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APPLICATION NUMBER: NDA 20773

PHARMACOLOGY REVIEW(S)

JUL 17 1997

NDA 20-773

SONORx

Bracco Diagnostics Inc.

1. Review and Evaluation of Pharmacology and Toxicology Data

**Division of Medical Imaging and Radiopharmaceutical Drug Products
HFD-160**

Nakissa Sadrieh, Ph.D.

2. Electronic File Number:

3. NDA Number: 20-773

4. Serial Number:

Submission Date: September 30, 1996

Type of submission: NDA

5. Information to Sponsor: Yes () No ()

6. Completion Date:

**7. Sponsor: Bracco Diagnostics Inc.
P.O. Box 5225
Princeton, NJ 08543-5225**

8. Manufacturer for drug substance:

9. Drug name: SONORx

1° drug (alternative names): Simethicone coated cellulose suspension 2° drug:

10. Chemical name: Simethicone (0.25%) coated cellulose

11. CAS number:

12. Structure:

13. Molecular Weight:

14. Relevant IND/NDA/DMF: IND DMF

15. Drug Class: Pharmacologically inert oral contrast agent for enhancement of ultrasound imaging. SONORx is expected to reduce ultrasound beam scattering and delineate the bowel wall from surrounding tissues and structures.

16. Indication: Enhancement of ultrasound imaging of the upper gastrointestinal tract, including the retroperitoneum. Intended for use in the delineation of anatomy and detection or exclusion of pathology in the upper abdomen, including the upper gastrointestinal tract and retroperitoneum. SONORx is designed to create an acoustic window by adsorbing and dispersing gas, thereby improving transmission of the ultrasound beam by producing a uniform echogenicity in the gastrointestinal tract.

17. Clinical Formulation (and components): SONORx is formulated as an orange-flavored aqueous suspension for oral administration. The active ingredient in the 400 ml dose is 7.5 mg simethicone-coated cellulose per ml (fiber length approximately 22 μ m) coated with 0.25% simethicone by weight. Human dose is 400 ml of SONORx which consists of 80 mg simethicone, 100 mg silicon and 10 gm cellulose (crystalline form manufactured from wood and considered to be generally regarded as safe, GRAS, by the FDA). The amount of simethicone in a single dose of SONORx is in the range of recommended daily doses for infants and children under 2 years of age, and is comparable or less than the maximum dose contained in antacids sold as over the counter tablets. SONORx is packaged as 10 or 16 fluid ounce clear glass bottles with a twist off vacuum indicating metal cap lined with plasticized PVC. SONORx is stable for 24 months at 25°-30°C. The maximum human dose used is 1000 ml which corresponds to 20 ml/kg for a 50 kg person.

| Component | Amount/ml | Amount/400 ml |
|------------------------------|-----------|---------------|
| Simethicone-coated cellulose | 7.5 mg | mg |
| Xanthan Gum, NF | mg | mg |
| Simethicone, USP | mg | mg |
| Sodium Lauryl Sulfate, NF | mg | mg |
| Fructose, USP | mg | mg |
| Orange Oil | mg | mg |
| FD&C Yellow No.6 | mg | mg |
| Sodium Benzoate, NF | mg | mg |
| Purified Water, USP | ml | ml |

18. Route of Administration: ORAL

19. Proposed clinical protocol:

20. Studies reviewed within this submission: This review will include a summary of all non-clinical animal data submitted by the sponsor. These data have previously been reviewed by the reviewing pharmacologists Dr. Norman See (March 10, 1994) and Dr. Ron Dundore (November 16, 1995 and January 25, 1996). These reviews will be summarized and evaluated together in order to provide a complete picture of the pre-clinical animal data submitted by Bracco in support of the safe use of SONORx. Therefore this review will comprise portions of the pharmacology reviews previously evaluated by other pharmacologists. The following is a table of all the preclinical studies that will be discussed in detail in this review. Also, a final summary and evaluation of all the preclinical data will be presented.

The following is a list of the pre-clinical animal data:

| Study # Vol. # | Study Date | Study Type | Species | Review Page |
|------------------------|---------------|--|---------|-------------|
| Ref. 1 Vol 4, p 174 | 12/92 | Acute oral toxicity | Mice | |
| Ref. 2 Vol 4, p 251 | 12/92 | Acute oral toxicity | Rats | |
| Ref. 3 Vol 4, p 328 | 12/92 | Acute intraperitoneal irritation/toxicity | Mice | |

| | | | | |
|---------------------------------------|----------------|--|------------------------------------|--|
| Ref . 4 Vol 5, p 1 | 12/92 | Acute intraperitoneal irritation/toxicity | Rats | |
| Ref . 7 Vol 5, p 118 | 12/92 | 28-day oral toxicity study | Dogs | |
| Ref. 5 Vol 6, p 1 | 9/95 | Acute intraperitoneal toxicity study with recovery | Rats | |
| Ref. 6 Study 698-001 Vol 7, p 1 | 12/92 | 28-day oral toxicity study | Rats | |
| Ref . 8 Vol 8, p 1 | 1/94 | Reproduction toxicity | Rats | |
| Ref. 9 Vol 9, p 1 | 4/94 | Developmental toxicity study | Rats | |
| Ref. 10 Vol 10, p 159 | 5/94 | Developmental toxicity study | Rabbits | |
| Ref. 11 Vol 11, p 1 | 4/94 | Perinatal and lactation study | Rats | |
| Ref. 12 Vol 12, p 176 | 12/92 | <i>Salmonella/E.coli</i> reverse mutation assay | TA98,100, 1535,1537 1538,WP2 | |
| Ref. 13 Vol 12, p 230 | 11/92- 2/93 | <i>In vitro</i> mammalian CHO/HGPRT forward mutation assay | CHO | |
| Ref. 14 Vol 12, p 277 | 11- 12/92 | CHO chromosomal aberrations assay | CHO | |
| Ref. 15 Vol 12, p 332 | 12/92 | Micronucleus cytogenetic assay | Mice | |

21. Studies not reviewed within this submission:

22. Disclaimer-use of sponsor's material: This review contains portions of the sponsor's submission.

23. Introduction/Drug History:

The IND for SonoRx[™] has previously been reviewed by Drs. See and Dundore. The NDA (20-773) is here reviewed by myself and includes sections from the pharmacology and toxicology reviews previously written by Drs. See and Dundore (March 10, 1994 and January 25, 1996, respectively). SonoRx is an orally administered ultrasound contrast agent for the visualization and detection of pathology of the abdominal organs and upper GI tract.

24. Previous clinical experience:

Please refer to the medical officer's review for details regarding the design and implementation of the clinical trials.

25. PHARMACOLOGY

26. Summary of Pharmacology:

27. Safety Pharmacology:

Summary of safety pharmacology:

SonoRx is not pharmacologically active.

28. Pharmacokinetics/Toxicokinetics

The sponsor claims that animal and human pharmacokinetic studies of simethicone and cellulose have been published in the literature and therefore no additional pharmacokinetic studies were performed in animals. Additionally, the sponsor states that cellulose (and its synthetic derivatives methylcellulose and carboxymethylcellulose) have been categorized as GRAS by the FDA, and simethicone has been included in the OTC monograph for Antacid Drug Products (published in the Federal Register on June 4, 1974). A maximum daily dose of 500 mg simethicone has been recommended by the OTC panel. The amount of simethicone in a 400 ml dose of SONORx is 80 mg. The dose of methylcellulose recommended for adults in the monograph for Laxative Drug Products for OTC use (Federal Register, October 1, 1986) is 6 gm daily (see table on page 3 for the composition of SONORx).

Summary of PK/TK:

29. TOXICOLOGY

The formulation used in the toxicology studies was the same formulation as that used in the clinical trials and that intended for marketing.

Table of dose multiples of SONORx as compared to the proposed human dose (400 ml) and the maximum human dose used in the clinical trials (1000 ml):

| <u>Species</u> | <u>Dose</u> | <u>Dose Multiple Based on Body Weight</u> | | <u>Dose Multiple Based on Surface Area</u> | |
|----------------|-------------|---|----------------|--|----------------|
| | | <u>400 ml</u> | <u>1000 ml</u> | <u>400 ml</u> | <u>1000 ml</u> |
| <u>Mice</u> | 10 | 1.25 | 0.5 | 0.1 | 0.04 |
| | 20 | 2.5 | 1 | 0.2 | 0.08 |
| | 40 | 5 | 2 | 0.4 | 0.16 |
| <u>Rats</u> | 2.5 | 0.31 | 0.125 | 0.05 | 0.02 |
| | 5 | 0.63 | 0.25 | 0.10 | 0.04 |
| | 10 | 1.25 | 0.5 | 0.2 | 0.08 |
| | 20 | 2.5 | 1 | 0.4 | 0.16 |
| | 40 | 5 | 2 | 0.8 | 0.32 |
| <u>Dogs</u> | 5 | 0.625 | 0.25 | 0.34 | 0.14 |
| | 10 | 1.25 | 0.5 | 0.68 | 0.27 |
| | 20 | 2.5 | 1 | 1.35 | 0.54 |
| <u>Rabbits</u> | 2.5 | 0.31 | 0.125 | 0.09 | 0.037 |
| | 5 | 0.625 | 0.25 | 0.19 | 0.074 |
| | 10 | 1.25 | 0.5 | 0.37 | 0.15 |

30. Single and repeat dose Toxicology

1. Reference 1. Acute oral toxicity study in mice with SONORx. Performing laboratory: Study completion date: March 10, 1993. Laboratory project identification: In-Life phase: December 4-18, 1992. This study is in volume 4, page 174. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR part 58).

