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Dr. Susan Wayland
Acting Assistant Administrator
Office of Prevention, Pesticides, and Toxic Substances TS-7101
Environmental Protection Agency
401 M Street, SW
Room 637, East Tower
Washington, DC 20460

MR 28030

Dear Dr. Wayland:

The Chemical Manufacturers Association makes available to the public and appropriate government agencies final reports of environmental, health, and safety research that it manages. In keeping with this policy, the following report that the CMA Ethylene Glycol Ethers Panel recently conducted is enclosed:

"Short-Term Studies to Evaluate the Dosimetry and Modes of Action of 2-Eutoxyethanol in B6C3F1 Mice"

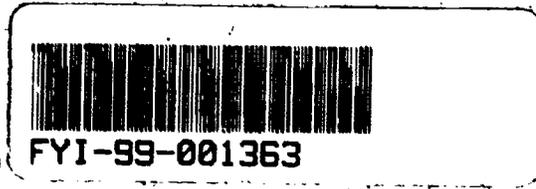
The report does not include confidential information.

If you have any questions, please call Dr. Susan Lewis of my staff at 703-741-5636.

Sincerely yours,

Courtney M. Price/HCS

Enclosure



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FINAL REPORT

Battelle Project No. 29752
CMA Agreement No. EGE-74.0-DOSE-BAT.NW – Amendment No. 8

July 13, 1999

**“Short-Term Studies to Evaluate the Dosimetry and Modes of Action of 2-
Butoxyethanol in F3C3F1 Mice”**

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EXECUTIVE SUMMARY

The draft NTP report on the chronic inhalation studies with 2-butoxyethanol (BE) identified the forestomach and liver of B6C3F1 mice as potential target organs for tumorigenicity (NTP, 1998). Based upon the general lack of genotoxicity and the known hemolytic mode of action of BE in rodents, it is likely that these potential treatment-related tumors occurred via indirect mechanisms involving chronic tissue injury. For the liver tumors, the hemolytic effects of BE and subsequent accumulation of degradation byproducts may have increased the levels of reactive oxygen species. Mice are peculiarly sensitive to oxidative stress, relative to rats and humans. Furthermore, humans are relatively insensitive to BE-induced hemolysis. For the forestomach tumors, chronic, contact irritation from grooming of BE deposited on the fur or mucociliary clearance of BE dissolved in the mucous layers of the respiratory tract may have been a contributing factor. Alternatively, systemic delivery of BE or, more likely, its major metabolite, butoxyacetic acid, at a high dose-rate may have caused chronic irritation in the mouse forestomach and subsequently increased the probability of spontaneously-initiated cells progressing to tumors. These potential indirect mechanisms were investigated in a series of studies conducted at Battelle Northwest and at the Indiana University School of Medicine (Dr. J. Klaunig, Principle Investigator).

At Battelle Northwest, the potential role of oral ingestion via grooming in the production of forestomach lesions of female mice exposed to BE via whole body vs. nose-only inhalation exposures was investigated. Small amounts of BE were removed from the fur of female mice exposed for 6 hr to target concentrations of 250 ppm BE vapor. The total dose that was available for oral consumption via grooming was 9.2 ± 2.9 mg/kg for the whole-body exposure (229.2 ppm BE) and 7.5 ± 2.3 mg/kg for the nose-only exposure (241.8 ppm BE). Very little difference was detected in the internal dose of BE resulting from whole-body vs. nose-only exposure as determined by analysis of BE and its major metabolite, BAA, in blood collected immediately post-exposure and in urine collected for up to 18 hr post-exposure. Small amounts of BE were detected in the urine which was attributed to residual BE on the fur and not from urinary elimination. Although this was not a mass-balance study, the amounts of BE deposited on the fur of mice represented 9-13% of the total BAA eliminated in the urine. BE deposited on the fur and consumed via grooming *during* the exposure as well as orally consumed via mucociliary clearance of BE deposited in the mucous layer lining the respiratory tract was not accounted for in this study. Nevertheless, there is evidence that at least a small amount of neat BE was available for oral consumption via grooming and that the potential role for chronic, daily oral doses in mouse forestomach toxicity cannot be ruled out.

To evaluate whether direct contact with BE can cause forestomach lesions and evaluate the potential role of hemolysis-mediated oxidative stress in the liver, neat BE (no vehicle) was administered to male and female mice at dose levels up to 1200 mg/kg/d for one week. Feed was withdrawn from each animal 4 hr prior to dosing and returned 2-3 hr post-dosing to mimic the conditions of the inhalation study. The dose levels used in this study resulted in severe hemolysis and mortality that was not predicted in a 1-week dose range finding study and the two-week study was terminated after only 4 doses. Neat BE

was clearly shown in this oral gavage study to cause irritation to the mouse forestomach similar to that observed by the NTP following inhalation exposure. Forestomach lesions consisted of focal areas of irritation and epithelial hyperplasia. The compensatory proliferative response was confirmed by PCNA immunohistochemistry. This clearly established that direct oral administration of neat BE is capable of causing lesions in the mouse forestomach.

To investigate whether or not parenteral administration of BE could also produce forestomach lesions in mice, additional mice were administered saline solutions of BE by either intraperitoneal (IP) or subcutaneous (SC) injection. Dose levels were 400 and 600 mg/kg/d for three consecutive days (probe study; n = 3/group) or 0 (saline control) and 400 mg/kg/d for five consecutive days (full study; n = 6/group). All three mice administered 600 mg/kg BE by IP injection for three days in the probe study showed clear evidence of focal irritation in the forestomach similar in appearance to the lesions observed in gavage and inhalation studies. One of three mice/group in each of the 400 mg/kg IP and 400 and 600 mg/kg SC groups in the 3-day probe study also had forestomach lesions, but these were considered minimal. Spontaneous lesions similar to these can occur in untreated mice. In the 5-day study at 400 mg/kg/d, 1/6 IP-dosed mice and 2/6 SC-dosed mice also had minimal lesions that were potentially treatment-related; none were observed in the controls. Together, these data suggest that forestomach lesions in the mouse may be induced by systemic administration and may not *require* direct contact with BE. It is further possible that the metabolite, BAA, may play a role in producing the forestomach lesions since the IP injection route results in first-pass liver metabolism while SC injection does not. Interestingly, these lesions had not been observed in previous drinking water studies conducted by NTP suggesting that the lesions may be dose-rate dependent.

In the liver evaluations, there was no evidence of degeneration and no corresponding compensatory cellular regeneration in mice administered neat BE by gavage. In addition, there was no remarkable accumulation of hemosiderin in Kupffer cells (H&E stain) although there was a clear dose-response increase in iron content in Kupffer cells (Perl stain) and to a much lesser degree in hepatocytes. The increase in iron may play a potentially significant role in oxidative stress in these cells. The potential for oxidative stress in the livers of these mice was evaluated and reported separately by the Indiana University School of Medicine (Kamendulis et al., 1999).

INTRODUCTION

2-Butoxyethanol (BE) was recently evaluated in a series of inhalation toxicity studies by the National Toxicology Program. While the technical report of this research program has not been reviewed and approved by the NTP Board of Scientific Counselors, the results have been prepared for public review and comment (NTP, 1998). Preliminary results from those studies have identified the forestomach and liver of B6C3F1 mice as potential target organs for tumorigenicity. These potential treatment-related tumors likely occurred via indirect mechanisms involving chronic tissue injury. For the liver tumors, the hemolytic effects of BE and subsequent accumulation of degradation byproducts may have increased the levels of reactive oxygen species. Mice are peculiarly sensitive to oxidative stress, relative to rats and humans. Furthermore, humans are relatively insensitive to BE-induced hemolysis which raises questions regarding the ability to extrapolate the liver effects observed in mice to human health risk. For the forestomach tumors, chronic, contact irritation from grooming of BE deposited on the fur or mucociliary clearance of BE dissolved in the mucous layers of the respiratory tract may have been a factor. The potential role for direct contact with neat BE in forestomach toxicity was also evaluated since exposure to BE via drinking water did not cause these effects (NTP, 1993). Alternatively, systemic delivery of BE or, more likely, its major metabolite, butoxyacetic acid, at a high dose-rate may have caused chronic irritation in the mouse forestomach and subsequently increased the probability of spontaneously-initiated cells progressing to tumors. Therefore, the specific aims of this study were to:

- Evaluate the potential role of oral ingestion (via grooming) of neat 2-butoxyethanol in the production of forestomach lesions of mice exposed to BE vapor (Aim 1)
- Evaluate the potential for increased oxidative stress associated with hemolysis in the production/progression of liver lesions in mice (Aim 2)
- Evaluate the potential role for systemic delivery of BE in producing forestomach lesions following routes of administration undergoing either first-pass metabolism (intraperitoneal injection) or systemic distribution prior to metabolism (subcutaneous injection) (Aim 3).

To accomplish these objectives, the amount of BE deposited on the fur of mice following either a single 6-hr, whole-body or nose-only exposure was determined. To evaluate the impact of fur deposition on the internal dose of BE, samples of blood and urine were analyzed for BE and its metabolite, butoxyacetic acid (BAA). In addition to the dosimetry studies, the direct effect of BE on the forestomach of mice was evaluated by administering neat BE daily by oral gavage for up to five days. The forestomach of each mouse was evaluated histologically for evidence of irritation and increased cell replication. These same orally-dosed animals were also used to evaluate whether the accumulation of iron in Kupffer cells, as a secondary event of hemolysis, is associated with an increased oxidative stress in the liver. This latter objective was conducted in collaboration with Dr. Jim Klaunig, Indiana University Medical Center. Finally, the indirect effect of BE on the forestomach of mice was evaluated histologically following

administration of saline solutions of BE by either intraperitoneal or subcutaneous injection for 3 or 5 days.

