

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of : BERNSTEIN, Joel E.
Serial No. : 13/197,581
Filed : August 3, 2011
For : COMPOSITIONS WITH REDUCED
: HEPATOTOXICITY
:
Examiner : DRAPER, Leslie A. Royds
Art Unit : 1629
Customer number : 23644
Confirmation No. : 6057
Attorney Docket No. : 41959-115246

APPEAL BRIEF

Commissioner of Patents and Trademarks
PO Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This appeal is from the Examiner's Office Action mailed March 27, 2013 with a Final Rejection, in which pending claims (namely, claims 30-37 and 40) were rejected. A timely Notice of Appeal was filed with the required fee August 27, 2013. An Amendment and Response to the Office Action of September 7, 2013 was entered.

A Petition to Make Special was granted on October 11, 2011.

A Petition for a four month extension of time until February 27, 2014 is filed concurrently with the required fee.

(i) Real Party of Interest

The Real Party of Interest is Winston Laboratories, Inc. The Assignment is recorded at Reel 015164, Frame 0716.

(ii) Related Appeals and Interferences

Appellant is not aware of any related appeals or interferences.

(iii) Status of Claims

On August 3, 2011, claims were filed in a Preliminary Amendment, for a patent application that is a divisional of U.S. Ser. No. 10/813,760, filed March 31, 2004, now abandoned. Claims 1-15 were cancelled, claims 38-39 were added, claims 16-39 were pending.

As a result of a restriction, claims 16-39, 38-39 were withdrawn, claim 40 was added, claims 30-37 were elected. Acetaminophen was the elected species.

(iv) Status of Amendments

An Office Action was mailed February 28, 2012.

In the Response mailed August 20, 2012, claim 30 was amended, dependent claims 40 and 41 were added.

Another Office Action was mailed September 7, 2012. In the response of March 7, 2013, claims 30, 31, 32, 33, 35, 36 and 40 were amended. Claim 41 was cancelled.

An Office Action with a Final Rejection ("Final Rejection") was issued on March 27, 2013. Claims 30-37 and 40 of March 7, 2013 are those on appeal.

(v) Summary of Claimed Subject Matter

Claims 1-15 and 41 are cancelled.

Claims 16-29, 38-39 are withdrawn.

Claim 30 relates a method of mitigating the hepatotoxicity of a hepatotoxic compound by formulating a composition containing a combination of a quantity of the

hepatotoxic compound, a quantity of nicotinamide, and a quantity of methionine, wherein the combination of the hepatotoxic compound, nicotinamide and methionine is administered contained together in the same individual dosage form selected from the group consisting of capsules, tablets and oral solutions. [0006, 0009, 0010, 0014, 0015, 0016, 0018]

Claim 31 is dependent on claim 30 wherein the composition is formulated such that for each dose of the hepatotoxic compound in the composition, the nicotinamide is present in the amount of 5-500 mg, and the methionine is present in the amount of 25-500 mg. [original claim]

Claim 32 is dependent on claim 31 wherein the nicotinamide is present in the amount of 25-200 mg per dose of the hepatotoxic compound. [original claim, 0010]

Claim 33 is dependent on claim 31 wherein the methionine is present in the amount of 10-100 mg per dose of the hepatotoxic compound. [original claim, 0010]

Claim 34 is dependent on claim 30 wherein the composition further comprises a quantity of folic acid. [0006, 0008]

Claim 35 is dependent on claim 34 wherein the folic acid is present in the amount of 50 mcg-5 mg per standard dose of the hepatotoxic compound. [original claim, 0010]

Claim 36 is dependent on claim 35 wherein said folic acid is present in the amount of 500 mcg-1mg per dose of the hepatotoxic compound. [original claim, 0010]

Claim 37 is dependent on claim 30 wherein the hepatotoxic compound is selected from the group consisting of acetaminophen, methotrexate, atorvastatin, simvastatin, niacin, flucanazole, divalproex sodium, and valproic acid. [original claim, 0011]

Claim 40 is dependent on claim 37, wherein the acetaminophen is present in the amount of 80 mg-1000mg per dose. [0010]

(vi) Grounds of Rejection To Be Reviewed on Appeal

1. Rejection of claims 35 and 40 under 35 U.S.C. §112 second paragraph as maintained.
2. Claims 30-37 and 40 as newly rejected in the Office Action of March 27, 2013 under 35 U.S.C. §112, second paragraph.
3. Claims 30-37 and 40 as rejected under 35 U.S.C. §103(a) over Kroger et al. in view of Ogata et al. (U.S. Pat. 5,478,815) and Murdock et al. (U.S. Pat. 4,526,788).

(vii) Argument

A. Comments Regarding Prosecution History, Public Need of the Invention

Appellant wishes to take this opportunity to point out to the Board several facts which make the prosecution of this case extraordinary. First, this case was filed in March 2004. There have been several primary examiners involved at various times, and the "relevant" prior art references have changed substantially over the course of the prosecution. Second, there has been, particularly over the last few years, a great deal of concern expressed by U.S. Government agencies (e.g. FDA and NIH) over the liver toxicity of certain widely available commercial drugs, especially acetaminophen. Despite pointing out to the U.S. Patent Office that the present invention helps address the problem of liver toxicity, the present examiner adopts the attitude of the character in the James Thurber story who huffed, "Mere proof won't convince me." In addition to citing specious arguments, and ignoring previous references submitted by the Applicant, the examiner makes a variety of mis-statements in her most recent rejection of the claims.

1. Longstanding Public Need

Acetaminophen liver toxicity accounts for by far the most deaths and hospitalizations in the U.S. of any over-the-counter (OTC) drug product. Although Johnson & Johnson and GlaxoSmithKline, which combined account for over 95% of the OTC acetaminophen sales in the world, and who monitor and assess all publications on acetaminophen world-wide have not solved the liver toxicity effect, the inventor submits that his invention cannot be obvious.

Confidentiality Agreements have been executed in 2013 with various global pain management companies in order to evaluate the product used in the present claimed invention. These are companies who have been trying to find ways to alleviate the toxic effects of acetaminophen. This demonstrates the long felt need for the present invention. Their recent interest indicates the problem of liver toxicity is still unsolved, and the present invention could be a solution. Proceeding to clinical trials necessitates a patent.

Governments in North America, Europe, and Asia have convened conferences to address acetaminophen liver toxicity, and none of the participants in these meetings have even remotely suggested the art of this patent application. If the thousands of scientists at Johnson & Johnson and GlaxoSmithKline are not skilled in the art, then who is?

In *Leo Pharmaceutical Products, Ltd. v. Rea*, 726 F.3d 1346 (Fed. Cir. 2013), the Court found a patent that combined vitamin D and corticosteroids to heal psoriasis was not obvious in light of prior art that used both separately to heal psoriasis. The Court found the patent's claimed combination would not have been obvious to try. "[W]here the prior art, at best gives only general guidance as to the particular form of the claimed invention or how to achieve it, relying on an obvious-to-try theory to support an obviousness finding is impermissible." **Furthermore, the Court stated that a long-felt need existed because a span of more than a decade existed between the prior art and the filing of the patent application at issue.** (*emphasis provided*)

2. Rejections Under 35 U.S.C. §112, 2nd Par. Are Due to Examiner's
Misstatements and Errors in the Record, Not to Indefiniteness

Claim 30 as amended March 7, 2013 reads:

30. A method of mitigating the hepatotoxicity of a hepatotoxic compound, the method comprising formulating a composition containing a combination of a quantity of the hepatotoxic compound, a quantity of nicotinamide, and a quantity of methionine, wherein the combination of the hepatotoxic compound, nicotinamide and methionine is administered **contained together** in the same individual dosage form selected from the group consisting of capsules, tablets and oral solutions. (*emphasis provided*)

On page 3 of the examiner's response ("Final Rejection") she states:

In claim 30, Applicant recites that the "combination of the hepatotoxic compound, nicotinamide and methionine is administered **contained together** in the same individual dosage form", which clearly states the use of a single dosage form to contain the recited combination. However, Applicant goes on to further define the individual dosage form as "capsules, tablets and oral solutions", **each option of which is plural** and, therefore, circumscribes multiple dosage forms, not a single dosage form. It is, therefore, wholly unclear whether Applicant intends for the claimed combination to be contained in a single or multiple dosage forms. Clarification is required. (*emphasis provided*)

The examiner totally ignored the phrase "contained together in." The examiner provided no argument for her belief that those of skill in the art would not understand that the **plural** form "capsules, tablets and oral solutions" does not negate "**contained together in,**" which means the combination of the hepatotoxic compound and nicotinamide and methionine are administered in the same capsule, or the same tablet or the same dose of an oral solution.

3. Claims 35 and 40 as Amended are Definite; the Examiner Misread
the Amendment of Claim 40

Claims 35 and 40 were rejected as indefinite under 35 U.S.C. §112, second paragraph because the modifier "standard" had no antecedent.

As the examiner pointed out, "Applicant states that the claims have been amended to remove the terms 'about' and 'standard!' (Office Action, page 3). That

deletion of "standard" from claim 35 was an inadvertent typographical error is clear by the Applicant's statement and deletion of "standard" from the other claims.

(Amendments of claims 31-33 and 36 deleted "standard".) Hopefully a decision on appeal won't depend on a claim 35 typographical error.

The examiner is clearly in error in her statement about claim 40 on page 3 of the Final Rejection:

In addition, Applicant completely fails to address the lack of antecedent basis for the term "the amount of about 80-1000 mg per standard dose."

The examiner evidently neglected to observe that the phrase she rejects was deleted by amendment.

B. In Addition to Secondary Consideration, the Publications Cited Do Not Support the Obviousness Rejection

Claims 30-37 and 40 were rejected as obvious over Kroger et al., in view of Ogata et al., and Murdock et al.

1. The publications cited related intraperitoneal administration which is not applicable to oral administration or intervenous injection

The definition of intraperitoneal from the reference in Goodman & Gilman, "The Pharmacological Basis of Therapeutics" Ninth Edition, page 8 (Exhibit A):

Intraperitoneal. The peritoneal cavity offers a large absorbing surface from which drugs enter the circulation rapidly, but primarily by way of the portal vein, first-pass hepatic losses are thus possible. **Intraperitoneal injection is a common laboratory procedure, but it is seldom employed clinically. The dangers of producing infection and adhesions are too great to warrant the routine use of this route in human beings.** (*emphasis provided*)

Note this cautionary explanation that IP "is seldom employed clinically" ... "dangers." Also, this publication indicates that drugs administered intraperitoneally not only "enter circulation rapidly, but primarily by way of the portal vein." Consequently, the amount of nicotinamide or methionine delivered in this fashion

would be very much higher than with an oral dose. Additionally, in the record Gerasimov et al. ("Pharmacology & Experimental Therapeutics," 295:51-57, 2000) (Exhibit B) showed that the differing effects of oral and IP administration are substantial and clinically significant.

In addition to the Goodman & Gilman reference, references from Remington's Pharmaceutical Sciences and the Merck Manual (Exhibits C and D) demonstrate that IP injection (intraperitoneal) is never used in humans except for cytotoxic treatment of intraperitoneal metastases from ovarian or uterine cancer.

2. Kroger Does Not Relate Oral Administration or Doses

In the Kroger paper acetaminophen was delivered directly into the stomach by **gavage**, and then later nicotinamide and methionine, are administered as separate intraperitoneal (IP) injections. ("gavage" is known in the art as administration directly by a tube into the stomach, e.g. force feeding.) In contrast, in the '760 application, all components (the hepatotoxic active drug agent and the hepatoprotective agents, nicotinamide, methionine, and folic acid) are provided in the same dosage form and administered together in this dosage form (e.g. a capsule, a tablet, a solution). Furthermore, the dosages of nicotinamide and methionine administered IP for protective effects in the Kroger publication are very substantially greater than those in the '760 application. IP dosages in the Kroger publication range from 25-100 mg/kg nicotinamide and 50-300 mg/kg methionine when each is given alone, to 12.5 mg/kg of each when they are both administered in separate IP injections. Based on the average body weight for adult Americans the dosage of nicotinamide in the claims of the '760 application ranges from .11 mg/kg to 5.7 mg/kg for males and from .33 mg/kg to 6.7 mg/kg for females. In contrast, IP injection results in much higher and much faster peak blood levels of drug. In the '760 application, these dosages are provided orally or by injection **not into** the peritoneum. Consequently, the dosages of nicotinamide and methionine in pending claims are miniscule compared to those in the Kroger publication.

On Page 6, the examiner maintains that intravenous administration is something new just introduced into the record by the applicant. In fact, the record

shows, for example in the original patent application and numerous reiterations over the years, that the composition and method claims repeatedly refer to intravenous solutions and intravenous injections.

Kroger's method in mice, which is administering very high dosages of nicotinamide and/or methionine separately by intra-peritoneal injection sometime after acetaminophen is delivered directly into the stomach by gavage, would not lead someone of ordinary skill in the art to the present claimed method of administering much lower dosages of nicotinamide and methionine simultaneously in a single dose form (e.g. tablet, capsule, solution) and in combination with acetaminophen.

3. Ogata Relates Intraperitoneal Administration

On Page 7 of the examiner's response to Appellant's arguments against the Ogata publication, she states:

Thirdly, Applicant avers that IP injection is "seldom employed clinically" (p.6, Reply) and cites again to Goodman & Gilman in support. This is unpersuasive. Applicant has failed to consider the teachings in Ogata et al., who clearly teaches that IP injection is routinely used in experimental animal testing to determine the effect of drugs to be administered systemically or orally to a human patient, such as by, e.g., tablets, granules, powders, capsules, or liquid preparations. See Ogata et al, Ex.2-3, cols.3-4 and col.2, l.36-40.

And at the bottom of Page 7 she states:

Ogata et al. teaches that IP injection is routinely used in experimental animal testing to determine the effect of drugs to be administered systemically or orally to a human patient, such as by, e.g., tablets, granules, powders, capsules, or liquid preparations. See Ogata et al., Ex.2-3, cols.3-4 and col.2, l.36-40.

Contrary to the examiner's assertions, Appellant has never denied what Ogata relates in these specific citations, that is, testing the effects of "TOCOPHERY-ASCORBYL-PHOSPHATE."

The examiner complains that

Applicant has again failed to consider the full scope of the teachings in Ogata et al.

and the examiner defines the teachings, "(See Ogata et al. Ex. 2-3, cols 3-4 and col 2, l 36-40)"

Contrary to the Examiner's Assertion (page 7 Final Rejection), applicant has considered the full scope of Ogata.

Also, the Examiner's interpretation of Ogata is inaccurate.

In terms of the examiner's statements as to what Ogata teaches regarding using IP injection to animals in order to "determine the effect of drugs to be administered systemically or orally to a human patient, such as by, e.g., tablets, granules, powders, capsules, or liquid preparations. See Ogata et al., Ex.2-3, cols.3-4 and col.2, l.36-40", Applicant cannot find a statement in the Ogata patent that even suggests this teaching.

Example 2 uses a rat model using intraperitoneal injection of rats to test the effect of substance "EPC-K," on induced fulminant liver disorders.

Results of Example 3 "... suggested that the compound is effective for acute liver disorder **in rats with vitamin E deficiency.**" (*emphasis provided*)

However, the present invention treats humans, not rats.

Col. 2, lines 36-40 cited by the examiner presents no data and only states a conclusion that:

The present compound can be used as an oral or a parenteral liver protectant ...

This is a standard boilerplate not proof. Because the compound of Ogata is not the compound in the presently claimed method, and this paragraph is an unsubstantiated statement, considering Ogata "in its entirety," as the examiner correctly required, this publication contributed nothing to an obviousness rejection. What Ogata **does** support is Appellant's argument that there is a long felt need to protect the liver from toxic medicaments.

4. The Examiner Misinterprets Murdock

Murdock is added to Kroger and Ogata. Further the examiner's arguments state:

Murdock demonstrated routine knowledge in the art of calculating a human dosage based upon the interrelationship of human/animal dosages.

Actually, Murdock just references Freireich (Exhibit E) for this dosage conversion. However, Freireich et al.'s article (Chemotherapy Reports, 50:219-244, 1966, Quantitative Comparison of Toxicity of Anticancer Agents in Mouse, Rat, Hamster, Dog, Monkey, and Man, Cancer) simply provides a conversation method based on toxicity for calculating the initial dose(s) for taking a new drug into human toxicity testing. The Freireich calculation indicates nothing about the effective dose or safety of effective doses in humans. This is made clear in the record by the "Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers" (Exhibit F). Consequently, the Examiner's calculation of the effective dose in human using the Freireich et al formula is completely flawed. The Ogata patent, in fact, supports Appellant by stating:

The dosage of the present indication, dosage form and treatment protocol may for example be about 1-100 mg/day/adult man in the daily, in the case of an oral preparation.

Appellant also challenges the Examiner's attributing the following statements as assertions of the Appellant: (1) "known doses of pharmaceutical compositions are not predictable from animal studies;" (2) "many treatments that appeared effective in animals have not proved to be effective in humans;" and (3) "drugs that have caused severe [drug-induced liver injury] in humans have not shown clear hepatotoxicity in animals." Rather these are statements by the FDA in two guidelines previously provided to the USPTO ("Guidance for Industry – Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers,"

(Exhibit F) and "Guidance for Industry – Animal Models – Essential Elements to Address Efficacy Under the Animal Rule" (Exhibit G)).

C. Contrary to the Examiner's Statement (Page 5) Appellant Considers the Combination of Publications When Addressing Obviousness

On page 5 of the Final Rejection, the examiner alleges that Appellant does not consider the combination of references when arguing a prima facie case of obviousness was not established, that Appellant argued against anticipation, not obviousness. Appellant never "advocated an improper standard." (Final Rejection, page 6)

On page 5 of Appellant's response filed March 7, 2013, all 3 publications, Kroger, Ogata and Murdock are acknowledged as offered to support the obviousness rejection. On page 6 of the March 7, 2013 Response, each publication is discussed as 'added' so clearly the combination is addressed.

Discussing each publication separately is acceptable, well established patent prosecution – to point out deficiencies in each publication, ending with a conclusion that their combination does not make up these deficiencies, and/or they would not be combined by those of skill in the art. The allegation that:

...Applicant believes the instant rejection is an anticipation rejection (Final Rejection, page 6, belies the record.

In the response filed August 28, 2012 to the Office Action of February 28, 2012 arguments were against Kroger as anticipated. That rejection was withdrawn.

On September 4, 2009, a response in the parent case:

The deficiencies of the Kröger et al 1997 publication are not cured by the Ogata et al. and Murdock et al. publications, as the Examiner stated that these references have been cited to demonstrate: 1) routine knowledge of using intraperitoneal (IP) injection as experimental animal testing; and 2) to demonstrate routine knowledge in calculating human dosage based upon the interrelationship of dosages for animals of various sizes and species, and humans, respectively. Ogata et al. and Murdock et al. neither teach nor suggest the effective

combination presently claimed. Therefore, Applicant continues to assert that the combined teachings of the cited publications do not render the aspects of the invention as set forth in independent claims 1 and 13, obvious.

A determination of obviousness requires that "the scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved." KSR International Co. v. Teleflex, Inc., U.S. , 127 S.Ct. 1727, 1734, 82 U.S.P.Q.2d 1385 (2007) quoting Graham v. John Deer Co., 383 U.S. 1, 17 (1966). In making a determination of obviousness by looking at the teachings of multiple patents, one should consider the effects of demands known to the design community or present in the market place; and the background knowledge possessed by a person having ordinary skill in the art, all in order to determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue. To facilitate review, this analysis should be made explicit.

KSR, 127 S.Ct. at 1740-41 (emphasis added). "[A] patent composed of several elements is not proved obvious merely by demonstrating the each of its elements was, independently, known in the prior art." Id. at 1741.

Reversal of the Examiner is therefore clearly in order and is solicited.