Study design: CD-1 (ICR)BR albino mice (30 animals, 15/sex), were administered SONORx (Lot # PRA-92-0904) by oral gavage at 10, 20 or 40 ml/kg. The animals were administered SONORx once, and then observed for 14 days. On day 15, the mice were sacrificed.

| Group | Dosage level (ml/kg) | Number of animals | |
|----------|-------------------------|-------------------|---------|
| | | Males | Females |
| 1 (Low) | 10 | 5 | 5 |
| 2 (Mid) | 20 | 5 | 5 |
| 3 (High) | 40 | 5 | 5 |

At initiation of dosing, the animals were approximately 6 weeks of age and weighed 26-30 gm (males) and 20-23 gm (females). The high dose of 40 ml/kg was reported to be the maximal volume that could be humanely administered as a single gavage dose. During the 14 day observation period, the mice were checked for mortality and moribundity twice daily. Body weights were measured and recorded on days 1, 2, 3, 8, 11 and 15, and food consumption was measured and recorded weekly. On day 15, the animals were given an intraperitoneal injection of sodium pentobarbital and exsanguinated. Necropsy and gross pathology was performed on all animals.

Results: All animals survived until necropsy. No clinical signs were reported. All animals gained weight during the 14-day observation period and mean food consumption was consistent for all animals, without apparent group difference. During gross pathology examination, one low-dose female had moderately distended horns (both) of the uterus, and one high dose female mouse had pale cortices of both kidneys. These effects were reported to be incidental, and not related to the drug. The data indicate that a single dose of SONORx administered at up to 40 ml/kg to mice does not lead to any gross pathology, weight loss or loss of food consumption.

Reviewer's comments: There was no control group in this study. Although tissues were

preserved in 10% formalin, no histopathological evaluation was done. No hematology or serum chemistry parameters were assessed in this study.

2. Reference 2. Acute oral toxicity study in rats with SONORx. Performing laboratory:

Study completion date: March 10, 1993. Laboratory project identification: In-Life phase: October 1-December 14, 1992. This study is in volume 4, page 251. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR part 58).

Study design: CD-BR Sprague-Dawley rats (30 animals, 15/sex), were administered SONORx (Lot # PRA-92-0904) by oral gavage at 10, 20 or 40 ml/kg. The animals were administered SONORx once, and then observed for 14 days. On day 15, the rats were sacrificed.

| Group | Dosage level (ml/kg) | Number of animals | |
|----------|-------------------------|-------------------|---------|
| | | Males | Females |
| 1 (Low) | 10 | 5 | 5 |
| 2 (Mid) | 20 | 5 | 5 |
| 3 (High) | 40 | 5 | 5 |

At initiation of dosing, the animals were 10 weeks of age with body weights ranging from 348-387 gm for the males and 219-241 gm for the females. No control animals were used in this study.

Results: All animals survived until necropsy. No clinical signs were reported. All animals gained weight during the 14-day observation period and mean food consumption was consistent for all animals, without apparent group difference. No observations were made during necropsy. The data indicate that a single dose of SONORx administered at up to 40 ml/kg to rats does not lead to any gross pathology, weight loss or loss of food consumption.

Reviewer's comments: There was no control group in this study. Although tissues were preserved in 10% formalin, no histopathological evaluation was done. No hematology or serum chemistry parameters were assessed in this study.

3. Reference 3. Acute intraperitoneal irritation/toxicity study in mice with SONORx.

Performing laboratory: _____ Study completion date: April 28, 1993. Laboratory project identification: _____ In-Life phase: November 15-December 28, 1992. This study is in volume 4, page 328. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR part 58). This study was reviewed by Dr. Norman See (March 10, 1994) and will be summarized here.

Study design: The study was conducted in order to determine the potential for SONORx to cause irritation/toxicity in the peritoneal cavity, if this agent were to leak out through a perforation of the GI tract. Male CD-1 mice (15 animals per injection volume) received a single IP injection of SONORx (Lot # PRA-92-0904). The injection volumes were 10, 20 or 40 ml/kg. Control animals received 40 ml/kg of sterile saline by intraperitoneal injection. Five animals in each dosage group were sacrificed on days 2, 7, and 14 after the intraperitoneal injection.

Results: None of the animals died during this study and no changes in clinical signs or body weights were noted. At necropsy, 2 mice had "raised" areas (connective tissue and/or edema) on their small intestines. On days 7 and 14, mice had "progressively increased incidences of enlarged liver with pale and/or raised areas, and to a lesser extent, enlarged spleens with raised areas. At day 14, interlobular adhesions of the liver and adhesions in the abdominal cavity were noted in one intermediate-dose mouse and four high-dose mice."

Histopathology showed dose-dependent and treatment-related changes including capsular granulomatous inflammation of the liver, spleen and small intestine. Phagocytosis of a "non-polarizing material" (presumably cellulose) by Kupffer cells was also observed.

Conclusion: A single IP injection of SONORx as low as 10 ml/kg (approximately half the maximum oral human dose) caused some degree of inflammation and proliferation of connective tissue (ie, adhesion) within the peritoneal cavity of the mouse.

Reviewer's comments: The following is a summary of the comments reported in Dr See's review. Dr. See suggests that the reason why the inflammatory response was principally observed in the liver and spleen is because these were the only tissues that were examined in all the animals. It is stated that histology of the inflammatory response is not well characterized and not all tissues within the peritoneal cavity were examined (eg. GI, pancreas, skeletal muscle). Also, it is thought that a more long term study should have been carried out in order to determine how long it would take for the inflammatory response to clear up. I am in agreement with Dr. See's comments. At present, the terminally sacrificed animals had significant inflammation of various organs even 14 days after the administration of SONORx, even at the lowest dose used (10 ml/kg). Therefore, based on the above-mentioned deficiencies in the study design, it cannot be determined if leakage of SONORx from a perforated GI would lead to significant adverse events in humans. However, it appears that a

delayed immune-mediated response is present at up to at least 14 days after a single intraperitoneal injection of SONORx to mice.

4. Reference 4. Acute intraperitoneal irritation/toxicity study in rats with SONORx.

Performing laboratory:) Study completion date: April 27, 1993. Laboratory project identification: In-Life phase: October 1-December 22, 1992. This study is in volume 5, page 1. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR part 58). This study was reviewed by Dr. Norman See (March 10, 1994) and will be summarized here.

Study design: The study was conducted in order to determine the potential for SONORx to cause irritation/toxicity in the peritoneal cavity, if this agent were to leak out through a perforation of the GI tract. Sprague Dawley rats (:CD-BR) received a single IP injection of SONORx (Lot # PRA-92-0904). Five rats per sex per group (4 groups total) were given injection volumes of 10, 20 or 40 ml/kg SONORx. Control animals received 40 ml/kg of sterile saline by intraperitoneal injection. Parameters that were monitored included mortality, clinical signs, body weigh and food consumption. Fourteen days after dosing, each animal was subjected to gross necropsy. Histopathology of the liver was carried out and gross lesions of the spleen were also examined microscopically.

Results: None of the animals died during this study. Clinical signs were limited to 2 low-dose male rats and one high-dose male rat that exhibited swelling of the right abdominal region. The mean body weight of the high-dose male rats was significantly reduced by day 14 after dosing. At day 2 after dosing, all SONORx-treated male rats had weight loss, but only high-dose rats maintained the weight loss until termination (day 14 after dosing). The body weight of the female rats was not affected. Additionally, food consumption was decreased in treated male rats. At necropsy, the findings included enlargement of the liver, "raised" areas of the liver, spleen, cecum and uterus, as well as adhesions in the abdominal cavity.

Histopathology showed dose-dependent and treatment-related changes such as capsular granulomatous inflammation of the liver and spleen. The liver and the spleen were the only tissues that were examined microscopically. Kupffer cells showed phagocytosis of a "non-polarizing material" (presumably cellulose).

Conclusion: A single IP injection of SONORx at 10 ml/kg (half the maximum human oral dose based on body weight) caused inflammation and proliferation of connective tissue within the peritoneal cavity in the rat. This effect was not resolved up to at least 14 days after dosing with SONORx.