METHODS

Test Materials. The test chemical, 99+% spectrophotometric grade 2-butoxyethanol (BE, lot number 00437AS for Specific Aim 1 and lot number 07847HN for Specific Aims 2 and 3), was purchased from Aldrich Chemical Co. (Milwaukee, WI). Certificates of analysis received from Aldrich Chemical Co. indicated that the test chemical was 99.59% and 99.87% pure by gas chromatography for lot numbers 00437AS and 07847HN, respectively. The test chemical was stored at controlled room temperature.

Test Animals. A total of 50 young adult female B6C3F1 mice (including extras for control blood and urine collection and matrix standards for analyses) were supplied by Taconic Laboratory Animals and Services (Germantown, NY), for use Specific Aim 1. Taconic Laboratory was the supplier of mice for the NTP inhalation studies. Animals were ordered to arrive at approximately 10-11 weeks of age so that the females were of sufficient size to fit in the nose-only exposure system. Animals were fed Purina 5002 certified pelleted diet and fresh tap water was provided *ad libitum*. Mice were housed individually in hanging wire stainless steel cages. Mice were observed for moribundity and mortality twice daily. Body weights were determined three days after arrival (for randomization and group assignment purposes) and prior to each exposure. The mice were assigned to their respective exposure group (20 each) prior to the initiation of the first exposure. Each animal designated for nose-only exposure was acclimated to the nose-only restraint device for four days prior to exposure. For Specific Aims 2 and 3, a total of 90 male and 90 female B6C3F1 mice (including extras) and 36 female B6C3F1 mice, respectively, were also supplied by Taconic. All animals were acclimated to the laboratory environment for at least one week prior to placing on study.

Specific Aim 1: Deposition of BE on the fur of mice.

Study Design. Female B6C3F1 were exposed by either whole-body (20 mice) or nose-only (20 mice) inhalation, for a single 6-hr exposure period to a target concentration of 250 ppm BE. This concentration was the highest concentration used in the NTP inhalation bioassay (NTP, 1998).

Immediately following each exposure (whole-body and nose-only), five mice were quickly sacrificed and their bodies washed two times each with hot tap water. Each water wash was analyzed for BE. Another 10 mice were transferred to metabolism cages for an 18-hr post-exposure urine collection. The urine and cage washes were analyzed for BE and its major metabolite, butoxyacetic acid (BAA). The remaining 5 mice were killed immediately post-exposure and their blood collected into heparinized tubes for analysis of BE and BAA.

Inhalation Exposures.

Vapor Generation System.

The test article was pumped by a precision metering pump (Model 302; Gilson Inc, Middleton, WI) into a 25 mm x 300 mm threaded-glass air sampling manifold (Ace Glass Inc., Vineland NJ) (Figure 1). BE was vaporized on the heated (~105°C) glass surface of the generator while a metered air stream flowed through the generator (~20 L/min) and then into the exposure chamber (whole body or nose-only). The buildup of vapor concentration in the exposure chambers at the beginning of exposure to 90% of its stable final concentration (T_{90}) was estimated to be 10 min for the whole body chamber (~85 L mixing volume) and 3 min for the nose-only system. Exposure duration was 6 hours + T_{90} from the time the exposure was initiated.

Whole Body Exposure Chamber.

The whole body exposure chamber was an 85 L polycarbonate box. Mouse cages were suspended in the center of the box above a stainless steel catch pan. BE vapor was introduced into the chamber from 4 ports along the top of the chamber and was exhausted from 4 ports below the catch pan. Eight sample ports were distributed across one face of the chamber; 4 of the ports were adjacent to the cage units and 4 ports were located above the cages. The generator airflow (~20 L/min) resulted in ~14 air changes per hour. The distribution of BE vapor in the chamber was determined prior to the start of the study by comparing the concentration from 3 different chamber positions.

Nose-Only Exposure Unit.

The nose-only exposure unit was a flow-past type consisting of two concentric stainless-steel manifolds; one supplied fresh vapor to the breathing zone of each animal and one removed the expired and excess vapor. Airflow through the unit was ~20 L/min from the generator (inlet) with an exhaust flow of ~18 L/min to prevent the animals from re-breathing their expired air. The exposure unit consisted of 3 tiers of 8 exposure ports. For concentration determination, three of the ports (one from each tier) were used to collect samples of the breathing atmosphere; samples were also collected from the inlet manifold using an additional port from the lowest tier. Uniformity of the test article concentration in the exposure unit was determined prior to the study.

Chamber monitoring.

The exposure concentrations of BE were determined by gas chromatographic analysis of atmosphere "grab samples" taken directly from the exposure chamber during animal exposure. Grab samples were collected on charcoal sampling tubes (ORBO-101). Known volumes of the chamber atmosphere were collected using a calibrated critical-orifice-controlled sampler at a constant flow rate of ~0.2 L/minute for 3 minutes each. An extraction solution was added to the primary charcoal bed after sample collection to desorb the test chemical from the charcoal. The extraction solution (toluene) contained an internal standard (1-phenylhexane) to correct for variations in injection volume and drift of instrument sensitivity. The sample extracts were analyzed using a HP-5890 gas chromatograph (GC) equipped with a flame ionization detector operated at 300°C and a

30-m x 0.53-mm ID, Rtx-5, 1.5-µm film thickness capillary column (Restek Corp., Bellefonte, PA). The carrier gas was helium at a head pressure of ~6 psi. The column was maintained at 60°C for 1.0 minute and then heated at 16°C/minute to a final temperature of 200°C. Under these conditions, BE had a retention time of ~4.4 minutes (Figure 2). Data were obtained using HP ChemStation software, version A.04.02.

Gravimetrically prepared standards made from high purity Aldrich Chemical Co. BE (lot number 07847HN) and the internal standard in toluene were used to calibrate the GC for quantitation of BE in the grab samples. Two independently weighed stock solutions were used each time the standard series was prepared. One stock solution was used to prepare the calibration standards. The quality control check standards were prepared with the second stock solution and were analyzed in the presence of the sampling medium using the same extraction procedure as the chamber grab samples. The quality control check standards were used to check the preparation of the standards, sample extraction from the sampling media, and any drift of instrument calibration throughout the analysis. The analyzed concentrations of the quality control check standards were required to be within ±10% of their prepared concentrations for a valid analysis.

Nominal exposure concentrations were calculated using the mass of test article pumped, the total time during which the metering pump was running and the dilution airflow into the chamber. This value was compared to the average exposure concentrations obtained by grab samples.

Collection of fur washes, blood and urine. At the end of each exposure (nose-only and whole-body), five mice were sacrificed using CO₂ and transferred to individual beakers and submerged in 50 ml of hot water. After rinsing in 50 ml of water, the mice were transferred to a second beaker containing an additional 50 ml of hot water. Each fur rinse was stored frozen (-70°C) until analyzed. This fur washing procedure was similar to that used by Tyl et al. (1995) to determine the deposition of ethylene glycol on the fur of rats and mice.

At the end of each exposure (nose-only and whole-body), five mice were immediately anesthetized using 70% CO₂ and samples of blood were collected via the retro-orbital sinus. The time of each blood sample was accurately recorded. Following collection, each blood sample was maintained on ice until placed in freezer (-70°C).

At the end of each exposure (nose-only and whole-body), 10 mice were placed in individual metabolism cages (Lab Products, Seaford, Delaware) for an 18-hour urine collection. The urine samples were collected over dry ice. Mice were provided with access to water but not food. Following urine sample collection, each metabolism cage was rinsed with ~25mL of distilled water. Each urine sample and cage rinse was stored frozen (-70°C) until analyzed.

Analyses of fur. Samples of each fur wash were analyzed directly using a Hewlett Packard (HP) 6890 GC equipped with a flame ionization detector (FID) (Hewlett Packard; Palo Alto, CA). Chromatography was achieved using a DB-Wax capillary column (15

meter x 0.53 mmid x 1.0 μ m film thickness, J &W Scientific, Folsom, CA.) in the splitless injection mode. Helium was used as the carrier gas at a constant column head pressure of 4.0 psi. One μ l of neat (water) sample was injected onto a large bore 4 mmid injection liner to facilitate the water vapor expansion generated at the constant injection temperature of 205°C. The oven temperature was initially maintained at 80°C for 2.0 min then ramping at 30°C/min to 240°C. FID temperature was 270°C. Under these conditions, BE had a retention time of ~3.2 minutes.