Respectfully submitted,

Date:

Feb. 25, 2014

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Claims Appendix

Claims 1-15 (Cancelled)

Claims 16.-29 (Withdrawn)

30. A method of mitigating the hepatotoxicity of a hepatotoxic compound, the method comprising formulating a composition containing a combination of a quantity of the hepatotoxic compound, a quantity of nicotinamide, and a quantity of methionine, wherein the combination of the hepatotoxic compound, nicotinamide and methionine is administered contained together in the same individual dosage form selected from the group consisting of capsules, tablets and oral solutions.

31. The method of claim 30 wherein said composition is formulated such that for each dose of the hepatotoxic compound in the composition, the nicotinamide is present in the amount of 5-500 mg, and the methionine is present in the amount of 25-500 mg.

32. The method of claim 31 wherein said nicotinamide is present in the amount of 25-200 mg per dose of the hepatotoxic compound.

33. The method of claim 31 wherein said methionine is present in the amount of 10-100 mg per dose of the hepatotoxic compound.

34. The method of claim 30 wherein said composition further comprises a quantity of folic acid.

35. The method of claim 34 wherein said folic acid is present in the amount of 50 mcg-5 mg per standard dose of the hepatotoxic compound.

36. The method of claim 35 wherein said folic acid is present in the amount of 500 mcg-1mg per dose of the hepatotoxic compound.

37. The method of claim 30 wherein said hepatotoxic compound is selected from the group consisting of acetaminophen, methotrexate, atorvastatin, simvastatin, niacin, flucanazole, divalproex sodium, and valproic acid.

38.-39. (Withdrawn)

40. The method of claim 37, wherein said acetaminophen is present in the amount of 80 mg-1000mg per dose.

Claim 41(Cancelled)

Evidence Appendix

Exhibit A	Goodman & Gilman's The Pharmacological Basis of Therapeutics, Ninth Edition, page 8.
Exhibit B	Gerasimov et al. (2000) "Pharmacology and Experimental Therapeutics," 295:51-57
Exhibit C	Remington's, Pharmaceutical Sciences, Chapter 39
Exhibit D	Merck Manual
Exhibit E	Freireich et al. Chemotherapy Reports, 50:219-244 (1966) "Quantitative Comparison of Toxicity of Anticancer Agents in Mouse, Rat, Hamster, Dog, Monkey, and Man, " Cancer 2007
Exhibit F	"Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers"
Exhibit G	Guidance for Industry – Animal Models – Essential Elements to Address Efficacy Under the Animal Rule," USHS, FDA, CDER, CBER, January, 2009

Related Proceedings Appendix

None.

Evidence Appendix

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Exhibit C	Remington's, Pharmaceutical Sciences, Chapter 39
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Exhibit E	Freireich et al. Chemotherapy Reports, 50:219-244 (1966) "Quantitative Comparison of Toxicity of Anticancer Agents in Mouse, Rat, Hamster, Dog, Monkey, and Man, " Cancer 2007
Exhibit F	"Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers"
Exhibit G	Guidance for Industry – Animal Models – Essential Elements to Address Efficacy Under the Animal Rule," USHS, FDA, CDER, CBER, January, 2009

U.S. Serial No. 13/197,581

Exhibit A

GOODMAN & GILMAN'S The PHARMACOLOGICAL BASIS OF THERAPEUTICS

Ninth Edition

McGraw-Hill

HEALTH PROFESSIONS DIVISION

New York St. Louis San Francisco Auckland Bogotá Caracas Lisbon London Madrid
Mexico City Milan Montreal New Delhi San Juan Singapore Sydney Tokyo Toronto

SECTION I GENERAL PRINCIPLES

ject insulin into their thigh may experience a precipitous drop in blood sugar that is not seen following injection into the arm or abdominal wall, since running markedly increases blood flow to the leg. Generally, the rate of absorption following injection of an aqueous preparation into the deltoid or vastus lateralis is faster than when the injection is made into the gluteus maximus. The rate is particularly slower for females after injection into the gluteus maximus. This has been attributed to the different distribution of subcutaneous fat in males and females, since fat is relatively poorly perfused. Very obese or emaciated patients may exhibit unusual patterns of absorption following intramuscular or subcutaneous injection. Very slow, constant absorption from the intramuscular site results if the drug is injected in solution in oil or suspended in various other repository vehicles. Penicillin often is administered in this manner. Substances too irritating to be injected subcutaneously may sometimes be given intramuscularly.

Intraarterial. Occasionally a drug is injected directly into an artery to localize its effect in a particular tissue or organ. However, this practice usually has dubious therapeutic value. Diagnostic agents are sometimes administered by this route. Intraarterial injection requires great care and should be reserved for experts. The first-pass and cleansing effects of the lung are not available when drugs are given by this route.

Intrathecal. The blood-brain barrier and the blood-cerebrospinal fluid barrier often preclude or slow the entrance of drugs into the CNS. Therefore, when local and rapid effects of drugs on the meninges or cerebrospinal axis are desired, as in spinal anesthesia or acute CNS infections, drugs are sometimes injected directly into the spinal subarachnoid space.

Intraperitoneal. The peritoneal cavity offers a large absorbing surface from which drugs enter the circulation rapidly, but primarily by way of the portal vein; first-pass hepatic losses are thus possible. Intraperitoneal injection is a common laboratory procedure, but it is seldom employed clinically. The dangers of producing infection and adhesions are too great to warrant the routine use of this route in human beings.

Pulmonary Absorption. Gaseous and volatile drugs may be inhaled and absorbed through the pulmonary epithelium and mucous membranes of the respiratory tract. Access to the circulation is rapid by this route, because the surface area is large. The principles governing absorption and excretion of anesthetic and other therapeutic gases are discussed in Chapters 13, 14, and 16.

In addition, solutions of drugs can be atomized and the fine droplets in air (aerosol) inhaled. Advantages are the almost instantaneous absorption of a drug into the blood, avoidance of hepatic first-pass loss, and, in the case of pulmonary disease, local application of the drug at the desired site of action. For example, drugs can be given in this manner for the treatment of bronchial asthma (see Chapter 28). The main disadvantages are poor ability to regulate the dose, cumbersome methods of administration, and the fact that many gaseous and volatile drugs produce irritation of the pulmonary epithelium.

Pulmonary absorption is an important route of entry of certain drugs of abuse and of toxic environmental substances of varied com-

position and physical states (see Section XVII). Both local and systemic reactions to allergens may occur subsequent to inhalation.

Topical Application. Mucous Membranes. Drugs are applied to the mucous membranes of the conjunctiva, nasopharynx, oropharynx, vagina, colon, urethra, and urinary bladder primarily for their local effects. Occasionally, as in the application of antidiuretic hormone to the nasal mucosa, systemic absorption is the goal. Absorption through mucous membranes occurs readily. In fact, local anesthetics applied for local effect sometimes may be absorbed so rapidly that they produce systemic toxicity.

Skin. Few drugs readily penetrate the intact skin. Absorption of those that do is proportional to the surface area over which they are applied and to their lipid solubility, since the epidermis behaves as a lipid barrier (see Chapter 64). The dermis, however, is freely permeable to many solutes; consequently, systemic absorption of drugs occurs much more readily through abraded, burned, or denuded skin. Inflammation and other conditions that increase cutaneous blood flow also enhance absorption. Toxic effects sometimes are produced by absorption through the skin of highly lipid-soluble substances (e.g., a lipid-soluble insecticide in an organic solvent). Absorption through the skin can be enhanced by suspending the drug in an oily vehicle and rubbing the resulting preparation into the skin. This method of administration is known as *unction*. Because hydrated skin is more permeable than dry skin, the dosage form may be modified or an occlusive dressing may be used to facilitate absorption. Controlled-release topical patches are recent innovations. A patch containing scopolamine, placed behind the ear where body temperature and blood flow enhance absorption, releases sufficient drug to the systemic circulation to protect the wearer from motion sickness. Transdermal estrogen replacement therapy yields low maintenance levels of estradiol while minimizing the high estrone metabolite levels observed following oral administration.

Eye. Topically applied ophthalmic drugs are used primarily for their local effects (see Chapter 65). Systemic absorption that results from drainage through the nasolacrimal canal is usually undesirable. In addition, drug that is absorbed after such drainage is not subject to first-pass hepatic elimination. Unwanted systemic pharmacological effects may occur for this reason when β -adrenergic antagonists are administered as ophthalmic drops. Local effects usually require absorption of the drug through the cornea; corneal infection or trauma may thus result in more rapid absorption. Ophthalmic delivery systems that provide prolonged duration of action (e.g., suspensions and ointments) are useful additions to ophthalmic therapy. Ocular inserts, developed more recently, provide continuous delivery of low amounts of drug. Very little is lost through drainage; hence, systemic side effects are minimized.

Bioequivalence. Drug products are considered to be pharmaceutical equivalents if they contain the same active ingredients and are identical in strength or concentration, dosage form, and route of administration. Two pharmaceutically equivalent drug products are considered to be bioequivalent when the rates and extents of bioavailability of the active ingredient in the two products are not significantly different under suitable test conditions. In the past, dosage forms of a drug from different manufacturers and even different lots of preparations from a single manufacturer sometimes differed in their bioavailability. Such differences were seen primarily among oral dosage forms of poorly soluble, slowly absorbed drugs. They result from differences in crystal form, particle size, or other physical characteristics of the drug that are not rigidly controlled in formulation.

and manufacture of the preparations. These factors affect disintegration of the dosage form and dissolution of the drug and hence the rate and extent of drug absorption.

The potential nonequivalence of different drug preparations has been a matter of concern. Strengthened regulatory requirements have resulted in few, if any, documented cases of nonequivalence between approved drug products. The significance of possible nonequivalence of drug preparations is further discussed in connection with drug nomenclature and the choice of drug name in writing prescription orders (*see* Appendix I).

DISTRIBUTION OF DRUGS

After a drug is absorbed or injected into the bloodstream, it may be distributed into interstitial and cellular fluids. Patterns of drug distribution reflect certain physiological factors and physicochemical properties of drugs. An initial phase of distribution may be distinguished that reflects cardiac output and regional blood flow. Heart, liver, kidney, brain, and other well-perfused organs receive most of the drug during the first few minutes after absorption. Delivery of drug to muscle, most viscera, skin, and fat is slower, and these tissues may require several minutes to several hours before steady state is attained. A second phase of drug distribution may therefore be distinguished; this is also limited by blood flow, and it involves a far larger fraction of the body mass than does the first phase. Superimposed on patterns of distribution of blood flow are factors that determine the rate at which drugs diffuse into tissues. Diffusion into the interstitial compartment occurs rapidly because of the highly permeable nature of capillary endothelial membranes (except in the brain). Lipid-insoluble drugs that permeate membranes poorly are restricted in their distribution and hence in their potential sites of action. Distribution also may be limited by drug binding to plasma proteins, particularly albumin for acidic drugs and α_1 -acid glycoprotein for basic drugs. An agent that is extensively and strongly bound has limited access to cellular sites of action, and it may be metabolized and eliminated slowly. Drugs may accumulate in tissues in higher concentrations than would be expected from diffusion equilibria as a result of pH gradients, binding to intracellular constituents, or partitioning into lipid.

Drug that has accumulated in a given tissue may serve as a reservoir that prolongs drug action in that same tissue or at a distant site reached through the circulation. An example that illustrates many of these factors is the use of the intravenous anesthetic thiopental, a highly lipid-soluble drug. Because blood flow to the brain is so high, the drug reaches its maximal concentration in brain within a minute after it is injected intravenously. After injection is concluded, the plasma concentration falls as thiopental dif-

fuses into other tissues, such as muscle. The concentration of the drug in brain follows that of the plasma, because there is little binding of the drug to brain constituents. Thus, onset of anesthesia is rapid, but so is its termination. Both are directly related to the concentration of drug in the brain. A third phase of distribution for this drug is due to the slow, blood-flow-limited uptake by fat. With administration of successive doses of thiopental, accumulation of drug takes place in fat and other tissues that can store large amounts of the compound. These can become reservoirs for the maintenance of the plasma concentration, and therefore the brain concentration, at or above the threshold required for anesthesia. Thus, a drug that is short acting because of rapid redistribution to sites at which the agent has no pharmacological action can become long acting when these storage sites are "filled" and termination of the drug's action becomes dependent on biotransformation and excretion (*see* Benet, 1978).

Since the difference in pH between intracellular and extracellular fluids is small (7.0 vs. 7.4), this factor can result in only a relatively small concentration gradient of drug across the plasma membrane. Weak bases are slightly concentrated inside of cells, while the concentration of weak acids is slightly lower in the cells than in extracellular fluids. Lowering the pH of extracellular fluid increases the intracellular concentration of weak acids and decreases that of weak bases, provided that the intracellular pH does not also change and that the pH change does not simultaneously affect the binding, biotransformation, or excretion of the drug. Elevating the pH produces the opposite effects (*see* Figure 1-2).

Central Nervous System and Cerebrospinal Fluid.

The distribution of drugs to the CNS from the bloodstream is unique, mainly in that entry of drugs into the cerebrospinal fluid and extracellular space of the CNS is restricted. The restriction is similar to that across the gastrointestinal epithelium. Endothelial cells of the brain capillaries differ from their counterparts in most tissues by the absence of intercellular pores and pinocytotic vesicles. Tight junctions predominate, and aqueous bulk flow thus is severely restricted. This is not unique to the CNS capillaries (tight junctions appear in many muscle capillaries as well). It is likely that the unique arrangement of pericapillary glial cells also contributes to the slow diffusion of organic acids and bases into the CNS. The drug molecules probably must traverse not only endothelial but also perivascular cell membranes before reaching neurons or other target cells in the CNS. Cerebral blood flow is the only limitation to permeation of the CNS by highly lipid-soluble drugs. The rate of diffusion of drugs with increas-

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Exhibit B



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Comparison between Intraperitoneal and Oral Methylphenidate Administration: A Microdialysis and Locomotor Activity Study¹

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► Abstract

The therapeutic and stimulant properties of methylphenidate (MP), a drug commonly prescribed for the treatment of attention deficit hyperactivity disorder, have been attributed to increases in synaptic dopamine (DA) concentrations

resulting from the blockade of DA transporters. In addition to obvious difficulties inherent in any interspecies comparison, interpretation of preclinical studies done with MP is further complicated by different routes of administration in animals (i.v. and i.p.) compared with humans (oral). In the present study we compared the effects of i.p. and intragastric (oral) MP both on rat nucleus accumbens DA assessed by in vivo microdialysis and on locomotor activity measured in a photocell apparatus. We also compared regional brain uptake and plasma levels of [³H]MP after administration of 5 mg/kg via both routes. Intraperitoneal MP (5 and 10 mg/kg) was approximately twice as potent as intragastric MP in terms of increasing extracellular DA levels and in stimulating locomotion. This

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was consistent with the higher brain uptake of [^{3} H]MP when given i.p. rather than intragastrically. The dose of 2 mg/kg produced significant increases in both measurements when administered i.p., but not intragastrically. This study shows that relatively low doses of MP (2 mg i.p. and 5 mg intragastric) significantly increase extracellular DA and locomotor activity and indicates that the differences in the neurochemical and behavioral effects of MP between the intragastric and the i.p. routes are due to central drug bioavailability.

► Introduction

Over the past decade increased recognition of attention deficit hyperactivity disorder (Swanson et al., 1998¹) has led to a dramatic increase in the use of methylphenidate (Ritalin, MP), a psychostimulant commonly prescribed to treat this disorder. Despite this widespread use, the mechanisms by which MP exerts its therapeutic effects remain poorly understood. Although a considerable number of preclinical studies have been completed, their interpretation is limited by the fact that i.p. and i.v. routes have been used, whereas the oral route is used clinically. Moreover, most studies have used doses significantly higher (2-15 mg/kg i.v. or 10-50 mg/kg i.p.) than those used clinically in humans (0.3-1 mg/kg; Sprague and Sleator, 1977²). Studies with doses that are therapeutically relevant (0.6-10 mg/kg i.p. or s.c.) have predominantly investigated sensitization and tolerance to motor-activating and stereotypic effects of MP (McNamara et al., 1993³; Gaytan et al., 1997⁴; McDougall et al., 1999⁵). Higher doses of MP lead to a greater incidence of side effects, including sleep disturbances and irritability in children (Cole, 1975⁶). To our knowledge, the only two studies that have investigated the effects of oral MP in rodents focused on its pharmacokinetic profile in brain and plasma, and not on the concomitant behavioral or neurochemical effects of the drug (Wargin et al., 1983⁷; Patrick et al., 1987⁸).

The therapeutic effects of MP as well as its psychostimulant properties are thought to be related to its ability to increase extracellular dopamine (DA) in the mesocorticolimbic system (Castellanos et al., 1996⁹), secondary to blockade of DA transporters (Ritz et al., 1987¹⁰). Similarly, the reinforcing effects of cocaine and cocaine-like drugs also are associated with their ability to block the DA transporter. This has led to serious concerns regarding the potential reinforcing or addictive properties of MP. However, despite the pharmacological similarities between MP and cocaine, including similar potency at the DA transporter (Volkow et al., 1995¹¹; Gatley et al., 1999¹²), its abuse is much less frequent (NIDA-CEWG, 1995¹³) and is mainly restricted to the i.v. or intranasal route of administration with very infrequent oral abuse (Parran and Jasinski, 1991¹⁴). The rare occurrence of oral abuse is probably related to the slow rate of DA transporter blockade achieved by oral MP because the reinforcing effects of psychostimulant drugs are thought to be related, at least in part, to a

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rapid rate of binding to DA transporters (Stathis et al., 1995) and subsequently, a rapid increase in synaptic DA (Balster and Schuster, 1973). Thus, although i.v. MP produces a "high", which cocaine abusers report to be similar to that induced by i.v. cocaine (Wang et al., 1997), oral MP with a slower onset of transporter blockade does not produce a high in normal subjects (Volkow et al., 1998).

Dose-related effects of MP were clearly demonstrated by Porrino and coworkers. They showed that local cerebral glucose utilization (LCGU) in the rat nucleus accumbens (NACC), a brain region associated with the reinforcing effects of drugs of abuse (Di Chiara, 1999), was stimulated by a low dose of MP (1.25 mg/kg i.v.), but not by a high dose (15 mg/kg). However, higher doses dramatically increased LCGU in the extrapyramidal system (Porrino and Lucignani, 1987). A similar dose-related pattern was observed with amphetamine (Porrino et al., 1984). These authors later demonstrated that behaviorally equivalent doses of i.v., but not i.p. cocaine, produced increases in LCGU in NACC (Porrino, 1993). This similarity in the distribution of changes in LCGU led the authors to propose a significant role of the NACC in the therapeutic response of hyperactive children to psychostimulant medications. Taken together, these data suggest the importance of determining the dose-response function for different routes of administration in evaluating the biochemical and behavioral effects of psychostimulants.

The purpose of the present study was to assess the effects of oral MP (intragastric administration) on extracellular DA in NACC and on locomotor activity. In addition, we directly compared the effects of oral versus i.p. MP to provide a context for evaluation of the findings from previous studies. The range of doses used (2-10 mg/kg) is, in general, lower than those investigated previously. Finally, we compared two methods of oral MP administration, a surgically implanted intragastric catheter versus gavage, to determine whether the stress associated with gavage would influence the effects of MP on NACC DA.

► Materials and Methods

Male Sprague-Dawley rats were used in all experiments (200-300 g; Taconic Farms, Germantown, NY) and were given food and water ad libitum. Temperature and humidity were kept constant. Each animal was housed individually on a 12/12-h light/dark cycle. All animals were used under an Institutional Animal Care and Use Committee-approved protocol and with strict adherence to National Institutes of Health guidelines.

Drug Treatment. MP hydrochloride (2, 5, or 10 mg/kg) (a racemic mixture of *d-threo*- and *l-threo*-MP; Research Biochemicals International, Natick, MA) was dissolved in saline and injected i.p.