Reviewer's comments: The following is a summary of the comments reported in Dr See's review. Dr. See suggests that the reason why the inflammatory response was principally

observed in the liver and spleen is because these were the only tissues that were examined in all the animals. It is stated that histology of the inflammatory response is not well characterized and not all tissues within the peritoneal cavity were examined (eg. GI, pancreas, skeletal muscle). Also, it is thought that a more long term study should have been carried out in order to determine how long it would take for the inflammatory response to clear up. I am in agreement with Dr. See's comments. At present, the terminally sacrificed animals had significant inflammation of various organs even 14 days after the administration of SONORx, even at the lowest dose used (10 ml/kg). Clearly, a more rigorous study would help to determine the amount of time necessary for total recovery of the lesions in the abdominal cavity.

5. Reference 5. Acute intraperitoneal irritation/toxicity study in rats with SONORx with extended recovery. Performing laboratory: _____ Study completion date: September 14, 1995. Laboratory project identification: _____ In-Life phase: July 15-October 12, 1994. This study is in volume 6 page 1. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR part 58). This study was reviewed by Dr. Ronald Dundore (November 16, 1995) and will be summarized here.

Study design: The study was conducted in order to determine the potential for SONORx to cause irritation/toxicity in the peritoneal cavity, if this agent were to leak out through a perforation of the GI tract. Sprague Dawley rats (CD-BR) received a single IP injection of SONORx (Lot # PRA-92-0904). Twenty rats per sex per group (4 groups total) were given injection volumes of 5, 10 or 20 ml/kg SONORx. Control animals (20 rats) received 20 ml/kg of sterile saline by intraperitoneal injection. Parameters that were monitored included mortality, clinical signs, body weight and food consumption. On days 15, 30, 60 and 90 after dosing, 5 animals per sex per group were sacrificed and subjected to gross necropsy. The following tissues were removed, preserved, embedded in paraffin, sectioned, stained and examined microscopically: adrenal, cecum, colon, gross lesions, duodenum, esophagus, ileum, jejunum, kidneys, liver, pancreas, rectum, spleen and stomach.

Results: None of the animals died during this study. No clinical signs of toxicity that could be attributed to the treatment were noted. Male rats showed significant weight loss (maximum loss was 5.4%) during the first 24 hours after IP injection of SONORx. During the first week after treatment, food consumption was also decreased in the male rats. No effects were noted on body weight or food consumption in female rats.

At necropsy, "raised" and pale areas were noted on a number of abdominal organs in particular on the liver, spleen and diaphragm. Histopathology showed capsular granulomatous inflammation surrounding a birefringent material and an accumulation of non-polarizing amorphous material within the phagocytic cells in the affected organs of the abdominal cavity.

The areas of capsular granulomatous inflammation correspond to the "raised" areas. An accumulation of non-polarizing amorphous material was also noted in the red pulp of the spleen and glomerulus of the kidney of some animals treated with SONORx. The incidence and severity of the histomorphological changes appeared to be related to the dose of the test agent but not to the amount of time following the administration of SONORx.

Conclusion: The administration of SONORx at 5, 10 and 20 ml/kg by single IP injection produced capsular granulomatous inflammation of the spleen, liver and kidneys, and an accumulation of non-polarizing amorphous material, presumably cellulose, in the red pulp of the spleen and glomerulus of the kidney. The inflammatory response persisted for 90 days after administration of SONORx, therefore no significant resolution of the inflammatory response is seen up to 3 months after administration of a single dose of the test agent. The lowest dose (5 ml/kg) at which this effect is seen is one fourth the maximum human dose (20 ml/kg for a 50 kg person, based on body weight). Therefore, because of the potential for inflammatory response from leakage into the peritoneal cavity, I am in agreement with Dr. Dundore that SONORx should not be administered to patients with suspected perforation of the GI tract.

6. Reference 6. 28-Day oral toxicity study in rats with SONORx. Performing laboratory: Study completion date: April 27, 1993. Laboratory project identification: In-Life phase: October 1, 1992-January 5, 1993. This study is in volume 7 page 1. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR part 58). This study was reviewed by Dr. Norman See (March 10, 1994) and will be summarized here.

Study design: Sprague Dawley rats (CD-BR) (20/sex/group for the control and high dose groups and 10/sex per group for the low and mid-dose group of rats) received a single daily dose of SONORx by oral gavage for 28 days at doses of 10, 20 or 40 ml/kg/day. The control rats received 40 ml/kg of deionized water. The dosing was according to the following table:

| Group | Dosage level (ml/kg) | Number of animals | |
|------------|-------------------------|-------------------|---------|
| | | Males | Females |
| 1(control) | 0 | 20 | 20 |
| 2 (Low) | 10 | 10 | 10 |
| 3 (Mid) | 20 | 10 | 10 |
| 4 (High) | 40 | 20 | 20 |

Ten animals per sex per group were sacrificed after 28 days of treatment and the remaining 10 animals per sex per group in the control and the high-dose group were maintained for an

additional 2 weeks without treatment; these animals were considered to be recovery animals. Parameters that were monitored included hematology, blood chemistry, urinalysis, organ weights and histopathology. Histopathology was performed on all control and high dose group rats. Animals in the low and mid-dose groups were subjected to histopathology of the lung, liver kidneys, and of gross lesions only. Histopathology was not performed on the animals in the recovery groups.

Results: No mortalities were observed and no changes in clinical observations and body weight were noted. Ophthalmology and hematology parameters were not affected. The only serum chemistry finding was a statistically significant increase in serum potassium in the low and high-dose female rats at termination. This effect was not seen in the high-dose recovery animals. This was considered to be incidental. Urinalysis, gross pathology and histopathology parameters were also unaffected by the treatment.

Conclusion: Oral administration of SONORx for 28 days at up to 40 ml/kg (twice the human dose based on body weight) had no effect on any of the parameters assessed in rats in this study. Therefore, orally administered SONORx is not toxic to rats at doses up to 40 ml/kg for 28 consecutive days.

7. Reference 7. 28-Day oral toxicity study in dogs with SONORx. Performing laboratory: Study completion date: April 27, 1993. Laboratory project identification: In-Life phase: October 1, 1992-December 4, 1993. This study is in volume 5 page 118. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR part 58). This study was reviewed by Dr. Norman See (March 10, 1994) and will be summarized here.

Study design: Beagle dogs (3/sex/group) received a single daily dose of SONORx by oral gavage for 29 or 30 days at doses of 5, 10 or 20 ml/kg/day. The control dogs received 20 ml/kg of deionized water. The dosing was according to the following table:

| Group | Dosage level (ml/kg) | Number of animals | |
|------------|-------------------------|-------------------|---------|
| | | Males | Females |
| 1(control) | 20 | 3 | 3 |
| 2 (Low) | 5 | 3 | 3 |
| 3 (Mid) | 10 | 3 | 3 |
| 4 (High) | 20 | 3 | 3 |

Parameters that were monitored included ECG, hematology, blood chemistry, urinalysis, organ weights and histopathology of all major tissues. Electrocardiographs were recorded

from all animals (animals were not anesthetized) prior to initiation of dosing and during week 4 of treatment. It is not specified if the ECGs were recorded immediately after the administration of the test agent, or whether the ECGs were recorded after a period of time following dosing.

Results: No mortality, changes in clinical signs or changes in body weight were noted. Ophthalmology, ECGs, hematology, blood chemistry, urinalysis, gross pathology and histopathology were also unremarkable.

Conclusion: Oral administration of SONORx for 28 consecutive days at dosages of up to 20 ml/kg/day had no apparent effect on the beagle dog. Therefore, orally administered SONORx is not toxic to dogs under the conditions of this study.

SUMMARY OF TOXICOLOGY:

Pre-clinical acute and repeat-dose toxicology studies have been carried out with SONORx. The data showed that a single dose of SONORx administered by gavage at up to 40 ml/kg to mice or rats does not lead to any gross pathology, weight loss or loss of food consumption.

Treatment of rats and mice with SONORx by a single intraperitoneal injection caused a significant inflammatory response in the animals. In mice, progressively increased incidences of enlarged liver with pale and/or raised areas with interlobular adhesions, and to a lesser extent, enlarged spleens with raised areas and adhesions in the abdominal cavity were noted. Histopathology showed a dose-dependent and treatment-related changes including capsular granulomatous inflammation of the liver, spleen and small intestine. Phagocytosis of a "non-polarizing material" (presumably cellulose) by Kupffer cells was also observed. Therefore a single IP injection of SONORx as low as 10 ml/kg (approximately half the maximum human oral dose) caused some degree of inflammation and proliferation of connective tissue (ie, adhesion) within the peritoneal cavity of the mouse, and this effect was not resolved up to at least 14 days after dosage with SONORx.

