

CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

**APPLICATION NUMBER
20-971**

Pharmacology Review(s)

Memorandum

To: Cynthia McCormick, M.D., Director, DACCADP

From: Lucy Jean, Ph.D., Pharmacologist, Team Leader 

Date: April 3, 2000

Subject: NDA 20-971

Trade Name (4% articaine HCl with 1:100,000 epinephrine). In this memo, Septanest[®], the Applicant's proposed original name is used

Summary of Nonclinical Pharmacology and toxicology

Introduction: Septanest is the product name for articaine HCl, an amide type of local anesthetic and a new molecular entity. Articaine HCl is indicated for dental use. Depending on the procedures, the recommended human doses range from 20 to 204 mg, or not to exceed a maximum dose of 7 mg/kg. Articaine HCl is formulated with epinephrine either at 1:100,000 (as Septanest[®]). In addition to epinephrine, both products contain sodium metabisulfite, with the pH adjusted to 5. Since 1988 articaine HCl has been approved and marketed for dental use in thirteen countries. The drug product is available in 1.7 mL glass cartridges containing articaine HCl at 68 mg.

Non-clinical data: The efficacy and reasonable safety of Septanest[®] have been shown in nonclinical studies. The local anesthetic effect was shown in guinea pigs following intradermal administration, in rabbit corneal model following instillation to eyes and in dogs following epidural administration. Epinephrine slightly prolonged the duration of anesthesia. The cardiovascular effects were studied in in vivo and in vitro studies. In general, articaine showed similar cardiodepressant activity as that of lidocaine. Articaine is mainly metabolized by plasma esterase to articainic acid in animal species and humans. The metabolite is reported in literature as inactive. Acute toxicity studies of articaine HCl with and without epinephrine following intravenous, subcutaneous, or intramuscular administration were conducted in mice, rats, rabbits and dogs. Addition of epinephrine increased the acute toxicity of articaine in mice and rats. Subchronic 4-week toxicity studies following subcutaneous administration of Septanest[®] (the to-be-marketed product) were conducted in rats and dogs. No target organ of toxicity was identified in rats and dogs. In rats, extramedullary hemopoiesis of the spleen with increased reticulocytes from 50 mg/kg was observed; but the significance is unknown, since the erythrocytes were not affected. In dogs these effects were not observed up to the highest dose tested (80 mg/kg). In both rats and dogs; elevated ALT and AST, without histopathological changes of the liver were observed at high doses of 100 mg/kg (rats) and 80 mg/kg (dog). The NOAELs are estimated to be 25 mg/kg for rats in terms of extramedullary hemopoiesis effects and 40 mg/kg for dogs in terms of convulsion and elevated liver function enzymes. The NOAEL C_{max} (µg/ml) and AUC (µg min/mL) are 1.9 and 58-98 (rats), and 2.5 and 127-150 (dogs), respectively. These NOAEL C_{max} data are 2- to 6-fold greater than the peak plasma levels of approximately 0.4 µg/mL and 0.9 µg/mL, obtained from human therapeutic doses of 68 mg and 204 mg, respectively. For the maximum human recommended dose of 7 mg/kg, the NOAEL level of 40 mg/kg in dogs is approximately 3X on a mg/m² basis, an adequate margin of safety for human use. Rats, however, showed extramedullary hemopoiesis at 50-100 mg/kg or 1X-2X the maximum dose of 7 mg/kg on a mg/m² basis. The significance is unknown, since erythrocytes were not affected. Furthermore, the effect may be a species-specific effect following a prolonged 4-week treatment,

April 3, 2000

since species concordance was not observed. Dogs did not show this effect up to the highest tested dose of 80 mg/kg, or 6X the maximum recommended human dose of 7 mg/kg on a mg/m². In addition, the available clinical data have not shown drug-related hematological effects in humans following twelve years of marketing experience, nor have these effects been observed in the clinical studies for this NDA. Thus considering the toxicokinetic data as the most relevant information from rats and dogs which showed the NOAEL levels at 2X and 6X, respectively, those of the recommended human doses of 68-204 mg, the animal data adequately support the reasonable safe use in humans. The NOAEL level from dogs at 3X the maximum recommended human dose of 7 mg/kg and the available clinical data showing no drug-related hematological effects also support the reasonable safe use of 7 mg/kg in humans. Local irritation at the injection site was observed in rats. Reproductive toxicity studies showed no adverse effects at a subcutaneous dose of 80 mg/kg on the fertility of male and female rats, nor were teratogenicity or fetotoxicity observed in rats or rabbits. In pre- and post-natal fetal development study in rats, increased incidence of stillbirth and an adverse effect on passive avoidance (learning) of F1 pups were observed in the 80 mg/kg group. Maternal toxicity, such as reduced activity, convulsions and/or deaths, was observed at 80 mg/kg. Articaine HCl alone or Septanest — with epinephrine) was not genotoxic in a standard battery of genotoxicity assays. Carcinogenicity studies are not required for occasional-use drugs like articaine HCl.

Conclusion and Recommendations:

The pharmacological and toxicological profiles generated in laboratory animals have shown reasonable safety and efficacy for the drug product. The application, therefore, is approvable based on the pharmacology. Before the application can be approved, however, revisions of the package insert should conform to those previously recommended.

Non-clinical issues:

There are no outstanding non-clinical issues; revision of the package insert has been made.

(prepared by Lucy Jean 3/31/2000/revised per CMc 4/3/2000memoarticaine.doc)

**APPEARS THIS WAY
ON ORIGINAL**

Memorandum

To: Cynthia McCormick, M.D., Director, DACCADP

From: Lucy Jean, Ph.D., Pharmacologist, Team Leader

LJ 3/31/00

Date: March 31, 2000

Subject: NDA 20-971

Trade Name (4% articaine HCl with 1:100,000 epinephrine). In this memo, Septanest — ; the Applicant's proposed original name is used

Summary of Nonclinical Pharmacology and toxicology

Introduction: Septanest is the product name for articaine HCl, an amide type of local anesthetic and a new molecular entity. Articaine HCl is indicated for dental use. Depending on the procedures, the recommended human doses range from 20 to 204 mg, or not to exceed a maximum dose of 7 mg/kg. Articaine HCl is formulated with epinephrine either at 1:100,000 (as Septanest —). In addition to epinephrine, both products contain sodium metabisulfite, with the pH adjusted to 5. Since 1988 articaine HCl has been approved and marketed for dental use in thirteen countries. The drug product is available in 1.7 mL glass cartridges containing articaine HCl at 68 mg.

Non-clinical data: The efficacy and reasonable safety of Septanest — have been shown in nonclinical studies. The local anesthetic effect was shown in guinea pigs following intradermal administration, in rabbit corneal model following instillation to eyes and in dogs following epidural administration. Epinephrine slightly prolonged the duration of anesthesia. The cardiovascular effects were studied in in vivo and in vitro studies. In general, articaine showed similar cardiodepressant activity as that of lidocaine. Articaine is mainly metabolized by plasma esterase to articainic acid in animal species and humans. The metabolite is reported in literature as inactive. Acute toxicity studies of articaine HCl with and without epinephrine following intravenous, subcutaneous, or intramuscular administration were conducted in mice, rats, rabbits and dogs. Addition of epinephrine increased the acute toxicity of articaine in mice and rats. Subchronic 4-week toxicity studies following subcutaneous administration of Septanest — (the to-be-marketed product) were conducted in rats and dogs. No target organ of toxicity was identified in rats and dogs. In rats, extramedullary hematopoiesis of the spleen with increased reticulocytes from 50 mg/kg was observed; but the significance is unknown, since the erythrocytes were not affected. In both rats and dogs; elevated ALT and AST, without histopathological changes of the liver were observed at high doses of 100 mg/kg (rats) and 80 mg/kg (dogs). The NOAELs are estimated to be 25 mg/kg (rats) and 40 mg/kg (dogs). The corresponding C_{max} (µg/ml) and AUC (µg min/mL) are 1.9 and 58-98 (rats), and 2.5 and 127-150 (dogs), respectively. In terms of general toxicity, these NOAEL C_{max} data are 2- to 6-fold greater than the peak plasma levels of approximately 0.4 µg/mL and 0.9 µg/mL, obtained from human therapeutic doses of 68 mg and 204 mg, respectively. The NOAELs in rats and dogs were approximately 0.6X and 3X the maximum recommended human dose of 7 mg/kg on a mg/m² basis, respectively. In rats, the factor of less than one compared with the maximum recommended human dose may indicate a species-specific sensitivity following a prolonged 4-week treatment. Increased reticulocytes and effects of extramedullary hematopoiesis on erythrocyte parameters in rats were not observed in dogs, nor have these been observed in humans following twelve years of marketing experience. Thus considering the PK data as the most relevant information from rats and

March 31, 2000

dogs and the human multiple relative to dogs on a mg/m² basis, the animal data support the reasonable safe use of the drug product in humans. Local irritation at the injection site was observed in rats. Reproductive toxicity studies showed no adverse effects at a subcutaneous dose of 80 mg/kg on the fertility of male and female rats, nor were teratogenicity or fetotoxicity observed in rats or rabbits. In pre- and post-natal fetal development study in rats, increased incidence of stillbirth and an adverse effect on passive avoidance (learning) of F1 pups were observed in the 80 mg/kg group. Maternal toxicity, such as reduced activity, convulsions and/or deaths, was observed at 80 mg/kg. Articaine HCl alone or Septanest™ (with epinephrine) was not genotoxic in a standard battery of genotoxicity assays. Carcinogenicity studies are not required for occasional-use drugs like articaine HCl.

Conclusion and Recommendations:

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Non-clinical issues:

There are no outstanding non-clinical issues; revision of the package insert has been made.

(prepared by Lucy Jean 3/31/2000/articainesummaryb.doc)

APPEARS THIS WAY
ON ORIGINAL

Memorandum

To: Lisa Rarick, M.D., Deputy Director
Office of Drug Evaluation II, CDER, FDA

Leah Ripper, Associate Director of Regulatory Affairs
Office of Drug Evaluation II, CDER, FDA

Cc: Cynthia G. McCormick, M.D., Director
Division of Anesthetic, Critical Care and Addiction Drug Products
Office of Drug Evaluation II, CDER, FDA

John Jenkins, M.D., Director
Office of Drug Evaluation II, CDER, FDA

From: Robin A. Huff, Ph.D., Pharmacology Team Leader *(initials)* 3-30-00
Division of Pulmonary and Allergy Drug Products
Office of Drug Evaluation II, CDER, FDA

Date: March 30, 2000

Re: NDA 20-971, Septanest (articaine HCl and epinephrine injection)

In the absence of Dr. Ken Hastings, ODE II Associate Director of Pharmacology, I was asked to complete a labeling review for this NDA. Labeling for the Carcinogenesis, Mutagenesis, Impairment of Fertility section is acceptable as presented in the facsimile dated March 20, 2000. However, the Pregnancy labeling should be revised as follows:

In developmental studies, no embryofetal toxicities were observed when TradeName was administered subcutaneously throughout organogenesis at doses up to 40 mg/kg in rabbits and 80 mg/kg in rats (approximately 2 times the maximum recommended human dose on a mg/m² basis). In rabbits, 80 mg/kg (approximately 4 times the maximum recommended human dose on a mg/m² basis) did cause fetal death and increase fetal skeletal variations, but these effects may be attributable to the severe maternal toxicity, including seizures, observed at this dose.

When articaine hydrochloride was administered subcutaneously to rats throughout gestation and lactation, 80 mg/kg (approximately 2 times the maximum recommended human dose on a mg/m² basis) increased the number of stillbirths and adversely affected passive avoidance, a measure of learning, in pups. This dose also produced severe maternal toxicity in some animals. A dose of 40 mg/kg (approximately equal to the maximum recommended human dose on a mg/m² basis) did not produce these effects.

Draft

Rationales for the requested changes are:

- 1) The first sentence of this section in the March 20, 2000 fax simply states that the drug was not teratogenic up to doses of 80 mg/kg, but the most recent approach to preclinical labeling is not to arbitrarily differentiate between teratogenic and other developmental effects.
- 2) The above language describes when effects might be appropriately ascribed to maternal toxicity rather than direct developmental effects of the drug.
- 3) The March 20, 2000 fax indicates that the perinatal study was performed with the drug product, when in fact the effects described occurred in a study performed with articaine alone (no epinephrine). A second study was performed with Septanest in which no effects on offspring were observed.
- 4) The effect of 80 mg/kg on eye opening in pups was left out because the effect was small and not clearly dose/treatment-related (14.7, 15.3, 15.4 and 15.5 days for Control, 20, 40 and 80 mg/kg, respectively). Statistical significance of the finding was not addressed in the review.
- 5) The effect of 80 mg/kg on pup weight was left out because it was only described as "slightly reduced" in the review and did not occur until after weaning. If the statement were included it would seem to imply low birth weight of pups, which is not the case.

Before finalizing labeling, the rabbit embryofetal development study should be reexamined to confirm the decrease in fetal viability and increase in skeletal variations. Decreased fetal viability is described in the review of the individual study, but not in the overall summary and evaluation of reproductive toxicity. The increase of skeletal variations is described in the overall summary and evaluation, but no effect is noted in the review of the individual study.

Other than labeling, there are two items worth mentioning. For accurate record keeping, it may be worth making a correction to the review in the division file in terms of the AUC units used in the Overall Summary and Evaluation. On page 30, AUC's are stated to be g.min/ml and ng.min/ml for rat and dog, respectively, but according to data in the review the units should be ug.min/ml for both species. The accuracy of these data is important when making comparisons to human exposure. Secondly, the pharmacology memo dated March 15, 2000 makes dose comparisons to a 68 mg clinical dose. It should be noted that this is not the maximum clinical dose. The maximum dose is stated in the labeling to be 7 mg/kg, which is 420 mg for a 60 kg person. Pharmacokinetic data may not be available for the maximum clinical dose; however, it is known that a dose of 204 mg produces a C_{max} of 0.9 ug/ml and an AUC of 92.5 ug.min/ml (reported as 1542 ng.hr/ml on page 31 of the pharmacology review), which are similar to the NOAEL values reported in the 4 week studies conducted in rat (1.9 ug/ml and 58 – 98 ug.min/ml) and dog (2.5 ug/ml and 127 – 150 ug.min/ml).

M E M O R A N D U M

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

DATE: March 15, 2000

FROM: Lucy Jean, Ph.D. *LJ*
Pharmacologist, Team Leader

TO: Cynthia McCormick, M.D.
Director, DACCADP

SUBJECT: NDA 20-971
Septanest™ (4% articaine HCl with 1:200,000 epinephrine) and
Septanest™ (4% articaine HCl with 1:100,000 epinephrine)

Summary of Nonclinical Pharmacology and toxicology

Introduction: Septanest is the product name for articaine HCl, an amide type of local anesthetic and a new molecular entity. Articaine HCl is indicated for dental use, generally at a dose of 1.7 mL (or 68 mg). Articaine HCl is formulated with epinephrine either at 1:100,000 (as Septanest™) or at 1:200,000 (as Septanest™). In addition to epinephrine, both products contain sodium metabisulfite, with the pH adjusted to 5. Since 1988 articaine HCl has been approved and marketed for dental use in thirteen countries.

Non-clinical data: The efficacy and reasonable safety of Septanest™ have been shown in nonclinical studies. The local anesthetic effect was shown in guinea pigs following intradermal administration, in rabbit corneal model following instillation to eyes and in dogs following epidural administration. Epinephrine slightly prolonged the duration of anesthesia. The cardiovascular effects were studied in vivo and in vitro studies. In general, articaine showed similar cardiodepressant activity as that of lidocaine. Articaine is mainly metabolized by plasma esterase to articainic acid in animal species and humans. The metabolite is reported in literature as inactive. Acute toxicity studies of articaine HCl with and without epinephrine following intravenous, subcutaneous, or intramuscular administration were conducted in mice, rats, rabbits and dogs. Addition of epinephrine increased the acute toxicity of articaine in mice and rats. Subchronic 4-week toxicity studies following subcutaneous administration of Septanest™ (the to-be-marketed product) were conducted in rats and dogs. No target organ of toxicity was identified in rats and dogs. In rats, extramedullary hematopoiesis of the spleen with increased reticulocytes from 50 mg/kg was observed; but the significance is unknown, since the erythrocytes were not affected. In both rats and dogs; elevated ALT and AST, without histopathological changes of the liver were observed at the high dose of 80 mg/kg. The NOAELs are estimated to be 25 mg/kg (rats) and 40 mg/kg (dog). The corresponding C_{max} (µg/ml) and AUC (µg min/mL) are 1.9 and 58-98 (rats), and 2.5 and 127-150 (dog), respectively. In terms of general toxicity, these NOAEL TK data compare favorably with those obtained from human therapeutic dose (68 mg): 4 µg/mL and 37 µg min/mL, and support the reasonable safe use of the drug product in humans. Local irritation at the injection site was observed in rats. Reproductive toxicity studies showed no adverse effects at a subcutaneous dose of 80 mg/kg on the fertility of male and female rats, nor were teratogenicity or fetotoxicity observed in rats or rabbits. In pre- and post-natal fetal development study in rats, increased stillbirth and slight effects on delayed eye-opening and passive avoidance (learning) were observed in F1 pups of the 80 mg/kg group. Maternal toxicity such as reduced activity, convulsion and/or deaths, was observed at 80 mg/kg. Articaine HCl alone or Septanest™ (with epinephrine) was not genotoxic in a standard battery of genotoxicity assays. Carcinogenicity studies are not required for occasional-use drugs like articaine HCl.

Conclusion and Recommendations:

The pharmacological and toxicological profiles generated in laboratory animals have shown reasonable safety and efficacy for the drug product. The application, therefore, is approvable based on the pharmacology. Before the application can be approved, however, revisions of the package insert should conform to those previously recommended.

Non-clinical issues:

There are no outstanding non-clinical issues; revision of the package insert has been made.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Division of Anesthetic, Critical Care & Addiction Drug Products

NDA: 20-971 (BL)

IND: _____

Submission: NDA Dated: March 9, 1999
Received by CDR: March 9, 1999
Received by Reviewer: March 19, 1999
Review Completed: March 22, 1999
Reviewer: M.A. Goheer, Ph.D.

Sponsor: Deproco Inc., 245-C Quigley Blvd., New Castle, DE 19720

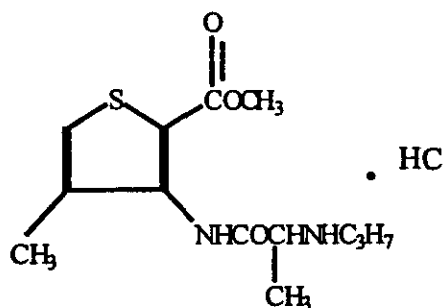
Information to be conveyed to the sponsor: Yes

Drug Name: Septanest[®] 1:200,000 and Septanest[®] 1:100,000 (4% Articaine plus 1/200,000 epinephrine and 4% Articaine plus 1/100,000 epinephrine)

Chemical Name: 2-Thiophene acid, 4 methyl-3-[[1-oxo-2-(propylamino) propyl]amino]-methyl ester, monohydrochloride.

Other Name: Articaine HCl with Epinephrine.

Structure:



Molecular weight: 320.84

Drug Product Manufacturer: Specialites Septodont, 58 rue du pont de Creteil, Saint-Maur des Fosses, 94107 Paris, France. (parent company of Deproco, Inc.)