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Intragastric administration (2, 5, or 10 mg/kg) was accomplished through the preimplanted catheter followed by a rinse with vehicle. Control animals received saline via both routes. These same methods were used with microdialysis and locomotor activity studies. In a separate microdialysis experiment MP (5 mg/kg) or vehicle was administered via a gavage needle that was gently passed down the esophagus to the stomach ($n = 6-8$ for each treatment group). All microdialysis and activity measures were obtained between 12:00 PM and 3:00 PM.

Microdialysis Studies. Microdialysis studies were completed as detailed previously (Gerasimov and Dewey, 1999). Animals were anesthetized with an i.m. injection of ketamine/xylazine mixture and siliconized guide cannulas were stereotaxically implanted into the right NACC (2.0 mm anterior and 1.0 mm lateral to bregma, and 7.0 mm ventral to the cortical surface). On completion of the brain surgery a polyethylene catheter was placed in the stomach of animals intended for intragastric studies by using aseptic surgical techniques. The catheter was exteriorized after anchoring with a suture in the back of the neck. Animals were allowed to recover for at least 4 days.

Microdialysis probes (2.0 mm; Bioanalytical Systems, West Lafayette, IN) were positioned within the guide cannulas and artificial cerebrospinal fluid (155 mM NaCl, 1.1 mM CaCl_2 , 2.9 mM KCl, and 0.83 mM MgCl_2) was administered through the probe by using a CMA/100 microinfusion pump (Bioanalytical Systems) at a flow rate of 2.0 l/min. Animals were placed in bowls, and probes were inserted and flushed with artificial cerebrospinal fluid overnight. On the day of study, a minimum of three samples was injected to determine baseline stability. Samples were collected for 20 min and injected on-line (Bioanalytical Systems). The average DA concentration of these three stable samples was defined as control (100%), and all subsequent treatment values were transformed to a percentage of that control. The HPLC system consisted of a BAS reversed phase column (3.0 μm C18), a BAS LC-4C electrochemical transducer with a dual glassy carbon electrode set at 650 mV relative to an Ag/AgCl reference electrode, a computer that analyzes data on-line by using a commercial software package (Chromgraph; Bioanalytical Systems), and a dual pen chart recorder. The mobile phase (flow rate 1.0 ml/min) consisted of 7.0% methanol, 50 mM sodium phosphate monobasic, 1.0 mM sodium octyl sulfate, and 0.1 mM EDTA, pH 4.0.

Locomotor Activity. Animals were individually placed in photocell activity boxes (San Diego Instruments, San Diego, CA). The boxes were 41.3 $\times$ 41.3 $\times$ 30.5-cm clear acrylic. The electronic system used to monitor the movements consists of 16 infrared beams projecting across the cages from left to right and 16 beams from back to back. All the beams are approximately 0.39 cm from the floor. After 100 min to allow initial exploratory behavior to decrease, animals were injected i.p. with vehicle or MP (2, 5, or 10 mg/kg). In a separate group of experiments, animals received saline or MP (2, 5, or 10 mg/kg) by intragastric infusion via a catheter. Beam crossings were recorded every

minute and the mean number of crossings for each group of animals was summed into 20-min intervals for graphical display.

***d*-[³H]threo-MP Uptake in the Brain.** Animals were treated with 5 mg/kg MP plus 2 μ Ci (per rat) of *d*-[³H]threo-MP either i.p. or via gavage. After 20 min animals were sacrificed by decapitation and their striata and cerebella dissected. Brain regions were weighed and dissolved in 1 ml of tissue solubilizer (Solvable; Packard, Meriden, CT). UltimaGold (Packard) liquid scintillation fluid was added and radioactivity was determined by scintillation counting with quench connection by external standard. Data are expressed as nanomoles of *d*-[³H]threo-MP per gram of tissue (wet weight).

Data Analysis. Peak increases in extracellular DA, expressed as a percentage of baseline values (the average of three predrug levels differing from each other by not more than 10%) were compared for every dose across both routes of administration by a one-way ANOVA and post hoc test. Significance levels were set at $P < .05$.

For comparison purposes, the increases in NACC DA levels were normalized to the highest value for each dose and expressed as a percentage of that value. Locomotor activity reflects the highest beam crossing count after subtraction of the baseline activity.

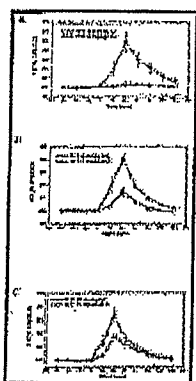
► Results

Microdialysis Studies. Intraperitoneal injections of MP dose dependently increased extracellular NACC DA above vehicle treatment values

($F = 26.75$; $P < .05$, $P < .01$, $P < .001$ for 2, 5, and 10 mg/kg, respectively). Intragastric administration also produced an increase in DA levels ($P < .05$ and $P < .001$ for 5 and 10 mg/kg, respectively). However, the measured response after intragastric administration of 2 mg/kg did not reach statistical significance. For both routes of administration and all three doses, the maximal effect of MP occurred at 40 min with levels returning to baseline values approximately 2.5 h postadministration (Fig. 1). During the first 20 min after administration the values approximately doubled for i.p. injection, but increased only by 30% after intragastric administration (Fig. 1, B and C), consistent with a faster rate of DA increase after the i.p. route.

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Fig. 1. Temporal profile of i.p. or intragastrical (p.o.) MP effect on extracellular NACC DA. A, effects of 2 mg/kg MP. B, effects of 5 mg/kg MP. C, effects of 10 mg/kg MP. Values are expressed as percentage of baseline DA and are mean $\pm$ S.E. ($n = 6-8$ /group). Drug or vehicle was administered at time 0. For clarity saline response is not shown.



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Administration of 5 mg/kg MP by gavage produced increases in DA levels that did not significantly differ from those produced by intragastric administration. The temporal profile of increases in extracellular DA after gavage administration is identical with the time course of increases after administration of the same dose via intragastric catheter (Fig. 2).

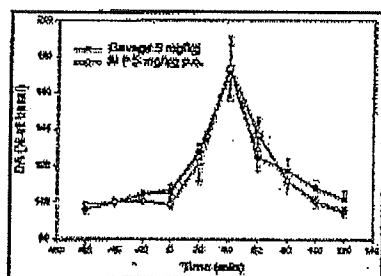


Fig. 2. Temporal profile of 5 mg/kg intragastrical (p.o.) and gavage MP effect on extracellular NACC DA. Values are expressed as percentage of baseline DA and are mean $\pm$ S.E. ($n = 6-8$ /group). Drug was administered at time 0.

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Locomotor Activity. Similar to our microdialysis data, i.p. injections of MP dose dependently increased gross locomotor activity above vehicle treatment values (Fig. 3) ($F = 36.68$; $P < .05$, $P < .01$, $P < .001$ for 2, 5, and 10 mg/kg, respectively). Intragastric administration, however, resulted in increased locomotion only after 5- and 10-mg/kg doses ($P < .01$, $P < .001$). On average, the maximal response for i.p. administration occurred at 20 min, whereas for intragastric administration it occurred at 40 min postdrug administration. For each dose examined, the DAergic and locomotor response to i.p. MP was significantly greater than the response after intragastric administration (Figs. 4 and 5).

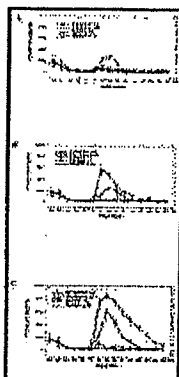


Fig. 3. Temporal profile of i.p. or intragastrical (p.o.) MP effect on gross locomotor activity. A, effects of 2 mg/kg MP. B, effects of 5 mg/kg MP. C, effects of 10 mg/kg MP. Values are expressed as the number of beam crossings and are mean $\pm$ S.E. ($n = 6-8$ /group). Drug or vehicle was administered at time 0. For clarity saline response is not shown.

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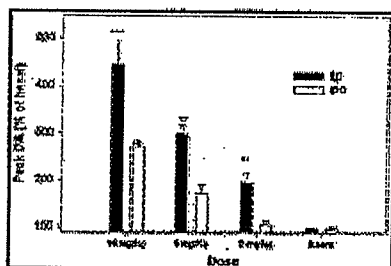


Fig. 4. Peak increase in NACC DA in response to i.p. or intragastrical (p.o.) administration of MP. Values are expressed as percentage of baseline DA and are mean $\pm$ S.E. ($n = 6-8$ /group). *** $P < .001$, ** $P < .01$ compared with the effect of the identical dose administered via different route [ANOVA ($F = 26.75$) and post hoc Tamhane test].

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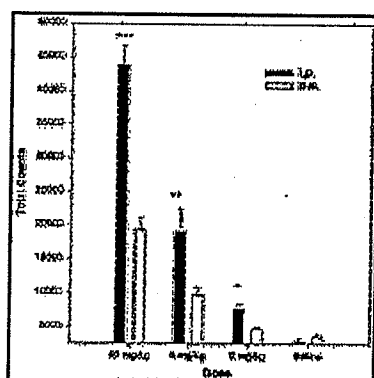


Fig. 5. Locomotor response to i.p. or intragastrical (p.o.) administration of MP. Values are expressed as total number of beam crossings over 200-min time interval and are mean $\pm$ S.E. ($n = 6-8$ /group). *** $P < .001$, ** $P < .01$, * $P < .05$ compared with the identical dose administered via different route [ANOVA ($F = 36.68$) and post hoc Tamhane test].

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The relationship between the temporal course of the changes in extracellular DA and locomotor activity is represented as a normalized response (Fig. 6). Because there was no change in either extracellular DA or locomotion after intragastric administration of 2 mg/kg, Fig. 5A shows only the response to i.p. injection.

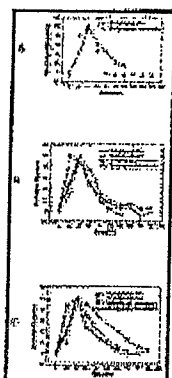


Fig. 6. Normalized NACC DA and locomotor temporal response to MP. Values are expressed as the percentage of the maximum for each given function and represent the average of six animals. Drug was administered at time 0.

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Brain and Plasma Levels of MP. Intraperitoneal administration of [^3H]MP resulted in higher levels of radioactivity in plasma and brain than the intragastric route (Table 1).

TABLE 1

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d- [^3H]threo-MP or metabolite(s) concentrations ($\mu\text{mol/g}$) in brain and plasma after administration of 5 mg/kg MP via gavage or intraperitoneal injection

Values are mean $\pm$ S.D. Superscript letters correspond to post hoc *t* tests showing significant difference of intraperitoneal injection from gavage (oral).

► Discussion

This study shows that intragastric and i.p. routes of administration differ significantly with respect to the absolute magnitude and the time course of increases in extracellular DA and locomotor response. Intraperitoneal MP was approximately twice as potent as oral MP both in increasing extracellular DA levels at the doses of 5 and 10 mg/kg (Fig. 1A) and in stimulating locomotor activity at these same doses (Fig. 1B). This is

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consistent with the apparent higher uptake of [^3H]MP measured in the brain after i.p. versus intragastric administration (Table 1). However, the interpretation of the brain uptake results is limited by the inaccuracy of assessing the total radioactivity counts without isolating [^3H]MP from ^3H -metabolites. Interestingly, at the lowest dose of 2 mg/kg given intragastrically, we did not observe an increase in DA levels above vehicle treatment nor was there any change in locomotor activity. In contrast, that same dose administered i.p. produced a significant increase above baseline values in extracellular DA and in locomotion. These data are in agreement with the notion that quantitatively different responses to identical doses of MP administered to humans via two systemic routes (i.v. and oral) are a function of bioavailability (Chan et al., 1980). The lower bioavailability for intragastric versus i.p. MP is presumably due to the slower absorption from the gastrointestinal tract and a greater degree of metabolism to ritalinic acid, a compound with negligible psychostimulant properties (Faraj et al., 1974).

Overall, quantitative and qualitative differences between oral, i.p., and i.v. routes of administration have been demonstrated for MP across species (Faraj et al., 1974; Wargin et al., 1983). However, the reports are varied and sometimes inconsistent. This may reflect considerable intersubject variability due to differences in drug absorption and metabolism. Studies by Faraj et al. (1974), Wargin et al. (1983), and Chan et al. (1980) provide evidence for presystemic metabolism of MP via deesterification in the gut and microsomal hydroxylation in liver as well as high intrinsic clearance due to plasma and/or tissue esterase activity. Reported estimates of the absolute systemic bioavailability of oral MP in children, rats, and monkeys vary between 10 and 50%.

Gross locomotor activity observed at 10 mg/kg i.p. does not seem to increase further with a higher dose of 20 mg/kg (O. Rice and S. J. Gatley, unpublished observations) even though extracellular NACC DA levels are increased further (860 versus 480% of baseline) (Rice et al., 1998). This is presumably due to focused stereotypies interfering with horizontal movements of the animal (Gaytan et al., 1996).

For both routes of administration gross locomotor activity responses to MP approximately track increases in NACC DA. This is consistent with the hypothesis that facilitation of DAergic transmission is involved in the locomotor response to psychostimulants (Beninger, 1983). The intragastric route exhibited a very close parallelism for the behavioral and neurochemical responses to MP, even though these measurements were obtained in different groups of animals. Normalized plots of extracellular DA and locomotor activity are almost identical (Fig. 6, A and B). However, for i.p. administration the quantitative features of MP-induced behavioral activation were dissociated from increases in extracellular DA. Peak hyperactivity occurred at 20 min postadministration, whereas the DA response took 40 min to reach its maximum. Between 20 and 40 min locomotor activity was

almost constant, whereas extracellular DA continued to increase. Although this could reflect a ceiling effect for locomotor activity, after which further DA increases result in stereotypies, it also could reflect acute tolerance to the locomotor-activating effects of synaptic DA, occurring after the fast initial increase in DA concentrations. A similar dissociation between plasma levels and therapeutic efficacy has been reported for oral MP in children (Swanson et al., 1999). The time course of plasma and brain MP concentration changes appears to be crucial in determining the efficacy of this drug (Srinivas et al., 1992). This notion was recently emphasized by Swanson et al. (1999) who demonstrated that the efficacy of a given total dose of MP is affected by its rate of administration.

A dissociation between DA and locomotor activity for the i.p. route of administration also was observed for the declining portion of the curves for the 10-mg/kg dose because at 120 min postinjection DA levels were already returning to baseline, whereas locomotion remained significantly elevated. This also could have reflected a ceiling effect and we cannot rule out the possibility that as DA levels fell stereotypy decreased concomitantly. New studies are now being designed with the goal of assessing the temporal course of stereotypies. Alternatively, this dissociation could reflect lingering downstream effects.

Another explanation is based on the partial involvement of the noradrenergic neurotransmitter system in the locomotor response to stimulants (Svensson and Ahlenius, 1983). We previously demonstrated that MP binds to norepinephrine transporters and is an effective *in vitro* inhibitor of norepinephrine uptake (Gatley et al., 1996). Kuczenski and Segal (1997) demonstrated that the temporal profile of the hippocampal norepinephrine response to i.p. MP in rats is significantly different from that of DA, with a slower onset and longer duration.

For the highest MP dose tested (10 mg/kg) the difference in locomotor activity between the two routes is better demonstrated by comparing the areas under the curve (total movements count over the sampling period) rather than the peak effects (Figs. 3 and 5) because the magnitudes of peak activity were similar, but i.p. MP had a much longer-lasting effect. This is likely to reflect almost complete DA transporter saturation at this dose for both routes, but the higher bioavailability achieved with the i.p. route may result in a longer duration of DA transporter blockade at this high level of occupancy.

In extrapolating the results of this study to the effects of clinical doses of MP the question of a proper dose for comparison arises. Matching of the peak MP plasma concentrations in humans and in rats does not seem to be appropriate for choosing a clinically relevant dose to be administered to animals. Concentrations that are considered therapeutic (8-10 ng/ml) (Swanson and Volkow, 2000) are reached at an average of 1 to 1.5 h after administration and roughly coincide with the peak changes in behavioral and somatic variables. The average half-life of oral MP is reported to be 2 to 3 h (Wargin

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et al., 1983; Volkow et al., 1998). However, similar plasma levels in the rat are only achieved for a short period of time (1.4-25 ng/ml at 15 min) and are almost undetectable at 30 min (0-4 ng/ml) (T. Cooper, personal communication). According to Patrick et al. (1984), oral administration of 1 mg/kg MP in rats results in peak serum concentrations of 40 ng/ml that are reached 10 min after dosing, but which fall to 15 ng/ml during the next 5 min. However, when 10 mg/kg oral MP is administered to rats, the half-life of MP appears to be 1 h with the plasma levels of 40 ng/ml occurring 3 h after drug administration (Wargin et al., 1983) or 10 to 20 ng/ml 4 h after 20 mg/kg (T. Cooper, personal communication). Based on these data one might argue that the lowest dose of oral MP (2 mg) used in the present study is not of clinical relevance due to its short-lived effective concentrations in plasma and subsequently, brain concentrations. However, the intragastric dose of 10 mg/kg leads to sustained plasma levels that are higher than those achieved therapeutically. The intermediate MP dose of 5 mg/kg administered intragastrically might mimic the therapeutic doses better. In terms of the magnitude of neurochemical and behavioral effects, the 5-mg/kg intragastric dose was roughly equivalent to the 2-mg/kg i.p. dose. Thus, by extrapolation one could suggest that i.p. MP doses of less than 5 mg/kg may be closer to those used clinically. However, one should keep in mind that therapeutic effect of MP in humans requires sustained brain levels, which are not achieved in rats.

In this study we did not observe a difference between the DAergic responses to MP administered intragastrically or via gavage. This indicates that both methods are adequate for testing the effects of oral MP.

Investigation of the relative effects of oral and i.p. MP is of basic and clinical significance. Low reinforcing effects of oral versus i.v. MP in humans have been linked to differences in pharmacokinetics rather than poor binding efficacy at the DA transporter (Volkow et al., 1998). Additionally, several studies have shown that the effects of MP are dependent on the behavioral state of the subject. This effect described as "rate dependence" has been documented both in rats and in humans (Rapport et al., 1984; Weber, 1985). Our results demonstrate that the route of administration is an important determinant of the behavioral and neurochemical consequences associated with MP administration in rodents. Additionally, we are currently conducting new studies investigating the changes in brain DA elicited by therapeutic doses of MP in humans.

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► Footnotes

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► Abbreviations

MP, methylphenidate; DA, dopamine; LCGU, local cerebral glucose utilization; NACC, nucleus accumbens.

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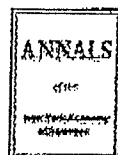
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Exhibit C

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consistent, and the presence of food may enhance or diminish the absorption of drugs. The most common type of interaction occurs when a food constituent binds the drug and the food-drug complex cannot pass through the gut wall. For example, complexation of tetracycline antibiotics may occur when these drugs are administered with dairy products or with antacids containing aluminum, calcium, or magnesium. The presence of a large meal in the stomach will delay gastric emptying. If a drug that is absorbed in the intestine is ingested with a large meal, the delay in gastric emptying may result in a delay in absorption of the drug. However, the presence of food in the stomach has also been shown to increase absorption of some drugs. For example, the bioavailabilities of the β -adrenergic blocking drugs, propranolol and metoprolol, are enhanced by the presence of food.³ Therefore, because of the difficulty in predicting the absorption pattern of a drug in the presence of food, it is usually advisable to administer drugs when the stomach is empty. An exception to this advice is with drugs which cause gastrointestinal irritation and nausea. These drugs must be given with food to prevent these side effects. It is recommended that such drugs always be taken with food to compensate for the differences in absorption that might occur if they were given one time with food and another time without food.