In rats, the administration of SONORx at 5, 10 and 20 ml/kg by single IP injection produced capsular granulomatous inflammation of the spleen, liver and kidneys, and an accumulation of non-polarizing amorphous material, presumably cellulose, in the red pulp of the spleen and glomerulus of the kidney. The inflammatory response persisted for 90 days after administration of SONORx, therefore no significant resolution of the inflammatory response was noted up to 3 months after administration of a single dose of the test agent. The lowest IP dose (5 ml/kg) at which this effect is seen is one fourth the maximum human dose oral (20 ml/kg for a 50 kg person, based on body weight).

In repeat-dose toxicity studies, oral administration of SONORx for 28 days at up to 40 ml/kg to rats (twice the maximum human dose based on body weight) had no toxic effect. In dogs, oral administration of SONORx for 28 consecutive days at dosages of up to 20 ml/kg/day had no apparent toxic effect on the animals.

31. Carcinogenicity:

32. IMMUNOTOX:

No formal immunotoxicity studies were carried out. However, immunotoxicity findings were noted upon IP administration of SonoRx to rats. These findings were characterized by capsular granulomatous inflammation of the spleen, liver and kidneys and an accumulation of non-polarizing amorphous material, presumably cellulose in the red pulp of the spleen and glomerulus of the kidney. The inflammatory response persisted for at least 90 days after the administration of SonoRx. This effect was not resolved and is not expected to resolve.

33. REPROTOX:

8. Reference 8. Reproduction study in rats. Performing laboratory: [redacted]
Study completion date: January 31, 1994. Laboratory project identification: 698-001. In-Life phase: March 2-June 26, 1993. This study is in volume 8 page 1. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR part 58).

Introduction: This study has previously been reviewed by Dr. Ronald Dundore (January 25, 1996) and will be presented in the following section. The following is taken directly from Dr. Dundore's review:

Methods: Male and female rats (CD, 25/sex/group) were given SONORx at doses of 2.5, 10 or 40 ml/kg/day orally by gavage. A control group of rats (25/sex/group) received distilled water, 40 ml/kg/day. Administration of the test agent to male rats began 60 days prior to mating and continued until euthanasia. Administration of the test agent to females began 14 days prior to mating and continued until day 7 of gestation. The animals were observed daily for mortality and signs of toxicity. Body weight and food consumption were measured periodically throughout the study. Each male was cohabited with one female of the same treatment group. Animals were observed daily for signs of copulation. The day that evidence of copulation was detected was designated as gestation day 0. On day 20 of gestation, all surviving females were subjected to euthanasia and cesarean section. The uterus was excised. The fetuses were removed. The location and/or number of viable and nonviable fetuses, early and late resorptions, implantations and corpora lutea were

determined. Individual fetuses were weighed, sexed and examined for malformations and variations. The fetuses were preserved for possible visceral and skeletal examinations but were not examined further. The males were sacrificed after completion of the cesarean sections.

Mortality and clinical observations: Nine animals died or were sacrificed in a moribund condition during the conduct of the study: 1 control female, 1 mid dose female, 2 high dose females and 5 high dose males. It is likely that the animals died as a result of injuries sustained during the dosing procedure since the animals exhibited perforations of the esophagus or damage in the thoracic and/or abdominal cavities at necropsy. No signs of toxicity related to the administration of the test agent were observed in surviving animals.

Body weight and food consumption: Body weight and food consumption were comparable between control and treated animals.

Fertility indices: The percentages of animals which copulated and conceived offspring were not affected by treatment with the test agent. The mean time to copulation was similar among treatment and control groups.

Reproductive indices: No significant effects related to treatment were observed with regard to number of corpora lutea, implantation sites, resorption sites, viable fetuses or pre- or postimplantation losses. Mean fetal and placental weights were similar among treatment groups.

Fetal morphological examinations: Four fetuses exhibited malformations. Micrognathia was observed in a fetus from the low dose group. A fetus from another litter in the low dose group exhibited exencephaly, omphalocele, microphthalmia, agnathia and rachischisis. A fetus from a litter in the vehicle control group exhibited cleft palate, omphalocele, micrognathia and microphthalmia. A short tail was observed in a second fetus from the same control group litter. It is probable that the fetal malformations were spontaneous and not related to treatment with the test agent since no malformations were observed in litters from the mid and high dose groups."

Reviewer's comments: The data presented in this rat reproduction study indicate that SONORx does not affect male or female fertility and early development at doses up to 40 ml/kg. Some mortality was noted in the animals but this is most likely due to the procedure for dosing since perforation of the esophagus and damage to the abdominal and thoracic cavities were noted upon necropsy. Additionally, fetal malformations were noted in 4 fetuses, however these were deemed to be incidental, since they were not dose-related.

9. Reference 9. Developmental toxicity study in rats. Performing laboratory: Study completion date: April 28, 1994. Laboratory project identification: 698-002. In-Life phase: March 15-September 2, 1993. This study is in volume 9 page 1. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR part 58).

Introduction: This study has previously been reviewed by Dr. Ronald Dundore (January 25, 1996) and will be presented in the following section. The following is taken directly from Dr. Dundore's review:

“Methods: Male and females rats (CD, 1:1 pairing) were cohabited for mating. The occurrence of copulation was determined by daily inspection for a copulatory plug. Gestation day 0 was defined as the day when a copulatory plug was detected. Mated females were consecutively assigned to 1 of 4 treatment groups (n=45/group). Animals received distilled water, 40 ml/kg/day, or SONORx, 2.5, 10 or 40 ml/kg/day, orally by gavage beginning on day 7 and continuing through day 17 of gestation. Animals were observed daily for signs of toxicity. Body weight and food consumption were measured periodically throughout the study. On day 20 of gestation, the first 23-25 animals in each group were euthanized to obtain 20 gravid animals. The animals were subjected to cesarean section. The uterus was excised. The fetuses were removed. The number and/or location of viable and nonviable fetuses, early and late resorptions, implantation sites and corpora lutea were determined. Placental weights were determined. Fetuses were weighed, sexed and examined for malformations and variations. Fetuses were preserved in Bouin's solution or alcohol for subsequent visceral or skeletal examinations, respectively.

The remaining F₀ females in each group were allowed to deliver. On lactation day 0, litters were examined for litter size, live births, stillbirths and gross anomalies. Eight pups of equal sex distribution, when possible, were randomly selected for inclusion in developmental studies. On day 4, the pups not selected to remain on study were weighed, examined and euthanized. Throughout lactation, dams and pups were observed daily. Pups were weighed periodically and observed for a number of developmental and behavioral indices including static righting reflex, pinna detachment, cliff aversion, eye opening, air drop righting reflex, neuropharmacological examination, auditory response and rotarod performance. On day 21 of lactation, the dams were euthanized and subjected to gross necropsy. At approximately 35 days after birth, one offspring per sex was chosen randomly from each litter to be included in tests of activity, learning and emotionality and to be mated. The remaining F₁ offspring were euthanized and necropsied. Motor activity and emotionality were evaluated on day 35 of age by use of an automated activity apparatus. A passive avoidance paradigm to test learning and memory was initiated between 70 and 85 days of age and was

conducted using an automated, computerized shuttle box apparatus. When the F₁ animals reached a minimum of 80 days of age, 1 male and 1 female from the same treatment group (avoiding sibling matings) were cohabited. The occurrence of copulation was detected as the presence of a copulatory plug or from a vaginal smear. On gestation day 20, F₁ females were euthanized and subjected to cesarean section. The uterus was excised. The F₂ fetuses were removed. The number and/or location of viable and nonviable fetuses, early and late resorptions, implantation sites and corpora lutea were determined. Placental weights were determined. Fetuses were weighed, sexed and examined for malformations and variations. After the cesarean sections were performed on the F₁ females, the F₁ males were subjected to euthanasia and necropsy.

F₀ mortality and clinical observations: One female receiving SONORx, 10 ml/kg, died on day 17 of gestation. Focal hair loss was the only clinical observation noted prior to death. Foci in the glandular stomach mucosa, a dilated renal pelvis and an enlarged renal lymph node were observed at necropsy. All other animals survived to study termination. No clinical signs of toxicity or abnormalities at necropsy which were related to treatment were observed in surviving animals.

F₀ body weight and food consumption: No treatment-related effects on body weight, body weight change or food consumption were observed during gestation and lactation.

F₀ reproductive indices: No treatment-related effects on the number of corpora lutea, implantation sites, postimplantation losses, resorptions or viable and nonviable fetuses were noted. The mean time of gestation and fetal and placental weights were also unaffected by treatment. The male to female ratio was statistically greater in litters from animals treated with SONORx, 40 ml/kg, when compared to litters from control animals; the biological significance of this increase in the male to female ratio is questionable.

