have the capability of metabolic reduction of I to both epimeric 6-hydroxy metabolites. However, considerable stereospecificity by the drug-metabolizing enzyme(s) favoring formation of the 6β-hydroxy metabolite was observed for all species. This result is consistent with metabolic data reported for other 6-oxo compounds containing the hydromorphone structure (2, 4).

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Generic Propoxyphene: Need for Clinical Bioavailability Evaluation

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Abstract □ Plasma level data on two investigational capsule formulations of propoxyphene with similar physicochemical parameters demonstrate that the formulations have different *in vivo* bioavailabilities. The potential for bioavailability problems with water-soluble drugs and the lack of correlation of *in vitro* and *in vivo* parameters for equivalent drug formulations are discussed.

Keyphrases □ Propoxyphene hydrochloride—different formulations, bioavailability compared □ Bioavailability—propoxyphene hydrochloride, different formulations compared □ Analgesics—propoxyphene hydrochloride, different formulations, bioavailability compared

With the recent expiration of the propoxyphene hydrochloride patent, several pharmaceutical manufacturers have marketed a generic formulation of this drug. These manufacturers were required to assess *in vitro* parameters such as drug content and dissolution rate to assure equivalence to the innovator's product. To date, no *in vitro* dissolution rate standard that has been shown to correlate with *in vivo* bioavailability has been developed.

However, with digoxin, for which *in vitro* standards were used to assure product equivalence (through batch certification), the Food and Drug Administration (FDA) clearly recognized that *in vitro* testing alone often does not ensure bioequivalence (1). This knowledge recently resulted in written bioavailability requirements for marketed digoxin products by the FDA (2).

Propoxyphene hydrochloride is a readily soluble drug. A simple capsule formulation of this drug would generally be considered as having a very low potential for bioavailability problems. Usually, only drugs with low water solubility are considered potential problems in formulating a readily available dosage form (3–5). The converse of this hypothesis (*i.e.*, soluble drugs have low potential for bioavailability problems) has not been investigated adequately. To ascertain possible bioavailability problems of drugs with high risk potential, the low risk potential drugs are rarely studied (6).

Although propoxyphene hydrochloride is a very soluble drug, comparative bioavailability data should be obtained prior to marketing any new formulation. Even the finding of equivalent *in vitro* parameters should not be sufficient justification for assuming bioequivalence of two different formulations of the same parent drug without *in vivo* data.

This paper reports the results of two bioequivalence studies in which two proposed marketed formulations with nearly identical *in vitro* parameters of potency and dissolution rate were compared to the recognized standard propoxyphene product.

EXPERIMENTAL

Bioavailability Studies—The design for both studies was identical. In Study 1, 21 healty adult volunteers[1] (14 males and seven females), averaging 23 years of age (range of 22–33 years) and weighing 68.6 kg (range of 52.2–86.4 kg), were selected. In Study 2, 24 healthy adult male volunteers, averaging 24.5 years (range of 19–51 years) and weighing 77.5 kg (range of 61.4–89.1 kg), were selected.

All subjects had normal screening vital signs (blood pressure, pulse, respiration, and temperature) and laboratory parameters (complete blood count, urinalysis, blood urea nitrogen, serum alkaline phosphatase, serum glutamic–oxaloacetic transaminase, serum bilirubin, and glucose). No subject received any barbiturates or other enzyme-inducing drugs for 30 days, or any other medication for 7 days, preceding the start of the study. All subjects received only the medication prescribed in the protocol for the duration of the study.

At zero time of each treatment period, each subject received single oral doses of medication[2] as follows: Treatment A, one 65-mg hard-filled capsule of propoxyphene hydrochloride (test formulation); or Treatment

[1] Study was initiated with 22 subjects, but one subject was dropped before completion of the clinical phase.

[2] Identity of products is available from authors upon request.

Table I—Physicochemical Parameters Obtained for the Different 65-mg Propoxyphene Hydrochloride Capsule Formulations

Property	Study 1		Study 2	
	Treatment A Formulation I	Treatment B Recognized Standard I	Treatment A Formulation II	Treatment B Recognized Standard II
Assay potency[a]	63.7 ± 0.85 mg	62.9 ± 1.4 mg	68.2 ± 1.7 mg	65.1 ± 0.6 mg
Dissolution rate[b] at 10 min, %	96.5 ± 8.9	89.9 ± 1.3	99.80 ± 1.0	99.68 ± 3.1

[a] Assay 10 capsules and report average of three or more assays for each lot. [b] Mean ± *SD* for six capsules. Unpublished assay procedure.