Water taken concomitantly with certain drugs may increase bioavailability. The administration of aspirin, erythromycin stearate, amoxicillin or theophylline with 250 mL of water results in greater bioavailability than if the same drugs are ingested with only 25 mL of water.⁴ It is probable that the increased amount of water enhances the amount of drug absorbed by improving drug dissolution as well as by hastening gastric emptying.

Diseases that affect the structure and function of the gastrointestinal tract are also capable of altering the absorption of drugs after oral administration. However, no consistent pattern develops; rather, there appears to be a complex relationship between the effect of the disease on stomach and intestinal functions and the absorption of the drug in question. For example, diseases such as diabetes mellitus or chronic renal failure, diseases that delay gastric emptying, will markedly delay the absorption and onset of effect of drugs that must reach the small intestine before they are absorbed. This has been a problem with the use of phenytoin in patients with chronic renal failure. Celiac disease and Crohn's disease, two diseases that alter the intestinal epithelium, have been studied in detail.⁵ In these diseases, absorption of some drugs is greatly affected, but there is no consistent pattern of altered drug absorption.

When a drug is to be administered orally to a patient with altered gastrointestinal motility, diseases of the stomach and small or large intestine, previous stomach or intestinal surgery, or gastrointestinal infection, there is a considerable probability that drug absorption characteristics in these patients will differ from those in healthy volunteers. This may result in a change in the time of peak blood level or the extent of absorption. It is advisable to observe such patients closely for clinical effect during initial drug administration and during chronic dosing in order to assess the influence of alterations in absorption and to correct dosing regimens accordingly. The monitoring of drug blood concentrations may be beneficial in adjusting dose.

Non-Oral Routes—Drugs are administered by a variety of non-oral routes. These include: subcutaneous, intramuscular, intravenous, inhalation, percutaneous, buccal, sublingual, rectal, vaginal, intra-arterial and intrathecal. In the cases of inhalation, topical application to the skin or mucous membranes, rectal, vaginal, intra-arterial or intrathecal administration, the route is often chosen to ensure that drugs reach a specific site with a minimum of systemic absorption.

The rationale is that the maximum concentration of drug will be at the site of action so that side effects will be lessened. Nevertheless, if large doses are administered by these routes, enough drug may reach the general circulation to produce side effects. Therefore, the dose and preparation should be such that limited quantities of drug reach the systemic circulation. The beta-adrenergic agonists, metaproterenol and albuterol, when administered by inhalation produce bronchodilation at doses that avoid serious systemic side effects. Similarly, the corticosteroid, beclomethasone, can also be administered by this route for the management of chronic asthma. Low doses of beclomethasone by inhalation are without the serious systemic side effects of oral steroids. However, as the dose is increased beyond two inhalations four times a day, for an average daily dose of 400 μ g, there is a greater incidence of side effects, including adrenal suppression.

The topical administration of drugs is rapidly becoming an important route of drug administration of systemic drugs. Previously used only for the application of drugs for local effects in diseases of the skin, it is now being explored as a means of administering drugs for their systemic effects. Nitroglycerin is commonly applied to the skin in the form of an ointment or transdermal patches; it is rapidly absorbed and provides sustained blood levels. Sublingual nitroglycerin is also employed to produce therapeutic blood levels; it produces a maximal effect on anginal pain within 3 to 5 mins but lasts only 20 to 60 min. In contrast, nitroglycerin ointment provides peak blood concentrations in about one hour and the effect on anginal pain may last for several hours. The sublingual tablets should be used to suppress acute angina attacks, whereas nitroglycerin ointment or transdermal patches may be useful to prevent recurrence of episodes of angina for prolonged periods, such as during the night. Whether or not the continuous administration of nitrates by this route will result in the development of tolerance is not clear at this time. There are several other drugs, such as those used to treat hypertension, for which percutaneous administration is being investigated as a means of attaining sustained plasma levels.

Close *intra-arterial* administration of drugs is used to get drugs directly to a target site or organ in high concentration. After it has passed through the target region it is distributed in the entire blood volume, which reduces the systemic levels of the drug and the consequent side effects. One example of this mode of drug administration is the use of cytotoxic drugs for the treatment of primary or metastatic tumors of liver. The infusion of drugs into the hepatic artery exposes the tumor to higher drug concentrations than can be tolerated with intravenous administration. If the drug is efficiently extracted by liver, the exposure of sensitive tissues such as bone marrow and gastrointestinal epithelium to the drug will be decreased. For example, after hepatic artery infusion of floxuridine (FUDR) hepatic vein concentrations are 2 to 6 times higher than comparable drug concentrations following intravenous infusion yet systemic blood concentrations are 75% less. Thus, the therapeutic index of FUDR in the treatment of liver cancer is considerably increased by hepatic arterial infusion. This type of selective drug administration may be beneficial with other drugs that have low therapeutic indices.

Intrathecal injection is used to deliver drugs to the brain in sufficient concentration to produce an effect but at the same time to reduce the incidence or severity of systemic side effects. The intrathecal administration of the cancer chemotherapeutic agent, methotrexate, is frequently employed in the management of leukemic involvement of the central nervous system. The epidural administration of morphine, which produces long-lasting (6–30 hrs) analgesia with minimal side effects, is proving to be of benefit in the management of chronic pain.

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Exhibit D

Drug Administration

Drugs are introduced into the body by several routes. They may be

- Taken by mouth (orally)
- Given by injection into a vein (intravenously), into a muscle (intramuscularly), into the space around the spinal cord (intrathecally), or beneath the skin (subcutaneously)
- Placed under the tongue (sublingually)
- Inserted in the rectum (rectally) or vagina (vaginally)
- Placed in the eye (by the ocular route)
- Sprayed into the nose and absorbed through the nasal membranes (nasally)
- Breathed into the lungs, usually through the mouth (by inhalation)
- Applied to the skin (cutaneously) for a local (topical) or bodywide (systemic) effect
- Delivered through the skin by a patch (transdermally) for a systemic effect

Each route has specific purposes, advantages, and disadvantages.

Oral route:

Many drugs can be administered orally as liquids, capsules, tablets, or chewable tablets. Because the oral route is the most convenient and usually the safest and least expensive, it is the one most often used. However, it has limitations because of the way a drug typically moves through the digestive tract. For drugs administered orally, absorption may begin in the mouth and stomach. However, most drugs are usually absorbed from the small intestine. The drug passes through the intestinal wall and travels to the liver before it is transported via the bloodstream to its target site. The intestinal wall and liver chemically alter (metabolize) many drugs, decreasing the amount of drug reaching the bloodstream. Consequently, these drugs are often given in smaller doses when injected intravenously to produce the same effect.

When a drug is taken orally, food and other drugs in the digestive tract may affect how much of and how fast the drug is absorbed. Thus, some drugs should be taken on an empty stomach, others should be taken with food, others should not be taken with certain other drugs, and still others cannot be taken orally at all.

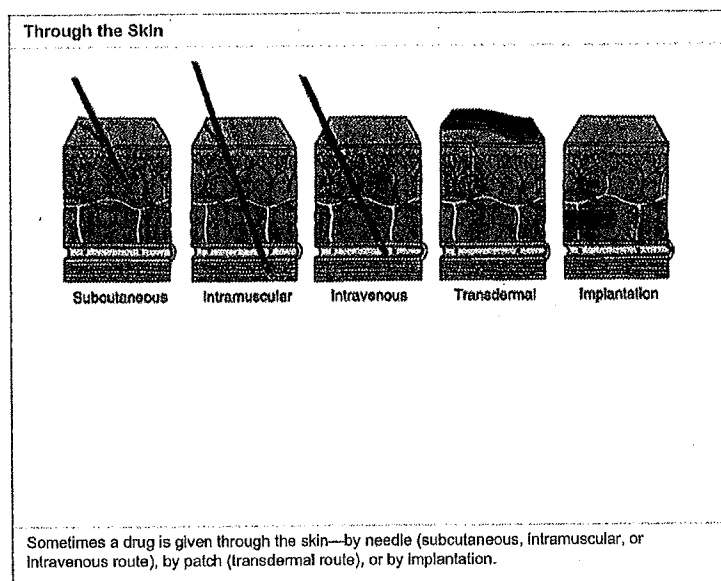
Some orally administered drugs irritate the digestive tract. For example, **aspirin** and most other nonsteroidal anti-inflammatory drugs (NSAIDs—see [Nonsteroidal Anti-Inflammatory Drugs](#)) can harm the lining of the stomach and small intestine to potentially cause or aggravate preexisting ulcers (see [Causes](#)). Other drugs are absorbed poorly or erratically in the digestive tract or are destroyed by the acid and digestive enzymes in the stomach.

Other routes of administration are required when the oral route cannot be used: for example, when a person cannot take anything by mouth, when a drug must be administered rapidly or in a precise or very high dose, or when a drug is poorly or erratically absorbed from the digestive tract.

Injection routes:

Administration by injection (parenteral administration) includes the subcutaneous, intramuscular, intravenous, and intrathecal routes. A drug product can be prepared or manufactured in ways that prolong drug absorption from the injection site for hours, days, or longer. Such products do not need to be administered as often as drug products with

more rapid absorption.



For the **subcutaneous route**, a needle is inserted into fatty tissue just beneath the skin. After a drug is injected, it then moves into small blood vessels (capillaries) and is carried away by the bloodstream. Alternatively, a drug reaches the bloodstream through the lymphatic vessels. Protein drugs that are large in size, such as **insulin**, usually reach the bloodstream through the lymphatic vessels because these drugs move slowly from the tissues into capillaries. The subcutaneous route is used for many protein drugs because such drugs would be destroyed in the digestive tract if they were taken orally.

Certain drugs (such as progestins used for birth control—see Contraceptive Implants) may be given by inserting plastic capsules under the skin (implantation). Although this route of administration is rarely used, its main advantage is to provide a long-term therapeutic effect (for example, etonogestrel that is implanted for contraception may last up to 3 years).

The **intramuscular route** is preferred to the subcutaneous route when larger volumes of a drug product are needed. Because the muscles lie below the skin and fatty tissues, a longer needle is used. Drugs are usually injected into the muscle of the upper arm, thigh, or buttock. How quickly the drug is absorbed into the bloodstream depends, in part, on the blood supply to the muscle: The sparser the blood supply, the longer it takes for the drug to be absorbed.

For the **intravenous route**, a needle is inserted directly into a vein. A solution containing the drug may be given in a single dose or by continuous infusion. For infusion, the solution is moved by gravity (from a collapsible plastic bag) or, more commonly, by an infusion pump through thin flexible tubing to a tube (catheter) inserted in a vein, usually in the forearm. Intravenous administration is the best way to deliver a precise dose quickly and in a well-controlled manner throughout the body. It is also used for irritating solutions, which would cause pain and damage tissues if given by subcutaneous or intramuscular injection. An intravenous injection can be more difficult to administer than a subcutaneous or intramuscular injection, because inserting a needle or catheter into a vein may be difficult, especially if the person is obese.

When given intravenously, a drug is delivered immediately to the bloodstream and tends to take effect more quickly than when given by any other route. Consequently, health care practitioners closely monitor people who receive an intravenous injection for signs that the drug is working or is causing undesired side effects. Also, the effect of a drug given by this route tends to last for a shorter time. Therefore, some drugs must be given by continuous infusion to keep their effect constant.

For the **intrathecal route**, a needle is inserted between two vertebrae in the lower spine and into the space around the spinal cord. The drug is then injected into the spinal canal. A small amount of local anesthetic is often used to numb the injection site. This route is used when a drug is needed to produce rapid or local effects on the brain, spinal cord, or the layers of tissue covering them (meninges)—for example, to treat infections of these structures. Anesthetics and analgesics (such as **morphine**) are sometimes given this way.

Sublingual route:

A few drugs are placed under the tongue (taken sublingually) so that they can be absorbed directly into the small blood vessels that lie beneath the tongue. The sublingual route is especially good for **nitroglycerin**—which is used to relieve angina (chest pain caused by an inadequate blood supply to the heart muscle)—because absorption is rapid and the drug immediately enters the bloodstream without first passing through the intestinal wall and liver. However, most drugs cannot be taken this way because they may be absorbed incompletely or erratically.

Rectal route:

Many drugs that are administered orally can also be administered rectally as a suppository. In this form, a drug is mixed with a waxy substance that dissolves or liquefies after it is inserted into the rectum. Because the rectum's wall is thin and its blood supply rich, the drug is readily absorbed. A suppository is prescribed for people who cannot take a drug orally because they have nausea, cannot swallow, or have restrictions on eating, as is required before and after many surgical operations. Drugs that can be administered rectally include **acetaminophen** (for fever), **diazepam** (for seizures), and laxatives (for constipation). Drugs that are irritating in suppository form may have to be given by injection.

Vaginal route:

Some drugs may be administered vaginally to women as a solution, tablet, cream, gel, suppository, or ring. The drug is slowly absorbed through the vaginal wall. This route is often used to give estrogen to women during menopause to relieve vaginal symptoms such as dryness, soreness, and redness.

Ocular route:

Drugs used to treat eye disorders (such as glaucoma, conjunctivitis, and injuries) can be mixed with inactive substances to make a liquid, gel, or ointment, so that they can be applied to the eye. Liquid eye drops are relatively easy to use but may run off the eye too quickly to be absorbed well. Gel and ointment formulations keep the drug in contact with the eye surface longer but they may blur vision. Solid inserts, which release the drug continuously and slowly, are also available, but they may be hard to put in and keep in place. Ocular drugs are almost always used for their local effects. For example, artificial tears are used to relieve dry eyes. Other drugs (for example, those used to treat glaucoma [see table on page see **Drugs Used to Treat Glaucoma**]), such as **acetazolamide** and **betaxolol** and those used to dilate pupils, such as **phenylephrine** and **tropicamide**) produce a local effect after they are absorbed through the cornea and conjunctiva. Some of these drugs then enter the bloodstream and may cause unwanted effects on other parts of the body.

Nasal route:

If a drug is to be breathed in and absorbed through the thin mucous membrane that lines the nasal passages, it must be transformed into tiny droplets in air (atomized). Once absorbed, the drug enters the bloodstream. Drugs administered by this route generally work quickly. Some of them irritate the nasal passages. Drugs that can be administered by the nasal route include **nicotine** (for smoking cessation), **calcitonin** (for osteoporosis), **sumatriptan** (for migraine headaches), and corticosteroids (for allergies).

Inhalation:

Drugs administered by inhalation through the mouth must be atomized into smaller particles than those administered by the nasal route, so that the drug can pass through the windpipe (trachea) and into the lungs. How deeply into the lungs they go depends on the size of the droplets. Smaller droplets go deeper, which increases the amount of drug absorbed. Inside the lungs, they are absorbed into the bloodstream.

Relatively few drugs are administered this way because inhalation must be carefully monitored to ensure that a person receives the right amount of drug within a specified time. In addition, specialized equipment may be needed to give the drug by this route. Usually, this method is used to administer drugs that act specifically on the lungs, such as aerosolized antiasthmatic drugs in metered-dose containers, and to administer gases used for general anesthesia.

Cutaneous route:

Drugs applied to the skin are usually used for their local effects and thus are most commonly used to treat superficial skin disorders, such as psoriasis, eczema, skin infections (viral, bacterial, and fungal), itching, and dry skin. The drug is mixed with inactive substances. Depending on the consistency of the inactive substances, the formulation may be an ointment, cream, lotion, solution, powder, or gel (see [Topical Preparations](#)).

Transdermal route:

Some drugs are delivered bodywide through a patch on the skin. These drugs are sometimes mixed with a chemical (such as alcohol) that enhances penetration through the skin into the bloodstream without any injection. Through a patch, the drug can be delivered slowly and continuously for many hours or days or even longer. As a result, levels of a drug in the blood can be kept relatively constant. Patches are particularly useful for drugs that are quickly eliminated

from the body because such drugs, if taken in other forms, would have to be taken frequently. However, patches may irritate the skin of some people. In addition, patches are limited by how quickly the drug can penetrate the skin. Only drugs to be given in relatively small daily doses can be given through patches. Examples of such drugs include [nitroglycerin](#) (for chest pain), [scopolamine](#) (for motion sickness), [nicotine](#) (for smoking cessation), [clonidine](#) (for high blood pressure), and [fentanyl](#) (for pain relief).

Last full review/revision July 2012 by Jennifer Le, PharmD, MAS, BCPS-ID

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Exhibit E

Equivalent Surface Area Dosage Conversion Factors

This table gives approximate factors for converting doses expressed in terms of mg/kg from one species to an equivalent surface area dose expressed as mg/kg in the other species tabulated. The assumptions and constants of the paper by Freireich EJ, et al, 1966 are used.

		TO				
		Mouse 20 g	Rat 150 g	Monkey 3 kg	Dog 8 kg	Man 60 kg
FROM	Mouse	1	1/2	1/4	1/6	1/12
	Rat	2	1	1/2	1/4	1/7
	Monkey	4	2	1	3/5	1/3
	Dog	6	4	1 2/3	1	1/2
	Man	12	7	3	2	1

Ex: Given a dose of 50 mg/kg in the mouse, what is the appropriate dose in the monkey assuming equivalency on the basis of mg/m²? $50 \text{ mg/kg} \times 1/4 = 13 \text{ mg/kg}$.

Representative Surface Area to Weight Ratios [km] for Various Species¹

Species	Body Weight [kg]	Surface Area [sq. m.]	km factor
Mouse	0.02	0.0066	3.0
Rat	0.15	0.025	5.9
Monkey	3.0	0.24	12
Dog	8.0	0.40	20
Human, Child	20	0.80	25
Human, Adult	60	1.6	37

¹ Freireich, EJ, et al. Quantitative comparison of toxicity of anticancer agents in mouse, rat, dog, monkey and man. *Cancer Chemother Rep.* 1966;50(4):219-244.

Ex: To express a mg/kg dose in any given species as the equivalent mg/sq.m. dose, multiply the dose by the appropriate km factor. In adult humans, 100 mg/kg is equivalent to $100 \text{ mg/kg} \times 37 \text{ kg/sq.m.} = 3700 \text{ mg/sq.m.}$

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Guidance Posted August 2007

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Exhibit F

Guidance for Industry

Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

July 2005
Pharmacology and Toxicology

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Guidance for Industry

Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers

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U.S. Department of Health and Human Services
Food and Drug Administration
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Guidance for Industry¹
**Estimating the Maximum Safe Starting Dose in Initial Clinical
Trials for Therapeutics in Adult Healthy Volunteers**

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This guidance outlines a process (algorithm) and vocabulary for deriving the maximum recommended starting dose (MRSD) for *first-in-human* clinical trials of new molecular entities in adult healthy volunteers, and recommends a standardized process by which the MRSD can be selected. The purpose of this process is to ensure the safety of the human volunteers.

The goals of this guidance are to: (1) establish a consistent terminology for discussing the starting dose; (2) provide common conversion factors for deriving a human equivalent dose (HED); and (3) delineate a strategy for selecting the MRSD for adult healthy volunteers, regardless of the projected clinical use. This process is depicted in a flow chart that presents the decisions and calculations used to generate the MRSD from animal data (see Appendix E).

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

¹ This guidance has been prepared by the Office of New Drugs in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration.

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II. BACKGROUND

The process identified in this guidance pertains to determining the MRSD for adult healthy subjects when beginning a clinical investigation of any new drug or biological therapeutic that has been studied in animals. This guidance is not pertinent to endogenous hormones and proteins (e.g., recombinant clotting factors) used at physiologic concentrations or prophylactic vaccines. The process outlined in this guidance pertains primarily to drug products for which systemic exposure is intended; it does not address dose escalation or maximum allowable doses in clinical trials.

Although the process outlined in this guidance uses administered doses, observed toxicities, and an algorithmic approach to calculate the MRSD, an alternative approach could be proposed that places primary emphasis on animal pharmacokinetics and modeling rather than dose (Mahmood et al. 2003; Reigner and Blesch 2002). In a limited number of cases, animal pharmacokinetic data can be useful in determining initial clinical doses.² However, in the majority of investigational new drug applications (INDs), animal data are not available in sufficient detail to construct a scientifically valid, pharmacokinetic model whose aim is to accurately project an MRSD.