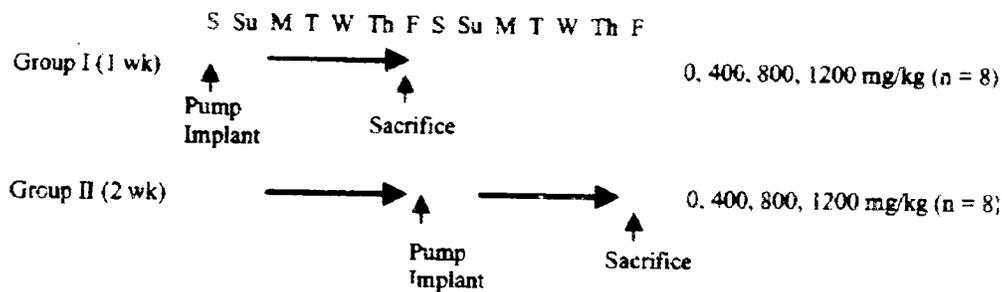
Analysis of blood and urine. A GC method developed previously to analyze for related glycol ethers and their alkoxyacetic acid metabolites was modified to simultaneously analyze for BE and the metabolite, BAA in blood (Corley et al., 1998). Urine samples and cage washes were also analyzed by this method for BE and BAA. The method involved the acidification of a weighed quantity (~0.25 g) of blood, urine or cage wash, with an equal volume of 0.9 M H₂SO₄, addition of 0.25 g Na₂SO₄ (to improve extraction efficiencies) and extracted with 0.25 ml ethyl acetate containing known concentrations of internal standards (ethoxyethanol [EE] and ethoxyacetic acid [EAA]) to correct for possible extraction/instrument variations during analysis. Separation and quantitation was achieved using a 30 m x 0.32 mmid x 0.5 μ m film thickness Stabilwax-DA capillary column (Restec, Bellefonte, PA) and a Hewlett Packard 6890 GC with flame ionization detection. Splitless injections of ~1.5 μ L of extract were made at an injector temperature of 200°C. The initial oven temperature was 70°C for 2.5 min then increased at 20°C/min to 240°C and held for one min. Hydrogen was used as the carrier gas at 18 psi constant pressure. The FID temperature was 270°C. For the preceding conditions EE, BE, EAA and BAA had retention times of ~3, ~4, ~7 and ~8 minutes, respectively (only approximate retention times are provided due to slight variations in retention times observed for each matrix).

Specific Aim 2: Effect of orally-administered neat BE on the forestomach and liver.

Probe Study. Male and female B6C3F1 mice, 5/sex/dose, were administered neat BE by oral gavage at 100, 400 or 800 mg/kg/day for 5 consecutive days. After two days of dosing at 800 mg/kg, no clinical signs of toxicity were observed. Therefore, animals assigned to the 100 mg/kg/day group were administered 1200 mg/kg/day for the remaining 3 days of the probe study. Feed and water were removed at ~7:00 a.m. and the dosing was initiated at approximately ~11:00 a.m. to mimic the fasting state associated with inhalation studies. Feed and water were returned to the animals by 2:00 p.m. Each animal was weighed on the morning of the first dose and the volume of BE delivered adjusted to deliver the appropriate mg/kg dose level for each animal. The animals were observed for overt signs of toxicity; animals not expected to survive the next dose were humanely sacrificed. The purpose of this probe study was to determine if the animals would survive the treatment regimen and select dose levels for the definitive study described below. Each surviving animal was weighed and sacrificed by cervical dislocation four hours after the final dose. Stomach (empty), liver and spleen weights were recorded and blood was drawn by cardiac puncture for determination of the hematocrit. Slices of esophagus, forestomach and glandular stomach, as well as liver

were fixed in neutral buffered formalin. Selected samples were evaluated as described below for the Full Study.

Full Study Design. Based on the results from the probe study, male and female B6C3F1 mice, 16/sex/dose, were initially treated by oral gavage with saline, 400, 800 or 1200 mg/kg of neat 2-butoxyethanol. Each animal was weighed at the beginning of the week to adjust the dosing volume to the appropriate mg/kg body weight. Prior to the first week of dosing a group of 8/sex/dose was implanted with 5-day osmotic minipumps delivering bromodeoxyuridine for determination of cells in S-phase in the liver. The dosing regimen was to have been 5 days/week according to the following schedule:



During the first two days of dosing, excessive mortality was observed across treatment groups that was not anticipated based on the results from the probe study. The mortality was observed in all groups, except controls, with and without implanted osmotic minipumps. Therefore, each dose level was reduced in half resulting in the following dose levels: saline control, 200, 400 or 600 mg/kg/day. During the next two days of administering the lower dose levels (total of four days of dosing) the mortality continued and the study was terminated on the fifth day. Each surviving animal was weighed and sacrificed by cervical dislocation the day after the final dose. Stomach (empty), liver and spleen weights were recorded and blood was drawn by cardiac puncture for determination of the hematocrit. Slices of esophagus, forestomach and glandular stomach, as well as liver were fixed in neutral buffered formalin for histopathology. The remaining liver tissues (~0.5 g) was snap frozen in liquid nitrogen for analysis of markers for oxidative stress by Jim Klaunig, Indiana University School of Medicine. After discussions with the sponsor, the tissues taken from the terminal sacrifice were analyzed as described below although the group sizes were significantly affected by the mortality. This action was considered necessary to complete the study prior to the meeting of the NTP Board of Scientific Councilors to review the NTP report and precluded repeating the study in the available time.

Forestomach Analysis. Paraffin-embedded tissues were serially sectioned and stained with hematoxylin and eosin (H&E) or antibodies against proliferating cell nuclear antigen (PCNA). Histological analysis (H&E) were conducted to determine evidence of necrosis and/or irritation of the gastric mucosa. The primary determination of cells in S-phase was accomplished by analyses of PCNA incorporation according to Eldridge and Goldsworthy (1996). Unit length labeling indices (ULLI) were determined by counting

the number of cells in S-phase per millimeter of the mucosa within the forestomach. The number of labeled epithelial cells were scored in at least four 0.25 mm random sections per animal.

Liver Analysis. Serial sections from liver slices were stained with either H&E, Perl's stain (ferrocyanide) or antibodies against bromodeoxyuridine (BrdU). Histological analysis (H&E) were conducted to determine if there was evidence of necrosis and whether the necrosis was diffuse or localized near iron-loaded Kupffer cells. Perl's stain was used to determine the extent of iron deposition in Kupffer cells and/or hepatocytes. Analysis of BrdU incorporation into S-phase nuclei was conducted by immunohistochemistry, using the procedure described by Stauber and Bull (1997). Visualization of BrdU-reactive cells was accomplished with the chromagen 3-amino-9-ethylcarbazole with a hematoxylin counterstain. A minimum of 2000 hepatocellular nuclei was scored by light microscopy in at least 10 randomly selected fields to determine the labeling index for cells in S-phase.

Specific Aim 3: Effect of IP vs SC-administered BE on the forestomach.

Probe Study. Female B6C3F1 mice, 3/group were administered saline solutions of BE by either intraperitoneal (IP) or subcutaneous (SC) injection at 400 or 600 mg/kg/d for 3 days. After two days of dosing, one 600 mg/kg IP mouse was sacrificed moribund.

Full Study. Groups of 6 female B6C3F1 mice were administered 0 or 400 mg/kg/d BE by either IP or SC injection for 5 days and necropsied on the afternoon of the 5th day of dosing. Terminal body weights and liver weights were measured and livers were flash-frozen and stored at -80°C for potential analysis by Indiana University. The stomach from each animal was dissected, inflated with formalin and fixed in neutral buffered formalin for histopathology.

Forestomach Analysis. Paraffin-embedded tissues were serially sectioned and stained with hematoxylin and eosin (H&E). Histological analysis (H&E) were conducted to determine evidence of necrosis and/or irritation of the gastric mucosa.

RESULTS AND DISCUSSION

Specific Aim 1: Deposition of BE on the fur of mice.

Whole-body chamber operation. The distribution of BE vapor concentrations in the exposure chamber was determined prior to the study. The concentrations were measured at 3 chamber positions during the first hour of a test generation and again during the last hour of the test generation. The concentrations were 238.1 ± 1.5 ppm (0.6% relative standard deviation) for the 6 samples. The analytical concentration averaged 95% of the nominal concentration (250.6 ppm).

During the animal exposure, the concentration of BE was measured at 3 chamber positions during the first hour of the exposure study and again during the last hour of the study. The analytical chamber concentration was 229.2 ± 1.0 ppm (92% of the target concentration with 0.4% relative standard deviation) for the 6 samples. The average analytical concentration was 92.4% of the nominal concentration (247.9 ppm).

Nose-only chamber operation. The distribution of BE vapor concentrations in the exposure chamber was determined prior to the study. The concentrations were measured at a port on each tier and an additional port on the lower tier (sampling from the inlet manifold) during the first hour of a test generation and again during the last hour of the test generation. The concentration was 241.8 ± 4.7 ppm (1.9% relative standard deviation) for the 8 samples. A nominal concentration was not determined due to a stoppage of the metering pump for an undetermined time in the period between the time the two sets of grab samples were taken. The measured mean concentration was 96.7% of the target concentration of 250 ppm.

During the animal exposure, the concentration of BE vapor was measured at a port on each tier and an additional port on the lower tier (sampling from the inlet manifold) during the first hour of a test generation and again during the last hour of the study. The average concentration was 245.7 ± 1.8 ppm (0.7% relative standard deviation) for the 8 samples. The analytical concentration averaged 99.4% of the nominal concentration (247.0 ppm).

Clinical observations. There were no early deaths from either exposure. There were no visible signs of overt toxicity during either the nose-only or whole-body exposure. However, a large number (7/10) of the urine samples collected from the mice following the nose-only exposure contained a red colored material, possibly blood. In addition 1/10 of the cage rinse samples were significantly tinted red.

Deposition of BE on the fur. An average of 205 μg of BE was detected on the fur of five female mice exposed whole-body to 229 ppm BE vapor (Table 1). Slightly less BE, 170 μg /mouse, was detected on the fur of female mice exposed nose-only to 242 ppm BE. Residual BE on the fur of the nose-only exposed mice was attributed to the use of a positive-pressure, flow-past exposure system. Most of the residual BE was detected in the first 50 ml wash (see Appendix A-1 for individual animal and fur wash data) thus the method used to wash the fur was considered to be effective. An example chromatogram from the fur wash is shown in Figure 3. However, evaporative losses of BE from the fur probably occurred during the time for the chamber to be shut down and evacuated and the animals sacrificed via CO_2 prior to immersion in 50 ml of hot water.