Table II—Average Plasma Propoxyphene Concentrations and Related Parameters Obtained from 21 Normal Volunteers after Receiving Single Oral Doses (65 mg) of Two Propoxyphene Formulations

Hours	Average Plasma Propoxyphene Level, ng/ml		Significance
	Treatment A	Treatment B	
0.0	0.20 (0.20)[a]	0.0 (0)[a]	N.S.
0.5	10.12 (2.40)	6.29 (2.43)	N.S.
1.0	32.11 (4.45)	48.84 (12.45)	N.S.
2.0	44.09 (4.51)	53.05 (3.84)	$p < 0.025$
3.0	40.75 (5.01)	44.52 (4.23)	N.S.
5.0	17.87 (1.61)	24.88 (2.47)	$p < 0.001$
7.0	12.98 (1.47)	20.84 (3.43)	$p < 0.05$
9.0	8.85 (1.02)	13.25 (2.10)	$p < 0.025$
12.0	5.77 (1.02)	8.46 (1.19)	$p < 0.005$
Average of individual peak plasma concentrations, ng/ml	51.58 (4.99)	69.81 (11.46)	N.S.
Average *AUC*, ng hr/ml (0–12 hr)	226.9 (19.95)	296.87 (25.39)	$0.001 < p < 0.005$
Average of individual peak times, hr	1.95 (0.21)	2.14 (0.28)	N.S.

[a] The ( ) represents the standard error of the mean.

B, one 65-mg hard-filled capsule of propoxyphene hydrochloride (recognized standard). Individual subjects were assigned treatments according to a randomized two-way crossover design. The studies were separated by 7 days.

Subjects were required to fast overnight and for 4 hr immediately after the medication was administered. The medication was taken at zero time with 180 ml of water. No beverages (except water) were permitted during the fasting period. The subjects were not permitted to engage in any strenuous or athletic activities during the days of drug administration.

Blood, 20 ml, was taken from each subject at zero time, *i.e.*, just before dosing with each medication and at 0.5, 1.0, 2.0, 3.0, 5.0, 7.0, 9.0, and 12 hr. In Study 2, a 16-hr postadministration sample also was drawn. Plasma was harvested from all samples as soon after drawing as possible and immediately frozen.

Assay Methodology—All plasma specimens were extracted using the procedure described by Wolen and Gruber (7). The extract residues were reconstituted in carbon disulfide for GLC analysis. All samples were chromatographed on U-shaped glass columns (0.31 m × 3 mm i.d.) of 3% (w/w) Apiezon-L and 2% (w/w) potassium hydroxide on 80–100-mesh Gas Chrom Q[3]. All newly prepared columns were preconditioned at 225° for 6 hr without carrier gas flow and for 16 hr with carrier gas flow of 10 ml/min.

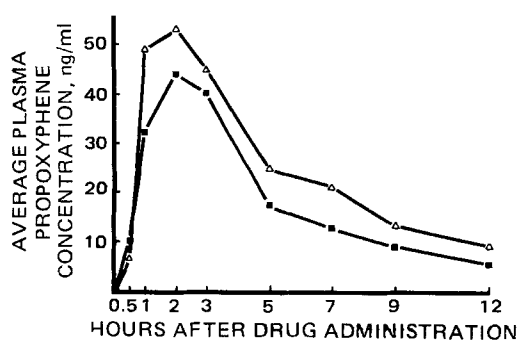

Figure 1—*Average plasma propoxyphene concentrations obtained from 21 normal volunteers after receiving a single 65-mg oral dose of propoxyphene. Key:* ■, *Treatment A; and* △, *Treatment B.*

[3] Applied Science Laboratories, State College, Pa.

During analysis, the column, injection port, and flame-ionization detector were maintained at 170, 175, and 205°, respectively. Helium, hydrogen, and air flow rates were 40, 50, and 350 ml/min, respectively. The retention times for propoxyphene, pyrroliphene, and norproxypheneamide were 3.2, 7.4, and 12 min, respectively.

Physical and Chemical Characteristics of Medication—The physical and chemical characteristics of the two formulations are presented in Table I. The test formulations and recognized standards were nearly identical in potency and dissolution rates.