Toxicity should be avoided at the initial clinical dose. However, doses should be chosen that allow reasonably rapid attainment of the phase 1 trial objectives (e.g., assessment of the therapeutic's tolerability, pharmacodynamic or pharmacokinetic profile). All of the relevant preclinical data, including information on the pharmacologically active dose, the full toxicologic profile of the compound, and the pharmacokinetics (absorption, distribution, metabolism, and excretion) of the therapeutic, should be considered when determining the MRSD. Starting with doses lower than the MRSD is always an option and can be particularly appropriate to meet some clinical trial objectives.

² If the parent drug is measured in the plasma at multiple times and is within the range of toxic exposures for two or more animal species, it may be possible to develop a pharmacokinetic model predicting human doses and concentrations and to draw inferences about safe human plasma levels in the absence of prior human data. Although quantitative modeling for this purpose may be straightforward, the following points suggest this approach can present a number of difficulties when estimating a safe starting dose. Generally, at the time of IND initiation, there are a number of unknowns regarding animal toxicity and comparability of human and animal pharmacokinetics and metabolism: (1) human bioavailability and metabolism may differ significantly from that of animals; (2) mechanisms of toxicity may not be known (e.g., toxic accumulation in a peripheral compartment); and/or (3) toxicity may be due to an unidentified metabolite, not the parent drug. Therefore, relying on pharmacokinetic models (based on the parent drug in plasma) to gauge starting doses would require multiple untested assumptions. Modeling can be used with greatest validity to estimate human starting doses in special cases where few underlying assumptions would be necessary. Such cases are exemplified by large molecular weight proteins (e.g., humanized monoclonal antibodies) that are intravenously administered, are removed from circulation by endocytosis rather than metabolism, have immediate and detectable effects on blood cells, and have a volume of distribution limited to the plasma volume. In these cases, allometric, pharmacokinetic, and pharmacodynamic models have been useful in identifying the human mg/kg dose that would be predicted to correlate with safe drug plasma levels in nonhuman primates. Even in these cases, uncertainties (such as differences between human and animal receptor sensitivity or density) have been shown to affect human pharmacologic or toxicologic outcomes, and the use of safety factors as described in this guidance is still warranted.

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The remainder of this guidance focuses on the recommended algorithmic process for starting dose extrapolation from animals to humans based on administered doses, since this method will likely be useful for the majority of INDs seeking to investigate new drugs in healthy volunteers. Some classes of drugs (e.g., many cytotoxic or biological agents) are commonly introduced into initial clinical trials in patient volunteers rather than healthy volunteers. Typically, patients are used instead of healthy volunteers when a drug is suspected or known to be unavoidably toxic. This guidance does not address starting doses in patients. However, many principles and some approaches recommended here may be applicable to designing such trials.

III. OVERVIEW OF THE ALGORITHM

The recommended process for selecting the MRSD is presented in Appendix E and described in this section. The major elements (i.e., the determination of the no observed adverse effect levels (NOAELs) in the tested animal species, conversion of NOAELs to HED, selection of the most appropriate animal species, and application of a safety factor) are all discussed in greater detail in subsequent sections. Situations are also discussed in which the algorithm should be modified. The algorithm is intended to be used for systemically administered therapeutics. Topical, intranasal, intratissue, and compartmental administration routes and depot formulations can have additional considerations, but similar principles should apply.

The process of calculating the MRSD should begin after the toxicity data have been analyzed. Although only the NOAEL should be used directly in the algorithm for calculating an MRSD, other data (exposure/toxicity relationships, pharmacologic data, or prior clinical experience with related drugs) can affect the choice of most appropriate species, scaling, and safety factors.

The NOAEL for each species tested should be identified, and then converted to the HED using appropriate scaling factors. For most systemically administered therapeutics, this conversion should be based on the normalization of doses to body surface area. Although body surface area conversion is the standard way to approximate equivalent exposure if no further information is available, in some cases extrapolating doses based on other parameters may be more appropriate. This decision should be based on the data available for the individual case. The body surface area normalization and the extrapolation of the animal dose to human dose should be done in one step by dividing the NOAEL in each of the animal species studied by the appropriate body surface area conversion factor (BSA-CF). This conversion factor is a unitless number that converts mg/kg dose for each animal species to the mg/kg dose in humans, which is equivalent to the animal's NOAEL on a mg/m² basis. The resulting figure is called a human equivalent dose (HED). The species that generates the lowest HED is called the most sensitive species.

When information indicates that a particular species is more relevant for assessing human risk (and deemed the *most appropriate species*), the HED for that species may be used in subsequent calculations, regardless of whether this species is the most sensitive. This situation is more applicable to biologic therapies, many of which have high selectivity for binding to human target

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proteins and limited reactivity in species commonly used for toxicity testing. In such cases, in vitro binding and functional studies should be conducted to select an appropriate, relevant species before toxicity studies are designed (refer to ICH guidance for industry *S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* for more details³). (However, if serious toxicities are observed in an animal species considered less relevant, those toxicities should be taken into consideration in determining the species to be used to calculate an HED. For example, in one particular case, dog was selected as the animal species used for calculation of an HED because of unmonitorable cardiac lesions, even though the rat was considered the most relevant species based on pharmacological activity data.) Additionally, a species might be considered an inappropriate toxicity model for a given drug if the dose-limiting toxicity in that species was concluded to be of limited value for human risk assessment, based on historical comparisons of toxicities in the animal species to those in humans across a therapeutic class (i.e., the dose-limiting toxicity is species-specific). In this case, data from that species should not be used to derive the HED. Without any additional information to guide the choice of the most appropriate species for assessing human risk, the most sensitive species is designated the *most appropriate*, because using the lowest HED would generate the most conservative starting dose.

A safety factor should then be applied to the HED to increase assurance that the first dose in humans will not cause adverse effects. The use of the safety factor should be based on the possibility that humans may be more sensitive to the toxic effects of a therapeutic agent than predicted by the animal models, that bioavailability may vary across species, and that the models tested do not evaluate all possible human toxicities. For example, ocular disturbances or pain (e.g., severe headaches) in humans can be significant dose-limiting toxicities that may go undetected in animal studies.

In general, one should consider using a safety factor of at least 10. The MRSD should be obtained by dividing the HED by the safety factor. Safety concerns or design shortcomings noted in animal studies may increase the safety factor, and thus reduce the MRSD further. Alternatively, information about the pharmacologic class (well-characterized classes of therapeutics with extensive human clinical and preclinical experience) may allay concerns and form the basis for reducing the magnitude of the default safety factor and increasing the MRSD. Although a dose lower than the MRSD can be used as the actual starting dose, the process described in this guidance will derive the maximum recommended starting dose. This algorithm generates an MRSD in units of mg/kg, a common method of dosing used in phase I trials, but the equations and conversion factors provided in this guidance (Table 1, second column) can be used to generate final dosing units in the mg/m² form if desired.

As previously stated, for purposes of initial clinical trials in adult healthy volunteers, the HED should ordinarily be calculated from the animal NOAEL. If the HED is based on an alternative index of effect, such as the pharmacologically active dose (PAD), this exception should be prominently stipulated in descriptions of starting dose calculations.

³ We update guidances periodically. To make sure you have the most recent version of a guidance, check the CDER guidance Web page at <http://www.fda.gov/cder/guidance/index.htm>.

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The remainder of this guidance provides a description of the individual steps in the recommended process and the reasoning behind each step.

IV. STEP 1: NO OBSERVED ADVERSE EFFECT LEVEL DETERMINATION

The first step in determining the MRSD is to review and evaluate the available animal data so that a NOAEL can be determined for each study. Several definitions of NOAEL exist, but for selecting a starting dose, the following is used: the highest dose level that does not produce a significant increase in adverse effects in comparison to the control group. In this context, adverse effects that are biologically significant (even if they are not statistically significant) should be considered in the determination of the NOAEL. The NOAEL is a generally accepted benchmark for safety when derived from appropriate animal studies and can serve as the starting point for determining a reasonably safe starting dose of a new therapeutic in healthy (or asymptomatic) human volunteers.

The NOAEL is not the same as the *no observed effect level (NOEL)*, which refers to any effect, not just an adverse one, although in some cases the two might be identical. The definition of the NOAEL, in contrast to that of the NOEL, reflects the view that some effects observed in the animal may be acceptable pharmacodynamic actions of the therapeutic and may not raise a safety concern. The NOAEL should also not be confused with *lowest observed adverse effect level (LOAEL)* or *maximum tolerated dose (MTD)*. Both of the latter concepts are based on findings of adverse effects and are not generally used as benchmarks for establishing safe starting doses in adult healthy volunteers. (The term *level* refers to dose or dosage, generally expressed as mg/kg or mg/kg/day.)

Initial IND submissions for first-in-human studies by definition lack in vivo human data or formal allometric comparison of pharmacokinetics. Measurements of systemic levels or exposure (i.e., AUC or C_{max}) cannot be employed for setting a safe starting dose in humans, and it is critical to rely on dose and observed toxic response data from adequate and well-conducted toxicology studies. However, there are cases where nonclinical data on bioavailability, metabolite profile, and plasma drug levels associated with toxicity may influence the choice of the NOAEL. One such case is when saturation of drug absorption occurs at a dose that produces no toxicity. In this instance, the lowest saturating dose, not the highest (nontoxic) dose, should be used for calculating the HED.

There are essentially three types of findings in nonclinical toxicology studies that can be used to determine the NOAEL: (1) overt toxicity (e.g., clinical signs, macro- and microscopic lesions); (2) surrogate markers of toxicity (e.g., serum liver enzyme levels); and (3) exaggerated pharmacodynamic effects. Although the nature and extent of adverse effects can vary greatly with different types of therapeutics, and it is anticipated that in many instances, experts will disagree on the characterization of effects as being adverse or not, the use of NOAEL as a benchmark for dose-setting in healthy volunteers should be acceptable to all responsible investigators. As a general rule, an adverse effect observed in nonclinical toxicology studies

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used to define a NOAEL for the purpose of dose-setting should be based on an effect that would be unacceptable if produced by the initial dose of a therapeutic in a phase I clinical trial conducted in adult healthy volunteers.

V. STEP 2: HUMAN EQUIVALENT DOSE CALCULATION

A. Conversion Based on Body Surface Area

After the NOAELs in the relevant animal studies have been determined, they are converted to HEDs. A decision should be made regarding the most appropriate method for extrapolating the animal dose to the equivalent human dose. Toxic endpoints for therapeutics administered systemically to animals, such as the MTD, are usually assumed to scale well between species when doses are normalized to body surface area (i.e., mg/m^2) (EPA 1992; Lowe and Davis 1998). The basis for this assumption lies primarily with the work of Freireich et al. (1966) and Schein et al. (1970). These investigators reported that, for antineoplastic drugs, doses lethal to 10 percent of rodents ($\text{LD}_{10\text{S}}$) and MTDs in nonrodents both correlated with the human MTD when the doses were normalized to the same administration schedule and expressed as mg/m^2 . Despite the subsequent analyses showing that the MTDs for this set of drugs scale best between species when doses are normalized to $W^{0.75}$ rather than $W^{0.67}$ (inherent in body surface area normalization) (Travis and White 1988; Watanabe et al. 1992), normalization to body surface area has remained a widespread practice for estimating an HED based on an animal dose.

An analysis of the affect of the allometric exponent on the conversion of an animal dose to the HED was conducted (see Appendix A). Based on this analysis and on the fact that correcting for body surface area increases clinical trial safety by resulting in a more conservative starting dose estimate, it was concluded that the approach of converting NOAEL doses to an HED based on body surface area correction factors (i.e., $W^{0.67}$) should be maintained for selecting starting doses for initial studies in adult healthy volunteers. Nonetheless, use of a different dose normalization approach, such as directly equating the human dose to the NOAEL in mg/kg , may be appropriate in some circumstances. Deviations from the body surface area approach, when describing the conversion of animal dose to HED, should be justified. The basis for justifying direct mg/kg conversion and examples in which other normalization methods are appropriate are described in the following subsection.

Although normalization to body surface area is an appropriate method for extrapolating doses between species, consistent factors for converting doses from mg/kg to mg/m^2 have not always been used. Given that body surface area normalization provides a reasonable approach for estimating an HED, the factors used for converting doses for each species should be standardized. Since body surface area varies with $W^{0.67}$, the conversion factors are dependent on the weight of the animals in the studies. However, analyses conducted to address the effect of body weight on the actual BSA-CF demonstrated that a standard factor provides a reasonable estimate of the HED over a broad range of human and animal weights (see Appendix B). The conversion factors and divisors shown in Table 1 are therefore recommended as the standard

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values to be used for interspecies dose conversions for NOAELs. (These factors may also be applied when comparing safety margins for other toxicity endpoints (e.g., reproductive toxicity and carcinogenicity) when other data for comparison (i.e., AUCs) are unavailable or are otherwise inappropriate for comparison.)

Table 1: Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area			
Species	To Convert Animal Dose in mg/kg to Dose in mg/m ² , Multiply by k _m	To Convert Animal Dose in mg/kg to HED ^a in mg/kg, Either:	
		Divide Animal Dose By	Multiply Animal Dose By
Human	37	---	---
Child (20 kg) ^b	25	---	---
Mouse	3	12.3	0.08
Hamster	5	7.4	0.13
Rat	6	6.2	0.16
Ferret	7	5.3	0.19
Guinea pig	8	4.6	0.22
Rabbit	12	3.1	0.32
Dog	20	1.8	0.54
Primates:			
Monkeys ^c	12	3.1	0.32
Marmoset	6	6.2	0.16
Squirrel monkey	7	5.3	0.19
Baboon	20	1.8	0.54
Micro-pig	27	1.4	0.73
Mini-pig	35	1.1	0.95

^a Assumes 60 kg human. For species not listed or for weights outside the standard ranges, HED can be calculated from the following formula:

$$\text{HED} = \text{animal dose in mg/kg} \times (\text{animal weight in kg/human weight in kg})^{0.33}$$

^b This k_m value is provided for reference only since healthy children will rarely be volunteers for phase I trials.

^c For example, cynomolgus, rhesus, and stump-tail.

B. Basis for Using mg/kg Conversions

The factors in Table 1 for scaling animal NOAEL to HEDs are based on the assumption that doses scale 1:1 between species when normalized to body surface area. However, there are occasions for which scaling based on body weight (i.e., setting the HED (mg/kg) = NOAEL (mg/kg)) may be more appropriate. To consider mg/kg scaling for a therapeutic, the available data should show that the NOAEL occurs at a similar mg/kg dose across species. The following circumstances should exist before extrapolating to the HED on a mg/kg basis rather than using

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the mg/m^2 approach. Note that mg/kg scaling will give a twelve-, six-, and twofold higher HED than the default mg/m^2 approach for mice, rats, and dogs, respectively. If these circumstances do not exist, the mg/m^2 scaling approach for determining the HED should be followed as it will lead to a safer MRSD.

1. NOAELs occur at a similar mg/kg dose across test species (for the studies with a given dosing regimen relevant to the proposed initial clinical trial). (However, it should be noted that similar NOAELs on a mg/kg basis can be obtained across species because of differences in bioavailability alone.)
2. If only two NOAELs from toxicology studies in separate species are available, one of the following should also be true:
 - The therapeutic is administered orally and the dose is limited by local toxicities. Gastrointestinal (GI) compartment weight scales by $W^{0.94}$ (Mordenti 1986). GI volume determines the concentration of the therapeutic in the GI tract. It is then reasonable that the toxicity of the therapeutic would scale by mg/kg ($W^{1.0}$).
 - The toxicity in humans (for a particular class) is dependent on an exposure parameter that is highly correlated across species with dose on a mg/kg basis. For example, complement activation by systemically administered antisense oligonucleotides in humans is believed to be dependent upon C_{max} (Geary et al. 1997). For some antisense drugs, the C_{max} correlates across nonclinical species with mg/kg dose and in such instances mg/kg scaling would be justified.
 - Other pharmacologic and toxicologic endpoints also scale between species by mg/kg for the therapeutic. Examples of such endpoints include the MTD, lowest lethal dose, and the pharmacologically active dose.
 - There is a robust correlation between plasma drug levels (C_{max} and AUC) and dose in mg/kg .

C. Other Exceptions to mg/m^2 Scaling Between Species

Scaling between species based on mg/m^2 is not recommended for the following categories of therapeutics:

1. Therapeutics administered by alternative routes (e.g., topical, intranasal, subcutaneous, intramuscular) for which the dose is limited by local toxicities. Such therapeutics should be normalized to concentration (e.g., mg/area of application) or amount of drug (mg) at the application site.
2. Therapeutics administered into anatomical compartments that have little subsequent distribution outside of the compartment. Examples are intrathecal, intravesical,

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intraocular, or intrapleural administration. Such therapeutics should be normalized between species according to the compartmental volumes and concentrations of the therapeutic.

3. Proteins administered intravascularly with $M_r > 100,000$ daltons. Such therapeutics should be normalized to mg/kg.

VI. STEP 3: MOST APPROPRIATE SPECIES SELECTION

After the HEDs have been determined from the NOAELs from all toxicology studies relevant to the proposed human trial, the next step is to pick one HED for subsequent derivation of the MRSD. This HED should be chosen from the most appropriate species. In the absence of data on species relevance, a default position is that the most appropriate species for deriving the MRSD for a trial in adult healthy volunteers is the most sensitive species (i.e., the species in which the lowest HED can be identified).

Factors that could influence the choice of the most appropriate species rather than the default to the most sensitive species include: (1) differences in the absorption, distribution, metabolism, and excretion (ADME) of the therapeutic between the species, and (2) class experience that may indicate a particular animal model is more predictive of human toxicity. Selection of the most appropriate species for certain biological products (e.g., human proteins) involves consideration of various factors unique to these products. Factors such as whether an animal species expresses relevant receptors or epitopes may affect species selection (refer to ICH guidance for industry *S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* for more details).

When determining the MRSD for the first dose of a new therapeutic in humans, absorption, distribution, and elimination parameters will not be known for humans. Comparative metabolism data, however, might be available based on in vitro studies. These data are particularly relevant when there are marked differences in both the in vivo metabolite profiles and HEDs in animals. Class experience implies that previous studies have demonstrated that a particular animal model is more appropriate for the assessment of safety for a particular class of therapeutics. For example, in the nonclinical safety assessment of the phosphorothioate antisense drugs, the monkey is considered the most appropriate species because monkeys experience the same dose limiting toxicity as humans (e.g., complement activation) whereas rodents do not. For this class of therapeutics, the MRSD would usually be based on the HED for the NOAEL in monkeys regardless of whether it was lower than that in rodents, unless unique dose limiting toxicities were observed with the new antisense compound in the rodent species.

VII. STEP 4: APPLICATION OF SAFETY FACTOR

Once the HED of the NOAEL in the most appropriate species has been determined, a safety factor should then be applied to provide a margin of safety for protection of human subjects receiving the initial clinical dose. This safety factor allows for variability in extrapolating from

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animal toxicity studies to studies in humans resulting from: (1) uncertainties due to enhanced sensitivity to pharmacologic activity in humans versus animals; (2) difficulties in detecting certain toxicities in animals (e.g., headache, myalgias, mental disturbances); (3) differences in receptor densities or affinities; (4) unexpected toxicities; and (5) interspecies differences in ADME of the therapeutic. These differences can be accommodated by lowering the human starting dose from the HED of the selected species NOAEL.

In practice, the MRSD for the clinical trial should be determined by dividing the HED derived from the animal NOAEL by the safety factor. The default safety factor that should normally be used is 10. This is a historically accepted value, but, as described below, should be evaluated based on available information.