F₁ fetal malformations and variations: Fetuses from F₀ dams subjected to cesarean section were examined for malformations and variations. The observed malformations occurred in such low frequencies that it is likely the malformations occurred spontaneously and were unrelated to treatment. Similarly, the number and types of variations appeared to be comparable among the treatment groups.

F₁ offspring viability and growth: The administration of the test agent did not affect the litter size, the number of viable and nonviable pups or the number of pups that survived to weaning. No significant differences in the mean body weights of the F₁ offspring were observed until week 11 of age. Beginning on week 11, the mean body weight of the F₁ males exposed to SONORx *in utero* were significantly lower than that of control animals. The toxicological relevance of this difference in mean body weight

is questionable since weight was not affected in a dose-dependent manner and no differences in weight were observed in F₁ females. The body weights in F₁ females were comparable among treatment groups. The observations of the F₁ offspring at cage side and at necropsy were unremarkable.

F₁ behavioral and developmental indices: No differences among the treatment groups were noted in the behavioral and developmental indices evaluated: static righting reflex, time to pinna detachment, cliff aversion, time to eye opening, air drop righting reflex, neuropharmacological observations, auditory response, rotarod performance, passive avoidance behavior and emotionality. Measurements of spontaneous motor activity obtained by use of an automated activity apparatus revealed no differences in horizontal, stereotypic, clockwise, anticlockwise or total motor activity. However, males exposed *in utero* to SONORx, 40 ml/kg/day, spent significantly less time in the vertical position than control animals. Since this effect on vertical time was not seen in females and was not corroborated by any other differences in motor activity among the groups, it was probably an incidental event not related to treatment.

F₁ reproductive function: The exposure to SONORx *in utero* did not affect the percentage of F₁ males or females that copulated and conceived offspring. Copulation time was similar among treatment groups. Body weight changes, fetal and placental weights as well as the number of corpora lutea, implantation sites, postimplantation losses, resorptions and viable fetuses were similar among the groups of F₁ females.

F₂ malformations: Fetuses from F₁ females were examined for malformations. The observed malformations occurred in such low frequencies that it is likely the malformations occurred spontaneously and were unrelated to treatment.

Reviewer's comments: In this study, F₀ females were administered SONORx from gestation day 7 through 17. A number of the females were allowed to deliver. The pups were then subjected to a number of tests to evaluate the developmental consequences of exposure to the test agent *in utero*. Current guideline for reproductive toxicity studies (59 FR 48746) recommend that, in studies designed to evaluate the developmental consequences of exposure *in utero*, females should be dosed from day 7 of gestation through day 21 of lactation. Therefore, the pups evaluated in this study were exposed to the test agent for a period of time less than that recommended in the current guidelines. Implications are discussed in the Summary and Evaluation section."

Reviewer's comments: Treatment of female rats with SONORx up to 40 ml/kg did not affect any maternal toxicity parameters, nor did it affect any reproductive indices in the dams. Additionally, no fetal malformations were noted in this study. The viability and growth of the offspring were not adversely affected by SONORx. Similarly, behavior and developmental

indices of rats exposed to SONORx at up to 40 ml/kg *in utero* were not adversely affected by the test article. The reproductive function of rats exposed *in utero* to SONORx as assessed by copulation time, fetal and placental weights, corpora lutea, implantation sites, resorptions and number of viable fetuses was not affected by the test article. Additionally, no malformations were noted in the F₂ generation fetuses of rats exposed to SONORx *in utero*. Therefore, SONORx does not lead to developmental toxicity in rats at doses up to 40 ml/kg.

10. Reference 10. Developmental toxicity study in New Zealand White rabbits.

Performing laboratory: _____ Study completion date: May 7, 1994.
Laboratory project identification: 698-003. In-Life phase: April 6-September 2, 1993. This study is in volume 10 page 159. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR part 58).

Introduction: This study has previously been reviewed by Dr. Ronald Dundore (January 25, 1996) and will be presented in the following section. The following is taken directly from Dr. Dundore's review:

"Methods: Female New Zealand white rabbits were artificially inseminated (the day of insemination was designated as gestation day) and randomly assigned to 1 of 4 treatment groups (n=20/group). The rabbits were given distilled water, 10 ml/kg/day, or SONORx, 2.5, 5 or 10 ml/kg/day, orally by gavage from day 6 through day 18 of gestation. The animals were observed daily for signs of toxicity. Body weight and food consumption was measured periodically throughout the study. On day 29 of gestation, the animals were sacrificed and subjected to cesarean section. The uterus was excised and the fetuses were removed. The number and/or location of viable and nonviable fetuses, early and late resorptions, implantation sites and corpora lutea were determined. Uterine weight was determined. Fetuses were weighed, sexed and examined for malformations and variations.

Mortality and clinical observations: One control group animal died on gestation day 10. A dilated renal pelvis, fluid and adhesions in the pericardial sac and abdominal cavity and a frothy material in the trachea were noted at necropsy. One animal in the mid dose group was euthanized after an injury which affected limb function. A fractured humerus was noted at necropsy. All other animals survived to study termination and exhibited no signs of toxicity. Observations noted at necropsy were unremarkable.

Body weight and food consumption: No differences in the body weight or the change in body weight were observed among the groups. The administration of test agent did not affect food consumption.

Reproductive indices: No treatment-related effects on the number of corpora lutea, implantation sites, pre- or postimplantation losses, resorptions or viable and nonviable fetuses were noted. Fetal and uterine weights were also unaffected by treatment. One animal in the control group spontaneously aborted on day 27 of gestation.

Fetal malformations and variation: Although malformations and developmental variations were observed in fetuses of each treatment group, the incidence of malformations and variations appeared to be similar among the groups.

Reviewer's comments: The maximum dose of SONORx evaluated in this study was 10 ml/kg/day. The maximum human clinical dose is 20 ml/kg. Implications will be discussed in the Summary and Evaluation section."

11. Reference 11. Perinatal and lactation study in rats. Performing laboratory f

Study completion date: April 28, 1994. Laboratory project identification: 698-003. In-Life phase: March 1-August 18, 1993. This study is in volume 11 page 1. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR part 58).

Introduction: This study has previously been reviewed by Dr. Ronald Dundore (January 25, 1996) and will be presented in the following section. The following is taken directly from Dr. Dundore's review:

Methods: Male and female rats (CD, 1:1 pairing) were cohabited for mating. The occurrence of copulation was determined by daily inspection for a copulatory plug. Gestation day 0 was defined as the day when a copulatory plug was detected. Mated females were consecutively assigned to 1 of 4 treatment groups (n=30/group). Animals received distilled water, 40 ml/kg/day, or SONORx, 2.5, 10 or 40 ml/kg/day, orally by gavage beginning on day 17 of gestation and continuing through day 21 of lactation. Animals were observed daily for signs of toxicity. Body weight and food consumption were measured periodically throughout the study. The females were allowed to deliver. On lactation day 0, litters were examined for litter size, live births, stillbirths and gross anomalies. Eight pups of equal sex distribution, when possible, were randomly selected for inclusion in developmental studies. On day 4, the pups not selected to remain on study were weighed, examined and euthanized. Throughout lactation, dams and pups were observed daily. Pups were weighed periodically and observed for a number of developmental and behavioral indices including static righting reflex, pinna detachment, cliff aversion, eye opening, air drop righting reflex, neuropharmacological examination, auditory response and rotarod performance. On day 22 of lactation, the dams were euthanized and subjected to gross

necropsy. At approximately 35 days after birth, one offspring per sex was chosen randomly from each litter to be included in tests of activity, learning and emotionality and to be mated. The remaining F₁ offspring were euthanized and necropsied. Motor activity and emotionality were evaluated on day 35 of age by use of an automated activity apparatus. A passive avoidance paradigm to test learning and memory was initiated between 73 and 77 days of age and was conducted using an automated, computerized shuttle box apparatus. When the F₁ animals reached a minimum of 100 days of age, 1 male and 1 female from the same treatment group were randomly cohabited for mating. The occurrence of copulation was detected as the presence of a copulatory plug or from a vaginal smear. On gestation day 20, F₁ females were euthanized and subjected to cesarean section. The uterus was excised. The F₂ fetuses were removed. The number and/or location of viable and nonviable fetuses, early and late resorptions, implantation sites and corpora lutea were determined. Placental weights were determined. Fetuses were weighed, sexed and examined for malformations and variations. After the cesarean sections were performed on the F₁ females, the F₁ males were subjected to euthanasia and necropsy.

F₀ mortality and clinical observations: One animal that received SONORx, 10 ml/kg/day, died on day 18 of gestation. At necropsy, discoloration of the urinary bladder wall and foci in the kidneys and stomach were observed. In addition, one animal in the control group died on day 1 of lactation. Fluid in the abdominal cavity, a perforation of the stomach and adhesions in the stomach, jejunum and liver were observed at necropsy. It is unlikely that these deaths were related to the administration of test agent since no deaths occurred in animals given SONORx, 40 ml/kg/day. All other animals survived to study termination. Observations at cageside and at necropsy were generally unremarkable.

