based upon the in vitro, preclinical and above mentioned data, the sponsor claims that the clinical picture is not consistent with an allergic sensitization, as there is no memory response and no associated symptoms. The sponsor goes on to conclude that, the formation of antibodies is a "simple manifestation of its bioavailabity and antigenicity and is not related to allergic sensitization", and the decline in antibody levels upon continued treatment reflects the tolerance of the antibody producing system. The sponsor deduces that the antibody is neutral: it does not interfere with the activity of the drug. The evidence supporting this claim comes from observation that (i)no matter how high the antibody levels, they do not interfere with the mechanism of action of the drug (activation of T-cells); and (ii)efficacy data reveal continued effectiveness with continued exposure to the drug even at highest levels of antibody levels.

The sponsor claims that no correlation was evident between antibody levels and episodes of "systemic reaction"s. Also there was no correlation between relapses and reactive antibody levels. However, in a somewhat inconsistent finding with the above statement, one small study revealed higher IgG levels among patients with systemic symptoms than those without adverse events. The sponsor has no explanation for this finding.

In this reviewer's opinion, the symptoms associated with "systemic reaction" are consistent with a generalized drug reaction. It is also apparent that there is activation of basophils and mast cells by Cop-1. The studies conducted and the many reported adverse events confirm these statements. To determine whether an immunologic process (such as systemic anaphylaxis) or a non-immunologic process (such as generalized anaphylactoid reaction) is responsible for the effects of the drug, more data is needed. There are studies and laboratory tests confirming the absence of IgE in the process. Hence, to refute the sponsor's claim (that the drug is not immunogenic) is difficult.

Another concern with this drug are the reports from animal studies (rats and monkeys) that, following chronic exposure, both drug and complement were found in the glomeruli of the kidney. No pathological effects of immune complex deposition were reported. However, in support of immune complex disease, there were reports of fibroid arterial lesions in a number of monkeys and anti-DNA and anti-histone antibodies in both rats and monkeys. There are no human studies that investigated autoimmune disorders or immune complex disease. There is no evidence that Cop-1 causes general immunosuppression, as there are no reports of increased

infections in the treated group.

There were no reported serious AEs under this system by the
sponsor, however there were two cases that were reported as
serious AEs and may be classified under this section: Patient 02-
1, study BR-1 "experienced sweating, anxiety, vasodilatation and
sensitivity at the injection site and syncope." Patient improved
with treatment for anaphylaxis and was not discontinued. This AE
could very well have been a "systemic reaction", but it did not
qualify as defined by the sponsor; and Patient 8428, study 1110-
1, was a 31 year old female that after 25 day of cop-1 treatment
experienced sysmptoms of injection site erythema and
hypersensitivity lasting 2 days. 8 days later experienced the
same symptoms and was given a diagnosis of "serum sickness
(arthus phenomenon)". Patient improved with discontinuation.

a.11.1 "systemic reaction"

"Systemic reaction" is the "adverse event" of greatest notoriety
in this submission. This is a term or rather a case definition
that the sponsor uses in an attempt to classify a confusing
event, which has defied clinical description. As mentioned
before, this "systemic reaction" was an arbitrary definition used
by the sponsor that attempts to group a series of adverse events
that are "transient, self-limited reactions immediately following
subcutaneous injection" of the drug. The term "systemic reaction"
was utilized as an umbrella for the concurrent AEs of
"vasodilatation or chest pain with palpitations, anxiety, and/or
dyspnea". Hence any patient with a reported adverse event of
vasodilatation or chest pain and a simultaneous report of
palpitations, anxiety, and/or dyspnea was classified as a patient
that experienced "systemic reaction."

Vasodilatation is a COSTART term that the sponsor has used as a
blanket term to describe a multitude of reported events, such as
"blood rushing to head, diffuse flush, face redness, flushed and
warm skin" and many other symptoms that impart the idea of
flushing, redness and warmth. Angioedema is not listed as a
COSTART term by the sponsor in the dictionary of adverse events
of this submission. Additionally, "angioedema" is not among the
patient or investigator reported adverse events, however there
are symptoms listed under "vasodilatation" and "facial edema"
that may be consistent with angioedema.

As presented in the ISS (using the sponsor's case definition), no
episodes of "systemic reaction" were reported in the clinical
pharmacology studies and of 844 patients in the clinical trials,
87 (10.31%) reported at least one such episode. Of these 87
patients, 52 reported only one episode, 17 had two episodes, 11
had three, 4 had four, 2 had five, no patient reported 6 episodes
and one patient reported a total of 7 episodes.

Table 10.b.1 documents the incidence of "systemic reaction"s in study # 01-9001.

Table 10.b.1

"systemic reaction"	Cop-1 (N = 125)	Placebo (N = 126)
Number of Patients	19 (15.2%)	4 (3.2%)
Number of Episodes		
1	10	4
2	4	0
3	3	0
4	1	0
7	1	0

The 4 placebo patients in this table also met the sponsor set criteria of "systemic reaction".

In this reviewer's opinion, the sponsor's arbitrary case definition for "systemic reaction" is restrictive in the number of symptoms used under its umbrella. The symptoms of "vasodilatation", chest pain, palpitations, anxiety, angioedema, flushing, urticaria, constriction of the throat and dyspnea may be all reflective of "systemic reaction" and relevant to this "adverse event". For example, if any three of these symptoms qualified as a "systemic reaction" the incidence then will be higher. Appendix 10.b.1 displays such a list of patients that could be designated aS having experienced "systemic reaction." This list was compiled from patient narratives of only two groups: premature terminations and hospitalizations. This list reveals a high frequency of recurrent episodes of this adverse event. Obviously, the list is not comprehensive.

It is apparent that these reactions may occur at any time interval during exposure and may occur only once or may have an irregular episodic pattern. Of special note, the time to first occurrence of most cases of the "systemic reaction" averages several months after initiation of cop-1, and as mentioned earlier, some experience only one episode while it is recurrent with others.

Aside from the case definition and the true etiology of this "systemic reaction," the question arises, as to whether the grouping of the individual adverse events that designate this "syndrome or systemic reaction" is misleading. The individual adverse events may completely be separate entities occurring together only coincidentally. This scenario is highly unlikely. But, in view of the seriousness of adverse events such as chest pain, it is only wise to consider this possibility. Also, the two death cases discussed in section 6, though can not be directly

linked to "systemic reaction", are worisome and a relationship can not be ruled out, in view of lack of details.

Although there is no evidence to support it, the sponsor puts forth a hypothesis that a possible trigger of the events may be secondary to injecting the drug into the wrong location (blood vessels instead of subcutaneously). Ascribing a causal relationship of the "systemic reaction" to the study drug is not in dispute. The difficulty lies in describing an etiology for it. The majority of cases may fall into the category as defined by the sponsor: "simple manifestation of its bioavailabity and antigenicity and is not related to allergic sensitization"--most likely mediated by non-immunologic mechanisms, i.e. direct activation of mediators.

There are few cases where an explanation of a true allergic manifestation (urticaria, angioedema, bronchospasm, etc.) of Cop-1 is plausible. In others, the possibility of immune-complex disease should also merit consideration. In some animal studies, there was evidence of immune complex formation and complement deposition. From the available human data, it is difficult to confirm this hypothesis, since there are no skin biopsies, renal tests, and autopsies provided on these patients. For immune-complex formation a high antigen load is necessary. There is evidence of rise in IgG antibody, but with continued treatment there is a decline in the levels. There are also conflicting reports of the association of IgG levels with the adverse event. Also, the almost always prevalent symptom of fever in immune-complex disease was missing in these patients.

The sponsor concludes that the "systemic reaction" is non-immunologic. I would venture that different patients may react differently: in some, drug allergy is a possibility, in the majority, it very well may be a non-immunologic process, and in others, immune-complex disease can not be ruled out. Currently, there is no convincing human data to support any of these hypotheses.

a.12 Skin--In the overall database the incidence rate for serious AEs related to this system was 0.4%(3/844) in the drug group and 0% in the placebo group. Most noteworthy issue here is the injection site reactions:

a.12.1 Injection Site Reaction

The most commonly occurring adverse events attributable to cop-1 were reactions at the site of injection (the incidence in study 01-9001/9001E was 90% of patients treated with cop-1 and 60% of patients treated with placebo). These are also the most common AEs associated with premature discontinuations. Injection site pain, erythema, pruritus and ecchymosis were the major complaints.

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The joint occurrence of injection site reactions to "systemic reaction" was examined to analyze a possible relationship. In study 01-9001/9001E, of the 19 patients that reported "systemic reaction" only 5 reported any moderate or severe local injection site reaction, and only one of the five reported the two events at the same time. It does not appear that experiencing a moderate or severe local injection site reaction is predictive of "systemic reaction".

The presentation of timing of symptoms and severity varied from immediate reactions post injection to reactions appearing with chronic exposure. As in the case of "systemic reaction" there is no evidence to support or refute the sponsor's claim that a possible trigger for this adverse event may be the injection of the drug into the wrong location (blood vessels instead of subcutaneously). It is the sponsor's claim that the immediate local reaction is most likely mediated by non-immunologic mechanisms, i.e. direct activation of mediators and release of histamine by Cop-1 without IgE release. Unfortunately, no skin biopsies were done on these cases to shed more light on this issue.

11. Laboratory Findings, ECG and Vital Signs

a. Laboratory Findings

The sponsor has submitted an analysis of the laboratory data and
tabulated the results. The sponsor has not used the analysis
approach recommended by this division: incidence tables,
tabulations of the statistical summary of mean changes from
baseline or other shift tables. Nonetheless, since there are no
significant abnormalities noted in my review, it was decided not
to make a request to reanalyze the data, but simply to document
the findings. In the controlled trials, laboratory testing was
performed at every visit (every three months), while in the other
studies laboratory testing was done at 3-6 month intervals. Under
th e laboratory section only one placebo patient was reported
with a serious chemistry AE. The data of the controlled trials
(as presented below) reflects the overall database and no
particular issues of concern were noted.

a.1 Serum Chemistry

Appendix 11.a.1.1 lists the criteria (used by DNDP) and incidence
of clinically significant chemistry laboratory abnormalities in
the controlled trials. As this table indicates, there are no
areas of concern regarding chemistry abnormalities in the
available data and none of the changes can be causally ascribed
to Cop-1.

a.2 Hematology

Appendix 11.a.2.1 lists the sponsor's criteria and incidence of
clinically significant hematology laboratory abnormalities in the
controlled trials. As this table indicates, there are no areas of
concern regarding hematology abnormalities in the available data
and the changes can not be causally ascribed to Cop-1.

a.3 Urine Analysis

There were no reports of serious adverse experiences or
premature terminations due to abnormalities in urinalysis
parameters. For this section, no individual cases were reviewed.
From the available data it is apparent that no particular urine
analysis abnormality can be attributed to Cop-1.

b. ECG Findings

ECGs, at baseline and termination were performed in the large
controlled trial 01-9001/9001E. A review of each ECG abnormality
reported, revealed no particular tendencies and no overall
increase of adverse events were noted when compared to placebo.

Cop-1 does not appear to induce heart rate, PR, QRS, or QTc

interval abnormalities.

c. Vital Signs

Appendix 11.c.1 lists the criteria and incidence of clinically
significant Vital Signs abnormalities in the controlled trials.
Evaluation of postbaseline shifts for vital signs disclosed no
differences between the Cop-1 and the placebo groups.

In animal studies, hypotensive effects were reported. Also, from
human cell culture studies, Cop-1 was shown to induce release of
interleukin-2, a cytokine that can initiate the release of other
cytokines that may destabilize the cardiovascular system. Despite
these findings, there is no clinical data to raise concern.

12. Effect of Age and Gender on Adverse Event Incidence

Age based analysis is not possible to perform. There was only one patient above the age of 65 enrolled in the clinical trials. No reliable analyses of adverse event incidences on the basis of gender were performed. Tabulations provided by the sponsor revealed that in the large placebo-controlled trial few more females receiving cop-1 reported "vasodilatation and lymphadenopathy".

13. Important Events Considered Not Drug Related

The definition of a serious adverse event is given above in section 9. All CRFs and patient narratives provided on serious adverse events and hospitalizations were reviewed and appendix 13.1 displays a listing of such adverse events for Cop-1 that in this reviewer's opinion are not attributed to treatment. Also, appendix 13.2 displays a listing of hospitalizations that in this reviewer's opinion are not attributed to treatment. Please note that fatalities have already been included in Appendix 6.1 and are not repeated in Appendices 13.1 and 13.2.

14. Human Reproductive Data

Pregnancy was an exclusion criterion for enrollment. Seven patients became pregnant while being treated with Cop-1 in the phase II-III studies.

Three of the patients electively terminated the pregnancies. Three other patients withdrew form the study after 424, 714 and 905 days of treatment and their pregnancies were uneventful resulting in births of normal healthy babies. No information is available regarding the seventh patient.

15. Overdose Experience

During the worldwide development of Cop-1 there was one attempted overdose using Cop-1 as the agent. Patient 08-813 from study 01-9001 injected four doses (80 mg total) of Cop-1 with no reported adverse events.

16. Withdrawal Phenomenon/Abuse Potential

No specific studies to evaluate the effects of withdrawal from Cop-1 were performed.

In addition, the sponsor does not report any studies to evaluate i ances of Cop-1 abuse or dependence. There was lack of voluntary and persistent dose escalation by patients. Overall, there seems to be no evidence of withdrawal phenomenon or abuse potential for this drug.

17. Summary of Drug Interactions

a. Drug-Demographic Interactions

The sponsor has not performed any studies to assess the effects of age on the pharmacokinetics of Cop-1.

b. Drug-Disease Interactions

The sponsor has not performed any studies to explore drug-disease interactions.

c. Drug-Drug Interactions

The sponsor has not performed any studies to explore interactions of Cop-1 with other drugs.

18. Labeling Review

The latest version of the annotated labeling (submitted 3/26/96), falls short on a clear discription and definition for the "systemic reaction", calling it a "transient, self-limited reaction". Also, there are no highlights of the commonly occurring AEs, except for the presentation of the >2% incidence AE table of AEs from study 01-9001/9001E.

19. Conclusions

Cop-1 is a synthetic basic copolymer of random amino acids that has been shown to be effective in suppression of EAE and is presented in this NDA as a candidate drug for the treatment of RR-MS.

Cop-1 is thought to initiate an immunomodulatory action at the site of injection. Therapeutic effects are then mediated by systemic distribution of locally activated T-cells. Based on animal studies, the drug is rapidly degraded at the site of injection and serum concentrations of the drug in humans are presumed to be low or undetectable following subcutaneous administration of 20 mg once-daily.

Ascribing a causal relationship to the treatment emergent adverse events grouped under the sponsor's definition of "systemic reaction" and injection site reaction seen with cop-1 is not in dispute, but describing an etiology is elusive. There are few cases where an explanation of a true allergic manifestation of Cop-1 is plausible. The majority of cases may fall into the category as defined by the sponsor "simple manifestation of its bioavailabity and antigenicity and not related to allergic sensitization": most likely mediated by non-immunologic mechanisms, i.e. direct activation of mediators. The sponsor concludes that the treatment emergent adverse events are non-immunologic.

Ascribing a causal relationship to the other commonly reported treatment emergent adverse events such as chest pain is not possible with the data and explanations available. In summary, the main safety concerns for this NDA are the AEs grouped by the sponsor as "systemic reaction" and injection site reactions. More data is needed to determine whether an immunologic process (such as systemic anaphylaxis) or a non-immunologic process (such as generalized anaphylactoid reaction) is responsible for the effects of the drug. Hence, to refute the sponsor's claim that the drug is not immunogenic is difficult.

20. Recommendations

In my opinion, the New Drug Application for Cop-1 is approvable from a safety standpoint if the efficacy review finds the drug to

be efficacious. However, to further support the safe and effective use of Cop-1, it is recommended that the following issues be explored by the sponsor:

(i)A cla. ification of the pharmacokinetics of the drug in humans. There is evidence from rat studies that with chronic exposure the systemic distribution of larger components of the drug increases;

(ii)Dose-response and dose-ranging studies should be performed. Is 20 mg the optimum dose? Are daily injections necessary?

(iii)A study to rule out autoimmune disease in humans. There were reports of fibroid arterial lesions in a number of monkeys and anti-DNA and anti-histone antibodies in both rats and monkeys;

(iv)A study to rule out immune complex disease in humans. In animal studies (rats and monkeys), following chronic exposure, both drug and complement could be found in the glomeruli of the kidney;

(v)A study to clarify the etiology of injection site reactions. This may be in the form of skin biopsies;
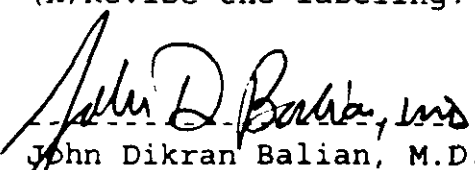
(vi)A study to characterize and understand the adverse event "chest pain/tightness" to rule out transient ischemic charges;

(vii)A study to better characterize and understand the ""systemic reaction"s" after an agreed upon case definition is formulated;

(viii)Postmarketing surveillance for evidence of vasculitis, immune complex disease, autoimmune disease, serum sickness glomerulonephritis, or other systemic effects of immune mediated diseases;

(ix)A discussion with the sponsor to reach an appropriate case definition for ""systemic reaction"". A broader grouping of adverse events under this umbrella may be necessary. This may facilitate future surveillance and reporting of the ""systemic reaction""; and

(x)Revise the labeling.

John Dikran Balian, M.D.   7/8/96
John Dikran Balian, M.D.   Date
Clinical Reviewer Safety Group,
Div. of Neuropharmacologic Drug Products

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APPENDICES

APPENDIX 5.b.1

Number of Patients with RR-MS Exposed to 20 mg Cop-1 Daily - Duration of Exposure (Trials 01-9001/9001E, BR-1, 01-9002, 1110-1, 1110-2, BR-3)

Months	Patients in Study at each interval
<6	779
≥6-<12	670
≥12-<18	490
≥18-<24	334
≥24-<30	290
≥30-<36	175
≥36-<42	87
≥42-<48	50
≥48-<54	33
≥54-<60	15
≥60-<72	15
≥72-<84	4
≥84-<96	4
≥96-108	4
≥108-<120	4
≥120	4
Total Patient Years	1092

APPENDIX 5.b.2
Duration of Exposure: 30 mg Cop-1 Daily
CP-MS*, Controlled Study BR-2

Months	Number of Patients in Study at each Interval	
	COP-1	Placebo
<6	51	55
≥6-<12	45	52
≥12-<18	41	43
≥18-<24	38	37
≥24-<30	21	18

·Total patient months were not calculated because
precise start/stop dates are not available for any patient.

APPENDIX 5.d.1
Demographics
All Studies in RR-MS Patients
(9001/9001E, BR-1, 9002, 1110-1, 1110-2 and BR-3)

Characteristic	COP-1	Placebo
Age (years) Mean ± SD Range	N = 779 36.8 ± 9.0 18 - 68	N = 151 33.6 ± 6.1 19 - 46
Weight (kg)[a] Mean ± SD Range	N=696 68.2 ± 14.9[a] 39.0 - 131.8[a]	N=151 67.4 ± 16.1 40.9 - 136.8
Sex Male N (%) Female N (%)	N=729 255 (33) 517 (67)	N=151 40 (27) 111 (73)
Race [b] Caucasian N (%) Non Caucasian N (%) Unknown [c] N (%)	N=426 366 (86) 20 (5) 40 (9)	N=151 143 (95) 8 (5)

[a] Data are not available in study BR-1 and BR-3

[b] Data are not available in studies 1110-1 and 1110-2.

[c] Study BR-3

APPENDIX 5.d.2

Demographics
Controlled Studies in RR-MS Patients
(9001/9001E and BR-1)

Characteristic	COP-1 (N=150)	Placebo (N=151)
Age (years) Mean ± SD Range	33.8 ± 5.6 19 - 46	8±6.1 19 - 46
Weight (kg)[a] Mean ± SD Range	70.5 ± 17.0 41.7 - 126.8	67.4 ± 16.1 40.9 - 136.8
Male N (%) Female N (%)	48 (32) 102 (68)	40 (26) 111 (74)
Caucasian (%) Non Caucasian (%)	141 (94) 9 (6)	143 (95) 8 (5)

[a] Data are not available in studies BR-1 and BR-3

APPENDIX 5.d.3

Demographics
Controlled Study in CP-MS Patients
(BR-2)

Characteristic	COP-1	Placebo
Age (years) Mean ± SD	N=51 41.6 ± 9.0	N=55 42.3 ± 8.2
Sex Male N (%) Female N (%)	N=51 23 (45.1) 28 (54.9)	N=55 25 (45.5) 30 (54.5)
Race Caucasian N (%) Non Caucasian N (%)	N=51 48 (94.1) 3 (5.9)	N=55 54 (98.2) 1 (1.8)

APPENDIX 6.1
SUMMARY OF PATIENT DEATHS

Study Number	Patient Number	Treatment Group	Age	Sex	Months in Study	Highest Dose (mg/day)	Cause of Death
BR-2	01-578	Cop 1	33	Male	11	30	Complications of neuroglioblastoma (6 months following premature termination)
BR-3	2038	Cop 1	46	Male	22	20	Complications of tracheostomy change
	2049	Cop 1	41	Female	36	20	Pneumonia
	2051	Cop 1	59	Female	36	20	Colon Malignancy
	2039	Cop 1	48	Female	19	20	Unknown
1110-1	8417	Cop 1	40	Female	796 Days	20	Unspecified
	8501	Cop 1	43	Female	-	20	Unspecified (Pneumonia and sepsis)

Adverse Experiences for which any Patient Discontinued Therapy

Body System	Adverse Experience	9001/9001E		BR-1	
		COP-1 N=125	Placebo N=125	COP-1 N=25	Placebo N=25
Body as a Whole	Bacterial Infection	1	0	0	0
	Chest Pain	1	0	0	0
	Face Edema	1	0	0	0
	Infection	1	0	0	0
	Injection Site Atrophy	2	0	0	0
	Injection Site Erythema	1	0	0	0
	Injection Site Induration	2	0	0	0
	Injection Site Inflammation	1	0	0	0
	Injection Site Pain	2	2	0	0
	Injection Site Urticaria	1	0	0	0
	Unspecified	2	0	1	0
Cardiovascular	Syncope	1	0	0	0
	Vasodilatation	2	1	0	0
Digestive	Nausea	1	0	0	0
	Vomiting	1	0	0	0
Hemic and Lymphatic	Lymphadenopathy	1	0	0	0
	Splenomegaly	1	0	0	0
Nervous	Depression	1	0	0	0
	Psychotic Depression	1	0	0	0
Respiratory	Dyspnea	2	1	0	0
Skin and Appendages	Rash	1	0	0	0
	Urticaria	2	0	0	0
Urogenital	Unintended Pregnancy	3	0	0	0

Body System	Adverse Experience	COP-1 (N=51)	Placebo (N=55)
Body as a Whole	asthenia	1	0
	injection site inflammation	2	0
	injection site pain	1	0
	injection site welt	1	0
	injection site mass	1	0
	neoplasm	1	0
	suicide attempt	1	0
Cardiovascular	hypotension	1	0
	palpitations	1	0
	tachycardia	2	0
	vasodilatation	1	0
Nervous	anxiety	1	1
	depression	1	0
	dizziness	2	0
	hypertonia	1	0
	tremor	2	0
Skin and Appendages	pruritus	1	0

*Chronic Progressive MS study

Body System Adverse Clinical Experience	Copolymer-1 (N=125)		Placebo (N=126)	
	N	%	N	%
Body as a Whole				
Abdominal Pain	16	12.8	14	11.1
Abscess	3	2.4	0	0
Allergic Reaction	2	1.6	2	1.6
Allergic Rhinitis	9	7.2	7	5.6
Asthenia	81	64.8	78	61.9
Back Pain	33	26.4	28	22.2
Bacterial Infection	11	8.8	9	7.1
Chest Pain	33	26.4	13	10.3
Chilis	5	4.0	1	0.8
Cyst •	5	4.0	1	0.8
Drug Reaction	2	1.6	1	0.8
Face Edema	11	8.8	2	1.6
Fever	15	12.0	13	10.3
Flank Pain	2	1.6	1	0.8
Flu Syndrome	38	30.4	34	27.0
Headache	76	60.8	75	59.5
Injection Site Atrophy	3	2.4	0	0
Injection Site Erythema	73	58.4	17	13.5
Injection Site Hemorrhage	9	7.2	4	3.2
Injection Site Induration	25	20.0	1	0.8
Injection Site Inflammation	35	28.0	9	7.1

Body System Adverse Clinical Experience	Copolymer-1 (N=125)		Placebo (N=126)	
	N	%	N	%
Injection Site Mass	33	26.4	10	7.9
Injection Site Pain	83	66.4	46	36.5
Injection Site Pruritus	48	38.4	5	4.0
Injection Site Reaction	4	3.2	1	0.8
Injection Site Urticaria	9	7.2	0	0
Injection Site Welt	19	15.2	5	4.0
Neck Pain	16	12.8	9	7.1
Pain	53	42.4	52	41.3
Cardiovascular				
Hypertension	3	2.4	1	0.8
Migraine	9	7.2	5	4.0
Palpitation	14	11.2	6	4.8
Syncope	8	6.4	4	3.2
Tachycardia	7	5.6	7	5.6
Vasodilatation	34	27.2	14	11.1
Digestive				
Anorexia	6	4.8	3	2.4
Bowel Urgency	3	2.4	1	0.8
Diarrhea	24	19.2	22	17.5
Dyspepsia	25	20.0	23	18.3
Dysphagia	7	5.6	6	4.8
Gastroenteritis	6	4.8	2	1.6
Gastrointestinal Disorder	10	8.0	8	6.3
Nausea	29	23.2	22	17.5

Body System Adverse Clinical Experience	Copolymer-1 (N=125)		Placebo (N=126)	
	N	%	N	%
Oral Moniliasis	3	2.4	0	0
Rectal Disorder	4	3.2	3	2.4
Salivary Gland Enlargement	2	1.6	0	0
Tooth Caries	3	2.4	0	0
Tooth Disorder	4	3.2	3	2.4
Ulcerative Stomatitis	2	1.6	0	0
Vomiting	13	10.4	7	5.6
Hemic and Lymphatic				
Ecchymosis	15	12.0	12	9.5
Lymphadenopathy	23	18.4	12	9.5
Metabolic and Nutritional				
Edema	5	4.0	1	0.8
Peripheral Edema	14	11.2	7	5.6
Weight Gain	7	5.6	0	0
Musculoskeletal				
Arthralgia	31	24.8	22	17.5
Nervous				
Abnormal Dreams	3	2.4	2	1.6
Agitation	7	5.6	4	3.2
Amnesia	7	5.6	7	5.6
Anxiety	30	24.0	29	23.0
Confusion	5	4.0	1	0.8
Emotional Liability	2	1.6	1	0.8
Euphoria	2	1.6	1	0.8

Body System Adverse Clinical Experience	Copolymer-1 (N=125)		Placebo (N=126)	
	N	%	N	%
Foot Drop	6	4.8	4	3.2
Hypertonia	44	35.2	37	29.4
L'hermittes Sign	3	2.4	3	2.4
Nervousness	4	3.2	2	1.6
Nystagmus	5	4.0	2	1.6
Sleep Disorder	2	1.6	2	1.6
Speech Disorder	5	4.0	3	2.4
Stupor	2	1.6	0	0
Tremor	14	11.2	7	5.6
Vertigo	12	9.6	11	8.7
Vestibular Disorder	2	1.6	1	0.8
Respiratory				
Bronchitis	18	14.4	12	9.5
Cough Increased	13	10.4	12	9.5
Dyspnea	23	18.4	8	6.3
Laryngitis	2	1.6	2	1.6
Rhinitis	29	23.2	26	20.6
Skin and Appendages				
Eczema	3	2.4	2	1.6
Erythema	8	6.4	4	3.2
Herpes Simplex	8	6.4	6	4.8
Herpes Zoster	2	1.6	1	0.8
Pustular Rash	2	1.6	1	0.8
Rash	21	16.8	19	15.1

Body System Adverse Clinical Experience	Copolymer-1 (N=125)		Placebo (N=126)	
	N	%	N	%
Skin Atrophy	2	1.6	1	0.8
Skin Disorder	5	4.0	2	1.6
Skin Nodule	4	3.2	1	0.8
Sweating	15	12.0	10	7.9
Urticaria	7	5.6	5	4.0
Wart	3	2.4	0	0
Special Senses				
Deaf	2	1.6	2	1.6
Diplopia	9	7.2	8	6.3
Ear Disorder	6	4.8	4	3.2
Ear Pain	15	12.0	12	9.5
Eye Disorder	8	6.4	1	0.8
Otitis Media •	7	5.6	7	5.6
Taste Perversion	3	2.4	3	2.4
Urogenital				
Amenorrhea	2	1.6	1	0.8
Breast Pain	2	1.6	2	1.6
Dysmenorrhea	12	9.6	9	7.1
Hematuria	2	1.6	1	0.8
Impotence	3	2.4	0	0
Menorrhagia	3	2.4	2	1.6
Pap Smear Suspicious	3	2.4	1	0.8
Unintended Pregnancy	4	3.2	0	0

Body System Adverse Clinical Experience	Copolymer-1 (N=125)		Placebo (N=126)	
	N	%	N	%
Urinary Urgency	20	16.0	17	13.5
Vaginal Hemorrhage	2	1.6	0	0
Vaginal Moniliasis	16	12.8	9	7.1

Appendix 9.a.2
Incidence of Adverse Clinical Experiences (≥2%)
Controlled Study BR-1

Body System Adverse Clinical Experience	Copolymer-1 (N=25)		Placebo (N=25)	
	N	%	N	%
Body as a Whole				
Fever	2	8.0	0	0
Headache	10	40.0	9	36.0
Injection Site Erythema	19	76.0	11	44.0
Injection Site Inflammation	22	88.0	4	16.0
Injection Site Pain	23	92.0	9	36.0
Injection Site Pruritus	3	12.0	0	0
Injection Site Reaction	2	8.0	0	0
Cardiovascular				
Palpitation	7	28.0	4	16.0
Vasodilatation	3	12.0	0	0
Digestive				
Anorexia	5	20.0	3	12.0
Constipation	10	40.0	6	24.0
Nausea	7	28.0	4	16.0
Vomiting	2	8.0	1	4.0
Nervous				
Dizziness	12	48.0	8	32.0
Hypesthesia	2	8.0	1	4.0

Body System Adverse Clinical Experience	Copolymer-1 (N=25)		Placebo (N=25)	
	N	%	N	%
Insomnia	2	8.0	0	0
Respiratory				
Dyspnea	3	12.0	0	0
Skin and Appendages				
Pruritus	18	72.0	7	28.0
Kash	6	24.0	5	20.0
Sweating	8	32.0	6	24.0

Appendix 9.a.3
Incidence of Adverse Clinical Experiences (≥ 2%)
Controlled Study BR-2

Body System Adverse Clinical Experience	Copolymer-1 (N=51)		Placebo (N=55)	
	N	%	N	%
Body as a Whole				
Accidental Injury	2	4.0	0	0.0
Arthralgia	16	31.0	11	20.0
Asthenia	2	4.0	0	0.0
Chills	3	6.0	1	2.0
Infection	4	8.0	1	2.0
Laryngysmus	10	20.0	7	13.0
Pain	3	6.0	0	0.0
Injection Site Hemorrhage	3	6.0	1	2.0
Injection Site Hypersensitivity	2	4.0	1	2.0
Injection Site Erythema	40	78.0	12	22.0
Injection Site Inflammation	41	80.0	9	16.0
Injection Site Pain	41	80.0	23	42.0
Injection Site Pruritus	29	57.0	7	13.0
Injection Site Welt	3	6.0	0	0.0
Injection Site Mass	19	37.0	9.0	16.0
Injection Site Reaction	2	4.0	0	0
Cardiovascular				
Palpitation	14	28.0	6	11.0

Body System Adverse Clinical Experience	Copolymer-1 (N=51)		Placebo (N=55)	
	N	%	N	%
Decreased BP	2	4.0	0	0.0
Chest Pain	10	20.0	9	16.0
Hematologic				
Lymphadenopathy	2	4.0	0	0.0
Nervous				
Anxiety	16	31.0	11	20.0
Respiratory				
Hyperventilation	2	4.0	0	0.0
Dyspnea	12	24.0	7	13.0
Skin and Appendages				
Rash	10	27.0	6	11.0

Other adverse experiences observed during clinical trials not already accounted for in the table of adverse events which occurred at an incidence of at least 1% in the Copolymer-1 group were as follows:

Body as a whole: abdomen enlarged, abdominal pain, accidental injury, allergic reaction, allergic rhinitis, bacterial infection, benign neoplasm, cellulitis, death, disease progression, drug reaction, fever, fever and chills, flank pain, fungal i: .on, generalized edema, headache, hernia, infection, injection site abscess, injection site edema, injection site ecchymosis, injection site fibrosis, injection site hematoma, injection site hypersensitivity, injection site hypertrophy, injection site melanosis, lack of drug effect, laparotomy, leg pain, Lyme Disease, malaise, moniliasis, moon face, mucous membrane disorder, neck rigidity, neoplasm, pain, photosensitivity reaction, polypectomy, reaction unevaluable, serum sickness, suicide attempt, surgery.

Cardiovascular: arrhythmia, atrial fibrillation, blood pressure unstable, bradycardia, cardiovascular disorder, decreased blood pressure, extrasystoles, fourth heart sound, hypertension, hypotension, midsystolic click, pallor, peripheral vascular disorder, postural hypotension, systolic murmurs, tachycardia, varicose vein, vascular disorders.

Gastrointestinal: appendectomy, bowel urgency, cholecystitis, colitis, constipation, diarrhea, dry mouth, dyspepsia, dysphagia, esophageal ulcer, esophagitis, fecal incontinence, flatulence, gastritis, gastrointestinal carcinoma, gastrointestinal discomfort, gastrointestinal disorder, gingivitis, glossitis, gum hemorrhage, hemorrhoidectomy, hepatomegaly, increased appetite, melena, mouth ulceration, nausea and vomiting, pancreas disorders, pancreatitis, periodontal abscess, rectal disorder, rectal hemorrhage, salivary gland enlargement, stomatitis, tenesmus, tongue discoloration, tooth disorder, ulcer duodenal, ulcerative stomatitis, viral hepatitis A.

Endocrine: Cushing's Syndrome, goiter, hyperthyroidism, hypothyroidism.

Hemic and Lymphatic: anemia, cyanosis, eosinophilia, leukopenia, lymphedema,

pancytopenia, splenomegaly.

Metabolic and Nutritional: alcohol intolerance, gout, healing abnormal, increased alcohol tolerance, weight decreased, xanthoma.

Musculoskeletal: arthritis, bone pain, bursitis, joint disorder, kyphoscoliosis, muscle atrophy, muscle disorder, myalgia, myasthenia, myopathy, osteomyelitis, tendon disorder, tenosynovitis.

Nervous: abnormal dreams, abnormal gait, amnesia, anxiety, ataxia, circumoral paresthesia, coma, depersonalization, depression, dizziness, dysesthesia, emotional lability, euphoria, facial paralysis, foot drop, hallucinations, hostility, hypesthesia, hypokinesia, incoordination, insomnia, L'hermittes Sign, libido decreased, manic reaction, memory impairment, meningitis, movement disorders, myoclonus, nervousness, neurosis, paranoid reaction, paraplegia, paresthesia, psychiatric disorder, psychotic depression, seizure, sleep disorder, somnolence, speech disorder, stupor, thinking abnormal, twitch, vertigo, vestibular disorder,.

Respiratory: asthma, cough increased, epistaxis, hyperventilation, hypoventilation, laryngismus, laryngitis, lung disorder, pharyngitis, pneumonia, respiratory disorders, sinusitis, voice alteration.

Skin and Appendages: acne, alopecia, angioedema, contact dermatitis, dry skin, dermatomycosis, eczema, erythema nodosum, fungal dermatitis, furunculosis, hair disorder, herpes simplex, herpes zoster, hirsutism, maculopapular rash, nail disorder, pruritus, psoriasis, pustular rash, rash, skin atrophy, skin benign neoplasm, skin carcinoma, skin disorder NOS, skin discoloration, skin hypertrophy, skin reaction, skin striae, urticaria, vesiculobullous rash.

Special Senses: abnormal vision, amblyopia, cataract, conjunctivitis, corneal lesion, corneal ulcer, deaf, diplopia, dry eyes, ear disorder, eye pain, lacrimation disorder, mydriasis, optic neuritis, otitis media, otitis externa, photophobia, ptosis, taste loss, taste perversion, tinnitus.

Urogenital: abortion, amenorrhea, breast engorgement, breast enlarge, breast pain, carcinoma cervix in situ, cervix disorder, cystitis, dysuria, endometrial disorder, fibrocystic breast, hematuria, hysterectomy, kidney calculus, kidney pain, menorrhagia, menstrual disorder, nocturia, ovarian cyst, Pap smear suspicious,

pregnancy, priapism, prostatectomy, prostatic disorder, pyelonephritis, sexual function abnormal, testicular disorder, urethritis, urinary frequency, urinary incontinence, urinary retention, urinary tract infection, urine abnormality, vaginal disorder, vaginal hemorrhage, vaginitis.

Study	Patient	Age	Sex	Dose mg/d	–Days	Comments
9001E	02-206	33	M	20	35	After 6 days of treatment, rashes on lower extremities and injection site lasting 1 month. On day 35 there was temporary (2 day) interruption of treatment due to tightness in the chest and syncope. With rechallenge-recurrence of the symptoms (chest tightness, flushing). With continued treatment no more adverse events were reported until two months later, when he reported hives. The medication was stopped again and rechallenged 6 days later with recurrence of the hives, this time he was removed from the study. Concomitant med-amoxicillin.
	02-214	32	F	20	48	PT** due to Syncope, chest tightness, flushing, N/V and SOB immediately following injection. Hx of PCN and sulfa allergy.
	07-707	26	F	20	120	PT due to enlarged lymph nodes. @ 4 months-vomiting, palpitations, chest tightness and SOB. A biopsy of the nodes revealed hyperplasia. Hx of PCN, shellfish and sulfa allergy.
	07-713	43	F	20	330	PT due to rash of 2 and1/2 month duration, also complained of angioedema and chest tightness.
	07-720	38	F	20	60	PT due to flushing, chest tightness and SOB. Hx of PCN allergy.
	07-727	33	F	20	90	One month into the study Pt** developed cervical and inguinal lymph node enlargement. At third month-hepatomegaly and later splenomegaly.
9002	020-002	30	F	20	90	PT due to rash and dyspnea. At one mo. she experienced a rash with interruption of therapy.
	01-007	40	F	20	60	PT due to allergic reaction (facial edema and SOB).
	012-003	47	F	20	90	PT due to chest tightness and SOB.
	005-007	35	F	20	210	PT due to itchy rash, flushing, chest tightness and SOB.

PT** = premature termination.

Study	Patient	Age	Sex	Dose mg/day	Days	Comments
BR-1	694	31	M	20	720	PT due to "systemic reaction". At 15 mo-SR***. A similar episode at 21 mo. Hx of a similar reaction post IVP.
	910	31	F	20	120	PT due to SR. Several months later rechallenged with recurrence, and reoccurring hives post discontinuation for several weeks.
BR-2	02-40	56	F	20	42	PT due to SR, two episodes 3 days apart.
	02-100	41	F	20	195	PT due to allergic like syndrome. @ 6 weeks-SR. @5.5mos SR. A brief interruption but reported welts at injection site after restarting and was discontinued.
	01-506	38	M	20	17	@ 14 days- SR- used two anaphylactic kits and symptoms lasted 45 min. 3 days later following injection a second episode . Was PT.
	01-2058	31	F	20	330	PT due to a series of "reactions" @ 1, 3, 10 and 11 months, characterized by allergic like symptoms.
1110-1	8005	44	M	20	160	PT due to a series of ""systemic reaction"s"
	8010	26	M*	20	216	PT due to a series of (3) ""systemic reaction"s," approximately a month apart.
	8038	23	F	20	105	PT due to a series of (6) ""systemic reaction"s," at first a month apart, then a week or 2 weeks apart.
	8048	31	F	20	427	PT due to a series of (4) ""systemic reaction"s," starting two weeks after study initiation, a month later, three months and a year later.

Study	Patient	Age	Sex	Dose mg/day	Days	Comments
1110-1	8059	39	F	20	174	PT due to a series of (5) ""systemic reaction"s" following the injection of the drug. The episodes started 5 mos into the study and each reaction lasted 7-10 min. Allergy skin tests were positive.
	8065	46	M	20	111	PT due to respiratory difficulty lasting 20 min on day 109, followed by a rash and peripheral edema the next day lasting a day.
	8080	39	F	20	126	PT due to welts at injection site lasting 3 mos and one episode of facial flushing lasting 10 min. Concomitant meds included antihistamine.
	8102	34	F	20	624	Injection site reactions (ISR****) a mo. into the study lasting 30 days. 3 mo into study more ISR and SR-chest tightness and dyspnes-lasting 15 min. A week and 2 yrs later more episodes of SR (the last episode lasting 2 hrs).
	8103	20	F	20	300	PT due to a series of (4) SRs-1st episode starting a mo after study initiation and then at different intervals usually symptoms lasting 10-20 min, but last episode lasted 4 hrs.
	8304	59	M	20	48	PT due to 2 episodes of weakness, shivering, fever and inability to walk.
	8401	24	F	20	282	PT due to a series of (5) SRs-1st episode starting 3 mos after study initiation.
	8402	25	M	20	173	2 episodes of SR at 2 mos and 3 mos of study.
	8419	42	F	20	183	ISR and 2 episodes of SR.
	8448	27	F	20	418	An episode of SR 2 mo into study. Treatment was stopped for 4 mos and then rechallenged. Upon rechallenge the pt experienced five more episodes and then PT.
	8451	31	F	20	108	P. or an episode of SR.
	9108	23	M	20	114	PT for an episode of SR.

Study	Patient	Age	Sex	Dose mg/d	Days	Comments
11101-1	9418	21	F	20	168	PT for an episode of SR.
BR-2	02-40	56	F	20	42	PT for 2 episodes of SR within 2 days.

PT* premature termination.
Pt** Patient
SR*** "systemic reaction" (includes at the minimum three of the following symptoms: chest tightness, palpitations, vasodilatation, angioedema, flushing, anxiety, constriction of the throat and SOB)
ISR **** Injection Site Reaction

APPENDIX 11.a.1.1
INCIDENCE OF CLINICALLY SIGNIFICANT BLOOD CHEMISTRY ABNORMALITIES
(9001/9001E BR-1 and BR-2)

Laboratory Test (Units)	Criteria for Clinically Significant Abnormal Values	Cop 1 (N=201)	Placebo (N=177)
BUN (mg/dL)	≥30 mg/dL	0	0
Calcium (mg/dL)	≤7 mg/dL	0	0
	≥12 mg/dL	0	0
Serum Chloride (mEq/L)	≤95 mEq/L	5(2.5%)	9(5.1)
	≥115 mEq/L	0	0
Creatinine (mg/dL)	≥2 mg/dL	3(1.5%)	2(0.6%)
Serum Glucose (mg/dL)	≤50 mg/dL	4(2.0%)	3(1.7%)
	≥300 mg/dL	1(0.5%)	0
Phosphorus (mg/dL)	≤7 mg/dL	8(4.9%)	2(1.1%)
	≥12 mg/dL	0	0
Serum Potassium (mEq/L)	≤3 mEq/L	0	0
	≥5.9 mEq/L	0	1(0.6)
AST (SGOT)(U/L)	≥150	0	3(1.7%)
ALT (SGPT) U/L)	≥165	3(1.5%)	6(3.4%)
LDH (U/L)*	≥750	0	0
Total Bilirubin (mg/dL)	≥2mg/dL	2(1%)	4(2.3%)

*LDH not done in 01-9001E

APPENDIX 11.a.2.1
INCIDENCE OF CLINICALLY SIGNIFICANT HEMATOLOGY ABNORMALITIES
(9001/9001E BR-1 and BR-2)

Laboratory Test (Units)	Criteria for Clinically Significant Abnormal Values	Cop 1 (N=201)	Placebo (N=177)
Hemoglobin (g/dL)	≤11.5 g/dL (male)	0	0
	≤ 9.5 g/dL (female)	0	1
Hematocrit (%)	≤37% (male)	0	0
	≤32% (female)	1(0.5%)	3(1.7%)
WBC (x10³/μL.)	≤2.8 x 10³/μL	5(2.5%)	1(0.4%)
	≥16 x 10³/μL	7(3.5%)	5(2.8%)
Platelets* (x10³/μL) N=292	≤75 x 10³/μL	0	2(1.13%)
	≥700 x 10³/μL	0	0

*Platelets not done in BR-1 and BR-2

APPENDIX 11.c.1
INCIDENCE OF CLINICALLY SIGNIFICANT VITAL SIGN ABNORMALITIES:
FOR 01/9001 and 9001E*

Vital Sign	Criterion Value	Change from Baseline	Cop 1 (N = 125)	Placebo (N = 126)
Systolic BP	≤ 90 mmHg	Decrease of ≥20	11(8.8%)	6(4.8%)
	≥180 mmHg	Increase of ≥20	0	1(0.8%)
Diastolic BP	≤ 50 mmHg	Decrease of ≥15	11(8.8%)	8(6.3%)
	≥105 mmHg	Increase of ≥15	0	0
Heart Rate	≤ 50 bpm	Decrease of ≥15	0	0
	≥120 bpm	Increase of ≥15	3(2.4%)	0

*Data not available for BR-1 and BR-2

Appendix 13.1*
Serious Adverse Experiences Considered Unlikely to be Related to Study Drug

Body System	Study Number	Patient Number	Age	Dose mg/day	Duration of Treatment (days)	Adverse Event
Body as a Whole	01-9001/9001E	403	34	20	914	Abdominal Pain
	01-9001/9001E	528	32	20	125	Back Pain
	01-9001/9001E	807	39	20	117	Benign Neoplasm
	01-9001/9001E	302	22	20	276	Suicide Attempt
	01-9001/9001E	813	27	20	109	Suicide Ideation
	01-9002	2/2	62	20	80	Accidental Injury
	01-9002	9/1	39	20	97	Asthenia
	01-9002	9/1	39	20	97	Fever
	01-9002	8/8	37	20	191	Infection
	1110-1	8053	36	20	225	Accidental Injury
	1110-1	8114	53	20	718	Accidental Injury
	1110-1	8320	43	20	780	Accidental Injury
	1110-1	8331	44	20	288	Accidental Injury
	1110-1	8441	44	20	212	Accidental Injury
	1110-1	8309	21	20	157	Laparotomy
	1110-1	8440	52	20	N/A	Subcutaneous swelling, left shoulder, possible Lipoma
Body as a whole (Continued)	1110-2	9401	42	20, every other day	684	Accidental Injury
	BR-2	01-578	35	30	454	Neoplasm
Cardiovascular	01-9001/9001E	212	31	20	613	Atrial Fibrillation
	01-9001/9001E	403	34	20	N/A	Hypertension
Digestive	01-9001/9001E	403	34	20	N/A	Gastritis
	1110-1	8106	58	20	1148	Appendectomy
	1110-1	8426	35	20	595	Hemorrhoidectomy
	1110-1	8427	35	20	364	Ulcer Duodenal

Body System	Study Number	Patient Number	Age	Dose mg/day	Duration of Treatment (days)	Adverse Event
	1110-1	8311	31	20	381	Viral Hepatitis A
Hemic and Lymphatic	1110-1	8114	53	20	81	Leucopenia
Musculoskeletal	01-9001/9001E	216	36	20	316	Arthralgia
	1110-1	8008	21	20	47	Osteomyelitis
Nervous	01-9001/9001E	403	34	20	312	Anxiety
	01-9001/9001E	403	34	20	163	Depression
	01-9001/9001E	1002	30	20	898	Significant Exacerbation of MS
	01-9001/9001E	1024	46	20	1022	Significant Exacerbation of MS
	01-9001/9001E	403	34	20	N/A	Terrible Sadness
	01-9001/9001E	126	25	20	806	Vertigo/Recurrent Vomiting
	01-9001/9001E	403	34	20	77	Faintness
	01-9001/9001E	403	34	20	496	Difficulty Walking and Fatigue
	01-9002	9/1	39	20	97	Ataxia
	01-9002	23/2	44	20	180	Depression
	01-9002	5/2	27	20	71	Dizziness, Nausea, Vertigo, Asthenia
	01-9002	1/6	39	20	93	Hallucinations
	01-9002	38/ 1		20	N/A	Loss of Consciousness
	01-9002	25/ 25	40	20	N/A	Optic Atrophy
Respiratory	01-9001/9001E	403	34	20	139	Bronchitis
	01-9002	9/1	40	20	97	Rhinitis
Skin and Appendages	01-9001/9001E	221	46	20	337	Skin Carcinoma
Urogenital	01-9001/9001E	424	29	20	388	Unintended Pregnancy

Body System	Study Number	Patient Number	Age	Dose mg/day	Duration of Treatment (days)	Adverse Event
	01-9001/9001E	905	30	20	732	Unintended Pregnancy
	01-9001/9001E	423	29	20	18	Unintended Pregnancy
	1110-1	8053	36	20	599	Hysterectomy
	1110-1	8122	38	20	355	Pregnancy
	1110-1	8106	58	20	1020	Prostatectomy
	1110-2	9413	35	20, every other day	268	Hysterectomy

*The same patient may appear more than once in appendices 13.1 and 13.2 and may appear in both appendices. However, every line represents a different event.

Body System	Study Number	Patient Number	Age	Dose mg/day	Duration of Treatment (months)	Adverse Event
Body as a Whole	9001/9001E	403	38	20	31	Abdominal Pain
	9001/9001E	528	32	20	4	Back Pain
	9001/9001E	403	34	20	12	Drug Intoxication
	9001/9001E	403	34	20	26	Headache, Asthenia
	9001/9001E	302	27	20	10	Suicide Attempt
	9001/9001E	813	27	20	4	Suicide Ideation
	9001/9001E	216	3C	20	26	Surgery
	9002	02/002	62	20	3	Accidental Injury
	9002	05/002	26	20	2.5	Asthenia
	9002	36/010	54	20	3	Gallstone surgery
	9002	12/005	32	20	3	Urticaria
	1110-1	8053	36	20	356 days	Accidental Injury
	1110-1	8304	59	20	31 days	Fever, Chills, Asthenia
	1110-1	8537	41	20	72 days	Hiatal Hernia
	1110-1	8315	25	20	204 days	Laparotomy
	1110-2	9408	55	20	24	Carcinoma Breast
Body as a Whole (Continued)	BR-3	01-1000	Unk	20	Unknown	Obesity
	BR-3	01-2030	20	20	7 yrs	Pain
	BR-3	01-2015	20	20	21	Surgery
	BR-2	01-578	35	30	25	Asthenia, Headache
	BR-2	01-184	32	30	2	Back Pain
Cardiovascular	9001/9001E	212	31	20	20	Atrial Fibrillation
	9001/9001E	0322	36	20	30	Atrial Fibrillation

Body System	Study Number	Patient Number	Age	Dose mg/day	Duration of Treatment (months)	Adverse Event
	9001/9001E	811	27	20	7 mos	Heart Murmur
	9001/9001E	609	46	20	20	Deep Vein Thrombosis
	9001/9001E	807	39	20	6	Thrombophlebitis
Digestive	9001/9001E	514	43	20	16	Gastroenteritis
	9002	08/008	37	20	6	Intestinal Infection
	9002	05/002	26	20	2.5	Nausea
	9002	05/002	26	20	2.5	Vomiting
	1110-1	8537	41	20	72 days	Esophagitis
Hemic and Lymphatic	1110-2	9401	42	20 every other day	unknown	Lymphadenopathy
Metabolic and Nutritional	9001/9001E	403	34	20	6	Dehydration
Musculoskeletal	BR-3	01-2030	20	20	5 yrs 9 mos	Muscle Disorder
	BR-2	01-578	35	30	25	Myasthenia
Nervous	9001/9001E	403	34	20	27	Depression
	9001/9001E	126	28	20	31	Depression
	9001/9001E	712	38	20	15	Depression
	9002	01/006	39	20	3	Agitation, Hallucination, Hostility
	BR-3	01-2018	36	20	17	Anxiety
	BR-3	01-2018	36	20	26	Psychiatric Disorder
	BR-3	01-2051	59	20	42	Somnolence, Stupor
Respiratory	9001/9001E	403	34	20	6	Bronchospasm
	9001/9001E	807	39	20	5	Lung Biopsy
	1110-1	8044	42	20	707 days	Lung Infection
	BR-3	01-2049	41	20	35	Pneumonia
	BR-3	01-2054	35	20	29	Pneumonia
Urogenital	1110-1	8053	36	20	615 days	Myoma

Body System	Study Number	Patient Number	Age	Dose mg/day	Duration of Treatment (months)	Adverse Event
	1110-2	9110	33	20 every other day	20	Abortion
	BR-2	02-136	45	30	2.5	Cystitis

*The same patient may appear more than once in appendices 13.1 and 13.2 and may appear in both appendices. However, every line represents a different event.

Appendix 13.3
Serious Adverse Experiences Considered Possibly Related to Study Drug

Body System	Study Number	Patient Number	Age	Dose mg/day	Duration of Treatment (days)	Adverse Event
Body as a Whole	01-9001/9001E	403	34	20	259	Chest Pain (musculoskeletal)
	01-9001/9001E	807	39	20	117	Injection site Staph infection
	01-9002	4/4	56	20	19	Rash
	01-9002	5/2	26	20	75	Asthenia
	01-9002	36/1	44	20	266	Syncope
	1110-1	8304	59	20	31	Fever/Chills, Asthenia
	1110-1	8428	31	20	-	Serum Sickness
	BR-2	1-184	32	30	60	Back Pain
	BR-2	02-1	33	30	91	Syncope
	BR-3	2058	31	20	609	"Severe Reaction"
Cardiovascular	01-9002	36/1	44	20	-	Loss of consciousness
Digestive	01-9002	4/4	56	20	19	Abscess
	01-9002	36/10	54	20	57	Cholecystectomy
	01-9002	5/2	26	20	74	Nausea/vomiting
	1110-1	8537	41	20	72	Esophagitis
Hemic and Lymphatic	01-9001/9001E	707	26	20	-	Lymphadenopathy
Nervous	01-9002	5/2	26	20	75	Dizziness/vertigo

REVIEW AND EVALUATION OF CLINICAL DATA

NDA:	20-622
SPONSOR:	Teva Pharmaceuticals, USA
DRUG:	Copaxone® (Copolymer-1 Injection)
PHARMACOLOGIC CATEGORY:	Acetate salts of synthetic polypeptides containing L-glutamic acid, L-Alamine, L-Fyrosine and L-Lysine
INDICATION:	Slowing progression of disability and reducing frequency of relapses in patients with relapsing-remitting multiple sclerosis.
DOSAGE FORM:	Sterile Lyophilized Powder for Reconstitution, 20mg Subcutaneous Injection
DESIGNATION:	Orphan (November 12, 1987)
DATE OF SUBMISSION:	June 15, 1995
DATE OF REVIEW:	December 5, 1995

1.0 Background

The present submission requests approval of an NDA for the orphan-designated drug Copolymer-1 (Copaxane) for Injection (20mg/vial) for reducing the frequency of relapses and slowing the progression of disability in patients with repasing-remitting multiple sclerosis. The recommended dose of Copaxone for the treatment of relapsing-remitting MS is 20 mg/day injected subcutaneously.

Copolymer-1 is the subject of the following INDs, which are cross-referenced for the supportive evidence of safety/efficacy for this new indication:

    IND
    IND   )
    IND

In addition, TEVA initiated a Treatment IND program (Protocol. 01-9002) in June 1993.

The total clinical program with copolymer-1 (excluding the Clinical Pharmacology trials) consists of 11 clinical trials in which a total of 857 with MS have been exposed to the drug (see Table 59, attached). Of these 857 patients, 670 were in the relapsing-remitting phase of the disease and received copolymer-1 by subcutaneous injection at a dose of 20 mg/day for at least 6 months; and 490 received the drug for at least 12 months.

The sponsor has presented the results of two placebo-controlled studies with one's extension to establish the efficacy and safety of Copaxone® (Copolymer-1) for the treatment of relapsing-remitting MS:

PROTOCOL   TITLE

BR-1   A pilot trial of copolymer in relapsing-remitting multiple sclerosis. Murray
           Bornstein, M.D., Albert Einstein College of Medicine, Bronx, NY..(N=51)
           Publication: Bornstein MW, Miller AJ, Slagel S, et al., 1987. A pilot trial of
           COP-1 in exacerbating-remitting multiple sclerodis. N ENG J MED 317: 408-
           14.

01-9001   Long-term, Double-Blind, Placebo-Controlled, Multicenter Phase III Study to
           Evaluate the Efficacy and Safety of Copolymer-1 Given Subcutaneously in
           Patients with Relapsing-Remitting Multiple Sclerosis. Principal Investigator:
           Kennet' P. Johnson, M.D., University of Maryland. (N=251).

01-9001E   Extension of Long-term, Double-Blind, Placebo-Controlled, Multicenter Phase
           III Study to evaluate the Efficacy and Safety of Copolymer-1 given
           subcutaneously in Patients with Relapsing-Remitting Multiple Sclerosis
           (N=125)

An original pr'   study report, case report tabulations were submitted for each pivotal
trial.

The focus of this review will be the controlled portion of each pivotal study, as this is the source of the efficacy claim; the open-label chronic experience will be integrated and examined for efficacy and safety in the Safety Review.

2.0   PIVOTAL CONTROLLED TRIALS
3.0   Protocol BR-1: A Pilot Trial of Copolymer-1 in Relapsing-Remitting Multiple
       Sclerosis. Dr. Murray Bornstein

This study was initiated February 13, 1980 and the last observation was February 22, 1985. The study was conducted under a physician sponsored IND (IND   ,. The results of the trial were published in 1987 (A Pilot Trial of Cop 1 in exacerbating-Remitting Multiple Sclerosis. Bornstein et al, NEJM 317:408-414 [August 13], 1987).

Background

The sponsor's report elaborates on the published account by including the detail expected in an integrated clinical and statistical report included in an NDA, an account of the sponsor's procedures for assuring data validity and accuracy, and a report of the applicant's reanalysis using the cohort presented in the publication ("Bornstein" cohort) as well as a cohort including all randomized patients ("All Patient" cohort).

An external advisory committee was established to monitor the ongoing progress of the trial. This group also served as a safety committee. Any decision for early termination of the trial or for breaking the treatment assignment codes would have been made by this committee. This group was also consulted in regard to changes in trial procedures.

Design

This was a two-year, placebo-controlled, randomized, parallel group, double-blind study involving 50 patients with relapsing-remitting MS in one US center. Patients were enrolled as matched pairs and were treated by daily subcutaneous self-injections of either copolymer-1 20mg (N=25) or placebo (N=25).

Study patients were matched according to sex, number of exacerbations per year within ± 1 exacerbation, and degree of disability as measured by the Kurtzke Scale in three strata: 0 to 2, 3 to 4, and 5 to 6. The random assignment of the first person of a pair determined the assignment of both.

Data from a personal and disease history and a neurological examination and status evaluation using Kurtzke's Disability Status Scale and eight Functional Groups were recorded at the time of screening and on the patient's entry into the study . Patients visited the clinic one month later and every three months thereafter for two years. At each visit, a neurologist unaware of the patient's treatment group completed a neurologic examination and status evaluation. The patient's self-evaluation of local or generalized side effects and changes in neurologic status were reported to the clinical assisstant, who was not blinded to treatment.

Patients were also seen at the times of suspected exacerbations, when reporting the rapid onset of new symptoms or a worsening of preexisting symptoms that persisted for 48 hours or more. The neurologist verified exacerbations on the basis of study criteria. An event was counted as an exacerbation only when the patient's symptoms were accompanied by observed ojective changes on the neurologic examination involving an increase of at least one grade in the score for one of the eight functional groups or the Kurtzke Scale. Sensory symptoms unaccompanied by objective findings or transient neurologic worsening were not considered to represent an exacerbation. Patients experiencing an acute exacerbation were evaluated at frequent intervals, usually every two weeks until a new, stable neurologic baseline had been established.

Patient Population

To be eligible for the study, patients had to be 20-35 years of age who met Poser's criteria

for clinically definite MS with an initial Kurtzke Disability Status Scale (DSS) score of 0-6.0 (ambulatory with assistance) and a history of at least two relapses in the 2 years prior to study entry, and who were determined to be emotionally stable by psychosocial evaluation. Initially the inclusion criterion required two or more relapses in each of the two years before randomization (i.e., at least four relapses overall). Recruitment difficulties forced relaxation of this criterion to two or more relapses in the two years before randomization (i.e., at least two relapses overall)

Questionnaires completed by 932 volunteers were reviewed; 140 of these candidates were evaluated in neurologic and psychosocial examinations. Ninety of the 140 were excluded-23 because of age; 21, low frequency of exacerbations; 19, lack of documentation; 15, psychosocial inadequacy; 8, transition to a chronic, progressive course; 3, distance from the clinic; and 1 pregnancy. Fifty patients were accepted into the trial.

Concomitant Medications
When clinically indicated, relapses were treated with all appropriate physical, therapeutic (including steroids), and supportive measures for the duration of the relapse. Seventy-four percent of 62 exacerbations in the placebo group and 75 percent of 16 exacerbations in the Cop 1 group were treated with steroids. Symptomatic medications such as cholinergic and spasmolytic drugs, were permitted.

Outcome Measures
The primary outcome measure was the proportion of relapse-free patients over the 24 month follow-up. Initially, a relapse was defined as the rapid onset of new symptoms or a worsening of preexisting symptoms that persisted for at least 24 hours. Relapses were objectively confirmed by the study investigator if the event produced an increase of at least one point in at least one Functional System score or an increase of at least one point in the DSS score. Sensory symptoms unaccompanied by objective findings or brief neurological worsening were not considered to represent a relapse.

In the course of the trial, the principal investigator and the external advisory committee lengthened the duration of the period of worsening to 48 hours in order to avoid a high rate of brief symptomatic episodes that did not represent true relapses. Data that had been previously collected were systematically subjected to the revised criteria and corrected retrospectively before the treatment assignment was broken.

Secondary outcome measures included frequency of relapses, change in DSS score from baseline, proportion of progression-free patients and time to progression. Progression was defined as an increase of at least one unit in the DSS score that persisted for at least 3 months.

Statistical Methods
The sample size was determined to have approximately 80% power to detect a difference of 40% in the proportion of patients who remained relapse-free over two years.

The study design included planned subgroup analyses according to the disability status of the patients when they were randomized (Kurtzke units 0 to 2, 3 to 4, and 5 to 6). However, only one patient entered with a score of 4, and three with a score of 5. Therefore, two of the three strata were combined (3 to 4 and 5 to 6), creating two strata (0 to 2 and 3 to 6) with approximately equal numbers of patients for subgroup analyses.

For the matched-pair analysis, the difference between treatment arms was tested with use of a McNemar's statistic for the 22 matched pairs. A two-tailed Fisher's exact test was used for other two-by-two contingency tables. The chi-square test was used to test two-by-three contingency tables for frequency of exacerbations.

Survival curves were ca! dated with life-table methods for the length of time before progression, with "progression" defined as an increase of at least one unit in the Kurtzke score. Progression was noted at the time of the visit during which it was observed; however, it had to be maintained for at least three months to be counted.

All statistical tests were conducted at the alpha=0.05 two sided level of significance. In addition to the cohort of patients analyzed in the publication (the "Bornstein" cohort), the sponsor conducted the same analyses using the "all patient" intent to treat cohort.

Results
Patient Disposition
Fifty patients were enrolled: 48 in matched-pairs and two unmatched. One unmatched patient was randomly assigned to each treatment group (Patient 726, copolymer 1; Patient 898, placebo). The disposition of the cohorts used in the efficacy and safety analyses is presented in Sponsor's Table 9 following

TABLE 9. DISPOSITION OF ALL PATIENTS WHO ENTERED THE TRIAL (MATCHED AND UNMATCHED)

PATIENTS	COP-1(N=25)	PBO (N=25)
Randomized	25	25
Matched	24	24
Unmatched	1	1
Efficacy and Safety Analysis(All Patient cohort)	25	25
Matched	24	24
Unmatched	1	1
Efficacy and Safety Analysis (Bornstein cohort)*	25	23
Matched	22	22
Unmatched	3	1

*Placebo patients #16 and #640 were excluded, as described in the publication.

For the Bornstein cohort, two placebo patients (#16 and #640) who did not complete the two-year follow-up and who were considered by the investigator to be unevaluable due to psychogenic reasons were excluded from the analysis. The exclusion of these two patients resulted in a sample including 22 matched pairs (44 patients) plus four unmatched patients, the additional two unmatched cases (#606 and #639, both on copolymer-1) being a consequence of the exclusions. Unmatched-pair analysis was used for the remaining 48 patients. In total, seven patients (3 copolymer-1 and 4 placebo) failed to complete 2 full years on their assigned treatments.

Summary statistics for demographic and baseline characteristics are presented for all 50 randomized patients in Table 10 (attached). For both the All Patient and Bornstein cohorts, there was no statistically significant difference at baseline between the treatment groups. Patients had an average duration of disease of approximately 5.6 years (range 1-13 years) with a two-year prior relapse rate of about 3.8. Baseline Kurtzke DSS scores were between 0 and 6 and almost half the patients had scores between 0 and 2. The extent of exposure was comparable for both groups. The total patient-months exposure in patients treated with copolymer-1 was 586 months compared to 559 months in the placebo group.

Premature Terminations

Seven patients failed to complete the two year trial. Of these, two patients, Patient 16 and Patient 640 (both placebo), were excluded from the Bornstein cohort efficacy analysis. Both patients, in the opinion of the investigator, had symptomatology considered psychogenic in nature that might interfere with evaluation of treatment effect on the disease, However, they were retained in the All Patient analyses of efficacy and in all safety summaries. Sponsor's Table 13 following summarizes the number of patients who prematurely withdrew prior to completing the trial and the reasons for premature termination. The rate of premature termination and time to withdrawal were similar for both groups.