Statistical Analysis—In both studies, the plasma data were submitted to a computer[4] programmed with an analysis of variance (ANOVA) program for crossover design. The variance components due to subjects, subjects per group (equal or unequal groups), time period, and treatments were estimated. The results of these estimations for plasma concentrations and the related parameters of peak concentration, peak time, and area under the plasma concentration–time curve are reported in Tables II and III.

RESULTS AND DISCUSSION

With any product, the rate and extent parameters of drug delivery to the general circulation are important in obtaining the desired pharmacologic or therapeutic response. If the drug is "absorbed" from two formulations at the same rate and to the same extent, the bioequivalence of these formulations is assured. The peak concentration, time of peak concentration, and area under the blood (plasma or serum) concentration–time curve are three parameters that allow estimation of the rate and extent of bioavailability.

Study 1—As shown in Fig. 1, both the test[5] and reference[6] formulations had measurable plasma levels at the 0.5-hr sampling time, with the average plasma propoxyphene curves peaking at 2 hr. Throughout the 12-hr sampling period, plasma levels of the test formulation were below those of the reference product. The statistical analysis from Table II indicates that the test formulation plasma levels were significantly lower than the recognized standard plasma levels at the 2-, 5-, 7-, 9-, and 12-hr sampling times.

The averages of the individual peak plasma concentrations for the test and reference products were 51.58 and 69.81 ng/ml, respectively, a 26% difference in peak concentration. The times of peak concentration were 1.95 hr for the test formulation and 2.14 hr for the recognized standard.

[4] IBM 360-50.
[5] Research lot 17,015-4.
[6] Lot 5HR70A.

Table III—Average Plasma Propoxyphene Concentrations and Related Parameters Obtained from 24 Normal Volunteers after Receiving Single Oral Doses of Two Different Propoxyphene Formulations

Hours	Average Plasma Propoxyphene Level, ng/ml		Significance
	Treatment A	Treatment B	
0.0	0.00 (0)[a]	0.00 (0)[a]	N.S.
0.5	2.22 (1.25)	0.32 (0.32)	N.S.
1.0	44.65 (4.44)	43.24 (5.23)	N.S.
2.0	64.36 (5.24)	64.59 (4.63)	N.S.
3.0	57.32 (4.39)	52.81 (3.99)	N.S.
5.0	35.58 (3.02)	34.73 (3.41)	N.S.
7.0	24.69 (2.98)	22.06 (3.03)	N.S.
9.0	17.22 (2.24)	14.75 (2.66)	N.S.
12.0	10.33 (1.84)	4.88 (1.47)	$p = 0.001$
16.0	5.52 (1.72)	2.29 (1.30)	$p = 0.01$
Average of individual peak plasma concentrations, ng/ml	69.03 (5.11)	68.67 (5.03)	N.S.
Average *AUC*, ng hr/ml, 0–12 hr	364.02 (30.8)	334.17 (32.8)	$p < 0.05$
0–16 hr	395.73 (33.9)	348.51 (35.28)	$p < 0.05$
Average of individual peak times, hr	1.96 (0.15)	2.04 (0.11)	N.S.

[a] The ( ) represents the standard error of the mean.

Neither the peak nor peak time differences were statistically significant.

The average areas under the plasma concentration–time curve (*AUC*) were 226.9 ng hr/ml for the test formulation and 296.87 ng hr/ml for the reference standard; this 24% difference was statistically significant ($p < 0.005$). A difference in this parameter indicates that the test formulation has approximately 76% the extent of availability of the reference standard. As previously noted, the *in vitro* parameters of assay potency and dissolution rate were quite similar for the test and reference formulations and did not detect the observed difference in bioavailability.

For the recognized standard, the plasma propoxyphene levels, peak concentration, peak time, and *AUC* for Studies 1 and 2 were quite similar to reported data (8, 9).

Study 2—As a result of the bioavailability findings of the first test formulation, the product was reformulated and tested for bioequivalence. As shown in Fig. 2 and Table III, test Formulation[7] II produced plasma levels essentially identical to a second lot of the recognized standard[8].