F₀ body weight and food consumption: No treatment-related effects on body weight, body weight change or food consumption were observed during gestation and lactation.

F₀ reproductive indices: The percentages of animals that copulated, conceived and delivered offspring were not affected by the treatment with SONORx. The number of implantation sites and the number of offspring delivered were also not affected by treatment.

F₁ fetal malformations and variations: Although malformations were observed in two fetuses exposed to SONORx, 10 ml/kg/day, *in utero*, it is likely that the malformations occurred spontaneously and were unrelated to treatment since malformations were not observed in fetuses exposed to 40 ml/kg/day of SONORx *in utero*. The incidence of developmental variations appeared to be similar among the treatment groups.

F₁ viability and growth: No differences in mean litter size or the mean number of dead and live offspring per litter existed between the treatment groups on day 0 of lactation. However, the percentage of live offspring to total implantation sites (birth index) was significantly lower in animals treated with SONORx, 10 ml/kg/day, than in control animals; one female in this mid dose group delivered 16 stillborn pups. Increased prenatal mortality was not observed in animals given 40 ml/kg/day of SONORx. Therefore, the difference in birth index was probably unrelated to treatment with the test agent. The ratio of male to female pups was greater in the low dose group than in the control group. The biological significance of this change in the male to female ratio is questionable. There were no differences among the treatment groups with regard to the percentage of pups surviving to day 35 of age. The body weight and the change in body weight of the F₁ offspring were not affected by the exposure to SONORx *in utero*. Observation of the F₁ offspring at cageside and at necropsy were generally unremarkable.

F₁ behavioral and developmental indices: No differences among the treatment groups were noted in the behavioral and developmental indices evaluated: static righting reflex, time to pinna detachment, cliff aversion, time to eye opening, air drop righting reflex, neuropharmacological observations, auditory response, rotarod performance, passive avoidance behavior and emotionality. Measurements of spontaneous motor activity were obtained by use of an automated activity apparatus. Although a few parameters of activity emerged as statistically significant among treatment groups, there was no consistent evidence to indicate that the exposure to SONORx *in utero* affected motor activity in the offspring.

F₁ reproductive function: The percentages of males and females that copulated and conceived offspring were not statistically different among the treatment groups and were within the normal historical limits of the laboratory. The mean time to copulation was also similar among the groups. Among the F₁ males that did not successfully conceive offspring, sperm motility and concentration and the percentage of abnormal sperm appeared similar and within historical limits. However, 1 male exposed to SONORx, 10 ml/kg/day, *in utero* had a sperm concentration of 0. Among F₁ females, the body weight and change in body weight during gestation was similar. Two F₁ females, 1 in the control group and 1 in the mid dose group, exhibited resorption of the entire litter. No treatment-related effects on the number of corpora lutea, implantation sites, pre- or postimplantation losses, resorptions or viable fetuses were noted. Fetal and placental weights were also unaffected by treatment.

F₂ external malformations and variations: Malformations were observed in 2 fetuses from the vehicle control group and 1 fetus from the low dose group. No external malformations were observed in F₂ fetuses from the mid and high dose groups. No

external variations were observed in any of the treatment groups.

Reviewer's comments: In this study, F₀ females were administered SONORx from day 17 of gestation through day 21 of lactation. The pups were then subjected to a number of tests to evaluate the developmental consequences of exposure to the test agent *in utero*. Current guideline for reproductive toxicity studies (59 FR 48746) recommend that, in studies designed to evaluate the developmental consequences of exposure *in utero*, females should be dosed from day 7 of gestation through day 21 of lactation. Therefore, the pups evaluated in this study were exposed to the test agent for a period of time less than that recommended in the current guidelines. Implications are discussed in the Summary and Evaluation section."

Reviewer's comments: The purpose of this study was to determine the effect of SONORx administration to pregnant female rats from day 17 of gestation to day 21 of lactation. Normally, in a "segment III" study (prior to ICH guidelines), the test agent was administered to animals from day 15 of gestation through parturition and lactation until weaning. However, the new ICH guidelines (4.2.1) recommend that the test agent be administered to animals from day 6 of gestation through day 21 of lactation. In the present "perinatal and lactation study in rats", SONORx was given at 2.5, 10 or 40 ml/kg by gavage from day 17 of gestation, rather than day 6 of gestation. With this study design, animals end up receiving 11 less doses of SONORx, than that recommended by the ICH guidelines (4.1.2). Since no significant adverse effects on development were noted in the study, this poor study design is of no great consequence. However, this type of study design is not desirable and is discouraged for future submissions.

The following is the summary and evaluation of the developmental toxicity studies previously reported in the review written by the pharmacologist, Dr. Ronald Dundore:

"SUMMARY AND EVALUATION OF DEVELOPMENTAL TOXICITY STUDIES:

The studies included in this submission seem to indicate that the oral administration of SONORx did not affect reproductive function or fetal development at doses up to 10 and 40 ml/kg/day in the rabbit and rat, respectively. The array of endpoints evaluated in these studies seems to include all examinations recommended by the current guidance (59 FR 48746). However, several inadequacies in experimental design and dose selection impact upon the interpretation and utility of the data obtained in these studies.

As stated above, current guideline for reproductive toxicity studies (59 FR 48746) recommend that, in studies designed to evaluate the developmental consequences of

exposure to test agents *in utero*, females should be dosed from day 6 or 7 of gestation through the end of lactation. Two of the studies discussed above were designed to assess the developmental consequences of exposure to SONORx *in utero*. In one study (study no. 698-002), dams were given SONORx from day 7 through day 17 of gestation. In a second study (study no. 698-004) dams were given SONORx from day 17 of gestation through day 21 of lactation. Therefore, the developmental consequences of continuous exposure to SONORx from day 7 of gestation to day 21 of lactation have not been evaluated. Since the active ingredient in SONORx is simethicone and since simethicone has been used safely in OTC products, this deficiency in the experimental design does not create a concern for the safety of the patients enrolled in the clinical trials. However, the deficiency in the experimental design may become an issue if the sponsor chooses to use the results of these studies to support labeling claims.

The maximum human clinical dose of SONORx is 1000 ml or 20 ml/kg (assuming a 50 kg human). In the studies discussed above, SONORx was administered at maximum doses of 10 and 40 ml/kg/day to rabbits and rats, respectively. Therefore, the doses utilized in these studies represent dose multiples of 0.5 and 2 times the human dose in the rabbit and rat, respectively. Since the maximum dose which can be practically administered to an animal is limited by the volume of test agent, the dose of SONORx administered to the rats, 40 ml/kg or 2 times the human clinical dose, seems reasonable. It was stated in the submission that 10 ml/kg was the maximum volume which could be practically administered to the rabbit. The sponsor should explore alternate means (alter concentration, split dose design, etc) to increase the exposure in rabbits to a level at least comparable to the maximum human clinical dose. This deficiency in experimental design does not create a safety concern but may become an issue if this study is used to support labeling claims. “

Reviewer's comments: I am in agreement with Dr. Dundore's evaluation. I would like to emphasize that the sponsor has combined pre-ICH and ICH guidelines in such a way that appropriate long-term dosing was never covered in the rat "segment III" study. Considering the nature of the drug product, this does not pose a significant safety issue, however, this particular study design is not desirable.

34. GENOTOXICITY:

12. Reference 12. Salmonella-Escherichia coli/mammalian microsome reverse mutation assay with a confirmatory assay. Performing laboratory:

Study completion date: April 16, 1993. Laboratory project identification: HWA 15327-0-409R. This study is in volume 12, page 176. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR part 58). This study was reviewed by

Dr. Norman See (March 10, 1994) and will be summarized here.

Study design: The study was conducted in order to determine the potential for SONORx (lot # PRA-92-0940) to cause genetic damage in *S. typhimurium* tester strains TA98, TA100, TA1535, TA1537, TA1538 and *E. coli* WP2 *uvrA*, in the presence and absence of Aroclor-induced rat liver S9. These tester strains are sensitive to basepair substitution (TA 1535 and WP2 *uvrA*) or frameshift mutations (TA 98, TA 1537, and TA 1538) or both (TA 100). A vehicle control (300 μ l deionized water) was used as well a negative control to determine spontaneous reversion frequencies. The concentrations of SONORx used were 10, 25, 50, 100, 200 and 300 μ l per plate. The final volume of test agent used was 300 μ l per plate, and the dilutions of SONORx were made with deionized water. Top agar was supplemented with 10 ml 0.5 mM biotin and 0.5 mM histidine per 100 ml agar for *S. typhimurium* strains, or containing 0.11 mg/ml tryptophan per 100 ml of agar for *E. coli* strain. The S9 mix contained: 10% S9, 5 mM glucose-6-phosphate, 4 mM β -nicotinamide-adenine-dinucleotide phosphate (NADP), 8 mM $MgCl_2$, and 33 mM KCl in 100 mM phosphate buffer at pH 7.4. The S9 mix was tested with 2-aminoanthracene as positive control mutagens. The Sham S9 mix contained 100 mM phosphate buffer at pH 7.4. Bottom agar consisted of Vogel-Bonner minimal medium E containing 1.5% (w/v) agar. On the day of the assay, the tester strains were tested for their correct genotype. The assay was performed in 2 phases: 1) dose-range finding study and 2) mutagenicity assay. Appropriate vehicle and positive controls were plated in triplicate with the tester strains. The sterility of the test article and the S9 mix was also appropriately tested. Plates were incubated for 48-72 hours at 37 ± 2 °C.