TABLE 13.   PREMATURE TERMINATION

	Copolymer-1 (N=25)		Placebo (N=25)	
	N	%	N	%
Number of Patients Who Withdrew	3	12.0	4	16.0
Principal Reason for Withdrawal				
Hospitalization for Relapse	0	0.0	1	4.0
Reaction to Injection	2	8.0	0	0.0
Termination by Investigator	0	0.0	2	8.0
Patient's Own Volition	0	0.0	1	4.0
Unspecified	1	4.0	0	0.0

Concomitant Medications

According to the publication, approximately 75% of relapses in both the placebo and

copolymer-1 groups were treated with steroids. Nearly half the placebo patients and one quarter of the copolymer-1 patients received anti-inflammatory agents, steroids and/or combination anti-inflammatory therapy

RESULTS: EFFICACY
Table 89 (attached) presents the results of all efficacy variables evaluated.

The primary outcome measure of the proportion of relapse-free patients significantly favored copolymer-1 (56% vs. 26.1% for placebo, p=0.039; Bornstein cohort).

The two-year relapse rate was 16/25 or 0.6 per patient for copolymer-1 and 59/23 or 2.6 per patient for placebo (p=0.002). The corresponding annualized rates were 0.3 for Copolymer-1 and 1.3 for placebo. The effect on relapse rate with copolymer-1 therapy was even greater in patients with baseline DSS scores of 0-2 (4/13 or 0.3 per patient vs. 24/10 or 2.4 per patient for placebo).

For patients with baseline DSS scores of 3-6 the relapses rates were 12/12 or 1.0 per patient for COPAXONE® and 35/13 or 2.7 per patient for placebo.

The proportion of patients with DSS scores which remained stable or improved when compared to baseline approached statistical significance in favor of COPAXONE®. (Fisher's exact probability test, p=0.066). Using a logistic regression, placebo patients were 3.67 times more likely to have a worsening in DSS score as compared with those patients on COPAXONE® (p=0.046).

The proportion of progression-free patients over the 24 month trial was 80% in the COPAXONE® group and 48% in the placebo group (p=0.034).

Patients receiving placebo were four times more likely to have progression than patients receiving COPAXONE®.

The adverse experience profile was similar to that observed in the other pivotal trial. No significant effects on laboratory evaluations were found in either COPAXONE® or placebo-treated patients.

COMMENT
The applicant's reanalysis of data using both the the cohort defined in the publication (Bornstein cohort) and a cohort consisting of all randomized patients (All Patient, ITT cohort) confirmed the conclusion of the publication.

For the primary end-point, the proportion of relapse-free patients, 56% of copolymer-1-treated patients compared with 26.1% of those on placebo were relapse-free (p=0.039, Bornstein cohort). An additional primary outcome measure for this trial was the number of relapses during the 24 month trial. Analysis revealed that for both the Bornstein and All Patient

cohorts, significantly more copolymer-1 treated patients had either none or fewer than 3 relapses compared to those on placebo, demonstrating that copolymer-1 is effective in reducing the frequency of relapses. For both DSS baseline categories (DSS of 0-2 and 3-6) there were fewer relapses in the copolymer treated patients. The most pronounced effect was observed in the low DSS category.

This study was reviewed statistically by FDA statistician Jay Levine when the publication first appeared. He concluded that Cop-1 appeared to reduce the frequency of exacerbations in patients with relapsing-remitting MS during the study, and the effect during the first year of the study is greater than the effect during the second year. Reviewer statistician Dr. Hoberman summarizes the results of the primary endpoints. The Fisher's Exact p-value was .004 for the sponsor's categorization of relapse frequencies. The p-value for proportion of relapse-free patients is .15 using Fisher's Exact test and .18 using McNemar's test. The p-value for time to progression was .023 using the log rank test. The p-value for the comparison of proportion of patients who worsened in Kurztke Scores from baseline was .13.

To summarize, the Bornstein study provides highly significant results of the efficacy of Copolymer-1 in the frequency of relapses and the proportion of relapse-free patients with relapsing-remitting multiple sclerosis.

4.0   Protocol 01-9001 Long-Term, Double-Blind, Placebo-Controlled, Multicenter Phase III Study to Evaluate the Efficacy and Safety of Copolymer-1 Given Subcutaneously in Patients with Relapsing-Remitting Multiple Sclerosis.
First patient enrolled October 23, 1991 and last observation May 25, 1994

This was a two-year, placebo-controlled, randomized, parallel group, double-blind study involving 251 patients with relapsing-remitting MS in 11 US centers ranging from 6 to 16 per cell, using daily subcutaneous self-injections of either Copaxone® 20mg (N=125) or placebo (N=126).

Patients, 18-45 years of age, who met Poser's criteria for clinically or laboratory-supported definite MS, with an initial Kurtzke Expanded Disability Status Scale (EDSS) score of 0-5.0 and a history of at least two relapses in the 2 years prior to study entry were eligible for the trial. In addition, patients were required to have objective evidence of neurologic disease reflecting predominantly white matter damage and a stable neurologic state for at least 30 days before entry. Patients who had received prior immunosuppressant therapy were excluded from the study. During the trial patients could receive corticosteroids for up to 28 days during relapses. Chemotherapeutic agents, chronic steroid therapy, or immunosuppressive drugs were not allowed during the study.

Randomization was centralized. The protocol was amended to include a double-blind extension phase that increased follow-up to a maximum of 35 months. (The extension phase is summarized separately as Trial 01-9001E).

The primary outcome measure was the mean number of relapses over the 24-month double-blind trial period. A relapse was defined as the appearance or reappearance of one or more neurologic abnormalities that lasted for at least 48 hours. Relapses were objectively confirmed by the study investigators if the event produced an increase of at least 0.5 point in the EDSS score or an increase of at least 2 points in one Functional System score or an increase of at least 1 point in at least two Functional System scores during the relapse. Patients were required to have a stable or improving neurologic state (or ≥ 30 days before a new relapse was confirmed.

A number of secondary outcome measures were also employed, including the proportion of relapse-free patients, median time to first relapse, change in disability (i.e., EDSS score) from baseline, Ambulation Index, proportion of progression-free patients, and time to progression. Progression was defined as an increase of at least one unit in the EDSS score that persisted for at least 3 months.

Efficacy Variables are summarized as follows:

Primary
  Number of relapses during treatment
Secondary
  Proportion of relapse-free patients
  Time to first relapse
  Proportion of progression-free patients
  Time to Progression (increase of at least one point in the EDSS score from baseline maintained for at least 3 months
  Change in Kurtzke EDSS score from baseline
  Change in Ambulation Index from baseline
  Change in Functional Systems score sum from baseline

Statistical Methodology
Before breaking the blind, a more detailed analytical plan was written as a companion to that originally specified in the protocol. It refers to various model fittings using ANOVA and ANCOVA with sex, duration of disease, prior 2-year relapse rate, and baseline Kurtzke score as potential covariates to predict relapse rate, i.e., the number of relapses per patients over 24 months. Using stepwise progression procedures, the sponsor identified prior 2-year relapse rate and baseline Kurtzke scale as the only statistically significant covariates. The final model upon which the reported p-values are based was a regression model with drug and center as factors and baseline Kurtzke score and prior 2-year response rate as covariates. Time to event analyses used the logrank test, Cox modeling and fitting the data to Wiebull and exponential distributions.

The all patients (intent to treat) cohort was considered the primary cohort for inferences. The "evaluable" cohort was included as a secondary cohort. Also, more of the data was analyzed, including LOCF, patients treated at least 24 months ("completed patients"),

retrieved dropouts, and patients treated for at least 6 months.

All statistical testing was conducted at the two-sided alpha = 0.05 level of significance.

Patient Disposition
Outcome was evaluated using the intent-to-treat population. Following screening (N=284), 251 patients were randomized. Thirty-six patients (19 [15%]COPAXONE® and 17 [13%] placebo) failed to complete 2 full years on their assigned treatments.

PATIENT DISPOSITION	NUMBER OF PATIENTS SCREENED=284			
	Copolymer-1		Placebo	
	n	%	n	%
Randomized	125	100.0	126	100.0
Completed[a]	106	84.8	109	86.5
Included in Safety Analysis	125	100.0	126	100.0
Included in Efficacy Analysis				
Intent to Treat Cohort	125	100.0	126	100.0
Evaluable Cohort[b]	105	84.0	115	92.0
Treated at Least 6 Months Cohort	119	95.2	119	95.2
Completed (≥730 days) Cohort				
All	99	79.2	109	87.2
Evaluable	90	72.0	106	84.8
[b] See Section 6.3.1 for definition				

Of the 284 patients screened, 251 eligible patients were identified. Of these, 125 were randomized to copolymer-1 and 126 to placebo. All 251 randomized patients were included in the intent-to-treat cohort for evaluation of efficacy. All patients received at least one dose of double-blind treatment and thus were included in the safety assessment. A total number of 220 patients (105 on copolymer-1 and 115 on placebo) were considered evaluable "per protocol", having not violated the exclusion criteria.

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Patient Demographics

The two treatment groups were well balanced with respect to demographic characteristics and MS history. Mean age across groups was 34.4 years, 73 percent of the patients were female. The duration of MS was 7.3 years for copolymer-1 patients vs. 6.6 for placebo patients. The two year relapse rate before randomization was 2.9 for cop-1 patients and 2.4 for placebo patients. Baseline Kurtzke EDSS score was 2.8 for cop-1 patients and 2.4 for placebo patients.

DEMOGRAPHIC CHARACTERISTICS: ALL PATIENTS (N=126)		
Parameter	Copolymer-1 (N=125)	Placebo (N=126)
Age Mean±SD Minimum-Maximum	34.6±6.0 19.0-46.0	34.3±6.5 19.0-46.0
Sex[n(%)] Male Female	37 (29.6) 88 (70.4)	30 (23.8) 96 (76.2)
Race [n(%)] Caucasian Black	118 (94.4) 7 (5.6)	118 (93.6) 8 (6.3)
Duration of Disease (yrs) Mean±SD Minimum-Maximum	7.3±4.9 0.6-21.2	6.6±5.1 1.0-23.0
Prior 2-Year Relapse Rate Mean±SD Minimum-Maximum	2.9±1.3 2.0-11.0	2.9±1.1 0.0-6.0
Baseline Kurtzke EDSS Score Mean±SD Minimum-Maximum	2.8±1.2 0.05-5.0	2.4±1.3 0.05-5.0

Efficacy Results

Efficacy results are listed in the attached table (page 8). The primary outcome measure of covariate-adjusted two-year relapse rate was significantly reduced by 29% in favor of COPAXONE®; 1.19 vs. 1.68 relapses per patient for placebo (p=0.007). The corresponding annualized rates were 0.60 for COPAXONE® and 0.84 for placebo.

Few patients in either treatment group had confirmed disease progression (21.6% v. 24.6%);

no significant differences between treatments were observed for the proportion of patients that progressed nor in the time to progression. Also, no significant differences were seen for the Ambulation Index.

Overall, 161 relapses were reported for COPAXONE® and 210 for placebo patients (Table 23, attached). The effect on relapses was apparent early over time but the overall rate of relapses declined during the second year of the study. Table 24 displays the distribution of patients by number of relapses. Two-thirds of the copolymer patients were equally divided between 0 and 1 relapse.

Sponsor's Table 21 tabulates the mean number of relapses by patient cohort. The results are significant for copolymer-1 across all the cohorts.

The positive effect of COPAXONE® was maintained across all levels of degrees of disability but was most pronounced in patients with baseline EDSS scores of 0-2, where the relapse rate was reduced by 33%.

The proportion of relapse-free patients was 33.6% in the COPAXONE® group, compared with 27% in the placebo group (p=0.098).

Compared with patients receiving placebo, the distribution of the number of relapses per patient was significantly different in favor of those patients treated with COPAXONE® (p=0.023). The relative risk of experiencing a relapse was 1.7 times greater for placebo patients.

The median time to first relapse was 287 days for the COPAXONE® patients and 198 days for placebo patients. The difference approached statistical significance (p=0.097). Approximately three-fourths of the patients in both groups were progression-free during the 24-month treatment period.

The change in EDSS score for each patient from baseline to each clinic visit was characterized as: improved (EDSS change $\leq$-1 point), no change (EDSS change $\pm$ 0.5) or worsened (EDSS change $\geq$ 1). Significantly greater number of COPAXONE® patients had improved EDSS scores and fewer COPAXONE® patients had worsening EDSS scores compared with patients who received placebo (p=0.037). At 24 months the change in EDSS score category from baseline also favored COPAXONE® over placebo (p=0.024).

Repeated measures analysis demonstrated a significant effect in favor of COPAXONE® for mean change in EDSS score (p=0.023). This difference was primarily due to consistent increases in mean EDSS score at each visit for placebo patients. This change was -0.05 at month 24 for COPAXONE® and +0.21 for placebo.

There were no statistical differences with respect to progression-free patients, time to progression, ambulation score, and functional systems score.

There were 14 patients (11%) with MS-related hospitalizations in the COPAXONE® treated group compared with 20 (16%) in the placebo group.

Serum samples were monitored every 3 months for the development of antibodies to COPAXONE®. COPAXONE® reactive antibodies developed in almost all COPAXONE® therapy and subsequently declined to a stable level over time. There was no correlation between a patient's antibody development and clinical outcome.

No clinically significant effects on vital signs, ECG or laboratory evaluations of hematology, blood chemistries and urinalysis were found in either COPAXONE® or placebo patients.

At the end of two years on their assigned treatment, trial patients had the option of continuing on their assigned treatment under blinded conditions (Protocol 01-9001E Extension). Ninety-four percent (94%) of the patients (99 COPAXONE® and 104 placebo patients) who completed the 24-month trial elected to continue into the extension.

Patients were treated for up to 35 months. Results of the core trial and the core trial plus extension are presented in Table 1 (page 7, attached) for the intent-to-treat cohort.

Through the end of the extension, the overall covariate-adjusted mean relapse rate was 32% lower for COPAXONE® patients (1.34) compared with placebo patients (1.98, p=0.002).

The proportion of relapse-free patients was significantly higher for COPAXONE® patients (33.6%) compared with placebo patients 24.6%, (p=0.035).

The time to first relapse approached statistical significance in favor of COPAXONE®. 287 vs. 198 days, (p=0.057).

While not statistically significant, the treatment difference in favor of COPAXONE® for the proportion of progression-free patients was greater at the end of the extension than at the end of the two-year core trial (76.8% vs. 70.6%).

The change in disability significantly favored COPAXONE® over time through the extension period (p=0.020). Including the extension period, the change in EDSS for COPAXONE® treated patients was -0.11 vs. 0.34 for placebo patients.

COMMENT
The reviewer statistician Dr. Hoberman examined the impact of imputation on the 36 premature dropouts, 19 in the drug group and 17 in the placebo group who failed to complete the two full years. The sponsor used a hybrid imputation rule: If a patient withdrew before 6 months, the patient was assigned the greater of the observed number of relapses or the overall average number of observed relapses per 24 months computed across treatment groups. If the patient completed 6 or more months of treatment, the observed number of relapses was multiplied by the inflation factor 730/actual number of days of treatment. The

relapse data was reanalyzed by applying each of the above methods separately to the "all patient" (ITT) cohort. The following three models were used:

1. Analysis of variance [drug (D), investigator (I), D x I interaction
2. Analysis of covariance (baseline Kurtzke EDSS, prior 2-year number of relapse, D, I, D x I interaction)
3. Analysis of covariance (baseline Kurtzke DSS, prior 2-year number of relapses, D and I main effects only)

Sponsor's following table highlights the p-values associated with the test of treatment effect using each imputation rule separately on all patients. In all cases, the mean (unadjusted and adjusted) number of observed relapses for the copolymer-1 group was less than that seen for the placebo group.

Algorithm	Model	P-value
>6 months of treatment (730/no.days on trt)	Drug(D),Investigator(I) D xI Interaction	0.037
	Baseline EDSS, prior 2-yr Relapses, D, I, DxI	0.006
	Baseline EDSS, prior 2-yr Relapses, D, I	0.005
<6 months of treatment (greater of either the observed number or the average across all patients)	Drug (D),Investigator (I), DxI Interaction	0.084
	Baseline EDSS, prior 2-yr relapses, D,I,DxI	0.040
	Baseline EDSS, prior 2-yr Relapses, D,I	0.013

If one does impute and put in a covariate, there is some data dredging performed to get a p-value of <.05. If one takes the imputed score with base model from the protocol, one does not reach $p=.05$. For every other group-completers, retrieved dropouts, no imputation-one does attain .05. If one does impute, the data barely makes it on drug center and center action. Imputation is not necessary if everyone drops out at the same rate randomly.

5.0   SUMMARY
Study 9001 has a small treatment effect. There is formal statistical significance, however, the

differences are very slim. The results are marginal but consistent. The Bornstein study demonstrated highly significant results. Could the difference between the studies be attributed to a difference in the patients? In the Bornstein study, even the placebo patients improved. In the multicenter study, th re were larger numbers of patients which are probably more representative of the whole of the MS diagnosis and how the drug would be used under conditions of real life. One remembers that the 50 Bornstein patients were recruited from an initial 932 questionnaires; 140 of these were evaluated in neurologic exams to yield the fifty paients. In the multicenter trial, 284 patients were screened, of which 251 eligible patients were identified. Also, the Bornstein patients were younger (20-35) v. (18-45) for the multicenter trial.

The question is which study is more representative. For the multicenter trial, the data is marginal but consistent. In the Bornstein study, for PBO patients, exacerbations were more prevalent in year 1 than in the second year. There were few exacerbations in the drug group, but many in the the PBO group.

Based on these two studies, Copolymer-1 appears to reduce the frequency of exacerbations in patients with exacerbating-remitting multiple sclerosis.

Janeth-Rouzer-Kammeyer, M.D.

cc:
Orig:NDA#20-622
HFD-120/Dr. Leber
    /Dr. Katz
    /Ms. Wheelous
12-8-95

ONE® - Copolymer-1 - For Injection   182
ion Summary
Overview   FINAL

Type/Trial Number	COPOLYMER-1					Placebo				
	RR-MS	CP-MS	MS-Unsp	Other	Total	RR-MS	CP-MS	MS-Unsp	Other	Total
CAL PHARMACOLOGY										
BR-OB	4	12			16					
BR-OA			4	3	7					
BR-OC		5			5					
BR-OD	6	15			21					
Subtotal	10	32	4	3	49					
MS TRIALS										
RR-MS CONTROLLED (US)										
01-9001/9001E	125				125	126				126
BR-1	25				25	25				25
Subtotal	150				150	151				151
RR-MS UNCONTROLLED										
US										
01-9002°	241				241					
01-9004°										
Subtotal	241				241					
NON-US										
1110-1	282				282					
1110-2	63				63					
1140°										
Subtotal	345				345					
MS CONTROLLED (US)										
BR-2		51			51		55			55
OMPASSIONATE USE & BR-PTP										
BR-3°	43	22	5		70					
1150°										
BR-PTP°										
Subtotal	43	22	5		70					
RAND TOTAL	789	105	9	3	906	151	55			206

ata available at time of this NDA
ven patients also participated in Trial 1110-1 and were subsequently transferred to this trial.
patient also participated in Trial BR-1 and 3 patients also participated in Trial BR-OB and were then enrolled in this trial

TABLE 59. IND SOURCE

Type/Trial	Country	IND Bornstein 14,115	IND Bornstein 20,583	IND Teva 27,998	Data Source CRFs	Data Source Publication	Data Source Other	Data Lock Date (dy-mo-yr)
CAL PHARMACOLOGY								
BR-OB	US	x		x*	x	x	x*	N/A*
BR-OA	Israel			x*		x		N/A
BR-OC	U*			x*		x		N/A
BR-OD	Germany			x*			x*	N/A
TRIALS								
RR-MS CONTROLLED (US)								
01-9001	US			x	x			7/22/94
01-9001E	US			x	x			1/31/95
BR-1	US	x		x*	x	x	x*	4/27/94
RR-MS UNCONTROLLED								
US								
01-9002	US			x	x			2/28/95
01-9004	US			x				N/A
NON-US								
1110-1	Israel			x	x			12/31/94
1110-2	Israel			x	x			12/31/94
1140	Europe							N/A
MS CONTROLLED (US)								
BR-2	US	x		x*	x	x	x*	2/8/95
COMPASSIONATE USE & PTP (US)								
BR-3	US		x	x*	x		x**	N/A
1150	Italy							N/A
BR-PTP	US	x						N/A

...orts prepared by TEVA were filed to IND 27,998 and are included in this NDA.
...er from the investigator
...gress notes and other source documents provided by Dr. Bornstein
...us reports filed by Dr. Bornstein to IND
...*- Not available

TABLE 10.   SUMMARY STATISTICS OF DEMOGRAPHIC AND BASELINE CHARACTERISTICS: ALL PATIENT COHORT

	Copolymer-1 (N=25)	Placebo (N=25)	P-Value
Sex			
Male	11	10	>0.99
Female	14	15	
Race			
White	23	25	0.49
Black/Other	2	0	
Age (years)			
Mean ± S.D.	30.0 ± 3.2	31.0 ± 3.5	0.34
Minimum	20.0	25.0	
Maximum	33.0	35.0	
Duration of Disease (years)			
Mean ± S.D.	4.9 ± 2.7	6.1 ± 3.9	0.22
Minimum	2.0	1.0	
Maximum	10.0	13.0	
Prior Relapse Rate (number over 2 years)			
Mean ± S.D.	3.8 ± 1.4	4.0 ± 1.2	0.59
Minimum	2.0	2.0	
Maximum	6.0	7.0	
Baseline Kurtzke DSS Score			
Mean ± S.D.	2.8 ± 1.9	3.2 ± 2.0	0.56
Minimum	1.0	0.0	
Maximum	6.0	6.0	
Baseline Kurtzke DSS Score			
0-2	13	11	
3-4	5	7	
5-6	7	7	

Cross Reference: Appendix I, Table 3, Appendix J, Outputs 10, 11 & 12, Appendix K Listings 2A & 2B

TABLE 23. OVERALL DISTRIBUTION OF RELAPSES BY TIME ON TREATMENT: ALL PATIENTS

Time Interval to Onset of Relapse (months)	Number of Relapses	
	Copolymer-1 (N = 125) n	Placebo (N = 126) n
0 ≤ 3	38	43
>3 - 6	20	29
>6 - 9	23	26
>9 - 12	21	30
>12 - 15	19	18
>15 - 18	13	25
>18 - 21	18	16
>21	9	23
Total	161	210

Source: Appendix 14.2.7.1

TABLE 24. DISTRIBUTION OF PATIENTS BY NUMBER OF RELAPSES: ALL PATIENTS

Number of Relapses	Copolymer-1 (N = 125) n	%	Placebo (N = 126) n	%
0	42	33.6	34	27.0
1	42	33.6	39	31.0
2	18	14.4	16	12.7
3	12	9.6	21	16.7
4	9	7.2	9	7.1
5	1	0.8	4	3.2
6	1	0.8	1	0.8
7	0	0	2	1.6

Source: Appendix 14.1.1.1, J4.1.1.1

TABLE 21.   COVARIATE ADJUSTED MEAN NUMBER OF RELAPSES BY PATIENT COHORT

Patients in Analysis	Copolymer-1 (N = 125) n	Copolymer-1 (N = 125) Adjusted Mean±SE	Placebo (N = 126) n	Placebo (N = 126) Adjusted Mean±SE	p-Value[a]
Primary Cohort:					
All Patients (ITT)	125	1.19±0.13	126	1.68±0.13	0.007
Secondary Cohorts:					
Evaluable Patients	105	1.27±0.14	115	1.75±0.13	0.013
Patients Treated at Least 183 Days	119	1.25±0.13	119	1.73±0.13	0.010
Patients Treated at Least 730 Days	99	1.23±0.15	109	1.74±0.14	0.015
Evaluable Patients Treated at Least 730 Days	90	1.21±0.16	106	1.76±0.15	0.011
All Patients with Imputation of Relapses	125	1.32±0.14	126	1.78±0.14	0.021
Evaluable Patients with Imputation of Relapses	105	1.39±0.15	115	1.86±0.15	0.026
Retrieved Dropouts: All Patients	125	1.22±0.13	126	1.68±0.13	0.011
Retrieved Dropouts: Evaluable Patients	105	1.30±0.14	115	1.75±0.14	0.021

[a]   p-value for ANCOVA between treatment group analysis.

Source: Appendix K4.2.1.1.1 - K4.2.5.9.2

...s – Core and Extension								Reference (Vol/Pg)	
	Core Trial (24 Months)				Core Trial including Extension (up to 35 Months)			Technical Section	Report
	COPAXONE (N=125)	Placebo (n=126)	Reduction vs. Placebo	P	COPAXONE (N=125)	Placebo (n=126)	Reduction vs. Placebo	P		
	1.19	1.68	-29%	0.007	1.34	1.98	-32%	0.002	157 001	042 107 084 074
	0.60	0.84			0.49	0.72				
	33.6%	27.0%		0.096	33.6%	24.6%		0.035	157 001	042 109 084 078
	287	198		0.087	287	198		0.057	157 001	042 111 084 079
	42 60 23	34 55 37		0.023	42 53 30	31 51 44		0.006	157 001	042 111 084 078
	78.4%	75.4%		0.476	78.8%	70.6%		0.189	157 001	042 112 084 080
	-0.05	0.21		0.023	-0.11	0.34		0.020	157 001	042 113 084 081
	0.28	0.28			0.28	0.36			157 001	042 115 084 084
	24.8%	15.2%		0.024	27.2%	12.0%		0.001	157 001	042 114 084 083
	54.4%	56.0%			54.4%	56.6%				
	20.8%	28.8%			18.4%	31.2%				

the extension phase.

Trial Results – Core and Extension

	Core Trial (24 Months)				Core Trial including Extension (up to 35 Months)				Reference (Vol/Pg)	
	COPAXONE (N=125)	Placebo (n=128)	Reduction vs. Placebo	P	COPAXONE (N=125)	Placebo (n=128)	Reduction vs. Placebo	P	Technical Section	Report
	1.19	1.88	-38%	0.007	1.34	1.88	-32%	0.002	157/ 001	042 107 / 084 074
	0.60	0.84			0.49	0.72				
	33.6%	27.0%		0.096	33.6%	24.6%		0.035	157 001	042 109 / 084 078
	287	198		0.097	287	198		0.057	157 001	042 111 / 084 079
0 / 1-3 / >3	42 / 60 / 23	34 / 56 / 37		0.023	42 / 53 / 30	31 / 51 / 44		0.008	157 001	042 111 / 084 078
	78.4%	75.4%		0.478	76.8%	70.8%		0.190	157 001	042 112 / 084 080
EDSS change from baseline	-0.05	0.21		0.023	-0.11	0.34		0.020	157 001	042 113 / 084 081
Ambulation Index Change from baseline	0.28	0.28			0.28	0.38			157 001	042 115 / 084 084
Improved disability (EDSS change ≤-1)	24.8%	15.2%		0.024	27.2%	12.0%		0.001	157 001	042 114 / 084 083
No change (EDSS change ±0.5)	54.4%	58.0%			54.4%	58.8%				
Worsened Disability (EDSS change ≥1)	20.8%	26.8%			18.4%	31.2%				

Up to 35 months in the extension phase.

<u>Statistical Review and Evaluation</u>

<u>NDA#</u>: 20-622

<u>Applicant</u>: TEVA Pharmaceuticals, USA

<u>Name of Drug</u>: Copolymer-1 for Injection

<u>Documents Reviewed</u>: Vols 1.47, 1.57, 1.58, 1.161, 1.236, amendment dated 11/30/1995

<u>Medical Input</u>: Janeth Rouzer-Kammeyer, M.D., HFD-120

<u>Background</u>

The sponsor has submitted two randomized, placebo-controlled, double-blind studies evaluating the effect of Copolymer-1 (cop-1) in patients with relapsing-remitting multiple sclerosis. Study 9001 is multicenter and Study BR-1 was conducted at a single center.

<u>Study 9001</u>

This study used randomization within center to assign 125 patients to cop-1 and 126 patients to placebo. Eleven (11) centers participated. The range of the number of patients in any treatment by investigator cell was from 6 to 16 with treatments groups well-balanced <u>within</u> center. **Table 1** displays the patient disposition over the trial, while **Table 2** displays baseline characteristics. All patients were ambulatory having baseline Kurtzke EDSS scores from 0-5. All patients were to have had at least 2 relapses in the previous 2 years. There was, however, 1 patient who had had none. The only statistically significant baseline differences were on Kurtzke EDSS score and Functional Systems score. Nineteen (19) patients on cop-1 and 17 on placebo prematurely terminated the 24 month treatment. There was no clear pattern in the reasons for dropping out except possibly for adverse experiences. See **Table 3**.

The primary endpoint was number of relapses over the 2 years of follow up. The definition of a relapse was the appearance of neurological abnormalities lasting at least 48 hours together with objective changes consistent with an increase of .5 on the EDSS score or one point in the score for two or more of the Functional Systems (FS) or two points in the score for one of the FS as compared with the previous evaluation. Other endpoints were 1) <u>time to first relapse</u>, 2) <u>time to progression</u> defined as one unit or greater increase in the Kurtzke EDSS from baseline sustained for at least 90 days, 3) <u>proportion of relapse-free patients</u> at 2 years, 4)<u>change in Kurtzke EDSS</u>, 5)<u>Ambulation Index</u>, and 6)<u>Functional Score Sum</u>.

The planned sample size of 120/group was based upon a relapse rate of 65% in the placebo group and 44% in the cop-1 group to achieve 85% power.

The statistical analysis plan was developed after the original protocol and before unblinding. It refers to various model fittings using ANOVA and ANCOVA with sex, duration of disease, prior 2-year relapse rate and baseline Kurtzke score as potential covariates to predict relapse rate, i.e., the number of relapses per patients over 24 months. Using stepwise regression procedures, the sponsor isolated prior 2-year relapse rate and baseline Kurtzke as the only statistically significant covariates. The final model upon which the reported p-values are based was a regression model with drug and center as factors and baseline Kurtzke score and prior 2-year response rate as covariates. Note that **treatment by center interaction was not in the model**. Time to event analyses used the logrank test, Cox modeling, and fitting the data to Weibull and exponential distributions.

Four (4) different cohorts were used:
a) observed cases
b) patients with at least 6 months treatment
c) completers
d) retrieved dropouts

There was also a distinction between an Intent to Treat (ITT) cohort and an 'evaluable' cohort defined as the ITT sample minus protocol violators. This review focuses on analyses which include protocol violators regardless of cohort. In addition, the sponsor used an imputation scheme for imputing values for non-completers: If a patient withdrew before 6 months, "the patient was assigned the greater of the observed number of relapses or the overall average number of observed relapses per 24 months computed across treatment groups. If the patient withdrew between 6 months and 730 days, the observed number of relapses was adjusted to account for 730 days of treatment using the multiplication factor 730/actual number of days of treatment."

The following table displays various p-values for treatment effect on relapse rate. The sponsor's report of least square means of **1.68 (placebo)** is stable over the analyses whereas the **1.19 reported for cop-1** rises to about 1.28 in some analyses. The p-values are cross-classified by the terms in the linear model and the data base used (D=Drug, C=Center).

	No Imputation (LOCF)	Completers	Imputed	Retrieved Drop Outs
D, C, DxC	.055	.03	.09	.07
D, C, DxC, bl EDSS, prior relapse	.02	.03	.03	.02
D, C, bl EDSS, prior relapse (sponsor's **reported** analysis)	.007	.015	.02	.01

2

Instead of depending solely on the sponsor's hybrid imputation rule (different ones for patients leaving before and after 6 months), the division requested the sponsor to submit supplementary analyses using each imputation rule separately on all patients. The first column of the table below displays the p-values using the **inflation factor of 730/#days on treatment**. The second column uses the **greater of either the observed number or the average across all patients.**

D, C, DxC	.037	.084
D, C, DxC, bl EDSS, prior relapse	.006	.04
D, C, bl EDSS, prior relapse	.005	.013

Table 4 displays the distribution of relapses over time and **Table 5** displays the distribution of patients over the number of relapses. Note that there are considerably fewer relapses overall in the second year of the study. **Table 6** lists results for different cohorts using the sponsor's model.

Time to first relapse was analyzed by logrank (p=.23) and by fitting a Weibull to get p=.097 which is the result that the sponsor reports in the text. The proportions of relapse-free patients (34%: cop-1 vs 27%: placebo) were not statistically significantly different using logistic regression with the same terms as the relapse rate analysis. A simple test of proportions yields p=.25. **The result of the trial differs markedly from the assumption in the design of a 56% relapse-free proportion in the cop-1 group and 35% in the placebo group.**

An ordinal logistic regression taking into account the whole distribution of relapses was significant (odds ratio 1.7).

Although the sponsor's ANCOVA on mean change from baseline in EDSS score was not significant using LOCF, the sponsor's repeated measures analysis (average over 24 months) was significant (p=.023).

There were no statistical differences with respect to progression-free patients, time to progression, ambulation score, functional systems score, and quality of life.

The main issues concerning the primary endpoint (relapse rate) are the use of covariance models and ways to characterize the putative difference between cop-1 and placebo.

First, the sponsor's use of a linear model may pose problems because 1) the model was found by data searching and 2) the assumption of no treatment by covariate interaction is essentially untestable due to the categorical nature of the EDSS score. Regarding 1), the table above indicates that the treatment effect is not significant without controlling for the 2 baseline covariates found by a data conditioned model. As for 2), when the treatment, baseline EDSS main effect and the interaction term are in the model, neither the treatment nor interaction term is significant. This is due to the fact that the correlation between the indicator variable for treatment and the interaction term is .85. Thus the linear model may be pathological for this kind of data.

As an alternative, this reviewer has found that a simple two-sample t-test is significant (p=.04). So is a CMH analysis using mean scores (P=.04). Controlling for center, the latter analysis yields p=.02. Alternatively, since there appears to be a higher mean EDSS score (which is positively correlated with relapse rate) in the cop-1 group at baseline, it seems reasonable to do a CMH analysis controlling for baseline EDSS. This is significant at p=.02. Thus, it appears that simple tests yield statistical significance without resorting to complicated linear or logistic models. Recall that there was no unique analysis specified in the protocol.

Although the groups were well-balanced for the mean number of prior relapses in the previous 2 years (2.9 in both groups), they were not balanced with respect to the frequencies in the two most populous categories: 2 and 3 relapses in the prior 2 years. Sixty-three (63) cop-1 and 51 placebo patients had had 2 relapses while 29 cop-1 and 40 placebo patients had had 3 relapses. However, it is not clear that this imbalance is important since the mean number of relapses on study in the cop-1 group was 1.24 in the category of 2 prior relapses and .90 in the category of 3 prior relapses (goes down), while in the placebo group, the respective means were 1.4 and 1.8 (goes up). Thus, the relation between number of previous relapses and mean number of relapses on study is seemingly reversed between the treatment groups.

The table below tabulates the number of patients who experienced a decrease, no change or increase in their frequencies of relapse:

	-7	-5	-4	-3	-2	-1	0	1	2	3	4
COP 1	1	5	7	22	27	42	9	7	4	0	1
PLACEBO	0	4	7	18	32	27	18	11	5	3	1

The mean decreases in the groups are 1.62 in the cop-1 group and 1.26 in the placebo group. This difference was not significant by either a t-test (p=.10) or Wilcoxon Rank Sum test (p=.17). Note that any differentiation between the distributions occurs only for the case of a decrease of 1 relapse/patient over 2 years (42 vs 27).

The Bornstein Study (BR-1)

This self-described two-year pilot study enrolled 50 patients. Patients were to have experienced at least 2 relapses in the previous 2 years and a disability of no greater than 6 on the Kurtzke DSS Scale. Foity-eight (48) belonged to randomized matched pairs. The other 2 patients were randomized separately. Matching was done on Kurtzke DSS scale: 0-2, 3-4, 5-6 and # of attacks in the previous two years (+ or - 2 years). An inspection of the data shows that 2 patients were not truly matched on one or both factors. **The sample size was determined to have approximately 80% power to detect a difference of 40% in the proportion of patients who remained relapse-free over 2 years.** A relapse was defined as a worsening lasting at least 48 hours (24 hours for an earlier period during the study, but all data was later revised in a blinded fashion to reflect the 48 hour definition). Worsening was defined as an objective change of at least 1 grade in the score for one of the eight Functional Systems or the Kurtzke DSS Scale. Note that this definition is somewhat different from that in Study 9001.

In a document written after the original protocol, the **major endpoints are stated to be # of relapses and proportion of relapse-free patients.** However, in the published report, only the latter was stated as a primary endpoint.

Table 7 displays the baseline comparisons for all patients. Seven (7) patients did not complete the two years. Two patients were deemed 'inevaluable' because symptomotology was judged to be psychogenic by the investigator. This review discusses only the 'all patients' analysis.

Table 8 displays the sponsor's categorization of **relapse frequencies.** The Fisher's Exact p-value was .004. **Figure 1** displays the frequency histogram. Note the long tail for the placebo group, only.

The p-value for **proportion of relapse-free** patients is .15 using Fisher's Exact test and .18 using McNemar's test.

The p-value for **time to progression** was .023 using the logrank test

Figure 2 displays the histogram of **change in Kurtzke Scores from baseline**. The p-value for the comparison of proportions of patients who worsened from baseline was .13.

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Conclusions

The **Bornstein** study produces a clear statistical difference between cop-1 and placebo. Study 9001's results are borderline with secondary endpoints going in the 'right' direction. The sponsor's covariate analysis was not really prespecified since it used a model to choose significant covariates. In addition, it was not possible to check the assumptions of the model. However, other analyses do produce p-values below .05. Thus, it is possible to argue that two studies produced statistically significant results for number of exacerbations. However, the overall experience in the two studies appears different. In **Study 9001**, 23/125, or 18% of the Cop-1 patients had 3 or more exacerbations whereas only 1 of the 25 patients on Cop-1 did in the **Bornstein Study**. The respective numbers in the placebo groups were 37/126 (29%) and 11/25 (44%). This accounts for the larger treatment difference in the **Bornstein** study relative to that in **Study 9001**.

This difference is also reflected in the **average decreases in relapses from the previous 2 years**. In the Cop-1 group in the **Bornstein** study, the average decrease was 3.2 relapses and in the placebo group the average decrease was 1.6 relapses. Note that the 1.6 for placebo is similar to that for placebo in **9001** (1.3). However the change in the Cop-1 group is quite different: 3.2 (**Bornstein**) vs 1.6 (**9001**). Thus, the change over the next 2 years was nearly the same in the placebo groups in the two studies, but different between the Cop-1 groups.

One indication that the studies' patients may have been drawn from different populations is that the **Bornstein Study**'s patients had a shorter duration of disease on average (5.5 vs 7 years) and a higher previous 2-year relapse rate (3.9 vs 2.9). Moreover, screening of patients was much more rigorous in the **Bornstein** study.

David Hoberman, Ph.D.
Mathematical Statistician

concur: Dr. Sahlroot   )TS   12-21-95

Dr. Chi   OK
12/22/95

cc:
Orig: NDA# 20-622
HFD-701/Dr. Anello
HFD-120/Dr Leber
HFD-120/Dr. Katz
HFD-120/Dr. Rouzer-Kammeyer
HFD-120/Mr. Purvis
HFD-120/Ms Wheelous

6

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

DATE: August 9, 1996

FROM: Glenna G. Fitzgerald, Ph.D. *gfr*
            Pharmacology Team Leader
            Division of Neuropharmacological Drug Products, HFD-120

TO: NDA 20-622, TEVA Pharmaceuticals
            Copolymer-1; Copaxone®
            Subcutaneous Injection

SUBJECT: Pharmacology and Toxicology Overview

The pharmacology and toxicology studies which have been submitted in support of this NDA
for injectable Copaxone, indicated for the treatment of patients with exacerbating-remitting
multiple sclerosis, are comprehensively summarized and evaluated in the excellent review by
Dr. John Jessop. The reproduction studies are reviewed by Dr. J. Edward Fisher in an
attachment to Dr. Jessop's review. It is Dr. Jessop's conclusion, as well as mine, that these
studies marginally support approval of this drug for this serious, chronic indication, as long
as a Phase 4 commitment to submit two valid lifetime rodent carcinogenicity studies is
honored.

Primary Issue Affecting Approvability:

The major concern about the adequacy of the preclinical package stems from the fact that the
carcinogenicity studies have not been completed, although studies in mice (3, 15, 30, 60
mg/kg/day) and rats (3, 7.5, 15, 30 mg/kg/day) using the subcutaneous route are in progress.
This deficiency exists even though the sponsor was informed in several meetings over the
years, and in written communications (letter of Dec. 1, 1993 attached as example), that
carcinogenicity studies would be required at the time of filing of the NDA unless they could
provide a compelling argument for why such a requirement should be waived. Their
argument (May 4, 1995 letter attached) was deemed inadequate. They have referred to the
then Step 2 ICH document which recommended that for life-threatening diseases
"carcinogenicity studies may be completed post-approval". In general, postponement of
carcinogenicity studies to Phase 4 completion has been allowed for drugs for serious or life-
threatening diseases with onset in the elderly, or for which there is no available therapy, or if
survival is altered by the drug. Multiple sclerosis is a disease of relatively young people
who could be exposed for a significant number of years, and there also is an available
therapy. It should also be noted that Copaxone is not used to alter survival or progression of

the disease. At the time the Division learned that Teva was going to submit the NDA without the carcinogenicity studies it was Dr. Temple's recommendation that the application be filed (May 26, 1995 E-mail to Dr. Leber), with the understanding that " We cannot, however, in advance of reviewing the data, including the evidence of clinical benefit, conclude that carcinogenicity studies will not be needed prior to approval."

The sponsor has included in the NDA a survey of tumors reported in their toxicology studies in support of their contention that there is no evidence that Copaxone possesses carcinogenic potential. In a fertility and reproductive performance study in rats, one middle dose dam (6 mg/kg/day) of 180 females in the study was found to have two malignant mammary epithelial tumors, one discovered on day 7 postpartum in the dorsum of the neck and one on day 20 in the mammary gland region. No tumors were observed in rats receiving 30 mg/kg for 6 months, and it is considered that the finding in the reproduction study is most likely not drug related. In a 4 week dog toxicity study, 10 oral papillomas were found, appearing in control and dosed animals. The incidence was 3/6 controls, 1/6 low dose, 4/6 middle dose, and 2/6 high dose. Six of the 10 spontaneously regressed during treatment. The sponsor has stated that these neoplasms are not uncommon in young dogs and that they usually are considered to be of viral etiology. These tumors are undoubtedly not drug related findings. It should, however, be noted that Copaxone was clastogenic in two *in vitro* human lymphocyte assays (according to FDA review but not according to the sponsor, vide infra). Copaxone was not mutagenic in the Ames test and the *in vitro* mouse lymphoma assay, and it was not clastogenic in the *in vivo* mouse bone marrow micronucleus assay. However, in light of the clastogenic response in lymphocytes, the possibility that Copaxone may be carcinogenic in lifetime bioassays must be considered.

Other Toxicology Issues:

1) Genetic Toxicology:

The findings in two *in vitro* chromosomal aberration assays in cultured human lymphocytes should be addressed, particularly since the sponsor has stated in the proposed labeling that the results were negative. The data from these assays are on pages 100 and 101 of Dr. Jessop's review. In the first assay a significant increase in "cells with aberrations excluding gaps" was seen in the presence of S9 rat liver microsomes at 20 hours, but not at 44 hours. The sponsor considered this to be a negative finding. Our experts have advised us that the 44 hour time point is used only if 20 hours is negative, and if one suspects that the drug might delay mitosis. With a positive finding at 20 hours, the assay is considered to be positive. In the second assay, a significant effect was reported for one of two replicates, and for the mean of the two replicates, at 20 hours in the presence of S9. The sponsor reported this as a negative finding as well. This would constitute a positive response by FDA standards. I have therefore considered the findings in both studies to provide positive evidence for clastogenicity in the human lymphocyte assay and recommend inclusion of the results in labeling.

2) Immunotoxicology:

Dr. Jessop's review provides an excellent, in-depth summary and discussion of the immunotoxicological characteristics of this drug (pages 125 through 139 and 144 through 146), to which I refer the reader. The sponsor has conducted a rather extensive battery of studies to characterize the immunotoxicity of Copaxone. It is apparent that, although anti-Copaxone antibodies are produced, they `re not neutralizing antibodies. Also, repeated administration to rats, monkeys and humans does not result in a general immunosuppressive effect. However, several hypersensitivity and potential autoimmunity type findings were reported in the toxicology studies, and the following sections have been added to labeling to describe them (Pharmacological Properties, following Clinical Pharmacology):

"Hypersensitivity: 1). In a 6-month rat and a 1-year cynomolgus monkey study at doses up to 30 mg/kg/day (15-times greater than the human dose in rat and 29 times greater in cynomolgus monkey on a mg/m² basis) injection site lesions and immune complex deposition in the glomeruli of the kidney occurred. The monkey study also revealed a low incidence of active fibrinoid arterial lesions in various highly perfused organs and inflammatory cell foci in brain (choroid plexus), spinal cord and heart. Although immune complex deposition in kidney did not result in detectable pathology, these results are consistent with a hypersensitivity response, most likely due, in part, to consistent antigenicity of the drug in all species tested. 2) In a study of Copaxone in mice by the subcutaneous route of administration, 59 of 600 treated animals died in the first 14 weeks of the study. The animals were dosed with a maximum of 60 mg/kg/day Copaxone, which is 15 times greater than the human dose on a mg/m² basis. A large proportion of these animals (62%) died within 5 hours of receiving drug. At necropsy the most consistent findings were at the injection site and in the vasculature and hematopoietic system, and the cause of death was reported to be a Type 1 hypersensitivity.

Anti-DNA and Anti-Histone Antibodies: In a 52-week study in cynomolgus monkeys receiving s.c. administration of 1, 10 or 30 mg/kg/day, a statistically significant increase in antibodies to double-stranded DNA occurred in male (10 and 30 mg/kg; weeks 8 and 13, p<0.01) and female (10 mg/kg; week 8, p<0.05) animals. In this same study, a statistically significant increase (p<0.05) in antibodies to histones was found in males (all doses at weeks 4, 8, and 13; 10 and 30 mg/kg/day a weeks 26, 39 and 52) and females (all doses at weeks 4, 8, 13 and 26; 30 mg/kg/day at week 2). Doses of 1, 10 and 30 mg/kg/day are in the same range, 10-fold and 29-fold greater, respectively, than the human dose on a mg/m² basis. These antibodies are often associated with autoimmune disease."

3

Dr. Jessop has directed several comments to the clinical reviewer about the potential problems associated with the immunotoxicological profile of Copaxone, as observed in animal studies (see page 148 of his review). I shall elaborate briefly on one of the issues, that of the lack of histopathological lesions in the kidney; f animals in which immune complex deposition occurred. Data (obtained by measurement of TCA precipitable drug) from the chronic rat (6 month) study indicated that there was a two-fold increase in large degradation products of Copaxone or of intact drug in plasma at the end of the study. Results obtained early in the study (day 28) showed a preponderance of small breakdown products. No measurements were taken between those two time points, so it is not known if increased exposure to high molecular weight products occurred early or late in the study. Since the large polymers or intact drug are the species associated with immune complex deposition, it is conceivable that, if that occurred late in the study, there was insufficient time for the renal pathology to develop. Drug was not measured in the one year monkey study, in which immune complex deposition also occurred, so it is not known if there was a correlation between these effects in that species as well. Dr. Jessop has therefore suggested that it would be appropriate to determine if systemic exposure to intact drug also increases with time in patients.

3) Cardiovascular Effects:
Copaxone produced hypotension in rats, rabbits, cats and dogs when administered intravenously. This effect appears to be mediated by histamine release, at least in part. It also caused the release of interleukin-2 from human blood cells, which can result indirectly in prolonged hypotension. Hypotension was not observed in the sub-chronic and chronic toxicology studies in which Copaxone was administered subcutaneously. The following "Cardiovascular Effects" section has been included in labeling under "Pharmacological Properties".

"**Cardiovascular Effects**: In vitro studies demonstrated that Copaxone directly induced histamine release from rat peritoneal cells and human peripheral blood basophils from healthy volunteers and multiple sclerosis patients. Safety pharmacology studies in rats, cats and Beagle dogs demonstrated that i.v. administration of Copaxone resulted in hypotension (decreased mean arterial pressure), and mechanistic studies revealed that the effect in rats and cats was probably due to histamine. Arrhythmias with increased T,R and S amplitudes occurred in dogs after intravenous dosing. The no effect dose in rats and dogs was 10 mg/kg and 5 mg/kg, respectively. This is 5 or 8 times greater than the human dose (20 mg), respectively, on a mg/m³ basis."

Recommendations:
This application may be considered to be approvable for pharmacology/toxicology, with the understanding that draft reports of the two ongoing carcinogenicity studies will be submitted as soon as they are available. Subsequent submission of final reports should include a complete listing of any changes noted between the draft and final reports. If it is determined that the validity of the mouse study has been compromised by excessive early mortalities, a second mouse study may be required.

4

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
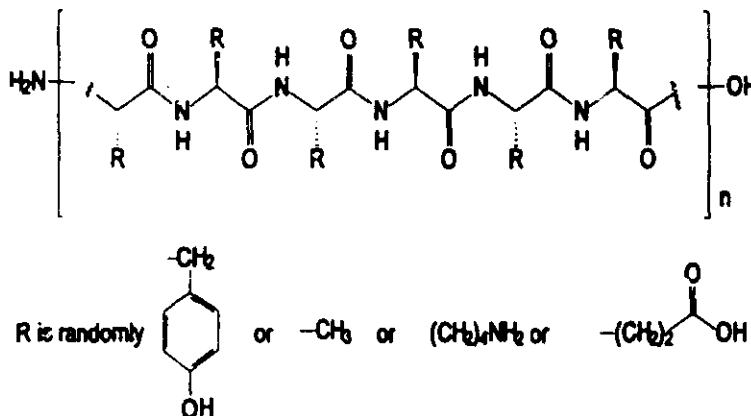## NDA Original Review

NDA #:   20-622

Review Date: February 21, 1995

Date of Submission: October 11, 1995

Sponsor: TEVA Pharmaceuticals USA
1510 Delp Drive
Kulpsville, PA 19443

Drug: Copolymer-1 for Injection (Copaxone®)

Structure:

Chemical Name: Acetate salts of synthetic polypeptides containing L-Glutamic Acid, L-Alanine, L-Tyrosine and L-Lysine with an average molar fraction of 0.141, 0.427, 0.095 and 0.338, respectively.

Molecular Formula: $Poly[L\text{-}Glu^{13\text{-}15}, L\text{-}Ala^{39\text{-}46}, L\text{-}Tyr^{8.8\text{-}10}, L\text{-}Lys^{30\text{-}37}] nCH_3CO_2H$

Molecular Weight:

Pharmacological Category: Immunomodulator (blocks myelin-specific autoimmune response).

Indication: Slowing progression of disability and reducing frequency of relapses in patients with relapsing-remitting multiple sclerosis.

Related INDs/NDAs: IND

Proposed Clinical Use:

The recommended dose of Copaxone is 20 mg/day injected subcutaneously for slowing progression of disability and reducing the frequency of relapses in patients with relapsing-remitting MS. 20 mg/day for a 50 kg patient is about 0.4 mg/kg/day. (I used 50 kg because the majority of MS patients are female).

Previous Human Experience:

The total clinical program with Copolymer-1 (excluding the Clinical Pharmacology trials) consists of 11 clinical trials in which a total of 857 patients with MS have been exposed to the drug. Of these 857 patients, 670 were in the relapsing-remitting phase of the disease and received Copolymer-1 by subcutaneous injection at a dose of 20 mg/kg/day for at least 6 months. 490 received the drug for at least 12 months.

REVIEW AND EVALUATION OF PHARMACOLOGY TOXICOLOGY DATA
Original NDA Review

PHARMACOLOGIST: John J. Jessop, Ph.D., M.P.H.

NDA #:   20-622

DRUG: Copolymer-1 for treatment of relapsing-remitting multiple sclerosis.

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Attachment: Review of Reproductive Toxicology Studies by Dr. Edward Fisher.

PHARMACOLOGY

Introduction
The pharmacology in this NDA is submitted in three parts, the first including information and studies pertaining to animal models of efficacy with respect to multiple sclerosis (MS), the second detailing studies to delineate the mechanism of action of the drug in treatment of MS, and the third describing the safety pharmacology. The pharmacology section of the NDA is quite extensive. The studies described in this section were performed over a period of about 25 years, primarily at the   The majority of the studies were carried out by   wrote the rather lengthy summary section included in the NDA concerned with the the mechanism of action and animal efficacy studies associated with Copolymer-1. For the purpose of this regulatory review, I will briefly summarize the more important pharmacological points included in the submission.

I. *Copolymer-1 and Animal Models of Efficacy for Treatment of MS*
MS is a chronic inflammatory disease, affecting the central nervous system (CNS). In this disease, lymphocytes, predominantly T cells and macrophages, infiltrate the CNS and induce damage to the neuronal myelin sheath. Although the precise etiology of the disease has yet to be determined, there are at least two major hypotheses proposed to explain the pathogenesis of the disease:

1. MS results from a viral infection of the CNS and the resulting inflammatory condition is, mainly, an antiviral response.

2. MS is an autoimmune disease in which infiltrating T cells recognize self-antigens and attach normal nerve tissue.

These two hypotheses are not mutually exclusive, in that the autoimmune disease may actually be triggered by environmental factors, including viral infection or chemical or drug induction.

Multiple sclerosis and the EAE model

The putative autoantigen has not been identified in patients with certainty. Certain myelin-associated proteins are suspect, such as Myelin Basic Protein (MBP), Proteolipid Protein (PLP) and Myelin Oligodendrocyte glycoprotein (MOG). The animal model of human MS is "experimental allergic encephalomyelitis", or EAE (also termed "autoimmune encephalomyelitis"). This is widely recognized as a valuable model for studying MS.

There are basically two EAE animal models, the acute model and the chronic-relapsing model. In the acute model, EAE may be induced in animal species by the injection of CNS material, purified encephalitogenic proteins or their peptide fragments in Freund's adjuvant (CFA). Clinical signs such as paralysis of the hind legs generally appear in 10 to 21 days after challenge. This treatment usually ends in death, although some of the animals may recover spontaneously. In the chronic-relapsing EAE (CR-EAE) model, EAE is induced by injection of (SJL/J x BALB/C) F1 mice with mouse spinal cord homogenate (MSCH). Other CR-EAE models include the use of PLP in mice and in the juvenile strain 13 guinea pigs.

Immunological mechanisms in EAE and MS

In the EAE model, systemic injection of CNS tissue, purified encephalitogenic proteins, or their peptide fragments to experimental animals stimulates a population of autoreactive T cells which recognize the encephalitogenic determinants in association with Major Histocompatibility Complex (MHC) class II molecules. It is these autoreactive immune cells that migrate into the CNS and mediate the pathologic processes. The role of CD4+ cells in this process has been demonstrated by the fact that transfer of MBP and PLP-specific T cell lines and clones to naive recipients will induce EAE.

Immunological processes in EAE are similar to those shown in human MS patients. Several studies have implicated MBP-specific T cells as pathogenic in MS patients. Patients with MS as well as normal volunteers have been shown to respond to myelin autoantigens, suggesting MS may be related to a defect in immune regulation. These similarities indicate that the EAE animal model is a valuable one for human MS and for testing various immunomodulators as potential therapeutic agents.

Copolymer-1 is thought to interfere with the immunological processes presumed to induce MS in patients. Therefore, to determine the potential utility of this drug for the treatment of MS, the Sponsor first studied the efficacy of the drug in the EAE animal model.

Study of Copolymer-1 in the EAE model

The Sponsor states that there are basically three different possible effects of Copolyer-1 cn both acute and CR-EAE, a) suppression, b) prevention and c) blocking. According to the Sponsor, suppression occurs when the drug is given to the animals after the challenge with CNS material, and involves a combination of the drug blocking autoantigen:MHC-II interactions and the drug treatment resulting in activation of T suppressor cells that specifically inhibit the function of autoantigenic T helper cell population. Prevention occurs when the drug is given to the animals prior to the myelin challenge, and probably involves generation of antigen-specific T suppressor cells. This is purported to be the most specific mechanism by which Copolymer-1 acts. Finally, blocking of EAE occurs when the drug is co-injected with the encephalitogenic CNS tissue and is most probably mediated by competition between Copolymer-1 and the autoantigen for binding to MHC-II molecules on the APC. This has been shown to be a relatively non-specific mechanism.

According to the Sponsor, adminstration of Copolymer-1 to animals in which EAE was induced by a number of antigens (e.g. MBP, MSCH or PLP) and in a number of different species (mice, rats, guinea pigs, rabbits and monkeys) resulted in preventing, blocking or suppressing of the disease, depending on the schedule of administration relative to the progression of EAE. These encouraging results were apparently found with both acute and CR-EAE. Apparently a number of other synthetic polypeptides also shared similar activity. However, Copolmer-1 was apparently not encephalitogenic and was the most active of the lot at mediating a protective effect on the EAE animals.

Reviewer's comments:

The fact that the drug demonstrated efficacy in the EAE animal model provides a valid scientific rationale for further study of the drug for use in the treatment of MS. However, one desirable characteristic of an immunosuppressive drug for the treatment of disease is a specificity for suppression of only the specific immune mechanism responsible for the disease. The sponsor acknowledges in this section that by at least one mechanism of action, that of blocking the binding of antigen to MHC-II molecules on APC, the drug action is not specific for myelin basic protein. Therefore, one might predict that the drug would act, at least in part, as a general immunosuppressant, which over time could impair the ability of the patients to resist infectious disease.

Theoretical Mechanism of Copolymer-1 in MS therapy

One theoretical approach to the treatment of MS is the autoantigen-based approach, which is aimed at the trimolecular complex formed by the antigen, MHC and the T cell receptor (TCR). The theory is that some, as yet undefined, autoantigen interacts with the MHC molecule on the surface of the Antigen Presenting Cell (APC) and, concomitantly, with the TCR of the autoreactive T cell, thus stimulating the autoreactive T cell to act against "self" tissue, such as the CNS. The therapeutic approach based on autoantigens is designed 1) to interfere in the activation of the T cell by preventing binding of the autoantigen to the MHC-II molecule on the APC, 2) to allow binding of the autoantigen to the TCR, but without providing the necessary signal for T cell activation (anergy) or 3) to allow binding to both MHC-II and TCR, but inducing T-suppressor cells instead of T helper cells. Number 1 and 2 would inhibit the activation of autoreactive T cells that damage the CNS, while number 3 would activate a population of T cells that would act to specifically inhibit the autoreactive immune response to the CNS.

II. *Studies to determine the actual mechanism of action of Copolymer-1 in EAE*

Studies relating to the mechanism of action of Copolymer-1 in the blocking, suppression and prevention of EAE related to five areas, 1) cross reactivity between MBP and Copolymer-1, 2) activation of T suppressor cells specific for inhibition of autoantigen-specific T helper cells, 3) effects of Copolymer-1 on cellular responses to various antigens, 4) competition between Copolymer-1 and other antigens for binding to the MHC-II molecules and 5) studies carried out on human lymphocytes *in vitro*.

1) Cross-reactivity: One possibility is that Copolymer-1 could affect MBP-induced EAE through some cross-reaction with MBP. In fact, Copolymer-1 and MBP cross-reacted in delayed-type hypersensitivity reactions in guinea pigs, in which animals were sensitized with one antigen and then challenged with the heterologous antigen. Cellular cross-reactivity was demonstrated by direct cross-stimulation of lymphocytes *in vitro* in several species (mice, guinea pigs and rabbits). Finally, cross-reactivity between Copolymer-1 and MBP was demonstrated using both polyclonal and monoclonal antibodies to these antigens. A large proportion of the anti-MBP mouse monoclonal antibodies cross reacted with Copolymer-1, and a few of the anti-Copolymer-1 antibodies also cross-reacted with MBP.

Reviewer's comments:
Although the Sponsor contends that there are these immunological cross-reactions between Copolymer-1 and MBP, which could explain how Copolymer-1 might inhibit the induction of EAE by MBP administration, they also insist that Copolymer-1 is not, itself, encephalitogenic.

2) Induction of T suppressor cells: Adoptive transfer studies demonstrated that the ability to suppress EAE induced in mice by pretreatment with Copolymer-1 could be transferred to naive untreated animals by transfusion of spleen cells from treated animals. The cells that mediated this suppressive state were identified as suppressor T cells sensitive to low doses of cyclophosphamide and to anti-thy 1 antibodies plus complement. Further characterization was achieved using hybridoma technology. Hybridomas were established from the spleen cells of Copolymer-1-treated mice by fusion with a lymphosarcoma T cell line (BW), and some of these T-cell hybridomas were able to transfer and confer unresponsiveness to encephalitogenic stimulus in naive animals. These T-cell hybridomas were also capable of inhibiting antigen-specific proliferation of MBP-specific T cell lines *in vitro* and inhibiting the MBP-specific induction of T cell line proliferation and IL-2 secretion. These data all point to a T suppressor cell line with a specificity for inhibition of MBP-induced immune response in T cells.

3) Effects of Copolymer-1 on cellular responses to various antigens:

The immunological assays used to examine the ability of Copolymer-1 to inhibit T cell response (cellular response) included the delayed-type hypersensitivity reaction (DTH), both *in vivo* and *in vitro*, and the MBP-induced secretion of interleukin-2 and gamma interferon. Copolymer-1 treatment caused inhibition of the MBP-induced delayed-type hypersensitivity (DTH) reaction in mice and rats using different doses, schedules and routes (oral, s.c., i.v.), when drug was administered at the same time as the antigen. The drug failed to inhibit development of DTH response to MBP under similar conditions in the guinea pig. Copolymer-1 also inhibited the *in vitro* sensitization of guinea pig and rabbit isolated lymphocytes to MBP by blocking recognition of the antigen during the primary macrophage-lymphocyte interaction. Finally, Copolymer-1 inhibited the MBP-induced secretion of cytokines such as interleukin-2 and gamma interferon in a dose-dependent manner. It also inhibited the proliferation of MBP-specific and PLP-specific T cell lines of different MHC restrictions and epitope specificities in response to their homologous antigen.

4) Competition between Copolymer-1 and other antigens for binding to MHC Class II molecules:

The other mechanism of action proposed by the Sponsor for Copolymer-1 involved the ability of the drug to interfere with the interaction between the autoantigen and the MHC-II molecules on the APC. A number of studies were carried out to test this theory. First of all, the inhibitory effect of Copolymer-1 on MBP and PLP specific T cell lines was dependent on the number of APC, suggesting competition for the MHC complex. In an ovalbumin (OVA)-specific T cell hybridoma, addition of Copolymer-1 could not inhibit antigen-dependent stimulation when the drug was added after the APC were fixed following prolonged exposure to OVA, indicating that no effect of drug was found when the drug could no longer compete for MHC class II molecules.

Studies using biotinylated proteins and peptides confirmed the specific binding of Copolymer-1, MBP and MBP-derived peptides to MHC class II molecules of a number of different APC populations *in vitro*. Neither Copolymer-1 nor MBP bound to APC that did not express MHC class II. Furthermore, treatment with anti-A (MHC class II molecules) but not anti-H2K or anti-H2D (MHC class I molecules) abolished the binding, confirming that Copolymer-1 and MBP both bind to MHC class 2 molecules. Finally, Copolymer-1 showed competition for MBP binding on APC. However, Copolymer-1 was also able to compete with other myelin-associated proteins (PLP, MOG) for binding to MHC class II on APC, again emphasizing the lack of specificity for this particular mechanism of action.

Reviewer's comments:

These data outlined in #1-4 above are consistent with an immunological mechanism of action for Copolymer-1 in which the drug 1) induces a population of T suppressor cells that inhibit the function of the MBP-specific T helper cells and 2) interferes with the interaction between MBP and MHC class II molecules on APC. While the T suppressor cells induced by the drug are probably specific in their inhibition of only the T helper cell population that specifically recognizes MBP, the immunosuppression due to the blocking of binding of the MBP to MHC class II on APC appears to be fairly non-specific. These data, again, raise the question of whether or not Copolymer-1, by at least one of its proposed mechanisms of action, is a general immunosuppressant, and whether or not this drug might decrease the patients ability to resist infections.

5) Studies carried out in human lymphocytes *in vitro*.

Apparently, a number of studies demonstrated that human peripheral blood mononuclear cells (PBMNC) from both healthy donors and MS patients with different HLA haplotypes proliferated and released interleukin-2 (IL-2) and interferon-gamma (IFN-γ)-like activity in response to Copolymer-1, in the absence of prior sensitization. As the Sponsor concludes, **this suggests a cross-reaction between Copolymer-1 and some common undefined natural antigen**.

Reviewer's comment:

The Sponsor suggests that release of IL-2 and IFN-γ upon treatment of human PBMC with Copolymer-1 supports the theory that a cross-reaction exists between Copolymer-1 and some common undefined naturally occurring antigen. They further indicate that these data support the theory that the mechanism of action for Copolymer-1 in the treatment of MS or EAE (animal model) somehow involves this cross-reaction. However, the results of these studies in human PBMC raise a number of concerns.

First, these data in human PBMC suggest that initial administration of the drug might actually induce release of IL-2 and IFN-γ *in vivo*, even in patients that have never received the drug (no prior sensitization). It is interesting that administration of Copolymer-1 early in clinical trials often results in what the Sponsor terms a "systemic response", characterized by vasodilatation, chest tightness with palpitations, anxiety and/or dyspnea. It is known that the administration of IL-2 to patients also results in a "systemic response", in this case described as including fever, chills, fatigue, nausea and vomiting. These effects of IL-2 are thought to be due to its induction of production of a whole cascade of other cytokines, including interleukin-1 and TNF-α, which are also known to mediate hypotension and decreased cardiac output. Therefore, one must wonder if the "systemic effects" associated with Copolymer-1 administration might be, at least in part, due to it's ability to induce release of these cytokines.