The manufacturer of _____

The manufacturer of _____

Pharmacologic Class: Local anesthetic of the amide type

Related NDAs: 16-964 - Bupivacaine, 17-751 - Etidocaine, 20-533 - Naropin

Indications and Dosages: Articaine hydrochloride is indicated for infiltration anesthesia and nerve block anesthesia in clinical dentistry. The maximum recommended dose of articaine hydrochloride for normal healthy adults should not exceed 7 mg/kg (259 mg/m²) of body weight.

Previously Reviewed Submission: Original NDA submitted on March 30, 1998 was reviewed on August 28, 1998.

Background: This submission contains the response from the sponsor regarding the issues raised by the FDA in its Approvable letter dated January 29, 1999.

Recommendation: The sponsor has incorporated our proposed changes in the labeling. Therefore, an amended package insert on the Preclinical labeling under Carcinogenesis, Mutagenesis, Impairment of Fertility and Pregnancy headings is acceptable.

S

M. Anwar Goheer

Concur by Team Leader:

cc:

IND ORIG. _____

NDA - 20-971

HFD-170

/Goheer

/Hblatt

/Samanta

S
Dou Huey (Lucy) Jean

3/22/99

Drug Product Manufacturer: Specialites Septodont, 58 rue du pont de Creteil,
Saint-Maur des Fosses, 94107 Paris, France. (Parent company of Deproco,
Inc.)

The manufacturer

The manufacturer

Pharmacologic Class: Local anesthetic of the amide type

Related IND:

Related NDAs: 16-964 - Bupivacaine, 17-751 - Etidocaine, 20-533 - Naropin

Indications and Dosages: Articaine hydrochloride is indicated for infiltration anesthesia and nerve block anesthesia in clinical dentistry. The maximum recommended dose of articaine hydrochloride for adults should not exceed 7 mg/kg (259 mg/m²) of body weight.

Previously Reviewed Submissions: Original IND submitted on Oct. 18, 1996 was reviewed on April 11, 1997.

Studies Reviewed: Toxicology

- A. Carticaine 4 per cent Without Adrenaline - Toxicity After a Single Administration Subcutaneously in the Mouse. Study No. 870975, 1988, page 132, vol. 6.
- B. Carticaine (Articaine 4 per cent Adrenaline 1/100,000) in comparison with Alphacaine SP (Articaine 4 per cent, Adrenaline 1/100,000) - Comparison of Safety in the Mouse Using the Subcutaneous Route. Study No. 870973, 1987, page 188, vol. 6.
- C. Carticaine (Articaine 4 per cent, Adrenaline 1/200,000) in comparison with Alphacaine N (Articaine 4 per cent, Adrenaline 1/200,000) - Comparison of Safety in the Mouse Using the Subcutaneous Route. Study No. 870974, 1987, page 234, Vol. 6.
- D. Aged Articaine Solution (3 months) in Comparison with "Fresh" Articaine Solution - Safety in the Mouse Via the Subcutaneous Route. Study No. 880001, 1988, page 279, vol. 6.
- E. Aged Articaine Solution (6 months) in Comparison with "Fresh" Articaine Solution - Safety in the Mouse Via the Subcutaneous Route. Study No. 871299, 1988, page 343, vol. 6.
- F. Articaine 4% without Vasoconstrictor - Study of the Skin Sensitivity According to Magnusson and Kligman. Study No. 871225. 1988, page 403, vol. 6.

First Annual Report (Serial No. 057) was submitted on Jan. 14, 1998.

NDA was submitted on March 30, 1998.

Studies Reviewed Within This NDA Submission:

- A. Repeat Dose Toxicity

1. 4-week subchronic toxicity study of Septanest 1/100,000 adrenaline by subcutaneous administration to Sprague-Dawley rats. (Report No. 10652/97, vol. 10, page 1).
2. 14-day dose-range finding study for a 4-week subchronic toxicity study of articaine hydrochloride by subcutaneous administration to beagle dogs. (Report No. 10376/97, vol. 12, page 1).
3. 4-week subchronic toxicity study of Septanest 1/100,000 adrenaline by subcutaneous administration to beagle dogs. (Report No. 10653/97, vol. 11, page 1).

B. Reproductive Toxicity:

1. Examination of the influence of Septanest 1/100,000 adrenaline on the fertility and early embryonic development to implantation of Sprague-Dawley rats by subcutaneous administration to the animals of the F₀ generation (Segment 1). (Report No. 10654/97, vol. 12, page 190).
2. Dose-range finding study to determine the dose levels for an examination of the influence of articaine hydrochloride in the pregnant rabbit and the fetus by subcutaneous administration. (Report No. 10379/97, vol. 13, page 322).
3. Dose-range finding study to determine the dose levels for an examination of the effects of articaine hydrochloride on the pre- and postnatal development of the rat embryo toxicity by subcutaneous administration to the dams of the F₀ generation. (Report No. 10137/96, vol. 12, page 64).
4. Examination of the influence of Septanest 1/100,000 adrenaline on the pregnant rabbit and the fetus by subcutaneous administration (embryo toxicity study/Segment II). (Report No. 10656/97, vol. 15, page 1).
5. Examination of the influence of Septanest 1/100,000 adrenaline on the pregnant rat and the fetus by subcutaneous administration. (Report No. 10655/97, vol. 13, page 1).
6. Examination of articaine hydrochloride for effects on the pre- and postnatal development (including maternal functions) following subcutaneous administration to the dams of the F₀ generation (Segment III study). (Report No. 10138/96, vol. 14, page 1)
7. Examination of Septanest 1:100,000 adrenaline for effects on the pre- and postnatal development (including maternal functions) following subcutaneous administration to the dams of the F₀ generation (Segment III study). (Report No. 10657/97, vol. 16, page 1)

C. Mutagenic Potential

In Vitro

1. Mutagenicity study of the articaine hydrochloride in the Salmonella typhimurium reverse mutation assay (in vitro). (Report No. 10089/96, vol. 17, page 1).
2. In vitro assessment of the clastogenic activity of the articaine hydrochloride in cultured CHO-cells. (Report No. 10090/96, vol. 17, page 50).
3. Mutagenicity study of articaine hydrochloride in mammalian cells (V79) in the in vitro gene mutation assay (HPRT Test). (Report No. 10373/97, vol. 17, page 217).

In Vivo:

4. Micronucleus test of articaine hydrochloride in bone-marrow cells of the NMRI mouse by subcutaneous administration. (Report No. 10374/97, vol. 17, page 160).

5. Micronucleus test of Septanest 1/100,000 adrenaline in bone marrow cells of the NMRI mouse by subcutaneous administration (base set). (Report No. 10651/97, vol. 17, page 104).

D. Pharmacokinetics:

1. 4-week subchronic toxicity study of Septanest 1/100,000 adrenaline by subcutaneous administration to Sprague-Dawley rats. (Report No. 10652/97, vol. 10, page 1).
2. 4-week subchronic toxicity study of Septanest 1/100,000 adrenaline by subcutaneous administration to beagle dogs. (Report No. 10653/97, vol. 11, page 1).

E. Biotransformation:

1. Metabolism of articaine by human isoenzymes, protein binding, and stability of articaine in human plasma in vitro validation of the analytical method. (Vol. 21).

Studies not Reviewed Within This Submission: Old published studies performed with different formulations of articaine than formulation proposed for marketing in the United States. These abstracts and full reports are discussed in the Overall Summary and Evaluation section.

Notes - (1) Portions of this review were excerpted directly from the sponsor's submission.

- (2) All preclinical and clinical studies performed with Septanest^R did not contain An Articaine hydrochloride investigated by Hoechst A.G. and others did contain this preservative.
- (3) All preclinical studies, performed by — for Specialites Septodont, were conducted in accordance with FDA Good Laboratory Practice (GLP), as specified in 21CFR Part 58, dated April 1, 1991.
- (4) Sodium metabisulfite was included in the formulations used in the toxicity studies (see page 1 for complete formulation).

A. Repeat Dose Toxicity

1. 4-week subchronic toxicity study of Septanest 1/100,000 adrenaline by subcutaneous administration to Sprague-Dawley rats. (Report No. 10652/97, vol. 10, page 1).

Study N^o: 10652/97

Compound: Septanest 1/100,000 adrenaline

Batch No. RD 100/11

Appearance: Colorless, clear liquid, pH 3.6.

Route: Subcutaneous

Dose Levels: 0 (control), 25 (low dose), 50 (medium dose) and 100 (high dose) mg/kg/day

Administration volumes: 0.625, 1.25 and 2.5 ml/kg/day

Strain: Crl:CD^RBR, Sprague-Dawley rat

Source: _____

Number: 10/sex/group for toxicology and 18 animals/sex/group for toxicokinetic
[3 animals/sex/sampling time/group]

Weights: 70-78 g, 27 - 30 days old.

Study Site: _____

Sponsor: Septodont, France

GLP/QAU: Submitted with signatures.

Study Dates: Aug. 6 - Nov. 10, 1997

Results:

Observation: High dose - Pilo-erection for 24 hours from day 16. Dose-related haematomas in all animals.

Mortality: Control - zero, Low dose - zero
Intermediate dose - 2/10 males on days 16 and 27 respectively
High dose - 3/10 males and 2/10 females between days 6 and 10.

Body weights: Intermediate dose - 7%↓ in ♂ High dose - 9%↓ in ♂

Food and water consumption: No difference

Physical examination: No change of the eyes and the auditory acuity.

Hematology: Intermediate dose - Hemoglobin (6% in males), leucocytes (18% ↑ in ♂ and 30% in ♀) and reticulocyte ↑ (55% in ♂ and 109% in ♀)
High dose - Hemoglobin ↓ (7% in ♂ and 3% in ♀), leucocytes ↑ (47% in ♂ and 62% in ♀), reticulocytes ↑ (118% in ♂ and 199% in ♀) and platelets ↑ (8% in ♂ and 29% in ♀).

Clinical chemistry: Intermediate dose - Blood urea ↑ (24% in ♂ and 11% in ♀)
High dose - Total bilirubin ↑ (26% in ♂ and 10% in ♀), blood urea ↑ (22% in ♂ and 15% in ♀), ALAT ↑ (30% in ♂ and 42% in ♀) and ASAT ↑ (77% in ♂ and 96% in ♀).

Urinalysis: No influence

Pathology: No difference. (eschar formation and subcutaneous haematomas at the injection sites in all treated animals).

Organ weights: The absolute and relative weights were within control range

Histopathology: Skin ulceration with eschar formation, epidermal/dermal and subcutaneous necrosis, inflammatory cell infiltration, granulation tissue, hemorrhage, oedema, muscular necrosis, atrophy and regeneration of adjacent skeletal muscle, epidermal hyperplasia and alopecia at the injection sites as compared to the control group. Extra-medullary haematopoiesis was also increased in all treated animals.

Toxicokinetics:

Mortality observed for the animals scheduled for kinetics:

| Group | Animal No. | Sex | Day of Death |
|-------------------|------------|--------|--------------|
| 3 (50 mg/kg/day) | 148 | female | 16 |
| 3 | 152 | male | 22 |
| 4 (100 mg/kg/day) | 165 | male | 14 |
| 4 | 167 | male | 25 |
| 4 | 183 | female | 7 |
| 4 | 184 | female | 28 |
| 4 | 185 | female | 23 |
| 4 | 187 | female | 6 |

Mean values in ng articaïne (free base) per ml plasma on the test day 1.[3 animals/sex/sampling time/group]

| T (min) | 25 mg/kg/day | | 50 mg/kg/day | | 100 mg/kg/day | |
|---------|--------------|----------|--------------|----------|---------------|----------|
| | Males | Females | Males | Females | Males | Females |
| 10 | 911±625 | 918±373 | 1501±74 | 1848±752 | 2821±604 | 2623±954 |
| 20 | 1349±492 | 1170±232 | 2075±418 | 2384±254 | 4362±167 | 4846±203 |
| 40 | 1779±92 | 1636±590 | 3588±198 | 3898±112 | 5678±186 | 7388±285 |
| 90 | 499±58 | 708±106 | 1624±38 | 1973±325 | 3767±375 | 3542±454 |

Mean articaïne values (ng, free base) per ml plasma on test day 28
[3 animals/sex/sampling time/group]

| Time (min) | 25 mg/kg day | | 50 mg/kg/day | | 100 mg/kg/day | |
|------------|--------------|----------|--------------|-------------------|---------------|-------------------|
| | Males | Females | Males | Females | Males | Females |
| 10 | 1912±195 | 2052±221 | 2065±199 | 1582 ¹ | 1963±296 | 1595 ¹ |
| 20 | 102±46 | 177±126 | 297±102 | 375 ¹ | 5732 | 3 |
| 40 | 789±676 | 962±796 | 1070±778 | 500 ² | 1567±108 | 2113 ¹ |
| 90 | 550±137 | 679±453 | 742±249 | 834 ¹ | 6532 | 3 |

1 - 2 surviving animals
3 - no surviving animal
Limit of quantification - \lesssim ng/ml

2 - 1 surviving animal
Limit of detection - \rightarrow ng/ml

Area under the curve ($\mu\text{g}\cdot\text{min}/\text{ml}$) [mean values]

| Dose level (mg/kg/day) | Day 1 | | Day 28 | |
|---------------------------|-------|---------|--------|---------|
| | Males | Females | Males | Females |
| 25 | 99.5 | 97.1 | 52.5 | 63.6 |
| 50 | 204.8 | 230.8 | 70.8 | 51.9 |
| 100 | 372.4 | 372.4 | 89.6 | 81.9 |

In general, there was a large scatter between the individual animals.

2. 14-day dose-range finding study for a 4-week subchronic toxicity study of articaine hydrochloride by subcutaneous administration to beagle dogs. (Report No. 10376/97, vol. 12, page 1).

Study No: No. 10376/97

Compound: Articaine hydrochloride, batch No. 96.444

Appearance: White to almost white powder

| | | | | |
|--------------|---|-------------------|----------------------------|---------------------|
| Dose Levels: | 1 | Control | 1 σ and 1 φ | 1 ml of aqua/kg/day |
| | 2 | Low dose | 1 σ and 1 φ | 25 mg/kg/day |
| | 3 | Intermediate dose | 1 σ and 1 φ | 50 mg/kg/day |
| | 4 | High dose | 1 σ and 1 φ | 100 mg/kg/day |

Route: Subcutaneous

Duration: 14 days

Administration Volume: 1 ml/kg/day

Species: Dog/Beagle

Source: _____

Number: 8 animals

Weights: 8.3 - 14.0 kg, 10 - 11 months old

Study Site: _____

Sponsor: Septodont, France

GLP/QAU: Statements submitted with signatures

Results:

Observation: 50 & 100 mg/kg/day - vomiting, defecation, scratching at injection site, convulsions and tremor within 5 to 60 min of injection.

Mortality: None

Body weights: No effect

Food and Drinking Water Consumption: No difference

Electrocardiography: No substance related effect on the heart rate, P-Q, Q-T and QRS intervals.

Conclusion: 25 mg/kg/day of articaine hydrochloride by subcutaneous administration in dogs for 2 weeks may be the NOEL(no-observed-effect level) dose.

3. 4-week subchronic toxicity study of Septanest 1/100,000 adrenaline by subcutaneous administration to beagle dogs. (Report No. 10653/97, vol. 11, page 1).

Study N^o: 10653/97

Compound: Septanest 1/100,000 adrenaline, Batch Numbers RD 100/10 & RD 100/11

Appearance: Clear colorless liquid

Volume Given: Ready-to-use solution as supplied by the sponsor

| | |
|-----------------------------|----------------|
| 1 - Control group | 2 ml. water/kg |
| 2 - Low dose group | 0.5 ml/kg |
| 3 - Intermediate dose group | 1 ml/kg |
| 4 - High dose group | 2 ml/kg |

Route: Subcutaneous injection

Dose Levels:

| | | |
|-------------------------|--------------|---------------|
| Control group | 0 mg/kg/day | 3 animals/sex |
| Low dose group | 20 mg/kg/day | 3 animals/sex |
| Intermediate dose group | 40 mg/kg/day | 3 animals/sex |
| High dose group | 80 mg/kg/day | 3 animals/sex |

Source: _____

Number: 24 animals (12 males and 12 females)

Weights: Males - 11.2 - 14.7 kg, Females - 10.9 - 13.2 kg

Age: 10 - 12 months

Study Site: _____

Sponsor: Septodont, France.

Monitor: _____

Experimental Period: July 30 to Aug. 27, 1997

GLP/QAU Statements: Submitted with signatures.

Results:

Local tolerance (injection sites): Subcutaneous indurations and palpable masses in all animals.

Observation: High dose group - Vomiting, salivation, ataxia, sedation, defecation, and clonic or tonoclonic convulsions.

Mortality: None

Body weights: Not effected.

Food and water consumption: No effect

Physical examination: (eyes and hearing): No changes

ECG: No substance-related changes in the heart rate, P-Q, Q-T, QRS intervals and QTC value.

Blood pressure: Within normal range

Hematology: No substance-related changes

Clinical chemistry: High dose group - ALAT ↑ (130% in ♂ and 134% in ♀), ASAT ↑ (112% in ♂ and 80% in ♀)

Urinalysis: No difference

Pathology: No substance-related findings

Organ weights: Within control range

Histopathology: No dose-related findings.

Toxicokinetics

Mean values in ng articaïne (free base) per ml plasma on test day 1.
[3 animals/sex/group]

| Time (min) | 20 mg/kg/day | | 40 mg/kg/day | | 80 mg/kg/day | |
|------------|---------------|---------------|--------------|--------------|--------------|---------------|
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| 10 | 1750± 1194 | 1048± 1315 | 1120± 235 | 590± 276 | 1378± 892 | 3749± 4490 |
| 20 | 2187± 1356 | 1393± 1178 | 1786± 355 | 930± 125 | 2083± 928 | 4340± 4373 |
| 40 | 2168± 1029 | 1537± 625 | 2508± 311 | 1690± 143 | 2940± 787 | 4162± 2718 |
| 90 | 1379± 206 | 880± 88 | 1481± 226 | 1235± 187 | 2736± 708 | 2542± 607 |

Mean values in ng/ml plasma on test day 28.

| Time (min) | 20 mg/kg/day | | 40 mg/kg/day | | 80 mg/kg/day | |
|------------|---------------|--------------|--------------|--------------|---------------|---------------|
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| 10 | 2676± 1211 | 1273± 598 | 1566± 941 | 1900± 680 | 8840± 2356 | 3747± 1142 |
| 20 | 2665± 1260 | 1820± 574 | 2071± 701 | 2314± 815 | 8605± 1119 | 4714± 906 |
| 40 | 2256± 692 | 1679± 523 | 1882± 545 | 2101± 398 | 5759± 1785 | 4492± 534 |
| 90 | 1176± 326 | 1003± 158 | 1021± 94 | 1372± 170 | 3023± 875 | 2607± 283 |

There was a large scatter between the individual animals as shown below.