Results: In the dose-ranging study, no cytotoxicity was seen. In the mutagenicity assay, no positive responses were noted for any of the tester strains. It is therefore concluded that SONORx is not mutagenic in the *Salmonella/Escherichia coli* reverse mutation assay.

Reviewer's Comments: SONORx is negative in the *Salmonella/Escherichia coli* reverse mutation assay.

13. Reference 13. Mutagenicity test on SONORx in the CHO/HGPRT forward mutation assay with an independent repeat. Performing laboratory:

Study completion date: April 29, 1993. Laboratory project identification:

This study is in volume 12, page 230. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR part 58). This study was reviewed by Dr. Norman See (March 10, 1994) and will be summarized here.

Study design: The study was conducted in order to determine the potential for SONORx (lot # PRA-92-0940) to cause forward mutations at the HGPRT locus of CHO cells under conditions with and without metabolic activation (Aroclor-induced rat liver S9 metabolic activation system). Six doses of SONORX were used ranging from 3.13 μ l/ml to 100 μ l/ml. 5-Bromo-

2'-deoxyuridine (50 $\mu\text{g}/\text{ml}$) (BrdU) and 3-methylcholanthrene (5 $\mu\text{g}/\text{ml}$) were used as positive controls. The vehicle control consisted of deionized water (300 μl). An initial cytotoxicity test was performed for the purpose of selecting dose levels for the mutation assay, and consisted of evaluation of SONORx on colony forming efficiency. Cells were exposed to 10 concentrations of the test article for 4 hours, in the presence and absence of a metabolic activation system. After the treatment, the cells were washed, detached, reseeded and incubated for 7-10 days. The colonies were fixed, stained with Giemsa and counted manually. For evaluation of cytotoxicity, cell survival was determined. For cloning efficiency and mutant selection, replicates from each treatment group were trypsinized and replated with 10 μM thioguanine and incubated for 7-10 days, after which time, the colonies were fixed, stained and counted. The activation assay was identical except for the addition of the S9 fraction.

Results and conclusion: CHO cells were assessed for their capacity for becoming resistant to the purine analog (6-thioguanine), as a result of a mutation at the X-chromosome-linked hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus, as a result of exposure to 6 concentrations of SONORx. In both cytotoxicity and mutagenicity assays, no treated cultures exhibited mutant frequencies above 15 mutants per 10^6 clonable cells, either with or without metabolic activation.

Reviewer's comments: Under the conditions and the criteria set forth in this assay, SONORx was found to be negative in the CHO/HGPRT mutation assay both in the presence and absence of metabolic activation.

14. Reference 14. Mutagenicity test on SONORx measuring chromosomal aberrations in chinese hamster ovary (CHO) cells with and without metabolic activation with a confirmatory assay with multiple harvests. Performing laboratory:

Study completion date: April 9, 1993. Laboratory project identification:

This study is in volume 12, page 277. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR part 58). This study was reviewed by Dr. Norman See (March 10, 1994) and will be summarized here.

Study design: The study was conducted in order to determine the potential for SONORx (lot # PRA-92-0940) to induce chromosomal aberrations in cultured CHO-WBL cells, with and without metabolic activation with Aroclor-induced rat liver S9. In the S9-activated system, exposure to the test agent was for 6 hours, after which time the culture medium was removed, the cells were washed, and fresh medium was added to the cells, before incubation at 37 °C for an additional 17.83 hours. The doses of SONORx used in the study were 10, 25, 50, 75 and 100 $\mu\text{l}/\text{ml}$, with water used as the negative control. The positive control agents were mytomycin C for the non-activation series, and cyclophosphamide in the metabolic activation series. Twenty four hours after initiation of treatment, the cells were harvested and metaphase spreads were prepared after incubation with Colcemid for 2.5 hours. The cells were the

trypsinized, harvested and differentially stained for analysis of cell cycle delay using a modified fluorescence-plus Giemsa technique. Metaphase spreads were assessed for the number of cell cycles and mitotic index.

Results and conclusion: Exposure to SONORx did not increase the occurrence of chromosomal aberrations. Therefore SONORx is not clastogenic.

15. Reference 15. Mutagenicity test on SONORx: *in vivo* mammalian micronucleus assay. Performing laboratory: _____ Study completion date: April 9, 1993. Laboratory project identification: _____ This study is in volume 12, page 332. This study was conducted in compliance with Good Laboratory Practice (GLP) Regulations (21 CFR part 58). This study was reviewed by Dr. Norman See (March 10, 1994) and will be summarized here.

Study design: The study was conducted in order to determine the potential for SONORx (lot # PRA-92-0940) to induce micronuclei in bone marrow polychromatic erythrocytes of ICR mice. SONORx was administered once by gavage at 10, 20 and 40 ml/kg to 5 males and 5 female rats per dose, per harvest time group. The vehicle control consisted of sterile deionized water at a volume of 40 ml/kg. The positive control was cyclophosphamide which was administered by oral gavage at 80 mg/ml.

The dosing scheme for the micronucleus assay was:

| | Animals per sex sacrificed after dose administration | | |
|-----------------------------|---|----------|----------|
| | 24 hours | 48 hours | 72 hours |
| Vehicle control (10 ml/kg) | 5 | 5 | 5 |
| Low test dose (10 ml/kg) | 5 | 5 | 5 |
| Mid test dose (20 ml/kg) | 5 | 5 | 5 |
| High test dose (40 ml/kg) | 5 | 5 | 5 |
| Cyclophosphamide (80 mg/kg) | 5 | | |

The treated mice were sacrificed by CO₂ asphyxiation, and bone marrow was aspirated from the femurs into a syringe. The collected bone marrow cells were spread onto 2-4 clean slides for each animal. The slides were fixed with methanol, stained with May-Gruenwald-Giemsa, and mounted. One thousand polychromatic erythrocytes per animal were scored for the presence of micronuclei. The frequency of micronucleated cells was expressed as percent micronucleated cells based on the total polychromatic erythrocytes present in the scored optic

field. The test was considered to be positive if a dose-responsive increase in micronucleated polychromatic erythrocytes was observed.

Results and conclusion: SONORx was tested for its ability to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow of male and female ICR mice. The positive control cyclophosphamide, induced a significant increase in the number of micronucleated polychromatic erythrocytes in both male and female mice relative to the vehicle control. In the vehicle control group and in the SONORx-treated groups of animals, there did not seem to be an increase in the number of micronucleated polychromatic erythrocytes at any dose level, regardless of the bone marrow collection time and in either sex. The PCE:NCE ratio was significantly higher in the 20 and 40 ml/kg dose groups at the 48 hour time point compared to the corresponding vehicle control groups. There was no explanation for this finding and it was not thought to be significant. I agree that this finding is not an indication that SONORx is positive in the mouse micronucleus test. Therefore, SONORx is negative in the *in vivo* mouse micronucleus assay.

Reviewer's comments: SONORx was shown to be negative in the following assays: 1) Salmonella-Escherichia coli/mammalian microsome reverse mutation assay, 2) the CHO/HGPRT forward mutation assay, 3) chromosomal aberration assay in chinese hamster ovary (CHO) cells with and without metabolic activation, and 4) *in vivo* mammalian micronucleus assay. It is therefore concluded that SONORx is not genotoxic.