Second, I am concerned that administration of Copolymer-1 will sensitize the immune system, resulting in induction of release of increasing amounts of these cytokines with repeated Copolymer-1 administration. Another adverse effect of repeated IL-2 administration is potentially life-threatening capillary leak syndrome. This is the result of endothelial cell destruction and perturbation of the vasculature, possibly due to either a direct action of IL-2 activated host cells and/or the result of an IL-2-mediated cascade of other cytokines (e.g. IL-1, TNF). Among other symptoms, vascular leak syndrome can lead to a dramatic decrease in blood pressure, shock, and eventually death.

Consistent with animal data was the fact that Copolymer-1 competed for binding sites on MHC-class II molecules on human-derived APC with MBP. However, also consistent with animal data was the fact that Copolymer-1 also competed with MOG and PLP-derived peptides for these binding sites, indicating that this action of the drug is non-specific in nature.

Reviewer's comments:

These data in human PBMC are consistent with animal data in suggesting that, at least with the mechanism of action involving inhibition of binding of autoantigen with MHC-class II molecules on APC, Copolymer-1 could be expected to act as a non-specific immunosuppressant. This again raises the concern that repeated administration of the drug might decrease the patient's resistance to infection. Also, induction of interleukin-2 production and release is of concern because the resulting cytokine cascade could potentially result in vascular leak syndrome.

Reviewer comments:

No data were submitted to evaluate binding of Copolymer-1 to the standard battery of receptor types, including adrenergic, cholinergic, etc.

Potential interaction between Copolymer-1 and interferon-beta (IFN-ß)

Apparently IFN-ß, a know modulator of the immune response, was shown to inhibit various T cell lines of human origin with respect to inhibition of proliferation and cytokine release. Its effects on MBP-specific T cell lines were found to be additive to those of Copolymer-1. This is consistent with the fact that Copolymer-1 has been shown to inhibit binding of antigen to MHC-class II molecules, while IFN-ß has been shown to decrease expression of MHC-class II molecules on the surface of APC. It is, therefore, likely that the two compounds would act synergistically to inhibit immune function mediated through MHC-class II molecules. The Sponsor suggests that the two drugs might be used together to treat MS at some point.

III. Safety Pharmacology

Cardiovascular pharmacology

Pharmacology data indicate that Copolymer-1 could potentially affect the cardiovascular system by a couple of different mechanisms. First of all, studies involving Copolymer-1 treatment of human PBMC revealed that the drug could induce release of IL-2 from immune cells that were not previously exposed to the drug. IL-2 has been shown, in turn, to induce release of IL-1 and TNF-α, cytokines that are known to induce hypotension, decreased cardiac output, and in the extreme, capillary leak syndrome. The Sponsor also demonstrated that Copolymer-1 administration could directly induce release of histamine, by a purported "non-immune" mechanism. Histamine is also known to mediate hypotension, probably at least in part through increasing vascular permeability. Therefore, the Sponsor was concerned about the potential effects of drug administration on the cardiovascular system, and they carried out a number of preclinical safety pharmacology studies to examine this issue. The following is a Table summarizing the *in vivo* safety pharmacology studies carried out by the Sponsor to determine the effects of Copolymer-1 on the cardiovascular system.

Table 22. Summary of In Vivo Studies on the Cardiovascular Effect of Copolymer-1

Species/Strain	No. Animals/dose	Dose mg/kg	Route of Admin.	Results	Ref
Rats, Wistar NON-GLP	9 males	10, 20 (Batch #TEVA/29021)	i.v.	1. No effect on H.R. or respiratory rate. 2. Hypotensive effect max at 20 mg/kg (↓34mmHg) 3. Hypotension blocked by combo H1 and H2 blockers 4. No tachyphylaxis 5. NOEL 10 mg/kg	TUP-1 Vol. 16, pg. 310
Cats, T/? NON-GLP	11 females	1, 2, 5, 10, 20, 40 (Batch #TEVA/29021)	i.v.	1. No effect on HR 2. Hypotension, max at 5 mg/kg. 3. Hypotension blocked by combo H1 and H2 blockers. 4. Tachyphylaxis. 5. Early pressor response at ≥20 mg/kg) 6. Histamine blockers increased pressor response. 7. NOEL 1 mg/kg	TUP-1 Vol. 16, pg. 310.
Rabbits NON-GLP	4 females	approx. 7 (Batch #TEVA/123-115)	i.v. bolus	1. Slight decrease (5%) in MAP between 2-3 hours after admin. 2. Increase in HR (13%) during same time period.	TUP-2, Vol. 17, pg. 008.
Beagle dogs	2 males, 2 females	20 (Batch # TEVA/12+13+17B)	s.c.	Slight decrease in blood pressure within 15 minutes of drug admin.	TEV/042/COP, Vol. 16, pg. 181.
Beagle dogs	3 males	0.4, 2, 5, 10, 20 (Batch #TEVA/RE-6781/1 and RE-6645)	i.v	1. Decreased MAP (about 20% @ 10 mg/kg for 30 min.). 2. Decreased H.R. (about 10%). 3. NOEL of 2-5 mg/kg.	TEV/048/COP, Vol. 16, pg 221
Beagle dogs	3 moles	0.4, 2.5, 5, 10, 20 (Batch #TEVA/9016/1)	i.v	1. Decreased MAP (about 67% @ 10 mg/kg, 37% at 10 mg/kg) 2. Decreased H.R. about 21%. 3. Arrythmias in all dogs at 10 and 20 mg/kg. NOEL of 5 mg/kg	TEV/051/COP, Vol. 16, pg. 267.

1. Cardiovascular effects of copolymer-1 in rats and cats, TUP-1 (016 304), February 1994 (report),
          NOT GLP, Batch #TEVA/29021.

Objective: This was a non-GLP study in which the Sponsor examined the effects of Copolymer-1 administration on the cardiovascular system in rats and cats.

Study Description: Chronic indwelling catheters were implanted into the caudal artery of anesthetized Wistar rats and femoral artery and vein of anesthetized cats to measure blood pressure. Copolymer-1 was administered i.v. and blood pressure and respiration rates were recorded. The experiments also included use of histamine H1 and H2 receptor antagonists to determine if the cardiovascular effects were due to Copolymer-1-induced histamine release.

Results:
Rats:
        Copolymer-1 was tested on two different batches of Wistar Rats at doses of 10 or 20 mg/kg. 20 mg/kg i.v. Copolymer-1 induced a 20.7 mmHg (first batch of rats) and 34 mmHg (second batch) drop in MAP in rats with latency periods for maximum effect of 112.9 and 79.6 seconds, respectively. There was little tachyphylaxis to this depressor effect with repeated administration of drug. Histamine blockers mepyramine (H1 receptors) and famotidine (H2 receptors) were shown to block histamine-related drops in MAP. 10 mg/kg i.v. mepyramine blocked the Copolymer-1 (20 mg/kg)-induced fall in blood pressure by about 54%. A combination of 10 mg/kg i.v. mepyramine and 35 mg/kg cimetidine (H2 blocker) blocked the response to Copolymer-1 completely. 4 mg/kg i.v. famotidine reduced the depressor response to Copolymer-1 (20 mg/kg) about 65%. A combination of i.v. famotidine (4 mg/kg) and mepyramine (5 mg/kg) blocked the response to 20 mg/kg Copolymer-1 about 94%.

Reviewer's comments:
        Histamine is known to mediate a drop in blood pressure due to action of both H1 and H2 receptors found in various vascular beds. The H1 receptors are reported to be responsible for a more rapid drop in blood pressure, while the H2 receptors reportedly mediate a more long-term drop in blood pressure with slower onset. The data reported in this study are consistent with a histamine-mediated drop in blood pressure due to Copolymer-1 administration, in that administration of either H1 or H2 receptor antagonists alone only partially inhibited the Copolymer-1-induced drop in blood pressure, while use of the two antagonists together completely inhibited the depressor response.

Cats:

Depressor responses to i.v. administration of Copolymer-1 were considerably greater in cat than rat, with an i.v. administration of 1 mg/kg drug resulting in a mean fall in blood pressure from 38 to 95 mmHg. In the cat there was pronounced tachyphylaxis, with the depressor effect almost completely disappearing with repeat administration of Copolymer-1. With respect to use of histamine antagonists, results were similar to those in rat, with partial block of the Copolymer-1-induced depressor response with either H1 or H2 antagonist alone, and complete block with the use of a combination of the two.

In the cats, an immediate **pressor response** to Copolymer-1 administration was also found. Repeated i.v. dosing with 10 or 20 mg/kg of the drug resulted in transient increases in MAP of 25-35 mmHg. When animals were pre-treated with H1 or H1 and H2 antagonists, the increase in MAP was found to be up to 55 mgHg, and therefore, blocking histamine receptors actually increased the pressor response. In further studies to attempt to determine the mechanism for this pressor response, the Sponsor tried the use of an α-blocker (phentolamine) 1 mg/kg, which successfully blocked the pressor response to phenylephrine but not Copolymer-1. They were unable to determine the mechanism for the pressor response to Copolymer-1 administration in the cat.

Reviewer's comments:

The depressor response to Copolymer-1 in this NON-GLP study appeared to be mediated by the release of histamine, while the Sponsor was unable to determine the mechanism for the pressor response. However, data in human PBMC from both normal volunteers and MS patients demonstrated that treatment with Copolymer-1 resulted in production and release of IL-2. Release of this lymphokine can, in turn, cause release of a whole cascade of other cytokines, including the inflammatory cytokines IL-1 and TNF-α. Additionally, since Copolymer-1 is thought to interact with MHC-II molecules on APC (monocytes), it would not be surprising to discover that the drug directly induces release of IL-1 and TNF-α from monocytes. It is known that administration of TNF-α can result in an initial pressor response, followed by a depressor response thought to be due to induction of vascular leak syndrome. Administration of this inflammatory cytokine has also been shown to result in a decrease in peripheral blood neutrophils, as was reported in both subchronic rat (3-month) and monkey (28-day) studies. Therefore, it is possible that, in addition to histamine-mediated effects of Copolymer-1 on the cardiovascular system, administration of Copolymer-1 might also result in cardiovascular effects due to induction of release of TNF-α. This may be of concern, because long-term effects of continued TNF-α are known to be at least partially responsible for such serious conditions as the vascular leak syndrome and severe hypotension associated with septic shock.

It might be a good idea to recommend to the Sponsor that they monitor for plasma TNF-α in patients receiving the drug on a daily basis for life, as increased production of this cytokine can result in severe hypotension and dea h.

2. Effect of a single intravenous injection of COP-1 (20 mg) in the conscious rabbi NON-GLP, July 1994, Batch #TEVA/123-115.

Objective: To assess the acute efect of an intravenous injection of Copolymer-1 on mean arterial pressure (MAP) and heart rate in the rabbit.

Study description: Copolymer-1 was injected i.v. by bolus into the marginal ear vein of 4 conscious female rabbits at a dose of 20 mg/rabbit (about 7 mg/kg). Blood pressure was monitored with a pressure transducer, implanted (under local anesthesia) into the central ear artery via a catheter. Heart rate was monitored by the same system. Rabbits were monitored for 3 hours after drug injection.

Results: No effects were seen up to 2 hours after drug injection. Between 2 and 3 hours after drug administration, MAP decreased about 5% (8 mmHg) and HR tended to increase slightly (300 beats/min in Control versus 339 in Treated animals). The Sponsor concluded that these effects were not drug-related, but were due to restraint of the conscious rabbits for an extended period of time.

Reviewer's comments:
I disagree that these effects were due to extended restraint of the rabbits. The Control animals were also restrained, and yet there were differences between Control and Treated animals. The decrease in MAP was minimal, but only a single dose of drug was used.

3. Cop-1 acute physiology study in beagle dogs by subcutaneous injection, June 1988, NON-GLP, Batch #TEV/12+13+17B (a mixture).

Objective: To assess the acute effects of Copolymer-1 on the cardiovascular system in Beagle dogs, as a result of a high dose subcutaneous injection.

Study description: Copolymer-1 dissolved in saline was injected subcutaneously into two male and two female dogs at a dose of 20 mg/kg body weight. Blood pressure and heart rate were recorded in the conscious dogs with the aid of a pressure transducer, at scheduled intervals for a period of 24 hours. Values were compared to those obtained before injection. Dogs were connected to the pressure transducer through their cannulated carotid artery.

Results: The Sponsor reports that there were no effects of the drug on blood pressure or heart rate. However, in three of the four animals (two males and one female) the blood pressure decreased about 7, 13 and 16% in the first 15 minutes, and in two of these animals remained at the lower level for at least 6 hours after drug injection.

Reviewer's comments:

This study is NON-GLP and includes only four animals, and therefore it is difficult to form any conclusions with respect to the results. However, it would appear that blood pressure did decrease slightly about 15 minutes after drug injection in three of the animals, and remained at these decreased levels for at least 6 hours. These data are consistent with cardiovascular effects in other animal species.

4. Copolymer-1: effects of i.v. injection on the cardiovascular system and respiration of Beagle dogs,
ION-GLP, November 1989, Batch #TEV/RE-6781/1 and RE-6645.

Objective: To assess the acute effects of Copolymer-1 on selected cardiovascular parameters and respiratoin in conscious Beagle dogs following intravenous injection of various doses.

Study description: Copolymer-1 dissolved in saline was administered i.v. to 3 conscious male dogs at doses of 0.4, 2, 5 and 10 mg/kg. Drug was injected successively to each dog at the various doses, after stabilization of the baseline. Direct blood pressure, heart rate, respiratory rate and ECG (lead II) were measured at time 0 (before treatment) and at 0-5, 15, 30, 45 and 60 minutes after treatment.

Results: Administration of 5 mg/kg resulted in marginal effects including a 17% decrease in H.R. at 30 minutes after injection, and virtually no effect on MAP. 10 mg/kg Copolymer-1 resulted in a 21% decrease in MAP lasting for about 30 minutes following drug administration and a 19% decrease in H.R. lasting for about the same period of time. Two of the dogs also exhibited increases in the T waves and in the R and S amplitutes at 10 mg/kg.

The Sponsor concluded that the results showed a "transient reduction in mean arterial pressure and heart rate and increases in the T, R and S amplitudes of ECGs."

The NOEL was listed by the Sponsor as 2-5 mg/kg for this study.

Reviewer's comments: These data are consistent with other studies in demonstrating that administration of Copolymer-1 results in a decrease in blood pressure. No attempt was made in this study to determine whether these effects were due to histamine release or some other mecnanism of action. These data further demonstrated ECG effects of the drug.

5. COP-1: effects of intravenous injection on the cardiovascular system and respiration in conscious Beagle dogs,
NON-GLP, January 1990, Batch #TEVA/99016/II.

Objective: To assess the acute effects of Copolymer-1 on selected cardiovascular parameters and respiration in conscious Beagle dogs as a result of intravenous injection of various doses.

Study description: Copolymer-1 dissolved in saline at 10 mg/ml was administered i.v. to 3 conscious male dogs at doses of 0.4, 2, 5, 10 and 20 mg/kg. The various doses were injected successively to each dog, after baseline stabilization. Direct heart rate, blood pressure, respiratory rate and ECG (lead II) were measured at time 0 (before treatment) and at 5, 15, 30, 45 and 60 minutes after treatment.

Results:

Following injection of Copolymer-1, reduced mean arterial blood pressure (MAP) and heart rate were observed at 10 and 20 mg/kg for about the first 15 minutes after drug administration. At 10 mg/kg, MAP dropped an average of 63% below baseline, while heart rate decreased about 21% below baseline. At 20 mg/kg MAP dropped about 37% and H.R. about 24%.

Furthermore, ECG results demonstrated that all dogs exhibited increases in the T-wave and, in some cases, in R and S amplitudes at 10 and 20 mg/kg. And finally, arrhythmia was noted in all dogs at doses of 10 and 20 mg/kg, mostly up to 15 minutes after administration.

The Sponsor estimated the NOEL for Copolymer-1 in this study to be 5 mg/kg.

Reviewer's comments:

It is of some concern that these animals experienced arrythmias after a single i.v. injection of the drug. The Sponsor did not attempt to determine the mechanism of action for this phenomenon. However, in keeping with their hypothesis that the drug is a direct inducer of histamine release, it is known that histamine can directly affect the heart. Apparently, H1 receptors can be involved in the slowing of AV conduction, H2 receptors can be involved in both heart contractility and electrical conduction. High dose histamine is also known to induce arrhythmias. Therefore, one possible explanation for these effects could be a direct induction of histamine release by the drug.

Analysis of dose at which cardiovascular effects occurred compared to human dose

The clinical dose of Copolymer-1 proposed for use in this NDA is subcutaneous injection of 20 mg/day of drug for the life of a patient with relapsing-remitting MS. For a 50 mg patient (MS is predominantly found in women) this translates into about 0.4 mg/kg/day. The NOEL for the safety pharmacology studies in dogs was reported to be 5 mg/kg. Since there are few pharmacokinetics data presented for this drug, we are left with a comparison on either a mg/kg or a mg/m^2 basis. With respect to mg/kg, the NOEL in dogs is about 12.5-fold greater than the proposed clinical dose. With respect to a surface area comparison, thought by some to be a more accurate means of comparing comparable doses between the species, a 5 mg/kg dose in dog is equivalent to about a 2.5 mg/kg dose in man. Therefore, on a surface area basis, the NOEL in the dog (5 mg/kg) is about 6.25-fold greater than

the proposed clinical dose. The major concern is that, in the dog, a two-fold increase in dose (from 5 mg/kg to 10 mg/kg) took us from the NOEL to dramatically decreased blood pressure, alterations in ECG patterns, and arrythmias. These data do not provide for much of a safety margin for the drug.

However, one must also consider the fact that the route of administration in the dog studies was different from the proposed clinical dosing. In the dog studies, i.v. bolus administration was used, while subcutaneous administration is proposed for the clinic. It is often true that i.v. administration of a drug results in greater toxicity than other routes, usually due to the fact that drug reaches the plasma more rapidly by i.v. administration, and plasma Cmax levels are usually higher by this route of administration, often resulting in greater toxicity. In fact, in the one dog study in which s.c. administration was used (2 males, 2 females), only a slight decrease in blood pressure was reported, and there were no direct effect on the heart seen.

Effects of Copolymer-1 on Smooth Muscle Preparations

The effects of Copolymer-1 on smooth muscle preparations were examined in a NON-GLP study (Study #TUP-3, January 1994). The smooth muscle preparations tested included ileal strips derived from male guinea pigs, tracheal strips derived from male guinea pigs, and stomach fundic tissue extracted from CR rats.

Results in guinea pig ileum demonstrated that Copolymer-1 showed a phasic response that developed within a few seconds, followed by a tonic contraction that built up and lasted for about 30-50 minutes. In most cases the supramaximal dose of Copolymer-1 for this response was 1.6 mg/ml, while the contractile potency in terms of an EC50 was about 0.4-0.8 mg/ml. These contractions were inhibited by H1 nd H2 histamine antagonists and by inhibitors of the prostaglandin/leukotrienes pathways. A combination of H1 plus H2 blockers accomplished a maximum inhibition of about 54%. Ultimately, the contractile response most likely depended on calcium mobilization, as complete inhibition of contraction was mediated by calcium blockers such as verapamil and nifedipine.

In rat stomach strips, Copolymer-1 treatment resulted in a contractile response which generated a pattern of mixed phasic contractions superimposed upon tonic contractions. The response was strongly suppressed by atropine (0.1 µM) and indomethacin (1 µM).

Copolymer-1 at a concentration of up to 1.6 mg/ml failed to induce a contractile response in tracheal preparations from non-sensitized animals. However, a contractile response, though somewhat inconsistent, could be evoked in preparations from animals that had been sensitized to the drug in advance. Furthermore, in the presence of indomethacin (3 µM), a consistent and uniform contractile response could be elicited from preparations from sensitized animals. The Sponsor states that this response, though not particularly relevant with respect to drug efficacy, could be important when the drug is administered in high dose by the i.v. route of administration.

Effects of Copolymer-1 on histamine release in two types of cells: rat peritoneal mast cells and human peripheral blood basophils

In a NON-GLP study to assess the effect of Copolymer-1 to stimulate release of histamine from rat peritoneal mast cells (study #HAP-1, Hadessah Medical Center, Jerusalem, Israel, August 1994), mast cells were incubated in the presence of various concentrations of Copolymer-1, with 3 μg/ml of compound 48/80 as a positive control and with vehicle as a negative control. The cells were exposed to the drug for 20 minutes at 37° C., pelleted, and histamine was measured in the cell pellet after sonication and in the supernatant through the use of a radioenzymatic assay. The percent histamine release was calculated, and results are shown in the following table:

Histamine release from rat peritoneal cells

Inducer	Conc. (μg/ml)	Net histamine release [a] %	SD
48/80	3	57.4	8.2
Copolymer-1	0.01	Lower than Control	
	0.1	6.5	1.2
	1.0	8.9	3.8
	5.0	19.7	8.8
	10.0	27.1	5.9
	100	43.9	3.9
	250	58.8	4.2
	500 [b]	75.0	4.7

[a] Net histamine release was calculated by subtraction of the value obtained in vehicle control incubations. The latter was below 5%.
[b] At 500 μg/ml, a cytotoxic effect of 10-20% was noted by staining with trypan-blue.

As seen in this table, Copolymer-1 directly induced release of histamine from rat peritoneal cells, with measurable histamine beginning at about 0.1 μg/ml drug. Histamine release was induced by Copolymer-1 in a dose-related manner, with over 50% release at 250 μg/ml drug. The Sponsor states that according to the scientific literature, rat peritoneal mast cells resemble human mast cells of the connective-tissue type and it can therefore be inferred from these data that Copolymer-1 will also directly induce release of histamine from human mast cells.

Effects of Copolymer-1 on histamine release from human peripheral blood basophils was also examined in PBMC from either healthy volunteers or Copolymer-1 treated MS patients. Apparently some measurable histamine release was seen at 100 μg/ml Copolymer-1, with about 20% release at 1000 μg/ml. Therefore, these human basophils were not as sensitive to Copolymer-1 induced histamine release as the rat mast cells. The Sponsor reported that this histamine release was found in human PBMC from both healthy volunteers and Copolymer-1 treated MS patients.

ADME

Copolymer-1 is a synthetic "immunoregulatory" peptide, designed to prevent the autoimmune response in MS in which T cells are primed to specifically destroy myelin. In fact, although not specifically stated by the Sponsor, Copolymer-1 is actually a "peptide vaccine". Therefore, the most appropriate drug bioavailability is probably that intact Copolyme'-1 drug that reaches the local lymph nodes that drain the tissues of the subcutaneous injection site (see Summary and Evaluation Section—ADME for further discussion). However, the Sponsor did not address this issue, but rather they presented data describing the systemic exposure of drug in animals.

The PK of Copolymer-1 was examined in terms of systemic exposure by administering radiolabelled drug. Data with respect to systemic exposure were problematic in that ^{125}I-labelled Copolymer-1 was used in the studies. Two problems are associated with this methodology, 1) ^{125}I-labelling is known to change the PK properties of a peptide and 2) this methodology did not allow them to clearly differentiate between plasma concentrations of intact parent drug, degradation products, or free radiolabelled iodide (see Summary and Evaluation Section—ADME for further discussion).

Absorption

Absorption studies after subcutaneous administration were carried out two ways, 1) extent of absorption was determined indirectly by measuring residual radioactivity at the injection site over time and 2) the rate of absorption was evaluated based on the plasma concentration-time curves for drug-related radioactivity. With respect to the extent of absorption, in mice, <14% of the total radioactivity remained at the injection site 1 hour after s.c. injection, and <5% of the dose remained after 8 hours at the injection site. This low residual radioactivity at the injection site within 1 hour following s.c. administration was interpreted by the Sponsor to mean that ^{125}I-Copolymer-1 was rapidly absorbed, and the 8 hour data indicated that the drug was extensively absorbed.

With respect to rate of absorption and elimination, plasma concentration-time curves in Sprague-Dawley rats administered Copolymer-1 by the s.c. route of administration were constructed based on "total plasma radioactivity". These data revealed K_{ab} (absorption rate constant) of about 0.1min^{-1} and a T_{l2} of about 10-20 minutes. Maximum plasma radioactivity after s.c. injection (both rats and monkeys) was attained in most animals in 2 hours (see Table 41 below). Therefore, based on these data, the drug was apparently both absorbed and eliminated fairly rapidly.

Table 41. Mean absorption parameters based on total plasma radioactivity after subcutaneous administration of ^{125}I-Copolymer-1.

Species/Strain	Group (M/F)[a]	Sampling Time	Copolymer-1 Dose (mg/kg)	Tmax (H)	K_a (Min^{-1})	$T_{1/2a}$ (Min)
Rat/ Sprague Dawley	8/0	0.25, 0.5, 1, 2, 4, 6, 8, 10 12 h	0.5 / 50	1.2 / 2.5	0.09 / 0.04	9.0 / 18.6
Rat/ Sprague Dawley	14/0	3, 5, 8, 12, 15, 20, 30, 45, Min, 1, 1.5, 2, 3, 4, 5, 8, 7, 8 h	0.5	1.3	0.06	9.6
Rat/Drl:CD (SD)BR	5/5	2, 5, 10, 20, 30, Min, 1, 2, 4, 6, 8, 24 h	3 / 10 / 30	2[e] / 2[e] / 2[e]	ND	ND
Monkey/Cynomolgus	1/1	2, 5, 10, 20, 30, Min, 1, 2, 4, 6, 8, 24, 72 h	20 / 40 / 60-h[h] / 60-iso[i]	2[e] / 4[e] / 1 / 2	2[e] / 2[e] / 1 / 2	ND	ND

[a] M=Male; F=Female.
[b] T_max=time to maximal plasma concentration.
[c] K_a= Absorption rate constant.
[d] T_1/2a=Absorption half-life.
[e] Median.
[f] ND=Not determined in this study.
[g] Data from individual animals.
[h] 60-h=Hyperosmotic formulation.
[i] 60-iso=Isosmotic formulation.

Pharmacokinetics

Due to the fact that it was impossible to know the proportion of the "total plasma radioactivity" that was made up of intact parent drug versus various degradation products versus free radiolabelled iodide, pharmacokinetics parameters were calculated based on: 1) the total radioactivity in plasma, and 2) the radioactivity in TCA-precipitable fractions of plasma.

Total radioactivity in plasma

Data for studies in rat and monkey, based on total radioactivity, are summarized in the following Table 44.

Table 44. Absolute values for pharmacokinetic parameters based on total plasma radioactivity following single subcutaneous doses of ^{125}I-Copolymer-1.

Report	Species	Gender	Dose (mg/kg)	Cmax [a] (µg/ml)	AUC$_{0-24}$ [a] (µg.h/ml)	AUC$_\infty$ [a] (µg.h/ml)
1028/21-1050	Monkey	Male	20	46.7	606.7	1391
(037 217)			40	96.4	1513	3581
			60	141.5	2324	5425
		Female	20	50.3	691.9	1555
			40	78.0	1106	2930
			60	138	2289	5467
1028/19-1011	Rat	Male	3	5.1	56.3	61.4
(037 114)			10	20.1	235.9	267.7
			30	54.4	642.3	699.1
		Female	3	5.5	66.5	71.9
			10	20.9	258.3	283.3
			30	60.9	719.6	766.6
BS/PK-2	Rat	Male	0.5	0.969	-	9.7S
(037 034)			50	73.96	-	773.9
BS/PK-3	Rat	Male	0.5	0.64	-	5.36 [b]
(037 062)						

[a] Cmax=maximum plasma concentration; AUC=area under the concentration-time curve.
[b] Orginal data were 322 µgxmin/ml; 322 µgxmin/ml converts to 5.36 µgxh/ml

Data shown in Table 44 above are pharmacokinetics data for total radioactivity found in plasma. These data show that, with respect to total radioactivity, plasma C_{max} and AUC levels after ^{125}I-Copolymer-1 s.c. injection in both rats and monkeys were linear and dose-dependent. No sex differences were found.

The Sponsor also carried out studies by the oral, i.v. and i.m. routes of administration. They state that the PK profiles for i.m. and oral administration were similar to that for s.c., although absorption was somewhat lower. They report the **absolute bioavailability for Copolymer-1 in the rat by the s.c. route of administration to be 46%** (relative to i.v. administration). However, this bioavailability figure is based on total radioactivity, and is therefore somewhat misleading.

Reviewer's comment:

The Sponsor gives a bioavailability figure for ^{125}I-Copolymer-1 of 46%. However, this figure pertains to total radioactivity determined in plasma. This figure is not really relevant to the mechanism of action because 1) as shown in the following "metabolism" section of this review, most of the total radioactivity found in plasma is actually metabolized/degraded drug 2) the drug is proposed by the Sponsor to act at the injection site, and therefore the systemic exposure is not required for the drug to work. As stated previously, my assessment is that Copolymer-1 is acting as a peptide vaccine, and the appropriate bioavailability is constituted by the intact drug that reaches the immune system through the lymph nodes that drain the area of the local s.c. injection site.

TCA-precipitable fraction

A problem with using radiolabelled drug is that the radiolabelled iodide can become dissociated from the drug and incorporated into other plasma proteins or remain free in the plasma. Furthermore, the intact peptide drug can become extensively degraded and still maintain the radiolabel. Therefore, determination of total plasma radioactivity will most likely yield an overestimation of systemic exposure to intact drug. The Sponsor reports that Copolymer-1 is approximately 80% TCA precipitable, and it is the high molecular weight drug that is detected by this methodology. Therefore, in an attempt to obtain a more meaningful value for systemic exposure, the Sponsor also determined plasma drug concentration using TCA precipitation. This methodology is also limited, in that the radiolabelled Copolymer-1 is incompletely precipitated by TCA and TCA will also precipitate some portion of the larger MW degraded drug as well as the parent. Furthermore, some free iodide will be incorporated into plasma proteins that are also TCA-precipitable.

The Sponsor reported that the TCA-precipitable radiolabelled material in plasma accounted for 20-40% of the total radioactivity at C_{max}. When the Sponsor used the TCA-precipitable radiolabelled material in plasma for PK calculations and extrapolated values for 0.3 mg/kg dose (the human dose; the Sponsor arrived at this figure by dividing the 20 mg/day dose by 70 kg person), they determined the predicted plasma C_{max} to be between 52 and 240 ng/ml. This extrapolation assumed linearity of PK and similarities across species (rat, monkey, human).

The Sponsor also pointed out that the $T_{1/2}$ for the TCA-precipitable material was about 50 hours, much longer than for total radioactivity (about 10 hours in rat). The Sponsor's interpretation of these results is that "...the elimination of total radioactivity from plasma is probably of no relevance to Copolymer-1, since it detects primarily the disposition of free iodide or of label which was further incorporated into other, unrelated molecules..." They point out that "...the elimination from plasma of TCA-precipitable radioactivity, with $T_{1/2}$ greater than 50 hours, supports a secondary association or incorporation of the radiolabel into other macromolecules..." Therefore, the Sponsor seems to suggest that, while TCA-precipitable material probably provides the best estimation of PK of the drug, this methodology is also flawed.

Extrapolation to therapeutic dose using "total radioactivity"

The Sponsor also used the "total radioactivity" values to predict the plasma C_{max} and AUC for the proposed human dose, 20 mg/day, which they calculate to be 0.3 mg/kg/day using a 70 kg patient. This extrapolation was accomplished by dividing the pharmacokinetic parameters (plasma C_{max} and AUC) for the PK data reflecting total radioactivity by the ratio of the experimental dose to the human therapeutic dose. Using this methodology, the Sponsor reported a predicted plasma C_{max} of 380-710 ng/ml for the proposed human dose of 20 mg/day (0.3 mg/kg/day for 70 kg person) (Table 45, 001 206, not included here).

However, then the Sponsor makes the following statement, "While a 50 mg/kg dose of ^{125}I-Copolymer-1 resulted in detection of radioactivity by means of HPLC paired with gamma counts (see figure 3, page 152) the same dose of unlabelled Copolymer-1 produced no detectable levels via HPLC fluorescence detection (Study GAD/PK-6). **Based on these animal studies, serum concentrations of Copolymer-1 are presumed to be low or not detectable following subcutaneous administration of 20 mg once daily to man.**" This is a somewhat confusing statement, in light of the fact that they spent a great deal of time and effort in predicting the plasma C_{max} and AUC for a human dose of 0.3 mg/kg and arrived at the values mentioned above. However, I interpreted this statement to mean that the drug is not detectable in plasma, even when 50 mg/kg is administered s.c., unless the drug is radiolabelled. Normal HPLC fluorescence detection techniques are apparently not sufficient to detect drug, even when 50 mg/kg (which should result in about 150 µg/ml in plasma by radiolabelled studies) of drug is administered s.c.

Distribution

There were basically three NON-GLP studies submitted with the NDA that dealt with the issue of tissue distribution of Copolymer-1. Those studies are summarized in the following:

1. Copolymer-1, a single dose pharmacokinetic study, tissue distribution of ^{125}I-Copolymer-1 in healthy and experimental autoimmune encephalomyelitis (EAE) mice
 NON-GLP, 1991.

Objective: Compare the tissue disposition pattern of Copolymer-1 related radioactivity in a murine model of MS with that of healthy mice after a single s.c. dose of ^{125}I-Copolymer-1.

Study Description: Healthy (21) and EAE-induced (16) female mice were given a single s.c. dose of 0.5 mg/kg ^{125}I-Copolymer-1, and one group was sacrificed 1 hour after dosing and a second group 8 hours after dosing. After decapitation, blood

(plasma), stomach, intestine, kidney, liver, spleen, brain, diaphragm, lung, heart, thymus, adrenal, urinary bladder and skin were all collected and examined for radiolabelled drug. Radioactivity was determined in plasma (total radioactivity), in TCA-precipitated plasma (TCA-precipitable drug), and in tissue homogenates using a gamma counter. Results were expressed as percentage of dose and as µg equivalents of intact Copolymer-1.

Results:

One hour after administration of rabiolabelled drug, the mean total concentration of Copolymer-1 related radioactivity in plasma was 43.15 µg/ml in healthy mice and 46.99 µg/ml in EAE mice. These concentrations declined to 28.2 and 17.4 µg/ml, respectively, after 8 hours. The TCA precipitate radioactivity, representing free drug and macromolecule-bound label, was 12.7 and 17.3% at 8 hours, for healthy and EAE mice, respectively.

Among the tissues examined, the stomach showed highest levels of radioactivity at both time points. The mean concentrations at 1 hour (272.7 and 282.2 µg/g in healthy and EAE mice, respectively) were about 6-fold higher than those for plasma. All other tissues showed levels lower than plasma. The brain exhibited the lowest uptake of drug-related radioactivity, and the difference in brain concentration between healthy and EAE mice (2.07 µg/g versus 45.5 µg/g) was statistically significant at the 1-hour time point.

The Sponsor stated that the fairly high levels of radioactivity in the stomach were probably due to a sequestration of free radiolabelled iodide released from the radiolabelled drug upon administration. The Sponsor concluded that there were no major differences between the tissue disposition pattern for drug-related radioactivity between the healthy and EAE-induced mice.

2. Copolymer-1, a single dose pharmacokinetic study, disposition of ^{125}I-Copolymer-1 in the rat,

NON-GLP, December 1994.

Objective: To assess the absorption, distribution, metabolism and excretion pattern of Copolymer-1 related radioactivity in rats after a single subcutaneous dose of ^{125}I-Copolymer-1.

Study description:

A single s.c. dose of 50 mg/kg ^{125}I-Copolymer-1 was administered to each of 24 rats. The rats were divided into three groups of eight rats each and sacrificed at 4, 8 or 12 hours after dosing. Blood, stomach, intestine, kidney, liver, spleen, brain, diaphragm, lung, heart, thymus, testicles, adrenals, and urinary bladder were collected for radioactivity determination in plasma, TCA-precipitated plasma and tissue homogenates.

Results:

The only organs demonstrating higher concentrations of the drug were the stomach and the intestines, consistent with the mouse study. 11.4% and 6.1% of the dose was found in the stomach and intestine, respectively, after 4 hours. This declined to about 1% of the dose after 12 hours in both organs. These were the only organs to demonstrate greater concentrations of the drug-related radioactivity (stomach, 308.8 µg equiv 'g; intestines, 62.3 µg equiv/g; both at 4 hours) than plasma (59.5 µg equiv/g at 4 hours). The brain contained the lowest concentration of drug-related radioactivity. The Sponsor stated that this is probably due to drug difficulty crossing the blood-brain barrier.

3. Copolymer-1, a single dose PK study in the rat: an 8-hour monitoring of plasma radioactivity and tissue distribution after i.v., s.c., i.m. and oral administration,
NON-GLP, December 1994.

Objective:

To assess the bioavailability and tissue distribution pattern of Copolymer-1 and its metabolites in rats after oral, s.c., i.m. or i.v. administration of a single dose of ^{125}I-Copolymer-1.

Study description: 41 male rats were cannulated in their left femoral vein and artery, divided into four groups and administered a single dose of 0.5 mg/kg ^{125}I-Copolymer-1 by one of the following routes: i.v., i.m., s.c. or oral. Blood was withdrawn from the arterial cannula at 3, 5, 8, 12, 15, 20, 30 and 45 minutes and 1, 1.5, 2, 3, 4, 5, 6, 7, and 8 hours after dosing. Plasma was prepared and TCA-precipitated, and the TCA-soluble fraction was further precipitated using silver nitrate. Animals were sacrificed at the end of the 8 hours and organs collected for tissue determination of drug-related radioactivity.

Results:

In this study, the Sponsor also examined the thyroid. A large proportion of the total drug-related radioactivity was found in the thyroid (>400 ng equiv/ml versus 220 ng equiv/ml in plasma after s.c. administration). The Sponsor makes the point that this is consistent with iodide concentration in the thyroid, and is probably due to a large amount of free radiolabelled-iodide that is released from the drug as the result of extensive metabolism associated with s.c. administration. Consistent with the other rat and mouse studies, the only other organ to demonstrate concentration of the drug was the stomach. In this study, by the s.c. route the stomach contained 4-fold higher concentrations of drug-related radioactivity than plasma. Brain contained the lowest concentration of drug-related radioactivity.

Metabolism

Studies were carried out both *in vivo* (rats and monkeys) and *in vitro* (rat and human tissues) to examine the metabolism of Copolymer-1. Various methodologies were used. In one study, the metabolism of Copolymer-1 was investigated using HPLC and combined HPLC/radiotracer techniques to monitor the disappearance of intact drug. In other studies, methods including radiotracer techniques and protein precipitation with TCA and precipitation of the TCA soluble fraction with silver nitrate ($AgNO_3$) were employed. The radioactivity in the TCA precipitate reflects high molecular weight material (including parent drug), the $AgNO_3$-soluble fraction contains the low molecular weight fraction, and the free iodide is contained in the $AgNO_3$ precipitate.

In Vivo Studies

Results from the studies in rats demonstrated that Copolymer-1 undergoes rapid degradation *in vivo*. The chromatographic profile of total radioactivity in plasma shown in Figure 3 below (001 218) demonstrates that at 3 minutes after s.c. administration of ^{125}I-Copolymer-1, a large proportion of intact drug, eluting with a retention time from about 30-45 minutes, was still present in plasma. However, at the 5 and 8 minute time points, additional plasma sampling demonstrated that the majority of the intact Copolymer-1 drug was already degraded to distinctly smaller fragments and free iodide. It is unclear whether these smaller species are Copolymer-1 metabolites or other unrelated species iodinated as the result of iodide exchange.

FIGURE 3. CHROMATOGRAPHIC PROFILE OF TOTAL RADIOACTIVITY IN PLASMA AFTER
SUBCUTANEOUS ADMINISTRATION OF A 50 mg/kg SINGLE DOSE OF
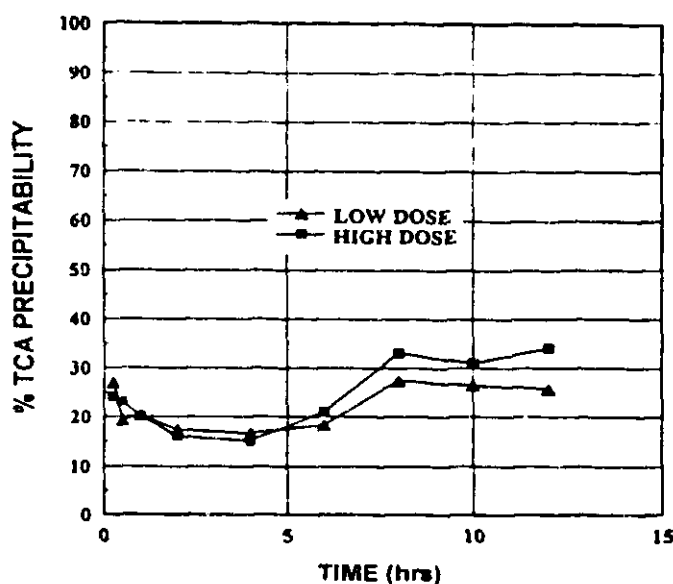^{125}I-COPOLYMER-1 TO RATS
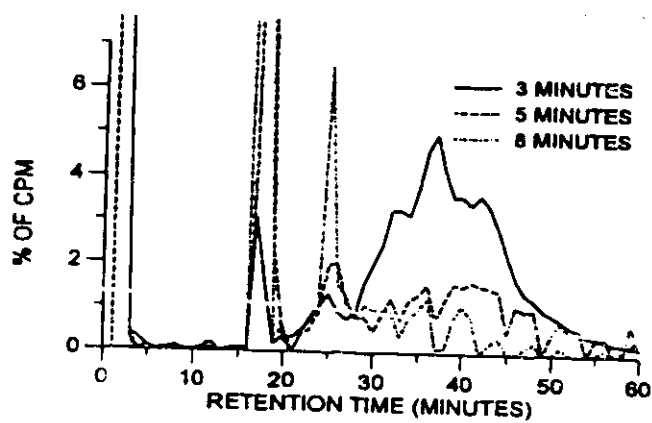
In another rat study including s.c. administration of the radiolabelled drug, it was found that 4 hours after s.c. injection of ^{125}I-Copolymer-1 that only 20% of the total plasma radioactivity was TCA-precipitable (see Figure 5 below, 037 054). These data are consistent with degradation of the intact drug to smaller, non-precipitable species. However, the percentage of total radioactivity that was TCA-precipitable steadily increased over the next 41 hours to a maximum of about 80% of plasma radioactivity being TCA-precipitable (see Figure 6 below, 037 055). The Sponsor explains this phenomenon as incorporation of degraded radiolabelled peptide and amino acids into newly formed plasma proteins and binding of these radiolabelled degradation products as well as free radiolabelled iodide to plasma proteins that are also TCA-precipitable.

PK-2/1   21

FIGURE 5   TCA PRECIPITABILITY OF RADIOACTIVITY IN THE PLASMA AFTER S.C. INJECTION OF ^{125}I-COPOLYMER-1 TO RATS

FIGURE 6 TCA PRECIPITABILITY OF RADIOACTIVITY IN THE PLASMA AFTER S.C. INJECTION OF
¹²⁵I-COPOLYMER-1 TO RATS
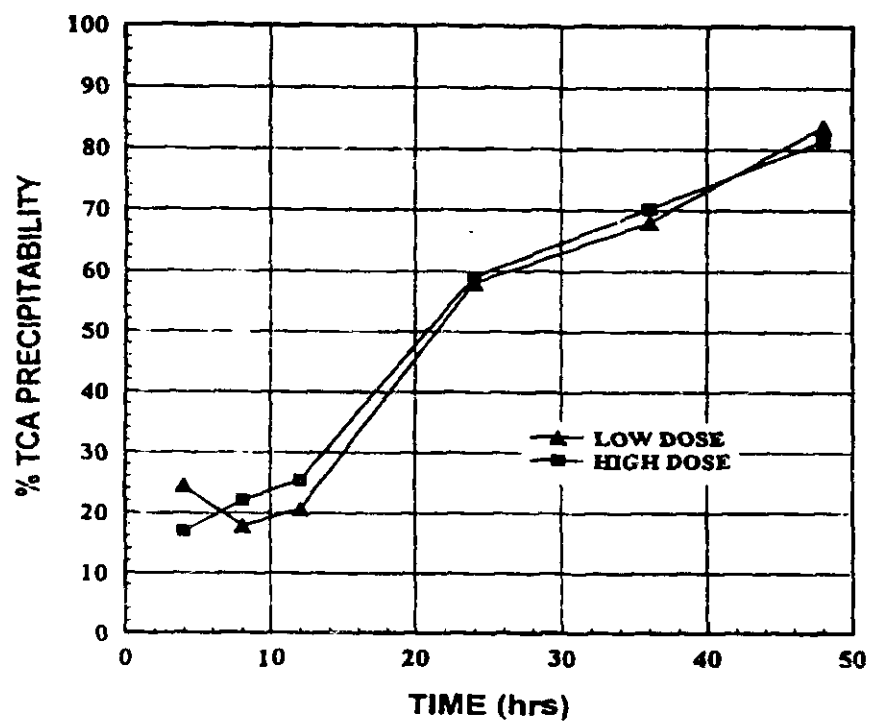
037 055

In Vitro Studies

In one *in vitro* study, ^{125}I-Copolymer-1 was incubated with Sprague-Dawley rat plasma or tissue homogenates, and TCA-precipitable radioactivity was subsequently determined to examine the extent to which these various tissues were able to degrade the drug. Data, shown in the following table, demonstrated that rat plasma actually had a somewhat stabilizing effect on the drug (70% radioactivity recovered in TCA-precipitate from Control incubation versus about 77% from rat plasma incubation). However, subcutaneous tissue (19.4% TCA-precipitable radioactivity recovered), striated muscle (35.4%) and other tissues were shown to result in rapid degradation of the drug.

Table: In vitro stability of Copolymer-1 in rat plasma and tissue homogenates

Tissue	TCA Precipitate (% radioactivity recovered)
Control	70
Plasma	76.97
Liver	63.28
Stomach	53.41
Subcutaneous tissue	19.39
Small intestine	26.2
Striated muscle	35.37
Kidney	38.9

These data indicate that the drug was metabolized or degraded by enzymes associated with various tissue homogenates, with the greatest effect occurring in subcutaneous tissue. The involvement of a protease in the hydrolytic degradation of Copolymer-1 was examined by coincubation of small intestine and subcutaneous tissue homogenates in presence or absence of a protease inhibitor. PMSF, a peptidase inhibitor, was reported to reduce the degradation seen with subcutaneous tissue homogenates but did not effect the degradation of drug associated with small intestine homogenate, suggesting that different enzymes were involved in the two tissues.

Similar effects were seen with human plasma and tissue homogenates, as seen in the table below:

In vitro stability of Copolymer-1 in human plasma and tissue homogenates

Tissue	Radioactivity Recovered (% recovered[c])		
	TCA precipitate	Soluble [b]	Free Iodide [a]
Control	62.78	17.38	19.84
Plasma	89.18	2.52	8.30
Subcutaneous tissue	16.06	36.41	47.51
Striated muscle	18.66	48.3	33.04
[a] Results are presented only for a 10 µg/ml concentration of Copolymer-1.			
[b] Silver nitrate soluble fraction.			
[c] Silver nitrate precipitable fraction.			

Both rat and human plasma appeared to have somewhat of a stabilizing effect on Copolymer-1, as evidenced by the fact that a greater percentage of total radioactivity was associated with TCA-precipitated plasma (intact drug) in plasmas from both species than from Controls in the respective studies. Also, subcutaneous tissue from both rat and human appeared to be the tissue that demonstrated the largest effect to degrade Copolymer-1 *in vitro*.

The Sponsor states that these data are consistent with the *in vivo* finding that higher TCA precipitability and slower disappearance of characteristic HPLC profile occurs following i.v. injection compared to s.c. injection.

Excretion
Excretion of radioactivity after s.c. administration of radiolabelled Copolymer-1 to rats occurred primarily in the urine, with almost no radioactivity detected in the feces. The Sponsor proposes that the radioactivity found in the urine constitutes mainly the excretion of free iodide, as intact Copolymer-1, as with most high molecular weight peptides, is too large to be filtered through the kidney glomeruli.

The residual radioactivity in the rat carcass after 24 hours was about 16-20%, which the Sponsor concludes is probably from the incorporation of the degradation products into newly-synthesized peptides or from accumulation of the released iodide in the thyroid and stomach. The Sponsor concludes that, once radiolabelled Copolymer-1 is injected s.c., it is rapidly degraded in the subcutitus to a combination of smaller peptides, amino acids and free radiolabelled iodide. They state that the free radiolabelled iodide is excreted in the urine or incorporated into newly synthesized proteins, while the smaller peptides and amino acids bind to plasma protein and other tissues in the body. However, they state that, due to the nature of the breakdown products, it is virtually impossible to track the pathway of the breakdown products.

Finally, twenty-four hours after each radiolabelled dose, the injection site contained <2% of the dose. The Sponsor stated that this suggests that a major portion of the dose was systemically available in some form.

TOXICOLOGY

Acute

I. S"bcutaneous route of administration

Following is a summary table of the acute toxicology studies by the subcutaneous route of administration submitted in support of this NDA:

Table 1. Summary table of acute toxicology studies and results by the subcutaneous (s.c.) route of administration.

Species	Lab ª/Report #/GLP status/ start date	No. Animals per group (M/F)ᵇ	Dosing Regimen/ Duration	Cop-1 Dose (mg/kg) and Batch #	Effects	NOEL/LD₉₀
Rat (Sprague-Dawley)	TEVA/B37/1/ 92, 21 004. NOT GLP 7-21-92	4/4	s.c., single dose, observe 14 days	0, 400 mg/kg	No deaths. No effects.	None calculated.
Dog (Beagle)	WIS/WZT/3, 21 022. NOT GLP 6-76	1/1	½ dose s.c. ½ dose i.m. observe 48 h	100 mg/kg #4, 5, 7, 8.	No deaths. No effects.	None calculated.

ª Lab= Laboratory where study conducted:
    TEVA = TEVA Pharmaceutical Industries, Ltd., Netanya, Israel.
    WIS = Weizmann Institute of Science, Rehovot, Israel.
ᵇ M/F=Male/Female

Following is a summary of the parameters that were examined for each of the acute toxicology studies listed in Table 1 above:

1. Toxic response of rats to COP-1 after subcutaneous injection of 400 mg/kg, report #B37/1/92.

The following parameters were examined: mortality, clinical signs and body weight.

2. Acute intramuscular and subcutaneous toxicity to beagle dogs of COP-1, report #WZT/3.

The following parameters were examined: gross pathology, histology (brain, hypophysis, lungs, liver, spleen, lymph nodes, kidneys, adrenals, intestines, muscles, gonads, tissue removed from injection site and cytological smear of bone marrow), body weights, clinical signs, mortality. The differential count of bone marrow included myeloid cells, lymphocytes, monocytes and eosinophils.

Reviewer's comments:

1. Neither of these studies (rat or dog) were done under GLP guidelines.
2. No attempt was made to find a lethal dose of the drug.
3. In the dog study, only one animal/sex/group was used at 100 mg/kg, and there were no Control animals included in the study.
4. In the dog study, half of the dose of drug was given s.c., but the other half of the dose was administered i.m.

Due to these inadequacies, these studies are essentially worthless as acute toxicology studies for the purpose of determining NOEL or LD_{50} or for calculating a margin of safety with respect to the human dosing regimen. It is encouraging that at 400 mg/kg, about a 1000-fold higher dose than the 0.4 mg/kg human dose, no mortality or toxic effects were seen in the rats. (Note: by surface area, 400 mg/kg is equivalent to about 57 mg/kg in man, which is about 142-fold higher than the human dose). However, the lack of GLP compliance, small number of animals, lack of Control dogs, and fact that dogs were administered half the dose by the i.m. route render these studies invalid from the Agency's perspective.

II. Other than subcutaneous route of administration

Following is a summary table of the acute toxicology studies and results by routes of administration other than s.c. that were submitted in support of this NDA:

Table 2. Summary table of acute toxicology studies and results by routes of administration other than the subcutaneous (s.c.) route.

Species	No. Animals per group (M/F)[b]	Dosing Regimen/ Duration	Cop-1 Dose (mg/kg) and Batch #	Effects	NOEL/LD$_{50}$
Mouse, ICR strain	5/5	i.m.[c], single dose, observe 14 days	0, 100, 500, 2500 mg/kg #12	No deaths. No effects.	None calculated. 2500 mg/kg=6000-fold>human (520-fold by surface area)
Mouse, ICR out-bred CD-1	3/3	i.p., single equal doses on two consecutive days	41.42-2000 mg/kg #19035A	Deaths: all animals @>232.1 mg/kg died within 2 h after last treatment.[e]	LD$_{50}$=232 mg/kg 580-fold> human dose[f] (by surface area is 48-fold>man)
Rat, Lewis	5/5	i.v., single dose, observed 14 days	0, 1500 mg/kg #3	No deaths. No clinical or pathological effects	None calculated. 1500 mg/kg is 3750-fold> human (125-fold by surface area)
Rat, ICR Sprague-Dawley	5/5	i.v., single dose[g], observed for 14 days	Copolymer-1 0, 40, 200 #99016/II Bromo-contaminated Cop-1: 40, 200. #99020/II	Deaths: (see Table 2B below) Cop-1: tremor, hepatic discoloration. Bromo Cop-1 unconscious, ataxia, bradyapnea, gastric mucoid congestion, duodenal, jejunal and pulmonary congestion, splenic enlargement and hemorrhage.	COP-1 NOEL=40 (100-fold>human; 14-fold>human by surface area) LD$_{50}$=>200 (500-fold> human, 71-fold>human by surface area) Bromo NOEL-none LD$_{50}$=between 40 and 200.
Rat, Sprague-Dawley	5/5	i.v., single dose, observed 14 days	0, 40, 200 mg/kg #00593 #06492	Deaths: 2F, 1M@200 Dead by 2h, showed ataxia, bradyapnea, gasping, dec activity before death. Lower wt gain@200 in females.	LLD (lowest lethal dose)=200 (500-fold> human; 71-fold by surface area) NOEL 40 mg/kg (100-fold> human; 14-fold by surface area)
Dog, Beagle	1/1	½ dose i.m. ½ dose s.c. Observe 48 hrs	100 mg/kg #4, 5, 7, 8	No deaths. No effects.	None calculated.

ous.

[d] Note: this study was actually designed as a combination range-finding study and mouse micronucleus test. The sponsor chose to also present as an acute toxicology study in mice.

[e] Cause of death was not determined. No toxicities were determined, as animals were examined as part of mouse micronucleus test.

[f] Human dose=20 mg = 0.4 mg/kg for 50 kg patient (majority MS patients female).

[g] This experiment also included a group of animals receiving a COP-1 preparation containing 12-14% bromotyrosine, a bromide-containing contaminant, to examine the toxic effects of this contaminant.

Table 2B. Mortality associated with rat study TEV/050/COP

Dose level	Mortality [b]		
(mg/kg)	Male	Female	Combined
Saline	0/2	0/2	0/4
COP-1 40	0/5	–	0/5
200	2/5	0/5	2/10
Bromo Cop-1			
40	0/5	0/5	0/10
200	3/5	4/5	7/10

[b] Deaths occurred within 3 hours after treatment.

Following is a summary of the parameters that were examined for each of the acute toxicology studies listed in Table 2 above:

1. Acute intramuscular toxicity to mice of "COP-1", report   January, 1976.

The sponsor examined body weights and looked for "signs of toxicity". No further details were given in the report of this NON-GLP study.

2. Study to evaluate the potential of COP-1 to induce micronuclei in the polychromatic erythrocytes of CD-1 mice, report   January 14, 1992.

The study was designed as a combination range-finding study to determine the LD_{50} and as a mutagenicity study (mouse micronucleus assay). Mortality, body weights and micronuclei were examined.

3. Acute intravenous toxicity to rats of "COP-1",   June 1973.

The report is very sketchy for this NON-GLP study. Apparently animals were observed for 14 days after treatment, and mortality, body weights, and gross pathology and histology were determined. Organs examined histologically included brain, heart muscle, thymus, lungs, intestinge, liver, spleen, kidneys, adrenals and gonads.

4. COP-1 and its impurity: acute intravenous toxicity study in rats, report   , November 19, 1989.

Animals were observed for 14 days after treatment, inspected four times on the day of dosing and once daily thereafter. The type, time of onset and duration of reactions to treatment were recorded. Body weights were recorded on the day of dosing and on Days 2, 5, 8 and at sacrifice. Animals were killed at termination of the study and examined at necropsy to detect pathological changes. All body cavities were opened and larger organs were narrowly sectioned and the G.I. tract was opened at intervals for examination of the mucosal surface.

5. Comparative study in rats of the acute toxicity of two batches of COP-1 drug substance, report   **May 12, 1993.**

Experimental observations for 14 days after treatment for this GLP study included clinical signs, mortality, body weights, gross pathology and organ weights (heart, lungs, liver, kidneys, thymus, spleen, brain and adrenals from each rat).

6.   Acute intramuscular and subcutaneous toxicity to beagle dogs of COP-1, repon

The following parameters were examined: gross pathology, histology (brain, hypophysis, lungs, liver, spleen, lymph nodes, kidneys, adrenals, intestines, muscles, gonads, tissue removed from injection site and cytological smear of bone marrow), body weights, clinical signs, mortality. The differential count of bone marrow included myeloid cells, lymphocytes, monocytes and eosinophils.

Subchronic

Table 3a. Summary table of rodent subchronic toxicology studies and results by the subcutaneous (s.c.) route of administration.

Species	Lab [a]/Report #/GLP status/ start date	No. Animals per group (M/F)[b]	Dosing Regimen/ Duration	Cop-1 Dose (mg/kg) and Batch #	Effects	NOEL/LD$_{50}$
Mouse (Crl:CD-1)		12/12	s.c., 13 weeks, daily injection into dorsal skin shoulder/thigh-rotate 4 sites.	0, 20, 40 or 60 mg/kg/day #05994	Not available.	None available.
Rat (CD Sprague Dawley)		12/12	s.c., 4 weeks, daily injection into supra-scapular region.	0, 2, 10, 20, 40 mg/kg/day #RT-1	Deaths: None Blood chem: slight ↑ ALPH, ALT, AST; slight ↑ urea. Macroscopic: slight liver pallor; red and swollen ears, swollen face, nose and limbs; injections site lesions (due to antigenicity?).	No deaths. No NOEL, but minor symptoms.
Rat, CR		5/5	i.m./s.c., 3+3 months	0, 250 (i.m.) or 200 (s.c.) mg/kg #36	Deaths: None. Macroscopic: edema at injection site. Bone marrow: ↓ lymphocytes. Histopathology: ↑ red pulp activity.	No deaths. Only single dose used in study; no NOEL calculated.
Rat, Crl:CD Charles River		20/20	s.c., daily injection for 26 week study; 4 sites: left & right shoulder; left & right thigh.	0, 3, 10, 30 mg/kg/day. #03992	Deaths: None treatment-related. Clin. Chem.: slight ↑ creatinine and urea. Immunotoxicology: antigenicity, evidence of immune complex deposition at kidney glomeruli, production of anti-nuclear antibodies. Macro/Micropath: injection site wounds/inflammation.	No deaths. No NOEL with respect to antigenicity or anti-nuclear antibody production. NOEL for immune complex deposition=3 mg/kg/day (7.5-fold > proposed human dose by mg/kg; in same range by mg/m³)

Table 3b. Summary table of dog subchronic toxicology studies and results by the subcutaneous (s.c.) route of administration.

Species	Lab [a]/Report #/GLP status/ start date	No. Animals per group (M/F) [b]	Dosing Regimen/ Duration	Cop-1 Dose (mg/kg) and Batch #	Effects	NOEL/LD₅₀
Dog, beagle		3/3	s.c., 4 weeks, daily injection into dorsal region of dog, 3 different sites.	0, 2, 10, 20 mg/kg/day RE 6551/I and RE 6551/II	Deaths: None Ophthalmoscopy: hyper-reflective points in the eye. Blood chemistry: increased gamma globulin, indicating antibody production. Micropath: lesion site inflammation.	No LD or LD₅₀. NOEL for eye effects=2 mg/kg/day=5-fold>human dose. No NOEL for injection site lesions.
Dog, beagle		5/5	s.c., daily injection for 36 (1/sex/group) or 90 days 4/sex/group)	10 mg/kg/day 4, 5, 7, 8.	Study was NOT GLP, and data was in narrative form only. Reported no histological effects.	None

male and 1 female were

sacrificed at 36 days and the remainder of the animals at 90 days.

Table 3c. Summary table of monkey subchronic toxicology studies and results by the subcutaneous (s.c.) route of administration.

Species	Lab [a]/Report #/GLP status/ start date	No. Animals per group (M/F) [b]	Dosing Regimen/ Duration	Cop-1 Dose (mg/kg) and Batch #	Effects	NOEL/LD$_{50}$
Monkey, Cyno		1/1	Dose-ranging study, s.c., 28 days, daily injection into 4 different sites (left and right shoulder, left and right back)	0, 20, 40, 60 mg/kg/day #00593	Deaths: None. Cardiovascular: ↓ H.R. HD animals. Hematology: HDM & HDF decreased WBC, lymphocytes, neutrophils. Urinalysis: ↓ urine volume HDM & HDF. Organ wts.: ↑ kidney weights HDM. Pathology: injection site lesions.	NO LD$_{50}$ NOEL 40 mg/kg for ↓H.R., ↓lymphocytes (males), and injection site lesions. (100-fold>human dose by mg/kg; 33-fold>by mg/m²) No NOEL for lymphocytes (Female), kidney weights.
Monkey, Cyno		1/4	s.c., 52 weeks, daily single injection into one of 7 sites (right & left upper and lower back, left and right flank of abdomen, area between shoulders)	0, 3, 10, 30 mg/kg/day #02093, 04493, 01394.	Deaths: 1 F@10 mg/kg/day. Antigenicity: anti-COP-1 antibodies; anti-DNA- and anti-histone antibodies. Urine analysis: ↓ creatinine clearance (GFR)-HDF. Histopathology: fibrinoid arteritis in several visceral organs; inflammatory cell foci in several organs including eye, heart, kidney, CNS and spinal cord. Immunohistochem: HDM positive stain in glomeruli for COP-1 and Complement C3; HDF positive stain in glomeruli for COP-1. Symptoms consistent with immune complex formation and deposition.	No LD$_{50}$. No NOEL determined. Antibody response is not dose-related phenomenon.

1. COP-1: 13 week subcutaneous range-finding toxicity study in the mouse, study 1028/2   started January 4, 1995, UK GLP regs.

Study Description

Animals: Mouse, Crl: CD-1

Treatment: Drug was administered by daily s.c. injection into dorsal skin shoulder/thigh-rotate 4 sites. Duration of the experiment was 4 weeks.

Observations

The following parameters were examined as part of the study:

Clinical signs, morbidity and mortality, body weights, food consumption, necropsy (macroscopic examination), immunohistochemistry, and histopathology. Immunohistochemistry included sectioning the poles of the left kidneys of all animals and evaluation of COP-1, complement C3 and IgG antibody complexes.

Results

Note: The Experimental Results and Report were listed as "Not Available".

Conclusions

No conclusions could be made, as no results were reported.

2. COP-1 repeated dose subcutaneous toxicity to rats, stu⎵ January 25, 1995, GLP.

Study Description

Animals: CD Sprague Dawley Rats

Treatment: Drug was administered by daily s.c. injections into supra-scapular region. Animals received 2, 10, 20 or 40 mg/kg/day of drug. The duration of the study was 4 weeks.

Observations: The following parameters were evaluated as part of the study: clinical signs, body weight, food and water consumption, clinical pathology, hematology, blood chemistry, macroscopic and microscopic pathology, organ weights. Histopathology was done mainly on animals from Groups 1 and 5, with the exception of kidneys, liver, lungs, and injection site tissue, which were processed and examined from animals of all groups. Also, processing and examination of all tissues was done for animals with obvious abnormalities and animals that died during the study.

Hematology included packed cell volume, hemoglobin, erythrocyte count, leukocyte count, neutrophils, lymphocytes, eosinophils, basophils, monocytes, normoblasts and platelets. Blood chemistries included urea nitrogen, fasting glucose, creatinine, alkaline phosphatase (ALPH), alanine aminotransferaise (ALT), aspartate

aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), protein, total bilirubin, sodium, potassium, chloride and calcium.

Results

Mortality
No deaths.

Clinical signs
The clinical signs and symptoms are summarized in the following table:

Dose (mg/kg/day) [*]	0	2	10	20	40
MALES					
No abnormalities detected	9	4	5	8	9
OBSERVATION					
Red ears	0	8	7	2	3
Swollen ears	1	0	0	0	0
Red skin	0	1	1	2	0
Swollen face	0	5	5	1	1
Periorbital staining	2	0	0	0	0
Swelling of limbs	0	0	0	1	0
Swollen nose	0	3	2	2	2
Wound	1	0	0	1	0
FEMALES					
No abnormalities detected					
OBSERVATION					
Hair loss on head	0	0	0	0	1
Red ears	0	4	4	1	2
Swollen face	0	3	3	0	3
Swollen nose	0	1	0	1	2
Wound	0	0	0	0	3
Abrasion	0	0	0	0	1

[*] There were 12 animals per group.

Reviewer's Comments:
The symptoms of red and swollen ears, swollen face, nose and limbs are mainly confined to animals treated with Copolymer-1 and are therefore most likely the result of drug treatment. The symptoms are probably the result of a drug allergic reaction as the result of the antigenicity of Copolymer-1 and are consistent with symptoms of a Type I hypersensitivity response.

Body weight and food intake
No effect on body weight or food consumption.

Hematology

A 5-6% decrease in packed cell volume, hemoglobin and erythrocyte counts was reported in both male and female animals. These effects appeared to be dose-related. No effects were seen on either white blood cell (WBC) number or neutrophil, lymphocyte, eosinophil or monocyte numbers.