Area under the curve (ng.min/ml) [individual values]

| Dose Level | Dog No. | Sex | Test Day 1 | Test Day 28 |
|--------------|---------|-----|------------|-------------|
| 20 mg/kg/day | 7 | M | | |
| | 8 | M | | |
| | 9 | M | | |
| | 10 | F | | |
| | 11 | F | | |
| | 12 | F | | |
| 40 mg/kg/day | 13 | M | | |
| | 14 | M | | |
| | 15 | M | | |
| | 16 | F | | |
| | 17 | F | | |
| | 18 | F | | |
| 80 mg/kg/day | 19 | M | | |
| | 20 | M | | |
| | 21 | M | | |
| | 22 | F | | |
| | 23 | F | | |
| | 24 | F | | |

Area under the curve (ng.min/ml) [mean values]

| Dose (mg/kg/day) | Test day 1 | | Test day 28 | |
|---------------------|------------|--------|-------------|--------|
| | ♂ | ♀ | ♂ | ♀ |
| 20 | 151913 | 101932 | 175098 | 123920 |
| 40 | 157185 | 106930 | 136227 | 162030 |
| 80 | 209425 | 293055 | 494760 | 331368 |

Dose-related increase in plasma C_{max} and area under the curve (AUC) was exhibited in this subcutaneous dog study. In general, there was a large scatter between the individual animals. The maximum articaïne plasma levels were reached ~20 min after dosing.

B. Reproductive Toxicity:

1. Examination of the influence of Septanest 1/100,000 adrenaline on the fertility and early embryonic development to implantation of Sprague-Dawley rats by subcutaneous

administration to the animals of the F₀ generation (Segment 1). (Report No. 10654/97, vol. 12, page 190).

Study No: No. 10654/97

Compound: 1/100,000 adrenaline, batch no. RD 100/12

Appearance: Clear, colorless liquid

Route: Subcutaneous

Duration of treatment: Males - Daily from 4 weeks before mating to end of mating period.

Females - Daily from 2 weeks before mating to the 7th day of pregnancy

Dose Levels: 20, 40 and 80 mg/kg/day

Volume given: 0.5, 1.0 and 2.0 ml/kg

Strain: Crl:CD^R BR: Sprague-Dawley rats

Source:

| | | |
|---------|--------------|-------------------------|
| Number: | Control | 20 males and 20 females |
| | Low dose | 20 males & 20 females |
| | Intermediate | 20 males & 20 females |
| | High | 20 males & 20 females |

Weights: Males - 251-278 g, Females - 165-192g

Age: 8 weeks

Study Site:

Sponsor: Septodont, France

Monitor:

GLP/QAU Statements: Both submitted with signatures

Observations:

| | |
|-------------------|--|
| Clinical Signs: | Daily |
| Viability: | Twice daily |
| Body weight: | Males - weekly, Females - daily from gestation |
| Food Consumption: | Daily during pregnancy |

Examination of fertility:

The ovaries and uteri were removed on day 13 of pregnancy and following parameters were determined:
number of fetuses and placentas
number and size of resorptions
corpora lutea, implantation sites, resorptions, placentae and fetuses
external examination of fetuses

Autopsy (F₀-generation parent animals): Ovary, uterus, testis, epididymis, prostate gland, seminal vesicle and coagulating gland.

Statistical evaluation: Student's test and Dunnett test

Results:

1. F₀ Maternal Observations: Dose-related scab formation at the injection sites.
2. Mortality: High dose - 3 males and 1 female.
3. Clinical Signs: Intermediate dose - Reduced motility, tonic/tonoclonic convulsion in 1 ♂
High dose - vomiting, reduced motility, tonic/tonoclonic convulsions,
increased respiratory rate and/or abdominal position in 15/20 ♂ and
7/20 ♀.
4. Body weight: High dose - Males (5-9 % ↓), females (2-4 % ↓)
5. Food consumption: No effect
6. Fertility: No dose-related effect
7. Sperm number, motility and viability: No effect
8. Early embryonic development - No drug-related effect
9. Macroscopic post mortem findings: None
10. Uterine and testicular weight within the normal range.

2. Dose-range finding study to determine the dose levels for an examination of the influence of articaine hydrochloride in the pregnant rabbit and the fetus by subcutaneous administration. (Report No. 10379/97, vol. 13, page 322).

Study No: — No. 10379/97

Compound: Articaine hydrochloride, batch no. 96,444

Appearance: Clear, white powder

Route: Subcutaneous

Duration of treatment: Sixth to 18th day of pregnancy

Strain: Himalayan rabbit

Source: _____

| | | | | |
|---------|---------|------------------|-----|---------------|
| Number: | Group 1 | Control | 2 ♀ | |
| | Group 2 | Low dose | 2 ♀ | 20 mg/kg/day |
| | Group 3 | Low intermediate | 2 ♀ | 40 mg/kg/day |
| | Group 4 | Intermediate | 2 ♀ | 80 mg/kg/day |
| | Group 5 | High | 2 ♀ | 120 mg/kg/day |

Study Site: _____

Sponsor: Septodont, France.

Monitor: _____

GLP/QAU Statements: Both submitted with signatures

Observations:

Clinical signs, viability, body weight, and food consumption: Daily

Examination of fertility:

The ovaries and uteri were removed on day 29 of pregnancy and following parameters were determined:
number of fetuses and placentae
number and size of resorptions

corpora lutea, implantation sites, resorptions, placentae and fetuses
external examination of fetuses

Statistical evaluation: None due to low number of animals per group.

Results:

1. F₀ Maternal Observations: Low dose - No effect
Group 3 -5 - Dose-related scab formation at the injection sites.
2. Mortality: High dose - Both animals died on gestation days 13 and 14.
3. Clinical Signs: Intermediate dose - Reduced motility, tonic/tonoclonic convulsion in both ♀.
High dose - Reduced motility, tonic/tonoclonic convulsions, slight tremor, increased or decreased respiratory rate.
4. Body weight: High dose - Decreased
5. Food consumption: High Dose - Reduced
6. Early embryonic development - No drug-related effect
7. Macroscopic post mortem findings: None

3. Dose-range finding study to determine the dose levels for an examination of the effects of articaine hydrochloride on the pre- and postnatal development of the rat embryo toxicity by subcutaneous administration to the dams of the F₀ generation. (Report No. 10137/96, vol. 12, page 64).

Study No: 10137/96

Compound: Articaine hydrochloride, batch no. 96,444

Appearance: White powder

Volume Given: 2 ml/kg

Route: Subcutaneous

Duration of treatment: Sixth to 17th day of pregnancy

Strain: Mol: — Sprague-Dawley rat

Source:

| | | | |
|------------------|---------|---------------|-----|
| Dose and Number: | Group 1 | Control | 2 ♀ |
| | Group 2 | 25 mg/kg/day | 2 ♀ |
| | Group 3 | 50 mg/kg/day | 2 ♀ |
| | Group 4 | 100 mg/kg/day | 2 ♀ |
| | Group 5 | 200 mg/kg/day | 2 ♀ |
| | Group 6 | 300 mg/kg/day | 2 ♀ |
| | Group 7 | 500 mg/kg/day | 2 ♀ |

Study Site:

Sponsor: Septodont, F-94110 Saint-Maur des Fosses, France

Monitor:

GLP/QAU statements: Both submitted with signatures

Examination: The rats were sacrificed on 20th day of gestation and examined for:

- (1) Number of fetuses and placenta
- (2) Determination of sex and viability of fetuses
- (3) Number and size of resorptions.
- (4) Number of corpora lutea
- (5) Weight of fetuses and placentae
- (6) Examination of the fetuses for malformations

Results:

Observation: Dose dependent induration at the injection sites in all animals.

Mortality: 300 mg/kg - 1 (50%), 500 mg/kg - 2 (100%)

Clinical Signs: 200 mg/kg - Pilo-erection

300-500 mg/kg - - Pilo-erection, tremor and reduced motility.

Body weights: 300 & 500 mg/kg - Reduced

Food and water consumption: Reduced in 300 & 500 mg/kg animals.

Autopsy Findings: Dark-red discolored lungs of deceased animals

Influence on the fetus: 25, 50 and 100 mg/kg - No effect

200 and 300 mg/kg - Dose-dependent reduction in fetal and placental weights.

No malformation or external variations

4. Examination of the influence of Septanest 1/100,000 adrenaline on the pregnant rabbit and the fetus by subcutaneous administration (embryo toxicity study / Segment II). (Report No. 10656/97, vol. 15, page 1).

Study No: — No. 10656/97

Compound: Septanest 1/100,000 adrenaline, batch # RD100/12

Appearance: Clear, colorless liquid

Volume Given: 0.5, 1.0 and 2 ml/kg

Route: Subcutaneous

Dose Levels: 20, 40 and 80 mg/kg

Treatment Period: from the 6th to 20th day of pregnancy

Strain: Himalayan rabbit

Source: _____

| | | | | |
|---------|-----------|-------------------|----------|------------|
| Number: | Group I | Control | 0 mg/kg | 16 females |
| | Group II | low dose | 20 mg/kg | 16 females |
| | Group III | Intermediate dose | 40 mg/kg | 16 females |
| | Group IV | high dose | 80 mg/kg | 16 females |

Weights: 2.55 - 3.70 kg.

Age: 6 - 6.5 months

Study Site: _____

Sponsor: Septodont, France

Monitor: _____

GLP/QAU Statements: Submitted with signatures.

Examination: The animals were sacrificed on 29th day of gestation and examined for:

- (1) Number of fetuses and placenta
- (2) Determination of sex and viability of fetuses
- (3) Number and size of resorptions.
- (4) Number of corpora lutea
- (5) Weight of fetuses and placentae
- (6) Examination of the fetuses for malformations

Results:

Observation: Dose related red area around the injection sites

Mortality: 80 mg/kg - 2 (one on gestation day 6 and other on gestation day 18).

Body Weights: No effect

Clinical Signs: 40 mg/kg - tremors (1 animal), tono-clonic convulsion (1 animal)

80 mg/kg - reduced motility, lateral and/or abdominal position, tono-clonic convulsions and slightly increased respiratory rate in most animals.
Tremor, increased salivation, sedation and dyspnoea in some animals.

Food consumption: Normal (21% ↓ in high dose group on gestation days 5 and 6 only) -

Water consumption: No effect

Autopsy Findings: No drug-related systemic pathological findings.

Reproduction data of dams: No differences

Influence on the Fetus:

Corpora leutea, implantation sites, resorptions and placentae - No prenatal effect.

Body weight: No differences

Viability - Within normal range except significantly decreased at 80 mg/kg within 6 hours.

Malformations - Skeletal and external examinations showed no malformed fetus

Variations - No drug-related variations

Retardations - No difference

5. Examination of the influence of Septanest 1/100,000 adrenaline on the pregnant rat and the fetus by subcutaneous administration (Embryotoxicity study / Segment II study). (Report No. 10655/97, vol. 13, page 1).

Study N^o: 10655/97 -in accordance with ICH guideline 4.1.3.

Compound: Septanest 1/100,000 adrenaline, used as supplied by the sponsor.

Appearance: Clear, colorless liquid

Volume Given: 0.5, 1 and 2 ml/kg

Route: Subcutaneous

Dose Levels: 20, 40 and 80 mg/kg

Administration Volume: 0.5, 10 and 2.0

Duration of Dosing: From 6th to 17th day of pregnancy

Species / Strain / Stock: Rat / Sprague-Dawley / _____

Source: _____

| | | | | |
|---------|-------------------|----------|-----------|------|
| Number: | Control | 0 mg/kg | 2.0 ml/kg | 20 ♀ |
| | Low dose | 20 mg/kg | 0.5 ml/kg | 20 ♀ |
| | Intermediate dose | 40 mg/kg | 1.0 ml/kg | 20 ♀ |
| | High dose | 80 mg/kg | 2.0 ml/kg | 20 ♀ |

Weights: 197 - 300 g

Study Site: _____

Sponsor: Septodont, France.

Monitor: _____

Date: Oct. 29, 1997.

GLP/QAU Statements: Both present with signatures

Examination: The animals were sacrificed on 20th day of gestation and examined for:

- (1) Number of fetuses and placenta
- (2) Determination of sex and viability of fetuses
- (3) Number and size of resorptions.
- (4) Number of corpora lutea
- (5) Weight of fetuses and placentae
- (6) External inspection of fetuses for damage, especially malformations
- (7) Examination of fetuses and determination of number and type of retardation, variations or malformations.

Results:

Observation: Dose-related reddened area around the injection sites

Mortality: None

Clinical Signs: 80 mg/kg - reduced motility and tremor in one animal on gestation day 12.

Body weights: 80 mg/kg - 28% ↓ on gestation day 18 to 20

Food and water consumption: 80 mg/kg - food consumption was reduced on gestation days 12 and 15. Water consumption was not effected.

Autopsy Findings: None

Influence on the Fetus:

Corpora lutea/implantation sites/resorption/weight and number of fetuses
alive/placental weight: None

External, skeletal and soft tissue examination: No malformed fetuses

Variations: None

Retardations: No differences in skeletal retardations

6. Examination of articaine hydrochloride for effects on the pre- and postnatal development (including maternal functions) following subcutaneous administration to the dams of the F₀ generation (Segment III study). (Report No. 10138/96, vol. 14, page 1)

Study N^o: 10138/96

Compound: Articaine hydrochloride, batch no. 96.444

Volume Given: 2 ml Route: Subcutaneous

Treatment of F0-generation: from implantation (6th day of gestation) to weaning (22nd day of lactation)

Observation Period: F1- and F2-generations

Species: Rat Strain: Sprague-Dawley

Breeder: _____

Number of animals used for the Fo-generation:

| | | | |
|---------|-------------------|------|-----------------|
| Group 1 | Control | 24 ♀ | Control vehicle |
| Group 2 | Low dose | 24 ♀ | 20 mg/kg |
| Group 3 | Intermediate dose | 24 ♀ | 40 mg/kg |
| Group 4 | High dose | 24 ♀ | 80 mg/kg |

Weights: 179-218 g

Study Site: _____

Sponsor: Septodont, F-94100 Saint-Maur des Fosses, France

Monitor: _____

GLP/QAU statements: Both submitted with signatures

Observation:

Clinical signs and Viability: Daily

Body weights:

F0 -, F1- generations: Daily during gestation and then on days 1, 7, 14, 21, and 22 of lactation.

F2 litters: On days 1, 4, 7, 14, and 21 of lactation.

Food consumption: weekly

Examination: The animals were allowed to deliver normally and examined for:

- (1) Number of pups at birth and 4, 7, 14, and 21 days after birth
- (2) Number of pups per dam
- (3) Determination of sex and viability of fetuses
- (4) Number of pups with stillbirths
- (5) Number of pups with malformations

Reproductive indices: Gestation, birth, live birth, viability, lactation, and overall survival.

Post natal physical and functional development::

| <u>Functional test</u> | <u>age of pups(days)</u> | <u>pups to be examined</u> |
|------------------------------|--------------------------|-----------------------------|
| Mid-air righting reflex | 14 | each |
| Auditory startle reflex | 14 | each |
| Pupillary reflex | 21 | each |
| Open field | 27±1 | first half of each litter . |
| Passive avoidance (learning) | 27±1 | second half of each litter |
| Passive avoidance (memory) | 34±1 | second half of each litter |

Morphological landmarks

| | | |
|--|----|------|
| Pinna detachment | 1 | each |
| Ear opening | 12 | each |
| Eye opening | 11 | each |
| Cleavage of the balanopreputial gland | 25 | each |
| Vaginal opening | 33 | each |
| Upper incisor eruption | 7 | each |

Termination / Autopsy:

- Fo-generation - Day 22 of lactation
- F1 pups not selected - Day 22 of lactation
- F1 parents - Males at the end of mating period
 - Females at the end of lactation period
- F2 offspring - After 3 lactation weeks

Results:**Fo-dams**

- Local tolerance: Dose-related scab formation at the injection site.
- Clinical signs: High dose - Tremor (1/24), reduced motility (2/24), vomiting (1/24), increased respiration (1/24), convulsion (1/24).
- Mortality: High dose - One (15 th day of gestation)
- Body weights: No effect (3% ↓ in high dose animals)
- Food consumption: High dose - 7 -19% ↓
- Examination of the dams at termination: No influence at 20, 40 and 80 mg/kg/day.
- Reproduction: No influence on duration of pregnancy and number of live pups.
 - Ten stillbirths (control 3, low dose 0, middle dose 0) at 80 mg/kg/day.
- Macroscopic post mortem finding: No systemic change

F1-generation (until weaning)

- Sex distribution: No difference
- Body weight: No effect
- External examination: No differences
- Morphological landmarks, functional tests, open-field test: Eye opening was delayed and reduced ability to pass the passive avoidance test in high dose animals.

| Criteria | Group/dose level (mg/kg) | | | |
|---|--------------------------|-----------|-----------|-----------|
| | 1(control) | 2 (20) | 3 (40) | 4 (80) |
| Eye opening: mean day of life | 14.7±0.6 | 15.3±0.9 | 15.4±0.9 | 15.5±0.6 |
| Passive avoidance test - memory % of negative findings | 9.0±22.9 | 7.6±12.7 | 3.0±9.8 | 9.8±16.3 |
| Passive avoidance test -learning % of negative finding | 16.4±25.5 | 10.0±12.1 | 12.0±19.3 | 32.6±28.7 |

Macroscopic post mortem findings: None

F1-dams and male F1-partners

Mortality: None
 Clinical signs: Nothing
 Body weight: High dose - Slightly reduced
 Food consumption: No differences
 Reproduction: No drug-related influence
 Macroscopic post mortem finding: No differences

F2-generation (until weaning)

Sex distribution: No differences
 Body weight: Within normal range
 External examination: No differences

7. Examination of Septanest 1:100,000 adrenaline for effects on the pre- and postnatal development (including maternal functions) following subcutaneous administration to the dams of the F₀ generation (Segment III study). (Report No. 10657/97, vol. 16, page 1)

Study N^o: 10657/97

Compound: Septanest 1/100,000 adrenaline; Batch No. RD100/11

Administered Volume: 0.5, 1.0, and 2.0 ml

Route: Subcutaneous

Treatment of Fo-generation: from implantation (6th day of gestation) to end (22nd day) of lactation

Observation Period: F0, F1- and F2-generations

Species: Rat

Strain: Sprague-Dawley / Cri:CDRBR

Breeder: _____

Number of animals used for the Fo-generation:

| | | | |
|---------|-------------------|------|-----------------|
| Group 1 | Control | 24 ♀ | Control vehicle |
| Group 2 | Low dose | 24 ♀ | 20 mg/kg |
| Group 3 | Intermediate dose | 24 ♀ | 40 mg/kg |
| Group 4 | High dose | 24 ♀ | 80 mg/kg |

Weights: 186-238 g

Study Site: _____

Sponsor: Septodont, F-94100 Saint-Maur des Fosses, France

Monitor: _____

GLP/QAU statements: Both submitted with signatures

Observation:

Clinical signs and Viability: Daily

Body weights:

F0 -, F1- generations: Daily during gestation and then on days 1, 7, 14, 21, and 22 of lactation.

F2 litters: On days 1, 4, 7, 14, and 21 of lactation.

Food consumption: weekly

Examination: The animals were allowed to deliver normally and examined for:

- (1) Number of pups absolute at birth and 4, 7, 14, and 21 days after birth
- (2) Number of pups per dam
- (3) Determination of sex and viability of fetuses
- (4) Number of pups with stillbirths
- (5) Number of pups with malformations

Reproductive indices: Gestation, birth, live birth, viability, lactation, and overall survival.

Post natal physical and functional development::

| <u>Functional test</u> | <u>age of pups(days)</u> | <u>pups to be examined</u> |
|------------------------------|--------------------------|----------------------------|
| Mid-air righting reflex | 14 | each |
| Auditory startle reflex | 14 | each |
| Pupillary reflex | 21 | each |
| Open field | 27±1 | first half of each litter |
| Passive avoidance (learning) | 27±1 | second half of each litter |
| Passive avoidance (memory) | 34±1 | second half of each litter |

Morphological landmarks

| | | |
|--|----|------|
| Pinna detachment | 1 | each |
| Ear opening | 12 | each |
| Eye opening | 11 | each |
| Cleavage of the balanopreputial gland | 25 | each |
| Vaginal opening | 33 | each |
| Upper incisor eruption | 7 | each |

Termination / Autopsy:

Fo-generation - Day 22 of lactation

F1 pups not selected - Day 22 of lactation

F1 parents - Males at the end of mating period

- Females at the end of lactation period

F2 offspring - After 3 lactation weeks

Results:

Fo-dams

Local tolerance: Dose-related scab formation at the injection site.