When corrected for the actual BE exposure concentration, whole body exposures averaged 25% more BE on the fur than the corresponding nose-only exposures. While there was clear evidence that residual BE adsorbs to the fur of mice, the total dose measured post-exposure that could be available to grooming and ultimately come into contact with the forestomach was less than 10 mg/kg.

BE and BAA in blood and urine. The concentration of BE in the blood of five female mice at the end of each exposure averaged 3.0 and 3.9 mg/l for the whole-body and nose-only exposures, respectively (Table 2; Appendix A-2). The concentrations of the major metabolite, BAA, in blood were 235 and 390 mg/l for the whole-body and nose-only exposures respectively (Table 2; Appendix A-2). These concentrations of BE and BAA were similar to the levels reported in the NTP chronic toxicokinetic study (Dill et al., 1998). An example chromatogram from the blood analyses is shown in Figure 4.

Low levels of unmetabolized BE (<100 µg) were found in the urine of mice collected 0-18 hr after both whole-body and nose-only exposures (Table 3; Appendix A-3). The BE in urine was presumed to have come from the fur of the mice since no BE is expected to have been excreted unmetabolized in urine based upon data obtained from rats (Corley et al., 1994). The total amounts of free BAA in the urine indicate little difference between the two methods of exposure and were similar to levels reported in the NTP chronic toxicokinetic study (Dill et al., 1998). An example chromatogram from the urine analyses is shown in Figure 5.

Specific Aim 2: Effect of orally-administered neat BE on the forestomach and liver.

Probe Study. After two consecutive days of dosing, no overt signs of toxicity were observed in male or female mice dosed as high as 800 mg/kg/d. Therefore, after consultation with the sponsor, the mice from the lower dose group (100 mg/kg/d) were administered 1200 mg/kg/d for the remaining three days of dosing. One mortality occurred (high dose male) that was due to gavage error. All mice were necropsied approximately 4 hr after the final dose. Body weights, organ weights (stomach, liver and spleen) and hematocrits from each of the mice are summarized in Table 4. Individual animal data are presented in Appendix B-1. Both absolute and relative organ weights were increased over controls for both sexes. Hematocrits were also significantly affected with the top dose group (1200 mg/kg/d) decreased 15-30% from control levels. Based on these data, the dose levels for the full study were selected to be 400, 800 and 1200 mg/kg/d.

Full Study. After two consecutive days of dosing, mortality was observed in all treatment groups except controls. Therefore, dose levels for each group were reduced in half resulting in dose levels of 200, 400 and 600 mg/kg/d. Over the next two days of dosing, the mortality continued to be excessive and the study was terminated on the fifth day with no further dosing. Terminal body weights were significantly decreased from pre-study and a dose-response mortality was observed (Table 5). Hematocrits were also decreased by as much as 23-29% from controls (Table 5). Relative liver weights and both absolute and relative stomach and spleen weights were increased over controls (Table 6). Individual animal body weights, organ weights and hematocrits are summarized in Appendix B-2.

Forestomach Histology.

Evaluation of H&E-stained slides from mice exposed to neat BE by gavage for four days resulted in lesions related to BE exposure in the forestomachs of male and female mice (see Appendix B-3). These lesions included epithelial hyperplasia and inflammation of the forestomach. In the low dose group, minimal to mild forestomach epithelial hyperplasia was observed in both male and female mice. The change was characterized by focal areas of increased layers of epithelial cells. In mice from the mid exposure group, minimal to moderate forestomach epithelial hyperplasia was observed in both sexes. The epithelial hyperplasia, although appearing somewhat focal, was more expansive than observed in low dose mice and the epithelial thickening due to increased epithelial cell layers was more prominent. There were down-growths of proliferating epithelial cells into the lamina propria. In this exposure group several mice had significant infiltrates of neutrophils and mononuclear inflammatory cells in the submucosa and muscularis. Both sexes of mice from the high exposure group had minimal to marked forestomach epithelial hyperplasia. Inflammation of the submucosa and muscularis was, again, commonly associated with the hyperplasia and in one high dose male appeared to have extended to the peritoneal surface. A very tiny ulcer with minimal forestomach epithelial hyperplasia at its border was observed in one female control and minimal focal necrosis and forestomach epithelial hyperplasia at the lesser curvature (junction of forestomach and glandular stomach) in another female control. These were considered spontaneous changes that are not uncommon in mice.

Initial efforts at calculating a unit length labeling index using PCNA immunohistochemistry in random sections of forestomach as originally planned was found to be an inappropriate technique due to the focal nature of the lesions. Therefore, analysis of labeling indices by PCNA was limited to a qualitative assessment of the relative amount of S-phase cells in histologically-normal forestomach epithelium versus the epithelium surrounding histological lesions. There was evidence of an increase in PCNA staining intensity in regions surrounding focal areas of hyperplasia and inflammation, as compared to either the forestomach epithelium of control mice or to histologically-normal regions within the forestomach of BE-exposed mice. The intensity of PCNA staining within each forestomach section was more closely associated with the apparent severity of the lesion rather than BE dose although the incidence and degree of lesions was associated with BE dose. Lesions with focal regions of inflammation and thickening of the epithelium showed greater overall PCNA staining as well as clearer evidence of S-phase cells.

Liver Histology.

Based on H&E-stained sections, liver morphology appeared normal in both male and female mice and at all BE dose groups. In addition, no treatment-related accumulation of hemosiderin in Kupffer cells was observed in any exposure group. Highly glycogenated-appearing liver cells were observed with low occurrence, lack of a dose response, and variable group distribution and were, therefore, not considered exposure-related.

There was a clear treatment-related increase in hepatic iron accumulation based on analysis of sections stained with Perl's stain. Enumeration of positive staining cells in random liver sections demonstrated that the iron accumulation in both male and female mice exposed to BE was dose-related. Although some minimal iron accumulation was occasionally observed in parenchymal cells, the predominant cells affected were Kupffer's cells. There was a trend for greater iron accumulation in male mice as compared to females (Figure 6). Although this sex-dependent trend was observed in both the probe and full study, it was not statistically significant based on the scoring methods used.

Analysis of proliferation indices by PCNA staining revealed no significant differences in the fraction of S-phase cells between control animals and BE-treated animals in either male or female mice (Table 7). The mean fraction of cells in S-phase in liver ranged between 0.14 and 0.16 % for males and between 0.16 and 0.19% for females. No treatment-related differences were observed in BE-exposed animals of either sex. Sex-dependent differences in PCNA labeling indices have been previously reported (Ellridge and Goldsworthy, 1996).

Specific Aim 3: Effect of IP vs SC-administered BE on the forestomach.

The dose level of 600 mg/kg IP resulted in the moribund sacrifice of one mouse after two doses in the probe study. Therefore, a dose of 400 mg/kg/d for five days was used in the 5-day study. All animals survived the 5-day dosing regimen with no clinical observations of treatment-related effects. Terminal body weights were similar to pre-study body weights in all dose groups, including controls (Appendix C-1). Liver weights were slightly elevated in both the IP and SC treatment groups (Appendix C-1).

Forestomach Histology

In the probe study, IP injection of 600 mg/kg for three days was associated with forestomach epithelial hyperplasia and inflammation (Appendix C-2). There were forestomach lesions in one mouse per group in the 400 mg/kg IP and the 400 and 600 mg/kg SC injection groups. Although suggestive of a treatment-related response, similar lesions occur spontaneously in mice.

In the 5-day study, 1/6 mice in the 400 mg/kg IP group had forestomach epithelial hyperplasia and 2/6 mice in the 400 mg/kg SC group had forestomach epithelial hyperplasia and inflammation (Appendix C-3). The lesion present in the IP-dosed mouse was similar to spontaneous lesions but those in the two SC-dosed mice appeared subjectively different from spontaneous lesions. Regardless, all three mice (IP and SC) had minimal lesions that could be identified when evaluated blindly; association with BE treatment was considered possible.

CONCLUSIONS

Small amounts of BE (134 - 318 μg) were removed from the fur of female mice exposed either whole-body or nose-only for 6 hr to target concentrations of 250 ppm BE vapor. The total dose that was available for oral consumption via grooming was 9.2 ± 2.9 mg/kg for the whole-body exposure and 7.5 ± 2.3 mg/kg for the nose-only exposure. Few differences were detected in the internal dose of BE resulting from whole-body vs. nose-only exposure as determined by analysis of BE and its major metabolite BAA in blood collected immediately post-exposure and in urine collected for up to 18 hr post-exposure. Although this was not a mass-balance study for the total fate of BE, the amounts of BE deposited on the fur of mice represented 9-13% of the total BAA eliminated in the urine. This calculation is, however, speculative since the amount of BAA found in the urine collected 18 hr post-exposure is likely an underestimate of the total absorbed dose. In addition, the amounts of BE deposited on the fur and consumed via grooming *during* the exposure as well as orally consumed via mucociliary clearance of BE deposited in the mucous layer lining the respiratory tract was not accounted for in this study. Nevertheless, there is evidence that at least a small amount of neat BE was available for oral consumption via grooming and that the potential role for chronic, daily oral doses in mouse forestomach toxicity cannot be ruled out.