Blood Chemistry

The response of the enzymes alkaline phosphatase (ALPH), alanine aminotransferase (ALT), and aspartate animotransferase (AST) differed between the sexes. ALPH was increased (42%@ HD) in the female high dose, with no effect in males. ALT was decreased in males (10%@ HD), and increased in females (45%@ HD). AST decreased in the male treatment group at 2 (14.7%) and 40 (20.5%) mg/kg/day. Urea concentrations were increased in female animals (15.3%). Phosphorus levels were increased in male rats at 10, 20 and 40 mg/kg/day (7, 2.2 and 10%, respectively).

Reviewer's Comments: The increased urea concentration in female rats could be a signal for effects of Cop-1 on the kidneys.

Organ weights
No effects.

Macroscopic findings
The only macroscopic findings of note were the following:
1. 2/12 HDM; accumulation of alveolar macrophages—multifocal.
2. 2/12 MDM and 2/12 HDM; hepatocytic pallor-centrilobular—slight.
3. Injection site lesions; data summarized in the following Table:

Group (12/group)	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
LESION subcutaneous inflammation-slight	4	1	3	2	0	7	7	6	3	1
Subcutaneous inflammation-moderate	6	7	6	4	3	3	4	4	6	3
Subcutaneous inflammation-marked	0	4	3	5	9	0	0	2	3	8
No abnormality detected	2	0	0	1	0	2	1	0	0	0

The number of animals responding to treatment with an increased "marked" severity of inflammatory reaction above Control was seen in both males and females.

Reviewer's Comments:

Copolymer-1 is antigenic, and this injection site inflammatory response could be due to Type III immune-complex mediated hypersensitivity. The formation of immune complexes can, with chronic exposure to the antigen (drug), result in circulating immune complex, and cause local (such as glomerulonephritis) or systemic immune complex disease. Therefore, while in this study the only symptoms of antigenicity appear to be injection site lesions, longer term studies may reveal more severe symptomology.

Summary and Conclusions

Four weeks of daily s.c. administration of Copolymer-1 to rats at 0, 10, 20 or 40 mg/kg/day resulted in minimal toxicity. The toxic symptomology probably relate mainly to the antigenicity of the drug. The clinical symptoms of red and swollen ears, swollen face, nose and limbs were confined to treated animals, and were most likely due to an allergic response to drug and are consistent with a Type I response. Lesions at the injection site could have been due to immune-complex formation (Type III hypersensitivity). Increased ALPH, AST and ALT could relate to a minimal effect on liver, as 4 animals (2MDM, 29HDM) demonstrated slight hepatocytic pallor. Increased urea concentration in female rats also suggest a minimal effect on kidney, which might be predicted for an antigenic drug. It is possible that effects on kidney could increase with a more chronic dosing regimen.

Overall, Copolymer-1 was fairly well tolerated at a s.c. dose up to 40 mg/kg/day in this 4 week study.

3. Subchronic intramuscular toxicity to rats of "COP-1", stud

December 1977, NOT GLP.

Study Description

Animals: Rat, CR

Treatment: Daily i.m. injection of Control (saline) or 250 mg/kg/day Copolymer-1 in saline for 3 months. Then s.c. injection of either Control (saline) or 200 mg/kg of Copolymer-1 in saline twice a week for an additional 3 months (total 6 month study). Throughout the study, drug was administered into the four legs of the animals.

Observations:

Mortality, clinical symptoms, necropsy, hematology, necropsy, histopathology, and bone marrow smears. Histopathology included brain, heart, thymus, lungs, intestines, liver, spleen, kidneys, adrenals, skeletal muscle, hypophysis and gonads. Bone marrow and blood smears were prepared for differential counts.

Results

Mortality: No deaths

Clinical symptoms: Edema of the injected areas of the four legs was apparent after 3 months of i.m. injection. The sponsor states that this subsided gradually with the implementation of the subcutaneous injection regimen for the last 3 months of the study.

Blood analysis: The sponsor concludes that there is no effect of Copolymer-1 on total WBC, RBC counts and other blood parameters.

Reviewer's Comment:

The sponsor mixed up the values for the means of blood analysis parameters (Table II, pg. 022 242) that is supposed to correspond to Table 1b (pg. 022 241), containing individual values for these same parameters. Due to this error in the submission, it is impossible to decipher the actual values with respect to correct treatment group (Male Controls, Female Controls, Male Copolymer-1, Female Copolymer-1). It is, therefore, impossible to determine from these data whether or not there is an effect of Copolymer-1 on total WBC, RBC counts, etc.

Bone marrow analysis:

Bone marrow differential counts showed a decrease in lymphocytes (M, 18%; F, 44%) and mast cells (M, 50%; F, 59%) with Copolymer-1 treatment when compared to Controls receiving saline injections (See Table VI below).

Reviewer's Comments:

The sponsor chooses to interpret these data by indicating that the Control animals (saline injection) have a higher number of lymphocytes than either Copolymer-1-treated or Normal (not receiving any injection) animals. They conclude that the number of lymphocytes in the Control (saline-injected) animals actually increased in relationship to Normal animals and Treated animals. However, I believe this to be an improper interpretation of these data.

In fact, the Normal (not injected) animal lymphocyte values fall between the values for Control (saline-injected) and Copolymer-treated animals, indicating that continued injection was not responsible for a general increase in lymphocyte number in either Treated or Control animals. And more importantly, the proper Controls for these experiments are the Control animals (placebo Controls) that also received injections (saline). Since both Controls and Treated animals received repeated injections, any difference due to injection "stress" is not a factor. When compared to the Control (saline injected) animals, there is a definite decrease in the number of lymphocytes and mast cells with treatment with Copolymer-1. This effect appears to be drug-related and of a magnitude (18-44%, M vs. F) that merits concern.

Furthermore, there was an increase in neutrophilic myelocytes (M, 13%; F, 39%) and neutrophilic granulocytes (M, 21%; F, 42%) (see Table 6 below).

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Reviewer's Comments: The sponsor interprets these data as an increase in neutrophil production in bone marrow to compensate for the inflammatory response resulting from repeated injection of Copolymer-1. I concur with this interpretation.

Table VI. Bone marrow differential counts (%)--mean values

Treatment	Lymphocytes	Mast Cells	Neutrophilic myelocytes	Neutrophilic granulocytes
Control males	26.7	0.83	19.73	21.66
Cop-1 males	21.76	0.40	22.33	26.2
Control females	28.43	0.85	15.1	19.1
Cop-1 females	16.03	0.35	21.04	27.16
Normal males	23.66	0.33	21.33	17.16
Normal females	24.16	0.16	18.5	23.5

<u>Peripheral blood analysis</u>

Data in Table VIII below show a small increase in peripheral blood lymphocytes in Copolymer-1 treated males (6%) and females (15%). The sponsor concludes that this increase is not biologically significant, and I concur.

Data from this same table demonstrates a more substantial decrease in peripheral blood neutrophilic granulocytes in Copolymer-1 treated males (42%) and females (26%). The sponsor concludes that this decrease in peripheral blood neutrophils is probably due to neutrophil participation in the inflammatory lesions at the injection sites. I would concur that this is probably the case, although this certainly doesn't rule out the potential for neutrophils to act at other inflammatory sites as well.

Table VIII. Averages of blood differential counts (as %).

Treatment	Lymphocytes	Neutrophilic granulocytes
Control I males	76.2	15.2
Cop-1 males	80.5	8.75
Control females	70.8	11.2
Cop-1 females	81.5	8.25
Normal males	81.0	10.66
Normal females	80.66	9.33

Histopathological exams

A description of histopathological findings was included, but no data to support this description were submitted. The only apparent drug-related histopathological finding reported was an alteration in the spleen. According to the sponsor, "Spleens of all treated animals showed changes in the ratio of red/white pulp." These changes were characterized by the sponsor as follows: "The red pulp was hyperplastic mainly due to excessive myeloid activity. The white pulp however, showed a narrowing of the corona areas of malpighian bodies and broadening of the perifollicular zone." According to the sponsor, "These changes in the spleen are a "physiological" reaction to the local inflammatory injury in the site of injection."

Reviewer's Comments:

Without data, pictures or a more detailed description, it is difficult to interpret these results. However, the effects as described in the spleen could be consistent with an antigen in the blood contacting the lymphocytes in the white pulp of the spleen and inducing an immune response. This could certainly be explained by the antigenicity of Copolymer-1.

Summary and Conclusions

Since this is not a GLP study, it's usefulness in supporting an NDA is negligible. A further confounding factor is the fact that animals were administered drug for 3 months i.m. and for an additional 3 months s.c. I.M. injection is one of the routes of administration that often results in the greatest immunogenicity of a given molecule. Furthermore, no SOPs for methodology for evaluating immune cells is bone marrow or peripheral blood were included, and therefore it is impossible to evaluate the validity of the sponsor's claims.

Irrespective of the fact that this is not a GLP study, it may be important to note the decrease (18%, M; 44%, F) in lymphocytes in the bone marrow of Copolymer-1 treated animals. A 44 % decrease could very well be a biologically significant effect. I disagree with the sponsor's contention that these effects are due to the stress of repeated injection for the reasons stated above.

The decrease in peripheral blood neutrophils could be explained by the inflammatory response at the injection sites. This is further supported by the increased neutrophilic myelocytes and granulocytes in the bone marrow. Unfortunately, in the absence of data, the reported histopathological effects in the spleen are impossible to interpret.

The effects on neutrophils and inflammatory effects at the injection sites are consistent with the administration of an antigenic drug.

4. COP-1: 26 week subcutaneous administration chronic toxicity study in the
i   _   . February 9, 1993, UK GLP regs.

Study Description

    <u>Animals</u>: Rat/Charles River Crl:CD(SD)BR strain, 28 day old weanlings, housed 5/cage, 12-day acclimatization period before starting study.

    <u>Treatment</u>: Rats (20/sex/group) were treated with Control (0.9% saline) or one of three doses of Copolymer-1 in saline (3, 10, 30 mg/kg/day). Rats were administered s.c. injections of 2 ml/kg constant dose volume of drug daily for 26 weeks into one of four sites (the left and right shoulder (sites 1 & 2) and above the left and right thigh (sites 3 & 4)). The injection sites were rotated daily whenever possible, commencing with the left shoulder.

    <u>Observations</u>: The following parameters were examined during the study: morbidity and mortality, clinical observations, body weight, food consumption, ophthalmoscopy, hematology, anti-Cop-1 antibody determination (weeks 5, 13 and 26), clinical chemistry, immunotoxicological assessment, urine analysis, and pathology (necropsy, organ weights, macro and microscopic pathology evaluation).

    Hematology included Hb conc., mean cell volume, packed cell volume, total and differential white blood cell count, platelet count, prothrombin time, and activated partial thromboplastin time.

    Clinical chemistry included determination of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, sodium, chloride, inorganic phosphorus, urea, creatinine, albumin, total cholesterol, potassium, calcium, glucose, total bilirubin, total protein, albumin/globulin ratio, protein fractions (by electrophoresis).

    Immunotoxicological assessment included obtaining blood samples from the remaining males and females in each main study group in Weeks 5, 13 and 26 and examination for the following: B lymphocytes, T lymphocytes, CD4$^+$ T lymphocytes, CD8$^+$ lymphocytes, CD4$^+$/CD8$^+$ T lymphocyte ratios, natural killer cells, anti-nuclear antibodies (ANA), anti-histone antibody analysis, and immunoglobulin G (IgG) and immunoglobulin M (IgM) anlysis.

    Urine analysis inlcuded volume, specific gravity, protein, ketones, blood, reducing substances, pH, total bilirubin, urobilinogen and microscopy of deposits.

    Organ weights included adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thyroids.

    Histopathology included microscopic examination of all the tissues specified below in Control and High Dose group along with all tissues from animals that died or were killed <u>in extremis</u>:

adrenals, aorta, blood smear, bone marrow smear, brain, caecum, colon, duodenum, eyes, femur, Harderian gland, head, heart, ileum, injection sites, jejunum, kidneys, lachrymal gland, liver, lungs, lymph nodes, mammary gland, nasal turbinates, esophagus, optic nerves, ovaries, pancreas, peripheral nerves, Peyer patches,

pituitary, prostate, rectum, salivery glands, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, testes, thymus, thyroids, tongue, trachea, urinary bladder, uterus, vagina, Zymbal's gland, all gross lesions.

Results

Mortality

Following is a summary table of animal deaths:

Group and sex	Animal number	Week of study	Reason for unscheduled death
1M	4	13	Eye damage during bleed
2M	39	20	Moribund removal
3M	`2	27	Found dead
3M	ა8	14	Moribund removal
4F	158	19	Found dead
4F	159	26	Eye damaged during bleed.

The sponsor stated that there were no histopathological findings to suggest that these animal deaths were related to drug treatment.

Observations in animals in groups 3 and 4 included sores at the injection sites sufficiently serious to cause those sites to be abandoned. Also rough hair coat and stained fur were noted.

Body weight

There were no statistically significant differences in body weight, with the exception of an about a 10% increase in the body weight of the High Dose Females from weeks 0-4. This increase was statistically significant by the statistical dose-response test. Also, at all time points, the High Dose Male animals had slightly lower body weights (about 2-3%) than Control animals. While not statistically significant, since this slight decrease occurred at all time points, it could suggest some mild toxicity in this dosing group.

Food consumption

No effect.

Ophthalmoscopy

No effect.

Hematology

The sponsor states that there were no drug-related changes in hematological parameters.

Reviewer's Comments

Although not statistically significant due to the large variability in white blood cell (WBC counts), the data reflect a **consistent increase** in the number of WBC, lymphocytes, and neutrophils in the peripheral blood of High Dose Male and Female animals at all times examined with the exception of the High Dose Females at Week 4 (see following Table). These data are consistent with the rat data from the Non-GLP study   reviewed above. In that study, the increase in peripheral blood lymphocytes corresponded with a decrease in bone marrow lymphocytes.

The data from these two studies, though by no means conclusive, do suggest that Copolymer-1 is affecting the immune system, possibly by decreasing the number of lymphocytes in the bone marrow while inducing a corresponding increase in peripheral blood lymphocytes. The mechanism for this effect, and whether or not it is tied to the immunogenicity of the drug, are unknown at this time. The major lymphocyte population in the bone marrow consists of B cells, which are the antibody-producing cells of the immune system.

Table: Effects of Copolymer-1 on total WBC, lymphocyte and neutrophil counts in rat peripheral blood in a 26 week study.

| Group | % Increase in High Dose Group | | | | | | | | |
| | Week 4 | | | Week 13 | | | Week 26 | | |
	WBC	L	N	WBC	L	N	WBC	L	N
MALES	45	38	58	15.5	18.8	0	8.2	10.8	0
FEMALES	0*	0	0	0	7.5	0	29.0	23.0	62.5

* actually a 22% decrease. * actually a 22% decrease.

These data are not consistent with those from the previous rat study, in that in that study the number of neutrophils in the peripheral blood decreased. The sponsor stated that this was probably due to the participation of peripheral blood neutrophils in the local injection site inflammatory responses seen in the treated animals. This discrepancy in effects on neutrophils could be explained partially by the fact that the previous rat study   , was carried using both i.m. and s.c. injections (3 months of each) and by a difference in timing for collection of peripheral blood for analysis. (It is unclear when bloods were collected in study   the Non-GLP study).

Anti-COP-1 antibody analysis

Antibodies to Copolymer-1 were determined, following 1, 3 and 6 months of s.c. treatment, by solid state radioimmunoassay using ^{125}I-labelled anti-rat IgG.

The sponsor stated that, "in general the level of anti-COP-1 antibodies peaked at Week 13 and declined at Week 26, except for Groups 2F and 3F, where the level peaked at Week 5. In all groups, the maximal number of responders was observed after 5 weeks and was lowest after 26 weeks."

Reviewer's Comments

Copolymer-1 proved to be a very antigenic drug in the rat in this study. Data indicate that the anti-Copolymer-1 antibody levels did peak at the 3 month time point, as did the number of responders (defined by the sponsor as any animal with an antibody titer two-times greater than the mean Control value for animals at that time point) (See Table 4 below).

Table 4. Summary: Anti-Copolymer-1 antibodies during 6-month study (at a 1:1000 dilution).

GROUP	COP 1 DOSE mg/kg	1 MONTH Mean±(S.D.)	Responders	3 MONTHS Mean±(S.D.)	Responders	6 MONTHS Mean±(S.D.)	Responders
1M	0	95 (29)	0/10	116 (34)	0/10	107 (10)	0/10
2M	3	458 (303)	6/10	597 (843)	4/10	218 (195)	3/9
3M	10	682 (623)	6/10	2105 (2010)	8/10	633 (697)	6/9
4M	30	851 (755)	9/10	1850 (1112)	9/10	691 (572)	7/10
1F	0	123 (40)	0/10	112 (26)	0/10	107 (24)	0/10
2F	3	975 (740)	9/10	669 (916)	4/10	309 (342)	3/10
3F	10	505 (437)	4/10	320 (268)	3/10	131 (53)	1/10
4F	30	747 (801)	4/10	1532 (1700)	5/10	506 (569)	4/9

In examining the antibody titers of the animals that were reported to die during the study, it was interesting that the dead animals were ones who had expressed the highest antibody titers in their respective groups. Animal #52 in the males 10 mg/kg group died, and its anti-Copolymer-1 antibody titer was 2045 (1 month), 5495 (3 months) and 2046 (6 months), compared to corresponding treatment group means for this treatment group of 683, 2105 and 633 for the corresponding times. Animal #158 in the females 30 mg/kg group died, and its anti-Copolymer-1 antibody titer was 2078 (1 month) and 4255 (3 months) (dead at 6 months), compared to corresponding treatment group means of 747 and 1532 at the corresponding times.

A number of other animals were destroyed due to their moribund condition, but these were the two animals that were actually found dead. These data do not conclusively prove that animal deaths were somehow related to an exaggerated antigenic response to drug, but they do suggest that some correlation between antibody response and death may be present.

The fact that s.c. Copolymer-1 administration to rats resulted in antigenicity does not necessarily mean that a similar response will be found in man. This depends on how the immune system processes the antigen and presents it to the T and B lymphocyte populations. However, the fact is that the sponsor also looked for

anti-Copolymer-1 antibodies in patients treated with this drug by the s.c. route of administration, and a similar pattern of antibody production was reported.

The chronic administration of an antigenic drug to patients raises two major concerns. First, if the anti-drug antibody is neutralizing to the drug, then chronic administration of the drug may be impractical. Second, if the drug forms immune complexes with the antibody, then those immune complexes can deposit in various organs such as kidney, vasculature, or heart and result in the type of inflammatory tissue damage characteristic of serum sickness.

Clinical Chemistry
There were few changes in clinical chemistry values with drug treatment. The sponsor stated that there were no drug-related effects on the renal functions of the animals as assessed by urea, creatinine and electrolytes.

Reviewer's Comments
A review of the individual data revealed the following:

MALES
Week 4 HDM (high dose males) had 5% increase in creatinine.
Week 26 HDM had a 6% increase in urea and a 4% increase in creatinine.

FEMALES
Week 26 HDF (high dose females) had 12.6% increase in urea and 10% increase in creatinine.

The 5% increase in creatinine in HDM on Week 4 and the 10% increase in creatinine in HDF on Week 26 were statisticaly significant by dose-response statistics.
While these effects are admittedly small, they nevertheless do represent increases in parameters that are used as markers of altered kidney function.

There was no way to do an analysis of anti-Copolymer-1 antibody titer versus effects on urea or creatinine (kidney parameters) because a different group of animals was used for determination of antibody production than for determination of urea/creatinine levels.

Immunotoxicology assessment (as part of 26-week rat study by s.c. administration)

Immunohistochemical staining for complement C3, COP-1 and antibody in the glomeruli of Control and High Dose animals:

From the examination of anti-Copolymer-1 antibody titers in the Control and Test animals, it was determined that Copolymer-1, administered by the s.c. route, is antigenic. Administration of an antigenic drug can lead to formation of antigen-antibody complexes in the blood that can be deposited in various sites around the body, one of the most prevalent sites often being the glomeruli of the kidneys. Upon deposition of such antigen-antibody complexes in these tissues, the complement cascade is activated, and complement (including C3) acts to destroy the tissue where the antigen-antibody complexes are deposited. Therefore, with the knowledge that Copolymer-1 was antigenic, the sponsor responded appropriately by examining the glomeruli of animals treated with Copolymer-1 for deposition of Copolymer-1 drug, anti-COP-1 antibody, and the presence of C3 complement.

Results

Copolymer-1 detection in the glomeruli of treated animals

By immunohistochemical staining, Copolymer-1 drug was demonstrated in the glomeruli of 3 of 20 High Dose (30 mg/kg) animals (animal #68M, 71M and 75M), with a fourth animal (78M) demonstrating "moderate staining" and therefore constituting a possible positive response as well. None of the Control Males showed any staining of the glomeruli. Therefore, it was found that the drug concentrated in the glomeruli of the kidneys of three or four treated animals at detectable levels by immunohistochemical staining techniques. It is possible that other animal glomeruli also contained drug, but in levels that were not detectable by these techniques. The sponsor chose to look only at the Control and High Dose animals.

Presence of C3 complement in the glomeruli of treated animals

PHARMACON carried out the portion of the study in which the kidneys were examined for the presence of C3 complement. The presence of this complement component is indicative of concurrent presence of antigen-antibody complex, as it is the presence of this complex that induces activation of the complement cascade. TEVA reports in this section of the NDA that positive immunohistochemical staining for C3 was found in the glomeruli basement membranes of the same three High Dose Male animals (68M, 71M and 75M) that were reported to show positive staining for Copolymer-1 drug in the glomeruli.

Reviewer's Comments:

Upon careful examination of the . . .   report (see pg. 53 of this review   Study Results"), I discovered that, in fact, 7 of 20 HDM, 5 of 20 HDF, 3 of 20 Males from Group 3 (10 mg/kg/day) and 1 of 20 Females from Group 3 demonstrated positive staining for C3 complement, indicating quite a

high incidence of this phenomenon. Therefore, according to the   data, it was fairly common to find animals in which immune complex had formed and deposited in the glomeruli of the kidneys in this study.

Presence of anti-Copolymer-1 antibodies in the glomeruli

No positive staining was reported for presence of anti-Copolymer-1 antibodies in the glomeruli of any of the Control or High Dose animals. The sponsor acknowledges the fact that antibodies are probably present in the glomeruli, but are undetectable due to insufficient sensitivity of the immunohistochemical staining technique used. It is reasonable to assume with presence of antigen (Copolymer-1) and complement C3 in the glomeruli that anti-Copolymer-1 antibodies are present.

Anti-Copolymer-1 antibody production in these animals

Two of these same three High Dose Male animals, #71M and 75M, also showed the highest levels of anti-COP-1 IgG in the radio-immuno assay at termination. The third animal, 68M, was not assessed for anti-Copolymer-1 IgG.

Summary of immunohistochemical evaluation results from TEVA report

At least three of the twenty High Dose Male animals treated with Copolymer-1 demonstrated positive staining for both Copolymer-1 and complement C3 in their glomeruli, while none of the Control animals demonstrated this staining. Two of these three animals were evaluated for the presence of anti-Copolymer-1 antibody in the peripheral blood, and were found to have the highest titers of those animals tested. Furthermore, a total of 7 of 20 HDM, 5 of 20 HDF, 3 of 20 Group 3 Males and 1 of 20 Group 3 Females demonstrated positive staining for C3 complement in the glomeruli of the kidneys. No anti-Copolymer-1 antibody was found in the glomeruli of any of the Control or High Dose animals, but this may be due to insufficient sensitivity of the assay.

Therefore, Copolymer-1 is antigenic in the rats, and this antigenicity does appear to result in the formation of immune complex (antigen-antibody complex) formation and deposition in the glomeruli of the kidneys. The deposition of immune complex in turn results in the presence of C3 complement. This is the mechanism by which immune complex disease, resulting in kidney damage, is mediated.

study results: HE study no 1028/18, June 3, 1994, Study sponsor   (Note the following aspects of the immunotoxicological analysis for Copolymer-1 were carried out and reported by   and some of these results may be redundant from the TEVA report in the previous section of this review).

*Lymphocyte subset analysis (CD5+, CD4+, CD8+, CD4+CD8+, and B
lymphocytes), serum IgM and IgG levels, ano anti-nuclear antibody levels.*

Study objectives

To determine the potential effects of Copolymer-1 on the immune system by
using the following parameters:

- assessment of lymphocyte subsets (T lymphocytes, B lymphocytes, CD4+,
  CD8+, and CD4+CD8+ lymphocytes), serum IgM and IgG levels, and anti-
  nuclear antibody levels.

- evaluation of the tissue deposition of immune complexes on kidney sections by
  immunohistochemistry.

- examination of lymphoid organs sections.

IgG and IgM levels

No treatment-related differences in IgG or IgM levels were found for the
Treatment Animals compared to the Controls. However, **the sponso: only chose to
look at the plasma Ig levels at week 5.**

Lymphocyte subset counts

No treatment-related differences in lymphocyte subset counts were observed in
the Treatment Groups compared to Controls. However, **the sponsor only chose to
look at the lymphocyte subset levels at week 5 of this 26-week study.**

Reviewer's Comment: While examining IgG/IgM levels at 5 weeks may be
appropriate due to the well-characterized time schedule for antibody production in
response to various antigens, this is not necessarily appropriate for examination of
lymphocyte subset counts. There are a number of mechanisms by which lymphocyte
subset counts could be altered, and there is no rational reason to expect such a
change to be limited to Week 5 of a study. Data from the 6-month i.m./s.c. study in
rats   reported alterations in bone marrow lymphocyte number,
peripheral blood neutrophil number and histopathological alterations in spleen after 6
months of treatment with Copolymer-1. Therefore, in this 26-week study, it would
seem appropriate to examine lymphocyte subset counts at 26 weeks (about 6
months) as well rather than limit their examination of this parameter to 5 weeks.
Therefore, with respect to lymphocyte subset data, the negative results may simply
be due to the fact that the sponsor looked at a single time point.

Anti-nuclear antibody assays

The sponsor states in the IND that there was no significant increase in the total
anti-nuclear antibodies assayed by immunofluorescence. They also state that there
was no significant increase in anti-DNA (double strand) and anti-histone antibodies
assayed by   This is consistent with   conclusion.

However, th　　　　　　　data demonstrates a number of animals in which
the plasma tested positive for anti-nuclear antibodies (see Table, Appendix 3 below):

Table, Appendix 3. Evaluation of total anti-nuclear antibodies (immunofluorescence)

MALES				FEMALES			
	Week 5	Week 13	Week 26		Week 5	Week 13	Week 26
Group 1 *				Group 1			
11	0	0	0	91	0	0	0
12	0	0	0	92	0	0	0
13	0	0	0	93	0	0	0
14	0	0	0	94	0	0	0
15	0 Cy**	0	0	95	0	0	0
16	0	0	0	96	0	0	0
17	0	0	0	97	0	0	0
18	0	0	0	98	0	0	0
19	0	0	0	99	0	0	0
20	0	0	0	100	0	0	0
Group 2				Group 2			
31	+++nu	0	0	111	0	0	0
32	0	0	0	112	0	0	0
33	+r	++r	+r	113	0	+nu	0
34	0	+r	0	114	0	0	0
35	0	0	0	115	0	0	0
36	0	++nu, *	0	116	0	0	0
37	0	0	0	117	0	0	0
38	0	0	0	118	0	0	0
39	0	0	NS	119	0	++r	0
40	0	0	0	120	0	0	0
Group 3				Group 3			
51	0	0	0	131	0	0	0
52	0	0	0	132	0	0	0
53	0	0	0	133	0	0	0
54	0	0	0	134	0	0	0
55	+hr	+r, *	0	135	0	0	+r
56	+r	0	0	136	0	0	0
57	0	0	++r	137	0	0	0
58	0	0	NS	138	0	0	0
59	0	0	0	139	0	0	0
60	0	++r	-r	140	0	0	0
Group 4				Group 4			
71	0	0	0	151	0	0	0
72	±ce	0	0	152	0	0	0
73	0	0	0	153	0	+r	0
74	0	0	0	154	0	0	0
75	0	0	0	155	0	0	0
76	0	0	0	156	0	0	0
77	0	0	0	157	0	0	0
78	0	0	0	158	Ce	0	NS
79	0	0	0	159	0	0	0
80	0	0	0	160	0	0	0

Group 1=Control, Group 2=3 mg/kg/day, Group 3=10 mg/kg/day, Group 4=30 mg/kg/day.

** Abbreviations: cy=cytoskeleton; nu=nucleolus; r=reticulate; hr=homogenous reticulate; ce=centriole; * =mitotic spindle;
=cytoplasmic granulations; 0=negative; ±=doubtful; +=positive (slight); ++= positive (moderate); +++=positive (marked).

Reviewer's Comment:

The sponsor concludes that these data describing the presence of anti-nuclear antibodies in plasma are not significant. While I am under the impression that they used statistical methodology to determine significance, this is unclear in the submission.

The data in the above Table, taken from Appendix 3 (024 359 and 024 360) show that at least 4 of 10 Group 2 Males and 4 of 10 Group 3 Males demonstrated positive staining for anti-nuclear antibodies. 2 of 10 Group 2 Females and 1 each of 10 Group 3 and Group 4 Females also demonstrated positive staining. Therefore, a number of animals treated with Copolymer-1 did demonstrate the presence of anti-nuclear antibodies in their plasma. Since antinuclear antibodies are often associated with autoimmune disease such as systemic lupus erythematosis (SLE), these results could be interpreted to indicate the potential for this drug to induce autoimmunity, at least in this species of rat. This is not a surprising finding in light of the demonstrated antigenicity of the drug in rats.

Immunohistochemical staining of kidney sections

In the review of the   study including immunohistochemical staining of kidney sections for C3 complement, it was reported that a minimal to slight reaction for C3 was associated with the basement membrane of the glomerulus in 7 of 20 High Dose (30 mg/kg/day) Male rats, 5 of 20 High Dose Female rats, 3 Male rats from Group 3 (10 mg/kg/day), and 1 Female rat from Group 3.

concluded that "these deposits are considered to be probably related to treatment."

Reviewer's Comments:

These data indicate that in this 26 week rat study it was fairly common (35% of HDM; 25% of HDF) for the antigenicity of the drug to result in production of immune complex and deposition in the glomerulus of the kidneys.

Histopathology of lymphoid organs

There were apparently no changes observed in the lymphoid organs examined. Also there were no differences in the number of secondary follicles in the mesenteric lymph node and the proportion of thymic medulla between Control and High Dose treated rats.

Overall summary Immunotoxicology Data in the 26-week rat study

The repeated s.c. dosing of rats with Copolymer-1 for 26 weeks resulted in immunotoxicological effects that raise the level of concern for the chronic administration of Copolymer-1 proposed in this NDA. The drug was shown to be highly antigenic in rats, resulting in relatively high titers of anti-COP-1 antibody in all Treatment Groups. Glomeruli of the kidney of a number rats treated for 26 weeks stained positive for the presence of complement component C3. In at least three HDM animals, kidneys stained positive for both the presence of Copolymer-1 drug and C3 complement, and furthermore two of these animals produced the highest anti-COP-1 antibody titers. Finally, while the sponsor concluded that the data for presence of anti-nuclear antibodies was not significant, in fact, anti-nuclear antibodies were present in the p'asmas of a number of Treated animals.

These data demonstrate that rats receiving repeated s.c. treatment with Copolymer-1, a highly antigenic drug, showed signs of immune complex deposition in the glomeruli of the kidneys and the production of anti-nuclear antibodies. While it is true that these effects did not lead to histopathological lesions in the kidneys of these animals, this could simply be due to the length of the study. Had this study been continued for more than 26 weeks, pathological lesions may have appeared. As previously stated, there was some evidence in this study for some minor effects on the kidney, in terms of increased urea and creatinine. Furthermore, the kidney is not the only potential site for immune complex deposition. This can also occur systemically, including vascular damage, heart lesions, and damage to other highly perfused organs. The Sponsor stated in their "Interpretation Section" that "The most probable explanation of these observations is that antibody-COP-1 complexes were formed, deposited in the glomerulus **(and other sites not intensively examined),** and fixed complement."

Urine analysis

Only urine volume and specific gravity were examined. Urine volume increased in a dose-related fashion in male rats on Week 5 and decreased in a dose-related fashion in male rats on Week 25. No other effects were observed.

Organ weights

No effects of Copolymer-1 on organ weights were seen.

Macroscopic and Microscopic Pathology

The only finding of significance was the presence of injection site lesions, that could be explained by either Type III or Type IV hypersensitivity response at the injection site. This is due to the high level of circulating antibodies to Copolymer-1 as the result of the antigenicity of the drug.

Upon histopathological examination, it was determined that these lesions, which were more prominent at posterior injection sites, included myositis, fibrosis and cellulitis. The following Table 1, taken from the NDA, contains a list of injection site lesions by grade:

Table 1: Incidence of selected injection site lesions by grade.

Sex		Male				Female			
Group		1	2	3	4	1	2	3	4
Number		20	20	20	20	20	20	20	20
Injection site 1									
Fibrosis	Grade 1	4	0	2	6	1	5	2	4
	Grade 2	0	1	0	1	0	0	0	2
Injection site 2									
Fibrosis	Grade 1	4	2	0	3	5	2	2	3
	Grade 2	0	1	0	2	0	0	2	1
Injection site 3									
Myositis	Grade 1	1	2	1	6	2	3	2	5
	Grade 2	0	0	0	0	0	0	0	3
Cellulitis	Grade 2	0	0	1	0	0	0	0	1
	Grade 3	0	0	0	0	0	0	1	2
Fibrosis	Grade 1	7	5	2	3	2	11	8	2
	Grade 2	0	5	10	6	0	2	7	8
	Grade 3	0	0	2	10	0	0	0	9
	Grade 4	0	0	0	1	0	0	0	1
Injections site 4									
Myositis	Grade 1	0	1	8	2	0	2	5	7
	Grade 2	0	0	1	5	0	0	0	2
	Grade 3	0	0	0	0	0	0	0	1
Cellulitis	Grade 1	1	0	0	2	0	0	2	0
	Grade 2	0	2	1	2	0	0	1	0
	Grade 3	0	0	0	2	0	0	0	0
Fibrosis	Grade 1	9	9	6	0	5	10	6	4
	Grade 2	1	1	9	8	0	2	9	8
	Grade 3	0	0	1	10	0	0	0	8
	Grade 4	0	0	0	1	0	0	0	0

Key: Grade   1 = minimal; 2 = slight; 3 = moderate; 4 = moderately severe; 5 = severe (NB no findings graded 5)

The injection site lesions appeared macroscopically as sore areas and microscopically as treatment-related lesions resulting in slight to moderate myositis, cellulitis and fibrosis. The sponsor states that these changes noted at the injection site "...would prevent dose levels of above 30 mg/kg/day from being selected in longer term rodent studies."

Reviewer's Comments: Since the drug is antigenic in humans as well, one might expect similar injection site injury to that found in the rat. Since the drug is proposed for s.c. administration essentially for the life of the patient, one might predict that some discomfort from injection site inflammation would be experienced by patients as well.

<u>Dose comparison with proposed human dose</u>

No drug-related mortality was found with Copolymer-1 treatment at s.c. doses up to 30 mg/kg/day. The proposed human dose is 20 mg/day (0.4 mg/kg/day for 50 kg human; based on female). 30 mg/kg/day is about 75-fold higher than the proposed human dose by mg/kg, and about 10-fold higher on a mg/m^2 basis.

With respect to toxic effects, no NOEL was found for antigenicity or anti-nuclear antibody formation. The NOEL for immune complex deposition was 3 mg/kg/day, which is 7.5-fold greater than the proposed human dose (0.4 mg/kg/day) on a mg/kg basis and in the same dose range on a mg/m^2 basis.

5. COP-1 four week study in beagle dogs by subcutaneous injection, Study

September 29, 1988, GLP.

Study description

<u>Animals</u>: Beagle dogs

<u>Treatment</u>: Dogs (3/sex/group) were administered daily s.c. injections of study drug for 4 weeks. Animals received 0, 2, 10 or 20 mg/kg/day, injections distributed among three sites in the dorsal region of the dog.

<u>Observations</u>: Clinical signs, physical examinations (teeth and gums, mucous membranes and skin, ears, superficial lymph nodes, abdomen, external genitalia, chest, gait and stance, general behavior and appearance), food consumption, body weight, ophthalmoscopy, neurology (cranial nerve reflexes, segmental reflexes, postural reactions, general observations), clinical pathology, hematology (PCV, Hb, RBC, platelets, mean corpuscular volume, total leucocyte count, differentials, prothrombin time), blood chemistry (urea, blood creatinine, fasting glucose, CPK, LDH, ALT, AST, GGTP, ALPH, bilirubin, total protein, sodium, potassium, chloride, calcium, phosphorus), urinalysis, macroscopic pathology, organ weight, microscopic pathology (including auricular and ventricular sections of the heart, brain (cerebellum, cerebral cortex, thalamic nuclei, mid-brain and medulla), spinal cord (cervical, thoracic and lumbar) and abnormal and visible lesions, adrenals, aorta, bone, brain, caecum, colon, duodenum, epididymidis, eye, gall bladder, heart, ileum, injection sites, jejunum, kidneys, liver, lungs, lympn nodes, female mammary glands, esophagus, optic nerve, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, skeletal muscle, skin, spleen, spinal cord, stomach, testes, thymus, thyroids, tongue, trachea, urinary bladder, uterus, vagina.

Results

<u>Mortality</u>
No deaths.

<u>Clinical signs</u>
Scratching of the injection site, certainly treatment related and seemingly dose-related.

Physical examinations

Swelling and hair loss at the injection site due to scratching was found for all treated animals. Other signs included dull coat, congested conjunctiva, oral papillomatosis and small bilateral testes.

Body weights
No effects.

Food consumption
No effects.

Ophthalmoscopy

Hyper-reflective points on the border between the Tapetum lucidum and negrum were observed in one male dog and one female out of 3 in the High Dose groups, respectively and in 2 of 3 males of the Intermediate Dose group when examined at 4 weeks. 2 of 3 HDM and 2 of 3 HDF also presented with congested bilateral eyes. It is not known whether or not these effects are drug related, but they did occur at the high dose.

Hematology

The **hematology data are uninterpretable** because the total WBC, neutrophil, lymphocyte and monocyte counts are decreased 36, 35, 35 and 95% in the High Dose Male animal group BEFORE the animals are treated with Copolymer-1. These values continue to be decreased when these same animals are examined after 2 and 4 weeks of treatment with Copolymer-1, but it is impossible to determine whether the decreases are due to drug treatment or to the fact that the animals in Group 4 had much lower values at the start of the experiment.

Blood chemistry

A small decline in sodium (1%) and phosphorus (26%) was found in HDF at 2 weeks of treatment. This effect disappeared at the 4 week time point. There was also an increase in gamma globulin in female animals receiving 10 or 20 mg/kg/day, which could reflect an increase in antibody production, possibly due to the antigenicity of the drug. The sponsor never examined the animals directly for anti-COP-1 antibody production.

Urinalysis
No effects.

Organ weights
No effects.

Macroscopic pathology

Injection site pathology only was reported, including subcutaneous congestion or hemorrhage (in Controls to same extent as Treated Animals), subcutaneous edema, and hair loss as shown in the following Table 1 (pg. 025 039 of NDA):

Text Table 1: Macroscopic lesions observed at the injection site:

Group and sex (3/sex/group)	1M	2M	3M	4M	1F	2F	3F	4F
LESIONS								
subcutaneous congestion or hemorrhages	2	1	2	3	2	0	2	3
subcutaneous edema	0	0	1	1	1	1	2	3
hair loss	0	0	0	1	0	0	0	1

Subcutaneous edema was observed at a higher incidence in the Treated Groups, while areas of hair loss, most probably resulting from itching and scratching, were observed in the high dosage group only.

Microscopic pathology

There was one of three Females at 10 mg/kg that presented with chronic cortical inflammation in both right and left kidney. It is unknown whether or not this was related to drug antigenicity.

The main microscopic pathological effects were again related to injection site wounds, as delineated in the following table:

Table 13: Summation of graded scores for micropathology at the injection site

| | Group and sex | | | | | | | |
	1M	2M	3M	4M	1F	2F	3F	4F
LE SION								
recent hemorrhage	5*	3	12	6	4	1	7	8
chronic inflammation	4	7	17	15	3	7	19	16
acute inflammation	-	3	10	7	3	-	1	5
edema	-	4	25	14	2	7	20	18
mononuclear cell infiltrate	-	3	12	13	-	9	19	12
multinucleate giant cells*	-	-	8	1	-	3	4	3
hematoma	-	-	-	2	-	-	2	-
subcutaneous fibrosis	-	-	-	-	-	-	-	2

*The injection site effects were graded 0-4, and the values in this table reflect the addition of the grade for each lesion at the three sites for the three dogs in each sex group. The maximum possible score for scored criteria is 36.

These data indicate that there is a treatment relationship at the injection sites in all three treatment groups, in the criteria of recent hemorrhage, chronic and acute inflammation, edema, mononuclear cell infiltrate and the presence of multinucleate giant cells. The effect appears to be largely dose-related.

The presence of these injection site lesions is indicative of the fact that the drug was antigenic in the dogs as well.

Dose considerations
There was no NOEL with respect to injection site lesions. These occurred in all Treatment Groups. The only other toxicological finding, hyper-reflective points in the eyes, had a NOEL of 2 mg/kg/day, which is 5-fold higher than the human dose on a mg/kg basis and 2.5-fold greater on a mg/m^2 basis. The clinical significance of this finding is unknown. There were no animals deaths, so or LD$_{50}$ was determined.

Overall summary
An increase in gamma globulin levels in the female animals receiving 10 or 20 mg/kg/day may suggest antibody production to the drug. The sponsor did not look specifically for anti-COP-1 antibodies in these animals.

Hyper-reflective points in the eye were observed in High Dose and Intermediate Dose animals. It is not known whether or not this effect is drug related, not is the clinical significance of this effect known.

The hematology data, which based on findings in the rat are important to this study, are uninterpretable because of unacceptably low values for blood cell counts in the Group 4 animals before treatment commenced.

The only other findings of significance in this study were the injection site lesions, which included hemorrhage, chronic inflammation, edema, mononuclear cell infiltrate, hematoma and subcutaneous fibrosis. These effects appeared to be both drug-related and dose-dependent. The presence of these injection site lesions suggests that the drug was antigenic in the dogs as well. The presence of mononuclear cell infiltrate and multinucleate giant cells is consistent with Type IV (delayed-type hypersensitivity) response.

These dog studies did not include an examination of the effects of Copolymer-1 administration on the cardiovascular system.

6. Subacute subcutaneous toxicity to beagle dogs of "COP-1", Study
<p align="right">**June, 1976, NOT GLP.**</p>

--and--

7. Subchronic subcutaneous toxicity to beagle dogs of "COP-1", Study
<p align="right">**, June, 1976, NOT GLP.**</p>

Study description
Five beagle dogs, ranging in weight from 8.5 to 16 kg, were administered daily s.c. injection of 10 mg/kg Copolymer-1 in saline for 90 days, with one male and one female being sacrificed after 36 days (hence #6 above, the so-called "Subacute" study). Batch #4, 5, 7 and 8 of Copolymer-1 were used.

Results
Clinical, necropsy and histological results were reported in narrative form only. The sponsor reported no clinical signs and no abnormal histopathological findings for brain, thymus, lungs, intestines, liver, spleen, kidneys, adrenals, pituitary, lymph nodes, testes and ovaries.

Reviewer's Comments: These studies are of no value in determining the toxicity of Copolymer-1 because they were not carried out under GLP guidelines and because insufficient information with respect to methodology or data were submitted to allow any scientific evaluation.

**8. COP-1: 28 day subcutaneous sub-chronic toxicity study in the monkey,
Study #1028/21-1050,** May 24, 1993, UK GLP regs.

Study description

Animals
Cynomolgus monkeys, 1/sex/group. Males 2.25-2.5 kg, Females, 1.95-2.15 kg.

Treatment
Monkeys were administered s.c. injections of study drug into four different sites (left and right shoulder, sites 1 and 2, respectively; left and right lower back, sites 3 and 4, respectively). The injection sites were rotated sequentially on a daily basis. Drug was administered for 28 days.

One week prior to dosing, all monkeys randomized to receive Copolymer-1 were administered ^{125}I-radiolabelled Copolymer-1 at the intended dose of 20, 40 or 60 mg/kg for pharmacokinetic evaluation. Blood samples were taken at predose, 2, 5, 10, 20, and 30 minutes postdose and 1, 2, 4, 6, 8, 24 and 72 hours postdose.

5

Observations

Clinical condition and behavior, body weights, food consumption, ophthalmoscopy, electrocardiogram (prior to dosing and on Week 4 prior to dosing), hematology (Hb, MCV, RBC, differentials, platelets, reticulocytes, prothrombin times), clinical chemistry (AST, ALT, GGT, ALP, sodium, chloride, phorphorus, urea, creatinine, albumin, cholesterol, IgG, IgA, potassium, calcium, glucose, total bilirubin, total protein, IgM), Urine analysis (volume, pH, glucose, urobilinogen, blood, microscopy, specific gravity, protein, ketones, bilirubin), macropathology, organ weights (adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes and epididymides, thyroids), histology (adrenals, aorta, blood smear, brain, caecum, colon, duodenum, epididymides, ovaries, pancreas, peripheral nerves, pituitary, prostate, rectum, salivary gland, seminal vesicles, eyes, femur, gall bladder, heart, ileum, injection sites, jejunum, kidneys, lachrymal gland, liver, lung, lymph nodes, esophagus, all gross lesions, skeletal muscle, skin and mammary gland, spinal cord, spleen, sternum, stomach, testes, thymus, thyroids, tissue masses or tumors, tongue, trachea, urinary bladder, uterus, vagina). **Note: Although all of the above tissues were prepared for microscopic examination, only tissues from gross lesions from all animals were observed. Therefore, the sponsor chose not to examine the other tissues histologically.**

Results

Mortality and clinical signs
No deaths. The only clinical signs reported were minor sores at the injection site, and no specific data were presented for this observation.

Body weights
No effect.

Food consumption
Animal numbers 510 (Group 5F; 25%) and 509 (Group 4F; 20%) had slightly decreased food consumptions in Weeks 4. There were no other effects on food consumption. With only 1 animal/sex/group, data are hard to interpret.

Ophthalmoscopy
No effects.

Electrocardiology
The electrocardiology determination was not planned for optimal evaluation of the effects of Copolymer-1 administration on heart rate or ECG parameters, because ECG readings were taken at 4 Weeks, **before drug administration.** It would have been preferable to examine ECG parameters over time directly after drug administration.

At 4 weeks, the male animals demonstrated what appears to be a dose-related

decrease in heart rate. The High Dose Male animal demonstrated about a 15% decrease in heart rate at Week 4. With only 1 animal per group, statistical analysis is impossible. No effects cn heart rate or other ECG parameters were seen in females.

Hematology
At 4 Weeks of treatment with Copolymer-1, High Dose male peripheral blood lymphocyte counts were down 59% with only a 14% decrease in total WBC counts. High Dose female total WBC counts were down 34%, Neutrophil counts down 24% and Lymphocyte counts down 50%. The sponsor states that "...since the Male Control values are similar to the Female Treated animal counts, that this effect is not real..."

Reviewer's Comments: It is true that it is inappropriate to place too much weight on results of a study that only includes a single animal per sex per group. However, I disagree with the premise that it is appropriate to compare the Female Treated Animal values with the Male Control Values. If one appropriately compares Male Treated to Male Control and Female Treated to Female Controls, then these decreases in WBC, lymphocyte and neutrophil counts are probably real, and decreases of this magnitude are more than likely biologically significant. I agree with the sponsor that these results raise the level of concern for effects of Copolymer-1 on mmune cells and suggests that one must very carefully consider these data in the 52-week Monkey study to be reviewed in the next section.

Anti-Copolymer-1 antibody formation
All animals treated with Copolymer-1 in all treatment groups developed anti-COP-1 antibodies following a 4 week s.c. treatment, as shown in the following Table (from the ND/

Table:Development of anti-COP-1 antibodies following a 4-WK treatment with COP-1.

Sample No.	COP-1 dose (mg/kg)	plasma dilution 1:100	plasma dilution 1:250	plasma dilution 1:1000	plasma dilution 1:5000
MALES					
3501	c	3708	1071	861	402
3502	20	39061	35309	25293	8569
3503	40	39038	32847	19442	5440
3504	60	39663	33802	20880	5957
3505	60 (in 50% saline)	33511	28500	13404	4263
FEMALES					
3506	0	3521	2399	1311	934
3507	20	30322	20527	7686	2283
3508	40	31987	30039	14015	3791
3509	60	33412	26342	9856	2383
3510	60 (in 50% saline)	29913	19638	7841	1969

Results presented (in cpm) are from a solid-phase RIA utilizing COP-1 as the coating agent.

Reviewer's Comments: These data are consistent with data in the rat, in which all Treated Animals also developed antibodies to COP-1. As with the rat, there is also the possibility of immune complex formation and deposition in kidneys, cardiovascular system and other organ systems followed by development of inflammation. There is also the question of whether or not the antibodies formed as the result of Copolymer-1 are "neutralizing" antibodies. The sponsor does not appear to attempt to answer this question in this animal study.

Clinical chemistry
No effects.

Urine analysis
On Week 4 of treatment, urine volume dropped 57% in High Dose Male animal and 76% in the High Dose Female animal. Urine volume dropped somewhat in the other Treated animals as well. These data could suggest some effect of the drug on the kidney. However, in the animals tested 2 Weeks before commencement of treatment, the High Dose male had a decreased urine volume of 76% compared to Male Control, and the High Dose female animal's urine volume dropped 88%. Therefore, it is more likely that this decreased urine volume is not due to drug treatment.

Organ weights
High Dose Males had kidney weights that were increased from 19-24% and thyroid weights that were decreased by 49-66%. No such effects were seen in Female animals. The significance of these alterations in organ weight are unknown. However, the effects on kidney weights are of some interest in light of the antigenicity of the drug and potential effects of immune complex deposition.

Macroscopic/m   ~opic pathology
The Group 2 ai . . . Males and Group 4 and 5 Females demonstrated injection site lesions. These lesions included dermatitis, cellulitis, myositis, and fibrosis with both macrophage and in some cases neutrophil infiltration. These lesions also included edema, hemorrhage and tissue necrosis.

The sponsor concluded the following: "Histopathological evaluation of the injection sites detected a chronic inflammatory response at 20 mg/kg/day and above. At 60 mg/kg/day this was accompanied in one animal by subcutaneous thickening and a gelatinous swelling. Therefore, in conclusion, although doses of up to 60 mg/kg/day of COP-1 appeared systemically well tolerated, changes at the injection site suggested that dose levels of less than 40 mg/kg/day should be considered for a subsequent 52 week study by the subcutaneous route."

Pharmacokinetics
Methodology
The pharmacokinetics of COP-1-related material was evaluated in male and female monkeys at dose levels of 20, 40 and 60 mg/kg by s.c. administration. A single dose was administered with a radiotracer (^{125}I)-COP-1 (Batch #00593-01). Blood samples were taken at predose, 2, 5, 10, 20, and 30 minutes postdose and 1, 2, 4, 6, 8, 24 and 72 hours postdose. In addition, the effect of changing the dose formulation from hyperosmotic (0.9% (w/v) saline) to isoosmotic (0.45% (w/v) saline) has been investigated at 60 mg/kg body weight.

Results
Results of the pharmacokinetics study with respect to total plasma radioactivity and TCA-precipitable ratioactivity (plasma protein-bound) are shown in the following Table 7.1 and 7.2, respectively:

Table 7.1 Total plasma radioactivity pharmacokinetic parameters following single subcutaneous doses of (^{125}I)-COP-1 to monkeys.

Animal group	Dose level (mg/kg)	Formulation saline (%w/v)	Cmax (µg/ml)	Tmax (h)	AUC (0-24) (µg.h/ml)	$t_{1/2}$ (h)
M(2)	20	0.9	46.67	2.00	606.7	32.31
F(2)	20	0.9	50.32	2.00	691.9	30.96
M(3)	40	0.9	96.39	4.00	1513.3	30.81
F(3)	40	0.9	78.01	2.00	1103.2	37.60
M(4)	60	0.9	141.5	2.00	2323.8	30.29
F(4)	60	0.9	136.0	4.00	2286.7	31.60
M(5)	60	0.45	133.9	2.00	1860.2	34.28
F(5)	60	0.45	132.8	2.00	2004.1	31.03

Table 7.2 Total plasma TCA precipitable radioactivity pharmacokinetic parameters following single subcutaneous doses of (^{125}I)-COP-1 to Monkeys

Animal group	Dose level (mg/kg)	Formulation saline (%w/v)	Cmax (µg/ml)	Tmax (h)	AUC (0-24) (µg.h/ml)	$t_{1/2}$ (h)
M(2)	20	20	14.37	1.00	206.1	98.7
F(2)	20	20	15.86	1.00	255.9	70.71
M(3)	40	0.9	20.78	1.00	466.7	86.19
F(3)	40	0.9	20.81	1.00	420.3	112.1
M(4)	60	0.9	41.70	1.00	832.2	58.41
F(4)	60	0.9	49.76	2.00	827.5	77.11
M(5)	60	0.45	46.13	0.50	761.2	70.50
F(5)	60	0.45	38.60	2.00	752.2	66.26

Radio-labelled drug-related material exposure (AUC) increased in a linear fashion with dose. Within the limits of an experiment that only includes 1 animal/sex/group, there appears to be no difference in exposure with respect to sex of the animal. About 25-30% of the exposure (AUC_{0-24}) to total drug-related radioactivity appeared to represent TCA-precipitable radiolabel. TCA-precipitable radiolabel most likely represents a combination of intact Copolymer-1 drug, large degradation products, and some free radiolabelled amino acids that have been reincorporated into new TCA-precipitable plasma proteins as well as free radiolabelled iodide that has become bound to TCA-precipitable plasma proteins. No bioavailability was calculated for COP-1 by s.c. route.

Overall summary

Major findings in this toxicology study included possible decreased heart rate (15%) in HDM on Week 4. It is noted that this decrease was found even though ECG determinations were made before administering the drug on this day.

High Dose Male peripheral blood lymphocyte counts were decreased 59% (only 14% decrease in total WBC), while High Dose Females had total WBC counts down 34%, neutrophil counts down 24% and lymphocyte counts down 50%. These are probably biologically significant effects.

Anti-Copolyer-1 antibodies were formed in all Treated animals, with similar levels being produced irrespective of dose. Urine volume dropped 57% in High Dose Males and 76% in High Dose Females, although it is difficult to tell if this is drug-related, since these animals also had decreased urine volumes before commencement of treatment.

High Dose Males had increased kidney weights (19-24%) and thyroid weights that were decreased 49-66%. No such effects were seen in Females. Although the significant of these effects are unknown, effects on kidney are of special interest when the administered drug is antigenic and provides the potential for immune complex formation.

Pathology included injection site lesions, that presented with edema, hemorrhage and tissue necrosis.

Pharmacokinetics data demonstrated that the radioactive drug-related material exposure (AUC) increased in a linear manner with dose, with no sex differences. TCA-precipitable drug (plasma protein bound) made up about 25-30% of the total radioactive exposure.

Dosage considerations compared to proposed human dose

There were no animal deaths, so no LD_{50} was determined. With respect to toxicity, it is difficult to determine a NOEL in a study in which only a single animal per sex per group was utilized, because there are no statistical considerations to help delineate effect versus no effect. However, by common sense estimate upon examination of the data, the NOEL for decreased heart rate, decreased lymphocyte count (males), and injection site lesions appeared to be about 40 mg/kg/day (100-fold>proposed human dose by mg/kg; 33-fold> by mg/m^2). There was no NOEL for

decreased lymphocyte count (females) or decreased kidney weights, as these effects occurred in all Treatment Groups irrespective of dose.

9. COP-1: 52 week subcutaneous chronic toxicity study in the monkey, Study 1028/26-1050 , September, 1994.

Study Description

Animals
Cynomolgus monkey (Macaca fascicularis), 16 males (2.15-3.6 kg) and 16 females (2.0-2.4 kg).

Treatment
The monkeys were divided into four treatment groups, 4/sex/group. Each animal received Control (normal saline) or 3, 10 or 30 mg/kg/day Copolymer-1 as a single daily s.c. injection. Injections were initially administered into four different sites, the right and left upper and lower back. Due to thickening and fibrous swelling at the injection sites, the number of sites in the 10 and 30 mg/kg group was increased to seven, to include the left and right flanks of the abdomen and the area between the shoulders. The sites were rotated but not always in specific order. The study drug was administered daily for 52 weeks.

Observations
Clinical condition and behavior, body weight, food consumption, ophthalmoscopy, electrocardiography, hematology (Hb, MCV, RBC, differential and total WBC counts, platelets), clinical chemistry (AST, ALT, Gamma GT, Alk Phos, sodium, chloride, inorganic phosphorus, urea, creatinine, alph1 globulin, beta globulin,albumin, cholesterol, IgA, potassium, calcium, glucose, total bilirubin, total protein, alpha 2 globulin, gamma globulin, IgG, IgM), anti-Copolymer-1 antibodies, anti-nuclear antibodies, anti-histone and anti-single and double-stranded DNA antibodies, urine analysis (volume, specific gravity, protein, ketones, blood, creatinine, pH, glucose, total bilirubin, microscopy), organ weights (adrenals, brain, kidneys, liver, ovaries, pituitary, spleen, testes and epididymides, thyroids), histopathology (adrenals, aorta, blood smear, brain, caecum, colon, duodenum, epididymides, eyes, femur, gall bladder, heart, ileum, pancreas, peripheral nerves, Peyer's patches, pituitary, prostate, rectum, salivary glands, seminal vesicles, skeletal muscle, skin and mammary glands, spinal cord, spleen, injection sites, jejunum, kidneys, lachrymal gland, liver, lungs, lymph nodes, esophagus, ovaries, gross lesions, sternum, stomach, testes, thymus, thyroids, tissue masses or tumors, tongue, trachea, urinary bladder, uterus, vagina)—all tissues from all animals were examined.

Results

Mortality

Animal #636, a Group 3 (10 mg/kg/day) Female, was killed during Week 14 of the study. The animal exhibited poor food consumption and lost body weight. Although receiving electrolyte solutions orally, it did not recover and was killed for humane reasons.

Histologically, this animal revealed lymphoid and bone marrow atrophy and adrenal cortical hypertrophy. There were non-specific inflammatory lesions in the skin of the tail and paw and in the rectum. In three visceral organs (pancreas, ileum and colon) there was evidence of minor active focal fibrinoid arteritis (which the sponsor states probably arose due to "stress"). An inactive fibrosed arterial lesion (which the sponsor states was probably pre-existing in origin) was found in the heart. The sponsor states that, "No specific cause of the morbidity could be established." They further state that "The combination of non-specific inflammatory changes may have caused the debility."

Reviewer's Comments:

The toxicological results with respect to this animal may be consistent with a systemic inflammatory response as a cause of morbidity and death. The animal had active focal fibrinoid arteritis in three organs, pancreas, ileum and colon. A fourth, inactive lesion, was found in the heart.

Also in support of a systemic inflammatory response are the following toxicology results with respect to animal #636:

Hematology

Animal 636 had a neutrophil count 158% higher than Control and a lymphocyte count 71% below Control, and well out of line with the other three Female animals in this dosing group.

Antibody to double stranded DNA

During Week 4, animal #636 had an antibody titer for anti-double stranded DNA that was increased 138% over Control, and was almost two-fold greater than the other three Female animals in this dosing group. Results were also similar on Week 8.

Antibody to single stranded DNA

During Week 4, animal #636 had an antibody titer for anti-single stranded DNA that was increased 100% over Control, and was 1.5-fold higher than the other three animals in this dosing group.

Total IgG

Animal #636 responded to Copolymer-1 treatment with an IgG level 140% higher than Controls, and an IgG level 2-fold greater than the other three animals in the same dosing group.

Anti-Copolymer-1 antibody

Animal #636, on Week 13, had a 20-fold increase in anti-Copolymer-1 antibody over Control levels, and a titer that was about 2-fold higher than the other three animals in this dosing group.

Reviewer's Comments: These data support a scenario where Animal #636, a Female receiving 10 mg/kg s.c. Copolymer-1, happened to mount a very strong antibody response to this antigenic drug. The results may be consistent with a systemic inflammatory response, possibly due to immune complex deposition, or even to autoimmune response.

Body weight

There was an approximately 35% decrease in body weights in High Dose Males and all Treated Females.

Food comsumption
No effect.

Ophthalmoscopy
No effects.

Electrocardiography
No effect.

Hematology

The only effect was a 13% decrease in APTT (activated partial thromboplastin time) in High Dose Males on Week 13.

Anti-single- and double-stranded DNA antibodies, anti-histone antibodies, and anti-nuclear antibodies

There was an increase in anti-single- and double-stranded DNA and anti-histone antibodies in Male and Female treated monkeys that was often dose-related. Anti-double-stranded DNA antibodies increased as much as 70% (Week 8) in males and 54% (Week 8) in females. Anti-single-stranded DNA antibodies increased as much as 30% (Week 8) in males and 20% (Week 4) in females. Anti-histone antibodies increased as much as 500% (Week 4) in males and 600% (Week 4) in females. Increased antibody levels continued out to Week 52 in all but anti-single-stranded DNA in females.

Reviewer's Comments: These anti-DNA and anti-histone antibodies are thought to be associated with autoimmune disease, and are present in cases of systemic lupus erythrematosus (SLE) for example.

Clinical chemistry

AST (aspartate aminotransferase), ALT (alanine aminotransferase) and IgG levels were increased at 13, 26 and 52 weeks in High Dose Males and Females. At 52 weeks, AST was increased 60% in Males and 26% in females, ALT 56% in males and 29% in females, and IgG levels were increased 53% in males and 63% in females. The reason for increased ALT and AST levels was unclear, although these enzymes usually fluctuate due to effects on the liver. Increased IgG levels are consistent with increased anti-Copolymer-1 antibody as well as increased anti-single- and double-stranded DNA antibodies and anti-histone antibodies. Increased IgG levels and increased anti-DNA and anti-histone antibodies are consistent with the development of autoimmunity.

Urine analysis

Although not statistically significant, the creatinine clearance (and thus the glomerular filtration rate (GFR)) decreased approximately 50% in High Dose Female animals. This could be indicative of some loss of renal function. These same animals had a loss of urine volume of about 32%.

Anti-Copolymer-1 antibodies

Copolymer-1 was highly immunogenic, and high levels of antibodies were present in all monkeys from all treatment groups (Table 11 below; 027 116). The antibody response appeared to be independent of dose, with a great deal of variability from animal to animal. Certain animals, such as #643 (the Group 3 female that died), 634F and 617M (from the 3mg/kg/day group), and 623M and 640F (from the 30 mg/kg/day group) all had persistently high levels of anti-COP-1 antibody throughout the study.

Reviewer's Comments:

While the sponsor states that "...peak antibody response appeared to occur for the majority of the animals at Week 13...", data in Table 11 below demonstrate that, in fact, when one considers the variability of the responses, there is probably no difference between the antibody titers at 13 Weeks and 52 Weeks. It is likely that the increased presence of high titers of anti-COP-1 antibody for a prolonged period would tend to increase the chances of development of immune complex disease.

Table 11. Anti-Copolymer-1 antibody levels at a 1:5000 dilution of serum.

Group and sex	COP-1 dose (mg/kg)	Week 4 Mean(SD) R	Week 13 Mean (SD) R	Week 26 Mean(SD) R	Week 39 Mean(SD) R	Week 52 Mean(SD) R
1M	0	261(57) 0/4	1656(1560) 2/4	667(294) 2/4	714(211) 2/4	717(194) 2/4
2M	3	6660(6926) 4/4	16047(3715) 4/4	9403(4184) 4/4	8901(2147) 4/4	10527(3305) 4/4
3M	10	5112(4351) 4/4	13465(7437) 4/4	5534(4073) 4/4	7031(2990) 4/4	7814(3080) 4/4
4M	30	2609(1224) 4/4	12334(8872) 4/4	9539(11083) 4/4	7613(6063) 4/4	8111(6525) 4/4
1F	0	703(355) 0/4	1022(368) 0/4	1156(642) 0/4	1395(871) 1/4	1053(293) 0/4
2F	3	7084(6103) 3/4	18675(6760) 4/4	15803(6429) 4/4	12053(912) 4/4	17073(5134) 4/4
3F	10	10650(5130) 4/4	13511(5565) 4/4	7312(6366) 3/3	7166(1181) 3/3	10014(1758) 3/3
4F	30	6326(4406) 3/4	13712(7565) 4/4	9195(6762) 4/4	8509(4858) 4/4	10571(8603) 4/4

R=Responders; responses were considered positive when the value was at least 2-fold higher than that of the mean±S.D. of the Control Group (1M or 1F) at Week 4 at the same dilution.

Organ weights

The only effect on organ weight of consequence was a decrease in High Dose Female heart weight of about 23%. This did not occur in Male animals, and the significant of this effect in unknown.

Macropathology

Treatment-related findings were confined to the injection sites. Most injection sites showed reddening. In the four initial sites, in all Treatment Groups, there was thickening and, in the Intermediate and High Dose Groups, gelatinous change.

Histopathology

Deceased animal 636F (Intermediate Dosing Group) presented with lymphoid and bone marrow atrophy and adrenal cortical hypertrophy. Inflammatory lesions were found in the skin of the tail and paw and in the rectum. There was fibrosing chronic inflammation at the injection sites. There were also "minor" active focal fibrinoid arteritis in pancreas, ileum and colon, in addition to an inactive fibrosed arterial lesion in the heart (the sponsor states that this is probably of pre-existing origin). The sponsor stated that "...no specific cause of the morbidity could be established. The combination of non-specific inflammatory changes may have caused the debility..."