Clinical signs: None.

Mortality: Intermediate dose - One

High dose - Three (18 ,9 18 th days of gestation)

Body weights: No effect
Food consumption: High dose - 8 - 18% ↓
Reproduction: No influence (pregnancy, parturition, birth index, viability index, lactation and overall survival)
Macroscopic post mortem finding: Unremarkable

F1-generation (until weaning)

Sex distribution: No difference
Body weight: No effect
External examination: No abnormalities.
Morphological landmarks, functional tests, open-field test: No differences in pinna detachment, upper incisor eruption, ear and eye opening, and vaginal opening.
No differences in functional tests
Macroscopic post mortem findings: None

F1-dams and male F1-partners

Mortality: None
Clinical signs: Nothing
Body weight: Within normal range
Food consumption: No differences
Reproduction: No drug-related influence
Macroscopic post mortem finding: No differences

F2-generation (until weaning)

Sex distribution: No differences
Body weight: Within normal range
External examination: No differences

C. Mutagenic Potential

In Vitro:

1. Mutagenic study of the articaïne hydrochloride in the Salmonella typhimurium reverse mutation assay (in vitro). (Report No. 10089/96, vol. 17, page 1).

Study: — Protocol No.10089/96

Study Site: _____

Sponsor: Septodont, F-94100 Saint-Maur des Fosses, France

Monitor: _____

GLP/QAU Statements: Both submitted with signatures

Final Report: Oct. 20, 1997

Compound: Articaïne hydrochloride, batch no. 96442

Appearance: White to almost white clearance

Cells: Strains: Five strains of Salmonella typhimurium

Concentrations: five /independent experiment (100 to 10,000 ug articaïne HCl/plate)

Plates: 3 per concentration and experiment

Data: Two independent experiments with and without metabolic activation using five tester strains, TA 98, TA 100, TA 102, TA 1535, TA 1537

Positive control substances:

Without metabolic activation

| | |
|----------------------------------|-----------------|
| Sodium azide in water | TA 1535, TA 100 |
| 2-nitro-9H-fluorene in DMSO | TA 98 |
| 9-aminoacridine in ethanol | TA 1537 |
| methyl methane sulfonate in DMSO | TA 102 |

with metabolic activation (S9)

2-anthraceneamide in DMSO all test strains

Result

No mutagenic effect was observed in any of the five tester strains in the presence or absence of activator.

2. In vitro assessment of the clastogenic activity of the articaïne hydrochloride in cultured CHO-cells. (Report No. 10090/96, vol. 17, page 50).

Study N°: No. 10090/96

Study Site: _____

Sponsor: Septodont, F-94100 Saint-Maur des Fosses, France

Monitor: _____

GLP/QAU statements: Both submitted with signatures

Final Report: Oct. 15, 1997

Compound: Articaïne hydrochloride, batch no. 96,442

Appearance: White to almost white powder

Results:

| Treatment (µg/ml medium) | 4-hours exposure | | | 20-hours exposure | | |
|--------------------------------|------------------|----------------------------------|--|-------------------|----------------------------------|--|
| | Mitotic index | Number of Metaphases Score | % of cells with aberration excluding gaps | Mitotic index | Number of Metaphases cored | % of cells with aberration excluding gaps |
| without metabolic activation | | | | | | |
| Test medium (vehicle) | | | | | | |
| 0 | 1.00 | 200 | 3.5 | 1.00 | 200 | 2.5 |
| Articaine hydrochloride | | | | | | |
| 125 | - | - | - | 0.99 | 200 | 3.0 |
| 250 | 0.85 | 200 | 3.5 | 0.94 | 200 | 2.5 |
| 500 | 0.78 | 200 | 4.0 | 0.51 | 200 | 4.0 |
| 1000 | 0.87 | 200 | 5.5 | 0.19 | 95 | 6.3 |
| 2000 | 0.58 | 200 | 10.0 | - | - | - |
| Mitomycin C | | | | | | |
| 0.1 | 0.41 | 200 | 11.5 | 0.56 | 200 | 20.5 |
| With metabolic activation | | | | | | |
| Test medium (vehicle) | | | | | | |
| 0.0 | 1.00 | 200 | 3.5 | - | - | - |
| Articaine hydrochloride | | | | | | |
| 500 | 0.82 | 200 | 6.0 | - | - | - |
| 1000 | 0.84 | 200 | 6.0 | - | - | - |
| 2000 | 0.85 | 200 | 6.5 | - | - | - |
| 3000 | 0.50 | 124 | 7.3 | - | - | - |
| Cyclophosphamide | | | | | | |
| 5 | 0.75 | 200 | 17.0 | - | - | - |

Articaine hydrochloride up to cytotoxic concentration did not reveal any indication of mutagenic properties with respect to chromosomal or chromatid damage.

3. Mutagenicity study of articaine hydrochloride in mammalian cells (V79) in the in vitro gene mutation assay (HPRT test). (Report No. 10373/97, vol. 17, page 217).

Study N^o: — No. 10373/97

Study Site: _____

Sponsor: Septodont, F-94100 Saint-Maur des Fosses, France

Monitor: _____

Final Report: Oct. 6, 1997

GLP/QAU statements: Both submitted with signatures

Compound: Articaine hydrochloride, batch no. 96.444

Appearance: White to almost white powder.

Cells: V79 (derived from lung tissue of fetal hamsters).

Results:

Preliminary test - Articaine at 4000 µg/ml produced severe cytotoxicity. According to ICH guidelines (ICH S2, April 1996), highest concentration should produce at least 80 percent toxicity (no more than 20 percent survival). Therefore, this dose was selected to investigate the mutagenic potential of articaine in main studies.

Main Studies: In two independent experiments, Articaine up to 4000 µg/ml with and without metabolic activation (S9) did not induce mutation. The positive controls (ethyl methanesulfonate and 9,10-dimethyl-1,2-benzanthracene) generated a pronounced increase in the mutation frequencies.

In Vivo:

4. Micronucleus test of articaine hydrochloride in bone-marrow cells of the NMRI mouse by subcutaneous administration. (Report No. 10374/97, vol. 17, page 160).

Study N^o: — No. 10374/97

Study Site: _____

Sponsor: Septodont, F-94100 Saint-Maur des Fosses, France

Monitor: _____

Final Report: May 23, 1997

GLP/QAU statements: Both submitted with signatures

Compound: Articaine hydrochloride, batch no. 96.444

Appearance: White to almost white powder.

Species/ Strain/ Stock: Mouse/ NMRI/ Cri:NMRI BR

Breeder: _____

Number of Animals: 50 (25 males and 25 females), 5 animals/sex/group

Age and Body Weight: 25 - 27 days, 20 - 24 g body weight

Route of Administration: Subcutaneous injection

Dose level: 75 mg/kg b.w.

Vehicle: Aqua ad injectabilia

Administration volume: 20 ml/kg

7 pages redacted from this section of
the approval package consisted of draft labeling

Positive Control: Cyclophosphamide, 27 mg/kg, by intraperitoneal injection
 Sampling times: 24, 48, and 72 hours after treatment.

The study was carried out according to ICH2-safety - S2 - Genotoxicity, dated June 24, 1993.

Number of polychromatic erythrocytes scored per group; 10,000

Results:

| Compound (mg/kg) | Sam-pling time (h) | Ratio PCE/NCE | | | micronucleated polychromatic erythrocytes | | | Signi-fican-ce |
|-------------------------|--------------------|---------------|---------|-----------------|---|---------|-----------------|----------------|
| | | males | Females | Treatment group | males | Females | Treatment group | |
| Articaine hydrochloride | | | | | | | | |
| 0 | 24 | 1.31 | 1.15 | 1.23 | 0.6 | 1.0 | 0.8 | - |
| 75 | 24 | 1.12 | 1.53 | 1.32 | 0.6 | 1.6 | 1.1 | n.s. |
| 75 | 48 | 0.96 | 1.03 | 0.99 | 1.2 | 0.4 | 0.8 | n.s. |
| 75 | 72 | 1.18 | 1.20 | 1.19 | 1.8 | 1.2 | 1.5 | n.s. |
| Cyclophosphamide | | | | | | | | |
| 27 | 24 | 1.23 | 1.29 | 1.26 | 13.6 | 11.4 | 12.5 | S |

5. Micronucleus test of Septanest 1/100,000 adrenaline in bone marrow cells of the NMRI mouse by subcutaneous administration (base set). (Report No. 10651/97, vol. 17, page 104).

Study N^o: No. 10651/97

Study Site: _____

Sponsor: Septodont, F-94100 Saint-Maur des Fosses, France

Monitor: _____

GLP/QAU statements: Both submitted with signatures

Final Report: Oct. 29, 1997

Compound: Septanest 1/100,000 adrenaline, batch no. RD 100/10

Appearance: Clear, colorless liquid

Species/ Strain/ Stock: Mouse/ NMRI/ Crl:NMRI BR

Breeder: _____

Number of Animals: 50 (25 males and 25 females), 5 animals/sex/group

Age and Body Weight: 25 days (males), 27 days(females); 19 - 26 g body weight

Route of Administration: Subcutaneous injection

Dose level: 75 mg/kg b.w.

Vehicle: Aqua ad injectabilia

Administration volume: 20 ml/kg

Positive Control: Cyclophosphamide, 27 mg/kg, by intraperitoneal injection

Sampling times: 24, 48, and 72 hours after treatment.

The study was carried out according to ICH2-safety - S2 guidelines.

Number of polychromatic erythrocytes scored per group; 10,000

Results:

| Compound (mg/kg) | Sam- pling time (h) | <u>Ratio PCE/NCE</u> | | | <u>micronucleated polychromatic erythrocytes</u> | | | Signi- fican- ce |
|-------------------------|------------------------------|----------------------|---------|--------------------|--|---------|--------------------|------------------------|
| | | males | Females | Treatment group | males | Females | Treatment group | |
| Articaine hydrochloride | | | | | | | | |
| 0 | 24 | 0.87 | 1.04 | 0.95 | 1.6 | 2.8 | 2.2 | - |
| 75 | 24 | 1.04 | 1.0 | 1.02 | 1.4 | 2.2 | 1.8 | n.s. |
| 75 | 48 | 1.14 | 1.04 | 1.09 | 2.4 | 2.2 | 2.3 | n.s. |
| 75 | 72 | 1.09 | 1.27 | 1.18 | 2.4 | 3.8 | 3.1 | n.s. |
| Cyclophosphamide | | | | | | | | |
| 27 | 24 | 0.55 | 0.47 | 0.51 | 10.4 | 13.4 | 11.9 | S |

D. Pharmacokinetics:

1. 4-week subchronic toxicity study of Septanest 1/100,000 adrenaline by subcutaneous administration to Sprague-Dawley rats. (Report No. 10652/97, vol. 10, page 1). Reviewed under repeat dose toxicity studies section (page 6).

2. 4-week subchronic toxicity study of Septanest 1/100,000 adrenaline by subcutaneous administration to beagle dogs. (Report No. 10653/97, vol. 11, page 1). Reviewed under repeat dose toxicity studies section (page 8).

E. Biotransformation:

1. Metabolism of articaine by human isoenzymes, protein binding, and stability of articaine in human plasma, in vitro validation of the analytical method. (Vol. 21).

Study No: [REDACTED] No. 10337/97

Study Site: [REDACTED]

Sponsor: Septodont, F-94100 Saint-Maur des Fosses, France.

Monitor: [REDACTED]

GLP/QAU statements: Both submitted with signatures

Compounds: Articaine hydrochloride (batch no. 96-444) and articainic acid (batch no. 95527X)

Methods and Results:

Metabolism. Human liver microsomes were incubated with test substance at two concentrations (1 and 4 µg/ml) at 37 °C for 3 hours. A positive (ethoxyresofurin deethylase) and negative (without cytochrome P450 isoenzymes) controls were also performed. Articaine and articainic acid were measured by [REDACTED] method.

Result: The results indicated an almost quantitative recovery of the metabolized articaine as articainic acid.

Protein binding: Articaine was incubated at approx. 500 and 2000 ng/ml with human serum albumin, human α -globulins and a fraction of human α - and β -globulins. Protein bindings were determined after 6 and 24 hours incubations at 37 °C.

Result: A low protein binding was observed to α -globulin and higher binding to albumin and β - and γ -globulins.

Stability: The stability of articaine in pooled human serum was determined at two concentrations for 24 hours and 7 days at room temperature and -20 °C.

Result: Articaine was unstable without metabolizing enzyme inhibitor (sodium fluoride). The samples stored at -20 °C with esterase inhibitor was stable for one week.

2. In a published article [Pharmakokinetische untersuchungen mit ^{35}S -markiertem articaine] by R.E. Hoffer and H. Altmann (Prakt Anasth 1974;9:157-161), ^{35}S -labeled articaine was rapidly absorbed in the dwarf pig (100%) and in the human (85%) after IM injection.

The curves for blood levels in man following a single administration of articaine showed two distinct phases of different half-life.

| | I.V. administration | I.M. administration |
|---------|---------------------|---------------------|
| Phase 1 | 1.2±0.1 hours | 2.9±0.1 hours |
| Phase 2 | 69.7±16.7 hours | 31.5±6.6 hours |

Distribution of the radioactivity in dwarf pig (28 kg average body weight) after single administration expressed in Ci ³⁵S/gram of fresh weight is given below.

| Organs | After I.V. Injection | | After I.M. Injection | |
|---------------|----------------------|----------------|----------------------|----------------|
| | 15 min (n=1) | 48 hours (n=2) | 60 min. (n=1) | 48 hours (n=2) |
| Brain | 49.6 | 0.38 | 21.0 | 0.39 |
| Heart | 39.2 | 1.01 | 25.0 | 0.76 |
| Lung | 119.8 | 1.37 | 132.2 | 2.35 |
| Liver | 145.0 | 2.74 | 59.6 | 3.30 |
| Spleen | 76.7 | 1.09 | 35.6 | 1.40 |
| Right Kidney | 498.4 | 3.86 | 208.6 | 3.16 |
| Left Kidney | 470.4 | 2.97 | 157.1 | 3.25 |
| Diaphragm | 35.3 | 0.62 | 16.6 | 0.62 |
| Muscle | 40.6 | 0.42 | 34.7 | 0.50 |
| Abdominal Fat | 2.5 | 0.95 | 8.3 | 0.95 |
| Dorsal Fat | 30.0 | 1.26 | 16.1 | 0.67 |
| Whole Blood | 27.2 | 1.18 | 16.0 | 0.81 |

Carticaine and its metabolites were eliminated rapidly mainly in the urine in both species. The metabolism of Carticaine was different in man than in pig; in man no intact Carticaine was found in the urine.

Overall Summary and Evaluation

Septanest, an injectable local anesthetic for dental procedures, is 4% articaine hydrochloride with either 1:200,000 epinephrine (0.5 mg/100 ml Septanest; Septanest ^R or 1:100,000 epinephrine (1.0 mg/100 ml Septanest; Septanest ^R, plus sodium metabisulphite (0.05 g/100 ml) and sodium chloride (0.16 g/100 ml). Articaine hydrochloride has the chemical name 4-Methyl-3-[2-(propylamino)propion-amido]-2-thiophenecarboxylic acid, methyl ester hydrochloride. The second active component, epinephrine bitartrate, is a vasoconstrictor. The product is clear colorless solution and is provided in auto-injectable single-use glass cartridges with a total volume of 1.7 ml. Septanest is administered as a submucosal

infiltration or nerve block for routine dental procedures. Articaine is classified as a local anesthetic of the amide type similar to lidocaine, prilocaine, mepivacaine, and bupivacaine. Septanest has been registered in 13 European countries (France, 1988; Belgium, Holland, 1990; Germany, 1993; Spain, Switzerland, Russia, 1994; Italy, 1995; Austria, Poland, Czech Republic, _____ Hungary, 1997) and Canada (1994). The Septanest formulations currently marketed in Europe and Canada also contains 0.025 g/100 ml EDTA (preservative for epinephrine) and sodium metabisulphite concentration is 0.1g/100ml.

All preclinical toxicity studies performed by the _____ for the sponsor were performed with Septanest 1/200,000 and Septanest 1/100,000 without _____ An articaine hydrochloride investigated by Hoechst A.G. and others did contain this preservative. This preservative may increase the possibility for contamination and may decrease the prospect for related hyper sensitization and allergic reactions. The Hoechst studies had been published by Baeder et al. in 1974 (Prakt Anasth. 9:147-152)

Pharmacology

The results from preclinical studies demonstrate that articaine has a similar mechanism of action to other local anesthetics (lidocaine, procaine, prilocaine, mepivacaine, and bupivacaine). It reversibly blocks the conduction of painful sensations by diminishing the sodium ion influx during the action potential period. The duration of anesthesia was slightly increased after the addition of epinephrine or norepinephrine. The average duration of action in dogs by epidural administration was 120 ± 8 minutes for analgesia and 120 ± 6 minutes for motor block. Effects of articaine on the cardiovascular system are similar to those of other local anesthetics. All doses of standard anticonvulsant (sodium hexobarbital [16 and 30 mg/kg], propranolol [5 mg/kg], thiopental [20 mg/kg], caffeine [80 mg/kg], phenobarbital [40 and 80 mg/kg], and hexyltheobromine [80 mg/kg]) reduced convulsions caused by IV articaine (7.5, 10, and 12.5 mg/kg) in the rat.

Acute Toxicity

During single subcutaneous administration of Septanest without adrenaline, the LD₄₀ was 500 mg/kg for male mice (LD₅₀ >500 mg/kg). In the female mice, the LD₅₀ was 440 mg/kg. The LD₅₀ of articaine without epinephrine by intravenous administration was approximately 38 mg/kg in the mouse, 23 mg/kg in the rat and 20 mg/kg in the rabbit. The acute minimal lethal dose by intravenous administration in the dog was 56 mg/kg. The toxicity of I.V. articaine was greater in the presence of 1:100,000 epinephrine than without as determined in two species. The LD₅₀ was 3.7 mg/kg in mice that received articaine with epinephrine compared to 38 mg/kg in mice that received articaine without epinephrine. Similarly the LD₅₀ was 11.4 mg/kg in rats that received articaine with epinephrine compared to 23 mg/kg in rats that received articaine without epinephrine. Thus the lethality of articaine with epinephrine increased 10-fold in the mouse and 2-fold in the rat. The minimum lethal dose by intramuscular administration was 160 mg/kg in the dog. The animals had trembling, vertigo, and tonic and clonic convulsions independent of the route of administration and species of animals used. Lethal doses of articaine caused pulmonary edema.

Subcutaneous administrations of aged articaine solutions, stored for three or six months at 37⁰ C, in Swiss mice did not cause more toxicity in comparison with a freshly prepared solution with the same composition. Articaine 4% without vasoconstrictor was non-allergenic when analyzed by the Maximisation test of Magnusson and Kligman.