A safety factor of 10 may not be appropriate for all cases. The safety factor should be raised when there is reason for increased concern, and lowered when concern is reduced because of available data that provide added assurance of safety. This can be visualized as a sliding scale, balancing findings that mitigate the concern for harm to healthy volunteers with those that suggest greater concern is warranted. The extent of the increase or decrease is largely a matter of judgment, using the available information. It is incumbent on the evaluator to clearly explain the reasoning behind the applied safety factor when it differs from the default value of 10, particularly if it is less than 10.

A. Increasing the Safety Factor

The following considerations indicate a safety concern that might warrant increasing the safety factor. In these circumstances, the MRSD would be calculated by dividing the HED by a safety factor that is greater than 10. If any of the following concerns are defined in review of the nonclinical safety database, an increase in the safety factor may be called for. If multiple concerns are identified, the safety factor should be increased accordingly.

- **Steep dose response curve.** A steep dose response curve for significant toxicities in the most appropriate species or in multiple species may indicate a greater risk to humans.
- **Severe toxicities.** Qualitatively severe toxicities or damage to an organ system (e.g., central nervous system (CNS)) indicate increased risk to humans.
- **Nonmonitorable toxicity.** Nonmonitorable toxicities may include histopathologic changes in animals that are not readily monitored by clinical pathology markers.
- **Toxicities without premonitory signs.** If the onset of significant toxicities is not reliably associated with premonitory signs in animals, it may be difficult to know when toxic doses are approached in human trials.

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- **Variable bioavailability.** Widely divergent or poor bioavailability in the several animal species, or poor bioavailability in the test species used to derive the HED, suggest a greater possibility for underestimating the toxicity in humans.
- **Irreversible toxicity.** Irreversible toxicities in animals suggest the possibility of permanent injury in human trial participants.
- **Unexplained mortality.** Mortality that is not predicted by other parameters raises the level of concern.
- **Large variability in doses or plasma drug levels eliciting effect.** When doses or exposure levels that produce a toxic effect differ greatly across species or among individual animals of a species, the ability to predict a toxic dose in humans is reduced and a greater safety factor may be needed.
- **Nonlinear pharmacokinetics.** When plasma drug levels do not increase in a dose-related manner, the ability to predict toxicity in humans in relation to dose is reduced and a greater safety factor may be needed.
- **Inadequate dose-response data.** Poor study design (e.g., few dose levels, wide dosing intervals) or large differences in responses among animals within dosing groups may make it difficult to characterize the dose-response curve.
- **Novel therapeutic targets.** Therapeutic targets that have not been previously clinically evaluated may increase the uncertainty of relying on the nonclinical data to support a safe starting dose in humans.
- **Animal models with limited utility.** Some classes of therapeutic biologics may have very limited interspecies cross-reactivity or pronounced immunogenicity, or may work by mechanisms that are not known to be conserved between (nonhuman) animals and humans; in these cases, safety data from any animal studies may be very limited in scope and interpretability.

B. Decreasing the Safety Factor

Safety factors of less than 10 may be appropriate under some conditions. The toxicologic testing in these cases should be of the highest caliber in both conduct and design. Most of the time, candidate therapeutics for this approach would be members of a well-characterized class. Within the class, the therapeutics should be administered by the same route, schedule, and duration of administration; should have a similar metabolic profile and bioavailability; and should have similar toxicity profiles across all the species tested including humans. A smaller safety factor might also be used when toxicities produced by the therapeutic are easily monitored, reversible, predictable, and exhibit a moderate-to-shallow dose-response relationship with toxicities that are

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consistent across the tested species (both qualitatively and with respect to appropriately scaled dose and exposure).

A safety factor smaller than 10 could be justified when the NOAEL was determined based on toxicity studies of longer duration compared to the proposed clinical schedule in healthy volunteers. In this case, a greater margin of safety should be built into the NOAEL, as it was associated with a longer duration of exposure than that proposed in the clinical setting. This assumes that toxicities are cumulative, are not associated with acute peaks in therapeutic concentration (e.g., hypotension), and did not occur early in the repeat dose study.

VIII. STEP 5: CONSIDERATION OF THE PHARMACOLOGICALLY ACTIVE DOSE

Selection of a PAD depends upon many factors and differs markedly among pharmacological drug classes and clinical indications; therefore, selection of a PAD is beyond the scope of this guidance. However, once the MRSD has been determined, it may be of value to compare it to the PAD derived from appropriate pharmacodynamic models. If the PAD is from an in vivo study, an HED can be derived from a PAD estimate by using a BSA-CF. This HED value should be compared directly to the MRSD. If this *pharmacologic* HED is lower than the MRSD, it may be appropriate to decrease the clinical starting dose for pragmatic or scientific reasons. Additionally, for certain classes of drugs or biologics (e.g., vasodilators, anticoagulants, monoclonal antibodies, or growth factors), toxicity may arise from *exaggerated pharmacologic* effects. The PAD in these cases may be a more sensitive indicator of potential toxicity than the NOAEL and might therefore warrant lowering the MRSD.

IX. SUMMARY

A strategy has been proposed to determine the maximum recommended starting dose for clinical trials of new therapeutics in adult healthy volunteers. In summary, usually NOAELs from the relevant animal studies should be converted to the HEDs using the standard factors presented in Table 1. Using sound scientific judgment, a safety factor should be applied to the HED from the most appropriate species to arrive at the MRSD. This process is meant to define the upper limit of recommended starting doses and, in general, lower starting doses can be appropriate. The process described in this guidance should foster consistency among sponsors and Agency reviewers.

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GLOSSARY

b: Allometric exponent

Body surface area conversion factor (BSA-CF): A factor that converts a dose (mg/kg) in an animal species to the equivalent dose in humans (also known as the *human equivalent dose*), based on differences in body surface area. A BSA-CF is the ratio of the body surface areas in the tested species to that of an average human.

Human equivalent dose (HED): A dose in humans anticipated to provide the same degree of effect as that observed in animals at a given dose. In this guidance, as in many communications from sponsors, the term HED is usually used to refer to the human equivalent dose of the NOAEL. When reference is made to the human equivalent of a dose other than the NOAEL (e.g., the PAD), sponsors should explicitly and prominently note this usage.

K: A dimensionless factor that adjusts for differences in the surface area to weight ratio of species because of their different body shapes.

k_m: Factor for converting mg/kg dose to mg/m² dose

Lowest observed adverse effect level (LOAEL): The lowest dose tested in an animal species with adverse effects.

Maximum recommended starting dose (MRSD): The highest dose recommended as the initial dose in a clinical trial. In clinical trials of adult healthy volunteers, the MRSD is predicted to cause no adverse reactions. The units of the dose (e.g., mg/kg or mg/m²) may vary depending on practices employed in the area being investigated.

Maximum tolerated dose (MTD): In a toxicity study, the highest dose that does not produce unacceptable toxicity.

No observed adverse effect level (NOAEL): The highest dose tested in an animal species that does not produce a significant increase in adverse effects in comparison to the control group. Adverse effects that are biologically significant, even if not statistically significant, should be considered in determining an NOAEL.

No observed effect level (NOEL): The highest dose tested in an animal species with no detected effects.

Pharmacologically active dose (PAD): The lowest dose tested in an animal species with the intended pharmacologic activity.

Safety factor (SF): A number by which the HED is divided to introduce a margin of safety between the HED and the *maximum recommended starting dose*.

W: Body weight in kg

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**APPENDIX A:
Analysis of Allometric Exponent on HED Calculations**

An analysis was conducted to determine the effect of the allometric exponent on the conversion of an animal dose to the HED. One can derive the following equation (see Appendix C) for converting animal doses to the HED based on body weights and the allometric exponent (b):

$$\text{HED} = \text{animal NOAEL} \times (W_{\text{animal}}/W_{\text{human}})^{(1-b)}$$

Conventionally, for a mg/m^2 normalization b would be 0.67, but a number of studies (including the original Freireich data) have shown that MTDs scale best across species when $b = 0.75$. The Interagency Pharmacokinetics Group has recommended that $W^{0.75}$ be used for interspecies extrapolation of doses in carcinogenicity studies (EPA 1992). There are no data, however, to indicate the optimal method for converting NOAELs to HEDs. Conversion factors were calculated over a range of animal and human weights using $(W_{\text{animal}}/W_{\text{human}})^{0.33}$ or $(W_{\text{animal}}/W_{\text{human}})^{0.25}$ to assess the effect on starting dose selection of using $b = 0.75$ instead of $b = 0.67$. The results are shown in Table 2. Using an allometric exponent of 0.75 had a big effect on the conversion factor for the smaller species mice and rats. Nonetheless, mice are not commonly used for toxicology studies to support the first-in-human clinical trials. In addition, there is evidence that the area under the plasma concentration versus time curves in rats and humans correlates reasonably well when doses are normalized to mg/m^2 (Contrera et al. 1995). We conclude that the approach of converting NOAEL doses to an HED based on body surface area correction factors (i.e., $b = 0.67$) should be maintained for selecting starting doses for initial studies in healthy volunteers since: (1) mg/m^2 normalization is widely used throughout the toxicology and pharmacokinetic research communities; (2) mg/m^2 normalization provides a more conservative conversion; (3) there are no data to suggest a superior method for converting NOAELs; and (4) CDER has significant experience in establishing safe starting doses based on mg/m^2 , and it is readily calculated.

Table 2: Effect of Allometric Exponent on Conversion Factor ^a					
Species	Weight Range ^b (kg)	Conversion Factors ^c			Ratio of 0.75 to 0.67
		Standard	$b = 0.67$	$b = 0.75$	
Mouse	0.018-0.033	0.081	0.075	0.141	1.88
Rat	0.09-0.40	0.162	0.156	0.245	1.57
Rabbit	1.5-3	0.324	0.33	0.43	1.30
Monkey	1.5-4	0.324	0.37	0.47	1.27
Dog	6.5-13.0	0.541	0.53	0.62	1.17

^a conversion factor = $(W_{\text{animal}}/W_{\text{human}})^{(1-b)}$

^b human weight range used was 50-80 kg (110-176 lb)

^c mean conversion factor calculated across entire animal weight range and human weight range

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The following summarizes the analysis of the effects of the allometric exponent on HED calculations:

- Changing the allometric exponent from 0.67 to 0.75 had a big effect on the conversion factor for the smaller rodent species; for mice the conversion factors differed by a factor of almost 2.
- Converting doses based on an exponent of 0.75 would lead to higher, more aggressive and potentially more toxic starting doses.
- The limited data available suggest that the most accurate allometric exponent for normalizing MTDs of antineoplastic agents for interspecies extrapolation is $b = 0.75$, but there are no data to indicate the optimal normalization method for interspecies extrapolation of NOAELs in a broad range of therapeutic classes. Using mg/m^2 is widely adopted throughout the drug development community.
- Unless evidence is provided to the contrary, HED calculations should be based on $b = 0.67$ (i.e., the standard conversions based on mg/m^2 relationships).
- There was no notable effect of body weight on calculation of the HED within the weight ranges examined.

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**APPENDIX B:
Analysis of Body Weight Effects on HED Calculations**

Accurate conversion of a mg/kg dose to a mg/m² dose depends on the actual weight (and surface area) of the test species. A popular formula for converting doses is:

- (i) $\text{mg/m}^2 = k_m \times \text{mg/kg}$
where $k_m = 100/K \times W^{0.33}$ where K is a value unique to each species (Freireich et al. 1966)
or $k_m = 9.09 \times W^{0.35}$ where a K value unique to each species is not needed (Boxenbaum and DiLea 1995; Burtles et al. 1995; Stahl 1956).

The k_m value is not truly constant for any species, but increases within a species as body weight increases. The increase is not linear, but increases approximately proportional to $W^{2/3}$. For example, the k_m value in rats varies from 5.2 for a 100 g rat to 7.0 for a 250 g rat. Strictly speaking, the k_m value of 6 applies only to rats at the *reference weight* of 150 g. For standardization and practical purposes, a fixed k_m factor for each species is preferred. An analysis was undertaken to determine the effect of different body weights within a species on the conversion of an animal dose to the HED using k_m factors. The k_m factor was calculated for a range of body weights using $k_m = 100/K \times W^{0.33}$. In Table 3, a working weight range is shown next to the reference body weight. This is the range within which the HED calculated by using the standard k_m value will not vary more than ± 20 percent from that which would be calculated using a k_m value based on exact animal weight. This is a relatively small variance considering dose separation generally used in deriving the NOAEL, in toxicology studies, which are often twofold separations. For example, suppose a NOAEL in rats is 75 mg/kg and the average rat weight is 250 g. The k_m value for a 250 g rat is 7.0.

$$\text{HED} = 75 \times (7/37) = 14 \text{ mg/kg in humans.}$$

Using the standard k_m value of 6 for rats,

$$\text{HED} = 75 \times (6/37) = 12 \text{ mg/kg in humans.}$$

The HED calculated with the standard k_m value of 6 is within 15 percent of the value calculated using the actual k_m value of 7. As shown in Table 3, the body weights producing k_m factors for which the nominal, integer conversion factor was within 20 percent of the calculated factor covered a broad range. This working weight range encompassed the animal weights expected for the majority of studies used to support starting doses in humans.

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Table 3: Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area						
Species	Reference Body Weight (kg)	Working Weight Range ^a (kg)	Body Surface Area (m ²)	To Convert Dose in mg/kg to Dose in mg/m ² Multiply by k _m	To Convert Animal Dose in mg/kg to HED ^b in mg/kg, Either	
					Divide Animal Dose By	Multiply Animal Dose By
Human	60	---	1.62	37	---	---
Child ^c	20	---	0.80	25	---	---
Mouse	0.020	0.011-0.034	0.007	3	12.3	0.081
Hamster	0.080	0.047-0.157	0.016	5	7.4	0.135
Rat	0.150	0.080-0.270	0.025	6	6.2	0.162
Ferret	0.300	0.160-0.540	0.043	7	5.3	0.189
Guinea pig	0.400	0.208-0.700	0.05	8	4.6	0.216
Rabbit	1.8	0.9-3.0	0.15	12	3.1	0.324
Dog	10	5-17	0.50	20	1.8	0.541
Primates:						
Monkeys ^d	3	1.4-4.9	0.25	12	3.1	0.324
Marmoset	0.350	0.140-0.720	0.06	6	6.2	0.162
Squirrel monkey	0.600	0.290-0.970	0.09	7	5.3	0.189
Baboon	12	7-23	0.60	20	1.8	0.541
Micro-pig	20	10-33	0.74	27	1.4	0.730
Mini-pig	40	25-64	1.14	35	1.1	0.946

^a For animal weights within the specified ranges, the HED for a 60 kg human calculated using the standard k_m value will not vary more than ±20 percent from the HED calculated using a k_m value based on the exact animal weight.

^b Assumes 60 kg human. For species not listed or for weights outside the standard ranges, human equivalent dose can be calculated from the formula: HED = animal dose in mg/kg x (animal weight in kg/human weight in kg)^{0.33}.

^c The k_m value is provided for reference only since healthy children will rarely be volunteers for phase I trials.

^d For example, cynomolgus, rhesus, and stump-tail.

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For the typical species used in nonclinical safety studies, Table 3 also shows the body surface area in m^2 for an animal at a particular *reference* weight. For example, a 400 g guinea pig has a body surface area of approximately 0.05 m^2 . These values come from published sources with surface area determined experimentally by various methods. Compilations of this type of data can be found in published references (Spector 1956).

For animal weights outside the working weight range in Table 3, or for species not included in the table, an alternative method is available for calculating the HED. In these cases the following formula can be used:

$$\text{HED} = \text{Animal dose (mg/kg)} \times [\text{animal weight (kg)} \div \text{human weight (kg)}]^{0.33}$$

For example, assume that a NOAEL of 25 mg/kg was determined in a study using rabbits weighing 4.0 kg. The 4.0 kg animals are outside the working range for rabbits of 0.9 to 3.0 kg indicated in Table 3.

$$\text{HED} = 25 \text{ mg/kg} \times (4.0 \div 60)^{0.33} = 25 \times (0.41) = 10 \text{ mg/kg}$$

Alternatively, if the standard conversion factor was used to calculate the HED

$$\text{HED} = 25 \text{ mg/kg} \div 3.1 = 8.1 \text{ mg/kg}$$

The value of 10 mg/kg for the HED is 25 percent greater than the value of 8.1 mg/kg that would be calculated using the standard conversion factor. For example, assume that a NOAEL of 25 mg/kg was determined in a study using rabbits weighing 4.0 kg. The 4.0 kg animals are outside the working range for rabbits of 0.9 to 3.0 kg indicated in Table 3.

$$\text{HED} = 25 \text{ mg/kg} \times (4.0 \div 60)^{0.33} = 25 \times (0.41) = 10 \text{ mg/kg}$$

Alternatively, if the standard conversion factor was used to calculate the HED

$$\text{HED} = 25 \text{ mg/kg} \div 3.1 = 8.1 \text{ mg/kg}$$

The value of 10 mg/kg for the HED is 25 percent greater than the value of 8.1 mg/kg that would be calculated using the standard conversion factor.

The k_m analysis addresses only half of the HED conversion process. The range of human sizes should also be considered to convert the mg/m^2 dose back to an HED dose in mg/kg. To examine the effect of both animal and human weights on the conversion factor, the principle of allometry was used. Interspecies biologic parameters are often related by the power function $Y = aW^b$ where W is body weight and b (allometric exponent) is the slope of the log-log plot, $\log y = b \times \log W + C$. Using algebraic manipulation (see Appendix C), one can derive an equation for converting an animal dose to the HED based on the body weights of the human and the animals for a given allometric exponent. For converting an animal NOAEL in mg/kg to the HED in mg/kg, the equation is:

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(ii) $HED = \text{animal NOAEL} \times (W_{\text{animal}}/W_{\text{human}})^{(1-b)}$

Since body surface area is believed to scale with an allometric exponent (b) of 0.67, one can explore how the animal and human body weights affect the conversion factor $(W_{\text{animal}}/W_{\text{human}})^{0.33}$.

The conversion factor was calculated over a range of animal weights and a range of human weights from 50-80 kg. The results are summarized in Table 4. Column B is the weight range of the animals used to calculate, in conjunction with the 50-80 kg range in humans, the conversion factor. The extremes of the conversion factors for the permutations chosen are shown in columns C and D. The proposed standard conversion factors are shown in column E. The percentage difference of these extremes from the standard is shown in column F. Finally, the range of animal weights that produced a conversion factor for a 60 kg human within 20 percent of the standard factor is shown in column G. The ± 10 percent and ± 20 percent intervals across the entire range of weights are graphically illustrated for rats in Table 5.