Injection site lesions were described as "...at the four initial injection sites, fibrosing chronic inflammation was characterized by areas of fibrosis, in which small mononuclear-cell accumulations, occasional giant cells and rarely germinal follicles occurred. In most animals a minor diffuse eosinophil infiltration was also present. A treatment-related increase in severity was evident...There were also minor and less prevalent edematous, hemorrhagic, and necrotic lesions at the injection sites, which showed no clear association with treatment.

Two High Dose Male and a single High Dose Female animal presented with fibrinoid arterial lesions as follows:

Animal	Group	Organ
623	4M	Gall bladder, duodenum, jejunum, seminal vesicles, testes, lung, epididymides. (Note: this animal also had inflammatory cell foci in muscle, sciatic nerve, liver, kidney, salivary gland, heart, pituitary, brain, spinal cord).
626	4M	Epididymides. (Note: this animal also had inflammatory cell foci in kidney, lung, lacrimal gland).
640	4F	Vagina. (Note: this animal also had inflammatory cell foci in liver, kidney, salivary gland, brain, lacrimal gland).

The sponsor states that "...the incidence of such arterial lesions is within the range of background pathology in terms of prevalence and severity for control primates in this laboratory..."

Furthermore, in two High Dose animals (623M and 640F), there were germinal follicles in the bone marrow. The sponsor states "...This change is recognized as an infrequent non-adverse finding in control animals in the laboratory, but this incidence in the high dose group may suggest a possible relationship to treatment..." Therefore, two High Dose Monkeys (30 mg/kg/day) presented with both minor chronic fibrosing arterial lesions and the presence of active germinal follicles in the bone marrow. Furthermore, a third monkey High Dose monkey (626M) showed only chronic focal arteritis.

Finally, of the three animals with arteritis included in the sponsor's Table shown above, those animals in addition to the arteritis, demonstrated a number of "inflammatory foci" in kidney, muscle, sciatic nerve, lung, brain, heart, spinal cord,liver and lacrimal glands. This large number of inflammatory foci are also of some concern.

Reviewer's Comments:
The presence of both fibrosing arterial lesions and active germinal follicles in bone marrow of these two High Dose monkeys, along with the fact that these were two of the animals that presented with consistently high antibody titers, present data that could be consistent with a profile of vasculitis, possibly as the result of immune complex formation. The activity in the bone marrow is consistent with production of additional inflammatory cells (monocytes, neutrophils), as might be seen with a systemic inflammatory response.
The sponsor states that "There was no evidence of any multicenter acute

fibrinoid arterial lesions suggestive of acute ongoing vasculitis." However animal 636F (the animal that died during the study) presented with "minor" active focal fibrinoid arteritis in pancreas, ileum and colon, in addition to an inactive fibrosed arterial lesion in the heart and animal 623 (High Dose Male; see above Table) presented with arterial lesions in gall bladder, duodenum, jejunum, seminal vesicles, testes, lung and epididymides. Therefore, there was, in fact, some evidence for multifocal arterial lesions in some of the treated animals that could be consistent with systemic vasculitis. There were also an inordinate number of "inflammatory foci" in a number of organs, including brain and spinal cord, that did not occur in Control Animals.

Finally, the description of the histopathology of the injection site lesions were consistent with that in the dog, in that the lesions included mononuclear-cell accumulation with occasional giant cells. This pathology is consistent with Type IV (delayed-type hypersensitivity) response.

Histopathological lesions in brain and spinal cord by dose

One major concern with Copolymer-1 is that, due to it's antigenicity as evidenced by the data in this monkey study, it has the potential to induce immune complex disease and the antibodies associated with autoimmunity (anti-DNA and anti-histone antibodies). Therefore, one has to be especially concerned that, in repeatedly administering the drug to treat M.S. patients, one is not actually causing CNS lesions. Data in the following Table show that in the monkey study, animals actually developed inflammatory cell foci in brain (choroid) (1 of 4 HDM; 2 of 4 M@3mg/kg; 2 of 4HDF) and spinal cord (1 or 4HDM; 1 of 4M@3 mg/kg). There was only a single such brain lesion in Control Animals (Female) and no such spinal cord lesions. Inflammatory cell foci in heart (1 of 4 HDM; 1 of 4 M@10 mg/kg; 1 of 4 M@3mg/kg; 1 of 4 HDF; 2 of 4 F@10mg/kg), another heavily perfused organ, also appeared with drug administration. There were no such foci in the heart of any Control Animals.

Table: Summary of histopathology data for 52-week cynomoigus monkey study with respect to development of inflammatory cell foci in brain, spinal cord and heart

Group/Dose	Animal #	Brain	Spinal Cord	Heart	Kidney
MALES 1 (0 mg/kg)	611M 612M 613M 614M	- * - - -	- - - -	- - - -	+ + + +
2 (3 mg/kg)	615M 616M 617M 618M	- - ++ (choroid) +	++ (choroid) - - -	+ - - -	- - + +
3 (10 mg/kg)	619M 620M 621M 622M	- - - -	- - - -	- ++ - -	+ - + -
4 (30 mg/kg)	623M 624M 625M 626M	+ - - -	+ - - -	+ - - -	+++ ++ + ++
FEMALES 1 (0 mg/kg)	627F 628F 629F 630F	+(choroid) - - -	- - - -	- - - -	- ++ + +
2 (3 mg/kg)	631F 632F 633F 634F	- - - -	- - - -	- - - -	++ +++ ++ -
3 (10 mg/kg)	635F 636F 637F 638F	- - - -	- - - -	++(aorta thick) + (arteritis)** - -	- - ++ +
4 (30 mg/kg)	639F 640F 641F 642F	- ++ (choroid) + (choroid) -	- - - -	- + - -	+ ++ ++ + (interstitial nephritis)

- = no inflammatory cell foci; + = minimal, ++ = slight; +++ = moderate.
**arteritis also included fibrosis

These data are somewhat disconcerting in that they suggest that repeat administration of Copolymer-1 for up to 52-weeks may have resulted in the formation of inflammatory foci in the CNS. There are no data presented to indicate that these inflammatory foci are due to immune complex deposition or autoimmunity. However, it is the CNS that is affected in the case of MS, and administration of a drug that could result in additional CNS inflammation would be unfortunate. Furthermore,

development of inflammatory cell foci in the heart would also constitute a rather serious side effect of drug administration.

As stated previously, the kidney is an organ of concern with respect to development of immune complex disease. As seen in the Table above, while it is true inflammatory foci occurred in Control as well as Treated Animals, the severity of these foci increased with increasing dose of drug.

Also of concern is the fact that inflammatory cell foci appeared in the eye of 2 of 4 M@3 mg/kg, 2 of 4 M@30 mg/kg, 1 of 4 F@3 mg/kg, 1 of 4F@10 mg/kg and 2 of 4F@30 mg/kg (not shown in Table above). None of these lesions were reported in Control Animals.

The lack of a clear-cut dose-relationship between the appearance of inflammatory cell foci and dose of drug is not particularly surprising, because the production of antibody in response to s.c. administration of Copolymer-1 is not a particularly dose-related response. It would appear from the anti-Cop-1 antibody data, shown in Table 11 of this review, that with administration of 3 mg/kg/day of drug, we are already at maximum antibody production. At this level of drug administration, the major variability is in the animal-to-animal variation in antibody response. Since it is antibody-production and immune complex formation that is most likely responsible for formation of these inflammatory cell foci in brain, spinal cord and various other organs, and since antibody production is not a dose-related response at this level of drug administration, then one might not expect development of inflammatory cell foci to constitute a dose-related phenomenon in this dose range either.

immunohistochemistry

High Dose Male (#623M) showed clear, linear staining of the glomeruli for Copolymer-1 and C3 complement. The staining was demonstrated to be specific by a study including absorption of the primary antibody with chemically pure specific antigen.

High Dose Female (#640F) showed minimal linear staining of a portion of the glomeruli for the presence of Copolymer-1, but no staining for C3 complement. This staining was also demonstrated to be specific for Copolymer-1 by absorption experiments. IgG was not demonstrated in glomeruli of any of the Treated Animals, although, as in the case of the rat study, this could be due to limitation of sensitivity of the assay.

Reviewer's Comments:

These data are confirmatory of the rat data, and demonstrate that repeated administration of Copolymer-1 and the resultant antigenicity can result in formation of immune complex and deposition in the glomeruli of the kidney and that the drug itself can be deposited in the kidney as well. However, while s.c. administration of Copolymer-1 to the rat appeared to result in a higher incidence of deposition of immune complex in the glomeruli of the kidney, probable vasculitis of highly perfused organs appeared to occur with higher incidence in the Cynomolgus monkey.

Morphometry of lymphoid tissue

There were no differences in the proportion of thymus occupied by the cortex between Treatment Groups and Controls. There were also no differences reported between the Treated and Control Groups with respect to the numbers of germinal follicles fond in the mesenteric lymph node of each terminal animal.

Dose with respect to proposed human dose

The proposed human dose for s.c. administration of Copolymer-1 is 20 mg/day for the life of the patient. This is about 0.4 mg/kg for a 50 kg human (most relevant to females, in which autoimmune diseases such as MS are more prevalent). There was only a single animal death, and that was at 10 mg/kg/day. No NOEL could be calculated for the histopathological lesions, with effects occurring in all Treatment Groups. Since these effects may be due to formation of deposition of immune complexes, which is in turn dependent on antibody titers, and since antibody response was not dose-dependent in the range of 3-30mg/kg drug, one might predict that effects would not occur in a dose-related fashion. Rather effects should occur in all Treatment Groups at about the same rate, with the variable being the degree of antibody response for a given Treated animal.

The lowest dose used in this study, 3 mg/kg, is 7.5-fold higher than the 0.4 mg/kg proposed for use in humans. From these data, there is no way to predict the degree of antibody production that might have occurred with administration of a 0.4 mg/kg dose of drug.

During Week 20 of the study the degree of thickening/fibrous swelling at the injection sites of monkeys treated at 30 mg/kg/day was so severe that it was necessary to introduce additional injection sites in order to continue the study up to 52 weeks. No NOEL was able to be determined for these injection site lesions.

The sponsor stated that, by their estimation based on two monkeys at 30 mg/kg/day showing active bone marrow germinal certers, deposition of immune complexes in the renal glomeruli and minor chronic focal fibrosing arterial lesions that the NOEL for systemic effects was set at 10 mg/kg/day. I obviously disagree with this determination.

Overall summary of Cynomolgus monkey study

There is no question that the drug is antigenic in Cynomolgus monkeys, resulting in high anti-COP-1 antibody titers in all treated animals. In this dose-range of drug (3-30 mg/kg/day) there is no dose-relationship to the antibody formation. There is a rather large animal-to-animal variability in antibody response.

The data in monkey are consistent with the rat study in demonstrating that immune complex is deposited in the kidney. Immunohistochemical data revealed that in at least one High Dose Male, the glomeruli stained positively for presence of both Copolymer-1 drug and complement C3, while one High Dose Female showed positive staining for the presence of Copolymer-1. These data indicate that immune complexes were most likely being deposited in the glomeruli. The lack of positive

staining in other animals could be due to the limitation of detection of the staining techniques. Consistent with effects of immune complex deposition on the kidney are data showing creatinine clearance (and thus glomerular filtration rate (GFR)) decreased approximately 50% in High Dose female animals, and the Table on page 51 of this review shows that inflammatory cell focal lesions in kidney of HD animals worsened with drug treatment.

In addition to effects in the kidneys, the monkey data also provide evidence that suggest the possibility of development of a more systemic immune complex disease and/or autoimmunity as a result of s.c. administration of Copolymer-1 for 52-weeks. The single animal that died in the study, a Female receiving 10 mg/kg/day, presented with fibrinoid arterial lesions in various visceral organs, including pancreas, ileum and colon, with evidence of another minor active lesion in heart. This animal also demonstrated a very high neutrophil count (consistent with inflammatory response), high titers of antibody to single- and double-stranded DNA, high titers of anti-histone antibody, and a very high titer of anti-Copolymer-1 antibody. These effects point to the possibility of a vasculitis. With respect to histopathology, in addition to the dead animal, fibrinoid arterial lesions (one possible effect of immune complex deposition) occurred in 2 HDM (gall bladder, duodenum, jujunum, seminal vesicles, testes, lung, epididymides) and one HDF (vagina). Furthermore, inflammatory cell foci occurred in Treated but not Control animals in the eye and heart. Finally, s.c. administration of Copolymer-1 resulted in inflammatory cell foci in brain of 3 of 12 Treated Male animals and 2 of 12 Treated Female animals and in spinal cord of 2 of 12 Treated Males. These lesions were apparently in the choroid plexus, where there is a very high perfusion of blood. This finding is especially disconcerting in that the drug is proposed for the treatment multiple sclerosis, an autoimmune disease causing lesions in the CNS,

It is not particularly surprising that that none of these lesions occur in a particularly dose-related fashion, since antibody production in this dose range is also not a dose-related phenomenon. Antibody titers are fairly similar in all Treated Animals in this dose range. The most important point is that these lesions are absent from Control Animals, with the exception of a single brain inflammatory cell focal point in Control Females.

Other immunotoxicological data, including WBC, lymphocyte, and monocyte counts and thymus and spleen weights and histopathologies suggest that Copolymer-1 has no other toxicological effects on the immune system other than those related to antigenicity and excessive immuno-stimulation, resulting in probable immune complex disease including arteritis and inflammatory cell foci in various organs including brain and spinal cord. These data indicate that Copolymer-1, as indica._J by the sponsor, it not a non-specific immunosuppressive agent, and repeated administration should not result in a decrease in resistance to infections.

10. Six-month subchronic toxicity study in the rat: three-month intramuscular administration followed by three-month subcutaneous administration, Study   December 1963, NON-GLP.

11. Subchronic toxicity study in rabbits: six-month intramuscular administration, Study   January, 1976.

These two studies (#10 and 11 above) are non-GLP studies carried out in 1963 and 1976 by routes of administration other than the subcutaneous route proposed in this NDA. Due to the fact that there are sufficient additional studies to evaluate the safety of the drug by the s.c. route and the fact that these are very old non-GLP studies, these studies were not evaluated.

Special Toxicology Studies

Antigenicity--Clinical Data
Whenever administration of a drug shows the drug to be antigenic, resulting in the production of anti-drug antibodies, one must be concerned with two possible aspects of this antigenicity, 1) the prosect that the antibody is neutralizing to the drug, thus potentially rendering repeated adminstration of that drug ineffective and 2) the possibility that the antibodies will mediate a hypersensitivity response. Such a hypersensitivity response can be in the form of a local inflammatory reactions at the injection site, an organ-specific inflammatory reaction, or a systemic inflammatory response consistent with immune complex disease and/or autoimmunity.

Neutralizing Antibodies to Copolymer-1
Preclinical studies in the rat (26-weeks) and the Cynomolgus monkey (52-weeks) demonstrated that s.c. administration of Copolymer-1 resulted in high titers of anti-Copolymer-1 antibody in the plasma of all Treated Animals. This type of result immediately raises the question of whether or not these antibodies might have the capacity to neutralize the drug, either by binding it up into an inactive conformation or by blocking the binding of the drug to it's receptor or action site. One must be concerned that once high antibody titers are established, any additional drug that is administered will simply be neutralized by the antibody, and therefore be rendered ineffective in terms of treating the prescribed illness. This is especially important when the clinical protocol proposes chronic administration of the drug, as does this NDA for Copolymer-1 for treatment of multiple sclerosis.

As there is no guarantee that animal and human immune systems will process a particular antigen in an identical manner, the demonstration of a neutralizing antibody in an animal model is not necessarily predictive of a similar condition in the human. Therefore, the sponsor in this NDA quite appropriately utilized the anti-Copolymer-1 antibodies formed in humans in the clinical trials to determine whether

or not these antibodies were neutralizing to the drug. The sponsor used two different means of determining the neutralizing capacity of the human anti-Copolymer-1 antibodies, one *in vivo* and one *in vitro*.

In Vivo studies--EAE blocking

When Copolymer-1 is added to the encephalitogenic emulsion used to induce EAE (Experimental Allergic Encephalomyelitis) in mice (mouse spinal cord homogenate (MSCH) + complete Freund's adjuvant (CFA)), the Copolymer-1 has been shown to block development of EAE in a dose-dependent manner. The sponsor first determined that 5µl of normal human serum (NHS) did not alter the induction of EAE by the MSCH, while 25µl of NHS alone blocked the induction of EAE. Therefore, a maximum of 5µl of human serum containing anti-Copolymer-1 was used in the final assay. The sponsor then collected serums from six different multiple sclerosis patients that had received Copolymer-1 treatment and had high titers of anti-Copolymer antibody in their serum (sponsor presented data to show high antibody titers in patient's sera, not included in this report). 5µl of each of these sera was added to the mixture of encephalitogenic emulsion + Copolymer-1 before it was administered to mice, to determine if the human Copolymer-1 antibodies contained in these sera could prevent the Copolymer-1 from blocking the induction of EAE in these animals. Results of this experiment are shown in Table 4 below.

These data show that the anti-Copolymer-1 antibodies contained in these six human sera had no ability to prevent Copolymer-1 from blocking the induction of EAE in mice by administration of this encephalitogenic emulsion, suggesting that these are non-neutralizing antibodies with respect to Copolymer-1.

Table 4. Effect of sera from Copolymer-1 treated patients on the EAE blocking activity of Copolymer-1

Treatment	EAE Clinical Incidence	Blocking (%)
Control-MSCH	9/10	
MSCH+25µl NHS*	0/10	100%
MSCH+5µl NHS*	9/10	0%
MSCH+Cop-1 b	0/10	100%
MSCH+Cop-1+Serum #1*	0/10	100%
MSCH+Cop-1+Serum #2*	0/10	100%
MSCH+Cop-1+Serum #3*	0/10	100%
MSCH+Cop-1+Serum #4*	0/10	100%
MSCH+Cop-1+Serum #5*	0/10	100%
MSCH+Cop-1+Serum #6*	0/10	100%

*NHS = normal human serum
b 250 µl Cop 1 batch No. 123094 was added to the MSCH inoculum in CFA
*5µl of the tested sera were added to the MSCH+Cop-1 inoculum in CFA

Reviewer's Comments:

1. The *in vivo* EAE model in the mouse is somewhat suspect, in that the mouse encephalitogenic emulsion is mixed with the Copolymer-1 **before** injecting into the animals in order for the Copolymer-1 to prevent the EAE induction. The fact that the Copolymer-1 blocks the encephalitogenic emulsion-induced EAE could be due to some action of the drug on the encephalitogenic emulsion in the test tube rather than an in vivo effect. To validate this model, the sponsor should ideally inject the encephalitogenic emulsion and the Copolymer-1 into the mice separately and at different injection sites. The optimum result would be for Copolymer-1 administration to prevent the development of EAE, even when injected separately and after administration of the encephalitogenic emulsion.

2. The problem with this test for the ability of the human anti-Copolymer-1 antibodies to block the effects of Copolymer-1 is that only 5μl of human serum could be used. It is possible that if a larger volume of human serum, and thus a higher concentration of antibodies, could have been added, that some neutralization may have been seen. One must also wonder why 25μl of normal human serum, added to an overall volume of 400μl test system, would itself block the formation of EAE in the absence of Copolymer-1.

Reviewer's Conclusion: Within the limits of the assay, these data do suggest that these anti-Copolymer-1 antibodies are not neutralizing. However, I would be much more convinced if the experiment included dose-response for antibody concentration.

In Vitro Study, effect on the proliferative response of a Copolymer-1 specific T-cell line

The sponsor used a T-cell line that was known to proliferate in the presence of Copolymer-1. The sponsor incubated this T-cell line in the presence of Copolymer-1 and 10μl of human serum (containing high titers of anti-Copolymer-1 antibody) from these same six patients. Proliferation was assessed by the incorporation of ^{3}H-thymidine. The results, shown in Table 5 below, demonstrate that Copolymer-1-induced T-cell proliferation was not affected by addition of 10μl of the antibody-containing human serum to the incubation mixture, as compared to Control samples containing normal human serum. These data, again, indicate that the anti-Copolymer-1 antibodies contained in the sera from these patients was not neutralizing to Copolymer-1 drug.

Table 5. Effects of human sera containing high titers of anti-Copolymer-1 antibody on Copolymer-1 induced proliferation of a T-cell line.

Serum	³H-Thymidine Incorporation (cpm)*
NHS*	9018±570
1	10315±835
2	8605±639
3	8469±420
4	8345±505
5	8994±495
6	10413±550

* Results are expressed as net cpm (after subtraction of cpm of cultures without antigen) and they represent the mean of triplicate cultures.
*S = Normal human serum

Reviewer's Comments:

These data are somewhat more convincing in demonstrating that the anti-Copolymer-1 antibodies are not neutralizing, in that the human serum was only diluted 1:20, and one might expect that neutralizing antibodies would at least partially block Copolymer-1-induced proliferation of this T-cell line under these conditions.

However, there is one caveat. The sponsor does not specify the sequence of events with respect to addition of the Copolymer-1 and human serum containing the anti-drug antibodies to the incubation. If the sponsor added either the Copolymer-1 and the human serum at the same time, or the human serum first, then the assay would be valid. However, if the sponsor chose to add the Copolymer-1 first and then the human serum, then it would be likely that once the Copolymer-1 induced T-cell proliferation, that no amount of anti-Copolymer-1 antibody added later would be able to inhibit.

Additional important immunological issues related to antigenicity and addressed by clinical data

There were two additional important immunological issues related to antigenicity that the sponsor addressed in patient populations.

Issue #1: anti-Copolymer-1 and anti-myelin basic protein antibodies formed in patients over a 25-month period

The first issue relates to the production of anti-Copolymer-1 antibodies and anti-myelin basic protein (MBP) antibodies over time in patients receiving the drug. Figure 1 (NDA page 137 009) below shows the profile for anti-Copolymer-1 and anti-MBP antibodies in patients receiving Copolymer-1 over a 25-month period in a clinical trial. The sponsor concludes that these data show that the antibody production peaks at 3 months and then declines in the subsequent 22 months. However, I disagree with this conclusion. In fact, if one carefully examines the error bars associated with

this figure, it becomes clear that there is most likely no statistical difference between the binding indexes for anti-Copolymer-1 antibody at any of these time points. It is only clear that the binding indexes are higher than Control at all time points. Therefore, I would argue that, just as in the case of the animal studies, there is no substantial evidence that the antibody response declines with time.

I agree with the Sponsor that the data shown in Figure 1 demonstrates that there is little or no antibody formed to myelin basic protein. This is a fortuitous situation, in that the production of anti-MBP antibody in response to administration of Copolymer-1 would most likely result in the very damage to the CNS that Cop-1 administration is being administered to prevent.

Figure 1.

Production of Anti-Copolymer-1 and Anti-MBP Antibodies in Patients Treated Daily With Copolymer-1 Doses of 20mg.
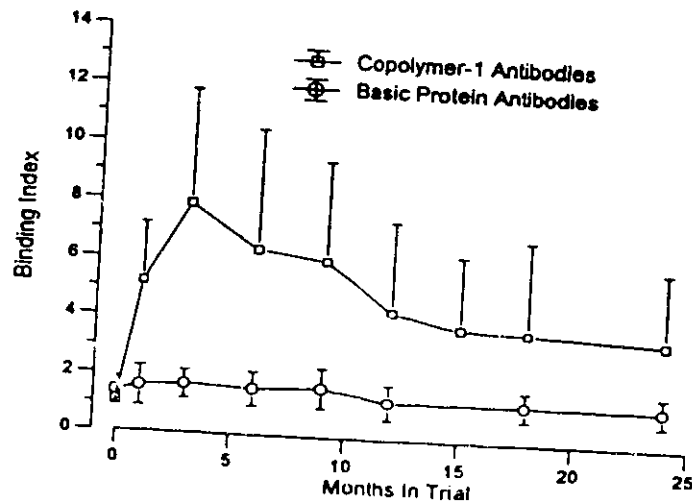
Issue #2: development of "immunological tolerance"

The sponsor examined the ability of human peripheral blood mononuclear cells (PBMC) taken from patients at different times during a 25-month Copolymer-1 clinical trial to proliferate *in vitro* in response to Copolymer-1 or MBP. Data from this study are shown in Figure 2 (NDA page 137 025) below. The sponsor concludes from these data that the proliferative response to Copolymer-1 decreased over time in patients receiving repeated administration of the drug. The sponsor argues that this diminution in proliferative response to Copolymer-1 with time demonstrates that the drug is bioavailable to the peripheral immune system and that this effect may suggest a development of "immunological tolerance". I disagree with this conclusion, in that when one considers the magnitude of the error bars at the earlier time points, one most likely cannot say there is any difference between the stimulation index at these time points and those at the later time points.

To further invalidate these particular data, the sponsor has difficulty explaining why PBMC from patients at time zero who have never been exposed to Copolymer-1 should proliferate to the same level as those from patients receiving Copolymer-1 injections for various times. Apparently, previous *in vitro* studies demonstrated that, for proliferation to occur, antigen-presenting cells are necessary, and those APC and T cells had to have previously been exposed to Copolymer-1 before proliferation would take place. Therefore, these particular clinical data are highly questionable.

The data with respect to myelin basic protein-induced proliferation are consistent with previous studies and support the premise that s.c. administration of Copolymer-1 does not activate the immune system with respect to myelin basic protein.

One important piece of information provided by these studies was the fact that, even at Week 25 of Copolymer-1 administration, response to the PPD antigen was high and remained high throughout the study (data not shown in the submission, only included in narrative). **These data supported the sponsor's contention that Copolymer-1 is not a general immunosuppressant.**

Figure 2.

Proliferation of PBMNC from Copolymer-1-Treated MS Patients in Response to Copolymer-1 and MBP.
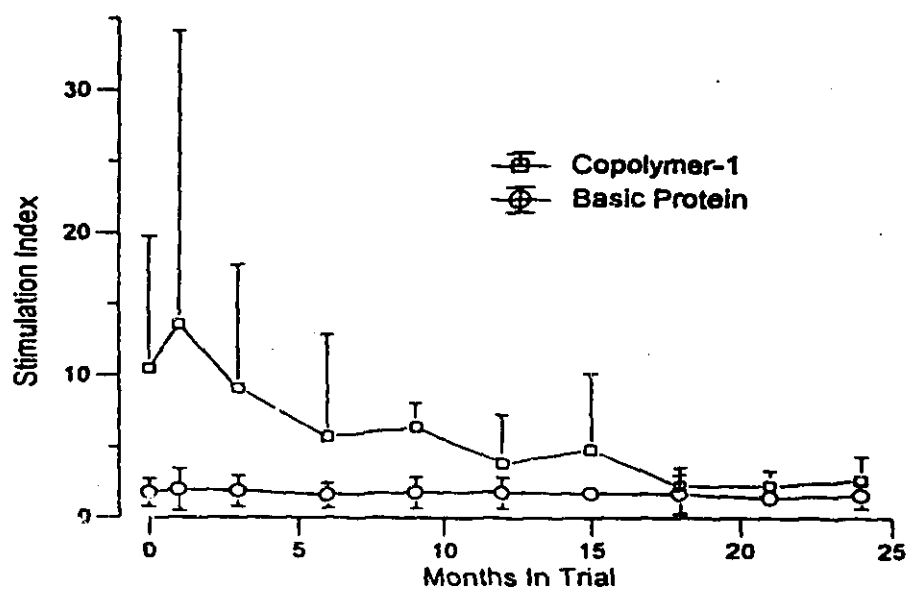
Additionally, the sponsor demonstrated that Copolymer-1 directly induced histamine release from basophils isolated from healthy volunteers and MS patients and that the anti-Copolymer-1 antibodies taken from patients receiving the drug were IgG and not IgE. They concluded from these studies that skin reactions from skin testing with Copolymer-1 done in patients receiving the drug were probably due to Copolymer-1 directly inducing histamine release, and not due to an immunological reaction involving IgE.

Reviewer's comments:

I agree that the drug does not appear to induce IgE production in animals or humans, and therefore Type I hypersensitivity reactions (including anaphylaxis) due to IgE-mediated histamine release may not occur with drug administration. However, the drug apparently directly induces the release of histamine from histamine-containing cells. The sponsor states that, "The antibodies formed are most probably non-IgE and non-neutralizing. Copolymer-1 can induce direct non-immunologic histamine release from various histamine containing cells. This effect, also described in animals and discussed in the Pharmacology section, may underlie the local injection site reactions reported during clinical trials." Therefore, I would argue that there is still the potential for induction of anaphylactoid response due to direct histamine release. Furthermore, animal studies demonstrate local injection site lesions with pathology consistent with Type IV (delayed type hypersensitivity) as well as evidence of possible immune complex deposition (kidney) and disease, a Type III (immune complex) response. Therefore, the absence of IgE antibodies does not rule out the possibility that such local injection site reactions or systemic inflammatory responses might occur in animals or humans.

The sponsor, as an argument that presence of anti-Copolymer-1 antibody production is not responsible for relapse of patients, makes the statement that "Clinically, antibody levels in patients are no different in relapse patients than in those that did not relapse". However, there are other pharmacokinetic and physiological factors in addition to antibody titer that might favor formation of immune complex and deposition in various tissues and organ systems. For example, an increase in the systemic exposure to intact Copolymer-1 drug with time could result in an increase in immune complex formation. This will be discussed further in the toxicokinetics section of this review.

Antigenicity--Preclinical Studies (Mice and Guinea Pigs)

1. An antigenicity study of FPF1300 (Copolymer-1) injection, Study #BOZO/B-2535
   GLP Regs, October 7, 1993.

This GLP study was actually a series of studies of the antigenicity of Copolymer-1 carried out in guinea pigs and mice. The guinea pig is a standard animal for examination of the antigenicity of a compound, as the guinea pig is especially sensitive to hypersensitivity reaction, especially anaphylaxis.

Active systemic anaphylaxis in guinea pigs

Study design: sixty guinea pigs were divided into four experimental and two control groups of 10/group. The experimental groups received 0.33 or 3.3 mg/kg dose of Copolymer-1 with or without Complete Freund's Adjuvant (CFA) as part of the sensitization protocol. Sensitization was carried out with a daily subcutaneous injection of Copolymer-1 for 21 consecutive days, while sensitization with Copolymer-1 + CFA involved 4 s.c. injections with a 7-day interval between injections. The two control groups received either ovalbumin + CFA (positive control) or mannitol with CFA (negative control). The control regimen was administered weekly.

On Day 14 after the final sensitization injection, five guinea pigs from each group, with the exception of the Ovalbumin + CFA group, received an i.v. antigen challenge of Copolymer-1 in a single 3.3 mg/kg dose. The Ovalbumin + CFA treated animals received a 2.0 mg/kg i.v. dose of ovalbumin. The guinea pigs were observed for 30 minutes for clinical signs, and anaphylactic signs were monitored and graded as negative (-); slight (+); moderate (++) or severe (+++).

Results

Following i.v. challenge with Copolymer-1, all Treated guinea pigs exhibited positive anaphylactic reactions characterized by piloerection, nose scratching, sneezing, cyanosis, labored breathing and often, death (See Table 25 below; NDA page 020 131). The anaphylactic response occurred in all Copolymer-1 sensitized animals without regard to presence or absence of CFA in the sensitization mixture. Positive and negative Controls responded as expected.

Table 25: Active systemic anaphylactic reaction in guinea pigs.

Sensitizing Antigen (Dose)	Challenge Antigen (Dose)	Anaphylaxis Grade	Number of Deaths
Copolymer -1 (0.33 mg/kg)	Copolymer-1 (3.3 mg/kg)	+++	5/5
Copolymer-1 (3.3 m/kg)	Copolymer-1 (3.3 mg/kg)	+++	2/5
Copolymer-1 + CFA (0.33 mg/kg)	Copolymer-1 (3.3 mg/kg)	+++	5/5
Copolymer-1 + CFA (3.3 mg/kg)	Copolymer-1 (3.3 mg/kg)	+++	5/5
Ovalbumin + CFA (2.0 mg/kg)	Ovalbumin (2.0 mg/kg)	+++	3/5
Mannitol + CFA (6.7 mg/kg)	Copolymer-1 (3.3 mg/kg)	-	0/5

These data showed that Copolymer-1 is clearly antigenic in the guinea pig test. Furthermore, Copolymer-1 sensitization followed by i.v. administration demonstrated that the drug caused anaphylaxis and death.

Homologous Passive Cutaneous Anaphylaxis

In this guinea pig model, anaphylaxis can be mediated through either IgG or IgE antibodies. To distinguish between the two, the sponsor carried out the homologous passive cutaneous anaphylaxis assay, also in the guinea pig.

This test involved drawing blood from five guinea pigs previously sensitized with Copolymer-1, separating serum, serially diluting, and dividing the sera for two separate tests of homologous passive cutaneous anaphylactic reaction.

For the first test, serum, heated at 56° C for 4 hours, was serially diluted and injected into the dorsal intracutis of naive guinea pigs. After 4-hour sensitization period, the guinea pigs were challenged i.v. with 3.3 mg/kg Copolymer-1 or 2 mg/kg ovalbumin administered in combination with Evans blue dye. 30 minutes later, the guinea pigs were sacrificed and the dorsal area skin abraded and examined for dye exudation. Positive responses were scored when the injection site displayed a consistent 5mm diameter of dye dispersion. This test was interpreted as indicative of an IgG mediated response.

In the second test, unheated serum dilutions in aliquots of 0.1 ml were injected into the dorsal intracutis of naive guinea pigs. After as sensitization period of 8 days, the guinea pigs received intravenously a fresh challenge of Copolymer-1 at 3.3 mg/kg along with Evans blue dye and were examined as described above. Positive responses were scored as above. This test was interpreted as indicative of an IgE mediated response. Results of these tests are shown below in Table 26 (NDA pg. 020 133).

Table 26. Homologous passive cutaneous anaphylaxis (PCA) serum titers in guinea pigs.

Group	Antigen for Sensitization (Dose)	Antigen for challenge (Dose)	No of donor animals	PCA titers[a] (Responders[b])	
				4 hours[c]	8 days
1	Copolymer-1 (0.33 mg/kg)	Copolymer-1 (3.3 mg/kg)	5	<5(2) 25(1) 125(2)	<5(5)
2	Copolymer-1 (3.3 mg/kg)	Copolymer-1 (3.3 mg/kg)	5	<5(4) 5(1)	<5(1)
3	Copolymer-1 + CFA (0.33 mg/kg)	Copolymer-1 (3.3 mg/kg)	5	25(4) 625(1)	<5(5)
4	Copolymer-1 + CFA (3.3 mg/kg)	Copolymer-1 (3.3 mg/kg)	5	<5(1) 25(3) 625(1)	<5(5)
5	OVA + CFA (2.0 mg/kg)	OVA (2.0 mg/kg)	5	3125(4) 5625(1) 78125(1)	D(5)
6	Mannitol + CFA (6.7 mg/kg)	Copolymer-1 (3.3 mg/kg)	5	<5(5)	<5(5)
		OVA (2.0 mg/kg)	5	<5(5)	<5(5)

[a] Highest dilution for given animal showing dye exudation to positive criterion.
[b] Number of animals with positive results at listed highest dilution; D=Deaths.
[c] heat inactivated serum

Results

4 of 5 guinea pigs sensitized at the low Copolymer-1 dose (0.33 mg/kg) and challenged with Copolymer-1 responded with titers of 25-125, and animals sensitized with low (0.33 mg/kg) or high (3.3 mg/kg) Copolymer-1 doses plus CFA responded to i.v. challenge with Copolymer-1 with high titers in the 25-625 range, all with 4 hours of sensitization (indicative of IgG response). None of the animals sensitized for 8 days (IgE response) responsed with increased titers. Positive and negative Controls responsed appropriately.

These data indicated that the guinea pigs were responding to sensitization and subseqent i.v. challenge with Copolymer-1 with the production of IgG, which mediated the anaphylactic response, and not IgE. The sponsor stated that clinical data gave similar results, that patients were responding to repeated Copolymer-1 administration by producing IgG antibodies, but not IgE antibodies.

The sponsor concluded that, in guinea pig, as in man, antibody response to Copolymer-1 was in the form of IgG, not IgE. In guinea pig, IgG1 can also mediate anaphylaxis, while in man, only IgE has been implicated in anaphylactic response.

Reviewer's Comments

These data demonstrate that Copolymer-1 is antigenic, and that antigenicity most likely results in IgG, but not IgE production in the guinea pig. These data do not, however, rule out the possibility that IgG production in man due to Copolymer-1 administration might mediate Type III hypersensitivity in the form of local injection site reactions and organ specific or systemic immune complex disease symptomology.

Immunotoxicology

The sponsor has set aside a separate section, entitled "Immunotoxicology", in which they reiterated the "antigenicity" and "immunotoxicology" study data taken from three animal studies previously presented in the "Subchronic Toxicology Section" of of this NDA submission. These studies included the 26-week rat study (#1028/18-1050), 28-day Cynomolgus monkey study (#1028/21-1050) and the 52-week Cynomolgus monkey study (#1028/26-1050). For review of these "antigenicity" and "immunotoxicology" data, please see the appropriate sections of this review under the "Subchronic Toxicology Section."

Possible Contaminants and Impurities

Three additional preclinical studies were implemented to examine the toxic potential of possible contaminants of Copolymer-1 as outlined in the following Table 28 (NDA pg. 020 137).

Table 28. Toxic Potential of Possible Contaminants

Species	Strain	Group (M/F)	Cop-1 Dose (Mg/kg)	Route	Duration of Treatment	Lab	Study #
Rat	Charles River CD, Sprague Dawley	5/5	40[f]	I.V.	SD	LSRI	TEV/078/COP (029 045)
Rat	Charles River, Sprague Dawley	2/2 5/5 5/5 5/0 5/5	0 40[g] 200[g] 40 200	I.V.	SD	LSRI[e]	TEV/050/COP (021 093)
Rat	Sprague Dawley	5/5 5/5 5/5 5/5 5/5	0 40 200 40[h] 200[h]	I.V.	SD	TEVA[e]	B37/2/93 (021 144)

[a] M/F = male/female
[b] IV = intravenous
[c] Lab = laboratory where study was conducted: LSRI = Life Science Research Israel Ltd., Ness Ziona, Israel; TEVA = TEVA Pharmaceutical Industries, Ltd, Netanya, Israel.
[d] SD = single dose
[e] Performed according to FDA regulations.
[f] Contained 0.73% fluorine contaminant
[g] 40 and 200 mg/kg of bromo-Copolymer-1.
[h] 40 and 200 mg/kg of Batch Number 06492 (rejected batch containing 0.28% of an unidentified impurity).

1. "High-fluor COP-1", acute intravenous toxicity study in rats, Study

December 6, 1990. Batch # RE 7159/3.

Study Description: Charles River CD, Sprague-Dawley rats, five/sex/group, were treated with a single i.v. injection of 40 mg/kg Copolymer-1 in saline containing 0.73% fluorine. The fluorine was present in an intermediate compound (TFA-Copolymer-1) formed during synthesis and was intentionally not removed. The limit of existing production specifications for Copolymer-1 at the time of this study was 0.2% fluorine. Rats were observed for 15 days, after which they were sacrificed and necropsies peformed.

Results: Mortality, clinical symptoms and macropathology were observed. There were no toxicological effects reported.

2. COP-1 and its impurity: acute intravenous toxicity study in rats, Study November 19, 1989, Batch #99020/II (Bromo-Copolymer-1).

Study Description

The acute intravenous toxicity of COP-1 and its impurity (Bromo-COP-1) was investigated in 4 groups of 5 male and 3 groups of 5 female Sprague-Dawley rats eath at doses of 0 (Control, doses with saline), 40 and 200 mg/kg. The test materials were administered as a solution in physiological saline. Mortality and other signs of reaction to treatment were observed for 14 days after dosing. Animals were necropsied and macroscopic pathology done.

Results

Two male rats receiving 200 mg/kg Cop-1 died during the study. Three male and four female rats dosed with Bromo-Copolymer-1 also died at 200 mg/kg drug as the result of treatment (See Table 1 below, page 021 106 of NDA). All deaths occurred within 3 hours of dosing.

Symptoms associated with death due to 200 mg/kg Copolymer-1 included ataxia and pigmented stain of snout prior to death. With Bromo-Copolymer-1 animals also presented with unconsciousness, prone posture, bradyapnea, gasping and cyanosis. Among the surviving rats receiving 40 mg/kg, the most common symptom was tremor.

Changes in macroscopic pathology associated with i.v. administration of 200 mg/kg Copolymer-1 included hepatic greyish discoloration and pulmonary hemorrhage. With i.v. administration of 200 mg/kg of Bromo-Copolymer-1, macroscopic lesions included serosal congestion of various intestinal segments; gastric mucoid congestion; duodenal, jejunal and pulmonary congestion, hemorrhages and slight splenic enlargement associated with darkening. Most surviving treated rats had reduced body weights during the observation period.

Conclusions

The sponsor stated that the acute intravenous median lethal dose (LD_{50}) of Copolymer-1 is greater than 200 mg/kg, and that of Bromo-Copolymer-1 is between 40 and 200 mg/kg. The sponsor also concluded that both Copolymer-1 and Bromo-Copolymer-1 at 40 mg/kg were non-toxic. They also concluded that, at doses of 200 mg/kg, Bromo-Copolymer-1 was more toxic than Copolymer-1 alone.

Table 1. Mortality in groups of male and female rats given a single i.v. dose of COP-1 and Bromo-COP-1 in saline at a volume-dosage of 10 ml/kg.

Dose level (mg/kg)		Male	Mortality Female	Combined
Saline	0	0/2	0/2	0/4
COP-1	40	0/5		0/5
	200	2/5	0/5	2/10
Bromo-				
COP-1	40	0/5	0/5	0/10
	200	3/5	4/5	7/10

Reviewer's Comments: It would appear that Bromo-Copolymer-1 is more toxic than Copolymer-1. Therefore, the specifications for bromo- contamination of the drug preparation should be as low as physically possible.

3. Comparative study in rats of the acute toxicity of two batches of COP-1 drug substan
GLP, May 12, 1993.

Study Description
50 rats (25 males, 25 females) were divided into five groups of 5/sex/group. Animals were administered either Control (sterile physiological saline) or a single i.v. dose of 40 or 200 mg/kg of Copolymer-1 Batch No. 00593 (a standard released batch) or Batch No. 06492 (a rejected batch containing 0.28% of an unidentified impurity) administered in sterile physiological saline. Study drug was administered into the lateral caudal vein. Rats were observed ato 30-60 minutes, 1 hour, and 4 hours after injection, and thereafter underwent clinical examinations at least once daily for 14 days. Animals were then necropsied, and macroscopic abnormalities were recorded and heart, lung, liver, kidney, thymus, spleen, brain and adrenals were weighed. The purpose of this study was to assess the potential toxic effects in rats of two batches of Copolymer-1 (one released and one rejected) following a single i.v. injection of 40 or 200 mg/kg.

Results
There were no deaths among the rats receiving 40 mg/kg. However, two female rats receiving 200 mg/kg of Copolymer-1 Batch No. 06492 and one male receiving 200 mg/kg of Copolymer-1 Batch No. 00593 died within 2 hours of dosing. Symptoms in the rats that died included ataxia, bradyapnea, gasping, cyanosis, lacrimation, decrease in spontaneous activity, and apathy. These symptoms appeared immediately upon injection of COP-1 from either Batch. In the surviving rats receiving 200 mg/kg of either Batch of drug, tremor, lacrimation, apathy and decrease in spontaneous motor activity were observed. In surviving rats receiving

both Batches of drug, a statistically significant loss of body weight was observed.

Statistically significant increases in kidney weight, compared to Controls, were seen for male and female rats receiving 200 mg/kg of Copolymer-1 from both Batches. Female rats in both treatment groups receiving Batch No. 00593 and female rats that received 40 mg/kg of Batch No. 06492 had statistically significant increased in liver weight compared to Controls.

Conclusions

40 mg/kg i.v. of either the accepted or the rejected Batches of drug product did not produce any toxic effects in Sprague-Dawley rats. A single i.v. administration of 200 mg/kg of Copolymer-1 caused 10% mortality for rats treated with Batch No. 00593 and a 20% mortality for rats treated with Batch No. 06492. The only difference in effects of administration of the two batches of drug product was a lower weight gain in female rats that received 200 mg/kg of Batch No. 06492 (the rejected batch).

Reviewer's Comments: The purpose of this study is somewhat vague, as the sponsor never reveals the identity or the incidence of finding this contaminant in various Batches of product. However, based on the female animal weight data, it does appear that the "rejected Batch No. 06492" may be somewhat more toxic than the batch meeting standard specifications.

Reviewer's Comments with respect to studies involving Copolymer-1 contaminants:

The contamination of Copolymer-1 with the bromo- compound appears to increase the toxicity of the product, as per the LD_{50} data in rats. It is unclear whether or not fluorine at 0.78% contaminant increased the toxicity of the drug. The data for female animal weights suggest that presence of the "unidentified contaminant" in the drug substance increases Copolymer-1 toxicity. The specifications for bromo-contaminant and this "unidentified contaminant" should be set as low as physically possible.

Mutagenicity

Following is Table 30 (NDA pg 020 151), which summarizes the mutagenicity studies included in this NDA:

Table 30. Mutagenicity Results

Test	Species/Cells	Results	Lab *	Report Number/Appendix
In Vitro				
Mutation frequency (AMES)	S. Typhimurium/E.coli	Negative	HM	2E6RETIP.001 Vol. 035 007
Mutation frequency	Mouse/lymphoma cells	Negative	HM	2TKRETIP.001 Vol. 035 070
Chromosomal changes	Human/lymphocytes	Positive	HM	1HLRETIP.001 Vol. 035 118
Chromosomal changes	Human/lymphocytes	Positive	HM	1HL2RETIP 001 Vol. 035 164
In Vivo				
Chromosomal changes	Mouse/micronucleate Erythrocytes	Negative	HM	WWMRETIP.001 Vol. 021 045

* Lab = laboratory where study was conducted; HM = Hazleton Microtest, York, UD; performed according to UK regulations.

1. Study to determine the ability of COP-1 to induce mutation in four histidine-requiring strains of Salmonella Typhimurium and two tryptophan-requiring strains of Escherichia Coli, Study
August 7, 1991, UK GLP Regs. Batch #19007/1 of Copolymer-1 was used in these studies.

Study Description

The sponsor carried out three separate AMES tests, using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 (histidine-requiring strains) and *Escherichia coli* strains WP2 pKM101 and WP2 uvrA pKM101 (tryptophan-requiring strains) to examine the mutagenic potential of Copolymer-1. To determine toxicity levels, a range-finding experiment was first performed with the *Salmonella* strain TA100 in which COP-1 ranged from 8µg/plate to 5000µg/plate in the presence or absence of S-9. From this preliminary experiment, five different Copolymer-1 concentrations were chosen for each of the three mutation experiments. The experiments were performed in the presence and absence of S-9 fraction.

Experiment #1 used 4, 20, 100, 500 and 2500 µg/plate of Copolymer-1 and Experiment 2 used 156.25, 312.5, 625, 1250 and 2500 µg/plate. Experiments 1 and 2 used the above-mentioned bacterial strains, while in experiment #3, designed to test the repeatability of a positive response found in Experiment 2 +S-9, 100, 200,

300, ·   ,0 µg/plate of Copolymer-1 was used, and only *Salmonella typhii*.   .train TA98 was tested.

For all assays, bacteria were cultured for 10 hours at 37°C. in nutrient broth (containing ampicillin for *S. Typhimurium* strains TA98 and TA100 and for both *E. Coli* strains). Since the results of the first experiment were negative, treatments in the presence of S-9 in Experiments 2 and 3 included a pre-incubation step in which the Copolymer-1 or control solutions were incubated for 1 hour at 37°C in the presence of bacteria and S-9 mix before adding molten agar at 46°C. All plates were then incubated in the dark for three days at 37°C.

Details of the AMES testing are shown in the following table:

Table: Summary of AMES testing.

Test Substance	Metabolic Activator (S-9)	Concentration (µg/plate)	Strains Used
Range-finding study Copolymer-1	+/-	8, 40, 200, 1000, and 5000	*Salmonella typhimurium* TA100
Negative control Water	+/-	NAP*	
Experiment 1 Copolymer-1	+/-	4, 20, 100, 500, 2500	*S. Typhimurium* TA98, TA100, TA1535, TA1537, *E. Coli* strain WP2 pkM101, WP2 uvrA PKM101
Experiment 2 Copolymer-1	+/-	156.25, 312.5, 625, 1250, 2500	*S. Typhimurium* TA98, TA100, TA1535, TA1537, *E. Coli* strain WP2 pkM101, WP2 uvrA PKM101
Experiment 3 Copolymer-1	+/-	100, 200, 300, 400, 500	*S. Typhimurium* strain TA98
Positive Control			
2-Nitrofluorene	-	50	*S. Typhimurium* strain TA98
Sodium Azide	-	2.0	*S. Typhimurium* strain TA100, TA1535
9-amin. acridine	-	50	*S. Typhimurium* strain TA1537
4-nitroquinoline 1-oxide	-	2.0	*E. Coli* WP2 pkM101, WP2 uvrA pKM101
2-aminoanthracene	+	5.0	At least one strain.

* NAP = not applicable
ᵇ Controls were not used for the range-finding experiment.

Results

The sponsor concludes that, "all Copolymer-1 treatments, with the exception of TA98 in the presence of S-9 in Experiment 2, failed to produce a statistically significant increase in revertant numbers." The sponsor carried out two separate Ames test experiments, and while all responses in all test strains were negative in the first experiment, in the second experiment in the +S-9 testing group with *Salmonella*

typhimurium strain TA98, a statistically significant (p≤0.01) positive response was reported (See Table below) in the samples treated with 312.5 μg/plate Copolymer-1. I would not even consider this a positive response by the F.D.A. criteria for a positive response in this assay, which includes "a response at least two-fold greater than the Control response" and "an increased response that is dose-related in nature". However, the sponsor was sufficiently concerned to repeat the experiment in this concentration range a third time in strain TA98. As can also be seen in the following table, the results were negative for this strain +S-9 in this concentration range of Copolymer-1. Positive and negative Controls responded appropriately in all cases.

Therefore, the overall conclusion is that Copolymer-1 tested negatively in the Ames test, and therefore is not mutagenic by this test.

Table: Summary of Three Experiments Involving Ames Testing of Copolymer-1 in *Salmonella typhimurium* strain TA98 +S-9

Experiment #	Concentration (μg/plate)	Number of Revertants (mean)
1	0	21.0
	4	22.0
	20	19.0
	100	20.3
	500	23.3
	2500	21.0
2	0	29.8
	156.25	37.3
	312.5	43.0*
	625.0	28.7
	1250.0	30.7
	2500.0	34.0
3	0	16.8
	100	21.0
	200	18.0
	300	11.0
	400	14.7
	500	17.0

* Statistically significant at p≤ 0.01.

Reviewer's Comments:

I would agree that Copolymer-1 tested negative in the Ames test. The sponsor should have done a range-finding study in each individual test strain to determine appropriate concentrations of drug to use in the assay, rather than doing the dose-ranging study in strain *S. typhimurium* strain TA100 only. However, this oversight was outweighed by the fact that the sponsor used concentrations of Copolymer-1 in the experiments that ranged up to 2500 μg/well, which should certainly be adequate to detect mutagenicity of the drug.

2. Study to determine the ability of Copolymer-1 to induce mutations at the thymidine kinase (tk) locus in mouse lymphoma L5178Y cells using a fluctuation assay, Stud, ... **February 14, 1992, UK GLP Regs.** Batch # 19035/A of Copolymer-1 was used.

Study Description

The purpose of this study was to assess the potential mutagenic activity of Copolymer-1 by examining its ability to induce TK mutations in L5178Y cells in the absence and presence of a rat liver metabolizing system (S-9). Initial range-finding studies resulted in the choice of maximum concentrations of 1000μg/ml in the absence of S-9 and 1250μg/ml in the presence of S-9. Five lower doses were also selected. Apparently precipitation was seen at the top dose (1250μg/ml) +S-9 only and high toxicity was evident a the top doses, with yields of only 1.9% relative survival (1000μg/ml) and 4.9% (1250μg/ml) relative survival, in cultures without and with S-9, respectively.

The evaluation criteria with respect to determination of whether or not the drug is mutagenic included the following:
1. The assay was valid.
2. The mutant frequency at 1 or more doses was significantly greater than that of the negative control.
3. There was a significant dose-relationship as indicated by the linear trend analysis.
4. The effects described above were reproducible.

Results

Following treatment with Copolymer-1, there were no increases in mutant frequency observed in Experiment 1 or Experiment 2 at any concentration level in presence or absence of S-9 that would suggest that the drug is mutagenic. Positive and negative Controls responded appropriately, indicating that the assays were valid.

3. Study to evaluate the chromosome damaging potential of COP-1 by its effects on cultured human lymphocytes using an *in vitro* cytogenetics assay, Stud **GLP Regs, August 12, 1991.** Batch #19007/1.

----and corresponding study

4. Study to evaluate the chromosome damaging potential of COP-1 by its effects on cultured human lymphocytes using an *in vitro* cytogenetics assay, Study **GLP Regs, February 20, 1992.**

Study Description

The objective of these studies was to assess the clastogenic potential of Copolymer-1 by examining its effects on the chromosomes (chromosomal aberrations) of the lymphocytes of a single human donor, cultured *in vitro* and treated with the drug in the absence and presence of a rat liver metabolizing system (S-9). Study   . to determine whether or not a positive response was repeatable. In the first study, dose levels up to 5000µg/ml were used. This dose level is considered to be an adequate top concentration for determination of clastogenicity of a drug in this assay. Treatments covering a broad range of doses separated by narrow intervals, were performed both in absence and presence of metabolic activation by a rat liver S-9 fraction (from Aroclor 1254-induced animals). The first experiment was carried out with treatment periods of both 20 and 44 hours. The second experiment included doses up to only 524 µg/ml, and only the 20 hour treatment point was examined. Appropriate negative (solvent) and positive (4-nitroquinoline 1-Oxide (NQO) and cyclophosphamide) were employed as part of the assays.

Results

Results of these two experiments are shown in Tables 27 and 28 shown below.

Table 27. Summary of data from human lymphocyte assay experiment #1.

CELLS WITH STRUCTURAL ABERRATIONS				
Test substance (μg/ml)	# of cells studied	Cells with aberrations Including gaps	Cells with aberrations Excluding gaps	Mitotic index[a] (Mean)
Without S-9				
20-Hour Sampling				
Negative Control				
(Saline)	200	7	3	7.0
Copolymer-1				
245.1	200	7	4	5.0
377.1	200	6	4	5.1
580.1	200	4	1	3.2
Positive Control				
4-nitroquinoline				
1-oxide, 5.0	50	18	17[b]	NP
44-Hour Sampling				
Negative Control				
(Saline)	200	5	5	7.5
Copolymer-1				
580.1	200	12	7	3.3
With S-9				
20-Hour Sampling				
Negative Control				
(Saline)	200	6	1	3.8
Copolymer-1				
580.1	200	8	3	3.5
892.5	200	8	2	2.5
1373	200	11	7[c]	1.6
Positive Control				
Cyclophosphamide				
25.0	50	18	16[b]	NP
44-Hour Sampling				
Negative Control				
(Saline)	199	4	3	4.9
Copolymer-1				
1373	200	4	3	3.6
[a] NP = Not performed. [b] Significantly different from control, p≤0.001. [c] Significantly different from control, p≤0.05.				

Table 28. Summary of data from human lymphocyte assay experiment #2.

CELLS WITH STRUCTURAL ABERRATIONS				
Test Substance (μg/ml)	Number of Cells Studied	Cells with aberrations including gaps	Cells with aberrations Excluding gaps	Mitotic index (Mean)
Without S-9				
Control	200	2	2	4.8
Copolymer-1				
288.4	200	4	2	4.2
355.5	200	2	1	3.8
419.4	190	3	2	1.8
Positive Control 4-nitroquinoline				
1-oxide, 5.0.	50	35	31	-
With S-9				
Control	200	4	1	5.8
Copolymer-1				
335.5	200	6	2	2.2
419.4	165	4	3	2.0
524.3	172	10	6*	2.2
Positive Control Cyclophosphamide				
25.0	50	17[b]	14	-
* significantly different from control, p≤0.05. [b] significantly different from control, p≤0.001.				

Results of the first experiment (Table 27 above) demonstrated a positive response at 1373 μg/ml Copolymer-1 in the presence of S-9 at the 20 hour treatment sampling time. This response of 7 "cells with aberrations excluding gaps" would be considered by the F.D.A. to be a positive response because it is 7-fold greater than the Negative Control response of 1. The criteria for a positive is that the response must be "at least two-fold higher than the Control" and "constitute a dose-related response. This response at 1372 μg/ml in presence of S-9 is 7-fold higher than Negative Control, and the response is dose-related in appearance.

The sponsor concludes that this positive response is "not of toxicological importance, because no statistically significant differences were obtained (p<0.05) following analysis of cultures treated at the same concentration but harvested after 44 hours...".

Reviewer's Comments:

I disagree with this conclusion. Normally, a positive response at the 20-hour time point is sufficient to conclude that the drug is clastogenic, and the 44-hour time point is only used as a further test for clastogenicity when the results at 20 hours are

negative and there is a suspicion that the test drug might prolong the mitotic interval and cause the assay to miss an effect at 20 hours. In fact, the 44-hour time point is normally only utilized in this assay when it is suspected that the tested chemical will prolong the mitotic interval. Therefore, I must conclude from the data in Experiment #1 that Copolymer-1 is clastogenic.

The sponsor completed Experiment #2 to determine if this result was repeatable. In this experiment, however, the sponsor chose to include a maximum concentration of 524.3 µg/ml, although the positive response in the previous experiment occurred at 1373 µg/ml. As shown in Table 28 above, a positive response was seen at the 524.3 µg/ml concentration, again in the presence of S-9. The sponsor again chose to conclude that "...no toxicological importance was attached to this observation since increases were small (normal range was exceeded only ba a single aberrant cell) and not reproducible (effect was seen in only one of two replicate culture; the 6 is a mean value)...".

Reviewer's Comments:
I must disagree with the sponsor's conclusions regarding the clastogenicity of Copolymer-1 in the human lymphocyte assay. First of all, since the positive response occurred at 1373 µg/ml Copolymer-1 in presence of S-9 in the first experiment, it would seem logical that the second experiment should have also included this drug concentration. Second, their data demonstrate positive responses (by the F.D.A. criteria) in two experiments in cultures in presence of S-9 in the dose range of 534.3-1373 µg/ml. As seen in Tables 27 and 28 above, these responses are both "at least two-fold greater than Negative Controls" and "are dose-related in nature." It is my opinion that their explanations are not sufficient to invalidate these positive findings, and **I must conclude based on these data that Copolymer-1 is clastogenic in the human lymphocyte assay.**

5. Study to evaluate the potential of COP-1 to induce micronuclei in the polychromatic erythrocytes of CD-1 mice, Stuc_
GLP Regs, January 14, 1992.

Study Description
The purpose of this study was to assess the potential clastogenicity of Copolymer-1 *in vivo* by examining micronuclei in polychromatic erythrocytes (PCE) of mouse bone marrow. The study consisted of a range-finding study and a micronucleus study. For the micronucleus study, 10 mice/sex/group were administered either negative control (saline, 0.9% NaCl) or a Copolymer-1 dose of 35 or 70 mg/kg/day in saline. 14 male and 15 female mice received a Copolymer-1 dose of 140 mg/kg and 5 male and 5 female mice received the positive control (40 mg/kg cyclosphophamide). The mice were administered an i.p. injection of study drug on two consecutive days. Animals were sacrificed after 24 or 48 hours, femurs removed, and bone marrow smears prepared and stained for determination of

polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE). At least 1000 total cells per slide were analyzed.

Results

In the range-finding experiment, the LD_{50} was found to be about 232 mg/kg. Ths sponsor stated that a dose equivalent to 50-80% of the LD_{50} is considered to be acceptable as a maximum dose level, so 140 mg/kg (60%) was chosen as an appropriate upper dose level for the micronucleus study. The following Table 4 (NDA pg. 035 204) shows results of the micronucleus test.

Table 4. Group mean frequency of micronucleated polychromatic erythrocytes.

Study Drug	Group Mean Frequency of Micronucleated PCE (per 1000 Cells)
24 hours	
Negative Control (saline)	1.09
Copolymer-1	
35	0.65
70	0.70
140	0.45
Positive Control	
Cyclophosphamide	
(40 mg/kg)	14.99
48 hours	
Negative Control (saline)	0.54
Copolymer-1 (mg/kg)	
35	0.74
70	0.99
140	0.45

Results at both 24 and 48 hours were negative when compared to Negative Controls. At the 70 mg/kg dose at 48 hours, the value of 0.99 micronucleated PCE per 1000 cells is somewhat higher than the Negative Control, but this is, as the sponsor claims, due to a single outlier out of an n of 10 animals. Therefore, the sponsor concludes that this is a negative response.

Reviewer's Comments:

I agree with the sponsor's conclusion that the data are negative for the mouse micronucleus assay. They are correct in stating that the value of 0.99 at the 70 mg/kg dose at 48 hours is somewhat larger than Control because of a single outlier. I agree that these data constitute a negative response, consistent with values at the other Copolymer-1 doses.

Carcinogenicity
Planned and ongoing carcinogenicity studies

Rat carcinogenicity study
According to the sponsor, carcinogenicity studies are ongoing in the rat and the mouse. The ongoing carcinogenicity study in the rat includes the use of 50 animals/sex/group and doses of Copolymer-1 of 0, 3, 7.5, 15 and 30 mg/kg/day to be administered by the s.c. route of administration. The study is being carried out in the GLP Guidelines.

According to the sponsor, 180 rats from this group will be used in an immunohistochemical satellite study. The study was apparently started in January of 1995 and will be completed in January of 1997.

Mouse carcinogenicity study
The ongoing study in mice began on August 8, 1995 at the
Mice are being treated by s.c. injection with 0, 3, 15, 30 or 60 mg/kg/day Copolymer-1. In a preliminary report (INL , Ser. No. 224, 12-01-95), the Sponsor states that during the first 14 weeks of the study, 61 of a total of 720 mice in the study died. All of these animals except for two were in the Copolymer-1 treated group. A large proportion of these animals (62%) apparently died within about 5 hours of receiving an injection of drug. At necropsy, apparently the most consistent findings were in the injection site, vasculature and hematopoietic system. Histopathologically, in the kidney, lung and liver of some of the decedent animals there was a generalized vascular congestion that was not present in the untreated animals. The two expert consultants hired by the Sponsor to review the necropsy data determined that the probable cause of these deaths was Type 1 hypersensitivity reaction. They concluded that, since no such findings occurred in the rat carcinogenicity study, this effect was fairly species-specific.

Reviewer's comments:

Without toxicokinetics data for the mouse, it is impossible to determine whether or not these mice received systemic exposure to intact drug, or for how long. Such exposure would promote immune complex formation and could also induce histamine release. The maximum dose of 60 mg/kg/day in the mouse is about 17-fold higher than the human dose of 20 mg/day in the human, on a mg/m^2 basis.

Tumor incidence in the various toxicology studies to date

Although none of the toxicology studies to date were designed as carcinogenicity studies, the sponsor prepared a section in the NDA in which they reported the incidence to tumor formation in the various animal toxicology studies carried out to date in support of this NDA. The sponsor states that 226 rats, 23 dogs and 32 monkeys were treated with Copolymer-1 in chronic and sub-chronic toxicity studies and 334 rats and 68 rabbits were treated with Copolymer-1 in reproduction studies. They do not differentiate the route of administration or dose of drug among these various studies.

They state that only two types of neoplastic lesions have been reported in animals from these studies to date, malignant mammary epithelial tumors in female CR/CD Rats (segment I reproductive toxicology study   and oral cavity papillomas in Beagle dogs (subchronic study in dogs   They basically conclude that these tumors were not related to drug treatment. Overall, they conclude that, "although one carcinogenicity study is ongoing with Copolymer-1, other available data do not appear to provide evidence for carcinogenic potential."

Reproductive Toxicology

See attached review by Dr. Edward Fisher.

TOXICOKINETICS

Pharmacokinetics studies usually include a single administration of drug (possibly at a number of different doses) to naive animals and examination of plasma-time concentration curves over a period of 24-72 hours related to that single administration. Toxicokinetics studies are usually carried out for the purpose of determining the exposure of study animals to the study drug during the course of a multiple-dosing toxicology study. Therefore toxicokinetics studies usually include examination of a number of different sets of 24-72 hour plasma-concentration time curves, constructed from data when drug is administered at various times during the course of a given toxicology study. In addition to examining the usual PK parameters (T_{max}, C_{max}, AUC) in naive animals, toxicokinetics studies also provide information pertaining to whether or not the pharmacokinetics of a drug change with multiple administration over time.

In this NDA submission, the majority of the studies in which pharmacokinetics were determined included only single-dose administration. Only a single study, done in Sprague-Dawley rats, was carried out as a true toxicokinetics study using multiple-dosing administration. Following is a review of that study:

1. (^{125}I)-Copolymer-1: pharmacokinetics of (^{125}I)-COP-1 and/or radiolabelled metabolites during chronic subcutaneous administration to the rat,
GLP, January 1995.

Objective: To determine the systemic availability, metabolic disposition, and retention of ^{125}I-Copolymer-1 at the injection site and in the carcass after chronic subcutaneous administration of nonradiolabelled Copolymer-1 to rats and to compare the results to those in naive rats.

Study description:
Sixty male and sixty female rats were included in this satellite study as part of a main 26-week toxicity study. The 120 rats were divided into four groups of 15/sex/group, one group receiving saline (0.9% NaCl) and the remaining three groups receiving one of three doses of nonradiolabelled Copolymer-1: 3, 10 or 30 mg/kg/day in saline (0.9% NaCl). The animals received s.c. injections of study drug daily into one of four sites, the left and right shoulder and above the left and right thigh. The injection sites were rotated daily, commencing with the left shoulder.