Oral dosing of neat BE to male and female mice 4 hr after feed was withheld resulted in clear dose-response irritation to the forestomach. No overt toxicity was observed by H&E stain in the liver. The dose levels used in this study were clearly excessive with severe hemolysis and mortality observed at all dose levels in the full study that were not predicted from the probe study. Dose levels used in this study were initially 0, 400, 800 and 1200 mg/kg/d for two days. After two days, each dose group reduced in half to 0, 200, 400 and 600 mg/kg/d for two additional days of dosing before the study was terminated on the fifth day. Overall mortality was 0, 6, 8 and 11 of sixteen male mice and 0, 6, 12 and 10 of sixteen female mice at the control, low, middle and high dose levels, respectively. Forestomach lesions consisted of focal areas of irritation and epithelial hyperplasia. The proliferative response was confirmed by PCNA immunohistochemistry of epithelial cells surrounding the lesions vs. normal cells in the forestomach. Similar forestomach lesions were observed following IP injection of 600 mg/kg for 3 days in 3/3 female mice. Minimal forestomach lesions were observed in 1/6 and 2/6 female mice following IP and SC injections of 400 mg/kg/d for 5 days, respectively. These data suggest that systemic administration of BE can result in forestomach lesions and the direct contact with BE may not be a requirement. It is further possible that the metabolite, BAA, may play a role in producing the forestomach lesions since the IP injection route results in first-pass liver metabolism while SC injection does not. Interestingly, these lesions had not been observed in previous drinking water studies conducted by NTP (NTP, 1993) suggesting that the lesions may be dose-rate dependent.

In the livers of mice orally-dosed with neat BE, there was no evidence of degeneration and no corresponding compensatory cellular regeneration. In addition, there was no remarkable accumulation of hemosiderin in Kupffer cells although there was a clear dose-response increase in iron content in Kupffer cells and to a much lesser degree in hepatocytes. The significant increase in iron may play a potentially significant role in oxidative stress in these cells. The potential for oxidative stress in the livers of these mice were evaluated and reported separately by the Indiana University Medical Center (Kemendulis et al., 1999).

In conclusion, these data coupled with the evaluations of Kemendulis et al. (1999) are consistent with the hypothesis that the tumors observed in the forestomachs and livers of B6C3F1 mice following chronic inhalation exposure are the result of indirect mechanisms associated with chronic tissue injury. The lesions in the forestomach appear to be dose-rate dependent and may not require direct contact with the test material. The lesions in the liver appear to be secondary to hemolysis and may result from increased oxidative stress associated with iron deposition.

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Figure 1. Vapor generating system used for whole-body and nose-only exposures to target concentrations of 250 ppm BE.

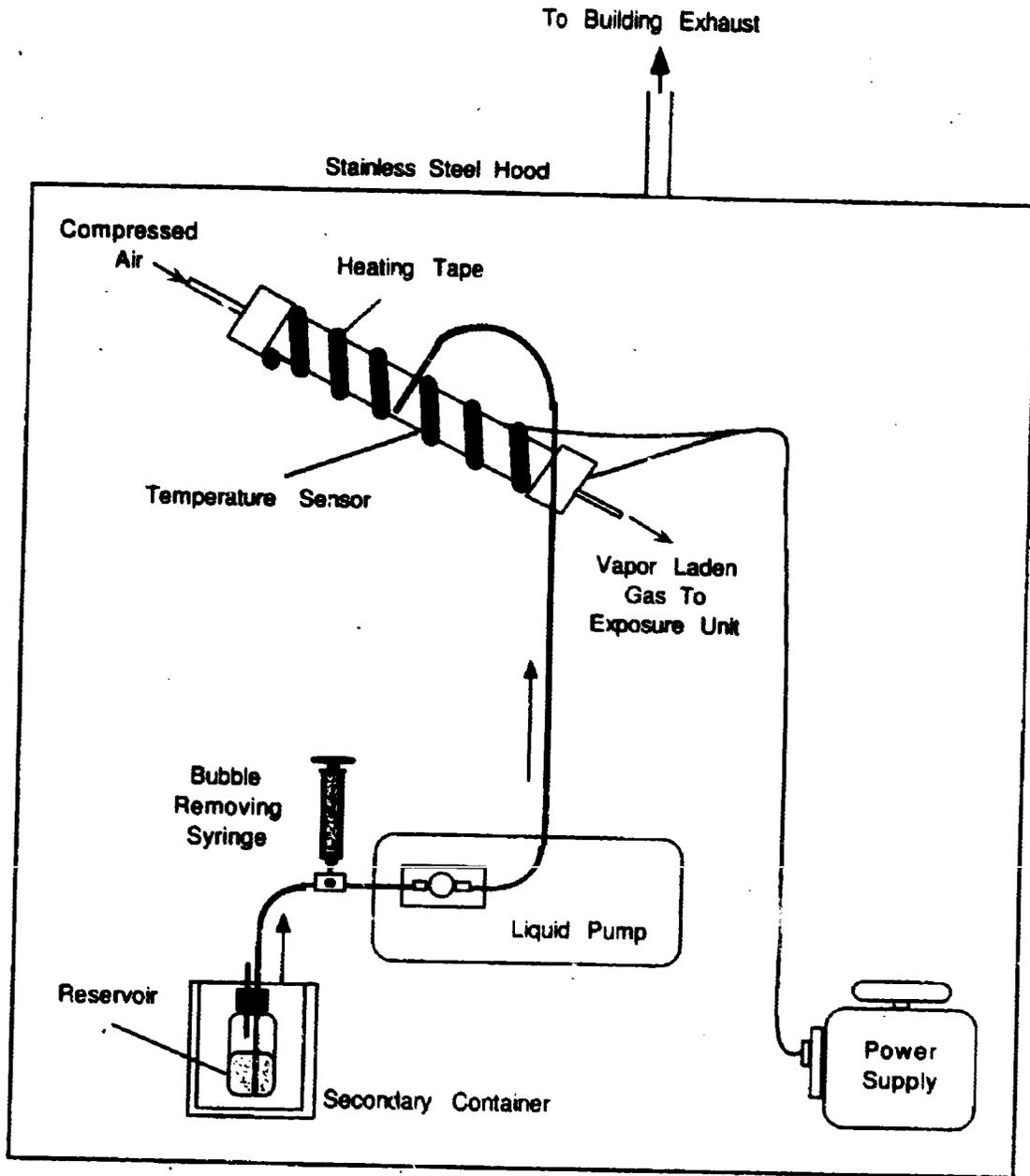


Figure 2. Example chromatogram of sample extract from nose-only exposure atmosphere. Peaks at ~4.4 minutes and ~7.7 minutes are BE and 1-phenylhexane internal standard, respectively.

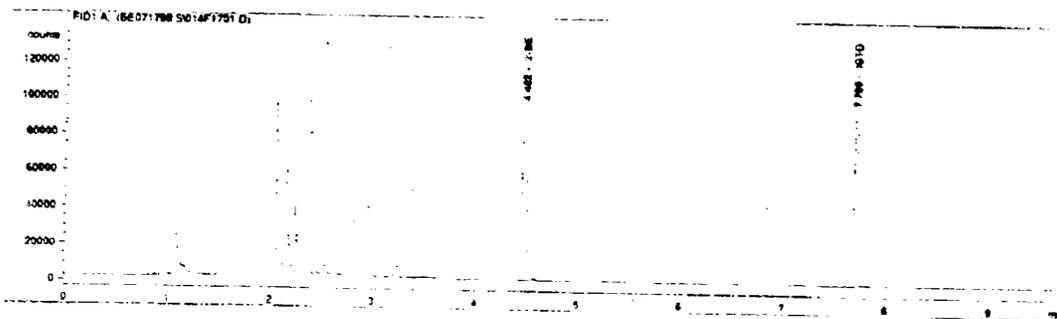
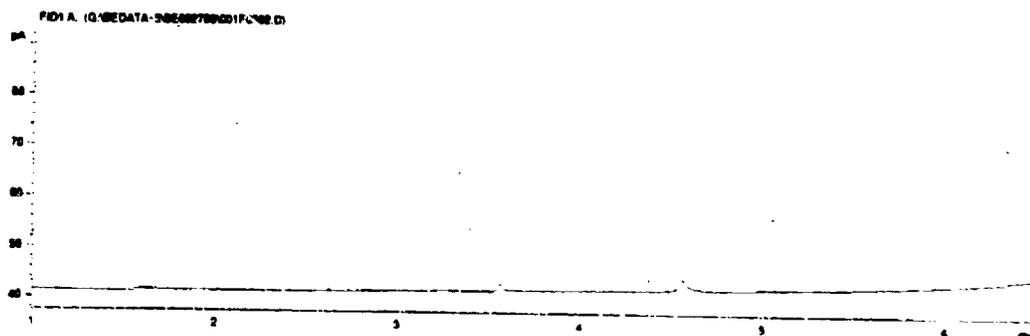


Figure 3. Example chromatograms from (a) control wash water and (b) fur wash from a mouse exposed whole body to 229 ppm BE for 6 hr.

(a) Control wash water



(b) Fur wash

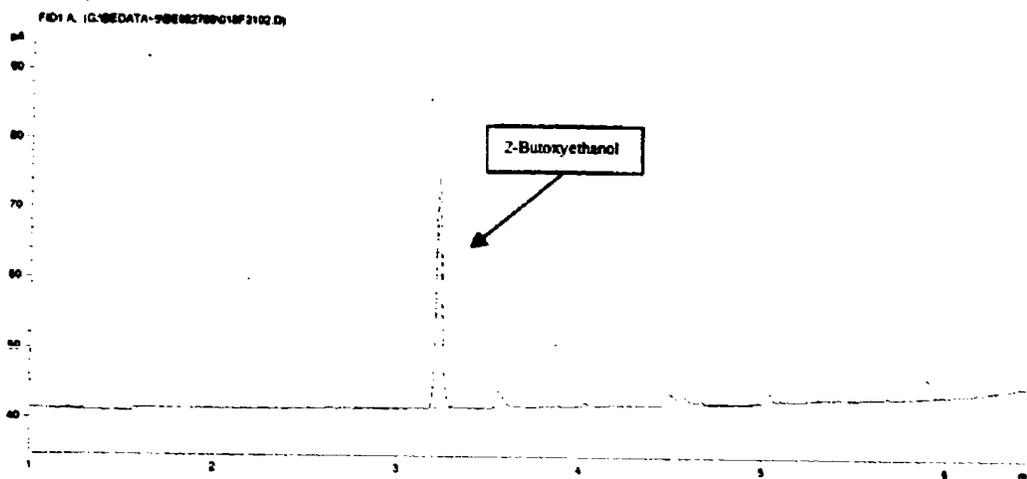
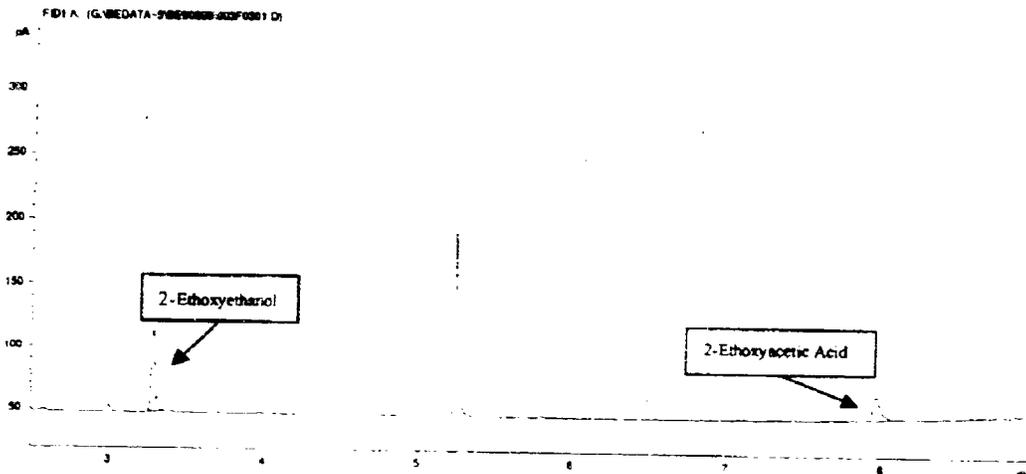


Figure 4. Example chromatograms from (a) control mouse blood and (b) blood from a mouse exposed whole-body to 229 ppm BE for 6 hr.

(a) Control mouse blood



(b) Blood sample from exposed mouse

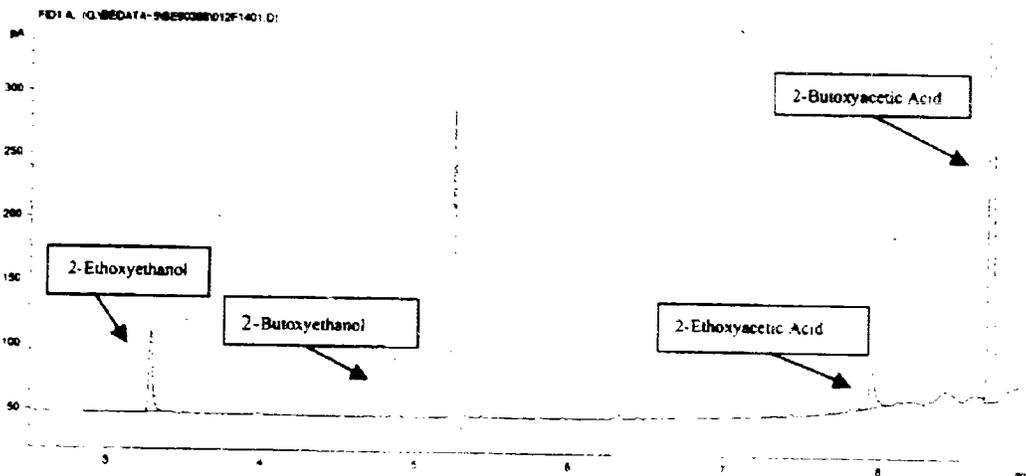
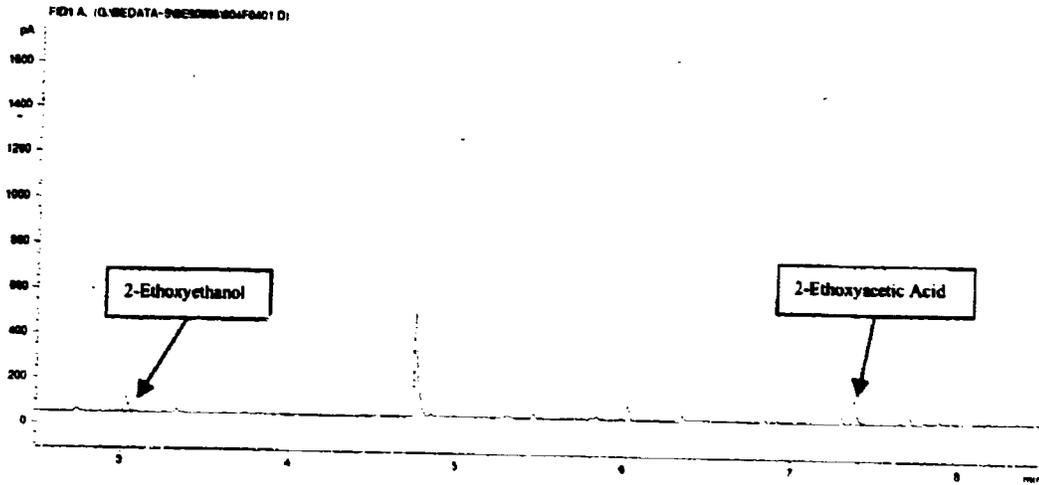


Figure 5. Example chromatograms from (a) control mouse urine and (b) urine from a mouse exposed whole-body to 229 ppm BE for 6 hr.

(a) Control urine



(b) Urine from exposed mouse

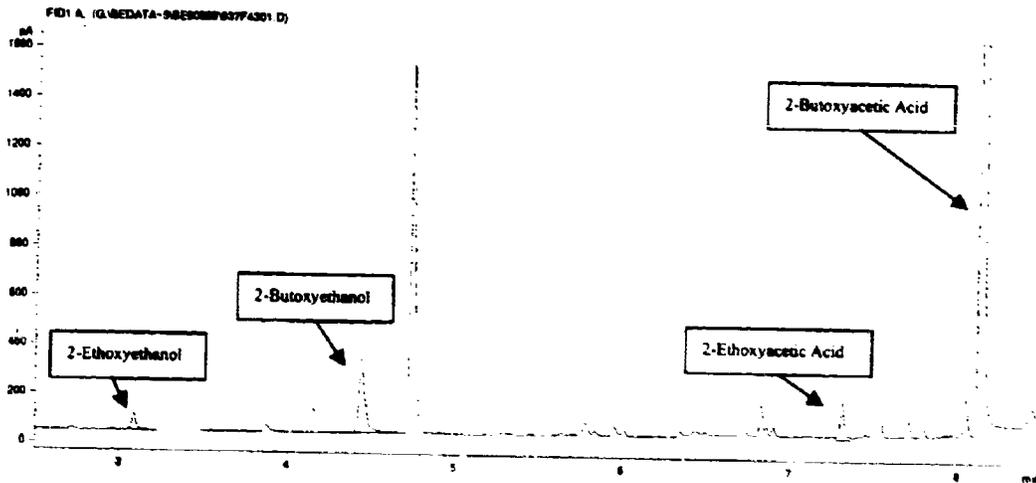


Figure 6. The relative intensity of Perl staining as a function of BE dose in male and female mice determined from the full study. Each value is the mean (\pm s.d.) number of positive cells per field (0.25 x 0.25 mm) from at least 4 mice, determined by counting 5 random fields per section.

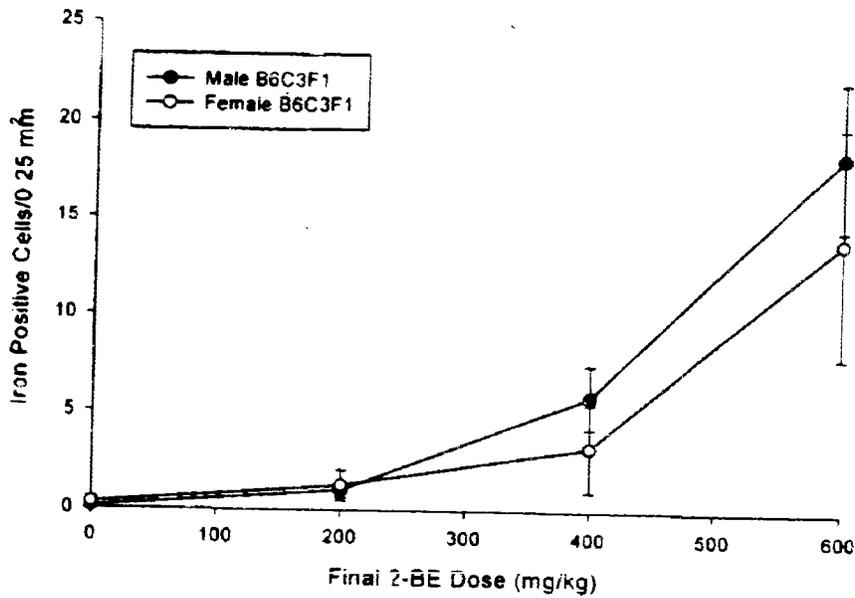


Table 1. Residual BE (mean \pm s.d.) removed from the fur of five female mice exposed either whole-body or nose-only for 6 hr to a target concentration of 250 ppm BE.