The averages of the individual peak plasma concentrations for the test and reference products were 69.03 and 68.67 ng/ml, respectively, while the averages of the individual peak times were 1.96 and 2.04 hr, respectively. The average *AUC* values (0–16 hr) were 395.73 ng hr/ml for the test formulation and 348.51 ng hr/ml for the recognized standard. This 12% difference in the *AUC* means tested statistically significant, but the formulations were judged equivalent.

The finding of statistically significant differences in the means of bioavailability parameters does not necessarily demand the declaration of bioinequivalence, nor does the lack of statistical significance imply bioequivalence. In many instances, small differences in mean values test statistically significant or large differences do not test statistically different. This result, of course, is related to the coefficient of variation (*CV*) observed in the individual studies and the power of the statistical analysis. In the first study, the test formulation produced plasma levels consistently lower than those of the recognized standard (as seen in the mean plasma levels of Fig. 1). This result was reflected in significant *AUC* differences despite high coefficients of variation for plasma level means and peak concentration.

In Study 2, the plasma level and bioavailability parameter coefficients of variation were generally lower than those of Study 1, and a much smaller (12 *versus* 24%) difference tested significant. The plasma levels in Study 2 were practically identical for test and standard formulations. The *AUC* differences were due to plasma level differences at the extreme tail-points of the serum level curve. It is very doubtful that these differences would have any clinical meaning.

Current federal regulations (10) for capsule formulations of propoxyphene hydrochloride require submission of an abbreviated NDA and proof of biological availability. However, the biological availability requirements were deferred for capsule formulations of propoxyphene hydrochloride when not in combination with aspirin or aspirin, phenacetin, and caffeine (11). Both the test formulations and the recognized standards were practically identical in the *in vitro* specifications of assay potency and dissolution rate. Although the lack of predictability of the *in vitro* parameters was unexpected, this point must be considered in the overall evaluation of propoxyphene formulations. Clearly, *in vitro* product equivalence does not assure *in vivo* bioequivalence. Thus, *in vitro* test parameters should not be used as the sole criterion in determining product bioequivalence. Confirmatory *in vivo* bioavailability testing should not be deferred for single-entity propoxyphene capsules in light of the data presented.

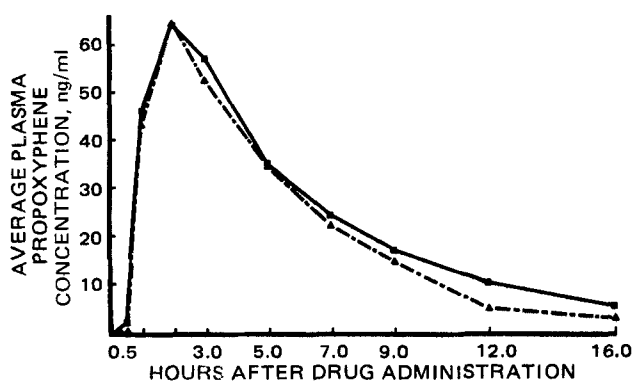

Figure 2—*Average plasma propoxyphene concentrations obtained from 24 normal volunteers after receiving a single 65-mg oral dose of propoxyphene hydrochloride. Key:* ■, *Treatment A; and* ▲, *Treatment B.*

[7] Research lot 17,320-1.
[8] Lot 5KL89C.

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Thebaine Content of Selections of *Papaver bracteatum* Lindl. at Different Ages

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Abstract □ Approximately 200 accessions of *Papaver* species were evaluated for identification as *P. bracteatum* Lindl. and for thebaine content. Fifteen authentic *P. bracteatum* accessions were selected on the basis of chromosome count. Statistical analyses of variation in alkaloid content were made for these accessions. Four of the 15 warranted further study based on the vigor of the plant and the total thebaine present in the tissue. Significant variability in thebaine content was found in wild strains of *P. bracteatum*, thus requiring genetic selection studies. The data showed that total yield potential of thebaine should be considered in selecting *P. bracteatum* strains for commercial growth rather than concentrating on strains that produce the highest thebaine concentrations. A method for the quantitative estimation of thebaine from *P. bracteatum* is presented.

Keyphrases □ Thebaine—GLC analysis, root and aboveground portions of various strains of *Papaver bracteatum* □ *Papaver bracteatum*—various strains, root and aboveground portions, GLC analysis of thebaine □ GLC—analysis, thebaine in root and aboveground portions of various strains of *Papaver bracteatum* □ Alkaloids—thebaine, GLC analysis in root and aboveground portions of various strains of *Papaver bracteatum* □ Narcotics—thebaine, GLC analysis in root and aboveground portions of various strains of *Papaver bracteatum*

Plants in the family Papaveraceae have been analyzed for alkaloids for more than a century. The genus *Papaver* and specifically *P. somniferum*, the opium poppy that produces thebaine, codeine, morphine, and other alkaloids, has received the most attention (1).