The four groups were further divided into three cohorts of 5/sex/group, receiving nonlabelled Copolymer-1 injections for 0, 28 or 177 days. These rats were administered a single s.c. dose of ^{125}I-Copolymer-1 on Day 0 (cohort 1), Day 29 (cohort 2) or Day 178 (cohort 3). The radioactive dose was included in the respective nonradiolabelled dose for the groups receiving Copolymer-1. In the case of Control animals, a dose of 3 mg/kg of ^{125}I-Copolymer-1 was given.

Following administration of radiolabelled drug, an aliquot of blood was sampled from a lateral caudal vein at 2, 5, 10, 20, and 30 minutes and 1, 2, 4, 6, and 8 hours postdose. Following measurement of radioactivity in the whole sample, plasma was precipitated with an equal volume of 25% TCA, and the radioactivity was determined in both supernatant and precipitate (pellet).

Results:

Results of this toxicokinetics study are given in the following tables 7.5, 7.6 and 7.7:

Table 7.5: Summary of plasma and pellet radioactivity PK parameters following a single (^{125}I-COP-1 dose to rats - Cohort 1 (naive animals)).

Dose group	Dose level (mg/kg)	Sex	Matrix	C_{max} (µg equiv/ml)	T_{max}[3] (h)	$AUC_{(0-t)}$ (µg equiv.h/ml)	T-1/2 (h)
A	3	Male	Plasma	5.130[2]	2.0	56.30[2]	6.768
B	3	Male	Plasma	5.684	2.0	69.16	9.159
C	10	Male	Plasma	20.06	2.0	235.9	7.900
D	30	Male	Plasma	54.41	2.0	642.3	6.654
A	3	Male	Pellet[1]	1.814[2]	2.0	22.26[2]	8.987
B	3	Male	Pellet[1]	2.955	2.0	37.78	10.06
C	10	Male	Pellet[1]	10.14	2.0	126.3	9.004
D	30	Male	Pellet[1]	24.3	2.0	293.5	8.047
A	3	Female	Plasma	5.540	2.0	66.46	6.680
B	3	Female	Plasma	6.043	2.0	65.74	5.884
C	10	Female	Plasma	20.90	2.0	258.3	6.922
D	30	Female	Plasma	60.92	2.0	719.6	6.030
A	3	Female	Pellet[1]	2.836	2.0	34.95	8.000
B	3	Female	Pellet[1]	2.219	2.0	19.95	6.780
C	10	Female	Pellet[1]	11.85	2.0	159.0	7.609
D	30	Female	Pellet[1]	24.74	2.0	314.9	7.886

1 = Plasma precipitated with 25% (w/v) TCA. Pellet = (Total - supernatant) radioactivity.
2 = Values multiplied by 1.36 to compensate for a systematic error in the dose administered.
3 = Median

Table 7.7: Summary of plasma and pellet radioactivity PK parameters following a single (^{125}I-COP-1 dose to rats - Cohort 3 (177 days treatment with COP-1)).

Dose group	Dose level (mg/kg)	Sex	Matrix	C_{max} (µg equiv/ml)	T_{max}[2] (h)	$AUC_{(0-∞)}$	T-1/2 (h)
A	3	Male	Plasma	5.476	2.0	72.81	10.04
B	3	Male	Plasma	5.871	2.0	66.94	9.618
C	10	Male	Plasma	18.91	2.0	280.2	16.15
D	30	Male	Plasma	51.33	2.0	787.7	10.32
A	3	Male	Pellet[1]	2.874	2.0	39.86	10.99
B	3	Male	Pellet[1]	3.017	1.0	37.44	10.99
C	10	Male	Pellet[1]	11.05	2.0	169.3	22.25
D	30	Male	Pellet[1]	29.76	3.0	476.1	11.68
A	3	Female	Plasma	7.615	2.0	101.8	9.289
B	3	Female	Plasma	6.925	2.0	92.32	8.954
C	10	Female	Plasma	21.94	2.0	266.3	7.905
D	30	Female	Plasma	60.11	2.0	872.0	12.03
A	3	Female	Pellet[1]	4.112	4.0	55.35	10.85
B	3	Female	Pellet[1]	3.812	2.0	53.10	10.53
C	10	Female	Pellet[1]	14.10	2.0	162.4	9.153
D	30	Female	Pellet[1]	36.15	2.0	510.5	13.10

1 = Plasma precipitated with 25% (w/v) TCA. Pellet = (total - supernatant) radioactivity.
2 = Median

Levels of total plasma radioactivity represent intact Copolymer-1 and other high and low molecular weight degradation products and free iodide. Metabolism data suggest that the majority of the total radioactivity at the earlier time points probably consists mainly of degradation products. Plasma levels of "pelleted radioactive species" represent all macromolecule-associated iodide as well as 80% of the intact Copolymer-1 drug and larger degradation products.

These data confirm that the plasma C_{max} and AUCs for total radioactivity and pelleted radioactivity increase linearly with dose in the naive animals and after 28 days of repeated drug administration. However, at 177 days (Cohort 3) there appears to be a increase in AUC for male and female plasma levels of total radioactivity, and an even greater increase in AUC for male and female pelleted radioactivity levels compared to time 0 or 28 days. These data suggest that exposure is increased (almost two-fold with respect to pelleted plasma) with time, indicating non-linear kinetics with time. A decrease in clearance with time, possibly due to a saturation of the clearance pathway (the subcutaneous degradation

pathway?), could explain this phenomenon. It is unlikely that this is due entirely to a saturation of the degradative enzymes in the subcutitus, as the injection sites were rotated periodically in the animals. It is important to note that the exposure in terms of "pelleted" plasma samples, apparently representing 80% of the parent drug, increases the most with time. The main point is that these data are consistent with an increase in plasma concentration of parent drug (as opposed to smaller metabolic species) with time.

The Sponsor also cites the following Table showing antibody formation for this 6-month study, as evidence for the formation of antibodies with repeat administration of Copolymer-1:

Table 4. Summary: Anti-Copolymer-1 antibodies during 6-month study (at a 1:1000 dilution).

GROUP	COP 1 DOSE mg/kg	1 MONTH		3 MONTHS		6 MONTHS	
		Mean±(S.D.)	Responders	Mean±(S.D.)	Responders	Mean±(S.D.)	Responders
1M	0	95 (29)	0/10	116 (34)	0/10	107 (10)	0/10
2M	3	458 (303)	6/10	597 (843)	4/10	216 (195)	3/9
3M	10	682 (623)	6/10	2105 (2010)	6/10	633 (697)	6/9
4M	30	851 (755)	9/10	1850 (1112)	9/10	891 (572)	7/10
1F	0	123 (40)	0/10	112 (26)	0/10	107 (24)	0/10
2F	3	975 (740)	9/10	669 (916)	4/10	309 (342)	3/10
3F	iu	505 (437)	4/10	320 (268)	3/10	131 (53)	1/10
4F	30	747 (801)	4/10	1532 (1700)	5/10	506 (569)	4/9

The Sponsor concludes, from these data, that the production of anti-Copolymer-1 antibodies does not affect the pharmacokinetics of Copolymer-1.

Reviewer's comments:
One would expect the production of anti-Copolymer-1 antibodies to increase clearance and therefore decrease T-1/2. However, data from these studies suggest that plasma exposure of the drug is increased at 177 days (about 6 months), possible due to a decreased clearance. **Therefore, the Sponsor is correct in that these results are not consistent with production of antibody increasing clearance of the drug.**

SUMMARY AND EVALUATION

PHARMACOLOGY

Etiology of Multiple Sclerosis (MS)
Unfortunately, although multiple sclerosis is classified as an autoimmune disease affecting mainly the central nervous system, the exact etiology of the disease remains unclear. The two prevalent theories at present are that 1) MS results from a viral infection of the CNS and the resulting inflammatory condition is, mainly, an antiviral response or 2) MS is an autoimmune disease in which infiltrating T cells, somehow activated by an as yet unknown autoantigen, recognize self-antigens and attack normal nerve tissue. It is also thought that perhaps the autoimmune disease may actually be triggered by environmental factors, including viral infection or chemical or drug induction, that are somehow responsible for the induction of an immune response to proteins that are normally identified as "self".

Scientific rationale for treating MS
Although the putative autoantigen responsible for the T cell-mediated response directed at the CNS in MS has not yet been identified with certainly, certain immunological approaches to treatment of MS have been proposed. One proposed approach involves the development of drugs that inhibit the interaction of the autoantigen:antigen-presenting cell (APC):T cell receptor (TCR)-complex, or trimolecular complex, that is theoretically required in order for the induction of a population of autoreactive T cells that will attack myelin, as is thought to occur in the case of MS. Among other possibilities, this can be accomplished by designing a drug that will 1) interfere with binding of the autoantigen to the MHC-II molecules on the APC, 2) allow binding of the autoantigen to the TCR, but without providing the necessary signal for T cell activation (anergy) or 3) allow binding of the autoantigen to both TCR on the T cells and MHC-II molecules on the APC, but with the induction of T suppressor cells rather than T helper cells. Numbers 1 and 2 would prevent activation of the autoreactive T cells that theoretically cause the MS pathology, and number 3 would induce a T suppressor cell population that would specifically inhibit the function of the specific autoreactive T cell population involved in the MS pathology.

One popular approach to the development of drugs designed to treat MS by one of these mechanisms has been to create a peptide vaccine that is capable of modulating the immune system to accomplish one of the above-enumerated goals. While the Sponsor does not specifically state that Copolymer-1 is designed to act as a peptide vaccine, the pharmacology and proposed mechanism of action for the drug as described in this submission are consistent with this approach.

The EAE animal model

While the putative autoantigen responsible for MS has not yet been identified in patients, certain myelin-associated proteins are suspect. In the EAE (experimental allergic encephalomyelitis) animal model of MS, certain known antigens such as Myelin Basic Protein (MBP), other purified encephalitogenic proteins, or their peptide fragments are injected systemically into experimental animals in an attempt to mimic MS by stimulating a population of autoreactive T cells which recognize these encephalitogenic determinants in association with MHC-class II molecules. It is these autoreactive immune cells that then migrate into the CNS and mediate the pathologic process. The role of CD4+ T cells in this process has been clearly demonstrated.

Copolymer-1 has been extensively studied in this generally accepted animal model of MS, and has been shown to effectively suppress (drug given to animals after antigen challenge), prevent (drug given prior to antigen challenge) and block (drug co-injected with antigen) symptoms of EAE in a number of different animal species (mice, rats, guinea pigs, rabbits and monkeys) when EAE was induced by a number of different encephalitogenic antigens. Based on these experimental results, it was predicted that Copolymer-1 might prove useful in the treatment of MS.

Potential problems associated with the use of Copolymer-1 to treat MS, based on preclinical studies into the actual mechanism of action of the drug

Lack of binding data for standard panel of receptors

No data were reported by the Sponsor for binding of Copolymer-1 or its degradation products to the standard panel of adrenergic, cholinergic, dopaminergic, etc. receptors that are usually examined for a drug submitted in an NDA. There is no way of predicting without such binding studies whether or not Copolymer-1 or its degradation products might activate these other receptor systems. It is true that the PK of the parent drug and degradation products are not very important from the perspective of efficacy, since the drug is most likely acting as a peptide vaccine acting at the local lymph nodes. However, the fact remains that the bioavailability of the parent drug and degradation products in some form have a fairly high bioavailability (46%) by the s.c. route of administration and are therefore available in plasma. From the perspective of potential toxicity of the drug, binding activity to the usual panel of receptors and potential biological activity of parent drug and degradation products are important issues.

Non-specific immunosuppression

In animal studies carried out to determine which of the proposed mechanisms of action for the treatment of MS were actually involved in the ability of Copolymer-1 to prevent EAE, it was discovered that there were probably two different mechanisms involved, 1) the induction of T suppressor cells that inhibit the functioning of the autoreactive T cells that attack myelin and 2) the ability to interfere with the binding and interaction of various proteins and peptides to MHC-class II molecules on APC.

While the induction of T suppressor cells by Copolymer-1 is theoretically a fairly specific mechanism for inhibiting autoreactive T cells, Copolymer-1 treatment interfered with the antigen:TCR interactions of a number of different antigens. Copolymer-1 competed for binding sites on MHC-class II molecules on human-derived APC with Myelin Basic Protein and a number of other antigens as well. Therefore, this mechanism of immunosuppression by the drug appears to be fairly non-specific in both animal and human systems, and one might predict that repeated administration of Copolymer-1 might result in general immunosuppression that could result in decreased ability to fight infection.

"Systemic reaction"

Administration of Copolymer-1 to humans has been associated with a "systemic reaction", that apparently includes vasodilatation, hypotension, chest tightness, palpitations, dyspnea and fatigue. Preclinical pharmacology studies have shown that the drug directly induced the release histamine from rat peritoneal cells and human mast cells and release of interleukin-1 (IL-1) and interferon-gamma (IFN-γ)-like activity from human peripheral blood mononuclear cells (PBMC) from both healthy volunteers and MS patients *in vitro*. The release of histamine was said to be "direct", not immune-related. Since none of the patients donating PBMC had been previously exposed to the drug, it was concluded by the Sponsor that the release of cytokines was also a direct effect of the drug, as the result of a cross-reaction with some undetermined natural antigen.

Histamine is known to cause constriction of pulmonary muscle as well as vasoconstriction and hypotension. It is also known that the release of IL-2 results in release of a cascade of other cytokines, including interleukin-1 (IL-1) and tumor necrosis factor (TNF). The release of these cytokines in response to Copolymer-1 administration could also result in hypotension, as well as aching, fever and fatigue. Therefore, the Copolymer-1-mediated release of these soluble mediators could certainly explain a number of the symptoms reported to be associated with the "systemic reaction". Furthermore, increased levels of IL-1 and TNF are associated with hypotension, decreased cardiac output, and vascular leak syndrome.

Synergistic effect with interferon-beta (IFN-ß)?

The Sponsor states that Copolymer-1 acts to block the interaction of antigen with MHC-class II on APC, while IFN-ß acts to inhibit expression of MHC-class II proteins on the surface of APC. Therefore, the Sponsor speculates that the two drugs might act in synergy in the treatment of MS.

Pharmacology conclusions

Based on the Sponsor's stated scientific rationale for use of Copolymer-1 for treatment of MS, I am of the opinion that this drug is actually a **peptide vaccine**. There are a number of such peptide vaccines that are under development at present for treatment of MS and other autoimmune diseases, and many of them propose a similar mechanism of action to Copolymer-1. In my opinion, the scientific rationale for use of Copolymer-1 to treat MS as indicated is a sound one. The drug was effective in preventing the symptomology associated with the EAE animal model of MS and various study results supported the Sponsor's proposed mechanism of action of the drug to 1) interfere with the interaction between MHC-class II on APC and the autoantigen and 2) induce a population of T suppressor cells that inhibit the cellular autoimmune response to myelin.

One of the known problems associated with development of peptide vaccines for treatment of autoimmune diseases is the potential hazard of altering the immune system to induce autoimmunity, the very disease one is trying to treat. In the case of these peptide vaccines, the immune mechanisms involved in their therapeutic effects are not well characterized. When attempting to modify immune mechanisms thought to be involved in autoimmunity, there is always the danger that one woud exacerbate the autoimmune effects. Also, in the case of synthetic peptides such as Copolymer-1, the issue of immunogenicity is often raised. Immunogenic drugs also have the inherent problem of potential induction of immune complex disease, which can take the form of Type III hypersensitivity and autoimmunity. This is further discussed in the "Toxicology Section" of my review.

Systemic exposure to Copolymer-1 includes exposure to both intact drug and various degradation products. However, no information is submitted in the NDA to describe the binding capabilities of either intact drug or degradation products to the normal panel of physiologically relevant receptors. Therefore, the normal binding studies against the standard panel of receptors should be completed.

The pharmacology study results related to the mechanism of action of the drug suggested that Copolymer-1 might act as a general immunosuppressant, which could impair the ability of the patient to resist infection. However, as discussed in the "Immunotoxicology" section of this review, the Sponsor carried out the appropriate immunotoxicology studies and demonstrated that Copolymer-1 probably does not act as a general immunosuppressant.

The "systemic reaction" associated with Copolymer-1 administration could be explained bv the release of histamine and a number of cytokines (IL-1, IL-2, TNF). Furthermore, there is a concern that repeated exposure to Copolymer-1 could lead to increased release of these inflammatory cytokines and histamine, and ultimately to lead to vascular leak and severe hypotension.

SAFETY PHARMACOLOGY

Cardiovascular studies

Pharmacology studies revealed at least two different mechanisms by which Copolymer-1 might affect the cardiovascular system, 1) induction of histamine release and 2) induction of IL-2-release. Histamine is hypotensive and also has direct effects on the heart, and IL-2 can induce release of a whole cascade of other cytokines, including IL-1 and TNF, which are also hypotensive and in the extreme can cause vascular leak syndrome.

Safety pharmacology studies were carried out in Wistar rats, cats, rabbits and Beagle dogs to examine the effect of Copolymer-1 on the cardiovascular system. These studies were NON-GLP and contained only a minimal number of animals, but certain consistencies were reported between species. Hypotensive effects were reported for all species tested. The dog was the most sensitive species with respect to this effect, demonstrating a decreased MAP of 67% and decreased heart rate of about 21% with the administration of 10 mg/kg i.v. Copolymer-1. These effects lasted for about 15 minutes after administration. In addition, all three dogs in this experiment presented with arrythmias about 15 minutes after drug administration at 10 and 20 mg/kg. No detailed description of the type or duration of the arrythmias was found in the submission. The NOEL in this experiment was reported to be 5 mg/kg. Arrhythmias were not reported in the other species tested.

Studies in rats and cats, in which the Sponsor examined the mechanism of action of this hypotensive effect, showed that the drug-induced hypotension was blocked by prior administration of a combination of H1 and H2 antagonists. These data indicated that histamine release was a likely candidate for mediation of the decrease in blood pressure. Histamine release could also explain the alterations in ECG patterns and arrythmias reported in the dogs, as H1 receptors have been reported to be involved in slowing AV-nodal conduction and H2 receptors have been shown to be involved in affecting both electrical conduction and contractility of the heart.

Additionally, in the cat an early pressor effect was found, and histamine did not appear to mediate this effect. It is also known that Copolymer-1 induces release of interleukin-2 (IL-2) from human T cells, and IL-2 can, in turn, induce release of a number of other cytokines such as the inflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α). These inflammatory cytokines, such as TNF and IL-1, have been shown to cause an immediate transient hypertensive effect, followed by more prolonged hypotension. In fact, TNF-α can cause vascular leak syndrome, which in it's most severe form, can result in dramatically decreased blood pressure and eventually shock. While no evidence of a role for these inflammatory cytokines was presented by the Sponsor, I would recommend that this area be investigated in the future.

Analysis of dose at which cardiovascular effects occurred compared to human dose

The clinical dose of Copolymer-1 proposed for use in this NDA is subcutaneous injection of 20 mg/day (0.4 mg/kg for 50 kg person) of drug for the life of a patient with relapsing-remitting MS. With respect to mg/kg, the NOEL in dogs is about 12.5-fold greater than the proposed clinical dose. With respect to a surface area comparison, thought by some to be a more accurate means of comparing comparable doses between the species, the 5 mg/kg NOEL dose in dog is equivalent to about a 2.5 mg/kg dose in man. Therefore, on a surface area basis, the NOEL in the dog (5 mg/kg) is about 6.25-fold greater than the proposed clinical dose. The major concern is that, in the dog, a two-fold increase in dose (from 5 mg/kg to 10 mg/kg) took us from the NOEL to dramatically decreased blood pressure, alterations in ECG patterns, and arrythmias. These data do not provide for much of a safety margin for the drug.

The i.v. route of administration was used in these dog safety pharmacology studies. However, Copolymer-1 is proposed for administration to patients by the s.c. route and therefore the majority of the animal toxicology studies were also done using s.c. administration. Based on the metabolism data showing a protective effect of plasma on intact drug, for short-term studies one would predict that systemic exposure by the i.v. route would consist mainly of intact drug. Subcutaneous administration would most likely result in systemic exposure to small degradation products. Since intact drug is most likely to induce histamine and cytokine release, while small degradation products would not, the greatest effect on the cardiovascular system would be expected to occur with i.v. administration of the drug. Of note is the fact that, in the safety pharmacology study in the dog in which s.c. administration was used (2 males, 2 females), only a slight decrease in blood pressure was reported, and there were no direct effects on the heart reported.

However, the picture with respect to long-term repeat s.c. administration of the drug may be somewhat different. Toxicokinetics studies in rat showed an increase in TCA-precipitable drug-related radioactivity, made up of intact drug and large degradation products, with time. Therefore, under these conditions of repeated administration over time, s.c. administration could also result in a higher systemic exposure to intact drug, which could mimic the i.v. route and result in increased cardiovascular problems. So due to the toxicokinetics of Copolymer-1, there may be potential for an increase in cardiovascular problems with long-term repeated s.c. administration of this drug. The risk of this can be better assessed by completing a toxicokinetics study in humans.

Safety Pharmacology Conclusions

Administration of Copolymer-1 can result in cardiovascular effects, including decreased blood pressure and cardiac arrythmias. These effects are probably more severe when the drug is administered i.v., which most likely results in a much higher systemic exposure to intact drug than with s.c. administration. Toxicokinetics studies (discussed in more detail in the "Toxicokinetics" section of this review) show that prolonged repeated s.c. administration of Copolymer-1 can result in an increase in systemic exposure to intact drug with time, suggesting that one could see an increase in cardiovascular effects over time with repeat s.c. administration. The risk in patients will be better evaluated when the Sponsor completes a toxicokinetics study in humans.

ADME Summary

With respect to the PK data focusing on systemic exposure, the Sponsor reported that Copolymer-1 was rapidly and extensively absorbed, as witnessed by a plasma T_{max} of about 2 hours for total drug-related radioactivity and total remaining radioactivity at the injection site in mice being 14% 1 hour after and 5% 8 hours after s.c. administration. The absolute bioavailability for Copolymer-1, based on AUCs calculated from total drug-related radioactivity in plasma, was reported to be 46% for s.c. administration relative to i.v. administration. However, it is impossible to tell whether or not some part of this 46% actually represents intact drug.

The Sponsor carried out PK studies in rats and monkeys and used the results to attempt by extrapolation to predict plasma C_{max} values one would expect in humans for the clinical dose of 20 mg/day, or 0.3 mg/kg/day by the Sponsor's calculation using a 70 kg patient. The Sponsor used two different methods for determining plasma drug concentrations, 1) total plasma radioactivity and 2) TCA-precipitable plasma radioactivity, which is supposed to reflect mainly the higher molecular weight radioactive material, including radiolabelled parent drug. There are inherent problems with both methods, and neither method results in values that are truly reflective of levels of intact drug in the plasma. Based on these methods, their predicted plasma C_{max} values for the clinical dose are 380-710 ng/ml when "total radioactivity " values are utilized and 52-240 ng/ml when "TCA-precipitable" radioactivity values are used. Finally, the Sponsor concluded that plasma levels of Copolymer-1 are not detectable, even when 50 mg/kg drug is administered s.c., unless radiolabelling techniques are used. HPLC fluorescence techniques could not detect any plasma drug under these conditions.

In **distribution** studies in rats and mice, the stomach appears to be the only organ in which the drug-related radioactivity is concentrated to levels greater than those found in plasma, while brain appears to demonstrate the least amount of drug-related radioactivity. In the one rat study in which the Sponsor examined the thyroid, a large proportion of the total drug-related radioactivity was found in thyroid. The Sponsor concluded that this radioactivity was due to concentration of free radiolabelled iodide in this organ. The problem with these distribution studies is that again there is no way for the Sponsor to determine whether the tissue-associated

radioactivity constitutes parent drug, a degradation product, or free rabiolabelled iodide. Therefore, it is impossible to predict the significance of these findings in terms of potential toxicity.

In vivo **metabolism** studies in rats demonstrated that Copolymer-1 undergoes rapid degradation. Total plasma radioactivity at 3 minutes after s.c. drug administration was composed mainly of parent drug (by HPLC), while at the 5 and 8 minute time-points the drug was already degraded to smaller species and free iodide. *In vitro* studies in rat and human plasma and tissues demonstrated that plasma from both species had a somewhat stabilizing effect on parent drug. However, subcutaneous tissue, striated muscle and other tissues were shown to result in rapid degradation of the drug. These data are consistent with *in vivo* data in that higher TCA precipitability and slower disappearance of characteristic HPLC profile occurs following i.v. injection, in which drug is introduced directly into the blood, compared to s.c. injection, where drug passes through the subcutaneous tissue before entering the bloodstream.

Excretion of drug-related radioactivity after s.c. administration of radiolabelled Cop-1 is found primarily in the urine. According to the Sponsor, this radioactivity is composed mainly of free radiolabelled iodide, as they contend that intact drug has a molecular weight too large to be filtered through the kidney glomeruli. I disagree with this conclusion, because peptides with a molecular weight (M.W.) below 30 kD can be filtered through the glomeruli and excreted. Due to tissue degradation, it is most likely that the majority of the drug reaching the plasma would be of a M.W. less than 30 kD.

Summary of Human Pharmacokinetics and Bioavailability

In this section of the NDA submission, the Sponsor essentially makes the case that study of pharmacokinetics of Copolymer-1 in patients "would be of limited value, and the use of isotopic tracers would submit patients to unacceptable risk due to prolonged radiation exposure." The Sponsor claims that Copolymer-1 exerts it effects locally at the s.c. injection site, and therefore its systemic distribution is irrelevant. They argue that the drug is rapidly degraded in peripheral tissues. Therefore, much of the drug-related material that is absorbed is degraded drug (smaller peptides and constituent amino acids) and free iodide, both of which become incorporated into endogenous polypeptides and proteins. Finally, the Sponsor argues that "...extrapolation of the animal pharmacokinetic findings to humans indicates that serum concentrations of Copolymer-1 will be low or not detectable following subcutaneous injection of a 20 mg dose of drug...". Therefore, they argue that they should not bother doing pharmacokinetics studies in humans, but rather they propose to demonstrate bioavailability based on 1) formation of antibody to Copolymer-1 in patients and 2) demonstration of the efficacy of the drug in treating patients for relapsing-remitting MS. They argue that the presence of anti-drug antibody formation and demonstration of efficacy of the drug in MS patients provide irrefutable evidence of drug bioavailability.

ADME Conclusions

Appropriate bioavailability

ADME data for this NDA submission are problematic. First of all, in my opinion Copolymer-1 is actually a "peptide vaccine", and therefore the most appropriate measure of bioavailability is not necessarily systemic exposure. A subcutaneously administered peptide vaccine must interact with cells of the immune system, and can do so by either being cleared with tissue fluid to the local draining lymph nodes or by entering the systemic circulation and being filtered through the spleen. While both of these avenues probably play some part in the mechanism of action of Copolymer-1, when considering the facts that 1) the majority of the drug is degraded to much smaller peptide fragments by the time it reaches the plasma and has therefore probably lost much of its immunogenicity and 2) the molecular weight range of the copolymer includes a significant percentage of the drug being over 20 kD in size and thus most likely preferentially absorbed through the lymphatics, it is likely that the appropriate bioavailability for this particular drug involves the Copolymer-1 that reaches the draining lymph nodes for the local injection site. However, the Sponsor submitted pharmacokinetics data that focused on systemic exposure to the drug.

Value of ADME data related to systemic exposure

As previously stated, the appropriate bioavailability for this drug is probably the drug that reaches the local lymph nodes draining the inection site. However, the Sponsor submitted PK data relating to systemic absorption. Furthermore, the data with respect to systemic exposure are flawed. The systemic exposure and bioavailability profile for Copolymer-1 is apparently complicated by the fact that upon administration to animals, the polypeptides that make up the drug are rapidly broken down to constituent peptides and amino acids, can subsequently be incorporated into physiological macromolecules, and are therefore not distinguishable from endogenous proteins and polypeptides unless the drug is radiolabelled. When the drug is radiolabelled, it can then be distinguished from endogenous proteins and peptides, but it is difficult to differentiate the parent drug from its metabolites or free radiolabel. This apparently can be done through the use of reverse-phase HPLC, which the Sponsor has done in a single study. However, for the majority of the ADME studies, the Sponsor has used TCA-precipitation techniques to attempt to separate "high molecular weight" from "low molecular weight" radioactive species. Unfortunately, these techniques are not able to clearly differentiate between parent drug, degradation products, and free radiolabelled iodide or radiolabelled iodide that has been incorporated into precipitable plasma proteins. The most valuable of the ADME data are probably the metabolism data, in that they clearly demonstrate that 1) drug is degraded very rapidly and 2) the majority of this degradation by the s.c. route probably occurs in the subcutaneous tissue. These data would therefore suggest that the majority of the drug reaching the systemic circulation would normally consist of small degradation products.

However, there is one area where systemic exposure data might prove useful. Toxicokinetics data in the 26-week repeat dose rat study demonstrated that the amount of "TCA-precipitable drug-associated radiolabel" found in plasma increased with time (117 Days). With many drugs by s.c. administration, intact drug is absorbed into the systemic circulation and then metabolized from there. However, in the case of Copolymer-1, data are consistent with a scenario in which the drug is normally metabolized at the s.c. tissue injection site, and only small degradation products are absorbed into the systemic circulation. However, these toxicokinetics data suggest that, with repeated s.c. administration of Copolymer-1, there is an increase in the absorption of intact drug over time, possibly due to saturation of the degradative enzymes at the injection site. Therefore, in the case of Copolymer-1, it appears that there is the potential for the systemic exposure to intact drug and large degradation products to increase with time. This could have significance in terms of adverse drug effects.

Excretion data do not give a clear picture of where the drug is eliminated. Apparently the radioactivity that is excreted is found exclusively in the urine, and the Sponsor speculates that this is mostly free iodide, since the parent drug is too large to be filtered in the glomeruli. However, since peptides less than 30 kD in size can be filtered through the glomeruli, I would predict that much of this radiolabel constitutes degradation products. The animal carcass also apparently maintains a certain percentage of the total drug radioactivity, presumable because the radiolabel is either incorporated into newly synthesized proteins or bound tightly to various tissue proteins.

Finally, when the pharmacokinetics of peptide drugs are examined, the peptide is often radiolabelled with ^{14}C- or ^{3}H- label. Labelling with these isotopes allows the peptide to remain identical to the unlabelled drug in its physicochemical and biological properties (Kompella, U and V. Lee. *Pharmacokinetics of peptide and protein drugs*, In Therapeutic Peptides and Proteins: Assessing the New Technologies, 1988 Cold Spring Harbor Laboratory Symposium). However, such products are often difficult to prepare, especially with sufficient radioactivity to detect in the systemic circulation. Therefore, classical radiotracer studies were used for the assessment of pharmacokinetics, using ^{125}I-Copolymer-1. Unfortunately, iodinated peptides are chemically distinct from the parent drug, and therefore, the PK may not be representative of the original peptide.

Copolymer-1, a peptide vaccine? Are PK studies needed in humans?

The Sponsor claims that the examination of the pharmacokinetics of Copolymer-1 in humans would require the use of radiolabelled drug, would be of limited usefulness, and the risks associated with the use of ^{125}I-labelled drug in humans should preclude the initiation of these studies. Human PK data are normally used to estimate systemic exposure to a drug. For many drugs, this systemic exposure data is, in turn, used for comparison to animal toxicology study data in the rational choice of a clinical dosing range that will hopefully maintain drug efficacy

while avoiding toxicity. These human systemic exposure data can also be used to adjust the human dose when necessary. However, Copolymer-1 is most likely acting as a peptide vaccine, and the appropriate bioavailability with respect to drug efficacy is not systemic exposure, but rather is drug exposure at the local lymph nodes that drain the s.c. injection site. Therefore, I would agree with the Sponsor that, in terms of drug efficacy, human PK data would most likely be of limited usefulness.

However, with respect to potential toxicity, I am somewhat reluctant to agree with the Sponsor that human PK data are unimportant. Animal data indicate that with short-term administration, systemic exposure to drug consists mainly of small degradation products, while the systemic exposure to larger degradation products and intact Copolymer-1 drug increased with time. Safety pharmacology and animal toxicology study results indicate that systemic exposure to intact drug can result in immune complex formation and deposition in the kidney, induction of histamine release with the potential to cause anaphylactoid reactions and cardiovascular effects including decreased blood pressure and heart rate as well as arrythmias. Taken together, these results are consistent with the conclusion that while short-term administration of drug may be safe, long-term s.c. administration may lead to rather serious adverse effects. Therefore, I do not think that it is appropriate to completely discount human PK studies concerning systemic exposure from the perspective of potential toxicity of the drug. It is my conclusion that the Sponsor should carry out the necessary studies to describe the toxicokinetics of Copolymer-1 in humans, to determine whether or not this time-related increase in systemic exposure to intact drug and large degradation products occurs in the clinic. It is imperative that these data are obtained, so that a proper risk assessment can be carried out with respect to long-term repeated administration of the drug as proposed in the NDA.

Are daily s.c. injections really necessary?
In light of the fact that Copolymer-1 is most likely acting as a peptide vaccine, it is unclear to me why it is necessary to inject the drug on a daily basis. This dosing regimen seems like it would subject the patient to an excessive amount of discomfort if it is not necessary to maintain efficacy. Furthermore, if there should be a problem in humans with saturation of the clearance mechanism, thus increasing the amount of intact drug in the systemic circulation over time, this problem might be lessened with intermittent rather than daily administration. I would recommend that the Sponsor evaluate the necessity of daily s.c. injections as opposed to more infrequent intermittent administration of the drug.

TOXICOLOGY

ACUTE TOXICOLOGY

Subcutaneous administration
There are no valid GLP studies presented in this NDA submission to adequately examine the acute toxicity potential of copolymer-1 administered by the subcutaneous route of administration indicated for the clinic. Therefore, there are no valid data with which to calculate an LD_{50} or NOEL, and no margin of safety can be determined with respect to the 0.4 mg/kg (20 mg) human dose.

In a NON-GLP study, 400 mg/kg Copolymer-1 was administered s.c. to 4 Sprague-Dawley rats of each sex with the sponsor reporting no mortality or toxicity. However, the report of this study was very sketchy, containing little data to support this claim, and only a minimal number of animals were included in the study. 400 mg/kg is a 1000-fold higher dose than that prescribed for man on a mg/kg basis, and about 142-fold higher on a surface area basis.

Other routes of administration
A number of other animal studies, some GLP, were submitted by other routes of administration (i.m., i.v.). However, these studies do not directly support the s.c. administration of Copolymer-1 outlined in this NDA, since they were not carried out by same route of aministration (s.c.) as proposed for the clinic. Two of these studies were GLP and were done by the i.v. route of administration in the rat. In one of the studies   ', the NOEL of 40 mg/kg is 100-fold greater than the human dose on a mg/kg basis, and about 14-fold greater by surface area considerations. In this same study, the LD_{50} of 200 mg/kg is about 500-fold greater than the human dose by mg/kg and about 71-fold greater by surface area. In study
(listed as GLP but no GLP statement found with the study report), the NOEL of 40 mg/kg and the lowest lethal dose of 200 mg/kg give the same margins of safety for toxicity and death as in stud .

Bromide contaminant
Excessive oral intake of bromide is known to cause some neurological symptoms in humans (headache, lethargy, ataxia, disorientation). Therefore, it is no surprise that in study   utilizing bromotyrosine-contaminated (12-14%) copolymer-1, the animals demonstrated ataxia, bradypnea and unconsciousness and a lower LD_{50} than when administered copolymer-1 without this contaminant. Also, bromide has been shown to affect thyroid function in animals, but not in humans (Stitch, G., and Kaferstein, H. (1988). Bromine in Handbook of Toxicity of Inorganic Compounds, edited by H.G. Sieler and H. Siege, pp 143-154, Marcel Dekker, N.Y.) In discussing this with the Agency chemist, Dr. Heimann, on the review team, she found that the sponsor has set a specification limiting the amount of this contaminant in the product to 0.2%. We agreed that the limit should be set as low as is physically possible with respect to removal of the contaminant.

Acute Toxicology Conclusions

The Sponsor did not submit any valid, GLP studies to support the safety of a single-dose administration of the drug by the s.c. route of administration. A single NON-GLP study in rats (4/sex/group) showed that 400 mg/kg of drug did not result in toxicity or death of the animals, suggesting a safety margin of about 1000-fold on a mg/kg basis, or 142-fold on a surface area basis. However, the lack of GLP compliance, the minimal number of animals, and the design of the study precluded any valid conclusions.

The Sponsor also submitted a number of acute animal toxicology studies by other routes of administration (i.v., i.m.). Two of these were rat studies in which the i.v. route of administration was utilized. Normally, I would conclude that acute studies done by the i.v. route of administration could be used to support the s.c. route for a drug, since i.v. administration usually results in higher plasma C_{max} than the s.c. route. However, in the case of Copolymer-1, the issue is a more complex one. S.C. administration of Copolymer-1 apparently results in systemic absorption of mainly degradation products of the drug, as a large proportion of the drug is degraded locally in the subcutaneous tissue. However, apparently i.v. administration of Copolymer-1 results in systemic exposure to mainly the parent drug, and plasma may even have a "stabilizing" effect on the drug, prolonging the life of the intact molecule. Furthermore, s.c. administration of an immunogen is known to result in a more robust immune response than i.v. administration. Therefore, for Copolymer-1, it is more difficult to evaluate the relevance of acute safety data by the i.v. route in terms of s.c. administration. Probably, the greatest potential danger with acute administration of the drug is through it's ability to directly induce histamine release. This effect could result in anaphylactoid response, and is optimized by i.v. administration.

There were two i.v. studies in the rat that were carried out under GLP guidelines. In one of the studies   the NOEL of 40 mg/kg is 100-fold greater than the human dose on a mg/kg basis, and about 14-fold greater by surface area considerations. In this same study, the LD_{50} of 200 mg/kg is about 500-fold greater than the human dose by mg/kg and about 71-fold greater by surface area. In study   (listed as GLP but no GLP statement found with the study report), the NOEL of 40 mg/kg and the lowest lethal dose of 200 mg/kg give the same margins of safety for toxicity and death as in stu   _ in, the relevance of these results to the s.c. route of administration are unclear.

SUBCHRONIC TOXICOLOGY

Requirements for subchronic animal studies in support of an NDA involving a drug to be administered by repeated administration for more than three months, as is the case with Copolymer-1, include a six-month rodent toxicology study and a 1 year toxicology study in a non-rodent species in which drug is administered by the same route of administration and similar dosing regimen as proposed for the clinic. A **sufficient number of appropriate subchronic animal toxicology studies utilizing**

the s.c. route of administration were submitted in support of this NDA.

The main GLP studies submitted included a 6-month study in rat and a 52-week study in Cynomolgus monkey. A 3-month study in beagle dogs was also included. Due to the fact that this drug was designed to affect the immune system, the Sponsor, quite appropriately, included studies to examine the direct immunotoxicology of the drug. Furthermore, the Sponsor included studies to examine the antigenicity of the drug and potential results of that antigenicity, including the production of "neutralizing" antibodies and the induction of hypersensitivity reactions (including immune-complex disease) and autoimmunity.

General toxicological effects (mortality, clinical signs, body wt/food consumption, clinical chemistry, ophthalmology, electrocardiography, macro- and micropathology)

Mortality
Repeated subcutaneous administration of Copolymer-1 to study animals resulted in very few animal deaths. A single male (10 mg/kg/day) and a single female (30 mg/kg/day) rat were found dead in the 26-week rat study, with an undetermined cause of death.

In the 52-week monkey study, a female Cynomolgus monkey (10 mg/kg/day; 4/sex/group) was killed early (Week 14) due to excessive morbidity. Histologically, this animal presented with lymphoid and bone marrow atrophy, inflammatory lesions in the skin of the tail and paw and rectum, and minor active focal fibrinoid arteritis in three visceral organs (pancreas, ileum, and colon). An inactive fibrosed arterial lesion was also found in the heart. These symptoms suggest the possibility of a systemic inflammatory response such as one might find with immune complex disease. This animal also demonstrated a neutrophil count 158% higher than Controls and plasma levels of anti-Copolymer- antibodies 20-fold higher than Controls and 2-fold higher than the other treated animals in this dosing group, findings that are also consistent with an animal demonstrating an inflammatory response.

Clinical signs
Clinical signs in all animal species were consistent with a hypersensitivity reaction. The rat presented with red and swollen ears, face, nose and limbs in the 4-week study and severe injection site lesions in the 26-week study. The dog demonstrated scratching of injection site and the monkey (28 days and 52 weeks) also demonstrated severe injection site lesions.

Blood chemistry
Effects on blood chemistry parameters were consistent with mild effects on liver d kidney. These included increased urea (15%, HDF, 4-week rat; 12.6%, HDF, 26-week rat), ALP (42%, HDF, 4-week rat), ALT (45%, HDF, 4-week rat; 56% HDM and 29% HDF, 52-week Cyno monkey), and AST (60% HDM, 26% HDF, 52-week Cyno monkey). Also, creatinine levels were increased, indicating decreased

creatinine clearance, in 26-week rat study (HDM 6%, HDF, 10%). These data would be consistent with mild to moderate kidney and liver effects.

Urine analysis
In the 52-week monkey study, HDF monkeys demonstrated a 50% decrease in creatinine clearance, consistent with effects of drug on the kidneys.

Ophthalmology
In the 4-week Beagle dog study, animals presented with congested bilateral eyes (2/3 HDM, 2/3 HDF) and hyperreflective points on the border between the Tapetum lucidum and negrum (1/3 HDM, 1/3 HDF, 2/3 MDM).

Macroscopic and microscopic pathology
Animals in all studies presented with injection site lesions and signs of possible immune complex disease that will be discussed in more detail in the following "Antigenicity" and "Immunotoxicology" section.

Electrocardiography
Although the study was not optimally designed to examine effects of Copolymer-1 administration on the heart (ECG readings were taken at 4 weeks, **immediately before** drug administration), male Cynomolgus monkeys (4-week study) demonstrated a dose-related decrease in heart rate, with a 15% decrease in HDM. No effects were seen in females. This study only contained a single animal per sex per group, so statistical analysis was impossible. No effects on the heart were reported in the dog studies.

IMMUNOTOXICOLOGY

The main safety concerns with respect to chronic s.c. administration of Copolymer-1 to MS patients are in the area of immunotoxicity. One would predict, based on the proposed mechanism of action of the drug and data presented in the "Pharmacology" section, that the drug could act as a general immunosuppressant. Copolymer-1 is also antigenic, inducing fairly high titers of anti-drug antibodies in rat, Cynomolgus monkey and man, which raises the question of development of a local or systemic inflammatory response. The production of anti-drug antibodies also raises the question of "neutralizing antibodies" that might interfere with long-term administration of the drug. Finally, both the antigenicity and the proposed mechanism of action indicate the possibility that this drug may have the capability of inducing autoimmunity in its own right. The Sponsor carried out the appropriate immunotoxicological studies in animals and man to address these important safety issues.

Immunosuppression

Copolymer-1 is a peptide drug that was designed to affect the immune system. Its mechanism of action is most likely as a vaccine that induces an immune response that interferes with the ongoing autoantigenic immune response against myelin basic protein thought to be the cause for the symptomology associated with multiple sclerosis (MS). It is proposed that Copolymer-1 specifically interferes with the interaction between MHC-II on the APC (antigen-presenting cell) and the T-cell that would otherwise produce a T-cell population primed to destroy myelin. However, data presented in the "Pharmacology Section" of the submission demonstrated that Copolymer-1 inhibited binding of a number of antigens in addition to myelin basic protein to MHC-II on APC, suggesting a more broad action for the drug. Therefore, one must be concerned with the possibility of a more general immunosuppressive effect, which could potentially impair the ability of the patient to fend off infections.

To determine whether or not Copolymer-1 might be a general immunosuppressant, the sponsor examined a number of immune parameters in the preclinical animal studies, such as WBC counts, differentials, spleen and thymus weights and pathology. Additionally, a general immunosuppressive effect in humans was assessed by evaluation of Copolymer-1-induced PBMC (peripheral blood mononuclear cell) proliferation from patients receiving s.c. administration of drug for up to 25 months.

With respect to preclinical data, in the **26-week rat study**, on Weeks 4, 13 and 26 there was an increase in peripheral blood WBC and lymphocytes in High Dose Males and an increase in neutrophils on Weeks 4 and 26. High Dose Females showed an increase in WBC, lymphocytes and neutrophils on Week 26. (An increase in neutrophils could be consistent with an inflammatory response). On Week 5, the only time the sponsor looked in this study, there were no changes in the lymphocyte subset counts (T-cells, B-cells, CD4+ cells, CD8+ cells, CD4+CD8+ cells). There were also no effects on spleen or thymus weights or pathologies. In the **52-Week study in Cynomolgus monkeys**, there were no effects overall on WBC counts, lymphocyte, monocyte or neutrophil counts, thymus or spleen weights, morphometry of lymphoid tissues (proportion of thymus occupied by cortex), or histopathology of immune tissues.

With respect to patients, the Sponsor examined Copolymer-1- and PPD-induced proliferation of PBMC isolated from patients receiving the drug over a period of 25 months. The PPD-induced response remained strong throughout the study, while the Sponsor concluded that the Copolymer-1 response may have decreased somewhat. Due to the extreme range of the error associated with these data, it is questionable whether or not data support this conclusion. (The Sponsor suggested that this decrease in Copolymer-1-induced response may suggest the development of immune tolerance, another possible mechanism of action for the drug).

Antigenicity

A major concern with respect to repeated s.c. administration of Copolymer-1 for a long period of time, as proposed in this NDA, is the profound antigenicity of the drug. Repea..ed subcutaneous administration of the drug resulted in high titers of anti-COP-1 antibodies in both rat and monkey, in all Treated animals in the dose range from 3-30 mg/kg (see Tables 4 (rat) and 11 (monkey) below).

Table 4. Summary: Anti-Copolymer-1 antibody titers during 6-month study in rats (at a 1:1000 dilution).

GROUP	COP 1 DOSE mg/kg	1 MONTH		3 MONTHS		6 MONTHS	
		Mean±(S.D.)	Responder*	Mean±(S.D.)	Responders	Mean±(S.D.)	Responders
1M	0	95 (29)	0/10	116 (34)	0/10	107 (10)	0/10
2M	3	458 (303)	6/10	597 (843)	4/10	218 (195)	3/9
3M	10	682 (623)	6/10	2105 (2010)	6/10	833 (697)	6/9
4M	30	851 (755)	9/10	1850 (1112)	9/10	691 (572)	7/10
1F	0	123 (40)	0/10	112 (26)	0/10	107 (24)	0/10
2F	3	975 (740)	9/10	669 (916)	4/10	309 (342)	3/10
3F	10	505 (437)	4/10	320 (268)	3/10	131 (53)	1/10
4F	30	747 (801)	4/10	1532 (1700)	5/10	506 (569)	4/9

*Responder=any animal with an antibody titer two-times greater than the mean Control value for animals at that time point.

Table 11. Anti-Copolymer-1 antibody levels for Cynomolgus monkeys at a 1:5000 dilution of serum.

Group and sex	COP-1 dose (mg/kg)	Week 4 Mean(SD) R	Week 13 Mean (SD) R	Week 26 Mean(SD) R	Week 39 Mean(SD) R	Week 52 Mean(SD) R
1M	0	261(57) 0/4	1658(1560) 2/4	687(294) 2/4	714(211) 2/4	717(194) 2/4
2M	3	6880(6926) 4/4	16047(3715) 4/4	9403(4184) 4/4	8901(2147) 4/4	10527(3305) 4/4
3M	10	5112(4351) 4/4	13465(7437) 4/4	5534(4073) 4/4	7031(2990) 4/4	7814(3080) 4/4
4M	30	2609(1224) 4/4	12334(8872) 4/4	9539(11083) 4/4	7613(6063) 4/4	8111(6525) 4/4
1F	0	703(355) 0/4	1022(368) 0/4	1156(642) 0/4	1395(871) 1/4	1053(293) 0/4
2F	3	7084(6103) 3/4	18675(6760) 4/4	15803(6429) 4/4	12053(912) 4/4	17073(5134) 4/4
3F	10	10650(5130) 4/4	13511(5565) 4/4	7312(6366) 3/3	7168(1181) 3/3	10014(1758) 3/3
4F	30	6326(4406) 3/4	13712(7565) 4/4	9195(6762) 4/4	8509(4858) 4/4	10571(8603) 4/4

R=Responders; responses were considered positive when the value was at least 2-fold higher than that of the mean±S.D. of the Control Group (1M or 1F) at Week 4 at the same dilution.

There was little or no dose-relationship between dose of Copolymer-1 administered and titer of antibody in the animal plasma, indicating that in this dose

range one is probably already at maximum antibody response. Antigenicity was also demonstrated in guinea pigs and in humans (data not shown here), and studies in these species indicated that the antibody being produced was predominantly IgG.

When a drug is antigenic, inducing production of high titers of anti-drug antibody, safety concerns include the potential for the development of local or systemic inflammation (hypersensitivity reactions), the issue of "neutralizing antibodies", and the potential for the drug itself to cause drug-induced autoimmunity.

Neutralizing Antibody

A "neutralizing antibody" is an antibody that is formed to a drug (antigen) that is then able to interact with any subsequently administered drug to either neutralize its biological activity or to increase its clearance from the body. Therefore, the formation of neutralizing antibodies can result in the destruction of any efficacious drug effect once the antibodies have accumulated, thereby negating drug efficacy and rendering the drug useless for long-term administration.

The sponsor appropriately examined the human anti-Copolymer-1 antibodies, formed as the result of s.c. administration of the drug in the clinic, for "neutralizing" properties. This was appropriate because development of neutralizing antibodies in an animal model is not absolutely predictive of a similar development in humans. The Sponsor examined the "neutralizing" ability of human anti-Copolymer-1 antibody *in vivo* by studying the ability of this human antibody to block the efficacy of Copolymer-1 in mouse EAE model and *in vitro* by studying the ability of these antibodies to block Copolymer-1-induced proliferation of a T-cell line. These studies are reviewed in the "Special Toxicology Studies, Antigenicity-Clinical" section of this review, and results show that the human anti-Copolymer-1 antibodies had no effect in these systems.

Inflammation/Type I Hypersensitivity

As previously stated, another concern with respect to antigenicity of a drug and antibody formation is the development of hypersensitivity, resulting in an inflammatory response such as Type I (anaphylaxis, due to IgE production and histamine release) or Type III (IgG, immune complex formation) response involving either local injection site, organ-specific, or more systemic inflammatory response due to deposition of immune (antibody-antigen) complex and subsequent activation of the complement cascade. These effects, ranging from anaphylaxis to formation of immune complex disease, can be life-threatening.

Guinea pig

The guinea pig is the animal most often used to determine whether or not a drug is antigenic and is capable of inducing a hypersensitivity response. This was accomplished by sensitizing the animals to the drug and then administering drug a second time by the s.c. route of administration, in the "active systemic anaphylasis test." Studies in the guinea pig indicated that s.c. administration of Copolymer-1 to previously sensitized animals resulted in antibody formation and induced a positive

anaphylactic response, including piloerection, nose scratching, sneezing, cyanosis, labored breathing and death in 5 of 5 animals tested at both 0.33 and 3.3 mg/kg. While it is true that guinea pigs are used in these studies because of their sensitivity to antigenic products, it is fairly unusual for all animals tested to die. This result probably reflects the somewhat extreme antigenicity of Copolymer-1.

The Sponsor then carried out a "homologous passive cutaneous anaphylaxis test" to determine whether or not the anaphylaxis mediated by Copolymer-1 was due to production of IgE or IgG antibodies. The results of these studies revealed that Copolymer-1 administration induced production mainly of IgG, and not IgE.

Mouse study

Results in mice were cause for more concern. In the mouse carcinogenicity study, begun in August of 1995, 61 of 720 total mice died in the first 14 weeks of the study, and all but two were in the Copolymer-1-treated groups. These animals were dosed s.c. with up to 60 mg/kg/day Copolymer-1. The probable cause of death was determined to be Type 1 hypersensitivity. It is unclear from preliminary results whether or not a) animals attained systemic exposure to high levels of intact drug or b) similar toxicokinetics to rat, in which systemic exposure to intact drug appeared to increase with time, also occured in mouse. Furthermore, it is unclear from the preliminary results whether or not these deaths were actually due to Type 1 hypersensitivity due to IgE-mediated release of histamine, or whether the ability of Copolymer-1 to directly induce histamine release may have been involved. However, these results must increase the level of concern with respect to repeated s.c. administration of Copolymer-1 to patients.

Local injection site reactions

Injection site lesions were reported in 26-week rat (Table 1 below), 4-week dog (Table 13 below) and 52-week monkey studies (no data shown). While the Sponsor insists that these lesions were most likely due to direct induction of histamine release from basophils by Copolymer-1, another plausible explanation would be a local inflammatory response due to either antigen-antibody complex accumulation or a delayed type hypersensitivity response. In the case of a drug demonstrating the level of antigenicity of Copolymer-1, it is possible that Type II (anti-cell or tissue antibody), Type III (immune complex) and Type IV (delayed-type hypersensitivity) reactions could all occur to various degrees. In the dog (4-week study) and Cynomolgus monkey (52-week study) studies, the micropathology associated with the injection site lesions described them as including mononuclear cell accumulation with the presence of multinucleate giant cells. This particular pathology is most consistent with Type IV (delayed-type hypersensitivity) response.

Table 1: Incidence of selected injection site lesions in rats by grade.

Sex		Male				Female			
Group		1	2	3	4	1	2	3	4
Number		20	20	20	20	20	20	20	20
Injection site 1									
Fibrosis	Grade 1	4	0	2	6	1	5	2	4
	Grade 2	0	1	0	1	0	0	0	2
Injection site 2									
Fibrosis	Grade 1	4	2	0	3	5	2	2	3
	Grade 2	0	1	0	2	0	0	2	1
Injection site 3									
Myositis	Grade 1	1	2	1	6	2	3	2	5
	Grade 2	0	0	0	0	0	0	0	3
Cellulitis	Grade 2	0	0	1	0	0	0	0	1
	Grade 3	0	0	0	0	0	0	1	2
Fibrosis	Grade 1	7	5	2	3	2	11	8	2
	Grade 2	0	5	10	6	0	2	7	8
	Grade 3	0	0	2	10	0	0	0	9
	Grade 4	0	0	0	1	0	0	0	1
Injections site 4									
Myositis	Grade 1	0	1	8	2	0	2	5	7
	Grade 2	0	0	1	5	0	0	0	2
	Grade 3	0	0	0	0	0	0	0	1
Cellulitis	Grade 1	1	0	0	2	0	0	2	0
	Grade 2	0	2	1	2	0	0	1	0
	Grade 3	0	0	0	2	0	0	0	0
Fibrosis	Grade 1	9	9	6	0	5	10	6	4
	Grade 2	1	1	9	8	0	2	9	8
	Grade 3	0	0	1	10	0	0	0	8
	Grade 4	0	0	0	1	0	0	0	0

Key: Grade   1 = minimal; 2 = slight; 3 = moderate; 4 = moderately severe; 5 = severe (NB no findings graded 5)

Table 13: Summation of graded scores for micropathology at the injection site in 4-week dog study.

	Group and sex							
	1M	2M	3M	4M	1F	2F	3F	4F
LESION								
recent hemorrhage	5*	3	12	6	4	1	7	8
chronic inflammation	4	7	17	15	3	7	19	16
acute inflammation	-	3	10	7	3	-	1	5
edema	-	4	25	14	2	7	20	18
mononuclear cell infiltrate	-	3	12	13	-	9	19	12
multinucleate giant cells*	-	-	8	1	-	3	4	3
hematoma	-	-	-	2	-	-	2	-
subcutaneous fibrosis	-	-	-	-	-	-	-	2

The injection site effects were graded 0-4, and the values in this table reflect the addition of the grade for each lesion at the three sites for the three dogs in each sex group. The maximum possible score for scored criteria is 36.

Type III (immune complex) hypersensitivity

Immune complex deposition in the kidney glomeruli

In both rat and monkey studies, a number of Treated animals demonstrated findings consistent with deposition of immune complex in the glomeruli. In the 26-week rat study, Copolymer-1 drug deposition was detected by immunohistochemical staining in the glomeruli of 3 of 20 High Dose (30 mg/kg/day) animals, with a fourth animal demonstrating "moderate" staining. No drug was detected in the glomeruii of Control animals (Sponsor only examined Control and High Dose). Presence of C3 complement fraction, indicative of concurrent presence of antigen-antibody complex, was also detected in the glomerular basement membranes of these same three High Dose (30 mg/kg/day) Male animals, and was also reported by   7/20 other HDM, 5/20 HDF, 3/20 Group 3 (10 mg/kg/day) Males and 1/20 Group 3 Females. Therefore, both drug and C3 complement were found together in kidneys of 3 High Dose animals as evidence of immune complex deposition. It was much more common to find animals in which Complement C3 alone was detectable in the glomeruli, and presence of Complement C3 alone is sufficient to indicate deposition of immune-complex. (While no anti-Copolymer-1 antibody was detectable in the glomeruli, this was probably due to the limitation of the assay rather than the absence of the antibody). Finally, two of the same three HDM animals that demonstrated both C3 complement and Copolymer-1 drug in their glomeruli also presented with the highest titers of plasma anti-Copolymer-1 IgG antibody.

In the 52-week Cynomolgus monkey study, clear staining of the glomeruli for both Copolymer-1 drug and C3 complement was seen in 1 of 4 High Dose male animals and 1 of 4 High Dose females. According to the Sponsor, these two monkeys also demonstrated active germinal follicles in the bone marrow, high titers of anti-Copolymer-1 antibodies, and above-background titers of several autoantibodies. They also showed minor histological signs of chronic fibrosing arterial lesions and, as stated, immunohistochemical evidence of Copolymer-1 and complement in the glomeruli. Although at a fairly low incidence (1/4 HDM, 1/4 HDF), these data confirm the rat data and indicate that repeated s.c. administration of Copolymer-1 can result in signs of immune complex formation in the glomeruli of the kidney and of a systemic inflammatory response

There were no histological findings in the kidneys of either the rats or the Cynomolgus monkeys to indicate that tissue damage had occurred in the kidneys as the result of immune complex deposition. However, although minimal inflammatory cell foci were found in kidneys of Control animals as well, there was some evidence of an increase in the incidence and severity of these inflammatory cell foci in the kidneys of Treated monkeys. Also, clinical chemistry results were consistent with mild to moderate effects on the kidneys of High Dose animals in the 4 and 26-week rat studies (increased urea, increased creatinine) and 52-week Cynomolgus monkey study (decreased creatinine clearance) (see general toxicology section reviewed above). Since these studies were only of 6-month to 1-year duration, it is possible that the effects of the immune complex deposition in the kidney could progress to

histopathologically detectable tissue damage with a longer duration of administration. This NDA proposes administering 20 mg/day for the life of the patient.

Histopathological lesions suggestive of vasculitis

In the 52-week Cynomolgus monkey study, three High Dose males and one High Dose female presented with active fibrinoid arterial lesions in a number of organ sites as shown in the following Table:

Animal	Group	Organ (active fibrinoid arterial lesions)
636	3F (deceased)	Pancreas, ileum and colon, and inactive lesion in heart.
623	4M (sacrificed)	Gall bladder, duodenum, jejunum, seminal vesicles, testes, lung, epididymides. (Note: this animal also had inflammatory cell foci in muscle, sciatic nerve, liver, kidney, salivary gland, heart, pituitary, brain, spinal cord).
626	4M (sacrificed)	Epididymides. (Note: this animal also had inflammatory cell foci in kidney, lung, lacrimal gland).
640	4F (sacrificed)	Vagina. (Note: this animal also had inflammatory cell foci in liver, kidney, salivary gland, brain, lacrimal gland).

Two of the High Dose animals also presented with active germinal follicles in the bone marrow, which would be consistent with production of additional inflammatory cells (macrophages, neutrophils) as might be expected in a systemic inflammatory response.

While the Sponsor states that "...the incidence of such arterial lesions is within the range of background pathology in terms of prevalence and severity for normal primates in this laboratory...", I would argue that 1) these effects are seen only in Intermediate or High Dose Treatment animals, not in Controls and 2) these results may suggest the development of a systemic inflammatory response in the vasculature (vasculitis). In light of the high level of antigenicity of Copolymer-1, it would certainly be imprudent to ignore these signs of a possible systemic inflammatory reaction.

Histopathological lesions in brain, spinal cord, and heart

Following is a Table summarizing the histopathology for the 52-week Cynomolgus monkey study with respect to the development of inflammatory cell foci in brain, spinal cord and heart of the study animals:

Table: Summary of histopathology data for 52-week cynomolgus monkey study with respect to development of inflammatory cell foci in brain, spinal cord and heart

Group/Dose	Animal #	Brain	Spinal Cord	Heart	Kidney
MALES 1 (0 mg/kg)	611M	-*	-	-	+
	612M	-	-	-	+
	613M	-	-	-	+
	614M	-	-	-	+
2 (3 mg/kg)	615M	-	++ (choroid)	+	-
	616M	-	-	-	-
	617M	++ (choroid)	-	-	+
	618M	+	-	-	+
3 (10 mg/kg)	619M	-	-	-	+
	620M		-	++	-
	621M	-	-	-	+
	622M	-	-	-	-
4 (30 mg/kg)	623M	+	+	+	+++
	624M	-	-	-	++
	625M	-	-	-	+
	626M	-	-	-	++
FEMALES 1 (0 mg/kg)	627F	+(choroid)	-	-	-
	628F	-	-	-	++
	629F	-	-	-	+
	630F	-	-	-	+
2 (3 mg/kg)	631F	-	-	-	++
	632F	-	-	-	+++
	633F	-	-	-	++
	634F	-	-	-	-
3 (10 mg/kg)	635F	-	-	++(aorta thick)	-
	636F	-	-	+ (arteritis)**	-
	637F	-	-	-	++
	638F	-	-	-	+
4 (30 mg/kg)	639F	-	-	-	+
	640F	++ (choroid)	-	+	++
	641F	+ (choroid)	-	-	++
	642F	-	-	-	+ (interstitial nephritis)

- = no inflammatory cell foci; + = minimal; ++ = slight; +++ = moderate.
**arteritis also included fibrosis

These data demonstrate that with repeated s.c. administration of Copolymer-1 for 52-weeks a number of Cynomolgus monkeys developed inflammatory cell foci in

the brain, spinal cord and heart. With t'ie sole exception of a single Control female monkey (brain foci), all of these inflamr,natory cell foci were found in Treated animals, indicating that the effects are most likely drug-related. 'The lack of a clear dose-effect relationship is not particularly surprising in light of the fact that the antibody response is similar at all Treatment doses. This is most likely because one has already reached the maximum antibody response at the lowest dose (1 mg/kg) of Copolymer-1 administered in this study. While some minimal inflammatory cell foci were found in the kidneys of Control animals as well, the severity of inflammatory cell foci in the kidney did appear to increase somewhat in treated animals.

Also of concern is the fact that, in the 52-week monkey study, inflammatory cell foci appeared in the eyes of 2 of 4 M@3 mg/kg, 2 of 4 M@30 mg/kg, 1 of 4 F@ 3 mg/kg, 1 of 4 F@10 mg/kg and 2 of 4 F@30 mg/kg (not shown in Table). None of these lesions were reported in Control animals.

Analysis of dose

In the rat study, there were no drug-related deaths. Detection of immune complex in the kidney glomeruli occurred with a NOEL of 3 mg/kg/day, about 8-fold greater on a mg/kg basis than the proposed human dose (0.4 mg/kg/day).

With respect to animal deaths, there was a single monkey death at 10 mg/kg/day, but no deaths at 30 mg/kg/day. Since the proposed human dose is about 0.4 mg/kg/day, this is about 75-fold higher than the human dose on a mg/kg basis. The NOEL for immune complex deposition in the monkey was 10 mg/kg/day, but it is not clear whether or not this is due to an actual absence of complement C3 in the kidneys of these animals or a limitation of the assay. However, 10 mg/kg/day is about 25-fold higher than the proposed human dose. No NOEL was determined for the histopathological effects described above, and the lowest dose used in this study was 3 mg/kg/day, about 8-fold higher than the proposed human dose on a mg/kg basis. Since there are no human PK data, no comparison of exposure rates (AUC) or plasma levels are possible.

The histopathological findings may be due to immune complex formation and deposition in various tissues, and immune complex formation is dependent to a large extent on antibody formation and the amount of antigen present. As seen in the antibody data for rat and monkey, antibody titers are not dose-dependent, at least in the dose ranges of Copolymer-1 used for these studies. The data suggest that, at the doses used in these studies, maximal antibody production has already been reached. Therefore, one might not expect to see dose-dependent toxicological effects related to antibody production. Unfortunately, without the appropriate data in hand for a dose range including 0.4 mg/kg/day, it is impossible to predict the extent of antibody response that would be seen at this dose of drug.

Depending on the proportion of antigen to antibody in the plasma, the formation of immune complex could also be dependent on the amount of antigen Copolymer-1 drug) present in the plasma. However, due to the fact that much of the Copolymer-1 that is absorbed systemically is enzymatically reduced to degradation products of fairly small size, it is unlikely that the majority of the systemic drug would

be likely to form antigen-antibody complex.

With respect to systemically absorbed drug that has the potential to act as antigen and form antigen-antibody (immune) complex, one potential problem does exist. The PK in the rat demonstrates that the systemic exposure to larger degradation products and intact drug increases with time with prolonged repeated s.c. administration. This could be explained by a saturation of the enzyme system that degrades drug at the subcutaneous injection site. It is more likely that this intact parent drug would form immune complex with anti-drug antibodies than the much smaller degradation products. Therefore, these toxicokinetics data suggest that the systemic exposure to intact drug could increase with time, and therefore the formation of immune complex in the systemic circulation could also increase with time.

Autoimmunity

The immune mechanisms involved in the autoimmune response are still not completely understood in the case of multiple sclerosis. Therefore, when one designs a drug with the intent to alter the cellular immune system in a manner that prevents the autoimmune response to myelin thought to be the basis for the etiology of the disease, one must also be concerned that one might, instead, induce or worsen the very autoimmune response one is trying to prevent. It has been reported that all T-cell responses are specific for a particular combination of peptide and MHC molecule, and, in fact, a helper determinant ideal for one individual in a populaiton might elicit a strong autoimmune response in another individual. To circumvent this problem, it has been suggested that the ideal peptide vaccine for any given individual would have to be custom made for each patient ("The value of synthetic peptides as vaccines for eliciting T-cell immunity", *In* Peptides as Immunogens, Ed. H. Koprowski and F. Melchers, Springer-Verlag, New York, 1987). This is obviously an impractical solution.