Exposure Group	Exposure Conc. (ppm)	Body Wt. (g)	Total BE (μ g)	Total BE (mg/kg BW)	Total BE (mg/kg/ppm)
Nose-Only	241.8	22.7 \pm 0.4	170 \pm 52	7.5 \pm 2.3	0.031 \pm 0.009
Whole-Body	229.2	22.0 \pm 1.3	205 \pm 69	9.2 \pm 2.9	0.040 \pm 0.013

Table 2. Concentration of BE and BAA in the blood of five female mice at the end of a 6-hr whole-body or nose-only exposure to a target concentration of 250 ppm BE.

Exposure Group	Exposure Conc. (ppm)	Body Wt. (g)	BE (mg/L)	BAA (mg/L)
Nose-Only	241.8	21.9 \pm 1.1	3.94 \pm 1.70	389.5 \pm 33.8
Whole-Body	229.2	21.6 \pm 1.2	3.02 \pm 1.25	235.4 \pm 52.3

Table 3. Amounts of free BE and BAA excreted in the urine of ten female mice 18 hr after the end of a 6-hr whole-body or nose-only exposure to a target concentration of 250 ppm BE.

Exposure Group	Exposure Conc. (ppm)	Total BE (μ g)	Total BAA (μ g)
Nose-Only	241.8	67.6 \pm 23.3	2021 \pm 537
Whole-Body	229.2	68.7 \pm 39.6	1783 \pm 645

Table 4. Body weights, organ weights (absolute and relative) and hematocrits from five mice/sex/dose gavaged with neat BE for five consecutive days (Probe Study). Data are expressed as the mean with the standard deviation on the next line.

(a) Males

Dose (mg/kg)	BW (g)	Stomach		Liver		Spleen		HCT (%)
		(g)	(%BW)	(g)	(%BW)	(g)	(%BW)	
0	26.36	0.182	0.685	1.099	4.158	0.066	0.248	47.8
	1.74	0.045	0.140	0.142	0.283	0.018	0.053	2.1
400	26.76	0.258	0.963	1.178	4.389	0.156	0.582	48.0
	1.91	0.058	0.196	0.149	0.305	0.051	0.181	4.1
800	28.20	0.262	0.931	1.220	4.320	0.170	0.606	N/A
	1.52	0.028	0.105	0.125	0.283	0.014	0.083	N/A
1200 ^a	23.18	0.228	0.978	0.925	3.988	0.075	0.325	40.8
	1.62	0.036	0.102	0.084	0.140	0.006	0.037	2.2

^a Mice were dosed with 100 mg/kg/d BE for two days followed by 3 days at 1200 mg/kg/d (see text).

(b) Females

Dose (mg/kg)	BW (g)	Stomach		Liver		Spleen		HCT (%)
		(g)	(%BW)	(g)	(%BW)	(g)	(%BW)	
0	20.4	0.174	0.858	0.810	3.979	0.074	0.363	50.3
	1.0	0.015	0.105	0.038	0.057	0.011	0.045	2.6
400	20.7	0.276	1.327	0.782	3.782	0.092	0.451	46.0
	1.6	0.052	0.193	0.061	0.353	0.019	0.129	5.4
800	19.8	0.248	1.250	0.818	4.124	0.158	0.797	N/A
	0.8	0.034	0.163	0.054	0.243	0.057	0.296	N/A
1200 ^a	19.0	0.302	1.619	0.766	4.061	0.092	0.491	35.8
	1.7	0.111	0.687	0.009	0.292	0.022	0.134	8.3

^a Mice were dosed with 100 mg/kg/d BE for two days followed by 3 days at 1200 mg/kg/d (see text).

Table 5. Body weights, mortality and hematocrits from 16 mice/sex/dose administered neat BE by gavage for four consecutive days (Full Study). Terminal body weights and hematocrits determined only in animals surviving until terminal sacrifice.

(a) Males

Dose (mg/kg/d)	Pre-Study Body Wt. (g)	Terminal Body Wt. (g)	Mortality	HCT (%)
0	26.8 ± 1.1	27.2 ± 1.0	0/16	52.1 ± 3.3
400/200 ^a	26.3 ± 1.2	26.9 ± 0.7	6/16	51.0 ± 2.6
800/400 ^a	27.1 ± 1.1	25.5 ± 2.5	8/16	46.6 ± 3.2
1200/600 ^a	26.9 ± 1.7	23.2 ± 2.9	11/16	40.0 ± 2.6

^a After two days of dosing the mortality was excessive; therefore, surviving mice from each dose group were administered half of their corresponding original target dose for two additional days prior to study termination.

(b) Females

Dose (mg/kg/d)	Pre-Study Body Wt. (g)	Terminal Body Wt. (g)	Mortality	HCT (%)
0	20.5 ± 1.8	20.8 ± 0.7	0/16	50.9 ± 2.5
400/200 ^a	19.5 ± 2.5	17.5 ± 4.3	6/16	47 ^b
800/400 ^a	20.4 ± 2.0	19.7 ± 2.2	12/16	37.5 ± 1.3
1200/600 ^a	20.8 ± 1.6	19.4 ± 2.1	10/16	36.3 ± 1.5

^a After two days of dosing the mortality was excessive; therefore, surviving mice from each dose group were administered half of their corresponding original target dose for two additional days prior to study termination.

^b Unable to obtain blood samples via orbital sinus puncture from all but one mouse.

Table 6. Terminal body weights and organ weights (absolute and relative) from up to 16 mice/sex/dose gavaged with neat BE for four consecutive days (Full Study). Terminal body weights and organ weights determined only in animals surviving until terminal sacrifice (see Table 5 for mortality data). Data are expressed as the mean with the standard deviation on the next line.

(a) Males

Dose (mg/kg)	BW (g)	Stomach		Liver		Spleen		Kidney	
		(g)	(%BW)	(g)	(%BW)	(g)	(%BW)	(g)	(%BW)
0	27.2	0.1723	0.6353	1.2285	4.5197	0.072	0.267	0.1926	0.7091
	1.0	0.0127	0.0548	0.1466	0.4528	0.012	0.050	0.0155	0.0432
400/200 ^a	26.9	0.1785	0.6566	1.4485	5.3887	0.0985	0.3680	0.1895	0.7067
	0.7	0.0213	0.0955	0.1893	0.6224	0.0231	0.0917	0.0105	0.0542
800/400 ^a	25.5	0.2075	0.8166	1.2956	5.0576	0.1158	0.4500	0.1895	0.7440
	2.5	0.0261	0.1014	0.2201	0.5120	0.0385	0.1262	0.0221	0.0657
1200/600 ^a	23.2	0.2036	0.8713	1.2168	5.1115	0.1154	0.4917	0.1712	0.7407
	2.9	0.0507	0.1568	0.2335	0.4185	0.0514	0.1770	0.0189	0.0462

(b) Females

Dose (mg/kg)	BW (g)	Stomach		Liver		Spleen		Kidney	
		(g)	(%BW)	(g)	(%BW)	(g)	(%BW)	(g)	(%BW)
0	20.8	0.1568	0.7562	0.9485	4.5588	0.075	0.3623	0.1254	0.6036
	0.7	0.0324	0.1707	0.0589	0.2056	0.009	0.0415	0.0127	0.0671
400/200 ^a	17.5	0.1443	0.8255	0.6350	3.9379	0.0665	0.3496	0.1165	0.6712
	4.3	0.0358	0.0734	0.2299	0.8666	0.0449	0.1655	0.0251	0.0820
800/400 ^a	19.7	0.1803	0.9176	1.0933	5.5288	0.1680	0.8723	0.1388	0.7016
	2.20	0.0175	0.0599	0.1869	0.4706	0.0493	0.3184	0.0243	0.0560
1200/600 ^a	19.4	0.1970	1.0184	1.0093	5.2250	0.1860	0.9549	0.1310	0.6795
	2.1	0.0213	0.0865	0.1655	0.2000	0.0400	0.1598	0.0062	0.0563

^a After two days of dosing the mortality was excessive; therefore, surviving mice from each dose group were administered half of their corresponding original target dose for two additional days prior to study termination.

Table 7. Percentage of S-phase cells in liver of mice treated with BE. Values (mean \pm s.d.) were determined by analysis of PCNA staining by scoring a minimum of 2000 hepatocytes from randomly selected fields, with a minimum of 4 animals scored per treatment group.

Dose Group	% S-Phase	
	Males	Females
Control	0.15 \pm 0.06	0.18 \pm 0.05
400/200 mg/kg ^a	0.15 \pm 0.06	0.19 \pm 0.08
800/400 mg/kg ^a	0.16 \pm 0.06	0.17 \pm 0.06
1200/600 mg/kg ^a	0.16 \pm 0.09	0.18 \pm 0.09

^a After two days of dosing the mortality was excessive; therefore, surviving mice from each dose group were administered half of their corresponding original target dose for two additional days prior to study termination. S-phase nuclei were scored based on the method reported by Eldridge and Goldsworthy (1996).