35. Special Toxicology Studies:

- Focal Toxicology
- Phototoxicology/photocarcinogenicity
- Dermal

36. Overall Summary:

SONORx is a pharmacologically inert oral contrast agent for enhancement of ultrasound imaging by reducing ultrasound beam scattering thus delineating the bowel wall from surrounding tissues and structures. SONORx is indicated for enhancement of ultrasound imaging of the upper gastrointestinal tract, including the retroperitoneum. SONORx is designed to create an acoustic window by adsorbing and dispersing gas, thereby improving transmission of the ultrasound beam by producing a uniform echogenicity in the gastrointestinal tract. SONORx is formulated as an orange flavored aqueous suspension for

oral administration. The active ingredient in the 400 ml dose is 7.5 mg simethicone-coated cellulose per ml (fiber length approximately 22 μm) coated with 0.25% simethicone by weight. Human dose is 400 ml of SONORx which consists of 80 mg simethicone mg silicon and gm cellulose (crystalline from manufactured from wood). Cellulose (and its synthetic derivatives methylcellulose and carboxymethyl cellulose) have been categorized as GRAS by the FDA, and simethicone has been included in the OTC monograph for Antacid Drug Products (published in the Federal Register on June 4, 1974). A maximum daily dose of 500 mg simethicone has been recommended by the OTC panel. The amount of simethicone in a 400 ml dose of SONORx is 80 mg. The dose of methylcellulose recommended for adults in the monograph for Laxative Drug Products for OTC use (Federal Register, October 1, 1896) is 6 gm daily (see table on page 3 for the composition of SONORx).

Since animal and human pharmacokinetic studies of simethicone and cellulose have been published in the literature, no additional pharmacokinetic studies were performed in animals. Neither cellulose nor simethicone are absorbed from the gastrointestinal tract and both agents are expected to be excreted unchanged in the feces.

Pre-clinical acute and repeat-dose toxicology studies have been carried out with SONORx. The data showed that a single dose of SONORx administered by gavage at up to 40 ml/kg to mice or rats does not lead to any gross pathology, weight loss or loss of food consumption.

Treatment of rats and mice with SONORx by a single intraperitoneal injection caused a significant inflammatory response in the animals. In mice, progressively increased incidences of enlarged liver with pale and/or raised areas with interlobular adhesions, and to a lesser extent, enlarged spleens with raised areas and adhesions in the abdominal cavity were noted. Histopathology showed a dose-dependent and treatment-related changes including capsular granulomatous inflammation of the liver, spleen and small intestine. Phagocytosis of a "non-polarizing material" (presumably cellulose) by Kupffer cells was also observed. Therefore a single IP injection of SONORx as low as 10 ml/kg (approximately half the maximum human dose) caused some degree of inflammation and proliferation of connective tissue (ie, adhesion) within the peritoneal cavity of the mouse, and this effect was not resolved up to at least 14 days after dosage with SONORx.

In rats, the administration of SONORx at 5, 10 and 20 ml/kg by single IP injection produced capsular granulomatous inflammation of the spleen, liver and kidneys, and an accumulation of non-polarizing amorphous material, presumably cellulose, in the red pulp of the spleen and glomerulus of the kidney. The inflammatory response persisted for 90 days after administration of SONORx, therefore no significant resolution of the inflammatory response was noted up to 3 months after administration of a single dose of the test agent. The lowest dose (5 ml/kg) at which this effect is seen is one fourth the maximum human dose (20 ml/kg

for a 50 kg person, based on body weight).

In repeat-dose toxicity studies, oral administration of SONORx for 28 days at up to 40 ml/kg to rats (twice the maximum human dose based on body weight) had no toxic effect. In dogs, oral administration of SONORx for 28 consecutive days at dosages of up to 20 ml/kg/day had no apparent toxic effect on the animals.

In developmental toxicology studies, SONORx did not affect male or female rat fertility and early development at doses up to 40 ml/kg. Treatment of pregnant female rats, during days 6 to 17 of gestation, with up to 40 ml/kg SONORx did not cause maternal toxicity, nor did it affect any reproductive indices in the dams. Additionally, no fetal malformations were noted. The viability and growth of the offspring were not adversely affected by SONORx. Similarly, behavior and developmental indices of rats exposed to SONORx at up to 40 ml/kg *in utero* were not adversely affected by the test article. The reproductive function of rats exposed *in utero* to SONORx (F₁ pups) as assessed by copulation time, fetal and placental weights, corpora lutea, implantation sites, resorptions and number of viable fetuses was not affected by the test article. Additionally, no malformations were noted in the F₂ generation fetuses of rats exposed to SONORx *in utero*. Therefore, SONORx does not lead to developmental toxicity in rats treated between days 6 and 17 of gestation with doses of the test article up to 40 ml/kg. In another study, SONORx administration to pregnant female rats from day 17 of gestation to day 21 of lactation given at up to 40 ml/kg by gavage did not lead to any significant adverse effects on development of the F₁ and F₂ generations of pups.

In rabbits, administration of SONORx at doses up to 10 ml/kg per day on days 6 to 18 of gestation did not lead to any signs of maternal toxicity. Additionally, no effect on any of the reproductive indices, such as number of corpora lutea, implantation sites, pre- or post-implantation losses, resorptions and number of viable fetuses, were seen with SONORx treatment. Therefore, SONORx does not lead to developmental toxicity in rabbits, at doses up to 10 ml/kg.

The studies included in this submission seem to indicate that the oral administration of SONORx did not affect reproductive function or fetal development at doses up to 10 and 40 ml/kg/day in the rabbit and rat, respectively. The maximum human clinical dose of SONORx is 1000 ml or 20 ml/kg (assuming a 50 kg human). In the reproduction studies, SONORx was administered at maximum doses of 10 and 40 ml/kg/day to rabbits and rats, respectively. Therefore, the doses utilized in these studies represent dose multiples of 0.5 and 2 times the human dose in the rabbit and rat, respectively, based on body weight. However, due to the paucity of toxicity associated with SONORx administration, it is unlikely that these small dose-multiples would present any significant risk to pregnant women.

In genotoxicity studies, SONORx was shown to be negative in the following assays: 1)

Salmonella-Escherichia coli/mammalian microsome reverse mutation assay, 2) the CHO/HGPRT forward mutation assay, 3) chromosomal aberration assay in chinese hamster ovary (CHO) cells with and without metabolic activation, and 4) *in vivo* mammalian micronucleus assay. It is therefore concluded that SONORx is not genotoxic.

37. Recommendations:

Based on preclinical animal data submitted in the NDA, I believe that there is no reason for concern regarding the safety of SonoRx in humans. Bracco has carried out appropriate preclinical studies to show that the oral administration of SonoRx in humans is safe. Therefore, I recommend **APPROVAL** of SonoRx for use with ultrasound in delineation of pathologies in the gastrointestinal tract.

- Internal Comments (info shared with MO, Chem, Stat, Biopharm, DSI)

I have been informed by the reviewing chemist, Dr. Sidney Gilman, that there are major chemistry and manufacturing concerns regarding the stability testing of SonoRx by Bracco. Brown precipitate has been found on the vial closures and apparently 20% of the sample vials that were put aside for stability testing never underwent the necessary analysis. Dr. Gilman has informed me that this is very serious and is grounds for **NON-APPROVAL**. It appears that this issue has been ongoing for several months, and the sponsor has been postponing a site inspection by FDA officials. On June 26, 1996, I was informed of the recent developments by Dr. Gilman, which I have summarized above. This does not impact on the Pharmacology/Toxicology study results. My recommendation of **APPROVAL** still stands, based on the preclinical animal data that was submitted for review.

- Future review issues

- External Recommendations (To Sponsor)

38. NDA Issues:

39. Labeling Reviews:

Carcinogenesis, mutagenesis, impairment of fertility:

No animal studies were conducted to evaluate the carcinogenic potential of SonoRx.

SONORx was shown to be negative in the following assays: 1) Salmonella-Escherichia coli/mammalian reverse mutation assay, 2) CHO/HGPRT forward mutation assay, 3) chromosomal aberration assay in (CHO) cells and 4) *in vivo* mammalian micronucleus assay.

It is therefore concluded that SONORx is not genotoxic.

Pregnancy:

Pregnancy category B. Reproduction and developmental toxicity studies have been performed in rabbits and rat at doses up to 0.4 and 0.8-times the recommended human dose based on surface area, and 1.25 and 5 times the recommended human dose based on body weight, respectively. At the doses tested, no evidence of impaired fertility or harm to the fetus due to SONORx was noted. There are however no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used in pregnancy only if clearly needed.

Nursing mothers:

It is not known whether SonoRx is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when SonoRx is administered to a nursing woman.

40. Investigator's Brochure/Informed Consent Review:

41. Reviewer Signature

IS

7/17/97

Team Leader Signature

IS

17 July '1997

I concur with Dr. Sadrieh's conclusions and recommendations.

42. CC:

- HFD-160/original NDA
- HFD-160/division files
- HFD-160/Sadrieh/Pharm/Tox
- HFD-160/Meyers/Pharm/Tox/team leader
- HFD-160/Gilman/Chemist
- HFD-160/Udo/Biopharm
- HFD-160/Al Osh/Stats
- HFD-160/Yaes/Medical Officer
- HFD-160/Jordan/CSO
- HFD-345/Compliance

43. Appendix:

NDA 20-773

SONORx

Bracco Diagnostics Inc.

44. Draft Date: May 1, 1997 (first draft)
July 1, 1997 (second draft)

45. Addendum: