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Dear :

Re: Guidance to Establish Equivalence or Relative Potency of Safety and Efficacy of a Second Entry Short-Acting Beta₂-Agonist Metered Dose Inhaler (MDI)

Please find attached the Therapeutic Products Programme (TPP) Guidance to Establish Equivalence or Relative Potency of Safety and Efficacy of a Second Entry Short-Acting Beta₂-Agonist Metered Dose Inhaler (MDI).

The attached guidance document deals only with the short-acting beta₂-agonist bronchodilator metered dose inhalers. The inhaled corticosteroids will be the subject of a separate guidance document to be issued in the future.

Please note that a draft guidance was in use since 1992. The principles outlined in the 1992 draft still apply i.e. equivalence could be established based on "bronchodilation equivalence protocol" and "bronchoprotection protocol". The main difference in the present revision is that either one may be used instead of the initial requirement for both.

.../2

- 2 -

This guidance is effective immediately. If you have

any questions or comments, please do not hesitate to contact Dr. Paul Roufail, Head, Endocrinology, Metabolism and Allergy Unit, Bureau of Pharmaceutical Assessment at (613) 941-3172. With respect to statistical issues, please contact Mr. Eric Ormsby at (613) 941-3694.

Dann M. Michols
Director General

Attachment

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DATE: April 16, 1999

**GUIDANCE TO ESTABLISH EQUIVALENCE OR RELATIVE
POTENCY OF SAFETY AND EFFICACY OF A SECOND ENTRY
SHORT-ACTING BETA₂-AGONIST METERED DOSE INHALER**

February, 1999

Endocrinology, Metabolism and Allergy Unit

Bureau of Pharmaceutical Assessment

Therapeutic Products Programme

Health Canada

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Guidance to Establish Equivalence or Relative Potency of Safety and Efficacy of a Second Entry Short-Acting Beta₂-Agonist Metered Dose Inhaler

1. INTRODUCTION

This guidance document details the recommendations to second entry applicants on methods to establish the equivalence or relative potency of the safety and efficacy of short-acting, beta₂-agonist bronchodilators delivered by inhalation aerosol (metered dose inhaler; MDI). The document takes into account the comments received from stakeholders including the Standards Committee of the Canadian Thoracic Society, the Canadian Pharmaceutical Manufacturers Association, the Canadian Drug Manufacturers Association and from many individuals. Although two specific study protocols are presented, other designs or types of studies may be considered provided the method is properly validated and upon prior consultation with the Therapeutic Products Programme (TPP).

2 PURPOSE

This guidance outlines the types of clinical studies which should be conducted in order to establish the equivalence of a second entry, short-acting, β₂- agonists, metered dose inhaler (MDI). Two different types of pharmacodynamic studies are recommended. Either of these study protocols may be used to satisfy the

comparative efficacy requirement provided the comparison is between identical β_2 -agonists with similar delivery systems. This document also outlines some issues which the sponsor should consider when planning such studies.

3. CONSIDERATIONS

It is agreed that β_2 -agonists act topically on specific receptors in the airways of the lungs and that systemic absorption does not necessarily reflect airway absorption or effect. Thus the usual bioequivalence determination via blood levels is not appropriate unless it can be shown that the analyte measured in the blood indicates what went through the lungs and its effect. Since *in vivo* and *in vitro* correlations have not been established, reliance on only *in vitro* data is not enough. Separate guidelines exist which address *in vitro* study issues.

The next practical method of showing therapeutic equivalence, is through the use of pharmacodynamic(PD) measures. Two potential PD measures are bronchodilation and bronchoprotection. The Canadian Asthma Practice Guidelines suggest that a short acting inhaled β_2 -agonist should be used only when needed. This recommendation was made because the need for a β_2 -agonist gives an indication of control (or lack of) and regular use several times daily leads to loss of its protective effect and possibly worsening asthma. This would indicate that bronchodilation is the most clinically relevant measure to use. However there appears to be no evidence that the receptors controlling dilation or protection response are in different locations

of the lung , thus suggesting either measure would be an indication of the amount of drug delivered to the appropriate location in the lungs.

The first generic salbutamol MDI was approved in 1989. This approval was based on *in vitro* measures of pharmaceutical equivalence and clinical studies including two period, two treatment crossover designs which compared bronchodilation responses from two puffs of the innovator's product with two puffs of the second entry (test) product. Criticism of this design was that there was no way to determine with just one dose whether the subjects were close to or on the plateau of the dose-response curve. If subjects were near the plateau, the ability of the study to discriminate differences (sensitivity) would be low and the comparison of duration of response from a plateau level may be subject to high variability. Thus it is recommended that more than one dose of both the test and reference products be measured in order to ensure sensitivity of the study. Subjects may be included or excluded based on a criterion defining an acceptable response to the doses to be used. The recommended design, therefore, has minimally four treatments - one and two puffs of both the reference and test products.

The choice of subjects is very important in these trials. Subjects should be in a stable state. Clearly, when testing for bronchodilation, there needs to be bronchodilation demonstrable and different degrees of this. However for bronchoprotective studies, the FEV₁ should ideally be normal or close to it and the degree of responsiveness should be measurable after β_2 -agonist so as to be able to

measure the degree of protection. For bronchoprotective studies it is also advisable that the patients be β_2 -agonist naive or at least did not use them on a daily basis. Medications listed in appendix 3 should not be used within the listed washout period.

The two recommended types of studies are given in Appendix 1 and 2. Appendix 1 outlines the basic protocol for executing the Bronchodilation study and Appendix 2 outlines the Bronchoprotection study.

4. SAFETY STUDY

The safety assessment should include monitoring for acute adverse events (heart rate, tremor, serum potassium etc). A dose response comparison of the acute adverse events between the test and reference drug products should be provided and should include the usually prescribed dose and doses higher than normally prescribed. The test product should not cause greater side effects than the reference product.

Long term safety testing is usually not required for a second entry bronchodilator aerosol inhaler unless there are changes which warrant such assessment i.e. changes in formulation and/or propellants etc. or there is cause for concern.

5. DESIGN, ANALYSIS AND EQUIVALENCE STANDARD

Data for declaring equivalency of inhaled short-acting β_2 -agonist bronchodilators should be collected according to a 4 sequence, 4 period, 4 treatment

crossover design. Each subject will receive 1 and 2 puffs (1 puff of 100 µg and 2 puffs amount to 200 µg) of each of the test and standard formulations. Analysis of variance (ANOVA) should be carried out including the effects given in Appendix 4. Least squares estimates and the residual variance required to calculate the 90% confidence interval for relative potency of test to reference products are also given in Appendix 4. The resulting confidence interval for the relative potency must be entirely contained within the interval (0.80, 1.25) in order to establish equivalence.

This protocol is acceptable for beta₂-agonist inhaler equivalence studies. If equivalence is not documented with these studies, then a 3 dose (e.g. 1, 2 and 4 puffs) study must be conducted to determine relative potency.

RELEVANT INFORMATION FOR DESIGNING MDI STUDIES

1. Report on the Working Groups for the Canadian Asthma Consensus Conference. *Can. Respir. J.* V 3 Sup B, Dec. 1996.
2. Commission of the European Communities-Committee for Proprietary Medicinal Products. Note for Guidance 111/5378/93 - Final.
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5. Guyatt GH, Walter S, Norman G. Measuring change over time; assessing the usefulness of evaluative instruments. *J Chron Dis* 1987; 40:171-8.
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8. Determining equivalence of inhaled medications - Rogers DF and Ganderton D. *Resp Med* 1995; 89:253-61.
9. Streiner DL, Norman GR. Reliability. In: *Health Measurement Scales*. Oxford University Press, 1989.
10. Nelson HS: Adrenergic therapy for bronchial asthma. *J Allergy Clin Immunol* 1986; 77:771-85.
11. Twentyman OP, Finnerty JP, Holgate ST, The inhibitory effect of nebulized albuterol on the early and late asthmatic reactions and increase in airway responsiveness provoked by inhaled allergen in asthma. *Am Rev Respir Dis* 1991; 144:782-87.
12. Phillips GD, Finnerty JP, Holgate ST. Comparative protective effect of the inhaled β_2 - agonist salbutamol (albuterol) on bronchoconstriction provoked by histamine, methacholine, and adenosine 5'-monophosphate in asthma. *J Allergy Clin Immunol* 1990; 85:755-62.

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16. Wong AG, O'Byrne PM, Lindbladh C, Inman MD, Ståhl E, Hargreave FE. The dose-response protective effect of salbutamol on methacholine airway responsiveness and a comparison between pressurized metered dose inhaler and turbuhaler. *Can Respir J* 1998; in press.
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18. Crane J, Burges C, Beasley R. Cardiovascular and hypokalemic effects of inhaled salbutamol, fenoterol and isoprenaline. *Thorax* 1989; 44:136-40.

APPENDIX 1

BRONCHODILATOR STUDY PROTOCOL

This protocol is an example of the considerations required in a single dose protocol.

A. Study Question

Are the relative potencies as measured by the magnitude and duration of increase in FEV₁ for the test to the reference product within 80-125%?

B. Study design

1. Measurement of the magnitude and duration of effect of 1 and 2 puffs for each of the test and reference products.
2. Four period, four treatment crossover study to allow within subject comparisons (2).
3. Randomization of treatment order. Ideally the same number of subjects receive the same order of treatments (balanced design). One of the four sequence designs of Williams is recommended (3).
4. Double-blind to minimize bias.
5. Appropriate test drug washout time between study periods to ensure no carry over effects.
6. Study completion over a short enough period of time to make variation in subject condition less likely.

C. Methods

1. FEV₁ should be performed with equipment and methods which meet ATS guidelines (1).

D. Preselection Evaluation

1. Complete medical history and physical examination performed within one week before study day 1.
2. Laboratory tests and screening procedures including:
 - a) FEV₁ (forced expiratory volume in 1 second) - see inclusion criteria.
 - b) Blood chemistries.
 - c) Urine analysis
 - d) CBC.
 - e) 12 lead ECG.
 - f) A urine pregnancy test for women of childbearing potential.

E. Selection of study subjects

Inclusion Criteria:

1. Subjects with airflow obstruction as demonstrated by FEV₁/VC of < 80% after withholding temporary relief medications for recommended time (Appendix 3). The airflow obstruction should represent a range of severity with a mixture of subjects with an FEV₁ between 40-60% of predicted FEV₁ and of between 60-79% of predicted. These former

subjects can be safely enrolled providing their condition is stable and they are on optimum corticosteroid treatment. There should be an acute reversibility as demonstrated by an improvement in FEV₁ of $\geq 15\%$ of their predicted FEV₁ , 15 minutes after two puffs of the reference product.

2. The condition causing the airflow obstruction should have been stable on the same regular treatment for at least 4 weeks.
3. Nonsmokers for a long enough period to avoid variable effects during the study eg. 4 weeks.
4. Males and non-pregnant females, ages 18-60 years.
5. Ability to be trained in the proper use of an MDI
6. Able to give written informed consent.

Exclusion criteria:

1. Intolerance to aerosolized β_2 -adrenergic agonists.
2. History of side effects to any of the MDI ingredients.
3. Evidence of respiratory tract infection in the 6 weeks before the study.
4. Seasonal or episodic exposure to an allergen or occupational chemical sensitizer to which the person is sensitized during the study.
5. History of cystic fibrosis or bronchiectasis.

6. Inability to withhold temporary withdrawal of medications which need to be withheld during the study.
7. Having received an investigational drug within 30 days before the current study.
8. Treatment with β_2 -adrenergic blocking drugs.
9. History of current cardiovascular, renal, neurologic, liver or endocrine dysfunction.
10. Inability to perform outcome measurements optimally.

F. Sample size

The sample size should be estimated by the investigator and is based on the most variable measure which must pass the bioequivalence standard. It is recommended that with a minimum of 80% power, the 90% confidence interval will fall within the 80-125% standard. Sample size is based on the intra-subject CV and the expected potency difference between test and reference products. Provision for dropouts should be included in the size calculations.

G. Pre-study Procedures

All subjects must be volunteers.

Preliminary evaluation should aim to satisfy subject inclusion and exclusion criteria and be determined within one week of the start of the study.

Preliminary measurements to be used for subsequent comparisons (e.g.

FEV₁) should be made at the same time of day as the study testing to minimize the effects of diurnal variation. Confounding factors which might influence airflow obstruction must be carefully avoided before and during the studies. These include ingestion of caffeine, vigorous exercise, inhalation of cold air and environmental smoke, dust or smells which might trigger airflow obstruction. The duration of avoidance prior to study depends on the likely duration of the effect of each factor.

Subjects should be assessed to ensure they are able to comply with study procedures.

H. Study Day activities

1. Pre-dose procedures.
 - a. Confirm that subjects to continue to meet inclusion and exclusion criteria and have withheld medications appropriately (Appendix 3).
 - b. The FEV₁ performed at the same time of day as the pre-study measurement should not differ by more than \pm 10%.

2. Drug administration

Care should be taken to maintain blinding.

Inhalers should be "primed" in a uniform fashion immediately before administration and away from any possibility of inhalation by the subject eg. discharged 2 times into a bag away from the patient.

The method of inhalation, use of add-on devices (eg. valved holding chamber with pressurized inhaler), duration of breath hold after inhalation and the time between repeated inhalations must be specified and regulated. For example, inhalation from the inhaler will be done as a slow and deep inhalation from FRC followed by a ten second breath hold. The slow inhalation should be at approximately 30 L/min and this can be assured by training with a placebo inhaler connected to a Vitalograph MDI modified compact spirometer (Vitalograph Limited, Buckingham, UK) which measures peak inspired flow, inspiratory volume and breath holding times. On each visit the patient can be allowed to practice their inhalation technique with a placebo inhaler to ensure proper inhalation technique.

3. Outcome measurements

- a. FEV₁. Measurements should be made in the sitting position. They should be made before and (repeated at appropriate times) after the inhaled drug is administered. This will identify the likely onset, maximum response and duration of action of reference and test medications. eg. suggested times for tests after administering salbutamol are 5, 10, 15, 20, 25, 30 then every 30 min for a total of 6 hours.

- b. Cardiovascular. Heart rate, 30 sec ECG rhythm strip (lead #2) and systolic and diastolic BP should be monitored at the same time as FEV₁ measurements.
- c. Other side effects. Hand tremor should be measured at the same time as FEV₁ measurements.

Generally these measurements should be performed at sufficient frequency to detect side effects.

I. Data Summary and Analysis

The FEV₁ data should be presented in tables for each subject and each treatment. The tables should contain subject id, period, sequence, pre-study FEV₁, baseline FEV₁ and FEV₁ for each time post dosing, maximum FEV₁, time of maximum FEV₁, FEV₁ as a % of baseline and an Area Under the FEV₁ Curve (AUFC). Means and standard deviations should be presented for all variables.

Confidence intervals for the relative potency should be constructed using the least squares means and the error mean squares from the appropriate Analysis of Variance (see Appendix 4 for more details).

J. Criteria

The criteria to enable the product to be marketed in Canada are:

1. Pulmonary response

The 90% confidence intervals for relative potency for maximum FEV₁ and AUFC must be contained entirely within 80-125%.

2. Cardiovascular Response

The test product must not produce a statistically significant worse response than the reference for vital signs.

3. Adverse Reactions

The incidence of adverse reactions with the test product must not be significantly greater than that seen with the reference product.

K. Ethical considerations

Individual studies should receive approval from the TPP for the study of new medications and the appropriate local ethics committee, receive signed informed consent from subjects taking part and be undertaken in accordance with the "declaration of Helsinki" and Canadian MRC guidelines concerning human investigation.

L. References

1. American Thoracic Society. Standardization of spirometry - 1994 update. Am J Respir Crit Care Med 1995; 152:1107-36.

2. Jones B, Kenward, MG.(1989) Design and Analysis of Crossover Trials. Chapman-Hall, London, England.
3. Finney DJ. Serially balanced sequences in bioassay. Proceedings of the Royal Society, B, 1956; 145:493-507.

APPENDIX 2

BRONCHOPROTECTION STUDY PROTOCOL

This protocol is an example of the considerations required in such a study.

A. Study question

Is the relative potency as measured by the magnitude of protection against methacholine airway constriction for the test to reference product between 80-125%?

B. Study design

1. Measurement of the magnitude of protection for 1 and 2 puffs of each of the test and reference products.
2. Four period, four treatment crossover study to allow within subject comparisons (3).

3. Randomization of treatment order. Ideally the same number of subjects receive the same order of treatments (balanced design). One of the four sequence designs of Williams is recommended (4).
4. Double-blind to minimize bias.
5. Appropriate test drug washout time between study periods to ensure no carry over effects.
6. Study completion over a short enough period of time to make variation in subject condition less likely.

C. Selection of study subjects

Inclusion criteria

1. Male and non-pregnant females, ages 18-60 years.
2. Subjects with asthma indicated by symptoms and airway hyperresponsiveness to methacholine with a $PC_{20} \leq 4$ mg/ml when the baseline FEV_1 is $\geq 70\%$ predicted or FEV_1/VC is $\geq 70\%$ after withholding temporary relief medicines for the recommended time (Appendix 3).
3. The asthma should be stable on the same regular treatment for at least 4 weeks, unlikely to exacerbate during the period of study because of, for example, seasonal allergen exposure.

4. The subjects could be on no regular treatment or on a regular dose of inhaled steroid which should be kept the same during the study. Subjects should be using an inhaled β_2 -agonist only when needed and preferably less than daily and no more than twice daily to minimize β_2 -agonist-loss of the bronchoconstriction protective effect of the drug.
5. The PC₂₀ methacholine should increase at least 4-fold after inhaling the dose (2 puffs) of the reference.
6. Non smokers for at least one month before the study to be more certain that smoking is unlikely to make the subject unstable during the study.
7. Ability to be trained to use an MDI properly.
8. Able to give written informed consent.

Exclusion criteria:

1. Intolerance to aerosolized β_2 -adrenergic agonists or methacholine.
2. History of side effects to any of the MDI ingredients.
3. Evidence of respiratory tract infection within 6 weeks before the study.
4. Seasonal or episodic exposure to an allergen or occupational chemical sensitizer to which the person is sensitized during the study.
5. History of cystic fibrosis or bronchiectasis.
6. Inability to tolerate temporary withdrawal of medication which need to be withheld during the study (Appendix 3).
7. Having received an investigational drug within 30 days before the study.

8. On treatment with β_2 -adrenergic receptor antagonist.
9. History of cardiovascular, renal, neurologic, liver or endocrine dysfunction.
10. Inability to perform outcome measurement optimally.
11. Inability to measure PC₂₀ methacholine after the highest dose of reference bronchodilator to be used in the study.

D. Sample size

The sample size should be estimated by the investigator and is based on the most variable measure which must pass the bioequivalence standard. It is recommended that with a minimum of 80% power, the 90% confidence interval will fall within the 80-125% standard. Sample size is based on intra-subject CV and the expected difference in potencies between test and reference products.

E. Procedures

There should be one or two pre-study subject evaluation days and four study days. On the first evaluation day subject characteristics should be documented by history, examination, spirometry and allergy prick skin tests with common allergen extracts. Other standard investigations may be needed to satisfy inclusion and exclusion criteria. Written informed consent must be obtained. On this day or a second evaluation day, two methacholine inhalation tests should be carried out. The second should be

done after recovery from the first at a specified time, eg. two hours, and ten minutes after inhalation of 2 puffs of the reference. The first methacholine test should be done at the same time of day as subsequent tests on study days to eliminate the effects of diurnal variation. Confounding factors which might influence airflow obstruction must be carefully avoided before and during these studies. These include ingestion of caffeine, vigorous exercise, inhalation of cold air and environmental smoke, dust or smells which can trigger airflow obstruction.

On each study day, the pretreatment FEV₁ should be performed at the same time of day as the FEV₁ obtained during the pre-study evaluation, and should not differ by more than \pm 10%. The test drug is then inhaled and, after ten minutes, the methacholine test begun. The second evaluation day and each study day should be separated by a long enough period to avoid an effect of the drug on PC₂₀ eg. 2 days, and short enough to complete the study as quickly as possible to minimize confounding factors, e.g. 7 days.

Drug side effects such as pulse, BP and tremor should be monitored just before each methacholine test.

The use of test drug inhalers must be standardized and closely supervised. The inhalers should be "primed" in a uniform fashion and away from any possibility of inhalation by the subject, e.g. discharged 2 times into a plastic bag immediately before administration. The method of inhalation, use of

add-on devices (e.g. valved holding chamber with pressurized inhaler), duration of breath hold after inhalation and the time between repeated inhalations must be specified and supervised during the study. For example, inhalation from the inhaler will be done as a slow and deep inhalation from FRC followed by a ten second breath hold. The slow inhalation should be at approximately 30 L/min and this can be assured by training with a placebo inhaler connected to a Vitalograph MDI modified compact spirometer (Vitalograph Limited, Buckingham, UK) which measures PIF, inspiratory volume and breath holding times. On each visit the patient can be allowed to practice their inhalation technique with a placebo inhaler to ensure proper inhalation technique.

F. Methods

1. FEV₁ should be performed with equipment and methods which meet ATS guidelines. (1)
2. Methacholine inhalation tests should be performed by a standardized method with concentrations increasing if necessary up to 256 mg/ml and PC₂₀ calculated or obtained by linear interpolation of the last two points (2). This test should be performed by experienced staff to avoid technical errors in its performance and interpretation.

3. In the pre-study evaluation, the methacholine test should be performed in the usual dose-response way to determine the PC_{20}
4. In the randomized control trial the methacholine tests can be started 10 minutes after the bronchodilator with a dose of methacholine two-fold dilutions below the prestudy PC_{20} . This will shorten the test and allow the PC_{20} after active drug inhalations to be determined at about 30 minutes.
5. The quality and stability of the methacholine solutions used in the study should be checked e.g. by determining the methacholine concentration at the beginning and end of the study by high-performance liquid chromatography.

G. Outcome measurements

1. Pulmonary. The primary outcome measurement is measured at the time of the expected maximum effect of the drug (about 30 minutes).
2. Cardiovascular. Heart rate and systolic and diastolic BP should be monitored before drug inhalation and before each methacholine test.
3. Other side effects. Hand tremor should be measured at the same times.

H. Data analysis

The FEV₁ and PC₂₀ data should be presented in tables for each treatment. The tables should contain subject id, period, sequence, pre-study FEV₁, each study day baseline FEV₁, FEV₁ for each time post challenge, and PC₂₀. Means and standard deviations should be presented for all variables. Confidence intervals for the relative potency should be constructed using the least squares means and the error mean squares from the appropriate Analysis of Variance (see Appendix 4 for more details).

I. Criteria

The criteria to enable the product to be marketed in Canada are:

1. Pulmonary Response

The 90% confidence interval for the relative potency for test to reference PC₂₀ must be contained entirely within 80-125%.

2. Cardiovascular Response

The test product must not produce a significantly worse response than the reference for vital signs.

3. Adverse Reactions

The incidence of adverse reactions with the test product must not be significantly greater than that seen with the reference product.

J. Ethical considerations

Individual studies should receive approval from the TPP for the study of new medications and the appropriate local ethics committee, receive signed informed consent from subjects taking part and be undertaken in accordance with the "declaration of Helsinki" and Canadian MRC guidelines concerning human investigation. Methacholine is the recommended substance for challenging the subjects. Although histamine has also been used it is not recommended since the potential for damaging the subjects lungs is too great.

K. References

1. American Thoracic Society. Standardization of spirometry - 1994 update. *Am J Respir Crit Care Med* 1995; 152:1107-36.
2. Juniper EF, Cockcroft DW, Hargreave FE. Histamine and methacholine inhalation tests: a laboratory tidal breathing protocol. 2nd ed. Astra Draco AB, Lund, Sweden 1994.
3. Jones B. And Kenward, M.G.(1989) Design and Analysis of Crossover Trials. Chapman-Hall, London, England.
4. Finney DJ. Serially balanced sequences in bioassay. *Proceedings of the Royal Society, B*, 1956; 145:493-507.

APPENDIX 3

DRUG WASHOUT TIMES

Recommended drug washout periods.

Subjects are permitted to take their current medications for asthma.

However, they must withhold the following preparations for the indicated period before each study day:

- | | |
|--|--------------------|
| - Inhaled β_2 agonist | at least 8 hours |
| - Inhaled long-acting β_2 -agonist | at least 48 hours* |
| - Oral β_2 -agonist | at least 12 hours |
| - Inhaled cromoglycate and nedocromil | at least 48 hours |
| - Xanthines a) taken 2 times daily | at least 24 hours |
| b) taken daily | at least 48 hours |
| - Aspirin and non-steroid antiinflammatory drugs | at least 48 hours |
| - Anticholinergic | at least 12 hours |
| - Antihistamines | at least 96 hours |
| except for astemizole | at least 6 weeks |
| - Antileukotrienes | at least 48 hours |

*or longer if the dose has been demonstrated to have a longer effect

APPENDIX 4.