With the growing shortage of medically useful codeine, an alternative source has been sought. *P. bracteatum*, a potential source of thebaine, reportedly contains as much as 3.5% of this alkaloid in mature capsules at a purity of 95% or greater (2, 3). Treating thebaine with hydrogen bromide results in a 76% yield of codeinone. Codeinone is reduced to codeine by the Meerwein–Pondorff process or by sodium borohydride in commercial preparations (4).

Extraction from *P. bracteatum* of reliable supplies of thebaine and chemical transformation to codeine and related derivatives for legitimate medical needs might reduce dependency on morphine. Morphine from opium now supplies about 95% of the codeine used in the United States (5) as well as 100% of the heroin, the most abused illicit drug in the United States[1].

[1] Dr. Quentin Jones, Staff Scientist in charge of Narcotics Program, National Program Staff, Agricultural Research Service, U.S. Department of Agriculture, personal communication.

EXPERIMENTAL

Plant Source—The origin of collections and chromosome numbers of the 15 accessions of *P. bracteatum* used are shown in Table I. The initial genetic stock of the *Papaver* species consisted of about 200 accessions from the Middle East and were reduced to the 15 accessions on the basis of chromosome counts of root tip cells, floral characteristics, and alkaloid profile. The remaining accessions (~185) were found not to be *P. bracteatum*. Chromosome counts were made[2] on immature plants to separate three closely related species found in the same geographic area where the seeds were collected from stands with no apparent prior cultivation, *i.e.*, *P. orientale* (2n = 28), *P. pseudo-orientale* (2n = 42), and *P. bracteatum* (2n = 14).

Plant Culture—Seeds from accessions were planted in 7.6-cm peat pots[3] in the greenhouse in composted potting soil with about 4.2% organic matter and 33% pit washed sand; the pH was 6.2–6.5. Greenhouse conditions were: illumination, ambient (September–November); temperature, 20–25°; fertilizer, none; and insecticides (applied once each month), (5-benzyl-3-furyl)methyl *cis-trans*-(+)-2,2-dimethyl-3-(2-methylpropenyl)cyclopropanecarboxylate for white fly control and tricyclohexylhydroxystannane for spider mite control.

Seedlings were successively thinned to five, three, and one per pot. Plants were sampled for thebaine content at 5, 7, and 11 months of growth. In September, after 11 months, the plants were transferred to the field; field soil was Elkton silt loam (typic ochraquult), pH 5.8. Soil analysis showed: magnesium, 143 kg/hectare; phosphate, 280 kg/hectare; and potash, 77 kg/hectare. Fertilizer amendments were: limestone, 840 kg/hectare, ammonium nitrate (34% N), 84 kg/hectare; phosphate (46% P_2O_5), 90 kg/hectare; and potash (60% K_2O in KCl), 269 kg/hectare.

Immature plants were analyzed for the thebaine content, fresh and dry weight yields of roots, and aboveground parts at time of harvest. The leaf, stem, capsule, and sometimes root tissue of mature plants were analyzed on a fresh and dry weight basis the following May, 2 weeks after petal fall. Approximately 80–90% of plants transferred to the field flowered. All plant harvesting was done at the same time of day to eliminate effects of diurnal variation on alkaloid concentrations.

Analytical—Analysis of variance was made of 11-month-old immature plants.

Thebaine yields from root, aboveground part, stem, and capsule tissues oven dried at 60° or freeze dried were compared. All tissues were ground to pass a 40-mesh sieve. Three 100-mg samples of each tissue dried by each method were taken. One sample was oven dried at 110° overnight for the determination of residual water content; the second was extracted in 50 ml of methanol–ammonium hydroxide (98:2 v/v) by rotation for 1 hr at 120 rpm; the third was extracted with 5% aqueous acetic acid, the reference extraction solvent, and purified as recommended by the United Nations (6). Routine analyses were from samples extracted in metha-

[2] M. L. Stiff, U.S. Department of Agriculture.

[3] Jiffy pots. (Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.)