Furthermore, along the same lines as hypersensitivity response, one must be concerned about the antigenicity of the drug and potential development of antibodies to "self" tissue or organs, which could result in organ-specific or systemic inflammatory lesions. Therefore, with the peptide vaccines, it is also important to look for signs of induction of autoimmunity, which can include the nervous system, vasculature, kidney, heart, and other highly perfused organ systems.

In the rat study, the sponsor reported that results of analysis for anti-nuclear antibodies in Copolymer-1-treated animals were insignificant. However, there were, in fact, a number of Treated animals that had expressed anti-nuclear antibodies. In the monkey study, the titer of anti-single-stranded DNA and anti-double-stranded DNA increased as much as 20-70% above Controls and anti-histone antibodies increased 500-600% in Treated animals compared to Controls. These antibodies, and especially the anti-double-stranded DNA antibodies, are known to occur in patients with autoimmune disease. While the animals in the rat study did not express all the symptoms of systemic autoimmune disease, the monkeys did show vascular lesions and inflammatory cell foci in a number of visceral organs. Whether or not these

lesions were due to an autoimmune response is unknown, but again, in light of the the immunotoxicology data in general one has to be somewhat suspicious.

Additional Studies, Either Planned or Ongoing, to Examine the Immunotoxicological Effects of Copolymer-1

The Sponsor is obviously concerned about the prosect of immune complex disease, as they also outlined two additional ongoing preclinical studies that included components for examination of the immunotoxicological potential of Copolymer-1 administration. Apparently there is an ongoing GLP study, a "13-Week Range-Finding Study in Mice by S.C. Administration," that will include immunohistochemical evaluation for Copolymer-1, C3 and IgG antibody complexes in kidney to determine immune complex deposition. There is also a "104-Week Chronic Toxicity Study in Rats, S.C. Administration," that is a life-span GLP carcinogenicity study. This study includes a satellite group of 180 rats (6 groups, 15/sex/group) for an immunohistochemistry study, in which kidneys will again be evaluated for localization of Copolymer-1, C3 and IgG to assess possible immune complex deposition.

Overall conclusions with respect to immunotoxicology

Based on preclinical pharmacology studies examining the mechanism of action of Copolymer-1, there was some concern that the drug might act as a general immunosuppressant, an undesirable side effect for a drug proposed for chronic administration. However, both preclinical and clinical immunotoxicology data (PPD-response) submitted by the Sponsor supports their conclusion that the drug does not act as a general immunosuppressant.

It is clear from preclinical data that s.c. administration of Copolymer-1 results in the production of anti-Copolymer-1 antibodies. The drug has also been shown to be antigenic in humans. In the dose range utilized in the preclinical studies (3-30 mg/kg/day), maximal antibody production was already attained, and therefore no dose-response with respect to antibody production was seen. Adoptive transfer studies demonstrated that the protective effects of Copolymer-1 in the rat EAE model are most likely mediated by the cellular immune system, and therefore the anti-Copolymer-1 antibodies probably have no role in the mechanism of action of the drug.

One major concern with respect to an antigenic drug is the formation of "neutralizing antibodies" that can limit the use of the drug for chronic administration. The Sponsor completed both *in vivo* and *in vitro* studies to examine the neutralizing potential of the anti-Copolymer-1 antibodies formed in humans, and the data were consistent with the conclusion that these antibodies were non-neutralizing in the systems tested. Therefore, there should be no limitation on the use of the drug for long-term repeated administration with respect to antibody neutralization of drug.

While the above conclusions with respect to Copolymer-1 administration regarding immunosuppression and neutralizing antibodies are somewhat reassuring from a safety perspective, results concerned with potential hypersensitivity response

raise a number of concerns. Results from studies in rat and Cynomolgus monkey show that repeated s.c. administration of Copolymer-1 can result in immune complex deposition in the glomeruli of the kidney, a condition that can lead to a local inflammatory response (Type III). Also, active fibrinoid arterial lesions associated with a number of highly perfused organs as well as histopathological lesions (inflammatory cell foci) in brain (choroid plexus), spinal cord and heart were reported in Treated Cynomolgus monkeys. Knowing the extent of the antigenicity of Copolymer-1 in rats and monkeys, it would be imprudent to ignore these findings. Systemic deposition of immune complex, circulating in the blood, would most likely occur either in the walls of the vasculature or in organs that are highly perfused with blood. Once immune complex is deposited in a given area, arterial lesions or inflammatory cell foci could form as the result of complement activation and an inflammatory response. Highly perfused areas including the choroid plexus of the brain cr the heart would be especially suspect. These lesions could, therefore, be explained by a systemic inflammatory response that could be consistent with evidence of immune complex deposition. Finally, a number of Treated rats and Cynomolgus monkeys also presented with increased titers of anti-nuclear antibodies, a phenomenon often found in animals that have developed autoimmune disease. These results, therefore, indicate that repeated s.c. administration of Copolymer-1 to animals resulted in signs and symptoms that could be explained by local and possibly systemic inflammatory response such as one might see with the development of immune complex disease (Type III hypersensitivity response) or autoimmune disease. Since a number of these symptoms are also seen in MS, a disease whose etiology involves autoimmune response to myelin, these findings are open to the interpretation that administration of this drug could potentially worsen the symptoms of MS.

The immune complex deposition in the glomeruli did not appear to result in any histopathological sequelae in the rat (26-weeks) kidney and while the severity of "inflammatory cell foci" in the kidneys of the Cynomolgus monkey (52-weeks) did appear to worsen in Treated animals, no data were presented that would suggest that this was the result of immune complex deposition. Furthermore, the incidence of immune complex deposition, inflammatory cell foci and arterial lesions in the animals was fairly low, with no evidence of animal death due to systemic inflammation (with the exception of a single animal). Therefore, while these results are consistent with signs and symptoms of immune complex disease, it was somewhat surprising that the disease had not progressed to a more severe state in 26- (rat) or 52-weeks (Cynomolgous monkey). It was for this reason that animal data were interpreted to support the safety of the drug for use in MS patients. However, it would be imprudent to completely ignore the potential for Copolymer-1 administration to induce immune complex disease and/or autoimmunity, as toxicokinetics study results in the rat may help to explain why symptoms had not progressed further by the end of these animal studies.

Pharmacokinetics and metabolism data demonstrated that, with repeated s.c. administration of Copolymer-1 to animals, systemic exposure consisted of mainly small degradation products that would most likely not contribute to the formation of

immune complex. However, with 177 days of repeated administration of Copolymer-1, the systemic exposure to "TCA-precipitable drug", representing larger degradation products and intact Copolymer-1, increased in a non-linear manner. Therefore, in the 26-week study in rats, animals may have actually only seen systemic exposure to intact Copolymer-1 drug for about the last one or two weeks of the study. This would explain why at the end of the rat study, only a few of the animals displayed evidence of immune complex deposition in the glomeruli. Furthermore, this immune complex deposition may not have been present long enough for sufficient inflammatory response to result in detectable kidney pathology. Also, no toxicokinetics studies were done in monkey, so we have no idea whether or not or for how long these animals experienced systemic exposure to intact drug. Therefore, 1) the lack of severe pathology associated with immune complex deposition in the animal studies may be explainable by toxicokinetics data and 2) the formation and deposition of immune complex with Copolymer-1 administration may only arise when, for some reason (possibly saturation of clearance at the injection site), systemic exposure to intact drug occurs. With this in mind, it is imperative that toxicokinetics studies be carried out in patients to determine whether or not such an increase in systemic exposure to intact drug with time also occurs in humans.

Data in rat, monkey, guinea pig and human consistently demonstrate the induction of high levels of anti-COP-1 antibodies that appear to be IgG. IgG antibodies are known to mediate both local injection site inflammatory reactions and organ-specific and systemic inflammatory reactions (Type III) through production of immune complex and deposition in various organs and tissues. These data further suggest that induction of Type I hypersensivity response such as anaphylaxis, a potentially life-threatening response to IgE, may not be much of a threat with Copolymer-1 administration. However, the preliminary results from a mouse carcinogenicity study showed that 61 of a total of 720 mice, all but two of which were in Treatment groups, died in the first 14 weeks of Type 1 hypersensitivity response. These data contradict those in the guinea pig and indicate that there is some danger of anaphylaxis occuring with s.c. injection of the drug. As previously stated, this does not rule out a non-immune related effect due to direct induction of histamine release by intact Copolymer-1 drug.

Data from the dog (4-week study), rat (26-week study) and Cynomolgus monkey (52-week study) demonstrated the formation of rather severe local injection site lesions. These lesions were so severe in the rat that the Sponsor concluded that "...the severity of these lesions would preclude the administration of a dose of 30 mg/kg repeatedly to rats..." In the case of a drug demonstrating the level of antigenicity of Copolymer-1, it is possible that a number of different hypersensitivity responses might occur concurrently. Data in kidney and other organs in rat and monkey are consistent with Type III hypersensitivity due to immune complex formation, as stated above. With respect to the local injection site lesions, the lesions were described as containing mononuclear cell accumulations accompanied by giant multinucleate cells. This pathology is consistent with Type IV (delayed-type hypersensitivity) response. Therefore, it would appear that administration of

Copolymer-1 to animals by the s.c. route of administration may have resulted in at least two types of hypersensitivity response. Type III and Type IV.

There are insufficient data to allow any meaningful comparison of the dose used in the preclinical animal studies in rats and Cynomolgus monkeys (3-30 mg/kg/day) to the proposed human dose (20 mg/day, or about 0.4 mg/kg/day). With respect to drug efficacy, it is probably the bioavailability at the lymph nodes draining the injection site that is relevant to the action of the drug, and there are no data to describe this exposure. With respect to adverse drug effects, immune complex formation and deposition and possibly the development of an autoimmune response are the effects of greatest concern. Immune complex formation in the systemic circulation is dependent on both the anti-drug antibody titer and the systemic exposure to intact drug and possibly to some of the larger degradation products as well. While anti-Copolymer-1 antibody formation has been examined both in animal studies and in patients receiving 0.4 mg/kg/day of drug over time, very little useful information is available regarding systemic exposure to the drug. What little toxicokinetics information that is available in animals, indicating an increase in exposure to intact drug over time, only serve to further confuse the issue and raise the question of whether or not similar kinetics occur in man. If one compares the doses based on a mg/m^2 basis, 30 mg/kg in the rat is about 14-fold higher than at the 20 mg/day human dose and in the Cynomolgus monkey is about 33-fold greater than the human dose.

It is true that the development of these immunotoxicological effects in the rat and Cynomolgus monkey do not definitively predict that humans receiving chronic s.c. administration of Copolymer-1 will develop local or systemic inflammatory symptoms of immune complex disease and/or autoimmunity. However, the immune system is highly conserved across species such that the organs and cells of the immune system in humans, mice and rats, for example, are similar. Also there are instances UV irradiation, ozone) where results of immune fuction studies in rodents have been accurate predictors of effects in humans (Selgrade, M K. et al., *Immunotoxicity-- bridging the gap between animal research and human health effects.* Fundamental and Applied Toxicology 24:13-21, 1995). Furthermore, the fact is that the drug has been shown to be antigenic in humans as well. Therefore, these data in the 6-month rat study and 52-week Cynomolgus monkey study have to increase the level of concern for s.c. administration of this drug to patients for their entire lifespan. It is imperative that the clinicians keep in mind the potential of this drug to induce immune complex disease and/or autoimmunity, and to therefore worsen the symptoms of autoimmune disease in the MS patients. While demonstration of efficacy for Copolymer-1 in treating relapsing/remitting MS would certainly argue against the possibility that administration of the drug itself is causing immune complex deposition or autoimmune effects that are damaging the CNS, this would not address the possibility of adverse effects on other highly perfused organ systems such as kidney, heart, or vasculature.

Mutagenicity

The following Table shows a summary of the mutagenicity testing completed by the Sponsor for Copolymer-1:

Table 30. Mutagenicity Results

Test	Species/Cells	Results	Lab *	Report Number/Appendix
In Vitro				
Mutation frequency (AMES)	S. Typhimurium/E.coli	Negative	HM	2E6RETIP.001 Vol. 035 007
Mutation frequency	Mouse/lymphoma cells	Negative	HM	2TKRETIP.001 Vol. 035 070
Chromosomal changes	Human/lymphocytes	Positive	HM	1HLRETIP.001 Vol. 035 118
Chromosomal changes	Human/lymphocytes	Positive	HM	1HL2RETIP.001 Vol. 035 164
In Vivo				
Chromosomal changes	Mouse/micronucleate Erythrocytes	Negative	HM	WWMRETIP.001 Vol. 021 045

* Lab = laboratory where study was conducted; HM = Hazleton Microtest, York, UD; performed according to UK regulations.

Overall Summary and Conclusions Regarding Mutagenicity Studies

The sponsor carried out the required battery of mutagenicity studies. Data submitted for the Ames test, mouse lymphoma assay, and mouse micronucleus test were negative and support the sponsor's conclusion that Copolymer-1 is not mutagenic. However, data submitted for the human lymphocyte assay (chromosomal aberration test) indicate that the drug is clastogenic.

The sponsor carried out two human lymphocyte experiments. In the first experiment, a positive response was found at 1373 μg/ml Copolymer-1 with the 20-hour sampling. The sponsor declared this finding was "...not of toxicological importance..." because the positive response did not repeat in this same experiment at the 44 hour sampling. This is an erroneous conclusion, because, a positive finding at 20-hours is indicative of clastogenicity. The 44-hour time point is normally only utilized if the 20-hour finding is negative and there is concern that clastogenicity may have been missed at 20 hours because the test drug/compound is known to delay mitosis. Therefore, my understanding of the correct interpretation of a positive response at 20-hours, irrespective of the finding at 44-hours, is that the drug is clastogenic.

In the repeat experiment the sponsor only included Copolymer-1

concentrations up to 524.3 μg/ml, even though the positive response in the first experiment was found at 1373 μg/ml. Irrespective of this experimental design, the sponsor reported a positive response at the 20-hour sampling time at a Copolymer-1 concentration of 524.3 μg/ml. The sponsor again concluded that "...no toxicological importance was attached to this observation since increases were small and not reproducible (effect was seen in one of two replicate cultures)..." However, the response was 6-fold higher than the Negative Control and was dose-related in nature, and therefore I must conclude that it constitutes a positive response.

Therefore, while I agree that data for the Ames test, mouse lymphoma assay and mouse micronucleus test are negative, I must conclude that the data for Copolymer-1 with respect to the human lymphocyte assay are positive and that Copolymer-1 is shown to be clastogenic by this methology.

Carcinogenicity

There is an ongoing "104-Week Chronic Toxicity Study in Rats, S.C. Administration," that is a life-span GLP carcinogenicity study. This study includes a satellite group of 180 rats (6 groups, 15/sex/group) for an immunohistochemistry study, in which kidneys will again be evaluated for localization of Copolymer-1, C3 and IgG to assess possible immune complex deposition. The Sponsor also reports an ongoing lifespan carcinogenicity study in the mouse, that began in August of 1995.

Carcinogenicity Conclusion

Based on the results of the mutagenicity testing that demonstrate that the drug is most likely clastogenic, in order that the patients might be sufficiently informed regarding the potential toxicity of the drug, the rodent carcinogenicity studies should

Reproductive Toxicology (see Attached for review by Dr. Edward Fisher)

Dr. Fisher reviewed the reproductive toxicology data submitted with the NDA, and his conclusions were as follows:

"The studies are adequate and indicate that COP-1 has little potential for reproductive toxicity. The Segment III finding (D-R decrease in offspring BW gain) is unexplained but does not appear to be toxicologically significant in the absence of any apparent underlying structural or functional deficits. In addition, slight reductions in offspring BW during lactation in the Segment I study were made up in the postweaning period, indicating that the effect on growth is not permanent. There were no apparent effects on embryo/fetal development in the Segment II studies. Therefore, this compound should probably be classified as Pregnancy Category B."

Toxicokinetics

Only a single true toxicokinetics study, examining the PK of the drug with repeated s.c. administration, was carried out. This study involved the inclusion of a satellite group of animals to the 26-week rat study. Animals were treated with a single s.c. injection of 3, 10 or 30 mg/kg/day ^{125}I-labelled Copolymer-1 on either Day 0 (naive animals), Day 29 or Day 177 of the 26-week study. Both total plasma radioactivity and precipitated (25% TCA) plasma radioactivity were determined. Radioactivity resulting from TCA precipitation of the plasma was reported to represent a combination of intact and larger degradation products of the parent drug and radiolabelled iodide that had dissociated from the drug and become associated with other TCA-precipitable macromolecules. Therefore, even with these additional data, it is impossible to determine the relative proportion of the plasma radioactivity that represents intact drug, degradation products or free radiolabelled iodide.

Results demonstrated linear PK with respect to plasma Cmax and AUC on Day 0 and Day 28, while at Day 177 the AUCs determined by total plasma radioactivity increased non-linearly with time and AUCs determined by "TCA-precipitable radioactivity" showed an even greater increase. A non-linear increase in drug exposure with time can often be explained by a decrease in drug clearance, possibly due to saturation of the pathway by which drug is cleared from the systemic circulation. However, the case of Copolymer-1 is somewhat different. Pharmacokinetics and metabolism data indicated that s.c. administration of Copolymer-1 resulted in systemic exposure mainly consisting of small degradation products. An increase in systemic exposure to "TCA-precipitable" drug-related radioactivity could indicate that, for some reason, the systemic exposure to larger degradation products and intact Copolymer-1 drug is increasing with time. This might be explained by a saturation of the enzyme pathway in the tissues at the injection site that is responsible for degradation of the drug, although this is more difficult to envision in light of the fact that injection sites were rotated on a daily basis. Alternatively, an increase in systemic exposure to "TCA-precipitable" drug-related radioactivity with time could be explained by an increasing incorporation of radiolabelled amino acids into TCA-precipitable endogenous plasma proteins, which would also decrease clearance of the radioactivity. It is most likely that the increase in "TCA-precipitable" radioactivity with time represents a combination of these two scenarios. With respect to Copolymer-1, from an efficacy standpoint, a greater amount of intact drug in the plasma is probably not all that important. The effect of such a "peptide vaccine" on the immune system probably occurs primarily through the local draining lymph nodes. However, the consequences are more difficult to predict with respect to adverse drug effects.

There is one potential adverse effect of increasing the amount of intact drug that reaches the systemic circulation. The plasma anti-Copolymer-1 antibody titer appears to remain fairly constant with s.c. administration of increasing doses of the drug (assuming one is at maximal antibody response). However, as more intact drug reaches the systemic circulation, there is the potential for an increase in the incidence

of immune complex formation, and therefore an increase in the potential for deposition of immune complex and induction of inflammatory response in the vasculature and various highly perfused organs. Without human pharmacokinetics, it is impossible to predict whether or not such a similar increase in systemic exposure to intact drug over time might occur in humans receiving 0.4 mg/kg/day drug as per the clinical dose.

Also, pharmacological data demonstrated that Copolymer-1 can directly induce release of histamine from both rat peritoneal cells and human mast cells. Therefore, while anaphylaxis due to an IgE-induced release of histamine may not be a concern with this drug, non-immune related response due to direct induction of histamine release may be possible, especially if the systemic exposure to intact drug increases with time.

Another concern with respect to a drug that induces the production of anti-drug antibodies is that these antibodies subsequently increase the clearance of the drug from the plasma, thus effectively reducing the efficacy of the drug. From the perspective of a peptide vaccine such as Copolymer-1, appropriate bioavailability involves presence of the drug at the local lymph nodes that drain the tissue of the injection site, and therefore plasma concentration of the drug is not as important. However, the Sponsor demonstrated significant plasma anti-drug antibody titers in these animals at up to 6 months, with an apparent decrease in drug clearance. These data are consistent with the conclusion that the anti-drug antibodies produced probably do not increase drug clearance from the plasma, since PK data are, in fact, consistent with a decrease in clearance with time. However, these data do not speak to the effects of the antibodies on drug concentration at the appropriate bioavailability site for a "peptide vaccine", the draining lymph nodes.

Overall conclusions with respect to toxicokinetics

Data support the conclusions that 1) AUCs for "total" and "TCA-precipitable" plasma radioactivity are non-linear at 177 Days, 2) there is an increase in "TCA-precipitable" plasma radioactivity with time, indicating a probable increase in systemic exposure to intact drug, and 3) anti-drug antibodies produced with repeated s.c. administration of drug probably do not result in an increased clearance of drug from the plasma. While it is unclear what proportion of the "TCA-precipitable plasma radioactivity" consists of precipitated plasma proteins containing free radiolabelled iodide versus intact drug, the increase in this radioactive fraction over time is somewhat disconcerting. In the case of Copolymer-1, an increase in systemic exposure to intact drug and possibly some of the larger degradation products could result in increase immune complex formation, which could exacerbate the adverse effects that are most likely associated with inflammatory response as the result of immune complex deposition. Furthermore, increased systemic exposure to intact drug could result in direct induction of histamine release and in cardiovascular effects not normally seen with systemic exposure to small Copolymer-1 degradation products.

OVERALL CONCLUSIONS

It is my conclusion, based on my review of the pharmacology/toxicology data included in this NDA, that these data support the approval of Copolymer-1 with respect to safety. However, this decision was a very close call, because study results raise a number of rather serious concerns regarding the daily subcutaneous administration of Copolymer-1 to MS patients for their entire lifetime.

On a positive note, the Sponsor presented data supporting their contention that the anti-Copolymer-1 antibodies produced as the result of drug administration to patients are not neutralizing antibodies, and therefore should not interfere with chronic administration of the drug. They also carried out experiments that show that repeated s.c. administration of the drug to animals or to patients does not result in a general immunosuppressive effect. However, various pharmacology, immunotoxicology and toxicokinetics results raise a number of concerns.

Type I hypersensitivity response (anaphylaxis)

Analphylaxis and other Type I hypersensitivity responses are usually mediated through IgE, and the Sponsor demonstrates that Copolymer-1 probably does not induce IgE production in most animal species or patients. However, the role of IgE in these hypersensitivity responses is to induce the release of histamine from mast cells and basophils, and therefore the chemical mediator of these often serious and life-threatening hypersensitivity responses is actually histamine. Pharmacology data in this NDA demonstrate that Copolymer-1 can directly induce release of histamine from rat peritoneal cells and human mast cells through a non-immune mediated mechanism. Furthermore, preliminary results from a lifetime s.c. carcinogenicity in mice demonstrated that a rather profound Type 1 hypersensitivity response did occur with Copolymer-1 administration in this species. Finally, clinical signs in a 4-week rat study, including swollen ears, face and limbs and red ears, are consistent with a histamine-induced response. Therefore, I am concerned that either a Type 1 (anaphylaxis) response or a non-immune related response due to direct release of histamine by Copolymer-1 administration cannot be ruled out. However, with respect to the major subchronic toxicology studies (6-month rat study, 52-week Cynomolgus monkey study) submitted in support of this NDA, there were no reports of Type-1 hypersensitivity response and/or animals dying from anaphylactic shock. Therefore, although I have a great deal of concern based on the overall study results, I am of the opinion that these study results support the safety of the drug with respect Type I hypersensitivity.

Type III hypersensitivity response and autoimmunity

The major concern with the proposed daily repeat s.c. administration of Copolymer-1 to MS patients for life lies in the antigenicity of the drug and in the potential for the drug (based on its mechanism of action) to induce autoimmunity. The drug was demonstrated to produce anti-Copolymer-1 antibodies in rats, monkeys and man. Studies in rats and monkeys demonstrated with repeated s.c.

administration of Copolymer-1 that both drug and complement could be found in the glomeruli of the kidney, indicating the deposition of immune complex. Furthermore, fibroid arterial lesions were reported in a number of monkeys in multiple heavily perfused organs and inflammatory foci were reported in brain, spinal cord and heart of a number Cynomolgus monkeys in the 52-week study. Finally, anti-DNA and anti-histone antibodies, often associated with autoimmune disease, were reported in Copolymer-1 treated rats and monkeys.

It is true that the incidences of these arterial lesions and inflammatory foci in Copolymer-1-treated rats (26-weeks) and monkeys (52-weeks) are fairly low, that no evidence is presented to clearly demonstrate that they are caused by immune complex deposition or autoimmune response, and no pathological effects directly attributable to immune complex deposition in rat or monkey kidneys were apparent. Therefore, I concluded that these data support the safety of the drug. However, based on the antige ..city of the drug and the mechanism of action that involves an attempt to alter the cellular immune response purported to be involved the autoimmune response to myelin, it would be quite imprudent to conclude that there is no risk of induction of immune complex disease or worsening of autoimmune response associated with the administration of Copolymer-1. In fact, the deposition of immune complex in the glomeruli and the appearance of arterial lesions and inflammatory cell foci in vasculature and heavily profused organs are quite consistent with the development of these adverse effects, and based on these findings it was somewhat surprising that symptoms had not progressed much further in these study animals. One possible explanation is found in the toxicokinetics data.

Results of the toxicokinetics study provide a possible explanation for the lack of kidney pathology in the rats after 26-weeks, in that these animals probably did not experience systemic exposure to the intact drug, most likely to form immune complexes, until about the last one or two weeks of the study. Up until about Day 177, the systemic exposure consisted mainly of small degradation products, that most likely would not form immune complex. Then, on about Day 177, the systemic exposure to large degradation products and intact drug began to increase in a non-linear fashion. Therefore, the immune complex deposition found in the kidneys may have only been present for one to two weeks, an insufficient amount of time to induce an inflammatory response of sufficient duration to result in pathological changes. This may explain why at 26-weeks in the rat, no definitive effects of immune-complex mediated glomerulonephritus or systemic inflammatory response were present. A lack of toxicokinetics data in the Cynomolgus monkey make it impossible to determine whether or not a similar systemic exposure pattern occurred in this study. Furthermore, it is impossible to predict, based on these animal data, whether or not these effects might occur in humans when this drug is administered at 0.4 mg/kg/day for more than 52-weeks. To better evaluate the situation, human PK studies should be carried out to determine whether or not systemic exposure to intact drug increases with time as is the case in the rat.

Cardiovascular effects

Safety pharmacology studies examined effects of Copolymer-1 administration on the cardiovascular system, and hypotensive effects were reported in Wistar rats, cats, rabbits and Beagle dogs, and decreased heart rate and arrythmias were seen in dogs as well. Studies in rats and cats using histamine H1 and H2 blockers demonstrated that the cardiovascular effects were, in part, mediated by histamine release. Furthermore, Copolymer-1 was shown to induce release of the cytokine interleukin-2 from human peripheral blood cells. Interleukin-2 release is known to result in a cascade of other cytokines, including interleukin-1 and TNFα, and these cytokines are known to also cause transient hypertensive effects followed by a more prolonged hypotension, in addition to sometimes causing vascular leak syndrome. Therefore, these data suggest a certain level of concern for adverse cardiovascular effects of the drug. However, in 4- and 52-week animal toxicology studies, cardiovascular effects were monitored and the only effect reported was in the High Dose Males in the 4-week study, that demonstrated about a 15% decrease in heart rate, with no other effects reported. Therefore, these animal toxicology studies carried out by s.c. drug administration did not reveal any serious adverse cardiovascular side effects.

However, of concern is the fact that toxicokinetics studies in the rat demonstrated that systemic exposure to intact drug probably increased repeated s.c. administration of drug over time. The i.v. studies in dogs demonstrated that systemic exposure to Copolymer-1 could induce cardiovascular effects, including arrythmias. Therefore, it is important that toxicokinetics studies be carried out in humans to determine whether or not this phenomenon of increased systemic exposure to the drug with time also occurs in patients.

Toxicokinetics

Toxicokinetics data in the rat show an increase in the plasma exposure of "TCA-precipitable" drug-associated radiolabel after 177 days of repeat s.c. drug administration. These data could be interpreted to mean that plasma exposure to intact Copolymer-1 drug increased with time. Based on the antigenicity of the drug and the demonstration of its ability to induce histamine release from human mast cells, an increase in systemic exposure to intact drug could increase the formation of immune complex in the systemic circulation, increase histamine release and result in similar cardiovascular effects as those reported with i.v. administration of the drug to Beagle dogs, including induction of arrythmias. Therefore, human PK data are necessary to determine whether or not this non-linear toxicokinetic effect also occurs in man.

RECOMMENDATIONS

Based on my review of the pharmacology/toxicology data submitted with NDA 20-622, I recommend that Copolymer-1 be approved for the proposed indication in the treatment of multiple sclerosis, with the understanding that the following recommendations be completed during Phase IV. These recommendations are categorized as either preclinical or clinical.

Preclinical recommendations

The following recommendations are directed to the attention of the clinical reviewer for consideration and determination of appropriateness for transmittal to the sponsor:

1. Short term s.c. administration of Copolymer-1 apparently results in systemic exposure to small degradation products that are most likely of little consequence. However, toxicokinetics studies in rats suggest that repeated s.c. administration of the drug such as proposed in this NDA can result in increased systemic exposure to intact drug and large degradation products over time. This could, in turn, result in increased immune complex formation, histamine release and possibly even adverse cardiovascular effects. Therefore, it is imperative that the Sponsor examine the toxicokinetics of s.c. Copolymer-1 administration in patients, to determine if systemic exposure to intact drug increases with time in humans as well.

2. Animal studies suggest that repeated s.c. administration of the drug may result in deposition of immune complex in the glomeruli of the kidney as well as the appearance of inflammatory lesions (fibroid arterial lesions, inflammatory foci) in the vasculature and in various highly perfused organs, results that are consistent with the induction of immune complex disease and/or autoimmune response. These adverse effects can be life-threatening, and may be indistinguishable from many of the symptoms of MS. While demonstration of clinical efficacy in MS patients would effectively rule out the possibility that administration of the drug is itself inducing damage to myelin, it is possible that damage to other organ systems may still occur. I, therefore, recommend that adverse events data collected during Phase IV be carefully monitored for evidence of vasculitus, renal impairment, cardiovascular effects or other effects that might suggest the induction of immune complex disease in organ systems other than the CNS.

3. Since Copolymer-1 is most likely acting as a peptide vaccine, it may not be necessary to subject patients to the discomfort and inconvenience of daily s.c. injections. The Sponsor should, therefore, determine whether or not daily s.c. injections are necessary to maintain efficacy.

4. The Sponsor should examine the effects of Copolymer-1 on interleukin-2, interleukin-1 and TNF-α release in patients, as these cytokines can cause hypotension and ultimately vascular leak syndrome. There is the potential for the production and release of this cytokine to increase with repeated administration of the drug.

LABELLING

The following are recommended changes to the Sponsor's proposed labelling for Copolymer-1: (Labelling is referenced by page number as it appeared in the NDA submission).

1. *Page 4*;

As stated,

"**Pharmacokinetics**: Based on animal studies, serum concentrations of copolymer-1 are presumed to be low or not detectable following subcutaneous administration of 20 mg once-daily to man. Consequently, pharmacokinetic information in patients receiving the recommended dose is not available."

Recommendation:

I recommend that the statement "Based on animal studies, serum concentrations of copolymer-1 are presumed to be low or not detectable following subcutaneous administration of 20 mg once-daily to man" be stricken from the labelling. The statement should just read "Pharmacokinetic information in patients receiving the recommended dose is not available" at present.

The Sponsor should be required to carry out a toxicokinetics study in humans to determine if the systemic exposure to intact drug increases with time as appears to be the case in the rat, and the labelling should be modified later according to results of those studies.

Reason:

The methodology used by the Sponsor to predict the C_{max} for patients receiving 20 mg (0.4 mg/kg) Copolymer-1 involves assumptions that are clearly not supported by data. For this prediction, they used pharmacokinetics data from rat studies, calculating plasma drug levels by two different methods. They used total drug-related radioactivity in one instance to calculate plasma C_{max}, and from these data predicted a human plasma level of 52-240 ng/ml drug at the proposed human dose of 20 mg (about 0.4 mg/kg). They also used TCA-precipitable plasma fraction of drug-related radioactivity to calculate plasma C_{max}, and with this methodology they predicted human plasma levels of about 380-710 ng/ml for the proposed human dose of 20 mg. One problem with this methodology is that neither "total plasma radioactivity" nor "TCA-precipitable plasma radioactivity" clearly define plasma level of either intact drug or degradation product. For example, "TCA-precipitable plasma" can represent larger intact drug degradation products, intact drug, and/or free radiolabelled amino acids that have reincorporated into TCA-precipitable plasma proteins. Therefore, it is unclear what actually constitutes the "plasma radioactivity" in either case. Furthermore, the prediction of the human plasma levels of drug at the proposed dose of 20 mr/patient is based on the assumptions that 1) the PK of the drug is linear down to 0.4 mg/kg dose and 2) that there are similarities in PK across species (rat, monkey, and man). There is no evidence presented to support these assumptions. Therefore, I conclude that the Sponsor actually has little or no idea what the plasma

drug concentration would be in humans with subcutaneous administration of Copolymer-1 at a dose of 0.4 mg/kg.

2. Page 4.

The Sponsor should carry out toxicokinetics studies in humans to determine whether or not an increase in systemic exposure to intact Copolymer-1 occurs over time with repeated s.c. administration, and the following statement needs to be added to the Pharmacokinetics section:

"*In vivo* metabolism studies demonstrated that short-term subcutaneous administration of Copolymer-1 to rats resulted in systemic exposure to mainly small degradation products, probably due to enzyme degradation of the drug at the local s.c. injection site tissue. However, results of toxicokinetics studies in the rat, with 177 days of daily repeated subcutaneous administration of 0, 3, 10 or 30 mg/kg/day, demonstrated that the AUC_{0-} for total plasma drug-associated radioactivity and for "TCA-precipitable" drug-associated plasma radioactivity increased in a non-linear manner. These results suggest that an increase in systemic exposure to intact Copolymer-1 drug and/or large degradation products occurs with repeated s.c. administration of drug over time. Therefore, clinical toxicokinetics studies are ongoing at present to determine if similar toxicokinetics effects occur with repeated subcutaneous administration of Copolymer-1 to patients."

3. Page 5.

As stated:
"In a cohort (N=104) from one open-label study using daily 20 mg subcutaneous COPAXONE®, the effect of copolymer-1 and MBP on the *in vitro* proliferation of peripheral blood mononuclear cells (PBMC) was evaluated. Before initiation of daily treatment with COPAXONE®, copolymer-1 stimulated PBMNC proliferation *in vitro*. During COPAXONE® treatment, the proliferative effect of copolymer-1 diminished over time suggesting that daily subcutaneous COPAXONE® therapy affects these systemic immune reactive cells."

Recommendation:
I recommend that this statement be removed from the labelling.

Reason:
I disagree with their conclusion. Data are shown on page 85 of this review ("Proliferation of PBMNC from Copolymer-1-Treated MS Patients in Response to Copolymer-1 and MBP). In light of the extremely large error bars at the earlier time points, it is impossible to make a valid conclusion with respect to these data.

4. Page 11.

As stated;

"**Carcinogenesis and Mutagenesis:** There is no evidence to suggest that either COPAXONE® or its potential breakdown products are structurally analogous to known carcinogens. Furthermore, the pharmacological and toxicological profiles of COPAXONE® do not indicate potential to induce carcinogenic responses theough either known non-genotoxic mechanisms or immunosuppressive mechanisms."

Recommended wording:

"**Carcinogenesis and Mutagenesis:** There is no evidence to suggest that either COPAXONE® or its potential breakdown products are structurally analogous to known carcinogens. However, results of two separate chromosomal aberration studies (human lymphocyte assay) indicate that this drug is clastogenic."

Reason:

Data from the mutagenicity battery demonstrated positive responses in two separate human lymphocyte assays (see Review page 98).

5. Page 11.

As stated:

"COPAXONE® did not induce structural chromosomal aberrations in cultured human lymphocytes."

Recommended wording:

"COPAXONE® induced structural chromosomal aberrations in cultured human lymphocytes in two separate studies."

Reason:
See #4 above.

6. Page 11.

As stated:

"Chromosomal aberrations or abnormalities did not occur in bone marrow cells of mice given 140 rng/kg, equivalent to approximately 60% of the LD50/kg, i.p."

Recommended wording:

"Chromosomal aberrations or abnormalities did not occur in bone marrow cells of mice given 140 mg/kg, equivalent to approximately 60% of the LD50/kg, i.p. and also about 40-fold greater than the human dose (20 mg/day) on a mg/m^2 basis."

Reason:
In the absence of exposure rates (AUC), it is appropriate to compare the animal dose to the human dose on a mg/m^2 basis.

7. Page 11.

As stated:
"Impairment of fertility: In a multi generational fertility and reproduction study in rats, COPAXONE® at doses of up to 36 mg/kg/day (i.e., 126 times the recommended dose of 20 mg/day) had no adverse effects on reproductive parameters."

Recommended wording:
"Impairment of fertility: In a multi generational fertility and reproduction study in rats, COPAXONE® at doses of up to 36 mg/kg/day (i.e., 18 times the recommended dose of 20 mg/day on a mg/m^2 basis) had no adverse effects on reproductive parameters."

Reason:
In absence of exposure rates (AUC) for comparison of animal and human exposure, comparison of doses on a mg/m^2 basis is the most appropriate.

8. Page 11.

As stated:
"Pregnancy-teratogenic effects: Pregnancy Category B:
Reproduction studies have been performed in rats and rabbits at doses up to 4587 times the human dose (20mg/70kg) and have revealed no evidence of impaired fertility or harm to the fetus due to copolymer-1. 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."

Recommended wording:
"Pregnancy-teratogenic effects: Pregnancy Category B:
Reproduction studies have been performed in rats and rabbits at doses of up to 36 mg/kg (18 times the human dose (20mg/70kg) on a mg/m^2 basis) and 37.5 mg/kg (41 times the human dose on a mg/m^2 basis), respectively, and have revealed no evidence of impaired fertility or harm to the fetus due to copolymer-1. 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."

Reason:
Appropriate comparison of human and animal exposure, in absence of AUC, is mg/m^2 surface area basis.

9. The following information on Cardiovascular effects should be added to the labelling, probably to the "Precautions" section:

Recommended wording:
"Cardiovascular effects: *In vitro* studies demonstrated that Copolymer-1 directly induced histamine release from rat peritoneal cells and human peripheral blood basophils from healthy volunteers and multiple sclerosis patients. Safety pharmacology studies in rats, cats and Beagle dogs demonstrated that i.v. administration of Copolymer-1 resulted in hypotension (decreased MAP; mean arterial pressure), and mechanistic studies revealed that the effect in rats and cats was probably due to histamine. The NOEL in rats and dogs was 10 mg/kg and 5 mg/kg, respectively. This is 5 or 9 times greater than the human exposure (20 mg/day), respectively, on a mg/m^2 basis."

10. The following information on Antigenicity/Hypersensitivity effects should be added to the labelling, probably to the "Precautions" section:

Recommended wording:
"Hypersensitivity: Results of 6-month rat and 1-year Cynomolgus monkey studies at doses up to 30 mg/kg/day (15 time greater exposure in the rat than human dose of 20 mg/day on a mg/m^2 basis; 35 times greater for the Cynomolgus monkey) demonstrated a high incidence of injection site lesions and a low incidence of immune complex deposition in the glomeruli of the kidney. The monkey study also revealed a low incidence of active fibrinoid arterial lesions in various highly perfused organs and inflammatory cell foci in brain (choroid plexus), spinal cord and heart. Although immune complex deposition in kidney did not result in detectable pathology, these results are consistent with hypersensitivity response (probably Type III and IV), most likely due, in part, to the consistent antigenicity of the drug in all species tested (including human).

11. The following information on Antigenicity/Hypersensitivity effects should also be added to the labelling, probably to the "Precautions" section:

Recommended wording:
"In a preliminary report of results of a lifetime carcinogenicity study of Copolymer-1 in mice by the subcutaneous route of administration, there were 61 deaths of a total of 720 animals in the first 14 weeks of the study, all but two of which were in the Copolymer-1 treated animals. The animals were dosed with a maximum of 60 mg/kg/day Copolymer-1, which is a 17 times greater dose than the human dose (20 mg/day) on a mg/m^2 basis. A large proportion of the animals (62%) died within 5

hours after receiving an injection. At necropsy the most consistent findings were at the injection site, vasculature and hematopoietic system, and the most probable cause of these deaths was reported to be a Type 1 hypersensitivity. Similar results were not seen in a two-year carcinogenicity study in rats."

John J. Jessop, Ph.D.

John J. Jessop, Ph.D., M.P.H.,
Pharmacologist

CC
NDA orig (#20-622)
Div file
HFD-120
        /G.Fitzgerald/J.J.Jessop/T. Wheelous
        ggt 8/6/96

NDA 20-622

REPRODUCTIVE TOXICITY OF COPOLYMER-1

A)   COP-1 EFFECTS ON FERTILITY AND REPRODUCTIVE PERFORMANCE IN RATS
GLP; Vols. 1.30-31)

1.   Treatment

Male and female rats (30/sex/grp) were dosed sc with 0 (saline control), 1, 6, or 36 mg/kg. Males were dosed for 10 weeks prior to mating, during mating with treated females (1:1 pairings, up to 21 days), and until sacrifice after weaning of F1 litters on Day 25 postpartum. Females were dosed for 14 days prior to mating, during mating, and until sacrifice on Day 20 of gestation (1/2) or Day 25 postpartum (1/2). Doses were based on the 4-week toxicity and teratology studies in rats. The HD is said to be about 100 times the human therapeutic dose on a mg/kg basis.
    Strain: Sprague-Dawley CD, Charles River, UK
    Drug batch #: 09005, 09008

2.   F0 Effects

a)   No treatment-related clinical signs were reported. There were no deaths.
b)   There were no group differences in BW, BW gain, or food consumption during treatment.
c)   There were no treatment effects on mating or fertility parameters.
d)   HD males had slightly lower blood packed cell volume and lower total hemoglobin concentrations than controls during Week 13 of treatment.
e)   Congestion and/or hemorrhage at the injection site was increased in HD group animals at necropsy.
f)   At necropsy, no differences in mean testicular or ovarian weights were observed between treated and control animals. One HD male had a testicular weight 80% lower than controls and failed to impregnate a female, but this was considered an isolated finding unrelated to treatment.

3.   Term Sacrifice Reproductive Parameters and Fetal Evaluations

(After examination of term fetuses for external abnormalities, 1/2 of each litter was cleared and stained for skeletal evaluation, and the other 1/2 was fixed and examined for visceral anomalies by the Wilson technique.)

a)   There were no group differences in corpora lutea, implantations, embryo/fetal viability, or fetal weight.
b)   The malformation and variation frequencies were similar among groups.

4.   Delivery and Offspring Developmental Parameters

a)   There were no treatment-related effects on reproductive parameters in dams allowed to deliver (gestation length, litter size, offspring viability).
b)   Offspring BWs at birth and during the lactation period were slightly decreased (NS, not D-R) in treatment groups (≤5% at HD), but there was evidence of recovery (catch up) in the postweaning period.
c)   There were no group differences in the emergence of air or surface righting

reflexes, in auditory startle response on Day 14, or in the pupillary light response at weaning.

d) No differences in F1 fertility and reproductive performance parameters were observed.

e) There were no T-R findings at necropsy of F1 pups.

B) TERATOGENICITY STUDY IN THE RAT
   GLP; Vol. 1.32)

1. Treatment

Pregnant rats (22/group) were treated with 0 (saline), 0.3, 1.5, 7.5, or 37.5 mg/kg sc on gestation Days 6 through 15. C-sections were performed on day 20. Doses were based on the results of a 4-week toxicity study in rats.
   Strain: Charles River CD
   Drug batch #: 99026/2

2. F0 Effects:

a) No T-R clinical signs were noted.
b) There were no effects on BW or food consumption.

3. Term Sacrifice Data and Fetal Evaluations

(After examination of term fetuses for external abnormalities, 1/2 of each litter was cleared and stained for skeletal evaluation, and the other 1/2 was fixed and examined for visceral anomalies using the Wilson technique.)

a) No differences in litter size, embryo/fetal viability, or fetal weight were observed between groups.
, b) No effects of COP-1 treatment on the incidences of fetal external, visceral, or skeletal anomalies were observed.

C) TERATOLOGY STUDY IN RABBITS
   GLP; Vol. 1.33)

1. Treatment

Pregnant rabbits (17/group) were treated with 0 (saline), 0.3, 1.5, 7.5, or 37.5 mg/kg sc on gestation days 6 through 19. C-sections were performed on day 29 of gestation.
   Strain: Charles River HY/CR New Zealand White
   Drug batch #: 09015

2. F0 Effects:

a) 1 HD animal had areas of necrosis and scabbing at the injection sites which were considered T-R. 1 LD animal was sacrificed after aborting on Day 27 and found to have necrotic fetuses in the uterus. This was not considered T-R. There were no other remarkable clinical observations.
b) Decreases (N.S.) in food consumption, BW gain (mean 50% below C), and BW

2

were observed at the HD during the dosing period.

3. Reproductive and Fetal Parameters; Fetal Evaluation

(Examination of viscera by fresh dissection method and skeletal evaluation were performed on each fetus.)

a) The numbers of corpora lutea, implantation sites, live, dead, and resorbed fetuses were comparable across groups. Fetal weight and C-R length were not affected by treatment.

b) Fetal morphology was not adversely affected by treatment.

E) PERINATAL-POSTNATAL STUDY IN RATS
GLP; Vol. 1.34)

1. Treatment

Female rats (25/group) were treated with 0 (saline), 1, 6, or 36 mg/kg sc from Day 15 of pregnancy until Day 21 postpartum. The dams were allowed to deliver and rear offspring. F1 offspring were evaluated for survival, growth, and reflex development.
Strain: Charles River CD
Drug batch #: 09005

2. F0 Effects:

a) No T-R clinical signs were noted during treatment. One MD female died during parturition; no cause of death was established.

b) Food consumption and BW gain were comparable among groups.

c) The duration of gestation was not altered by treatment.

d) There were no gross pathological findings at necropsy following weaning.

3. Parturition and F1 Parameters

a) All females produced live litters, and the numbers and viability of offspring were similar among groups.

b) Offspring body weights were similar at birth, but a D-R reduction in postnatal BW gain was observed in all treated groups compared to C (Table 1). BW was significantly lower in HD offspring (combined sex means) on PNDs 14, 18, and 21 compared to controls, although the difference was small (<10%). MD male BWs were also significantly lower during PNDs 18-21.

c) Reflex development (surface and air righting, auditory startle, pupillary closure) was similar among groups.

d) There were no T-R gross pathological findings in F1 groups at necropsy.

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TABLE 1

Offspring body weight (g); F1 pups -
group mean values and standard deviations

Group	:	1	2	3	4
Test material	:		COP-1		
Dosage (mg/kg/day)	:	0	1	6	36

Group No.		Day post partum						
		1	4	7	11	14	18	21
1	i	6.91	9.81	16.04	25.49	32.82	41.43	51.71
	ii	0.78	1.44	1.82	2.47	3.38	4.19	5.52
	iii	0.42	0.74	1.36	1.89	2.16	2.59	3.34
	N	22	22	22	22	22	22	22
2	i	6.94	10.05	16.42	25.65	32.50	40.86	50.88
	ii	0.54	^.90	1.36	2.00	2.32	2.87	3.73
	iii	0.49	0.78	1.25	1.75	1.97	2.34	3.13
	N	22	22	22	22	22	22	22
3	i	6.55	9.24	15.33	24.31	31.03	39.22	48.65
	ii	0.44	0.77	1.23	1.73	1.86,	2.80	3.77
	iii	0.48	0.82	1.52	2.31	3.20	3.10	4.00
	N	21	21	21	21	21	21	21
4	i	6.88	9.73	15.64	24.01	30.45[b]	38.43[b]	48.10[a]
	ii	0.56	1.06	1.65	1.97	2.09	2.53	3.67
	iii	0.42	0.79	1.42	2.12	2.49	2.86	3.68
	N	22	22	22	22	22	22	22

i   Group means
ii   Standard deviation
iii Pooled weighted within litter standard deviation
N   Sample size

a : Significantly different from control. $p < 0.05$
b : Significantly different from control. $p < 0.01$

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SUMMARY:

Segment I

Administration of COP-1 to CD rats prior to and during mating, gestation, and lactation at sc doses up to 36 mg/kg produced some parental toxicity (reduced PCV and HGB in HD males, injection site irritation in HD males and females) but had no adverse effects on fertility or reproductive outcome. The NOAEL for effects on reproduction was 36 mg/kg.

Segment II (rat)

Administration of COP-1 to pregnant rats during the period of organogenesis at doses up to 37.5 mg/kg produced no evidence of maternal toxicity and no apparent effects on embryofetal development. The NOAEL for maternal and developmental effects was 37.5 mg/kg.

Segment II (rabbit)

Administration of COP-1 to pregnant rabbits during the period of major organogenesis at sc doses up to 37.5 mg/kg caused slight maternal toxicity at the HD (decreased BW gain, necrosis at injection sites) but no adverse effects on embryofetal development. The NOAEL for developmental effects was 37.5 mg/kg.

Segment III

Administration of COP-1 to female CD rats during the last third of gestation and throughout lactation at sc doses up to 36 mg/kg produced small reductions in offspring preweaning BW gain (BW deficits statistically significant in HD males and females and in MD males) in the absence of maternal toxicity (indicating selective effect). There were no apparent adverse effects on offspring viability or functional development. The NOAEL for developmental toxicity was 1 mg/kg.

EVALUATION:

Because no evidence of maternal toxicity was observed at the high doses used in the rat studies (36 or 37.5 mg/kg), there is some question about whether or not dose selection was appropriate. However, the HDs are about 20-fold greater than the human dose on a mg/m2 basis, which should provide an adequate safety margin. The Segment III finding (D-R decrease in offspring BW gain) is unexplained but does not appear to be toxicologically significant in the absence of any apparent underlying structural or functional deficits. In addition, slight reductions in offspring BW during lactation in the Segment I study were made up in the postweaning period, indicating that the effect on growth is not permanent. There were no effects on embryo/fetal development in the Segment II studies. These studies indicate that COP-I has little potential for reproductive or developmental toxicity and should probably be classified into Pregnancy Category B.

cc:
NDA (20-622)
Div File
HFD-120/GFitzgerald/EFisher/TWheelous
JF 8/6/96

J.E. Fisher, Ph.D.

5

DIVISION OF NEUROPHARMACOLOGICAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA#: 20-622

CHEMISTRY REVIEW: # 1　　　　　　　　　　　　**DATE REVIEWED:** 05-APR-96

Submission Type	Document Date	CDER Date	Assigned Date
RESUBMISSION	10-OCT-95	11-OCT-95	18-OCT-95
AMENDMENT	10-JAN-96	16-JAN-96	18-JAN-96
AMENDMENT	12-JAN-96	16-JAN-96	18-JAN-96
AMENDMENT	29-MAR-96	01-APR-96	03-APR-96

NAME & ADDRESS OF APPLICANT:　Teva Pharmaceuticals USA
1510 Delp Drive, Kulpsville, PA 19443

DRUG PRODUCT NAME:
Proprietary:　　　　　　　　　　　　　　COPAXONE®
Nonproprietary/Established/USAN:　Copolymer-1 or COP-1
Chemical Type/Therapeutic Class:　1P

DESI / Patent Status: N/A

PHARMACOLOGY CATEGORY / INDICATION: Remitting / Relapsing MS
DOSAGE FORM:　　　　　　　　　　　Injection
STRENGTHS:　　　　　　　　　　　　20 mg
**ROUTE OF ADMINISTRATION:**　　　IM
DISPENSED:　　　　　　　　　　　　**XX** Rx　　___ OTC

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA:

L-Glutamic acid, polymer with L-alanine, L-lysine and L-tyrosine, acetic acid salt or
L-Tyrosine, polymer with L-glutamic acid, L-alanine and L-lysine, acetic acid salt
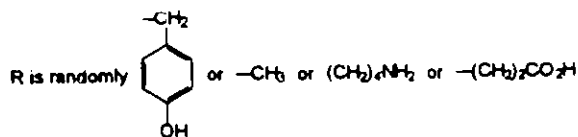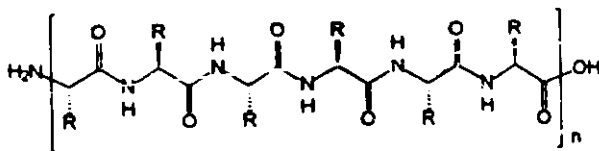
Poly[L-Glu$^{13-15}$, L-Ala$^{39-46}$, L-Tyr$^{8.6-10}$, L-Lys$^{30-37}$]•n CH$_3$CO$_2$H, n= 15 to 24 eq. HOAc/100 amino acid residues

NOTE: This is a totally random polymer and cannot be sequenced

CAS No.: Acetate salt, [147245-92-9]; Base, [28704-27-0]　　Mol. Weight: ca 4,700 to 13,000 Daltons

SUPPORTING DOCUMENTS: DMF

RELATED DOCUMENTS (if applicable):　　IND
　　　　　　　　　　　　　　　　　　　　　　IND

CONSULTS: The initial Environmental Assessment review (16-DEC-95) was returned to HFD-120 on
21-DEC-95. Microbiology: Reviewed by Dr. Patricia Hughes, HFD-160. Review returned to division on
05-JAN-96. Deficiencies were noted by both the EA and Microbiology reviewers.
The 29-MAR-96 (manufacture o'　　　　　　　　was sent to HFD-160 for review on 05-APR-96.

REMARKS/COMMENTS: Establishment Evaluations are still pending. Microbiology and
Environmental Assessment reviewers have identified deficiencies in the application. Methods
Validation cannot be initiated until we obtain a satisfactory response to deficiencies detailed in
this review [see review notes and draft letter]. There are several manufacturing deficiencies.
The most recent revision of DMF　　duplicates information submitted in the CMC portion of
this NDA and was not reviewed separately.

CONCLUSIONS & RECOMMENDATIONS: The NDA is Not Approvable for Chemistry at this time.

cc: Orig. NDA 20-622
　　HFD-120/Division File
　　HFD-120/MHeimann/05-APR-96
　　HFD-120/TWHEELOUS

Martha R. Heimann, Ph.D., Review Chemist

DIVISION OF NEUROPHARMACOLOGICAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA#: 20-622

CHEMISTRY REVIEW: # 2

Submission Type	**Document Date**	**CDER Date**	DATE REVIEWED: 02-DEC-96
Amendment	02-OCT-96	02-OCT-96	**Assigned Date**
Amendment	06-NOV-96	07-NOV-96	04-OCT-96
			25-NOV-96

NAME & ADDRESS OF APPLICANT: Teva Pharmaceuticals USA
1510 Delp Drive, Kulpsville, PA 19443

DRUG PRODUCT NAME:
Proprietary: COPAXONE®
Nonproprietary/Established/USAN: Copolymer-1 or COP-1
Chemical Type/Therapeutic Class: 1P

DESI / Patent Status: N/A

PHARMACOLOGY CATEGORY / INDICATION: Remitting / Relapsing MS
DOSAGE FORM: Injection
STRENGTHS: 20 mg
ROUTE OF ADMINISTRATION: IM
DISPENSED: XX_ Rx __ OTC

CHEMICAL NAME, STRUCTURAL FORMULA AND MOLECULAR FORMULA:

L-Glutamic acid, polymer with L-alanine, L-lysine and L-tyrosine, acetic acid salt or
L-Tyrosine, polymer with L-glutamic acid, L-alanine and L-lysine, acetic acid salt
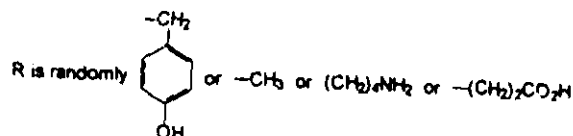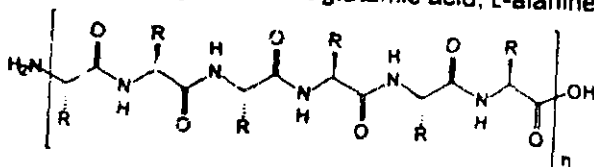
Poly[L-Glu$^{13-15}$, L-Ala$^{39-46}$, L-Tyr$^{8-10}$, L-Lys$^{30-37}$]•n CH$_3$CO$_2$H, n= 15 to 24 eq. HOAc/100 amino acid residues
NOTE: This is a totally random polymer and cannot be sequenced
CAS No.: Acetate salt, [147245-92-9]; Base, [28704-27-0] Mol. Weight: ca 4,700 to 11,000 Daltons

SUPPORTING DOCUMENTS: DMF

RELATED DOCUMENTS (if applicable): IND:
INI

CONSULTS: Microbiology: Reviewed by Dr. Patricia Hughes, HFD-160. Review returned to division on 08-NOV-96 with recommendation for approval.

REMARKS / COMMENTS: The 02-OCT-96 amendment contains responses to CMC and Microbiology deficiencies communicated to the firm in the Division's 07-AUG-96 deficiency letter. Many of the CMC deficiencies pertain to stability data submitted to support the firm's request for refrigeration storage of the drug substance and drug product. The sponsor has agreed to accept an 18 month expiry, with drug product stored frozen (-20°C to -10°C) and defer any request for alternate conditions or extended expiry until these deficiencies can be addressed fully. Acceptable responses were obtained for all of the remaining questions except the MW related specifications [refer to Draft Letter for revised specifications.] The 06-NOV-96 amendment is the most recent labeling revision. Establishment Evaluations have been completed and found to be acceptable and Environmental Assessment is complete. Methods Validation is being initiated.

CONCLUSIONS & RECOMMENDATIONS: The NDA is Approvable for Chemistry provided the revised Gel Permeation Chromatography (GPC) specifications [see Draft Letter] are accepted by the sponsor.

Martha R. Heimann, Ph.D., Review Chemist 12/2/96

ENVIRONMENTAL ASSESSMENT

AND

FINDING OF NO SIGNIFICANT IMPACT

FOR

Copaxone™

(Copolymer-1 for Injection)

TEVA Pharmaceuticals USA

NDA 20-622

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

Division of Neuropharmacological Drug Products
(HFD-120)

FINDING OF NO SIGNIFICANT IMPACT

NDA 20-622

Copaxone™

(Copolymer-1 for Injection)

The National Environmental Policy Act of 1969 (NEPA) requires all
Federal agencies to assess the environmental impact of their
actions. FDA is required under NEPA to consider the
environmental impacts of approving certain drug product
applications as an integral part of its regulatory process.

The Food and Drug Administration, Center for Drug Evaluation and
Research, has carefully considered the potential environmental
impact of this action and has concluded that this action will not
have a significant effect on the quality of the human environment
and that an environmental impact statement therefore will not be
prepared.

In support of their new drug application for Copaxone™, TEVA
Pharmaceuticals USA has prepared an abbreviated environmental
assessment in accordance with 21 CFR 25.31a(b)(3), (attached)
which evaluates the potential environmental impacts of the
manufacture, use and disposal of the product.

Copolymer-1 is a chemically synthesized drug which is
administered ʳ ₁ sterile solution in the treatment of multiple
sclerosis. T   ʳug substance will be manufactured by TEVA
Plantex Ltd., .. anya, Israel. The drug product will be
manufactured by TEVA pharmaceutical Industries Ltd., Kfar Sava,
Israel and Ben Venue Laboratories, Inc., Bedford, Ohio, USA. The
finished drug product will be used in hospitals, clinics and by
patients in their homes.

Copolymer-1 drug substance may enter the environment from
excretion by patients, from disposal of pharmaceutical waste or
from emissions from manufacturing sites.

Disposal of the drug may result from out of specification lots,
discarding of unused or expired product, and user disposal of
empty or partly used product and packaging. Returned or out-of-
specification drug substance and rejected or returned drug
product will be disposed of at licensed landfills. At U.S.
hospitals and clinics, empty or partially empty packages will be
disposed of according to hospital/clinic regulations. From home
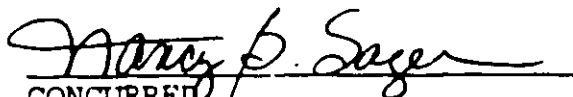
use, empty or partially empty containers will typically be disposed of by a community's solid waste management system which may include landfills, incineration and recycling, while minimal quantities of unused drug may be disposed of in the sewer system.

The Center for Drug Evaluation and Research has concluded that the product can be manufactured, used and disposed of without any expected adverse environmental effects. Precautions taken at the sites of manufacture of the bulk product and its final formulation are expected to minimize occupational exposures and environmental release. Adverse effects are not anticipated upon endangered or threatened species or upon property listed in or eligible for listing in the National Register of Historic Places.

3/25/96
DATE

PREPARED BY
Carl J. Berninger, Ph.D.
Environmental Scientist
Environmental Assessment Team
Center for Drug Evaluation and Research

4/14/96
DATE

CONCURRED
Nancy B. Sager
Acting Supervisor
Environmental Assessment Team
Center for Drug Evaluation and Research

Attachments: Environmental Assessment, FOI copy,
                 Material Safety Data Sheet included

PUBLIC

ENVIRONMENTAL ASSESSMENT

FOR

NDA 20-622

Copaxone™

(Copolymer-1 for Injection)

TEVA Pharmaceuticals USA

January 31, 1996

TABLE OF CONTENTS

1.0 DATE

January 31, 1996

2.0 NAME OF APPLICANT

TEVA Pharmaceuticals USA

3.0 ADDRESS

1510 Delp Drive
Kulpsville, PA 19443

4.0 DESCRIPTION OF PROPOSED ACTION

4.1 Requested Approval

TEVA Pharmaceuticals USA is requesting approval to manufacture, package, distribute, and market Copaxone®. Copaxone® is a sterile, non-pyrogenic drug product comprising the lyophilized dosage form and its solvent for reconstitution, Sterile Water for Injection USP. The recommended daily dose of Copaxone® is 20 mg active ingredient (Copolymer-1) in 1 ml of Water for Injection. The declared amount of active ingredient per vial, equivalent to the recommended daily dose, is 20 mg. However, each vial of the lyophilized dosage form will contain 22 mg of the active ingredient, Copolymer-1. This amount includes an overage of 2 mg of the active ingredient to allow for losses during preparation for injection. The lyophilized dosage form will be packaged in 2-ml amber Type I glass vials with rubber closures, aluminum seals, and plastic flip-off caps. Water for Injection will be packaged in 2-ml colorless Type I glass vials with rubber closures, and aluminum tear-off seals. The declared amount of Water for Injection per vial is 1 ml. However, each vial of Water for Injection for Copaxone® will contain 1.2 ml of Water for Injection. This includes an overage of 0.2 ml of Water for Injection to allow for losses during preparation for injection and provides for delivery of 20 mg of active ingredient in 1.0 ml of Water for Injection. The marketed product will be dispensed only on the order of a licensed physician.

4.2 Need for the Action

The active ingredient in Copaxone®, Copolymer-1, is a mixture of the acetate salts of synthetic polypeptides. It was developed to alleviate symptoms of multiple sclerosis in the exacerbating-remitting phase of the disease. With long-term use it has been shown to reduce both the frequency and the intensity of the attacks that the patients experience. Copolymer-1 was granted Orphan Drug designation for the treatment of multiple sclerosis on November 9, 1987.

4.3 Locations Where the Products will be Manufactured

The drug substance, Copolymer-1, will be manufactured by TEVA Plantex Ltd. in Netanya, Israel. The drug product, Copaxone®, will be manufactured and packaged at TEVA Pharmaceutical Industries Ltd. in Kfar Sava, Israel and at Ben Venue Laboratories, Inc. in Bedford, Ohio. Copaxone® will be shipped from the TEVA Pharmaceutical Industries Ltd. and the Ben Venue Laboratories, Inc. facilities for distribution in the United States.

Drug Substance Manufacturing site:

TEVA Plantex Ltd.
1 Haplada Street
P.O. Box 160
Netanya 42101
Israel

Drug Product Manufacturing and Packaging Sites:

TEVA Pharmaceutical Industries Ltd.
9 Hashikma Street, Industrial Zone
P.O. Box 353
Kfar Sava 44102
Israel

Ben Venue Laboratories, Inc.
270 Northfield Road
P.O. Box 46568
Bedford, Ohio 44146-0568

4.4 General Dispersement of Finished Product

Copaxone® will be dispensed by prescription and self-administered by patients at various locations, including private residences, hospitals, and clinics, throughout the United States. Copaxone® is intended for single dose subcutaneous injection. The lyophilized powder is reconstituted immediately prior to injection in the form of an aqueous solution with 1.0 ml of Water for Injection USP.

4.5 Sites of Disposal

Product introduced into patients is expected to be rapidly hydrolyzed into smaller polypeptides and free amino acids. Excretion occurs almost exclusively through the urine, resulting in distribution to wastewater treatment systems throughout the United States.

Returned product and packaging will be received by TEVA Marion Partners and transferred to

The waste materials that will be generated as a result of the packaging, storage, and distribution of Copolymer-1 will be cardboard cartons and also any product that is damaged during packaging or transporting. The cardboard waste that is generated will be placed into the recycling dumpster and transported to the recycling facility. Any product which needs to be disposed of will be collected in drums and transported to the landfill as non-hazardous waste. Incineration of the waste is also a disposal alternative.

Rejected drug substance and drug product manufactured at the TEVA Netanya and Kfar Sava sites in Israel and the Ben Venue Laboratories site in Bedford, Ohio will be disposed of at licensed and approved offsite facilities.

4.6 Types of Environments Present at and Adjacent to Corporate Locations

This section describes the general environment in the vicinity of the manufacturing facilities for Copolymer-1 and Copaxone®.