STATISTICAL ANALYSIS

Relative potency will be estimated from a 4 point parallel line assay (2 formulations at 2 doses) within a 4 sequence, 4 period crossover design. Each subject will receive 4 treatments consisting of 2 formulations, the test (T) and the standard (S), each at 2 dose levels (1 actuation and 2 actuations). These 4 treatments are designated as S_1 , S_2 , T_1 and T_2 . The sequence of the 4 treatments will be determined by randomly assigning each subject to one of the four sequences of a serially balanced crossover design (Williams Design) such as:

		Period			
		1	2	3	4
S e q u e n c e	1	S_1	T_2	S_2	T_1
	2	S_2	S_1	T_1	T_2
	3	T_1	S_2	T_2	S_1
	4	T_2	T_1	S_1	S_2

Statistical analysis of the data should include an analysis of variance (ANOVA) associated with the statistical model containing the factors: sequence, subject(sequence) nesting, formulation, dose, formulation * dose interaction and subject(sequence) * dose interaction

For example, the model to be used for initial testing is:

$$y_{ijkl} = \mu + \text{seq}_i + \text{subj}(\text{seq})_j + [\text{form}_l + \beta x + \text{form}_l * \beta x] + \{\text{subj}(\text{seq}) * \beta x\}_{ij} + \varepsilon_{ijkl}$$

$i = 1, 2, 3, 4$; $j = 1, 2, \dots, n$; $k = 1, 2, 3, 4$; and $l = s, t$ ($s = \text{standard}$, $t = \text{test}$) and

μ is the overall mean,

seq_i is the i^{th} sequence effect,

$\text{subj}(\text{seq})_j$ is the random effect of the j^{th} subject nested within the i^{th} sequence,

form_l is the overall l^{th} formulation effect,

β is the overall slope parameter associated with the continuous variable x
 x is $\log(\text{dose})$,

$\{\text{subj}(\text{seq}) * \beta x\}_{ij}$ is the random effect of the ij^{th} slope associated with the j^{th} subject (within the i^{th} sequence) (i.e., this fits a separate slope for each subject),

ε_{ijkl} is the random residual effect.

From the above model, estimates of dose effect for each subject can be obtained and testing their significance provides a preliminary screening for non-responders, i.e., a subject with a non-significant dose effect should be investigated as a potential non-responder.

From the ANOVA the validity of the model must be examined by testing the effects in the following table.

Effect	Result for model validity	Variance components involved in testing
sequence	nonsignificant	subject(sequence)
dose*formulation interaction	nonsignificant	residual
dose	significant	subject(sequence)*dose

Log(relative potency) can be estimated from the model,

$$y_{ijkl} = \mu + \text{seq}_i + \text{subj}(\text{seq})_j + [\text{form}_t + \beta x] + \varepsilon_{ijkl} ,$$

$$\text{as } R = (\text{form}_t - \text{form}_s) / b ,$$

where

R is the estimate of log(relative potency (p)),

form_t is the estimated overall form effect due to the test formulation

form_s is the estimated overall form effect due to the standard formulation

b is the estimate of the overall common slope β

The 90% confidence interval for log(relative potency) is :

$$C.I(\log(p)) = [R - g v_{12} / v_{22} \pm (t/b) \{v_{11} - 2R v_{12} + R^2 v_{22} - g (v_{11} - v_{12}^2 / v_{22})^{1/2}\}] / (1 - g) ,$$

where,

$$g = t^2 v_{22} / b^2$$

v₁₁ is the variance of (form_t - form_s)

v₁₂ is the covariance of (form_t - form_s) and b

v₂₂ is the variance of b

t is the 95th percentile of the t distribution .

The estimate and confidence interval for relative potency (p) is obtained by transforming back into the original dose units. The resulting confidence interval must be entirely contained within the interval (0.80, 1.25) in order to establish equivalence.