Repeat- Dose Toxicity

Histopathology of S-D rats treated for 4 week with subcutaneous administration of Septanest 1/100,000 adrenaline showed irritation effect (skin ulceration with eschar formation, epidermal/dermal and subcutaneous necrosis, inflammatory cell infiltration, granulation tissue, hemorrhage, oedema, muscular necrosis, atrophy and regeneration, epidermal hyperplasia and alopecia at the injection sites as compared to the control group) of the test compound. Extra-medullary haematopoiesis was Increased in the treated animals. The systemic no-toxic effect level [NOEL] was 25 mg/kg/day by subcutaneous injection. The C_{max} at the NOEL dose was approximately 1.9 µg/ml. Maximum articaine plasma levels were reached 20 - 40 min after administration on Day 1 and 10 - 20 minutes after administration on Day 20. The mean AUC values at NOEL dose were 98.3 and 58,0 g.min/ml on days 1 and 28 of dosing, respectively. Subcutaneous administration of Septanest showed a dose-related increase in the articaine plasma but there was a large variation in values. Articainic acid was one of the metabolites.

Subcutaneous administration of 80 mg/kg/day of Septanest for 4 weeks in beagle dogs resulted in an increase in plasma ALAT and ASAT activities. Increased salivation, ataxia, vomiting, sedation, defecation, clonic or tonoclonic convulsions, abdominal and/or lateral position at the higher dose were also observed. The non-toxic-effect-level [NOEL] was 40 mg/kg/day in this study. The C_{max} at NOEL dose was 2.2 - 2.7 µg/ml. The mean AUC values in dogs at NOEL dose were 126.9 and 149.5 ng.min/ml on day 1 and day 28 of drug administration, respectively. The large variation in individual values and small number of animals per group makes it difficult to make best use of animals PK data. Articainic acid was one of the metabolites.

In general, the PK values in rat and dog are so scattered [p 6 & 9] due to small number of animals, it is very difficult to compare these values with human PK values. In human, articaine is metabolized to articainic acid by plasma esterase.

Mean± Standard Deviation of Human Pharmacokinetic Parameters

| Parameters | Articaine dose | | | |
|--------------------------|----------------|-----------|-----------------|-----------|
| | 68 mg | 204 mg | 68 mg | 204 mg |
| | Articaine | | Articainic Acid | |
| C _{max} (ng/ml) | 385±165 | 899±363 | 1429±514 | 3793±796 |
| T _{max} (hr) | 0.4±0.1 | 0.8±0.2 | 0.9±0.3 | 1.3±0.2 |
| Kel (/hr) | 0.39±0.04 | 0.44±0.06 | 0.49±0.05 | 0.40±0.06 |
| T _{1/2} (hr) | 1.8 | 1.6 | 1.4 | 1.7 |

| | | | | |
|----------------------------------|----------|----------|----------|-----------|
| AUC ₀₋₁ (ng.hr/ml) | 631±135 | 1542±354 | 3751±885 | 11543±234 |
| Vd (L/kg) | 4.2±1.4 | 4.7±1.5 | 0.54* | 0.65* |
| CL (ml/min) | 1879±431 | 2323±561 | 316** | 309** |

* - Estimated Vd = (estimated Cl/Kel)/mean body weight

** - Estimated CL (articaic acid)=AUC (articaic acid) X CL (articaic acid)/AUC (articaic acid)

The primary metabolite, articaic acid, is further metabolized to articaic acid glucuronide. Peak plasma concentrations of articaic acid are related to dose and generally occur within 0.5 hour after administration

Reproductive Toxicity:

Segment I study was performed in according to ICH guideline 4.1.1. In this fertility and early embryonic development study, the fertility of rats was not impaired after treatment with 80 mg/kg of Septanest. Treatment of animals with 80 mg/kg led to 4 deaths (3 and 1). The vomiting, reduced motility, tonic convulsions and/or abdominal position were observed in animals before death. The NOEL dose were 40 and 20 mg/kg for female and males animals, respectively. There were no substance-related differences in number of corpora lutea, implantation sites and malformations of fetuses.

The influence of Septanest 1/100,000 adrenaline during critical phase of organogenesis was studied in rabbits. The no-observed-effect-level (NOEL) for the dams during Segment II study (embryotoxicity study) in rabbits by subcutaneous administration was 20 mg Articaic HCl. Forty mg/kg generated tremor, abdominal position and tono-clonic convulsions and 80 mg/kg produced mortality. The NOEL for the fetuses was 40 mg/kg. An increase in skeletal variations (13th ribs, a common variation) was observed at 80 mg/kg [control 23%, low dose 33%, medium dose 28% and high dose 38%].

During embryotoxicity/segment II study in rats, the NOEL of Septanest 1/100,000 adrenaline was 40 mg/kg for the dams and 80 mg/kg for the fetus. No substance-related influence on embryo fetal development was detected at 20, 40 and 80 mg/kg/day during the critical phase of development.

Articaic hydrochloride at 80 mg/kg during pre- and postnatal development caused slight maternal toxicity in Sprague-Dawley rats. Increased number of stillbirth (Control 3, low dose 0, mid dose 0, and high dose 10), the marginal delay in eye opening, and the influence on the passive avoidance (learning) in F1 generation were also observed. The NOEL dose during gestation, lactation, and reproduction in F0-dams and postnatal growth and survival in F1-generation was 40 mg/kg/day S.C. The NOEL in the F2 generation was 80 mg/kg/day.

During Segment III study in Sprague-Dawley rats, 80 mg/kg Septanest 1/100,000 adrenaline caused maternal toxicity (3 deaths). One animal also died at intermediate dose (40 mg/kg).

Marginal decrease of the pup weight after 14 and 21 lactation days was observed in high dose animals. The NOEL dose during gestation and lactation was 20 mg/kg/day S.C. The NOEL dose for reproduction (fertility and breeding system) and pups postnatal growth and survival (F1 generation) was 40 mg/kg/day S.C.

The PK data (metabolites, $t_{1/2}$, C_{max} , AUC etc.) was not collected during any reproductive toxicity studies. Therefore, it is impossible to compare animal PK data with human PK values.

Mutagenic Potential:

Articaine hydrochloride (100 to 10,000 $\mu\text{g}/\text{plate}$) did not show any mutagenic activity in bacteria with and without mammalian metabolic activator (Ames test). Articaine hydrochloride up to a cytotoxic concentration in the presence (3000 $\mu\text{g}/\text{ml}$) and absence (2000 $\mu\text{g}/\text{ml}$) of metabolic activator (human S9) did not show any clastogenic activity in cultured CHO cells. Mitomycin C and cyclophosphamide, positive controls, induced significant chromosome damage in the same test.

The maximum tolerated dose of Septanest 1/100,000 adrenaline (75 mg/kg) by NMRI mouse produced no increase in the incidence of micronucleated polychromatic erythrocytes in the mouse bone marrow micronucleus test by subcutaneous administration. Under similar experimental conditions, Articaine hydrochloride at 75 mg/kg revealed comparable results. Articaine hydrochloride up to cytotoxic concentration (4000 $\mu\text{g}/\text{ml}$ medium) in the presence and absence of metabolic activation did not induce any mutation in the HPRT-V79 mammalian cells

Absorption, Distribution, Metabolism, Excretion:

The kinetics of Septanest with 1:100,000 epinephrine in rats and dogs have been reviewed under repeat-dose toxicity section. These studies showed a dose-related increase in articaine plasma levels in both species. The C_{max} at the NOEL dose of 25 mg/kg/day by subcutaneous administration was approximately 1.9 $\mu\text{g}/\text{ml}$ in rats. The C_{max} in dogs at NOEL dose of 40 mg/kg/day was 2.2 - 2.7 $\mu\text{g}/\text{ml}$. The mean AUC values at NOEL dose in rats were 98.3 and 58.0 $\mu\text{g}\cdot\text{min}/\text{ml}$ on days 1 and 28 of dosing, respectively. The mean AUC values in dogs at NOEL dose were 126.9 and 149.5 $\mu\text{g}\cdot\text{min}/\text{ml}$ on day 1 and day 28 of drug administration, respectively. The final concentration of articaine and articainic acid, major metabolite in both species, were the same in several plasma samples. In general, the PK values in rat and dog are so scattered [pages 6 & 9] due to small number of animals it is very difficult to compare these values with human PK values. The detailed pathway, extent and site of metabolism are not studied and identified in animals. The activity of the metabolite(s) remains unknown. The excretion data are not available for rats and dogs.

In a published article by Hoffer and Altmann (Prakt Anasth 1974;9:157-161), S^{35} -labeled articaine was rapidly absorbed in the dwarf pig (100%) and in the human (85%) after IM injection. Preliminary tissue distribution data from dwarf pig showed the highest levels in

kidneys, liver and lungs following either intravenous or intramuscular administration. By 48 hours all tissue levels were low. Carticaine and its metabolites were eliminated rapidly mainly in the urine in both species. The metabolism of Carticaine was different in man than in pig; in man no intact Carticaine was found in the urine. These preliminary and old data measuring radioactivity in 1 - 2 pigs in 1974 are not considered appropriate for inclusion in the label as proposed by the Applicant.

Recommendations

This NDA is approvable from the pharmacology/toxicology point of view. Before the NDA can be approved, however, the amendment of the package insert should be made as follows:

- 1. Page 3 Clinical Pharmacology, second sentence in Distribution "

Draft

should be deleted.

This experiment was done in 1974 with small number of animals (n=1 or 2). The database does not have enough power to warrant the inclusion in the package insert.

- 2. Page 6 Carcinogenesis, Mutagenesis, Impairment of Fertility.

(a) In accordance with 21 CFR 201.57 on the reproductive data, the multiples of the doses comparing those of the animals and humans should be included in the label either in term of AUC or body surface area unit. This information has been incorporated in the label by this reviewer.

- (b) "

Draft

to read

Draft

(c) The following paragraphs should be changed as stated below and moved to Pregnancy Category in accordance with 21 CFR 201.57

Draft

to read

should be changed

2 pages redacted from this section of
the approval package consisted of draft labeling

1

DRAFT

LABELING

└

/s/

M. Anwar Goheer

^D
/s/

Dou Huey (Lucy) Jean

Concur by Team Leader:

cc:

IND ORIG. _____

NDA - 20-971

HFD-170

/Goheer

/Hblatt

/Knolan

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Division of Anesthetic, Critical Care & Addiction Drug Products

NDA: 20-971

IND: _____

Submission: NDA Dated: March 30, 1998
 Received by CDR: March 30, 1998
 Received by HFD 170: March 31, 1998
 Received by Reviewer: April 2, 1998
 Review Completed: Aug. 28, 1998
 Reviewer: M.A. Goheer, Ph.D.

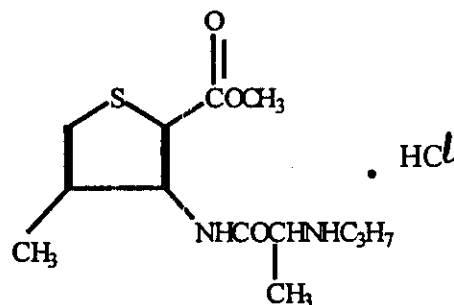
Sponsor: Deproco Inc., 245-C Quigley Blvd., New Castle, DE 19720

Information to be conveyed to the sponsor: Yes

Drug Name: _____ Septanest[®] — 1:100,000 (4% Articaine plus
 1/200,000 epinephrine and 4% Articaine plus 1/100,000 epinephrine)

Chemical Name: 2-Thiophene acid, 4 methyl-3-[[1-oxo-2-(propylamino)
 propyl]amino]-methyl ester, monohydrochloride.

Other Name: Articaine HCl with Epinephrine.

Structure:

Molecular weight: 320.84

Formulation: Formulations for Spécialites Septodont Articaine preparations

| | | |
|--------------------------------|--|--|
| Formula | | Septanest [®] SP 1:100,000 epinephrine |
| Articaine HCl | | 4.000 g |
| Epinephrine base | | 0.001 g |
| Sodium metabisulphite | | 0.050 g |
| Sodium chloride | | 0.160 |
| Sodium hydroxide solution q.s. | | to pH 5.0±0.2 |
| Excipient | | |

Drug Product Manufacturer: Spécialites Septodont, 58 rue du pont de Creteil,
 Saint-Maur des Fosses, 94107 Paris, France. (parent company of Deproco, Inc.)

The manufacturer of articaine hydrochloride:

The manufacturer of epinephrine:

Pharmacologic Class: Local anesthetic of the amide type

Related IND: _____

Related NDAs: 16-964 - Bupivacaine, 17-751 - Etidocaine, 20-533 - Naropin

Indications and Dosages: Articaine hydrochloride is indicated for infiltration anesthesia and nerve block anesthesia in clinical dentistry. The maximum recommended dose of articaine hydrochloride for normal healthy adults should not exceed 7 mg/kg (259 mg/m²) of body weight.

Previously Reviewed Submissions: Original IND submitted on Oct. 18, 1996 was reviewed on April 11, 1997.

Studies Reviewed: Toxicology

- include these in DSA page*
- A. Articaine 4 per cent Without Adrenaline - Toxicity After a Single Administration Subcutaneously in the Mouse. Study No. 870975, 1988, page 132, vol. 6.
 - B. Articaine (Articaine 4 per cent Adrenaline 1/100,000) in comparison with Alphacaine SP (Articaine 4 per cent, Adrenaline 1/100,000) - Comparison of Safety in the Mouse Using the Subcutaneous Route. Study No. 870973, 1987, page 188, vol. 6.
 - C. Articaine (Articaine 4 per cent, Adrenaline 1/200,000) in comparison with Alphacaine N (Articaine 4 per cent, Adrenaline 1/200,000) - Comparison of Safety in the Mouse Using the Subcutaneous Route. Study No. 870974, 1987, page 234, Vol. 6.
 - D. Aged Articaine Solution (3 months) in Comparison with "Fresh" Articaine Solution - Safety in the Mouse Via the Subcutaneous Route. Study No. 880001, 1988, page 279, vol. 6.
 - E. Aged Articaine Solution (6 months) in Comparison with "Fresh" Articaine Solution - Safety in the Mouse Via the Subcutaneous Route. Study No. 871299, 1988, page 343, vol. 6.
 - F. Articaine 4% without Vasoconstrictor - Study of the Skin Sensitivity According to Magnusson and Kligman. Study No. 871225. 1988, page 403, vol. 6.

First Annual Report (Serial No. 057) was submitted on Jan. 14, 1998.

NDA was submitted on March 30, 1998.

Studies Reviewed Within This NDA Submission:

A. Repeat Dose Toxicity

1. 4-week subchronic toxicity study of Septanest 1/100,000 adrenaline by subcutaneous administration to Sprague-Dawley rats. (Report No. 10652/97, vol. 10, page 1).
2. 14-day dose-range finding study for a 4-week subchronic toxicity study of articaine hydrochloride by subcutaneous administration to beagle dogs. (Report No. 10376/97, vol. 12, page 1).

3. 4-week subchronic toxicity study of Septanest 1/100,000 adrenaline by subcutaneous administration to beagle dogs. (Report No. 10653/97, vol. 11, page 1).

B. Reproductive Toxicity:

1. Examination of the influence of Septanest 1/100,000 adrenaline on the fertility and early embryonic development to implantation of Sprague-Dawley rats by subcutaneous administration to the animals of the F₀ generation (Segment 1). (Report No. 10654/97, vol. 12, page 190).

2. Dose-range finding study to determine the dose levels for an examination of the influence of articaine hydrochloride in the pregnant rabbit and the fetus by subcutaneous administration. (Report No. 10379/97, vol. 13, page 322).

3. Dose-range finding study to determine the dose levels for an examination of the effects of articaine hydrochloride on the pre- and postnatal development of the rat embryo toxicity by subcutaneous administration to the dams of the F₀ generation. (Report No. 10137/96, vol. 12, page 64).

4. Examination of the influence of Septanest 1/100,000 adrenaline on the pregnant rabbit and the fetus by subcutaneous administration (embryo toxicity study/Segment II). (Report No. 10656/97, vol. 15, page 1).

5. Examination of the influence of Septanest 1/100,000 adrenaline on the pregnant rat and the fetus by subcutaneous administration. (Report No. 10655/97, vol. 13, page 1).

6. Examination of articaine hydrochloride for effects on the pre- and postnatal development (including maternal functions) following subcutaneous administration to the dams of the F₀ generation (Segment III study). (Report No. 10138/96, vol. 14, page 1)

7. Examination of Septanest 1:100,000 adrenaline for effects on the pre- and postnatal development (including maternal functions) following subcutaneous administration to the dams of the F₀ generation (Segment III study). (Report No. 10657/97, vol. 16, page 1)

C. Mutagenic Potential

In Vitro

1. Mutagenicity study of the articaine hydrochloride in the Salmonella typhimurium reverse mutation assay (in vitro). (Report No. 10089/96, vol. 17, page 1).

2. In vitro assessment of the clastogenic activity of the articaine hydrochloride in cultured CHO-cells. (Report No. 10090/96, vol. 17, page 50).

3. Mutagenicity study of articaine hydrochloride in mammalian cells (V79) in the in vitro gene mutation assay (HPRT Test). (Report No. 10373/97, vol. 17, page 217).

In Vivo:

4. Micronucleus test of articaine hydrochloride in bone-marrow cells of the NMRI mouse by subcutaneous administration. (Report No. 10374/97, vol. 17, page 160).

5. Micronucleus test of Septanest 1/100,000 adrenaline in bone marrow cells of the NMRI mouse by subcutaneous administration (base set). (Report No. 10651/97, vol. 17, page 104).

D. Pharmacokinetics:

1. 4-week subchronic toxicity study of Septanest 1/100,000 adrenaline by subcutaneous administration to Sprague-Dawley rats. (Report No. 10652/97, vol. 10, page 1).
2. 4-week subchronic toxicity study of Septanest 1/100,000 adrenaline by subcutaneous administration to beagle dogs. (Report No. 10653/97, vol. 11, page 1).

E. Biotransformation

1. Metabolism of articaine by human isoenzymes, protein binding, and stability of articaine in human plasma in vitro validation of the analytical method. (Vol. 21).

Studies not Reviewed Within This Submission: Old published studies performed with different formulations of articaine than formulation proposed for marketing in the United States. These abstracts and full reports are discussed in the Overall Summary and Evaluation section.

Notes - (1) Portions of this review were excerpted directly from the sponsor's submission.

- which proteins!!!*
- (2) All preclinical and clinical studies performed with Septanest^R did not contain methyl parahydroxybenzoate, a preservative. An Articaine hydrochloride investigated by Hoechst A.G. and others did contain this preservative. (?)
- (3) All preclinical studies, performed by ~~—~~ for Specialites Septodont, were conducted in accordance with FDA Good Laboratory Practice (GLP), as specified in 21CFR Part 58, dated April 1, 1991.
- (4) Sodium metabisulfite was included in the formulations used in the toxicity studies (see page 1 for complete formulation). *↓ what about in clinical studies*

A. Repeat Dose Toxicity

1. 4-week subchronic toxicity study of Septanest 1/100,000 adrenaline by subcutaneous administration to Sprague-Dawley rats. (Report No. 10652/97, vol. 10, page 1).

Study N^o: 10652/97

Compound: Septanest 1/100,000 adrenaline

Batch No. RD 100/11

Appearance: Colorless, clear liquid, pH 3.6.

Route: Subcutaneous

Dose Levels: 0 (control), 25 (low dose), 50 (medium dose) and 100 (high dose) mg/kg/day

Administration volumes: 0.625, 1.25 and 2.5 ml/kg/day

Strain: Cr:CD^RBR, Sprague-Dawley rat

Source:

Number: 10/sex/group for toxicology and 18 animals/sex/group for toxicokinetic
[3 animals/sex/sampling time/group]

Weights: 70-78 g, 27 - 30 days old.

Study Site: _____

Sponsor: Septodont, France
 GLP/QAU: Submitted with signatures.

Study Dates: Aug. 6 - Nov. 10, 1997

Results:

Observation: High dose - Pilo-erection for 24 hours from day 16. Dose-related haematomas in all animals.

Mortality: Control - zero, Low dose - zero
 Intermediate dose - 2/10 males on days 16 and 27 respectively
 High dose - 3/10 males and 2/10 females between days 6 and 10.

Body weights: Intermediate dose - 7% ↓ in ♂, High dose - 9% ↓ in ♂

Food and water consumption: No difference

Physical examination: No change of the eyes and the auditory acuity.

Hematology: Intermediate dose - Hemoglobin ↓ (6% in ♂), leucocytes ↑ (18% in ♂ and 30% in ♀) and reticulocyte ↑ (55% in ♂ and 109% in ♀)
 High dose - Hemoglobin ↓ (7% in ♂ and 3% in ♀), leucocytes ↑ (47% in ♂ and 62% in ♀), reticulocytes ↑ (118% in ♂ and 199% in ♀) and platelets ↓ (8% in ♂ and 29% in ♀).

Clinical chemistry: Intermediate dose - Blood urea ↑ (24% in ♂ and 11% in ♀)
 High dose - Total bilirubin ↑ (26% in ♂ and 10% in ♀), blood urea ↑ (22% in ♂ and 15% in ♀), ALAT ↑ (30% in ♂ and 42% in ♀) and ASAT ↑ (77% in ♂ and 96% in ♀).

Urinalysis: No influence

Pathology: No difference. (eschar formation and subcutaneous haematomas at the injection sites in all treated animals).

Organ weights: The absolute and relative weights were within control range

Histopathology: Skin ulceration with eschar formation, epidermal/dermal and subcutaneous necrosis, inflammatory cell infiltration, granulation tissue, hemorrhage, oedema, muscular necrosis, atrophy and regeneration of adjacent skeletal muscle, epidermal hyperplasia and alopecia at the injection sites as compared to the control group. Extra-medullary haematopoiesis was also increased in all treated animals. †

Toxicokinetics:

50 µg/min

Mortality observed for the animals scheduled for kinetics:

| Group | Animal No. | Sex | Day of Death |
|-------------------|------------|--------|--------------|
| 3 (50 mg/kg/day) | 148 | female | 16 |
| 3 | 152 | male | 22 |
| 4 (100 mg/kg/day) | 165 | male | 14 |
| 4 | 167 | male | 25 |

*? cause of death
sacrifice?*

| Group | Animal No. | Sex | Day of Death |
|-------|------------|--------|--------------|
| 4 | 183 | female | 7 |
| 4 | 184 | female | 28 |
| 4 | 185 | female | 23 |
| 4 | 187 | female | 6 |

Mean values in ng articaïne (free base) per ml plasma on the test day 1.
[3 animals/sex/sampling time/group]

| T (min) | 25 mg/kg/day | | 50 mg/kg/day | | 100 mg/kg/day | |
|------------|--------------|----------|--------------|----------|---------------|----------|
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| 10 | 911±625 | 918±373 | 1501±74 | 1848±752 | 2821±604 | 2623±954 |
| 20 | 1349±492 | 1170±232 | 2075±418 | 2384±254 | 4362±167 | 4846±203 |
| 40 | 1779±92 | 1636±590 | 3588±198 | 3898±112 | 5678±186 | 7388±285 |
| 90 | 499±58 | 708±106 | 1624±38 | 1973±325 | 3767±375 | 3542±454 |

Mean articaïne values (ng, free base) per ml plasma on test day 28
[3 animals/sex/sampling time/group]

| Time (min) | 25 mg/kg day | | 50 mg/kg/day | | 100 mg/kg/day | |
|---------------|--------------|----------|--------------|-------------------|------------------|-------------------|
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| 10 | 1912±195 | 2052±221 | 2065±199 | 1582 ¹ | 1963±296 | 1595 ¹ |
| 20 | 102±46 | 177±126 | 297±102 | 375 ¹ | 573 ² | ³ |
| 40 | 789±676 | 962±796 | 1070±778 | 500 ² | 1567±108 | 2113 ¹ |
| 90 | 550±137 | 679±453 | 742±249 | 834 ¹ | 653 ² | ³ |

1 - 2 surviving animals
3 - no surviving animal
Limit of quantification - — ng/ml

2 - 1 surviving animal
Limit of detection - — ng/ml

Area under the curve ($\mu\text{g}\cdot\text{min}/\text{ml}$) [mean values]

| Dose level (mg/kg/day) | Day 1 | | Day 28 | |
|---------------------------|-------|---------|--------|---------|
| | Males | Females | Males | Females |
| 25 | 99.5 | 97.1 | 52.5 | 63.6 |
| 50 | 204.8 | 230.8 | 70.8 | 51.9 |
| 100 | 372.4 | 372.4 | 89.6 | 81.9 |

In general, there was a large scatter between the individual animals.

2. 14-day dose-range finding study for a 4-week subchronic toxicity study of articaine hydrochloride by subcutaneous administration to beagle dogs. (Report No. 10376/97, vol. 12, page 1).

Study N°: No. 10376/97

Compound: Articaine hydrochloride, batch No. 96.444

Appearance: White to almost white powder

| | | | | |
|--------------|---|-------------------|-----------|---------------------|
| Dose Levels: | 1 | Control | 1♂ and 1♀ | 1 ml of aqua/kg/day |
| | 2 | Low dose | 1♂ and 1♀ | 25 mg/kg/day |
| | 3 | Intermediate dose | 1♂ and 1♀ | 50 mg/kg/day |
| | 4 | High dose | 1♂ and 1♀ | 100 mg/kg/day |

Route: (Subcutaneous)

Duration: 14 days

Administration Volume: 1 ml/kg/day

Species: Dog/Beagle

Source: _____

Number: 8 animals

Weights: 8.3 - 14.0 kg, 10 - 11 months old

Study Site: _____

Sponsor: Septodont, France

GLP/QU: Statements submitted with signatures

Results:

Observation: 50 & 100 mg/kg/day - vomiting, defecation, scratching at injection site, convulsions and tremor within 5 to 60 min of injection.

Mortality: None

Body weights: No effect

Food and Drinking Water Consumption: No difference ✓

Electrocardiography: No substance related effect on the heart rate, P-Q, Q-T and QRS intervals. ✓

at what plasma levels?

Conclusion: 25 mg/kg/day of articaine hydrochloride by subcutaneous administration in dogs for 2 weeks may be the NOEL(no-observed-effect level) dose.

3. 4-week subchronic toxicity study of Septanest 1/100,000 adrenaline by subcutaneous administration to beagle dogs. (Report No. 10653/97, vol. 11, page 1).

Study N°: 10653/97

Compound: Septanest 1/100,000 adrenaline, Batch Numbers RD 100/10 & RD 100/11

Appearance: Clear colorless liquid

Volume Given: Ready-to-use solution as supplied by the sponsor

| | |
|-----------------------------|----------------|
| 1 - Control group | 2 ml. water/kg |
| 2 - Low dose group | 0.5 ml/kg |
| 3 - Intermediate dose group | 1 ml/kg |
| 4 - High dose group | 2 ml/kg |

Route: (Subcutaneous) injection

Dose Levels:

| | | |
|-------------------------|--------------|---------------|
| Control group | 0 mg/kg/day | 3 animals/sex |
| Low dose group | 20 mg/kg/day | 3 animals/sex |
| Intermediate dose group | 40 mg/kg/day | 3 animals/sex |
| High dose group | 80 mg/kg/day | 3 animals/sex |

Source:

Number: 24 animals (12 males and 12 females)

Weights: Males - 11.2 - 14.7 kg, Females - 10.9 - 13.2 kg

Age: 10 - 12 months

Study Site:

Sponsor: Septodont, France.

Monitor:

Experimental Period: July 30 to Aug. 27, 1997

GLP/QAU Statements: Submitted with signatures.

Results:

Local tolerance (injection sites): Subcutaneous indurations and palpable masses in all animals.

Observation: (High dose group - Vomiting, salivation, ataxia, sedation, defecation, and clonic or tonoclonic convulsions.)

Mortality: None

Body weights: Not effected.

Food and water consumption: No effect

Physical examination: (eyes and hearing): No changes

ECG: No substance-related changes in the heart rate, P-Q, Q-T, QRS intervals and QTC value.

Blood pressure: Within normal range

Haematology: No substance-related changes

Clinical chemistry: High dose group - ALAT ↑ (130% in ♂ and 134% in ♀), ASAT ↑ (112% in ♂ and 80% in ♀)

Urinalysis: No difference

Pathology: No substance-related findings

Organ weights: Within control range

Histopathology: No dose-related findings.

Toxicokinetics

Mean values in ng articaïne (free base) per ml plasma on test day 1.
[3 animals/sex/group]

| Time (min) | 20 mg/kg/day | | 40 mg/kg/day | | 80 mg/kg/day | |
|------------|---------------|---------------|--------------|--------------|--------------|---------------|
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| 10 | 1750± 1194 | 1048± 1315 | 1120± 235 | 590± 276 | 1378± 892 | 3749± 4490 |
| 20 | 2187± 1356 | 1393± 1178 | 1786± 355 | 930± 125 | 2083± 928 | 4340± 4373 |
| 40 | 2168± 1029 | 1537± 625 | 2508± 311 | 1690± 143 | 2940± 787 | 4162± 2718 |
| 90 | 1379± 206 | 880± 88 | 1481± 226 | 1235± 187 | 2736± 708 | 2542± 607 |

Mean values in ng/ml plasma on test day 28.

| Time (min) | 20 mg/kg/day | | 40 mg/kg/day | | 80 mg/kg/day | |
|------------|---------------|--------------|--------------|--------------|---------------|---------------|
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| 10 | 2676± 1211 | 1273± 598 | 1566± 941 | 1900± 680 | 8840± 2356 | 3747± 1142 |
| 20 | 2665± 1260 | 1820± 574 | 2071± 701 | 2314± 815 | 8605± 1119 | 4714± 906 |
| 40 | 2256± 692 | 1679± 523 | 1882± 545 | 2101± 398 | 5759± 1785 | 4492± 534 |
| 90 | 1176± 326 | 1003± 158 | 1021± 94 | 1372± 170 | 3023± 875 | 2607± 283 |

There was a large scatter between the individual animals as shown below.

Area under the curve (ng.min/ml) [individual values]

| Dose Level | Dog No. | Sex | Test Day 1 | Test Day 28 |
|--------------|---------|-----|------------|-------------|
| 20 mg/kg/day | 7 | M | | |
| | 8 | M | | |
| | 9 | M | | |
| | 10 | F | | |
| | 11 | F | | |
| | 12 | F | | |
| 40 mg/kg/day | 13 | M | | |
| | 14 | M | | |
| | 15 | M | | |
| | 16 | F | | |
| | 17 | F | | |
| | 18 | F | | |
| 80 mg/kg/day | 19 | M | | |
| | 20 | M | | |
| | 21 | M | | |
| | 22 | F | | |
| | 23 | F | | |
| | 24 | F | | |

Area under the curve (ng.min/ml) [mean values]

| Dose (mg/kg/day) | Test day 1 | | Test day 28 | |
|---------------------|------------|--------|-------------|--------|
| | ♂ | ♀ | ♂ | ♀ |
| 20 | 151913 | 101932 | 175098 | 123920 |
| 40 | 157185 | 106930 | 136227 | 162030 |
| 80 | 209425 | 293055 | 494760 | 331368 |

Dose-related increase in plasma C_{max} and area under the curve (AUC) was exhibited in this subcutaneous dog study. In general, there was a large scatter between the individual animals. The maximum articaine plasma levels were reached ~20 min after dosing.

B. Reproductive Toxicity:

1. Examination of the influence of Septanest 1/100,000 adrenaline on the fertility and

early embryonic development to implantation of Sprague-Dawley rats by subcutaneous administration to the animals of the F₀ generation (Segment 1). (Report No. 10654/97, vol. 12, page 190).

Study N°: No. 10654/97

Compound: Septanest 1/100,000 adrenaline, batch no. RD 100/12

Appearance: Clear, colorless liquid

Route: Subcutaneous

Duration of treatment: Males - Daily from 4 weeks before mating to end of mating period.

Females - Daily from 2 weeks before mating to the 7th day of pregnancy

Dose Levels: 20, 40 and 80 mg/kg/day

Volume Given: 0.5, 1.0 and 2.0 ml/kg

Strain: Cri:CD^R BR: Sprague-Dawley rats

Source:

Number: Control 20 males and 20 females

Low dose 20 ♂ & 20 ♀

Intermediate 20 ♂ & 20 ♀

High 20 ♂ & 20 ♀

Weights: Males - 251-278 g, Females - 165-192g

Age: 8 weeks

Study Site:

Sponsor: Septodont, France

Monitor:

GLP/QAU Statements: Both submitted with signatures

Observations:

Clinical Signs: Daily

Viability: Twice daily

Body weight: Males - weekly, Femlaes - daily from gestation

Food Consumption: Daily during pregnancy

Examination of fertility:

The ovaries and uteri were removed on day 13 of pregnancy and following parameters were determined:

number of fetuses and placentae

number and size of resorptions

corpora lutea, implantation sites, resorptions, placentae and fetuses

external examination of fetuses

Autopsy (F₀-generation parent animals) Ovary, uterus, testis, epididymis, prostate gland, seminal vesicle and coagulating gland.

Statistical evaluation: Student's test and Dunnett test

Results:

1. F₀ Maternal Observations: Dose-related scab formation at the injection sites.
2. Mortality: High dose - 3♂ and 1♀
3. Clinical Signs: Intermediate dose - Reduced motility, tonoclonic convulsion in 1♂
High dose - vomiting, reduced motility, tonic/tonoclonic convulsions, increased respiratory rate and/or abdominal position in 15/20 ♂ and 7/20 ♀.
4. Body weight: High dose - Males (5-9 % ↓), females (2-4 % ↓)
5. Food consumption: No effect
6. Fertility: No dose-related effect
7. Sperm number, motility and viability: No effect
8. Early embryonic development - No drug-related effect
9. Macroscopic post mortem findings: None
10. Uterine and testicular weight Within the normal range.

2. Dose-range finding study to determine the dose levels for an examination of the influence of articaine hydrochloride in the pregnant rabbit and the fetus by subcutaneous administration. (Report No. 10379/97, vol. 13, page 322).

Study N^o: — No. 10379/97
 Compound: Articaine hydrochloride, batch no. 96,444
 Appearance: Clear, white powder
 Route: Subcutaneous
 Duration of treatment: Sixth to 18th day of pregnancy
 Strain: Himalayan rabbit

Source: _____

| | | | | |
|---------|---------|------------------|----|---------------|
| Number: | Group 1 | Control | 2♀ | |
| | Group 2 | Low dose | 2♀ | 20 mg/kg/day |
| | Group 3 | Low intermediate | 2♀ | 40 mg/kg/day |
| | Group 4 | Intermediate | 2♀ | 80 mg/kg/day |
| | Group 5 | High | 2♀ | 120 mg/kg/day |

Study Site: _____
 Sponsor: Septodont, France.
 Monitor: _____
 GLP/QAU Statements: Both submitted with signatures

Observations:

Clinical signs, viability, body weight, and food consumption: Daily

Examination of fertility:

The ovaries and uteri were removed on day 29 of pregnancy and following parameters were determined:
 number of fetuses and placentae
 number and size of resorptions
 corpora lutea, implantation sites, resorptions, placentae and fetuses

- (3) Number and size of resorptions.
- (4) Number of corpora lutea
- (5) Weight of fetuses and placentae
- (6) Examination of the fetuses for malformations

Results:

Observation: Dose dependent induration at the injection sites in all animals.
 Mortality: 300 mg/kg - 1 (50%), 500 mg/kg - 2 (100%)
 Clinical Signs: 200 mg/kg - Pilo-erection
 300-500 mg/kg - - Pilo-erection, tremor and reduced motility.
 Body weights: 300 & 500 mg/kg - Reduced
 Food and water consumption: Reduced in 300 & 500 mg/kg animals.
 Autopsy Findings: Dark-red discolored lungs of deceased animals
 Influence on the fetus: 25, 50 and 100 mg/kg - No effect
 200 and 300 mg/kg - Dose-dependent reduction in fetal and placental weights.
 No malformation or external variations

4. Examination of the influence of Septanest 1/100,000 adrenaline on the pregnant rabbit and the fetus by subcutaneous administration (embryo toxicity study / Segment II). (Report No. 10656/97, vol. 15, page 1).

Study N°: — No. 10656/97
 Compound: Septanest 1/100,000 adrenaline, batch # RD100/12
 Appearance: Clear, colorless liquid
 Volume Given: 0.5, 1.0 and 2 ml/kg
 Route: Subcutaneous
 Dose Levels: 20, 40 and 80 mg/kg
 Treatment Period: from the 6th to 20th day of pregnancy
 Strain: Himalayan rabbit
 Source: _____

| | | | | |
|-------------|-----------------|-------------------|---------------------|------------|
| Number: | Group I | Control | 0 mg/kg | 16 females |
| | Group II | low dose | 20 mg/kg | 16 females |
| | Group III | Intermediate dose | 40 mg/kg | 16 females |
| | Group IV | high dose | 80 mg/kg | 16 females |
| Weights: | 2.55 - 3.70 kg. | | Age: 6 - 6.5 months | |
| Study Site: | _____ | | | |

Sponsor: Septodont, France
 Monitor: _____
 GLP/QAU Statements: Submitted with signatures.

Examination: The animals were sacrificed on 29th day of gestation and examined for:
 (1) Number of fetuses and placenta

- (2) Determination of sex and viability of fetuses
- (3) Number and size of resorptions.
- (4) Number of corpora lutea
- (5) Weight of fetuses and placentae
- (6) Examination of the fetuses for malformations

Results:

Observation: Dose related red area around the injection sites

Mortality: 80 mg/kg - 2 (one on gestation day 6 and other on gestation day 18).

Body Weights: No effect

Clinical Signs: 40 mg/kg - tremors (1 animal), tono-clonic convulsion (1 animal)

80 mg/kg - reduced motility, lateral and/or abdominal position, tono-clonic convulsions and slightly increased respiratory rate in most animals. Tremor, increased salivation, sedation and dyspnoea in some animals.

Food consumption: Normal (21%↓ in high dose group on gestation days 5 and 6 only)

Water consumption: No effect

Autopsy Findings: No drug-related systemic pathological findings.

Reproduction data of dams: No differences

Influence on the Fetus:

Corpora leutea, implantation sites, resorptions and placentae - No prenatal effect.

Body weight: No differences

viability - Within normal range except significantly decreased at 80 mg/kg within 6 hours.

Malformations - Skeletal and external examinations showed no malformed fetus

Variations - No drug-related variations

Retardations - No difference

5. Examination of the influence of Septanest 1/100,000 adrenaline on the pregnant rat and the fetus by subcutaneous administration (Embryotoxicity study / Segment II study). (Report No. 10655/97, vol. 13, page 1).

Study N°: 10655/97 -in accordance with ICH guideline 4.1.3.

Compound: Septanest 1/100,000 adrenaline, used as supplied by the sponsor.

Appearance: Clear, colorless liquid

Volume Given: 0.5, 1 and 2 ml/kg

Route: Subcutaneous

Dose Levels: 20, 40 and 80 mg/kg

Administration Volume: 0.5, 10 and 2.0

Duration of Dosing: From 6th to 17th day of pregnancy

Species / Strain / Stock: Rat / Sprague-Dawley / _____

Source: _____

| | | | | |
|---------|-------------------|----------|-----------|------|
| Number: | Control | 0 mg/kg | 2.0 ml/kg | 20 ♀ |
| | Low dose | 20 mg/kg | 0.5 ml/kg | 20♀ |
| | Intermediate dose | 40 mg/kg | 1.0 ml/kg | 20♀ |

High dose 80 mg/kg 2.0 ml/kg 20♀
Weights: 197 - 300 g
Study Site: _____

Sponsor: Septodont, France.

Monitor: _____

Date: Oct. 29, 1997.

GLP/QAU Statements: Both present with signatures

Examination: The animals were sacrificed on 20th day of gestation and examined for:

- (1) Number of fetuses and placenta
- (2) Determination of sex and viability of fetuses
- (3) Number and size of resorptions.
- (4) Number of corpora lutea
- (5) Weight of fetuses and placentae
- (6) External inspection of fetuses for damage, especially malformations
- (7) Examination of fetuses and determination of number and type of retardation, variations or malformations.

Results:

Observation: Dose-related reddened area around the injection sites

Mortality: None

Clinical Signs: 80 mg/kg - reduced motility and tremor in one animal on gestation day 12.

Body weights: 80 mg/kg - 28%↓ on gestation day 18 to 20

Food and water consumption: 80 mg/kg - food consumption was reduced on gestation days 12 and 15. Water consumption was not effected.

Autopsy Findings: None

Influence on the Fetus:

Corpora lutea/implantation sites/resorption/weight and number of fetuses
alive/placental weight: None

External, skeletal and soft tissue examination: No malformed fetuses

Variations: None

Retardations: No differences in skeletal retardations

6. Examination of articaine hydrochloride for effects on the pre- and postnatal development (including maternal functions) following subcutaneous administration to the dams of the F₀ generation (Segment III study). (Report No. 10138/96, vol. 14, page 1)

Study N°: 10138/96

Compound: Articaine hydrochloride, batch no. 96.444

Volume Given: 2 ml Route: Subcutaneous

Treatment of F₀-generation: from implantation (6th day of gestation) to weaning (22nd day of lactation)

Observation Period: F1- and F2-generations

Species: Rat Strain: Sprague-Dawley

Breeder: _____

Number of animals used for the Fo-generation:

| | | | |
|---------|-------------------|-----|-----------------|
| Group 1 | Control | 24♀ | Control vehicle |
| Group 2 | Low dose | 24♀ | 20 mg/kg |
| Group 3 | Intermediate dose | 24♀ | 40 mg/kg |
| Group 4 | High dose | 24♀ | 80 mg/kg |

Weights: 179-218 g

Study Site: _____

Sponsor: Septodont, F-94100 Saint-Maur des Fosses, France

Monitor: _____

GLP/QAU statements: Both submitted with signatures

Observation:

Clinical signs and Viability: Daily

Body weights:

F0 -, F1- generations: Daily during gestation and then on days 1, 7, 14, 21, and 22 of lactation.

F2 litters: On days 1, 4, 7, 14, and 21 of lactation.

Food consumption: weekly

Examination: The animals were allowed to deliver normally and examined for:

- (1) Number of pups absolute at birth and 4, 7, 14, and 21 days after birth
- (2) Number of pups per dam
- (3) Determination of sex and viability of fetuses
- (4) Number of pups with stillbirths
- (5) Number of pups with malformations

Reproductive indices: Gestation, birth, live birth, viability, lactation, and overall survival.

Post natal physical and functional development::

| <u>Functional test</u> | <u>age of pups(days)</u> | <u>pups to be examined</u> |
|------------------------------|--------------------------|----------------------------|
| Mid-air righting reflex | 14 | each |
| Auditory startle reflex | 14 | each |
| Pupillary reflex | 21 | each |
| Open field | 27±1 | first half of each litter |
| Passive avoidance (learning) | 27±1 | second half of each litter |
| Passive avoidance (memory) | 34±1 | second half of each litter |

Morphological landmarks

| | | |
|--|----|------|
| Pinna detachment | 1 | each |
| Ear opening | 12 | each |
| Eye opening | 11 | each |
| Cleavage of the balanopreputial gland | 25 | each |
| Vaginal opening | 33 | each |
| Upper incisor eruption | 7 | each |

Termination / Autopsy:

- Fo-generation - Day 22 of lactation
- F1 pups not selected - Day 22 of lactation
- F1 parents - Males at the end of mating period
 - Femlaes at the end of lactation period
- F2 offspring - After 3 lactation weeks

Results:**Fo-dams**

- Local tolerance: Dose-related scab formation at the injection site.
- Clinical signs: High dose - Tremor (1/24), reduced motility (2/24), vomiting (1/24), increased respiration (1/24), convulsion (1/24).
- Mortality: High dose - One (15 th day of gestation)
- Body weights: No effect (3% ↓ in high dose animals)
- Food consumption: High dose - 7 -19% ↓
- Exmination of the dams at termination: No influence at 20, 40 and 80 mg/kg/day.
- Reproduction: No influence on duration of pregnancy and number of alive pups.
 - Ten stillbirths (control 3, low dose 0, middle dose 0) at 80 mg/kg/day.
- Macroscopic post mortem finding: No systemic change

F1-generation (until weaning)

- Sex distribution: No difference
- Body weight: No effect
- External examination: No differences
- Morphological landmarks, functional tests, open-field test: Eye opening was delayed and reduced ability to pass the passive avoidance test in high dose animals.

| Criteria | Group/dose level (mg/kg) | | | |
|---|--------------------------|-----------|-----------|-----------|
| | 1(control) | 2 (20) | 3 (40) | 4 (80) |
| Eye opening: mean day of life | 14.7±0.6 | 15.3±0.9 | 15.4±0.9 | 15.5±0.6 |
| Passive avoidance test - memory % of negative findings | 9.0±22.9 | 7.6±12.7 | 3.0±9.8 | 9.8±16.3 |
| Passive avoidance test -learning % of negative finding | 16.4±25.5 | 10.0±12.1 | 12.0±19.3 | 32.6±28.7 |

Macroscopic post mortem findings: None

F1-dams and male F1-partners

- Mortality: None
- Clinical signs: Nothing
- Body weight: High dose - Slightly reduced
- Food consumption: No differences
- Reproduction: No drug-related influence

Macroscopic post mortem finding: No differences

F2-generation (untill weaning)

Sex distribution: No differences

Body weight: Within normal range

External examination: No differences

7. Examination of Septanest 1:100,000 adrenaline for effects on the pre- and postnatal development (including maternal functions) following subcutaneous administration to the dams of the F₀ generation (Segment III study). (Report No. 10657/97, vol. 16, page 1)

Study N°: 10657/97

Compound: Septanest 1/100,000 adrenaline; Batch No. RD100/11

Administered Volume: 0.5, 1.0, and 2.0 ml

Route: Subcutaneous

Treatment of Fo-generation: from implantation (6th day of gestation) to end (22nd day) of lactation

Observation Period: F0, F1- and F2-generations

Species: Rat

Strain: Sprague-Dawley / CrI:CD^RBR

Breeder: _____

Number of animals used for the Fo-generation:

| | | | |
|---------|-------------------|-----|-----------------|
| Group 1 | Control | 24♀ | Control vehicle |
| Grpup 2 | Low dose | 24♀ | 20 mg/kg |
| Group 3 | Intermediate dose | 24♀ | 40 mg/kg |
| Group 4 | High dose | 24♀ | 80 mg/kg |

Weights: 186-238 g

Study Site: _____

Sponsor: Septodont, F-94100 Saint-Maur des Fosses, France

Monitor: _____

GLP/QAU statements: Both submitted with signatures

Observation:

Clinical signs and Viability: Daily

Body weights:

F0 -, F1- generations: Daily during gestation and then on days-1, 7, 14, 21, and 22 of lactation.

F2 litters: On days 1, 4, 7, 14, and 21 of lactation.

Food consumption: weekly

Examination: The animals were allowed to deliver normally and examined for:

- (1) Number of pups absolute at birth and 4, 7, 14, and 21 days after birth
- (2) Number of pups per dam
- (3) Determination of sex and viability of fetuses
- (4) Number of pups with stillbirths
- (5) Number of pups with malformations

Reproductive indices: Gestation, birth, live birth, viability, lactation, and overall survival.
 Post natal physical and functional development::

| <u>Functional test</u> | <u>age of pups(days)</u> | <u>pups to be examined</u> |
|------------------------------|--------------------------|----------------------------|
| Mid-air righting reflex | 14 | each |
| Auditory startle reflex | 14 | each |
| Pupillary reflex | 21 | each |
| Open field | 27±1 | first half of each litter |
| Passive avoidance (learning) | 27±1 | second half of each litter |
| Passive avoidance (memory) | 34±1 | second half of each litter |

Morphological landmarks

| | | |
|--|----|------|
| Pinna detachment | 1 | each |
| Ear opening | 12 | each |
| Eye opening | 11 | each |
| Cleavage of the balanopreputial gland | 25 | each |
| Vaginal opening | 33 | each |
| Upper incisor eruption | 7 | each |

Termination / Autopsy:

Fo-generation - Day 22 of lactation
 F1 pups not selected - Day 22 of lactation
 F1 parents - Males at the end of mating period
 - Femlaes at the end of lactation period
 F2 offspring - After 3 lactation weeks

Results:

Fo-dams

Local tolerance: Dose-related scab formation at the injection site.
 Clinical signs: None.
 Mortality: Intermediate dose - One
 High dose - Three (18 ,9 18 th days of gestation)
 Body weights: No effect
 Food consumption: High dose - 8 - 18% ↓
 Reproduction: No influence (pregnancy, parturitionm birth index, viability index,
 lactation and overall survival)
 Macroscopic post mortem finding: No systemic change

F1-generation (until weaning)

Sex distribution: No difference
 Body weight: No effect
 External examination: No abnormalities.

Morphological landmarks, functional tests, open-field test: No differences in pinna detachment, upper incisor eruption, ear and eye opening, and vaginal opening.
No differences in functional tests
Macroscopic post mortem findings: None

F1-dams and male F1-partners

Mortality: None
Clinical signs: Nothing
Body weight: Within normal range
Food consumption: No differences
Reproduction: No drug-related influence
Macroscopic post mortem finding: No differences

F2-generation (untill weaning)

Sex distribution: No differences
Body weight: Within normal range
External examination: No differences

C. Mutagenic Potential

In Vitro:

1. Mutagenic study of the articaine hydrochloride in the Salmonella typhimurium reverse mutation assay (in vitro). (Report No. 10089/96, vol. 17, page 1).

Study: ~~---~~ Protocol No.10089/96

Study Site: _____

Sponsor: Septodont, F-94100 Saint-Maur des Fosses, France

Monitor: _____

GLP/QAU Statements: Both submitted with signatures

Final Report: Oct. 20, 1997

Compound: Articaine hydrochloride, batch no. 96442

Appearance: White to almost white clearance

Cells: Strains: Five strains of Salmonella typhimurium

Concentrations: five /independent experiment (100 to 10,000 ug articaine HCl/plate)

Plates: 3 per concentration and experiment

Data: Two independent experiments with and without metabolic activation using five tester strains, TA 98, TA 100, TA 102, TA 1535, TA 1537

Positive control substances:

Without metabolic activation

| | |
|----------------------------------|-----------------|
| Sodium azide in water | TA 1535, TA 100 |
| 2-nitro-9H-fluorene in DMSO | TA 98 |
| 9-aminoacridine in ethanol | TA 1537 |
| methyl methane sulfonate in DMSO | TA 102 |

with metabolic activation (S9)

2-anthraceneamide in DMSO all test strains

Result

No mutagenic effect was observed in any of the five tester strains in the presence or absence of activator.

2. In vitro assessment of the clastogenic activity of the artocaine hydrochloride in cultured CHO-cells. (Report No. 10090/96, vol. 17, page 50).

Study N°: — No. 10090/96

Study Site: _____

Sponsor: Septodont, F-94100 Saint-Maur des Fosses, France

Monitor: _____

GLP/QAU statements: Both submitted with signatures

Final Report: Oct. 15, 1997

Compound: Artocaine hydrochloride, batch no. 96,442

Appearance: White to almost white powder

Results:

| Treatment (µg/ml medium) | <u>4-hours exposure</u> | | | <u>20-hours exposure</u> | | |
|---|-------------------------|-----------------------------------|--|--------------------------|-----------------------------------|--|
| | Mitotic index | Number of Metaphases Scored | % of cells with aberration excluding gaps | Mitotic index | Number of Metaphases scored | % of cells with aberration excluding gaps |
| without metabolic activation Test medium (vehicle) | | | | | | |
| 0 | 1.00 | 200 | 3.5 | 1.00 | 200 | 2.5 |
| Artocaine hydrochloride | | | | | | |
| 125 | - | - | - | 0.99 | 200 | 3.0 |
| 250 | 0.85 | 200 | 3.5 | 0.94 | 200 | 2.5 |

| Treatment (µg/ml medium) | <u>4-hours exposure</u> | | | <u>20-hours exposure</u> | | |
|----------------------------------|-------------------------|-----------------------------------|--|--------------------------|-----------------------------------|--|
| | Mitotic index | Number of Metaphases Scored | % of cells with aberration excluding gaps | Mitotic index | Number of Metaphases scored | % of cells with aberration excluding gaps |
| 500 | 0.78 | 200 | 4.0 | 0.51 | 200 | 4.0 |
| 1000 | 0.87 | 200 | 5.5 | 0.19 | 95 | 6.3 |
| 2000 | 0.58 | 200 | 10.0 | - | - | - |
| Mitomycin C | | | | | | |
| 0.1 | 0.41 | 200 | 11.5 | 0.56 | 200 | 20.5 |
| With metabolic activation | | | | | | |
| Test medium (vehicle) | | | | | | |
| 0.0 | 1.00 | 200 | 3.5 | - | - | - |
| Articaine hydrochloride | | | | | | |
| 500 | 0.82 | 200 | 6.0 | - | - | - |
| 1000 | 0.84 | 200 | 6.0 | - | - | - |
| 2000 | 0.85 | 200 | 6.5 | - | - | - |
| 3000 | 0.50 | 124 | 7.3 | - | - | - |
| Cyclophosphamide | | | | | | |
| 5 | 0.75 | 200 | 17.0 | - | - | - |

Articaine hydrochloride up to cytotoxic concentration did not reveal any indication of mutagenic properties with respect to chromosomal or chromatid damage.

3. Mutagenicity study of articaine hydrochloride in mammalian cells (V79) in the in vitro gene mutation assay (HPRT test). (Report No. 10373/97, vol. 17, page 217).

Study N°: — No. 10373/97

Study Site: _____

Sponsor: Septodont, F-94100 Saint-Maur des Fosses, France

Monitor: _____

Final Report: Oct. 6, 1997

GLP/QAU statements: Both submitted with signatures
Compound: Articaine hydrochloride, batch no. 96.444
Appearance: White to almost white powder.
Cells: V79 (derived from lung tissue of fetal hamsters).

Results:

★ Preliminary test - Articaine at 4000 µg/ml medium produced severe cytotoxicity.
Main Studies: In two independent experiments, Articaine up to 4000 µg/ml with and without metabolic activation (S9 did not induce any mutation. The positive controls (ethyl methanesulfonate and 9,10-dimethyl-1,2-benzanthracene) generated a pronounced increase in the mutation frequencies.

In Vivo:

4. Micronucleus test of articaine hydrochloride in bone-marrow cells of the NMRI mouse by subcutaneous administration. (Report No. 10374/97, vol. 17, page 160).

Study N°: — No. 10374/97

Study Site: _____

Sponsor: Septodont, F-94100 Saint-Maur des Fosses, France

Monitor: _____

Final Report: May 23, 1997

GLP/QAU statements: Both submitted with signatures

Compound: Articaine hydrochloride, batch no. 96.444

Appearance: White to almost white powder.

Species/ Strain/ Stock: Mouse/ NMRI/ Cri:NMRI BR

Breeder: _____

Number of Animals: 50 (25 males and 25 females), 5 animals/sex/group

Age and Body Weight: 25 - 27 days, 20 - 24 g body weight

Route of Administration: Subcutaneous injection

Dose level: 75 mg/kg b.w.

Vehicle: Aqua ad injectabilia

Administration volume: 20 ml/kg

Positive Control: Cyclophosphamide, 27 mg/kg, by intraperitoneal injection

Sampling times: 24, 48, and 72 hours after treatment.

The study was carried out according to ICH2-safety - S2 - Genotoxicity, dated June 24, 1993.
Number of polychromatic erythrocytes scored per group; 10,000

Results:

| Compound (mg/kg) | Sam- pling time (h) | Ratio PCE/NCE | | | micronucleated polychromatic erythrocytes | | | Signi- fican- ce |
|-------------------------|------------------------------|---------------|---------|--------------------|--|---------|--------------------|------------------------|
| | | males | Females | Treatment group | males | Females | Treatment group | |
| Articaine hydrochloride | | | | | | | | |
| 0 | 24 | 1.31 | 1.15 | 1.23 | 0.6 | 1.0 | 0.8 | - |
| 75 | 24 | 1.12 | 1.53 | 1.32 | 0.6 | 1.6 | 1.1 | n.s. |
| 75 | 48 | 0.96 | 1.03 | 0.99 | 1.2 | 0.4 | 0.8 | n.s. |
| 75 | 72 | 1.18 | 1.20 | 1.19 | 1.8 | 1.2 | 1.5 | n.s. |
| Cyclophosphamide | | | | | | | | |
| 27 | 24 | 1.23 | 1.29 | 1.26 | 13.6 | 11.4 | 12.5 | S |

5. Micronucleus test of Septanest 1/100,000 adrenaline in bone marrow cells of the NMRI mouse by subcutaneous administration (base set). (Report No. 10651/97, vol. 17, page 104).

Study N°: — No. 10651/97

Study Site: _____

Sponsor: Septodont, F-94100 Saint-Maur des Fosses, France

Monitor: _____

GLP/QAU statements: Both submitted with signatures

Final Report: Oct. 29, 1997

Compound: Septanest 1/100,000 adrenaline, batch no. RD 100/10

Appearance: Clear, colorless liquid

Species/ Strain/ Stock: Mouse/ NMRI/ Cr:NMRI BR

Breeder: _____

Number of Animals: 50 (25 males and 25 females), 5 animals/sex/group

Age and Body Weight: 25 days (males), 27 days(females); 19 - 26 g body weight

Route of Administration: Subcutaneous injection

Dose level: 75 mg/kg b.w.

Vehicle: Aqua ad injectabilia

Administration volume: 20 ml/kg

Positive Control: Cyclophosphamide, 27 mg/kg, by intraperitoneal injection

Sampling times: 24, 48, and 72 hours after treatment.

The study was carried out according to ICH2-safety - S2 guidelines.