TEVA Plantex Ltd. (TEVA Plantex Facility)

The drug substance, Copolymer-1, will be manufactured at the TEVA Plantex Ltd. facility in Netanya, Israel, a town of approximately 140,000 people. TEVA Plantex is located in the Netanya industrial zone, located about 3 km east of the Mediterranean coast. It is bordered on the south, east, and west primarily by small workshops and commercial enterprises operated on a day-shift basis, and on the north by interurban road No. 56 which connects Netanya with the two parallel North-South highways of Israel. Currently, the Plantex facility is one of three plants in the industrial zone that operate 24 hours a day. The facility is served by municipal water and sewer systems. The local topography is flat. The area has a mild, Mediterranean climate with an average daily temperature of 25°C in the July and 12°C in the winter.

TEVA Pharmaceutical Industries Ltd. (TEVA Kfar Sava Facility)

The drug product, Copaxone®, will be manufactured and packaged at the TEVA Pharmaceutical Industries Ltd. facility in Kfar Sava, Israel. The facility is served by municipal water and sewer systems. TEVA Kfar Sava is located in the eastern industrial zone just outside the town of Kfar Sava. The facility is approximately 10 km east of the Mediterranean Sea. It is surrounded by other industrial and commercial facilities. The industrial zone is adjacent to residential areas on the west and rural agricultural areas (orange orchards) to the east. The local topography is flat. The area has a mild, Mediterranean climate with an average daily temperature of 28°C in the summer and 10°C in the winter.

Ben Venue Laboratories, Inc.

Copaxone® also will be manufactured and packaged at Ben Venue Laboratories, Inc. in Bedford, Ohio. A manufacturing site abbreviated environmental assessment for the Ben Venue Laboratories facility is provided in Appendix 1. Bedford is a city of approximately 15,000 people, located in Cuyahoga County, approximately 17 miles south of Cleveland, Ohio. Approximately 400 people are employed at the site. The surrounding land use consists of light industrial and chemical manufacturing and single family residential. The site is served by municipal water and sewer services. No additional construction or employment will result from the proposed action. The climate is typical of a northern temperate zone with an average summer time temperature of 26°C in June and -9°C in January. Precipitation in the form of rain and snow averages 35 inches and 56 inches, respectively.

5.0 IDENTIFICATION OF CHEMICAL SUBSTANCES SUBJECT TO THE PROPOSED ACTION

The chemical substances that are the subject of the proposed action can be divided into five categories: (1) drug substance, (2) drug substance impurities and degradants, (3) drug product excipients, (4) drug substance and drug product manufacturing waste products, and (5) packaging materials and package disposal waste products.

5.1 Drug Substance

Laboratory Name: Copolymer-1

Synonym: COP-1

Chemical Name/Molecular Formula: Copolymer-1 is the acetate salt of synthetic polypeptides prepared by chemically reacting the protected and activated derivatives of four amino acids: L-glutamic acid (L-Glu), L-alanine (L-Ala), L-tyrosine (L-Tyr), and L-lysine (L-Lys) in a specified ratio. The molar fraction of each amino acid residue ranges as follows: L-Glu (0.129-0.153), L-Ala (0.392-0.462), L-Tyr (0.086-0.10), L-Lys (0.30-0.374).

CAS Number: 28704-27-0

Structural Formula: Poly [L-Glu$^{13-15}$, L-Ala$^{39-46}$, L-Tyr$^{8.6-10}$, L-Lys$^{30-37}$) · nCH$_3$COOH, where the superscripts represent the range of amino acid residues present in the polypeptide expressed as a molar percent, and where the sequence of amino acids varies within each individual component; n is the range of acetic acid moieties per 100 amino acid residues (this value is between 15 and 29).

Physical Description: White to slightly yellowish lyophulized material.

5.2 Drug Substance Impurities and Degradants

Potential Impurities

Data concerning the potential impurities in Copolymer-1 were provided as Confidential Information.

Degradants

Copolymer-1 is stable for 6 months when stored at -20°C to -10°C.

5.3 Drug Product Excipients

The components of the drug product are listed below:

Lyophilized Product

Active ingredient: Copolymer-1

Excipients: Mannitol USP
Nitrogen NF

Solvent for Copolymer-1

Sterile Water for Injection USP

5.4 Manufacturing Waste Products

Drug substance wastes are materials that can potentially be released during the manufacture of Copolymer-1, and the materials used in cleaning and maintaining the production facilities. These substances include the drug substance, and a number of substances typically found in a pharmaceutical manufacturing facility, such as organic solvents, alcohols, reagents and chemical intermediates, purified water, commercial surfactants, cleaning agents, and detergents. Specific data concerning the chemicals used during the manufacture of Copolymer-1 at the TEVA Plantex Ltd. facility in Netanya, Israel were provided as Confidential Information.

Drug product manufacturing wastes are substances that can potentially be released during the lyophilized drug product manufacturing process. These include components of the drug product, as well as commercial cleaning agents, surfactants, and detergents. A list of the chemicals used during the manufacture of Copaxone® at both the TEVA Pharmaceutical Industries Ltd. facility in Kfar Sava, Israel and the Ben Venue Laboratories, Inc. facility in Bedford, Ohio was provided as Confidential Information.

5.5 Packaging Materials

The following materials will be used in packaging of the drug substance, Copolymer-1:

> polyethylene bags
> aluminum bag
> high density polyethylene (HDPE) container, 4.5 L
> HDPE screw cap

These packaging materials will enter the waste stream subsequent to manufacture of the drug product.

The lyophilized drug product (Copaxone®) and Sterile Water for Injection will be packaged individually in glass vials. The following materials will be used in packaging the drug product:

> 2-ml amber, Type I glass USP vials
> gray bromobutyl rubber stoppers (siliconized)
> gray aluminum seals with insert gray polypropylene flip-off caps

The following materials will be used in packaging Sterile Water for Injection:

> 2-ml clear, Type I glass USP vials
> gray bromobutyl rubber stoppers (siliconized)
> aluminum tear-off seals

These packaging materials will enter the waste stream as a result of product use, and when rejected or expired materials are returned. These are widely available and used pharmaceutical packaging materials.

6.0 INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

Copolymer-1 and the substances associated with its manufacture can potentially enter the environment from four major sources: (1) the site of manufacture of the drug substance, Copolymer-1; (2) the sites of manufacture and packaging of the drug product, Copaxone®; (3) the sites of use by patients; and (4) waste disposal sites for rejected, discarded, or returned product and packaging materials.

The manufacture of Copolymer-1 will take place at TEVA Plantex Ltd. in Netanya, Israel. The final drug product, Copaxone®, will be manufactured and packaged at TEVA Pharmaceutical Industries Ltd. in Kfar Sava, Israel and at Ben Venue Laboratories, Inc. in Bedford, Ohio. The drug product will be shipped to the LEMMON Company facility in Sellersville, Pennsylvania for packaging. The market packages will be shipped from LEMMON Company to TEVA Marion Partners in Kansas City, Missouri for distribution to hospitals, physicians, and pharmacies in the United States. Returned products will be transferred to LEMMON Company, Sellersville, Pennsylvania.

The expected emissions, emissions controls, and compliance with relevant environmental and occupational laws for each source of emissions associated with the proposed action are discussed below. Israeli law provides for the control of air and noise pollution, fresh water quality, marine pollution, hazardous substances and solid waste, and for the protection of nature and the health and safety of workers. General enabling legislation governs the national and local implementation of the existing environmental and occupational legislation. The national regulatory agencies include the Ministry of Environment, the Ministry of Labor, and the Ministry of Health; however, local municipal authorities typically are responsible for ensuring compliance with applicable environmental and occupational regulations. Specific requirements for individual manufacturing facilities typically are not listed in environmental regulations, guidelines, or permits; rather, many requirements for manufacturing facilities are developed, monitored, and enforced on a case-by-case basis. Appendix 2 contains a summary of Israeli environmental legislation that is applicable to operations at TEVA's Netanya and Kfar Sava manufacturing facilities.

Figures that outline the production processes for Copolymer-1 and Copaxone® were provided as Confidential Information.

6.1   Introduction of Substances From Manufacture of Copolymer-1

TEVA Plantex Ltd., Netanya, Israel

Introduction of the drug substance, Copolymer-1, and its manufacturing by-products into the environment potentially can occur from the TEVA Plantex facility in Netanya, Israel. The existing Copolymer-1 unit is being expanded to accommodate commercial production of the drug substance. The commercial production unit will be constructed and integrated within the existing Plantex production facilities. TEVA's manufacturing facilities operate under the environmental and occupational laws and regulations of Israel. A list of the substances expected to be emitted in the production of Copolymer-1 at the TEVA Plantex facility was provided as Confidential Information.

Control of Environmental Emissions

TEVA is committed to a strategy of environmental management based on complete cooperation with the relevant regulatory authorities having jurisdiction: the Ministries of Health, Labor, and Environment. All TEVA facilities attempt to minimize emissions of manufacturing wastes to the environment by optimizing process operations; collecting wastes for recovery, recycling, or destruction where this is technically and economically feasible; and maintaining a high standard of general housekeeping through standard operating procedures and good manufacturing practices.

Air Emissions

Air emissions associated with production of Copolymer-1 and with storage and transfer of raw materials will be controlled, as appropriate, by using a number of different types of control technologies and standard operating procedures. General ventilation in all Copolymer-1 production areas will be without air recirculation; only fresh filtered and one pass air conditioned air will enter these areas. Filtration efficiency for air entering the Copolymer-1 unit will be 90-95 percent, with the exception of the lyophilizer and ultrafiltration areas where HEPA filtration systems with greater than 99.97 percent final stage filtration efficiency will be used. Non-woven cotton fabric type filter systems with approximately 30 percent filtration efficiency will be used for air vented to the atmosphere.

Nitrogen blanketing will be used for all manufacturing operations involving solvents or volatile organic chemicals, and for solvent storage tanks. All vents on process reactors and vessels will be connected in series, as appropriate, to chilled water condensers, activated carbon adsorbers, and process scrubbers (dilute caustic, dilute acid, or water) before discharge to ambient air. The Copolymer-1 tank farm will be integrated within the Plantex tank farm. Solvent storage tanks will be underground and other chemical tanks will be within aboveground diked areas. Selected acid storage tanks will be in a separate diked area. The diked space around the tank will be protected by a high-capacity emergency system capable of eliminating all acid fumes in case of a spill. Air emissions from storage tanks will be controlled using (1) vapor volume exchange between tanks and tank-trucks or drums; (2) vent scrubbers on acid storage tanks; and (3) activated carbon adsorption on organic chemical tank vents.

Wastewater Generation and Disposal

Sanitary sewage will be discharged directly to the municipal sewer system. Concentrated, dilute, and saline process wastewaters will be pumped to separate dedicated wastewater holding tanks in the Plantex tank farm. Both concentrated and dilute wastewaters ultimately will be transported to TEVA's new Teva-Tech plant, currently under construction at the Ramat Hovav Industrial Park

This plant will have extensive neutralization and treatment facilities, with sludge thickening and dewatering. Treated wastewater will be discharged to the Ramat Hovav public process sewer system. Sludge cake will be sent to Environmental Services (Ramat Hovav) Ltd., known as the National Hazardous Waste Site. This is the only designated site in Israel for treatment and disposal of hazardous wastes. Reference is made to the National Hazardous Waste Site in the Licensing of Business Regulations (Disposal of Hazardous Substances), 1990 (see translation in Appendix 2, Item 1). Because the new Teva-Tech plant may not be completed prior to the initiation of Copolymer-1 production, wastewater will be disposed of, as necessary, at the Ramat Hovav National Hazardous Waste Site. Saline wastewater is transported to a central location, operated in cooperation with national environmental authorities, for chloride/salt recovery and treatment, with ultimate release of saline water to the sea.

Solid Waste Generation and Disposal

Hazardous solid waste will be generated from the Copolymer-1 production process and as sludge cake from wastewater treatment at the Ramat Hovav Teva-Tech site. These materials will be collected according to TEVA standard operating procedures and will be disposed of at the National Hazardous Waste Site.

General office and industrial waste will be collected and disposed of in local municipal sanitary landfills.

Compliance With Emissions Requirements

This section describes the specific regulations to which operations at the TEVA Plantex facility are subject and the corresponding regulatory authorities responsible for issuing permits and for enforcing the regulations

The Ministry of the Environment has primary authority for regulating industrial waste, air emissions, and the handling and disposal of hazardous substances. Compliance with environmental regulations is typically enforced by municipal authorities in cooperation with regional and national regulatory authorities. Specific regulatory requirements for individual manufacturing facilities typically are developed on a case-by-case basis. A primary mechanism for ensuring compliance with environmental regulations for the TEVA Plantex facility involves the issuance of a license under provisions of the Hazardous Substances Law, 1993 (see summary in Appendix 2, Item 2). This license, referred to as a Poisons Permit, provides the environmental regulators (Ministry of the Environment or authorized representatives, including local municipal regulators) with authority to enter and inspect facilities possessing a license. Under provisions of the Hazardous Substances Law, 1993, the Ministry of the Environment may make

recommendations to modify or may halt operations at the regulated facility to assure compliance with environmental regulations and proper handling of hazardous substances. The Poisons Permit for the TEVA Plantex facility is issued annually. The chemicals associated with production of Copolymer-1, identified in summary tables cited in previous sections of this Environmental Assessment, account for a very small number and volume of the chemicals actually listed in the Poisons Permit.

Air Emissions

Air emissions from the Plantex facility are in compliance with existing environmental regulations, and approval of the requested action is not expected to affect the facility's ability to comply with these regulations. No permits related specifically to air emissions are required for the TEVA Plantex facility. Currently, air emissions are regulated under the Abatement of Nuisances Regulations (Air Quality), 1992 (see summary in Appendix 2, Item 3). However, control of air emissions from the Copolymer-1 production process has been planned in accordance with new regulations expected to be promulgated within the next few years. These regulations will be based on the European Economic Community system which has adopted the German TA-Luft (see summary in Appendix 2, Item 4). The regulatory authority for air emissions, the Ministry of the Environment has, in effect, begun to use the European Community system in making decisions regarding industrial emissions; however, no formal licensing or permitting process has been established. TEVA Plantex is working with the Ministry of the Environment and the Netanya Municipal Authorities to incorporate control of emissions from the Copolymer-1 unit to TA-Luft standards.

The European Economic Community system for controlling air emissions addresses both the concentration of a given pollutant and its emission rate, and will be more stringent and address more pollutants than the existing regulations. If a pollutant is emitted in excess of the prescribed emission rate, it is necessary to comply with a specific concentration limitation. However, if the pollutant is emitted at a rate below the prescribed emission rate, the concentration limit will be developed based on a case-specific evaluation by the Ministry of the Environment. Not all materials emitted from TEVA facilities will exceed the regulatory emission rates; however, for consistency and to simplify compliance with the regulations, TEVA has designed its process controls to comply with the regulatory concentration limits for the maximum emission rates

The Ministry of the Environment works informally with facility engineers and designers to establish design criteria for new industrial operations. For modifications to an existing plant, it may be necessary to complete a questionnaire addressing issues that could affect environmental emissions. In addition, the Ministry of the Environment periodically may require an air emissions survey to be completed. Any information that may be required in this regard is determined on a case-by-case basis. For plants that use toxic chemicals in large amounts, the Ministry may require

preparation of a risk assessment. This risk assessment provides an analysis of emergency response plans to address scenarios selected by the regulatory authorities. In this regard, TEVA prepared an analysis of response activities at the Plantex facility for the following three scenarios: (1) a fire involving solvents at the tank farm; (2) an acid leak at the tank farm; and (3) release of selected process reagents due to a faulty process adsorber. The Copolymer-1 unit has been designed to provide for adequate controls to effectively respond to scenarios such as those evaluated in the risk assessment.

Currently, the Ministry of the Environment typically does not require control at the stack of sulfur dioxide, suspended particulates, and nitrogen dioxide emissions from small industrial boilers (less than approximately 80 megawatts) such as the main boiler at the TEVA Plantex facility. Concentrations of these emissions are controlled primarily by requiring use of the boiler according to proper operating specifications. Ultimately, low sulfur fuel will be required for use in specified industrial boilers; however, only very limited amounts of this type of fuel currently are available in Israel. The Plantex facility is in compliance with existing regulations and anticipates meeting the new regulations without changes in existing power generation or control equipment.

TEVA Plantex has designed Copolymer-1 process emissions controls to comply with the anticipated TA-Luft based air regulations. The controls will incorporate the following:

(1)   use of chilled water or brine for condensers on process vessels and storage tanks;
(2)   installation of an activated carbon adsorber on each process vessel and storage tank, as necessary, after the condenser, if a condenser is fitted;
(3)   connection of activated carbon adsorbers, if required, to an absorption tower operated either with water or dilute (3-10%) caustic soda solution, depending on the chemicals to be removed.

There are three adsorber/scrubber systems in the Copolymer-1 plant with design flow rates sufficient to meet anticipated production needs. Use of the systems described above will result in emissions of regulated air contaminants below concentration limits that will be specified in the air regulations.

Emissions from process chemical storage tanks during filling operations will be prevented through the use of a fill and draw system whereby a volume of vapor space is transferred from the tank being filled to the tank or drum being emptied at a flow rate equivalent to the flow rate of the liquid. Activated carbon adsorbers will be installed on the vents of tanks for solvents with relatively high vapor pressures to minimize releases through conservation vents due to tank breathing. The acid storage tank will be permanently refrigerated by chilled water. This tank also is attached to a 150 cu m /hour process scrubber operated with 10% caustic soda solution. In addition, an emergency containment and control system is in place to respond to spills at the acid storage tank

Fugitive emissions from process operations are not regulated. However, a number of controls are typically employed at TEVA manufacturing facilities. These include, where possible, enclosed pumps, appropriate mechanical seals, appropria'e connections on flexibles, diaphragm type valves, conservation vents, and activated carbon protected breathers.

Wastewater Generation and Disposal

Wastewater disposal is carried out by TEVA Plantex in accordance with the requirements of the Model Bylaw for Local Authorities (The Discharge of Industrial Wastes into the Sewage System), 1981 (see translation in Appendix 2, Item 5). Model bylaws are approved by the Minister of the Interior. They are not of themselves binding, but serve as a recommendation to Local Authorities. Disposal of liquid wastes from the Plantex facility to the Netanya Municipal Sewage System and offsite is in compliance with existing environmental regulations, and approval of the requested action is not expected to affect the facility's ability to comply with these regulations. Three types of wastewater will be associated with Copolymer-1 production at the Plantex facility.

Only sani'ary sewage, with a contribution of about 3 cu.m. per day from the Copolymer-1 plant, will be di charged directly to the Netanya Municipal Sewage System. The Netanya municipal authorities or Ministry of the Environment authorities occasionally request monitoring data on discharges to the municipal sewage treatment system; however, regular submissions are not required.

Concentrated (i.e., high COD), dilute, and saline process wastewaters will be collected and stored separately at the Plantex facility. The concentrated and dilute process wastewaters ultimately will be transported to TEVA's Teva-Tech plant currently being built at the Ramat Hovav Industrial Park. This plant will have extensive neutralization and physico-chemical treatment facilities, with sludge thickening and dewatering. All treated wastewater from the Teva-Tech site will be discharged to the Ramat Hovav public process sewer system. Saline wastewater will be treated and disposed of separately, under a program administered by a designated Industrial Organization and monitored by the Ministry of the Environment.

The Ramat Hovav public process sewer system incorporates special collection, treatment, and disposal systems designed to serve the numerous facilities in this industrial zone without allowing environmental deterioration. Its maintenance and administration is under the jurisdiction of the Ramat Hovav Industrial Local Council. This Industrial Local Council was created in 1988 by joint legislation from the Ministries of Interior, Industry and Commerce, Health, and the Environment. Delegates from these ministries along with members of industrial corporations at the Ramat Hovav Industrial Park have seats on the Council. Wastewater discharged to the process sewer system is treated by solar evaporation in high density polyethylene lined evaporation ponds with leak monitoring capabilities. Effluents to this system can be separated

into clean (i.e., biodegradable) wastewater and process (i.e., non-degradable) wastewater. The biodegradable effluents ultimately will be treated in a biological treatment plant, presently under pilot investigation, prior to discharge to the evaporation ponds.

The Teva-Tech facility may not be ready to accept wastes for treatment prior to startup of the Copolymer-1 production process. Concentrated and dilute process wastewaters will be transported to the Ramat Hovav National Hazardous Waste Site for disposal until the Teva-Tech facility is able to accept wastes. The National Hazardous Waste Site treats and disposes of all hazardous wastes generated in Israel, as required under the Licensing of Business Regulations (Disposal of Hazardous Substances), 1990 (see translation in Appendix 2, Item 1). Currently, all hazardous wastes are treated and disposed of by landfilling in double high density polyethylene lined cells, with waste segregation and monitoring. TEVA Plantex collects, transports, and disposes of hazardous wastes according to the Licensing of Business Regulations (Disposal of Hazardous Substances), 1990 and the Hazardous Substances Law, 1993 (see summary and translation in Appendix 2, Items 1 and 2). TEVA follows Standard Operating Procedures for waste and drug removal and destruction and complies with the waste tracking requirements of the National Hazardous Waste Site. Hazardous materials are transported for treatment and disposal by a hauler registered with the Ministry of Transport.

Solid Waste Generation and Disposal

The primary regulations governing storage, treatment, and disposal of hazardous wastes are the Licensing of Business Regulations (Disposal of Hazardous Substances), 1990 and the Hazardous Substances Law, 1993 (see summary and translation in Appendix 2, Items 1 and 2). Disposal of solid wastes from the Plantex facility is in compliance with existing environmental regulations, and approval of the requested action is not expected to affect the facility's ability to comply with these regulations. Hazardous solid wastes will be generated from the Copolymer-1 production process, primarily in the form of spent activated carbon; approximately 1,000 kg per year of these wastes will be generated. In addition, sludge cake from wastewater treatment ultimately will be generated at the Teva-Tech facility. These wastes will be disposed of at the Ramat Hovav National Hazardous Waste Site. Hazardous solid wastes are handled in accordance with formal Standard Operating Procedures for the Plantex facility. Hazardous materials are transported for treatment and disposal by a hauler registered with the Ministry of Transport. Currently, hazardous wastes are disposed of in landfills at the National Hazardous Waste Site. However, disposal of organic wastes by incineration is expected to begin at the Site in 1996.

Non-hazardous solid wastes will be disposed of in landfills operated by the local municipalities. No special permits are required for use of these landfills.

Compliance With Occupational Health and Safety Requirements

The Plantex facility operates in compliance with applicable occupational health and safety laws.
The Israeli Ministry of Labor has adopted, by reference, the American Conference of
Governmental Industrial Hygienists TLVs, Threshold Limit Values for Chemical Substances and
Physical Agents in the Work Environment. The TEVA Plantex Safety Committee works with the
facility's Medical Advisor to ensure the health and safety of workers.

In general, worker health and safety is protected by appropriate engineering controls that
eliminate or reduce worker exposure to potentially hazardous chemicals or situations. Personal
protective devices and clothing are used as necessary. In addition, medical testing is carried out
as necessary. All necessary steps are taken to assure compliance with Work Safety Ordinance
requirements of the Ministry of Labor. The Regional Superintendent of the Ministry of Labor has
the authority to halt operations at any manufacturing facility with major violations of health and
safety regulations or to recommend actions to improve conditions at a facility. In reality,
however, the Good Manufacturing Practices applied in the pharmaceutical industry tend to result
in more stringent protection of worker health and safety than the Ministry of Labor regulations.
Accordingly, health and safety violations in the pharmaceutical industry are extremely rare.
Appendix 4 contains a copy of the Material Safety Data Sheet (MSDS) for Copolymer-1.

Occupational health and safety requirements for the Ministry of Labor typically are determined on
a case-by-case basis. TEVA Plantex periodically submits data on industrial hygiene and medical
testing to the Ministry of Labor. In addition, representatives of the Ministry of Labor inspect the
facility, on average once per year. The Regional Superintendent of the Ministry of Labor has the
authority to halt operations at manufacturing facilities that are not in compliance with
occupational safety and health requirements. The continued operation of TEVA Plantex in good
standing with the Ministry of Labor indicates its compliance with existing occupational safety and
health regulations.

As part of the requirements for demonstrating the safety of a proposed manufacturing operation,
the Ministry of the Environment identifies three scenarios describing emergencies that could
threaten the health or safety of the plant workers or the neighboring population. In this regard,
TEVA carried out an evaluation of its response capabilities in the event of (1) a fire at the tank
farm, (2) an acid leak at the tank farm, and (3) a faulty process scrubber. Results of the risk
assessments for these scenarios indicated to the regulatory authorities that the Plantex facility
could adequately respond to these emergencies

Effects of Approval on Compliance

Approval of the proposed action is expected to have no effect on the ability of the Plantex facility to comply with any environmental or occupational safety and health laws or regulations currently in effect. The Plantex facility is designed to have adequate controls in place to allow for compliance with applicable air emissions regulations. Solid and liquid wastes generated as a result of Copolymer-1 production will be disposed of in compliance with existing regulations at licensed disposal facilities.

TEVA Plantex possesses a currently valid Poisons Permit (I.D. Number 3006135) under the Hazardous Substances Law, 1993 (Appendix 2, Item 1). The Plantex facility is subject to inspection by the Ministry of the Environment and the local environmental authorities under the requirements of the Hazard Substances Law, 1993. Possession of this permit indicates that the Plantex facility is in compliance with existing environmental regulations relating to air, water, and solid waste emissions.

6.2   Introduction of Substances From Manufacture of the Drug Product, Copaxone®

Manufacture of the drug product, Copaxone®, will take place at TEVA Pharmaceutical Industries Ltd. in Kfar Sava, Israel and at Ben Venue Laboratories, Inc. in Bedford, Ohio. Although emissions are expected to be minimal, potential discharges of Copolymer-1 may occur during manufacturing and packaging runs, and equipment and facility cleaning. Minimal emissions of other chemical substances typically used in a pharmaceutical manufacturing facility will also occur throughout the manufacturing and packaging activities. A list of the substances expected to be emitted in the production of Copaxone® was provided as Confidential Information.

TEVA Pharmaceutical Industries Ltd., Kfar Sava, Israel

Control of Environmental Emissions

TEVA is committed to a strategy of environmental management based on complete cooperation with the relevant regulatory authorities having jurisdiction: the Ministries of Health, Labor, and Environment. All TEVA facilities attempt to minimize emissions of manufacturing wastes to the environment by optimizing process operations; collecting wastes for recovery or destruction where this is technically and economically feasible; and maintaining a high standard of general housekeeping through standard operating procedures and good manufacturing practices.

Air Emissions

The heating, ventilation, and air conditioning (HVAC) system for the Kfar Sava Copaxone®
production unit incorporates the use of an air filtration system. Copolymer-1 and compounded
Copaxone® may enter the controlled air space within the Copaxone® production areas in very
limited amounts. All air effluent generated from the processes involved in the manufacture of
Copaxone® (e.g., weighing, mixing, and packaging) will pass through HEPA filters integrated into
the facility air filtration system. Air is recirculated in the Copaxone® manufacturing suite with
makeup air sufficient to account for leakage. The HEPA filters have collection efficiencies
greater than 99.97 percent for all the particle sizes normally encountered. Spent filters and waste
material collected in the air filtration systems will be handled as hazardous solid waste and will be
disposed of at the National Hazardous Waste Site at Ramat Hovav.

Wastewater Generation and Disposal

Wastewater will be generated from washing of empty vials before their use in the drug product
filling operation. Washing is carried out first with purified water, followed by a final rinse with
water for injection USP. Liquid effluent from the washing process is discharged to the Kfar Sava
plant's industrial drainage system for pretreatment prior to its release to the municipal sewer.
Wastewater containing minute quantities of process raw materials and drug product may be
generated during the Copaxone® manufacturing process, primarily during the cleaning of
equipment and manufacturing areas. This wastewater also flows first to the facility's industrial
drainage system for pretreatment prior to discharge to the municipal sewer system. Total product
loss is expected to be minimal.

The TEVA Kfar Sava industrial drainage system incorporates pretreatment in a series of four
reservoirs that consists of separation of grease and fats, neutralization, settling of particulates, and
collection of high chloride saline water. Treated water is subsequently discharged to the
municipal sewer system. Saline process wastewater is transported to a separate central treatment
location, operated in cooperation with national environmental authorities, for chloride/salt
recovery and treatment, with ultimate release of saline water to the sea. Collected particulates are
treated as solid waste and disposed of at the National Hazardous Waste Site at Ramat Hovav.
Liquid effluent from the industrial drainage system is monitored periodically by both plant
personnel and the municipality. Sanitary sewage from the TEVA Kfar Sava facility is discharged
directly to the municipal sewer without pretreatment.

Solvents and other potential hazardous wastes are collected in drums for disposal at the National
Hazardous Waste Site at Ramat Hovav. These substances are not discharged to the facility
industrial drainage system or the municipal sewer. Hazardous effluents are not associated with
the Copaxone® manufacturing process. Water is the solvent used in the production of Copaxone®
and hazardous substances are not used in the process.

the Copaxone* manufacturing process. Water is the solvent used in the production of Copaxone*
and hazardous substances are not used in the process.

Solid Waste Generation and Disposal

Solid waste generated at the TEVA Kfar Sava plant potentially will include small residual
amounts of raw materials, rejected raw materials, rejected batches and expired lots, broken filled
and empty vials, and spent filters from the air handling and dust collection units. These materials
will be collected at a designated holding area and sent to the National Hazardous Waste Site.
General manufacturing solid wastes such as paper and cartons are collected for disposal at the
Kfar Sava municipal landfill.

Compliance With Emissions Requirements

This section describes the environmental regulations to which operations at the TEVA Kfar Sava
facility are subject and the corresponding regulatory authorities responsible for monitoring
compliance and enforcing the regulations.

The Ministry of the Environment has primary authority for regulating industrial waste, air
emissions, and the handling and disposal of hazardous substances. Compliance with
environmental regulations is typically enforced by municipal authorities in cooperation with
regional and national regulatory authorities. The local Environmental Quality Unit with
jurisdiction for the TEVA Kfar Sava facility has provided a letter certifying that production of
Copaxone* now (as a pilot plant operation) and under anticipated future conditions will not cause
air, water, or soil pollution, and that the TEVA Kfar Sava facility is currently and is expected to
remain in compliance with existing environmental regulations. A copy of this compliance letter is
provided in Appendix 3.

Air Emissions

Air emissions from the Kfar Sava facility are in compliance with existing environmental
regulations, and approval of the requested action is not expected to affect the facility's ability to
comply with these regulations. No permits related specifically to air emissions are required for the
TEVA Kfar Sava facility. Currently, air emissions are regulated under the Abatement of
Nuisances Regulations (Air Quality), 1992 (see summary in Appendix 2, Item 3) However,
control of air emissions from the TEVA Kfar Sava facility is being planned in accordance with
new regulations expected to be promulgated within the next few years. These regulations will be
based on the European Community system which has adopted the German TA-Luft (see
summary in Appendix 2, Item 4). The regulatory authority for air emissions, the Ministry of the
Environment has, in effect, begun to use the European Community system in making decisions

regarding industrial emission; however, no formal licensing or permitting process has been established.

Control of air emissions from the Kfar Sava facility will be in accordance with new regulations to be promulgated within the next year. No emissions associated with the Copaxone® production process will be specifically regulated by these regulations. The primary emissions of potential concern are associated with losses of the drug substance, Copolymer-1, during manufacture of the drug product. However, emissions to air will be negligible and any losses would be captured by the HEPA filtration system or during normal cleanup activities.

Emissions of sulfur dioxide, suspended particulates, and nitrogen dioxide from the main boiler stack at the facility are regulated under the existing and anticipated new air regulations. Concentrations of these emissions are controlled primarily by requiring use of the boiler according to proper operating specifications. Two new boilers were recently installed at the Kfar Sava facility, replacing the previously used units. Diesel fuel is currently used in the TEVA Kfar Sava boilers. Emissions from the facility's boiler stack are tested periodically by the local Environmental Unit. The Kfar Sava facility is in compliance with existing regulations and anticipates meeting the new regulations without changes in existing power generation or control equipment.

Wastewater Generation and Disposal

Wastewater disposal is carried out by TEVA Kfar Sava in accordance with the requirements of the Model Bylaw for Local Authorities (The Discharge of Industrial Wastes into the Sewage System), 1981 (see translation in Appendix 2, Item 5). The discharge of wastewater from the Kfar Sava facility is in compliance with existing environmental regulations, and approval of the requested action is not expected to affect the facility's ability to comply with these regulations.

Sanitary sewage is discharged directly to the municipal sewer system. Process wastewater, which may contain minute quantities of Copolymer-1, will be pretreated prior to release to the municipal sewer system. Pretreatment includes separation of grease and fats, neutralization, settling of particulates, and collection of saline wastewater for disposal. Saline wastewater will be treated and disposed of separately, under a program administered by a designated Industrial Organization and monitored by the Ministry of the Environment. Sludge from the pretreatment facility will be transported to the Ramat Hovav National Hazardous Waste Site for treatment and disposal.

Hazardous wastewaters are not generated as part of the Copaxone® production process. However, TEVA Kfar Sava collects, transports, and disposes of any hazardous wastes generated at the plant according to the Licensing of Business Regulations (Disposal of Hazardous Substances), 1990 and the Hazardous Substances Law, 1993 (see summary and translation in

Appendix 2, Items 1 and 2). TEVA Kfar Sava follows Standard Operating Procedures for waste and drug removal and destruction and complies with waste tracking requirements of the National Hazardous Waste Site. Hazardous materials are transported for treatment and disposal by a hauler registered with the Ministry of Transport.

The Kfar Sava municipal authorities or local Ministry of the Environment representatives occasionally monitor water discharged from the facility to the municipal sewer system. TEVA Kfar Sava also monitors these effluents periodically. Regulatory authorities occasionally request monitoring data on discharges to the municipal sewer system; however regular submissions are not required.

Solid Waste Generation and Disposal

The primary regulations governing storage, treatment and disposal of hazardous wastes are the Licensing of Business Regulations (Disposal of Hazardous Substances), 1990 and the Hazardous Substances Law, 1993 (see summary and translation in Appendix 2, Items 1 and 2). Disposal of solid wastes from the Kfar Sava facility is in compliance with existing environmental regulations, and approval of the requested action is not expected to affect the facility's ability to comply with these regulations. Solid waste from the Copaxone³ production process, including remnants of the drug substance and drug product, and broken vials, and spent filters will be collected and transported to the Ramat Hovav National Hazardous Waste Site. Hazardous solid wastes are handled in accordance with formal Standard Operating Procedures for the Kfar Sava facility. Hazardous materials are transported for treatment and disposal by a hauler registered with the Ministry of Transport.

Non-hazardous solid wastes will be disposed of in landfills operated by the local municipalities No special permits are required for use of these landfills.

Compliance With Occupational Health and Safety Requirements

The TEVA Kfar Sava facility operates in compliance with applicable occupational health and safety laws. The Israeli Ministry of Labor has adopted, by reference, the American Conference of Governmental Industrial Hygienists TLVs, Threshold Limit Values for Chemical Substances and Physical Agents in the Work Environment.

In general, worker health and safety is protected by appropriate engineering controls that eliminate or reduce worker exposure to potentially hazardous chemicals or situations Personal protective devices and clothing are used as necessary. In addition, medical testing is carried out periodically. All necessary steps are taken to assure compliance with Work Safety Ordinance requirements of the Ministry of Labor. The Regional Superintendent of the Ministry of Labor has

the authority to halt operations at any manufacturing facility with major violations of health and safety regulations or to recommend actions to improve conditions at a facility. In reality, however, the Good Manufacturing Practices applied in the pharmaceutical industry tend to result in more stringent protection of worker health and safety than the Ministry of Labor regulations. Accordingly, health and safety violations in the pharmaceutical industry are extremely rare Appendix 4 contains a copy of the Material Safety Data Sheet (MSDS) for Copolymer-1.

Occupational health and safety requirements for the Ministry of Labor typically are determined on a case-by-case basis. TEVA Kfar Sava periodically submits data on industrial hygiene and medical testing to the Ministry of Labor. In addition, representatives of the Ministry of Labor inspect the facility, on average once per year. The Regional Superintendent of the Ministry of Labor has the authority to halt operations at manufacturing facilities that are not in compliance with occupational safety and health requirements. The continued operation of TEVA Kfar Sava in good standing with the Ministry of Labor indicates its compliance with existing occupational safety and health regulations.

Effects of Approval on Compliance

Approval of the proposed action is expected to have no effect on the ability of the TEVA Kfar Sava facility to comply with any environmental or occupational safety laws or regulations currently in effect. Emissions of potentially toxic substances, including the drug substance and drug product, associated with the manufacture of Copaxone® will be very small. Furthermore, adequate controls are in place to minimize emissions in air, wastewater, and solid waste.

The local representatives of the Ministry of the Environment have certified that the TEVA Kfar Sava facility is currently in compliance with existing environmental requirements and that it anticipates continued compliance after commercial production of Copaxone® is begun. A letter from the local Environmental Quality Unit documenting this certification is provided in Appendix 3.

Ben Venue Laboratories, Inc., Bedford, Ohio

A Manufacturing Site Abbreviated Environmental Assessment for the Ben Venue Laboratories Inc. facility is provided in Appendix 1. Information relevant to the Copaxone® production process is summarized in this section of the Environmental Assessment.

Control of Environmental Emissions

Air Emissions

Air emissions from the manufacture of Copaxone® at the Ben Venue facility may include trace particulates. All work areas used in the manufacture of the drug product are vented to High Efficiency Particulate Filters (HEPA). These filters are expected to capture at least 97 percent of any particulates entering the air in the work space. Filtered air is vented to the outside. The filters are monitored on a schedule that varies with production activity because of the multiproduct nature of the manufacturing site. Filters no longer meeting control specifications enter the solid waste stream from the facility.

Wastewater Generation and Disposal

Liquid waste from the Copaxone® production process is expected to consist of production equipment rinses and tailings left over from the filling operation. Prior to rinsing, all equipment is vacuumed to remove any solid material left. The vacuumed material is considered toxic and is handled as toxic waste. Both the vacuumed material and the rinse material from Copaxone® production are collected by Ben Venue Laboratories and disposed of by an EPA licensed contractor, Chemical Analytics, Inc.

Solid Waste Generation and Disposal

Non-hazardous solid wastes such as paper, aluminum, plastic, filters, and disposable suits and labware that cannot be recycled are disposed of in a state licensed landfill.

Solid wastes treated as toxic substances, including rejected raw materials and finished product, severely contaminated HEPA filters and quality control laboratory wastes are disposed of by incineration by a facility permitted to handle such waste streams. In order to minimize the amount of material for disposal, excess Copolymer-1 may be returned to the customer for further use.

Copaxone® (Copolymer-1 for Injection)
Environmental Assessment
Page 23

Compliance With Emissions Requirements

This sections discusses Ben Venue Laboratories, Inc.'s compliance with applicable environmental regulations and requirements.

Air Emissions

No air emission permits are required for the Ben Venue facility by either state or federal authorities due to the nature and low volume of emissions. No volatile organic solvents are used in the Copaxone* production process; all solvents are water based. A small amount of alcohol and bleach is used for sanitization. The emissions from this use are expected to be negligible. Three boilers are registered by the Ohio EPA. These registrations, Numbers 1318030726B001, 1318030726B002, and 1318030726B003, have no date of expiration.

Wastewater Generation and Disposal

Ben Venue Laboratories holds a permit for indirect discharge to the Bedford Publicly Owned Treatment Works (POTW). The permit (3PD00005101*BP) is administered by the Ohio EPA in accordance with the Clean Water Act and Rule 3745-33-06 of the Ohio Administrative Code. It requires the monitoring of discharge flow rate, pH, and cyanide concentrations at 3- to 6-month intervals. The discharge pH is limited to a pH of not less then 5.0 at any given time. Cyanide discharge concentrations are limited to not more then 1.63 mg/liter CN on a daily basis. Flow rate is not limited. The permit was updated on September 13, 1994, and expires on December 31, 1999. The proposed action is expected to have no effect on this permit because of the limited volume of waste entering the sanitary sewer and the fact that no cyanide is used in this production process.

Hazardous liquid wastes, including some liquid tailing wastes from the filling operation as well as liquid waste from the quality control laboratory will be collected in 55-gallon drums and disposed of by a licensed contract waste disposal firm. Ben Venue Laboratories, Inc. holds identification number 0HD091625749 under the Resource Conservation and Recovery Act. The contract waste disposal firms addresses and EPA identification numbers are given below:

The operations of Chemical Analytics are audited by Ben Venue personnel on an annual basis

In the event Chemical Analytics is unable to remove hazardous wastes, two secondary waste disposal firms have been identified:

Solid Waste Generation and Disposal

Non-hazardous solid wastes are either recycled or disposed of in a state licensed landfill.

Toxic solid wastes are disposed of by incineration at a facility permitted to handle such waste streams. The disposal is managed under contract by:

Compliance With Occupational Health and Safety Requirements

All necessary steps are taken to comply with the Occupational Safety and Health Act (1070), the Hazard Communication Standard (1983, 1987), and Title 29 of the Code of Federal Regulations Part 1910. Material Safety Data Sheets (MSDS) are available onsite for all chemicals used in the production process. Employees associated with production have the appropriate MSDSs

available for their review. Appropriate protective clothing including uniforms, gloves, safety glasses, hard hats, and personal respirators are available. Employees receive ongoing training on safe work habits, and adherence to safety protocols is strictly enforced. In the event of an emergency, the site specific emergency response plan would be implemented to protect workers and the environment.

Effects of Approval on Compliance

Ben Venue Laboratories, Inc. is in compliance with all applicable emissions requirements set forth in permits, consent decrees, and administrative orders, as well as with emissions requirements set forth in applicable federal, state, and local statutes and regulations.

Approval of the proposed action is expected to have no effect on the ability of the Ben Venue Laboratories facility to comply with any environmental or occupational safety laws or regulations currently in effect. Emissions of potentially toxic substances, including the drug substance and drug product, associated with the manufacture of Copaxone® will be very small. Furthermore, adequate controls are in place to minimize emissions to air, wastewater, and solid waste

6.3   Introduction of Substances From Product Use and Disposal

Copolymer-1 has been designated as an Orphan Drug by the FDA and small amounts of Copaxone® are estimated to be produced during the fifth year of production. Accordingly, very small amounts of Copolymer-1 are expected to be introduced into the environment as a result of product use and disposal. Most of the drug substance expected to be released to the consumer or medical waste disposal streams during the fifth production year will result from very small losses that can occur during preparation of Copaxone® for injection (i.e., approximately 2 mg per vial). Copolymer-1 is expected to be metabolized in the body prior to excretion. Fifth year drug substance use estimates (in kilograms), as well as estimates of drug substance introduction into the consumer or medical waste disposal streams, were provided as Confidential Information.

Packaging for the drug product also may enter either the general office waste stream or the medical waste stream. These components consist of materials used in a wide variety of products and none are specifically regulated by federal, state, or local authorities.

Secondary packaging of Copaxone® will take place at the LEMMON Company facility in Sellersville, Pennsylvania. Returned product also will be handled at the LEMMON Company facility in Sellersville for disposition. Waste materials that will be generated include cardboard cartons, any product and associated packaging materials damaged during transport or packaging, and returned product and associated packaging. The packaging materials for Copaxone® are used in a wide variety of products and none are specifically regulated by federal, state, or local authorities. Cardboard waste will be collected in the LEMMON Company recycling dumpster and transported to a local recycling facility. Damaged or returned product and associated packaging materials will be collected in drums and transported to a local landfill for disposal as

specifically regulated by federal, state, or local authorities. Cardboard waste will be collected in
th.   · recycling dumpster and transported to a local recycling facility
Damaged or returned product and associated packaging materials will be collected in drums
and transported to a local landfill for disposal as non-hazardous waste.
authorized by the Pennsylvania Department of Environmental Resources to use any sanitary
landfill for disposal of these types of wastes. The landfill currently employed b,
Company is:

6.4   Statement of Compliance

By signing this Environmental Assessment report, TEVA Pharmaceuticals USA states that it is in
compliance, or on an enforceable schedule to be in compliance, with all environmental laws and
regulations applicable to the production of Copaxone® at its facilities described above.

7.0 through 11.0   Not required for abbreviated environmental assessment
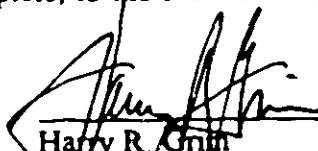
12.0   LIST OF PREPARERS

Ehud Marom
Director of Operations and Head of Ecology, TEVA Chemical Division, Israel
B.S., Chemical Engineering, Technion, Israel

More than 20 years experience in chemical and pharmaceutical industry; active participation on ultidisciplinary committees dealing with manufacturing related environmental issues.

13.0 CERTIFICATION STATEMENT

The undersigned responsible official certifies that the information presented is true, accurate, and complete, to the best of the knowledge of TEVA Pharmaceuticals USA..

Harry R. Smith
Vice President, Manufacturing

Feb. 27 1996
Date

14.0 REFERENCES

None cited.

15.0 APPENDICES

LIST OF APPENDICES

Appendix 1   Abbreviated Environmental Assessment, Ben Venue Laboratories, Inc.

Appendix 2   Summary of Israeli Environmental Legislation *Due to the volume of information provided, Appendix 2 has not been included in the FOI EA by FDA.*

Item 1   Licensing of Business Regulations (Disposal of Hazardous Substances), 1990

Item 2   Hazardous Substances Law, 1993 (Summary)

Item 3   Abatement of Nuisances Regulations (Air Quality), 1992 (Summary)

Item 4   Basis for Anticipated Future Air Emissions Regulations (Technical Instructions on Air Quality Control, TA-Luft)

Item 5   Model Bylaw for Local Authorities (The Discharge of Industrial Wastes Into the Sewage System), 1981

Appendix 3   Environmental Quality Unit Compliance Letter, TEVA Pharmaceutical Industries Ltd., Kfar Sava, Israel.

Appendix 4   Material Safety Data Sheet (MSDS) for Copolymer-1

Appendix 1

Abbreviated Environmental Assessment, Ben Venue Laboratories, Inc.

Manufacturing Site
Abbreviated Environmental Assessment

1.0   DATE

January 27, 1995

2.0   NAME OF APPLICANT

Ben Venue Laboratories, Inc.

3.0   ADDRESS

270 Northfield Road
P.O. Box 46565
Bedford, OH 44146

4.0   DESCRIPTION OF THE PROPOSED ACTION

Ben Venue Laboratories, Inc. proposes to formulate, aseptically fill and seal the drug
product Copaxone®, a novel new therapy for multiple sclerosis, for TEVA
Pharmaceuticals. This manufacturing site's environmental assessment information is
being supplied as part of the abbreviated environmental assessment for Copaxone®, as
required by 21 CFR 25.31. The product will be available in the United States on the
order of a licensed physician.

A.   Description of the general environment

Ben Venue Laboratories is located in Bedford, Ohio, a city of approximately
15,000. The city is located in Cuyahoga County and is approximately 17 miles
south of Cleveland, Ohio. Approximately 400 people are employed at the site.
The surrounding land use consists of light industrial and chemical manufacturing
and single family residential. The site is served by municipal water and sewer
services. No additional construction or employment will result from the proposed
action.

The climate is typical of a northern temperate zone with an average summer time
temperature of 79° F. in June and 15° F in January. Precipitation in the form of
rain and snow averages 35 inches and 56 inches, respectively.

B.   Air resources

Air Quality in this area is in compliance with the National Air Quality Standards
(NAQS) of the Clean Air Act for all air toxics except ozone. Cuyahoga County is
in non-attainment with the NAQS for ozone. The State of Ohio Environmental

Protection Agency (Ohio EPA) is responsible for implementing the state air quality implementation plan.

C.   Water resources

All process waters used at the site are supplied by the Bedford Municipal Water Authority. The facility uses approximately 150,000 gallons per day on average throughout the year. Daily water use varies depending upon production needs. Approximately 15 percent, or 22,500 gallons per day are lost through evaporation and the remaining 122,500 gallons discharged to the sanitary sewer. Wastewater discharged from the site is received by the City of Bedford's Publicly Owned Treatment Works (POTW). Ben Venue Laboratories holds a permit for indirect discharge to the POTW under the National Pollutant Discharge Elimination System. Storm water drainage from the site enters Tinkers Creek, a minor tributary of the Cuyahoga River. Ben Venue holds a Storm Water General Permit, number 3GR00298, administered by the Ohio EPA.

D.   Land Resources

The manufacturing facility is located on approximately 8.4 acres of land and comprises two major buildings with about 225,000 square feet of floor space. The topography of the site is generally flat to slightly sloping. Soil is a silt-clay typical of the northern Ohio region.

5.0   IDENTIFICATION OF CHEMICAL SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION

The preparation of this drug product involves the formulation of the liquid materials, sterile filing, lyophilizing the formulation and sealing the vials. The chemicals used in the formulation and filling operations are listed below:

Copolymer 1
Mannitol, USP
Water for Injection, USP
Nitrogen, NF

Cleaning agents used in the production process are water and industrial cleaners typical of a pharmaceutical manufacturing facility.

6.0   INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

Expected emissions, emission controls, occupational safety standards and compliance with environmental laws and regulations are discussed below.

A.   Air emission controls

Air emissions from the manufacture of the drug product may include trace
particulates. All work areas used in the manufacture of the drug product are
vented to High Efficiency Particulate Filters (HEPA). These filters are expected
to capture at least 97 percent of any particulates entering the air in the work space.
Filtered air is vented to the outside. The filters are monitored on a schedule that
varies with production activity because of the multiproduct nature of the
manufacturing site. Filters no longer meeting control specifications enter the
solid waste stream from the facility.

No air emission permits are required for the facility by either state or federal
authorities due to the nature and low volume of emissions. No volatile organic
solvents are used in this production process, all solvent are water based. A small
amount of alcohol and bleach is used for sanitization. The emissions from this
use are expected to be negligible. Three boilers are registered by the Ohio EPA.
These registrations, Numbers, 1318030726B001, 1318030726B002,
1318030726B003 have no date of expiration.

B.   Liquid wastes and emission controls

Liquid waste from the production process are expected to consist of production
equipment rinses and tailings left over from the filling operation. Prior to rinsing,
all equipment is vacuumed to remove any solid material left. The vacuumed
material is considered toxic and is handled as toxic waste. Both the vacuumed
material and the rinse material from Copaxone® production are collected by Ben
Venue Laboratories and disposed of by an EPA licensed contractor, Chemical
Analytics, Inc.

As noted above, Ben Venue holds a permit for indirect discharge to the Bedford
POTW. The permit (3PD00005101*BP) is administered by the Ohio EPA in
accordance with the Clean Water Act and Rule 3745-33-06 of the Ohio
Administrative Code. It requires the monitoring of discharge flow rate, pH, and
cyanide concentrations at 3 to 6 month intervals. The discharge pH is limited to a
pH of not less then 5.0 at any given time. Cyanide discharge concentrations are
limited to not more then 1.63 mg/liter CN on a daily basis. Flow rate is not
limited. The permit was updated on September 13,1994 and expires on December
31, 1999. The proposed action is expected to have no effect on this permit
because of the limited volume of waste entering the sanitary sewer and the fact
that no cyanide is used in this production process.

Hazardous liquid wastes, including some liquid tailing wastes from the filling
operation as well as liquid waste from the quality control laboratory will be
collected in 55 gallon drums and disposed of by a licensed contract waste disposal
firm. Ben Venue Laboratories Inc. holds identification number OHD091625749

under the Resource Conservation and Recovery Act. The contract waste disposal firms address and EPA identification numbers are given below:

The operations of Chemical Analytics are audited by Ben Venue personnel on an annual basis.

In the event Chemical Analytics is unable to remove hazardous wastes, two secondary waste disposal firms have been identified:

C.   Solid waste controls

Non-hazardous solid wastes such as paper, aluminum, plastic, filters and disposable suits and lab ware that cannot be recycled are disposed of in a state licensed land fill.

Toxic solid wastes, including rejected raw materials and finished product, severely contaminated HEPA filters and quality control laboratory wastes are disposed via incineration by a facility permitted to handle such waste streams. The disposal is managed under contact by:

In order to minimize the amount of material for disposal, excess Copolymer 1 may be returned to the customer for further use.

D.   Employee protection

All necessary steps are taken to comply with the Occupational Safety Act of 1971, the Hazards Communication Standard of 1985 and Title 29 of the Code of Federal Regulations Part 1910. Material Safety Data Sheets (MSDS) are available on-site for all chemicals used in the production process. Employees associated with production have the appropriate MSDS's available for their review. Appropriate protective clothing including uniforms, gloves, safety glasses, hard hats and individual respirators are available. Employees receive on-going training on safe work habits and adherence to safety protocols are strictly enforced. In the event of an emergency, the site specific emergency response plan would be implemented to protect workers and the environment.

E.   Compliance statement

By signing this environmental assessment, Ben Venue Laboratories, Inc. confirms that it is in compliance with all applicable emission requirements set forth in permits, consent decrees and administrative orders, as well as emission requirements set forth in applicable federal, state, and local statutes and regulations at its facility discussed above.

7.0   FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT

This item is intentionally omitted as permitted by 21 CFR 25.31 a(b)3(ii).

8.0   ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES

This item is intentionally omitted as permitted by 21 CFR 25.31 a(b)3(ii).

9.0   USE OF ENERGY AND RESOURCES

This item is intentionally omitted as permitted by 21 CFR 25.31 a(b)3(ii).

10.0   MITIGATION MEASURES

This item is intentionally omitted as permitted by 21 CFR 25.31 a(b)3(ii).

11.0   ALTERNATIVES TO THE PROPOSED ACTION

This item is intentionally omitted as permitted by 21 CFR 25.31 a(b)3(ii).

12.0   LIST OF PREPARERS

Valerie Baker, C.S.P. Ben Venue Laboratories, Inc., Safety, Health and Environmental
Engineer. Six years of experience in the development and administration of occupational
safety, health and environmental programs. She holds a Bachelor of Science degree from
Indiana University of Pennsylvania in Safety Sciences.

Carl G. Osborne, D.V.M., WEINBERG CONSULTING GROUP Inc., Consultant.
Sixteen years experience in the development and review of environmental assessments
and environmental impact statements.

13.0   CERTIFICATION

The undersigned responsible officials certify that the information presented in this
document is true, accurate, and complete, to the best of the knowledge of Ben Venue
Laboratories, Inc.

Robert V. Kasubick , Ph.D.   31. 27. 95
Vice President of Regulatory Affairs   Date
Ben Venue Laboratories, Inc.

Appendix 3

**Environmental Quality Unit Compliance Letter
TEVA Pharmaceutical Industries, Ltd., Kfar Sava, Israel**

November 21, 1994

TO WHOM IT MAY CONCERN

The Regional Division of Environmental Quality for Kfar-Sava, Raanana, Hod Hasharon, Ramot Hashavim and the Southern Sharon, acting by authority of the Ministry of the Environment in licensing the construction of new industrial plants and the operation of existing industrial plants, has examined the plans and documents that Teva Ltd. submitted to the Kfar-Sava Municipality in connection with Teva's request to build a production facility for the product Copaxone (COP-1) in the framework of the company's activities at the Teva Kfar-Sava plant.

On the basis of these documents and plans and the existing facilities at the Teva Kfar-Sava plant, the Division has determined that the normal production conditions of the product Copaxone (COP-1) at the Kfar-Sava plant will not cause any air, water, or ground pollution. These assumptions are founded on the Division's considerations, based on its familiarity with the facilities on the one hand, and on examination of the manufacturing process of the product Copaxone (COP-1) on the other.

The Division of Environmental Quality has recommended to the Kfar-Sava Local Planning and Construction Board to grant a building permit for the structure at the Teva Kfar-Sava plant.

In addition, the Division of Environmental Quality is aware that the product Copaxone (COP-1) has been manufactured for several years at the Kfar-Sava plant on a pilot scale in the existing production facility. The records and results of the periodic testing conducted by the Regional Division at the Teva Kfar-Sava plant indicate compliance of the plant with the environmental quality requirements and with environmental pollution prevention standards.

Based on its experience with the Teva Kfar-Sava plant, the Division expects the plant to continue to comply with these conditions at present and in the future, also after the new facility is completed and becomes operational for the production of Copaxone (COP-1) on a commercial scale.

Iehuda Olander
Environmental Quality Unit, Manager

Appendix 4

Material Safety Data Sheet (MSDS) for Copolymer-1

MATERIAL SAFETY DATA SHEET

ADDRESS

EMERGENCY AND INFORMATION
PHONE CALLS: TEL.: (972)-9-604336
FAX: (972)-9-339063

504336 PLANTEX LTD.
HAICADAR St. INDUSTRIAL ZONE
P.O. BOX 160, NETANYA 42101
ISRAEL   Date Prepared: /March, 1995
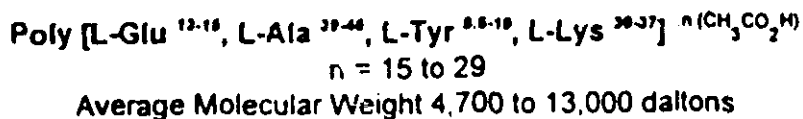WARNING STATEMENT   AVOID INGESTION, INHALATION, SKIN CONTACT

This material is a synthetic product.
Information on the chemical, physical and toxicological
properties is not readily available.

SECTION 1-IDENTITY

COMMON NAME   Copolymer-1
SYNONYMS   COP-1
CAS NUMBER   147245-92-9
RTECS NUMBER   Not registered
CHEMICAL NAME   L-glutamic acid, polymer with L-alanine,
(REPRESENTATIVE)   L-lysine and L-tyrosine, acetate (salt)

L-alanine, polymer with L-glutamic acid, L-lysine
and L-tyrosine, acetate (salt)

L-lysine, polymer, with L-alanine,
L-glutamic acid and L-tyrosine, acetate (salt)

L-tyrosine, polymer with L-alanine, L-glutamic acid and
L-lysine, acetate (salt)

CHEMICAL FAMILY   Synthetic Polypeptides

THERAPEUTIC CATEGORY   LO3A (ATC Classification)

FORMULA

Poly [L-Glu $^{12-18}$, L-Ala $^{39-46}$, L-Tyr $^{8.6-10}$, L-Lys $^{30-37}$] $^{n(CH_3CO_2H)}$
n = 15 to 29
Average Molecular Weight 4,700 to 13,000 daltons

SECTION 2 – HAZARDOUS INGREDIENTS

	NAME	PERCENT	THRESHOLD LIMIT VALUE (UNITS)
PRINCIPAL HAZARDOUS COMPONENTS (Chemical & Common name (s)]	COP-1	Pure Material	Not established

SECTION 3 - PHYSICAL AND CHEMICAL CHARACTERISTICS
Fire and Explosion Data

APPEARANCE AND ODOUR

White to slightly yellowish material with no specific odour.

PERCENT VOLATILE BY VOLUME (%)

0

SOLUBILITY IN WATER

Soluble

REACTIVITY IN WATER

Not reactive

FLAMMABLE LIMITS IN AIR % BY VOLUME

Not determined

EXTINGUISHER MEDIA

Water spray, dry chemical, carbon dioxide or foam appropriate for surrounding fire and materials.

TO - IGNITION TEMPERATURE

Not determined

SPECIAL FIRE FIGHTING PROCEDURES

As with fires evacuate personnel to safe area. Firefighters should use self-contained breathing equipment and protective clothing.

UNUSUAL FIRE AND EXPLOSION HAZARDS

This material is assumed to be combustible. As with all dry powders it is advisable to ground mechanical equipment in contact with dry material to dissipate the potential buildup of static electricity. When heated to decomposition material emits toxic fumes.

SECTION 4 - PHYSICAL HAZARDS

STABILITY

Stable

CONDITIONS TO AVOID

Material is stable from a safety point of view. Should be stored -20 C from the biological activity point of view.

INCOMPATIBILITY (MATERIALS TO AVOID)

Acids, Alkalies

HAZARDOUS DECOMPOSITION PRODUCTS

Emits toxic fumes under fire conditions.

SECTION 5 - HEALTH HAZARDS

THRESHOLD LIMIT VALUE   None established
SIGNS AND SYMPTOMS OF   A single dose of 400 mg /kg was well tolerated by
OVEREXPOSURE   rodents after subcutaneous injection.

ACUTE   n/a

CHRONIC   Possible hypersensitization

PRECAUTIONS TO CONSIDER   Persons developing hypersensitivity (anaphylactic)
                                 reactions must receive immediate medical
                                 attention. Material may be irritating to mucous
                                 membranes and respiratory tract. As a general
                                 rule, avoid all contact and inhalation of dust, fumes,
                                 mists and/or vapours associated with the material.
                                 Keep container tightly closed and use with
                                 adequate ventilation; wash thoroughly after
                                 handling. Individuals working with chemicals
                                 should consider all chemicals to be potentially
                                 hazardous, even if their individual hazards may be
                                 uncharacterized or unknown.

MEDICAL CONDITIONS
AGGRAVATED BY EXPOSURE   Hypersensitivity to material

CHEMICAL LISTED AS   National Toxicology Program ☐ Yes   ☐ No
CARCINOGEN OR POTENTIAL   I.A.R.C. Monographs   ☐ Yes   ☐ No
CARCINOGEN   OSHA   ☐ Yes   ☐ No
                                 Daily doses of up to 30 mg/day were not
                                 associated with side-effects other than those
                                 usually known.

                                 ACGIH   OTHER EXPOSURE
                                 TLB: n/a   LIMIT(S) USED: n/a

OSHA PERMISSIBLE EXPOSURE
LIMIT: Not established

OTHER EXPOSURE LIMIT USED   Not established

EMERGENCY AND FIRST AID
PROCEDURES   Remove from exposure. Remove contaminated
                                 clothing. Persons developing serious
                                 hypersensitivity reactions must receive immediate
                                 medical attention. Upon eye or skin contact, flush
                                 affected area with copious quantities of water
                                 Obtain medical attention. If not breathing give
                                 artificial respiration. If breathing is difficult give

SECTION 5 - HEALTH HAZARDS
(cont'd)

STEPS TO BE TAKEN IN CASE
MATERIAL IS SPILLED OR
RELEASED

Wear approved respirator and chemically compatible gloves. Vacuum or sweep up spillage. Avoid dust. Place spillage in appropriate container for waste disposal. Wash contaminated clothing before reuse. Ventilate area and wash spill site.

WASTE DISPOSAL METHODS

Dispose of waste in accordance with all applicable local laws.

1. INHALATION

May cause irritation of respiratory tract. Remove to fresh air.

2. EYES

May cause irritation. Flush with copious quantities of water.

3. SKIN

May cause irritation. Flush with copious quantities of water.

4. INGESTION

May cause irritation. Flush out mouth with water.

SECTION 6 - SPECIAL PROTECTION INFORMATION

RESPIRATORY PROTECTION
(SPECIFY TYPE)   NIOSH approved respirator
VENTILATION   Adequate
    LOCAL EXHAUST   Recommended
    MECHANICAL (GENERAL)   Recommended
    OTHER   n/a
PROTECTIVE GLOVES   Rubber
EYE PROTECTION   Safety goggles
OTHER PROTECTIVE CLOTHING
OR EQUIPMENT   Appropriate laboratory apparel; protect exposed skin.

SECTION 7 - SPECIAL PRECAUTIONS AND SPILL/LEAK PROCEDURES

PRECAUTIONS TO BE TAKEN
IN HANDLING AND STORAGE

Store in tight container at -20°C.
This material should be handled and stored per label and other instructions to ensure product integrity.

OTHER PRECAUTIONS

Avoid contact with eyes, skin or clothing. Avoid breathing dust or mist. Use with adequate dust control. Wash thoroughly after handling. Wear fresh clothing daily. Wash contaminated clothing before reuse. Do not permit eating, drinking or smoking near material.

4 1996

A. 1.   NDA 20-622

APPLICANT:

TEVA PHARMACEUTICALS USA
1510 Delp Drive
Kulpsville, PA 19443

2.   PRODUCT NAMES:

Copolymer-1 For Injection (20 mg/vial)
COPAXONE®

3.   DOSAGE FORM AND ROUTE OF ADMINISTRATION:
Copolymer-1 for Injection is a sterile, freeze-dried powder intended for
subcutaneous injection following reconstitution with Sterile Water for Injection
USP.

4.   METHODS OF STERILIZATION:
Copolymer-1 for Injection is an aseptically filled, freeze-dried product. The
reconstituted product contains no preservatives.

5.   PHARMACOLOGICAL CATEGORY:
Treatment of relapsing-remitting multiple sclerosis.

B. 1.   DATE OF INITIAL SUBMISSION: June 13, 1995
    2.   DATE OF RESUBMISSION: October 10, 1995
    3.   RELATED DOCUMENTS:

4. ASSIGNED FOR REVIEW: July 18, 1995

C. REMARKS: Copolymer-1 for Injection was initially submitted on June 14, 1995 and a refuse to file (RTF) letter issued by the Division of Neuropharmacological Drug Products on August 10, 1995. Teva responded to the RTF issues on October 10, 1995 and NDA volume 1 and the entire CMC section of the NDA, volumes 2-13 were resubmitted. The other sections of the NDA, including the microbiology section, remained unchanged and were not resubmitted.
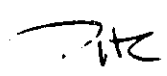
Two manufacturing sites are proposed for the drug product: Teva Pharmaceutical Industries, Kfar Sava, Israel and BenVenue Laboratories (BVL), Inc. Bedford, Ohio. Sterile Water for Injection (WFI) USP vials used as a diluent for administration of Copolymer-1 for Injection are manufactured at BVL and the manufacturing process for the diluent WFI is not presented and reviewed here. Drug product vials and WFI vials are shipped to Lemmon Company, USA from either Teva or BVL where the vials are packaged.

D. CONCLUSIONS: The NDA 20-622 for Copaxone® is not recommended for approval.

Letter to the Applicant".

12/26/95

Patricia F. Hughes, Ph.D.
Review Microbiologist

1/4/96

cc: Original NDA 20-622
      HFD-160/Consult File
      HFD-160/P.F.Hughes/12/26/95
      HFD-120/Division File
      HFD-120/CSO/T. Wheelous

      Drafted by P.F.Hughes, 12/26/95
      R/D initialed by P. Cooney, 12/26/95