Table 4: Effect of Body Weight on Human Equivalent Dose Conversions ^a						
A	B	C	D	E	F	G
Species	Animal Weight Range ^b (kg)	Conversion Factor ^c			% Difference of Extreme ^e from Standard	$\pm 20\%$ Range ^f for 60 kg Human (kg)
		sm animal lg human	lg animal sm human	Standard ^d		
Mouse	0.018-0.033	0.060	0.089	0.081	-22%	0.015-0.051
Rat	0.090-0.400	0.106	0.213	0.162	-35%	0.123-0.420
Rabbit	1.5-3.0	0.269	0.395	0.324	+22%	1.0-3.4
Monkey	1.5-4.0	0.319	0.435	0.324	+34%	1.0-3.4
Dog	6.5-13.0	0.437	0.641	0.541	-19%	4.7-16.2

^a conversion factor = $(W_{\text{animal}}/W_{\text{human}})^{0.33}$

^b human weight range used was 50-80 kg (110-176 lb)

^c HED in mg/kg equals animal dose in mg/kg multiplied by this value

^d See Table 1

^e extreme from column C or D

^f range of animal weights that produced a calculated conversion factor within 20 percent of the standard factor (column E) when human weight was set at 60 kg

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**Table 5: Human and Rat Body Weights Producing Body Surface Area Dose Conversion Factors
Within 10 Percent and 20 Percent of the Standard Factor (0.162)**

EFFECT OF BODY WEIGHT ON BSA-CF							
HED = animal NOAEL · (W _{animal} /W _{human}) ^b exp(1-b), b = 0.67 for mg/m ² conversion							
Standard conversion to mg/kg = 0.162				± 10%	0.146-0.178		
				± 20%	0.130-0.194		
Rat Body Weight (kg)	Human Body Weight (kg)						
	50	55	60	65	70	75	80
0.090	0.124	0.120	0.117	0.114	0.111	0.109	0.106
0.100	0.129	0.125	0.121	0.118	0.115	0.113	0.110
0.110	0.133	0.129	0.125	0.122	0.119	0.116	0.114
0.120	0.137	0.132	0.129	0.125	0.122	0.119	0.117
0.130	0.140	0.136	0.132	0.129	0.126	0.123	0.120
0.140	0.144	0.139	0.135	0.132	0.129	0.126	0.123
0.150	0.147	0.142	0.138	0.135	0.132	0.129	0.126
0.160	0.150	0.146	0.141	0.138	0.134	0.131	0.129
0.170	0.153	0.149	0.144	0.141	0.137	0.134	0.131
0.180	0.156	0.151	0.147	0.143	0.140	0.137	0.134
0.190	0.159	0.154	0.150	0.146	0.142	0.139	0.136
0.200	0.162	0.157	0.152	0.148	0.145	0.141	0.138
0.210	0.164	0.159	0.155	0.151	0.147	0.144	0.141
0.220	0.167	0.162	0.157	0.153	0.149	0.146	0.143
0.230	0.169	0.164	0.159	0.155	0.152	0.148	0.145
0.240	0.172	0.166	0.162	0.157	0.154	0.150	0.147
0.250	0.174	0.169	0.164	0.160	0.156	0.152	0.149
0.260	0.176	0.171	0.166	0.162	0.158	0.154	0.151
0.270	0.179	0.173	0.168	0.164	0.160	0.156	0.153
0.280	0.181	0.175	0.170	0.166	0.162	0.158	0.155
0.290	0.183	0.177	0.172	0.168	0.164	0.160	0.157
0.300	0.185	0.179	0.174	0.179	0.165	0.162	0.158
0.310	0.187	0.181	0.176	0.171	0.167	0.163	0.160
0.320	0.189	0.183	0.178	0.173	0.169	0.165	0.162
0.330	0.191	0.185	0.180	0.175	0.171	0.167	0.163
0.340	0.193	0.187	0.181	0.177	0.172	0.169	0.165
0.350	0.194	0.188	0.183	0.178	0.174	0.170	0.167
0.360	0.196	0.190	0.185	0.180	0.176	0.172	0.168
0.370	0.198	0.192	0.187	0.182	0.177	0.173	0.170
0.380	0.200	0.194	0.188	0.183	0.179	0.175	0.171
0.390	0.202	0.195	0.190	0.185	0.180	0.176	0.173
0.400	0.203	0.197	0.191	0.186	0.182	0.178	0.174
0.410	0.205	0.199	0.193	0.188	0.183	0.179	0.175
0.420	0.207	0.200	0.194	0.189	0.185	0.181	0.177
0.430	0.208	0.202	0.196	0.191	0.186	0.182	0.178
0.440	0.210	0.203	0.197	0.192	0.188	0.183	0.180
0.450	0.211	0.205	0.199	0.194	0.189	0.185	0.181
0.460	0.213	0.206	0.200	0.195	0.190	0.186	0.182

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The following are conclusions from these analyses:

- The ± 20 percent interval around the standard conversion factor includes a broad range of animal and human weights.
- Given that the human weights will vary broadly, it is not usually necessary to be concerned about the affect of the variation of animal weights within a species on the HED calculation.
- If an extreme animal weight is encountered in a toxicology study, one can calculate an accurate conversion factor using $(W_{\text{animal}}/W_{\text{human}})^{0.33}$.

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**APPENDIX C:
Derivation of the Interspecies Scaling Factor $(W_a/W_h)^{(1-b)}$**

Power equation $(mg) = aW^b$
 $\log(mg) = \log(a) + b \cdot \log(W) = b \cdot \log(W) + c$

Given the weights of animal and human, and animal dose in mg/kg, solve for HED in mg/kg:

Let H = mg/kg dose in humans
 A = mg/kg dose in animals
 W_h = weight of human
 W_a = weight of animal

for animal $\log(mg) = \log(a) + b \cdot \log(W_a) = b \cdot \log(W_a) + c$
replace mg $\log(A \cdot W_a) = b \cdot \log(W_a) + c$
solve for c $c = \log(A \cdot W_a) - b \cdot \log(W_a)$
 $= \log(A) + \log(W_a) - b \cdot \log(W_a)$
 $= \log(A) + (1-b)\log(W_a)$

likewise for human $c = \log(H) + (1-b)\log(W_h)$

equate two equations $\log(A) + (1-b)\log(W_a) = \log(H) + (1-b)\log(W_h)$
solve for $\log(H)$ $\log(H) = \log(A) + (1-b)\log(W_a) - (1-b)\log(W_h)$
 $= \log(A) + (1-b)[\log(W_a) - \log(W_h)]$
 $= \log(A) + \log[(W_a/W_h)^{(1-b)}]$
 $\log(H) = \log[A \cdot (W_a/W_h)^{(1-b)}]$

solve for H $H = A \cdot (W_a/W_h)^{(1-b)}$

For example, using mg/m^2 normalization ($b = 0.67$) the predicted human MTD in mg/kg based on a rat LD_{10} in mg/kg is $MTD = LD_{10} \cdot (W_a/W_h)^{0.33}$.

Likewise the HED in mg/kg based on a surface area conversion given an animal NOAEL is $HED = NOAEL \cdot (W_a/W_h)^{0.33}$.

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**APPENDIX D:
Examples of Calculations for Converting Animal Doses
to Human Equivalent Doses**

This appendix provides examples of specific calculations to be taken in deriving an HED based on standardized factors.

Tables 1 and 3 provide standardized conversion factors for changing animal or human doses expressed as mg/kg to doses expressed as mg/m². Tables 1 and 3 also have factors (and divisors) for converting animal doses in mg/kg to the human dose in mg/kg that is equivalent to the animal dose if both were expressed on a mg/m² basis. This human dose in mg/kg is referred to as the HED.

Example 1: Converting to mg/m² HED

To convert an animal or human dose from mg/kg to mg/m², the dose in mg/kg is multiplied by the conversion factor indicated as k_m (for mass constant). The k_m factor has units of kg/m²; it is equal to the body weight in kg divided by the surface area in m².

formula:	$\text{mg/kg} \times k_m = \text{mg/m}^2$
to convert a dose of 30 mg/kg in a dog:	$30 \times 20 = 600 \text{ mg/m}^2$
to convert a dose of 2.5 mg/kg in a human:	$2.5 \times 37 = 92.5 \text{ mg/m}^2$

Example 2: Converting to mg/kg HED in two steps

To calculate the HED for a particular dose in animals, one can calculate the animal dose in mg/m² by **multiplying** the dose in mg/kg by the k_m factor for that species as described in Example 1. The dose can then be converted back to mg/kg in humans by **dividing** the dose in mg/m² by the k_m factor for humans.

formula:	$(\text{animal mg/kg dose} \times \text{animal } k_m) \div \text{human } k_m = \text{human mg/kg dose}$
to calculate the HED for a 15 mg/kg dose in dogs:	$(15 \times 20) \div 37 = 300 \text{ mg/m}^2 \div 37 = 8 \text{ mg/kg}$

Example 3: Converting to mg/kg HED in one step

The calculation in Example 2 can be simplified by combining the two steps. The HED can be calculated directly from the animal dose by **dividing** the animal dose by the ratio of the human/animal k_m factor (third column in Table 1) or by **multiplying** by the ratio of the animal/human k_m factor (fourth column in Table 1).

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Division method

NOAEL	calculation	HED
	$\text{mg/kg} \div [k_{\text{mhuman}}/k_{\text{manimal}}]$	
15 mg/kg in dogs	$15 \text{ mg/kg} \div 1.8 =$	8 mg/kg
50 mg/kg in rats	$50 \text{ mg/kg} \div 6.2 =$	8 mg/kg
50 mg/kg in monkeys	$50 \text{ mg/kg} \div 3.1 =$	16 mg/kg

Multiplication method

NOAEL	calculation	HED
	$\text{mg/kg} \times [k_{\text{manimal}}/k_{\text{mhuman}}]$	
15 mg/kg in dogs	$15 \text{ mg/kg} \times 0.541 =$	8 mg/kg
50 mg/kg in rats	$50 \text{ mg/kg} \times 0.162 =$	8 mg/kg
50 mg/kg in monkeys	$50 \text{ mg/kg} \times 0.324 =$	16 mg/kg

APPENDIX E:
Selection of Maximum Recommended Starting Dose
for Drugs Administered Systemically to Normal Volunteers

Step 1

Determine NOAELs
(mg/kg) in toxicity
studies



Is there justification for extrapolating
animal NOAELs to human equivalent dose
(HED) based on mg/kg (or other
appropriate normalization)?

Yes



HED (mg/kg) = NOAEL (mg/kg)
(or other appropriate
normalization)

No



Convert each animal NOAEL
to HED (based on body
surface area; see Table 1)



Select HED from most
appropriate species



Step 2

Step 3

Step 4

Choose safety factor and
divide HED by that factor



**Maximum Recommended
Starting Dose (MRSD)**



Step 5

Consider lowering dose based on a
variety of factors, e.g., PAD

U.S. Serial No. 13/197,581

Exhibit G

Guidance for Industry

Animal Models — Essential Elements to Address Efficacy Under the Animal Rule

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document contact Rosemary Roberts, CDER, at 301-796-2210 or the Office of Communications, Training, and Manufacturers Assistance (CBER), 301-827-1800 or 800-835-4709.

**U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)**

**January 2009
Pharm/Tox**

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Guidance for Industry

Animal Models — Essential Elements to Address Efficacy Under the Animal Rule

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**U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
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Guidance for Industry¹
Animal Models — Essential Elements to Address
Efficacy Under the Animal Rule

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This guidance provides information to potential sponsors (industry, academia, and government) on the development of animal models to study efficacy. The guidance focuses on the identification of the critical characteristics (essential data elements) of an animal model to be addressed when developing drug or biological products for approval or licensure, respectively, under the Animal Rule (see 21 CFR 314.600 for drugs; 21 CFR 601.90 for biological products).

This guidance does not address:

- The preclinical pharmacology/toxicology studies necessary for early drug or biological product development
- The details of study design and conduct for either animal efficacy studies or human safety studies
- The development of animal models for other purposes, such as for assessment of toxicology
- The threshold for determining that human efficacy studies are not ethical and/or not feasible

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

¹ This guidance has been prepared by the Animal Model Characterization Working Group in the Center for Drug Evaluation and Research (CDER) in cooperation with the Center for Biologics Evaluation and Research (CBER) at the Food and Drug Administration.

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II. BACKGROUND

FDA's regulations concerning the approval of new drugs or biological products when human efficacy studies are neither ethical nor feasible are known as "the Animal Rule" (21 CFR 314.600 for drugs; 21 CFR 601.90 for biological products). The Animal Rule states that in selected circumstances, when it is neither ethical nor feasible to conduct human efficacy studies, FDA may grant marketing approval based on adequate and well-controlled animal studies when the results of those studies establish that the drug or biological product is reasonably likely to produce clinical benefit in humans. Demonstration of the product's safety in humans is still necessary (see section V.).

The purpose of this guidance is to identify the critical characteristics of an animal model that should be addressed when efficacy of the product under development will be established under the Animal Rule.

The critical characteristics discussed in section IV identify the essential elements to be considered and fully explored as part of the development of an animal model. All elements may not be achievable for each etiologic agent² and intervention³ being studied. Early and frequent interactions between FDA and the sponsor are recommended to discuss these elements and any issues encountered by the sponsor. Current FDA requirements for establishing the safety of a product in humans continue to apply. Although the discussion in this guidance touches on clinical safety, it is not meant to address all requirements for assurance of human safety.

III. ANIMAL RULE CONSIDERATIONS

To develop an animal model to demonstrate efficacy, the sponsor should obtain information on the natural history of the disease or condition in both humans and animals, on the etiologic agent, and on the proposed intervention. Data from the human experience with the etiologic agent and/or with the intervention, if available, may support applicability of the animal model.

The Animal Rule states that FDA can rely on the evidence from animal studies to provide substantial evidence of effectiveness only when:

1. There is a reasonably well-understood pathophysiological mechanism of the toxicity of the (chemical, biological, radiological, or nuclear) substance and its prevention or substantial reduction by the product
2. The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal

² For this document the terms *agent*, *threat agent*, or *etiologic agent* refer to lethal or permanently disabling toxic chemical, biological, radiological or nuclear (CBRN) substances regarding which efficacy studies in humans are neither ethical nor feasible. The term *challenge agent* refers to the CBRN material used in the animal studies.

³ The terms *treatment* and *therapy* refer to any intervention that prevents or mitigates the toxicity of these etiologic agents.

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species that represents a sufficiently well-characterized animal model⁴ for predicting the response in humans

3. The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity
4. The data or information on the (pharmaco) kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans
(21 CFR 314.610(a)(1)-(4); 21 CFR 601.91(a)(1)-(4))

If these criteria are met, it is reasonable to expect the effectiveness of the product in animals to be a reliable indicator of its effectiveness in humans.

The Animal Rule allows approval based on a single animal species, if the animal model is sufficiently well-characterized; however the usual expectation is that efficacy will be demonstrated in more than one species. In order to support approval based on one animal species, in general more than one efficacy study using that species should be conducted to demonstrate reproducibility of the results.

Data from animal studies to demonstrate dose-response and to support the dose selected for the animal efficacy studies are expected as is the case for traditional product development. Sponsors of products approved for other indications may be asked to provide additional nonclinical and/or clinical data to support approval/licensure of the proposed product for the indication under consideration.

If another regulatory pathway to approval (i.e., one using human data) is feasible and ethical, that pathway must be used (21 CFR 314.600 and 601.90). Although the Animal Rule allows development of products that would otherwise not have any route to approval, the rule reflects the Agency's recognition that many treatments that appeared effective in animals have not proved to be effective in humans. Consequently, developing animal models that will yield efficacy results that can be expected to be predictive for humans is challenging. The animal studies must be adequate and well-controlled (21 CFR 314.610 and 601.91), and should use the pertinent features of an adequate and well-controlled clinical study, such as a detailed protocol with randomization and adequate blinding and a statistical plan as described in 21 CFR 314.126.

Early and frequent interactions between FDA and the sponsor are recommended to discuss the applicability of the Animal Rule and specific areas of concern, as well as to enable the review of, and comment on, protocols prior to study initiation. FDA may seek Advisory Committee consultation before approval and/or early in the development process to discuss whether the concept of using certain animal data to support efficacy is reasonable.

All studies intended to support approval under the Animal Rule must be carried out under the procedures and controls outlined in FDA's Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies regulations (21 CFR Part 58). FDA recognizes that conforming to GLP regulations in the conduct of studies on CBRN agents may present challenges. Such issues and

⁴ A "sufficiently well-characterized animal model" is one for which the model has been adequately evaluated for its responsiveness.

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their possible impact on study results and conclusions, should be discussed with the review division prior to conduct of the studies. In addition, the studies must comply with the Animal Welfare Act (7 U.S.C. 2131). For certain infectious agents, sponsors must adhere to the Select Agent Rule⁵ and should comply with standards on the use of Biosafety Level (BSL) laboratory facilities.⁶

The animal efficacy studies conducted to support approval under the Animal Rule are likely to use a significant number of animals. Sponsors should submit detailed protocols (see 21 CFR 312.23(a)(6)) and provide for frequent monitoring throughout the study period. FDA strongly encourages sponsors to submit a development plan and to communicate frequently with the Agency when developing products under the Animal Rule. The protocols for the animal efficacy studies should be discussed with FDA, with sufficient time for FDA review and comment, prior to the study being conducted.

IV. DISCUSSION OF ESSENTIAL DATA ELEMENTS OF AN ANIMAL MODEL

This section provides further information on the Table, Essential Data Elements of an Animal Model, found in section VI.

A. Characteristics of CBRN Agent that Influence the Disease or Condition

Some characteristics of the specific chemical, biological, radiological, and/or nuclear (CBRN) agent that influence the disease or condition under study include: the challenge agent, pathogenic determinants, the route of exposure, and quantification of exposure.

I. The Challenge Agent

The challenge agent used in animal studies generally should be identical to the etiologic agent that causes the human disease. The purity of the challenge preparation should be documented when appropriate. If the challenge agent is different from the etiologic agent known to cause human disease, the sponsor should provide justification for the use of this challenge agent and explain why, when used in the proposed animal model, it should be considered suitable for establishing effectiveness of the intervention in humans. For example, for an animal efficacy study to support approval of a radiation countermeasure, a sponsor may not be able to predict the actual radiation exposure that would follow a nuclear detonation or the subsequent fallout. In such a case, the sponsor should provide a detailed explanation of the appropriateness of the type of radiation and dose used in the study and its relevance to the clinical situation. FDA strongly recommends that the scientific approach under consideration be discussed with FDA prior to the start of the animal studies.

⁵ See Select Agent Rule (42 CFR Parts 72 & 73) available at http://www.cdc.gov/od/sap/final_rule.htm.

⁶ See 5th Edition of Biosafety in Microbiological and Biomedical Laboratories (BMBL), available at <http://www.cdc.gov/od/ohs/biosfty/bmb15/bmb15toc.htm>.

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2. Pathogenic Determinants

It should be demonstrated that the pathogenic determinants of disease in the animal model are similar to those understood for humans. Pathogenic determinants can include toxin production, target organs or enzyme systems, or type of radiation. For example, although mice and guinea pigs are susceptible to *Bacillus anthracis*, the pathogenesis and mechanism of toxicity are different from those in humans, so that these rodent species may not be appropriate efficacy models for anthrax.⁷ Animal species that are not susceptible to the agent, or do not demonstrate the endpoint of interest (i.e., potential for mortality or major morbidity that might be reduced or prevented by sufficiently effective interventions) are not suitable for the efficacy studies.

3. Route of Exposure

In general, the animal models developed should use a route of exposure to the challenge agent that is the same as the anticipated human exposure route. This is especially important for conditions for which the route of exposure is directly related to pathogenesis. For example, human infection with *Yersinia pestis* through flea bite, the intravenous (IV) route, or aerosol exposure results in the development of bubonic, septicemic, or pneumonic plague, respectively. If a sponsor is proposing a route of exposure to the etiologic agent in animals that is different from what is expected in humans, adequate scientific justification should be provided. FDA strongly recommends that if such an approach is being considered, it should be discussed with FDA before the start of the animal studies.

4. Quantification of Exposure

Reliable quantification and reproducibility of the challenge dose should be demonstrated. When appropriate, the sponsor should describe the scalar relationship of the animal dose to that anticipated in human disease. If large differences are observed, then potential implications for interpretation of comparative pathogenesis, pathophysiology, and study results should be discussed with FDA. Standardization of the challenge dose may be a consideration in the future to ensure robust evaluation of data in the determination of effectiveness.

B. Host Susceptibility and Response to Etiologic Agent

The animal model chosen for development should be susceptible to the threat agent. FDA recognizes there may be species differences. For example, an animal species being used to study efficacy for a radiation countermeasure may require a different threshold of radiation exposure to develop acute radiation syndrome, but the animal species may still be appropriate for study if the resulting illness and course are similar in the animal species and humans. However, if this threshold differs greatly from the human threshold, the suitability of the animal model may be

⁷ Leffel, E.K. and Pitt, L.M., Anthrax. In *Biodefense: Research Methodology and Animal Models*. Swearingen, J.R. ed. Boca Raton, FL. CRC Press, 2006, 77-93.