Number of polychromatic erythrocytes scored per group; 10,000

Results:

| Compound (mg/kg) | Sam- pling time (h) | <u>Ratio PCE/NCE</u> | | | <u>micronucleated polychromatic erythrocytes</u> | | | Signi- fican- ce |
|-------------------------|------------------------------|----------------------|---------|--------------------|--|---------|--------------------|------------------------|
| | | males | Females | Treatment group | males | Females | Treatment group | |
| Articaine hydrochloride | | | | | | | | |
| 0 | 24 | 0.87 | 1.04 | 0.95 | 1.6 | 2.8 | 2.2 | - |
| 75 | 24 | 1.04 | 1.0 | 1.02 | 1.4 | 2.2 | 1.8 | n.s. |
| 75 | 48 | 1.14 | 1.04 | 1.09 | 2.4 | 2.2 | 2.3 | n.s. |
| 75 | 72 | 1.09 | 1.27 | 1.18 | 2.4 | 3.8 | 3.1 | n.s. |
| Cyclophosphamide | | | | | | | | |
| 27 | 24 | 0.55 | 0.47 | 0.51 | 10.4 | 13.4 | 11.9 | S |

D. Pharmacokinetics:

1. 4-week subchronic toxicity study of Septanest 1/100,000 adrenaline by subcutaneous administration to Sprague-Dawley rats. (Report No. 10652/97, vol. 10, page 1). Reviewed under repeat dose toxicity studies section (page 6).

2. 4-week subchronic toxicity study of Septanest 1/100,000 adrenaline by subcutaneous administration to beagle dogs. (Report No. 10653/97, vol. 11, page 1). Reviewed under repeat dose toxicity studies section (page 8).

E. Biotransformation

1. Metabolism of articaine by human isoenzymes, protein binding, and stability of articaine in human plasma, in vitro validation of the analytical method. (Vol. 21).

Study N°: — No. 10337/97

Study Site: _____

Sponsor: Septodont, F-94100 Saint-Maur des Fosses, France.

Monitor: _____

GLP/QAU statements: Both submitted with signatures

Compounds: Articaine hydrochloride (batch no. 96-444) and articainic acid (batch no. 95527X)

Methods and Results:

Metabolism. Human liver microsomes were incubated with test substance at two concentrations (1 and 4 µg/ml) at 37 °C for 3 hours. A positive (ethoxyresorufin deethylase) and negative (without cytochrome P450 isoenzymes) controls were also performed. Articaine and articainic acid were measured by ~~—~~ method.

Result: The results indicated an almost quantitative recovery of the metabolized articaine as articainic acid.

Protein binding: Articaine was incubated at approx. 500 and 2000 ng/ml with human serum albumin, human γ-globulins and a fraction of human α- and β-globulins. Protein bindings ~~were~~ ^{was} determined after 6 and 24 hours incubations at 37 °C.

Result: A low protein binding was observed to γ-globulin and higher binding to albumin and α- and β-globulins.

Stability: The stability of articaine in pooled human serum was determined at two concentrations for 24 hours and 7 days at room temperature and -20 °C.

Result: Articaine was unstable without metabolizing enzyme inhibitor (~~—~~) The samples stored at -20 °C with esterase inhibitor was stable for one week.

2. In a published article [Pharmakokinetische untersuchungen mit ³⁵S-markiertem articaine] by R.E. Hoffer and H. Altmann (Prakt Anasth 1974;9:157-161), ³⁵S-labeled articaine was rapidly absorbed in the dwarf pig (100%) and in the human (85%) after IM injection. The curves for blood levels in man following a single administration of articaine showed two distinct phases of different half life.

| | I.V. administration | I.M. administration |
|---------|---------------------|---------------------|
| Phase 1 | 1.2±0.1 hours | 2.9±0.1 hours |
| Phase 2 | 69.7±16.7 hours | 31.5±6.6 hours |

Distribution of the radioactivity in dwarf pig (28 kg average body weight) after single administration expressed in Ci ³⁵S/gram of fresh weight is given below.

| Organs | After I.V. Injection | | After I.M. Injection | |
|--------|----------------------|----------------|----------------------|----------------|
| | 15 min (n=1) | 48 hours (n=2) | 60 min. (n=1) | 48 hours (n=2) |
| Brain | 49.6 | 0.38 | 21.0 | 0.39 |
| Heart | 39.2 | 1.01 | 25.0 | 0.76 |
| Lung | 119.8 | 1.37 | 132.2 | 2.35 |
| Liver | 145.0 | 2.74 | 59.6 | 3.30 |

Pharmacology

(what pharmacology studies?)

The results from preclinical studies demonstrate that articaine has a similar mechanism of action to other local anesthetics (lidocaine, procaine, prilocaine, mepivacaine, and bupivacaine). It reversibly blocks the conduction of painful sensations by diminishing the sodium ion influx during the action potential period. The duration of anesthesia was slightly increased after the addition of epinephrine or norepinephrine. The average duration of action in dogs by epidural administration was 120 ± 8 minutes for analgesia and 120 ± 6 minutes for motor block. Effects of articaine on the cardiovascular system are similar to those of other local anesthetics. All doses of standard anticonvulsants (sodium hexobarbital [16 and 30 mg/kg], propranolol [5 mg/kg], thiopental [20 mg/kg], caffeine [80 mg/kg], phenobarbital [40 and 80 mg/kg], and hexyltheobromine [80 mg/kg]) reduced convulsions caused by IV articaine (7.5, 10, and 12.5 mg/kg) in the rat. *sp*

Acute Toxicity

During single subcutaneous administration of Septanest without adrenaline, the LD_{40} was 500 mg/kg for male mice ($LD_{50} > 500$ mg/kg). In the female mice, the LD_{50} was 440 mg/kg. The LD_{50} of articaine without epinephrine by intravenous administration was approximately 38 mg/kg in the mouse, 23 mg/kg in the rat and 20 mg/kg in the rabbit. The acute minimal lethal dose by intravenous administration in the dog was 56 mg/kg. The toxicity of i.v. articaine increased in the presence of 1:100,000 epinephrine, and the LD_{50} decreased from 38 mg/kg to 3.7 mg/kg in the mouse and from 23 mg/kg to 11.4 mg/kg in the rat. Thus the lethality of articaine with epinephrine increased 10-fold in the mouse and 2-fold in the rat. The minimum lethal dose by intramuscular administration was 160 mg/kg in the dog. The animals had trembling, vertigo, and tonic and clonic convulsions independent of the route of administration and species of animals used. Lethal doses of articaine caused pulmonary edema. *included*

Subcutaneous administrations of aged articaine solutions, stored for three or six months at 37° C, in Swiss mice did not cause more toxicity in comparison with a freshly prepared solution with the same composition. Articaine 4% without vasoconstrictor was non-allergenic when analyzed by the Maximisation test of Magnusson and Kligman.

Repeat-Dose Toxicity

Histopathology of S-D rats treated for 4 week with subcutaneous administration of Septanest 1/100,000 adrenaline showed irritation effect (skin ulceration with eschar formation, epidermal/dermal and subcutaneous necrosis, inflammatory cell infiltration, granulation tissue, haemorrhage, oedema, muscular necrosis, atrophy and regeneration, epidermal hyperplasia and alopecia at the injection sites as compared to the control group) of the test compound. Extra-medullary haematopoiesis was increased in the treated animals. The systemic no-toxic effect level [NOEL] was 25 mg/kg/day by subcutaneous injection. The C_{max} at the NOEL dose was approximately 1.9 μ g/ml. Maximum articaine plasma levels were reached 20 - 40 min - after administration on Day 1 and 10 - 20 minutes after administration on Day 20. The mean

subcutaneous injection

AUC values at NOEL dose were 98.3 and 58.0 $\mu\text{g}\cdot\text{min}/\text{ml}$ on days 1 and 28 of dosing, respectively. Subcutaneous administration of Septanest showed a dose-related increase in the articaine plasma but there was a large variation~~s~~ in values. Articainic acid was one of the metabolites.

Subcutaneous administration of 80 mg/kg/day of Septanest for 4 weeks in beagle dogs resulted in an increase in plasma ALAT and ASAT activities. Increased salivation, ataxia, vomiting, sedation, defecation, clonic or tonic convulsions, abdominal and/or lateral position at the higher dose were also observed. The non-toxic-effect-level [NOEL] was 40 mg/kg/day in this study. The C_{max} at NOEL dose was 2.2 - 2.7 $\mu\text{g}/\text{ml}$. The mean AUC values in dogs at NOEL dose were 126.9 and 149.5 $\text{ng}\cdot\text{min}/\text{ml}$ on day 1 and day 28 of drug administration, respectively. The large variation in individual values and small number of animals per group makes it difficult to make best use of animal~~s~~ PK data. Articainic acid was one of the metabolites.

In general, the PK values in rat and dog are so scattered [p 6 & 9] due to small number of animals, it is very difficult to compare these values with human PK values. In human, articaine is metabolized to articainic acid by plasma esterase.

Mean \pm Standard Deviation of Human Pharmacokinetic Parameters

| Parameters | Articaine dose | | | |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|
| | 68 mg | 204 mg | 68 mg | 204 mg |
| | Articaine | | Articainic Acid | |
| C_{max} (ng/ml) | 385 \pm 165 | 899 \pm 363 | 1429 \pm 514 | 3793 \pm 796 |
| T_{max} (hr) | 0.4 \pm 0.1 | 0.8 \pm 0.2 | 0.9 \pm 0.3 | 1.3 \pm 0.2 |
| Kel (/hr) | 0.39 \pm 0.04 | 0.44 \pm 0.06 | 0.49 \pm 0.05 | 0.40 \pm 0.06 |
| $T_{1/2}$ (hr) | 1.8 | 1.6 | 1.4 | 1.7 |
| AUC ₀₋₁ (ng.hr/ml) | 631 \pm 135 | 1542 \pm 354 | 3751 \pm 885 | 11543 \pm 234 |
| Vd (L/kg) | 4.2 \pm 1.4 | 4.7 \pm 1.5 | 0.54* | 0.65* |
| CL (ml/min) | 1879 \pm 431 | 2323 \pm 561 | 316** | 309** |

* - Estimated Vd = (estimated Cl/Kel)/mean body weight

** - Estimated CL (articainic acid) = AUC (articaine) X CL (articaine) / AUC (articainic acid)

The primary metabolite, articainic acid, is further metabolized to articainic acid glucuronide. Peak plasma concentrations of articaine are related to dose and generally occur within 0.5 hour after administration

Reproductive Toxicity:

Segment I study was performed in according to ICH guideline 4.1.1. In this fertility and early embryonic development study, the fertility of rats was not impaired after treatment with 80 mg/kg of Septanest. Treatment of animals with 80 mg/kg led to 4 deaths (3 ♂ and 1 ♀). The vomiting, reduced motility, tonic convulsions and/or abdominal position were observed in animals before death. The NOEL dose were 40 and 20 mg/kg for female and males animals, respectively. There were no substance-related differences in number of corpora lutea , implantation sites and malformations of fetuses.

The influence of Septanest 1/100,000 adrenaline during critical phase of organogenesis was studied in rabbits. The no-observed-effect-level (NOEL) for the dams during Segment II study (embryotoxicity study) in rabbits by subcutaneous administration was 20 mg Articaïne HCl. Forty mg/kg generated tremor, abdominal position and tono-clonic convulsions and 80 mg/kg produced mortality. The NOEL for the fetuses was 40 mg/kg. An increase in skeletal variations (13th ribs) was observed at 80 mg/kg [control 23%, low dose 33%, median dose 28% and high dose 38%].

During embryotoxicity/segment II study in rats, the NOEL of Septanest 1/100,000 adrenaline was 40 mg/kg for the dams and 80 mg/kg for the fetus. No substance-related influence on prenatal fetal development was detected at 20, 40 and 80 mg/kg/day during the critical phase of development.

Articaïne hydrochloride at 80 mg/kg during pre- and postnatal development caused slight maternal toxicity in Sprague-Dawley rats. Increased number of stillbirth (Control 3, low dose 0, mid dose 0, and high dose 10), the marginal delay in eye opening, and the influence on the passive avoidance (learning) in F1 generation were also observed. The NOEL dose during gestation, lactation, and reproduction in F0-dams and postnatal growth and survival in F1-generation was 40 mg/kg/day s.c. The NOEL in the F₂ generation was 80 mg/kg/day.

During Segment III study in Sprague-Dawley rats, 80 mg/kg Septanest 1/100,000 adrenaline caused maternal toxicity (3 deaths). One animal also died at intermediate dose (40 mg/kg). Marginal decrease of the pup weight after 14 and 21 lactation days was observed in high dose animals. The NOEL dose during gestation and lactation was 20 mg/kg/day s.c. The NOEL dose for reproduction (fertility and breeding system) and pups postnatal growth and survival (F1 generation) was 40 mg/kg/day s.c.

The PK data (metabolites, $t_{1/2}$, C_{max} , AUC etc.) was not collected during any reproductive toxicity studies. Therefore, it is impossible to compare animal PK data with human PK values.

Mutagenic Potential

Articaïne hydrochloride (100 to 10,000 ug/plate) did not show any mutagenic activity in bacteria with and without mammalian metabolic activator (Ames test). Articaïne hydrochloride up to a cytotoxic concentration in the presence (3000 µg/ml) and absence (2000 µg/ml) of metabolic activator (human S9) did not show any clastogenic activity in cultured CHO cells. Mitomycin C and cyclophosphamide induced significant chromosome damage in the same

? photo 4

test.

The maximum tolerated dose of Septanest 1/100,000 adrenaline (75 mg/kg) by NMRI mouse produced no increase in the incidence of micronucleated polychromatic erythrocytes in the mouse bone marrow micronucleus test by subcutaneous administration. Under similar experimental conditions, Articaine hydrochloride at 75 mg/kg revealed comparable results. Articaine hydrochloride up to cytotoxic concentration (4000 µg/ml medium) in the presence and absence of metabolic activation did not induce any mutation in the HPRT-V79 mammalian cells

Absorption, Distribution, Metabolism, Excretion

The kinetics of Septanest with 1:100,000 epinephrine in rats and dogs have been reviewed under repeat-dose toxicity section. These studies showed a dose-related increase in articaine plasma levels in both species. The C_{max} at the NOEL dose of 25 mg/kg/day by subcutaneous administration was approximately 1.9 µg/ml in rats. The C_{max} in dogs at NOEL dose of 40 mg/kg/day was 2.2 - 2.7 µg/ml. The mean AUC values at NOEL dose in rats were 98.3 and 58.0 µg.min/ml on days 1 and 28 of dosing, respectively. The mean AUC values in dogs at NOEL dose were 126.9 and 149.5 ng.min/ml on day 1 and day 28 of drug administration, respectively. The final concentration of articaine and articainic acid, major metabolite in both species, were the same in several plasma samples. In general, the PK values in rat and dog are so scattered [pages 6 & 9] due to small number of animals it is very difficult to compare these values with human PK values. The detailed pathway, extent and site of metabolism are not studied and identified in animals. The activity of the metabolite(s) remains unknown. The excretion data are not available for rats and dogs.

In a published article by Hoffer and Altmann (Prakt Anasth 1974;9:157-161), S^{35} -labeled articaine was rapidly absorbed in the dwarf pig (100%) and in the human (85%) after IM injection. Preliminary tissue distribution data from dwarf pig showed the highest levels in kidneys, liver and lungs following either intravenous or intramuscular administration. By 48 hours all tissue levels were low. Articaine and its metabolites were eliminated rapidly mainly in the urine in both species. The metabolism of Articaine was different in man than in pig; in man no intact Articaine was found in the urine. These preliminary and old data measuring radioactivity in 1 - 2 pigs in 1974 are not considered appropriate for inclusion in the label as proposed by the Applicant.

APPEARS THIS WAY
ON ORIGINAL

Recommendations

This NDA is approvable from the pharmacology/toxicology point of view. Before the NDA can be approved, however, the amendment of the package insert should be made as follows:

- 1. Page 3 Clinical Pharmacology, second sentence in Distribution

DRAFT

OK

This experiment was done in 1974 with small number of animals (n=1 or 2). The database does not have enough power to warrant the inclusion in the package insert.

- 2. Page 6 Carcinogenesis, Mutagenesis, Impairment of Fertility.

(a) In accordance with 21 CFR 201.57 on the reproductive data, the multiples of the doses comparing those of the animals and humans should be included in the label either in term of AUC or body surface area unit. This information has been incorporated in the label by this reviewer.

(b)

DRAFT

to read

(c) The following paragraphs should be changed as stated below and moved to Pregnancy Category in accordance with 21 CFR 201.57

Draft

in any, none?
Published
Literature?
Product

✓

2 pages redacted from this section of
the approval package consisted of draft labeling

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DRAFT

LABELING

└

/s/

M. Anwar Goheer

^D
/s/ ^

Dou Huey (Lucy) Jean

Concur by Team Leader:

cc:

IND ORIG. _____

NDA - 20-971

HFD-170

/Goheer

/Hblatt

/Knolan

MAY 12 1998

MEMORANDUM

Date: May 7, 1998

To: Mei-Ling Chen, Ph.D.

Through: John Hun. *5/12/98*

From: Venkata Ramana S. Uppoor, Ph.D. *5/11/98*

Subject: Filing meeting for NDA 20,971 for Septanest - and Septanest - (articaine) solution for injection, 4% articaine hydrochloride with epinephrine 1/200,000 solution for injection and 4% articaine hydrochloride with epinephrine 1/100,000 solution for injection, 1S NDA, submitted on March 30, 1998 by Deproco, Inc., New Castle, DE 19720

Septanest - solutions for injection contain 4% articaine along with epinephrine 1/200,000 and 1/100,000 solution respectively. Articaine hydrochloride is a local anesthetic that has a reversible effect of blocking the conduction of painful sensations. Epinephrine is a vasoconstrictor added to articaine to slow down its passage into the general circulation and thus ensure the prolonged maintenance of active tissue concentration of articaine. This is indicated for infiltration anesthesia and nerve block anesthesia in clinical dentistry in patients 4 years and older. The recommended oral dose depends on the indication and should not exceed 7 mg/kg body weight.

PHARMACOKINETIC / BIOAVAILABILITY STUDIES

This NDA contains several clinical and few pharmacokinetic studies along with literature reports. The formulation used throughout the drug development program in U.S. (U.S. clinical and PK studies), according to the sponsor, is same as the to-be marketed formulation. Supportive clinical studies (e.g. conducted in France) utilized a slightly different formulation. The following have been submitted, based on FDA's recommendation, to characterize the pharmacokinetics of articaine and articainic acid.

1. Single dose and multiple dose PK study using the 4% articaine/1/200,000 epinephrine product
2. Literature article to provide PK in pediatric population age 3 to 12 years old.
3. Other published articles to provide PK data.
4. In vitro metabolism of the drug
5. Protein binding study
6. Assay methodology

COMMENTS: The following review issues have been noted: 1. The multiple dose study utilizes multiple doses given almost one after another. Therefore, comparison of this data to the single dose data (5.4 ml vs. 1.7 ml) can provide dose proportionality information. 2. The package insert has some errors, in terms of units for elimination rate constant. 3. No mass balance study has been submitted. 4. While PK in both males and females has been studied, no analysis for gender effect has been carried out.

RECOMMENDATION: The Human Pharmacokinetics and Bioavailability section of this NDA is organized, indexed, and paginated in a manner to initiate review. Hence, the submission is fileable from OCPB point of view. The sponsor should analyze the PK data for any gender effect.

CC list: HFD-170: NDA 20,971; Division file; CSO; HFD-870: Venkata Ramana S. Uppoor, Mei-Ling Chen, John Hunt; CDR\Barbara Murphy.

APPEARS THIS WAY
ON ORIGINAL

10 pages redacted from this section of
the approval package consisted of draft labeling

Per CSET Team Leader, no abuse liability review required.

APPEARS THIS WAY
ON ORIGINAL