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called into question. The factor that determines differences in susceptibility to the threat agent should be described to the best extent possible (e.g., see the discussion of pyridostigmine and soman in section E.2).

The response to the etiologic agent (resulting illness or injury) manifested by the animal species exposed to that agent should be similar to the illness or injury seen in humans. For example, mustard gas typically produces extensive blistering to exposed human skin. If the animal species evaluated does not have blistering as a prominent feature of exposure to mustard gas, it is unlikely that this animal model would be acceptable to the Agency. If the sponsor believes that such a model is supportive to the study of its investigational drug, the model should be discussed with the Agency and a justification should be provided

C. Natural History of Disease: Pathophysiologic Comparability

The natural history of disease in animals and in humans should be characterized, compared, and discussed with the Agency before the sponsor initiates intervention studies in animals. In some instances, use of several different models in the same development plan can be considered. Experimental parameters may need to be modified to create a condition that more closely mimics the disease in humans. For example, variola virus causes human smallpox, and humans are the only known natural host. Nonhuman primate animal models that have been studied using variola virus as the challenge agent require a large inoculum, and often the IV route of administration is used. FDA recommends that compounds found to be active in vitro against orthopoxviruses be studied in several animal models using multiple different orthopoxviruses initially. Based on data from initial studies and availability of suitably characterized models, the next step may be to assess the appropriateness of additional study in an animal model using variola.⁸ Sponsors who plan to use an animal model that involves exposure to a challenge agent that is different from the known etiologic agent in humans should discuss this with the Agency along with their planned protocols and any major differences in, or limitations of, the animal model.

When comparing the disease in animals with the disease in humans, sponsors should include time to onset of disease/condition; time course of progression of disease; and manifestations, that is, signs and symptoms (severity, progression, clinical and pathologic features, laboratory parameters, the extent of organ involvement, morbidity, and outcome of disease). A single animal model may not reflect the entire spectrum of human disease. The time to onset of disease, progression of disease, and the manifestations/outcome can be influenced by many factors, including concentration and type of etiologic agent, virulence or lethal potential of the etiologic agent, route of exposure, and other host factors including immune status.

⁸ See FDA's draft guidance for industry *Smallpox (Variola) Infection: Developing Drugs for Treatment or Prevention*. Once finalized this guidance will represent the Agency's thinking on this topic. Also, we update guidances periodically. To make sure you have the most recent version of a guidance, check the appropriate (CDER or CBER) guidance Web site, listed on the second title page of the guidance.

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1. Time to Onset of Disease/Condition

The time to onset of disease/condition in animals should be reasonably similar to that in humans. Factors such as strain of the infective microorganism, route of exposure, and/or the level of exposure (i.e., concentration of the chemical, biological, radiological, or other etiologic agent) can influence time to disease/condition onset.

2. Time Course of Progression of Disease/Condition

The progression of the disease/condition in animals should be similar to that of the disease in humans to allow for observation of the effects of intervention. For example, hamsters challenged with anthrax have an extremely rapid disease progression. Thus, this species is not useful for testing the efficacy of products for the treatment of anthrax in humans. Furthermore, the clinical course of disease in the animal may be more rapid than that in the human as a result of experimental conditions, such as the route of exposure (e.g., an IV route of exposure may alter many characteristics including the time course of disease). The change in the clinical course may result in making disease recognition, intervention, and assessment of outcome more difficult. Showing the effect of an intervention may be more challenging when the time between onset of disease and death is short.

3. Manifestations (signs and symptoms)

The disease manifestations, including clinical signs and their known time course, laboratory parameters, histopathology, gross pathology, and the outcome (morbidity or mortality), should be compared between untreated animals and untreated humans (e.g., historical information). Differences should be clearly noted and explained based on the understanding of the pathophysiologic differences between the species, with due acknowledgment of the limitations that may arise where this level of understanding is limited. Because certain disease manifestations in humans (e.g., fever and shortness of breath) may be difficult to discern in animals through clinical observation, a sponsor may need to use more refined techniques, such as telemetry, to evaluate affected animals. Animals in the natural history studies as well as animals in the efficacy studies should be observed with greater frequency over the entire course of the day than would be typical of most nonclinical (pharmacology/toxicology) animal studies. This is especially true when the primary endpoint is mortality and animals are being evaluated in the context of prospectively-defined euthanasia criteria. With a mortality endpoint, animal welfare and sample integrity need to be addressed. Sample integrity (e.g., cultures, histology) may be compromised if not obtained just prior to or immediately after death or euthanasia. Study results may be influenced by the criteria used. Study personnel should be blinded to treatment and should follow observation and euthanasia criteria to minimize the possibility of unnecessary suffering of moribund animals.⁹

⁹Refer to Animal Welfare Act (7 U.S.C. 2131).

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D. Trigger for Intervention

Identification of the trigger for intervention in the animal studies is critical to defining the timing of the intervention. Because animals cannot simulate the health-seeking behavior manifested by humans, the trigger for intervention should be accurately defined in the animal model. If signs and symptoms in the animal model closely resemble those in humans, these can serve as the trigger for intervention when they are recognized in the individual animal. However, in the absence of disease-defining manifestations, certain biological parameters should be used to identify the time for initiation of treatment if they are known to be relevant to the diagnosis of human disease and if a relationship to the likely diagnostic process and timing in human use of the product can be shown. For example, presence of bacteremia has been used in some efficacy studies in humans for initiation of intervention with antimicrobial drug products.¹⁰ The utility of biological parameters/biomarkers should be demonstrated, including an analysis of the time course of the appearance of the biomarkers in animals and the onset of disease and availability of diagnostic information in humans.

When a biomarker is used as a trigger for intervention in animal studies, both the assay methodology for the biomarker and its performance characteristics should be adequately characterized. The materials and methods for the assay, as well as the raw data and results from the actual testing, should be provided for FDA review. Summary data are not sufficient. Sponsors are encouraged to initiate early discussion with FDA regarding the utility of the chosen triggers for intervention, particularly when the signs and symptoms of disease in the animal differ from those in humans.

E. Characterization of Medical Intervention

Efficacy studies should reflect the expected clinical use and indication. A particular dosage form may not be suitable for the proposed indication, so the product's dosage form should be considered in planning the development of the product. For example, an oral dosage form is preferred for postexposure prophylaxis for large populations, while an IV dosage form may be necessary for seriously ill patients. If the product is already approved for human use, there may be information on which to base the expected dose and regimen, but if there is no approved human use, the animal result will need to be translated for human use, generally requiring some PK/PD assessment. The following specific information should be submitted on the product and its characteristics in humans and in animals.

1. Product Class

The product's therapeutic class should be identified. Information that is available about other members of the class can be used to help identify potential animal models and predict/evaluate safety and efficacy issues in the proposed animal model.

¹⁰ Refer to package insert for Cubicin, NDA No. 021572, accessible at Drugs@FDA:
<http://www.accessdata.fda.gov/scripts/cder/drugsatfda/>.

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2. Mechanism of Action

Understanding the mechanism of action may help to identify specific safety and efficacy issues in the proposed animal model and to identify what additional studies should be performed. The animal studies to support the approval of pyridostigmine as a pretreatment for exposure to the nerve agent soman highlight the importance of understanding the mechanism of action of the drug and host factors in each animal species evaluated. Pretreatment with pyridostigmine was shown to decrease the lethality of soman in rhesus monkeys. However, pretreatment with pyridostigmine produced small and inconsistent effects on mortality in studies using rats, mice, and rabbits. The effect of pyridostigmine was masked in these latter species because of high serum levels of the enzyme carboxylesterase, which eliminates soman from the blood and makes these species naturally highly resistant to the nerve agent. Rhesus monkeys and humans have little or no carboxylesterase. To elucidate the mechanism of pyridostigmine and bridge the data to the human experience, a study was conducted in rats pretreated with pyridostigmine as well as a carboxylesterase inhibitor prior to exposure to soman. In this study, pyridostigmine demonstrated a mortality benefit in the rats similar to that seen in the rhesus monkeys.

3. In vitro Activity

Understanding the in vitro activity of the product will supplement known information on the mechanism of action and provide early screening information.

4. Activity in Disease/Condition of Similar Pathophysiology

If a candidate product is targeted at a common pathway in the pathophysiologic cascade, information may be available on the candidate product's use for diseases that possess a similar pathway. For example, information for a product approved for the treatment of neutropenia secondary to chemotherapy in cancer patients may provide useful data to support studying this product for the reduction of mortality in patients with neutropenia secondary to acute radiation syndrome. This information on the related condition, although not required, lends further support to the candidate product's efficacy for the indication to be studied.

5. Pharmacokinetics (PK) in Unaffected Animals/Humans

PK studies should be done in unaffected animals and humans to characterize the PK profile in each and to propose dosing regimens that provide comparable drug exposures in the animals and humans. Early interaction with FDA is critical to justify and establish the appropriate dosing regimen for the pivotal animal studies.

6. PK/PD (Pharmacokinetics/Pharmacodynamics) in Affected Animals/Humans

PK information in affected animals should be compared to PK information obtained from unaffected animals to establish whether the pathophysiology of a disease affects the PK

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(e.g., changes in metabolic parameters may alter the pharmacokinetics). Measures of treatment response (PD measurements such as clinical outcome or exploratory biomarkers) should be proposed for discussion based on both animal studies and any available human information. If a candidate product has been used in humans for other indications, PK/PD information for the alternate indications may be supportive. It should be noted that the animal model may not predict specific disease/drug interactions. Such interactions may not be observed until the disease is treated in humans, reinforcing the critical need for postmarket clinical studies in the event of human disease.

7. PK Interactions with Medical Products Likely to Be Used Concomitantly

The absorption, distribution, metabolism, and excretion (ADME)^{11, 12} of a candidate product should be studied and understood.¹³ The sponsor, with knowledge of the ADME of the investigational product, should discuss with FDA other medical products that are likely to be co-administered based on the clinical scenario. Potential combinations should be considered for interaction studies that may affect the PK of either product. For example, if a candidate drug is metabolized via the cytochrome P450 system, safety or efficacy of the candidate drug could be compromised by the concomitant use of cytochrome P450 inhibitors or inducers. Such drug/drug interactions should be evaluated.

8. Synergy or Antagonism of Medical Products Likely to Be Used in Combination

Candidate products should be evaluated within the context that reflects anticipated clinical use. The sponsor, in consultation with FDA, should consider other products that are likely to be used and evaluate whether the activity of either product, when used in combination, is affected (i.e., synergy or antagonism). Examples of potential interactions include drug/drug interactions and drug/vaccine interactions. For example, it should be known whether the use of an anthrax antitoxin monoclonal will have an effect on the activity of the antimicrobials used for the treatment of disseminated anthrax disease. This potential interaction should therefore be evaluated in the animal model. This information is especially important when the therapeutic intervention is expected to include more than one medical product.

F. Design Considerations for Animal Efficacy Studies

Assessment of efficacy in animals should be robust. Adequate and well-controlled animal efficacy studies, with endpoints that demonstrate substantial clinical benefit, generally the enhancement of survival or prevention of major morbidity, are required. The time course of observation should be optimized to assess the true treatment effect. At a minimum, placebo-

¹¹ See guidance for industry: *Drug Metabolism/Drug Interaction Studies in the Drug Development Process: Studies In Vitro*.

¹² See guidance for industry: *Drug Interaction Studies – Study Design, Data Analysis, and Implications for Dosing and Labeling*.

¹³ Biodistribution and elimination should be studied for products that are not biologically amenable to traditional ADME measures (e.g., many biologics such as vaccines, and cell and gene therapies).

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controlled animal studies should be performed. If a product approved for the same indication is available, it should be used as an active comparator in addition to the investigational drug and placebo arms. The study should also be blinded to the extent feasible; any situation in which study staff might become aware of treatment assignments should be discussed with FDA in advance in view of the potential for major effects on study interpretability. Animals of both sexes should be included. FDA recognizes that there are significant supply constraints on using mature or older animals of certain animal species. The issue of the age and the immune status of the animals used in efficacy studies as compared to the intended human population should be addressed by the sponsor, when relevant. Study procedures should be uniformly applied to all study groups, and potential bias should be reduced by prespecifying the criteria for euthanasia and discussing their potential effects on interpretation of results.

Studies should be designed to mimic the clinical scenario and achieve meaningful outcomes comparable to the endpoints desired in humans. In some instances, supportive care should be administered to the animals as part of the study design. In such cases, demonstration of a product's benefit over supportive care (i.e., supportive care plus investigational drug arm should be demonstrated to be superior to the supportive care plus placebo arm) will be necessary for approval or licensure. Early discussion between the sponsor and the review division regarding the type, timing, and choice of supportive care to be administered is highly recommended.

In addition to the design characteristics already discussed in this section, the following parameters should also be addressed in the study protocols:

1. Endpoints

The product studied in the animal model should demonstrate a beneficial effect analogous to the intended outcome in humans. Primary study endpoints, which should be specifically discussed with the review division, generally are the enhancement of survival or prevention of major morbidity. The dose response for these endpoints should be explored fully and established. Although secondary endpoints can provide useful information about the animal model and the activity of the product as studied in the animal model, ordinarily, only primary endpoints can serve as the basis of approval.

2. Timing of intervention

The time to initiate intervention should support the specific indication sought for a product. If the intent is to develop the product for a treatment indication, intervention before disease is established may overestimate the effect that is likely to be seen in humans and may indeed show an effect when none would be seen in humans. A reasonable understanding of the disease course and a trigger for intervention defined by the natural history studies will be needed to design the animal efficacy studies for a treatment indication; it is important to establish the relationship of time after exposure to effectiveness. With this information, the timing for intervention can be defined, thus differentiating postexposure prophylaxis from treatment. A product to be used for postexposure prophylaxis should be administered within a reasonable window after exposure to the threat agent, but before onset of disease, with a time relationship that is

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adequately justified with respect to administration of the product to humans. Proposals for pre-exposure prophylaxis should be described and discussed in advance on a case-by-case basis.

3. Route of Administration

The route of administration should reflect the indication being sought and the anticipated clinical scenario, such as mass casualty. For example, if a large number of people were exposed to anthrax, an oral dosage form would be preferred over an injectable for postexposure prophylaxis. It may be important to study multiple routes.

4. Dosing Regimen

Drugs, monoclonals, and small therapeutic proteins:

The determination of the dosing regimen should rely on sufficient PK and PD data or other relevant product information in animals and/or humans. The goals should be to (a) determine a regimen in animals that is safe and effective for the indication studied; (b) determine the corresponding exposure (i.e., AUC, C_{max}) in animals that is yielded by that dosing regimen; and (c) calculate a dosing regimen in humans that will give an equivalent exposure to that seen in the animal. This will enable initial extrapolation from a dosing regimen found to be efficacious in the animal model to one expected to produce a similar benefit in humans, assuming similar exposure–response relationships. Different dosing regimens in animals and humans may be needed to provide equivalent exposure to the product and thus should be discussed with the Agency.

Vaccines:

The goal should be to develop a regimen that provides a protective immune response and that is safe. For vaccines, the dose(s) used in the animal should induce an immune response that allows for appropriate extrapolation of the animal protection data to humans based on solid scientific principles. A shorter dosing interval between inoculations as compared to the proposed clinical dosing interval may be acceptable with appropriate scientific justification.

In summary, the indication being sought drives the study design. The desired outcomes of the study (i.e., product's effect) should be determined early and carefully factored into the study design to ensure that the study meets both scientific and regulatory objectives. The Agency recommends that study protocols be prepared and submitted to FDA with enough time for FDA to review the protocols and provide feedback to the sponsor before the animal studies are initiated. The sponsor can submit these protocols (i.e., the adequate and well-controlled animal efficacy studies) with a request for review under the Special Protocol Assessment (SPA) provisions.¹⁴

¹⁴ See guidance for industry: *Special Protocol Assessment*.

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V. HUMAN SAFETY INFORMATION

The body of available human safety data, including data from the product's evaluation and use in other indications, is a critical component of any product's development plan and influences the risk/benefit considerations. FDA may ask for additional human safety trials to complete the safety profile of the product. Healthy human volunteers should be enlisted when there is no known significant risk in the administration of the product. If the risk is significant, study in a patient population with a similar disease should be considered if a population can be identified for which the risk/benefit balance of the study is appropriate. Sponsors should propose selection and justification of the appropriate study population in advance for FDA review and feedback.

The size of the required clinical safety database depends on many factors. Existing safety data would generally be satisfactory for products that are already marketed for another indication and known to have an acceptable safety profile in the populations that would receive the product for the new indication. When the new indication requires a longer duration of use or higher dose, additional safety data must be obtained (21 CFR 314.50(d)(5)(vi)). The type of indication being sought is another factor. For example, a product that will be used as prophylaxis in large numbers of people should have a larger safety database than a product developed for treatment of patients who are symptomatic with a disease of known high mortality. In prophylaxis scenarios, it is likely that some proportion of humans will receive the product without having been exposed to the threat agent. An adequate safety database is needed to reduce the risk of serious harm in a healthy population.

The timing and design of clinical safety studies should be coordinated with exploration of the efficacious dose and regimen in animals, in order to plan adequate studies to characterize the safety of the intended human dose, formulation, route of administration, and duration of use. Preclinical safety information should guide the choice of additional safety assessments of interest in the human safety studies. This is particularly useful for products with no prior human safety data, or when the anticipated human dosing regimen has not been previously studied or approved.

VI. ESSENTIAL DATA ELEMENTS OF AN ANIMAL MODEL

The essential data elements for the development and evaluation of animal models are listed in the table below. These elements serve as a guide. They may be modified or revised as new scientific information relevant to the condition under study becomes available. Early and frequent interactions between the sponsor and FDA are critical for feedback on proposals and appropriate discussion of uncertainties and the risk/benefit balance.

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Table: Essential Data Elements of an Animal Model

DATA ELEMENTS	Animal(s)	Human
A. Characteristics of the CBRN Agent that Influence the Disease or Condition		
1. The challenge agent		
2. Pathogenic determinants		
3. Route of exposure		
4. Quantification of exposure		
B. Host Susceptibility and Response to Etiologic Agent		
C. Natural History of Disease: Pathophysiologic Comparability		
1. Time to onset of disease/condition		
2. Time course of progression of disease/condition		
3. Manifestations (signs and symptoms)		
D. Trigger for Intervention		
E. Characterization of the Medical Intervention		
1. Product class		
2. Mechanism of action		
3. In vitro activity		
4. Activity in disease/condition of similar pathophysiology		
5. PK in unaffected animals/humans		
6. PK/PD in affected animals/humans		
7. PK interactions with medical products likely to be used concomitantly		
8. Synergy or antagonism of medical products likely to be used in combination		
F. Design Considerations for Animal Efficacy Studies		
1. Endpoints		
2. Timing of intervention		
3. Route of administration		
4. Dosing regimen		
HUMAN SAFETY INFORMATION		

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ATTACHMENT A: ACRONYMS AND ABBREVIATIONS

553		
554		
555	ADME	Absorption, distribution, metabolism, and excretion
556		
557	AUC	Area under plasma concentration-time curve from zero to infinity
558		
559	BSL	Biosafety Level
560		
561	CBER	Center for Biologics Evaluation and Research
562		
563	CBRN	Chemical, Biological, Radiological, or Nuclear
564		
565	CDER	Center for Drug Evaluation and Research
566		
567	Cmax	Maximum (peak) plasma drug concentration after single dose administration
568		
569	FDA	Food and Drug Administration
570		
571	GLP	Good Laboratory Practices
572		
573	IV	Intravenous
574		
575	PD	Pharmacodynamics
576		
577	PK	Pharmacokinetics
578		
579	SPA	Special Protocol Assessment

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