Appendix A-1
Individual Animal Data -- Fur Deposition Study

Residual 2-Butoxyethanol on the Fur of Mice -- Nose Only Exposure (241.8 ppm)

Animal ID	Body Wt. (g)	Wash No.	Amount of Wash Water (g)	BE in Wash (µg/ml)	Total BE in Wash (µg)	Residual BE (mg BE/kg BW)	Residual BE (mg BE/kg BW /ppm Exposure)
306	22.5	1	48.982	4.63	226.92	---	---
		2	49.118	0.426	<u>20.94</u>	---	---
					247.9	11.02	0.0456
307	23.1	1	48.479	3.61	174.89	---	---
		2	48.721	0.49	<u>23.79</u>	---	---
					198.68	8.60	0.0356
308	22.1	1	48.988	2.24	109.91	---	---
		2	48.562	0.51	24.67	---	---
					<u>134.58</u>	6.09	0.0252
309	22.5	1	48.772	2.29	111.86	---	---
		2	49.026	0.47	<u>22.85</u>	---	---
					134.71	5.99	0.0248
310	23.1	1	49.114	2.29	112.3	---	---
		2	49.196	0.44	<u>21.68</u>	---	---
					<u>133.97</u>	5.80	0.0240
Mean:	22.66				169.97	7.50	0.0310
SD:	0.43				51.69	2.27	0.0094

Appendix A-1
Individual Animal Data – Fur Deposition Study
Residual 2-Butoxyethanol on the Fur of Mice – Whole Body Exposure (229.2 ppm)

Animal ID	Body Wt. (g)	Wash No.	Amount of Wash Water (g)	BE in Wash (µg/ml)	Total BE in Wash (µg)	Residual BE (mg BE/kg BW)	Residual BE (mg BE/kg BW /ppm Exposure)
106	23.8	1	48.741	3.88	189.08	---	---
		2	48.633	0.68	33.18	---	---
						9.34	0.0408
107	21.7	1	49.259	2.72	134.15	---	---
		2	49.403	0.59	29.29	---	---
						7.53	0.0329
108	22.6	1	49.163	5.72	281.00	---	---
		2	48.666	0.76	36.75	---	---
						14.06	0.0613
109	20.4	1	49.291	3.10	153.01	---	---
		2	49.206	0.43	21.3	---	---
						8.54	0.0373
110	21.6	1	49.159	2.66	130.93	---	---
		2	49.050	0.30	14.60	---	---
						6.74	0.0294
Mean:	22.02				204.66	9.24	0.0403
SD:	1.27				69.31	2.87	0.0125

Appendix A-2
Individual Animal Data – Fur Deposition Study

Concentration of 2-Butoxyethanol (BE)/Butoxyacetic Acid (BAA) in Blood

Animal No.	Exposure	Body Wt. (g)	BE (mg/l)	BAA (mg/l)
101	WB	22.9	4.68	195.0
102	WB	22.2	2.21	206.9
103	WB	21.4	2.84	215.5
104	WB	19.6	3.82	234.2
105	WB	21.7	1.57	325.5
Mean:		21.6	3.02	235.4
SD:		1.23	1.25	52.3
301	NO	20.8	5.95	409.7
302	NO	21.0	5.49	364.2
303	NO	21.8	3.18	401.1
304	NO	22.1	3.12	345.1
305	NO	23.6	1.99	427.2
Mean:		21.9	3.51	389.5
SD:		1.1	1.70	33.8

WB = 229.2 ppm Whole Body exposure.
NO = 241.8 ppm Nose Only exposure.

Appendix A-3
Individual Animal Data - Fur Deposition Study
2-Butoxyethanol (BE) and Butoxyacetic Acid (BAA) Levels in Urine collected 18 hr Post-Exposure

Animal No.	Exposure	Total BE in Urine (µg)	Total BAA in Urine (µg)
111	WB	104.4	2331.8
112	WB	80.1	2641.4
113	WB	130.7	2678.1
114	WB	74.6	1527.8
115	WB	43.8	1546.2
116	WB	5.9	813.6
117	WB	90.6	1395.5
118	WB	81.1	2165.7
119	WB	8.5	1051.0
120	WB	68.1	1674.8
Mean:		68.7	1782.6
SD:		39.6	645.0
311	NO	67.5	2695.0
312	NO	94.6	1709.7
313	NO	112.9	1143.5
314	NO	63.0	1583.8
315	NO	80.0	2103.5
316	NO	32.0	1948.6
317	NO	55.8	1949.5
318	NO	47.6	1674.8
319	NO	57.1	2825.3
320	NO	65.3	2571.6
Mean:		67.6	2020.5
SD:		23.3	537.4

Appendix B-1
Individual Animal Data – Gavage Study, Probe
Body Weights, Organ Weights and Hematocrits for Animals at Terminal Sacrifice

Animal #	Terminal			Saline Control – Males (Probe)			Relative		
	Body Weight (g)	Stomach Wt. (g)	Relative Stomach Wt. (% BW)	Liver Wt. (g)	Relative Liver Wt. (% BW)	Spleen Wt. (g)	Relative Spleen Wt. (% BW)	HCT (%)	
1	24.4	0.140	0.574	0.987	4.045	0.050	0.205	48	
2	27.9	0.190	0.681	1.200	4.301	0.080	0.287	NS	
3	28.1	0.220	0.783	1.280	4.555	0.090	0.320	45	
4	26.7	0.230	0.861	1.090	4.082	0.060	0.225	48	
5	24.7	0.130	0.526	0.940	3.806	0.050	0.202	50	
Mean:	26.36	0.182	0.685	1.099	4.158	0.066	0.248	47.8	
SD:	1.74	0.045	0.140	0.142	0.283	0.018	0.053	2.1	

NS=No Sample; NW=Not Weighed; N/A=Not Applicable.

Animal #	Terminal			400 mg/kg/d – Males (Probe)			Relative		
	Body Weight (g)	Stomach Wt. (g)	Relative Stomach Wt. (% BW)	Liver Wt. (g)	Relative Liver Wt. (% BW)	Spleen Wt. (g)	Relative Spleen Wt. (% BW)	HCT (%)	
11	23.4	0.220	0.940	0.920	3.932	0.120	0.513	48	
12	28.1	0.230	0.819	1.200	4.270	0.110	0.391	53	
13	27.3	0.310	1.136	1.290	4.725	0.240	0.879	47	
14	27.8	0.330	1.187	1.270	4.568	0.160	0.576	42	
15	27.2	0.200	0.735	1.210	4.449	0.150	0.551	50	
Mean:	26.76	0.258	0.963	1.178	4.389	0.156	0.582	48.0	
SD:	1.91	0.058	0.196	0.149	0.305	0.051	0.181	4.1	

NS=No Sample; NW=Not Weighed; N/A=Not Applicable.

800 mg/kg/d - Males (Probe)

Animal #	Terminal Body Weight (g)	Stomach Wt. (g)	Relative Stomach Wt. (% BW)	Liver Wt. (g)	Relative Liver Wt. (% BW)	Spleen Wt. (g)	Relative Spleen Wt. (% BW)	HCT (%)
16	29.1	0.290	0.997	1.370	4.708	0.160	0.550	NS
17	28.8	0.230	0.795	1.280	4.444	0.160	0.556	NS
18	25.5	0.260	1.020	1.040	4.078	0.190	0.745	NS
19	28.7	0.240	0.836	1.250	4.355	0.160	0.557	NS
20	28.9	0.290	1.003	1.160	4.014	0.180	0.623	NS
Mean:	28.20	0.262	0.931	1.220	4.320	0.170	0.606	N/A
SD:	1.52	0.028	0.105	0.125	0.283	0.014	0.083	N/A

NS=No Sample; NW=Not Weighed; N/A=Not Applicable.

1200 mg/kg/d - Males (Probe)

Animal #	Terminal Body Weight (g)	Stomach Wt. (g)	Relative Stomach Wt. (% BW)	Liver Wt. (g)	Relative Liver Wt. (% BW)	Spleen Wt. (g)	Relative Spleen Wt. (% BW)	HCT (%)
6	22	0.220	1.000	0.880	4.000	0.070	0.318	42
7	24.2	0.260	1.074	1.010	4.174	0.080	0.331	43
8	21.6	0.180	0.833	0.830	3.843	0.080	0.370	38
9	24.9	0.250	1.004	0.980	3.936	0.070	0.281	40
Mean:	23.18	0.228	0.978	0.925	3.988	0.075	0.325	40.8
SD:	1.62	0.036	0.102	0.084	0.140	0.006	0.037	2.2

NS=No Sample; NW=Not Weighed; N/A=Not Applicable.

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Saline Control - Females (Probe)

Animal #	Terminal Body Weight (g)	Stomach Wt. (g)	Relative Stomach Wt. (% BW)	Liver Wt. (g)	Relative Liver Wt. (% BW)	Spleen Wt. (g)	Relative Spleen Wt. (% BW)	HCT (%)
102	21.6	0.170	0.787	0.860	3.981	0.090	0.417	54
101	19.8	0.160	0.808	0.790	3.990	0.080	0.404	48
103	21.1	0.160	0.758	0.840	3.981	0.070	0.332	NS
104	20.3	0.190	0.936	0.790	3.892	0.070	0.345	49
105	19.0	0.190	1.000	0.770	4.053	0.060	0.316	50
Mean:	20.4	0.174	0.858	0.810	3.979	0.074	0.363	50.3
SD:	1.0	0.015	0.105	0.038	0.057	0.011	0.045	2.6

NS=No Sample; NW=Not Weighed; N/A=Not Applicable.

400 mg/kg/d - Females (Probe)

Animal #	Terminal Body Weight (g)	Stomach Wt. (g)	Relative Stomach Wt. (% BW)	Liver Wt. (g)	Relative Liver Wt. (% BW)	Spleen Wt. (g)	Relative Spleen Wt. (% BW)	HCT (%)
111	22.2	0.360	1.622	0.820	3.694	0.080	0.360	50
112	18.6	0.220	1.183	0.700	3.763	0.120	0.645	38
113	21.6	0.260	1.204	0.760	3.519	0.070	0.324	48
114	19.6	0.280	1.429	0.860	4.388	0.100	0.510	48
115	21.7	0.260	1.198	0.770	3.548	0.090	0.415	NS
Mean:	20.7	0.276	1.327	0.782	3.782	0.092	0.451	46.0
SD:	1.6	0.052	0.193	0.061	0.353	0.019	0.129	5.4

NS=No Sample; NW=Not Weighed; N/A=Not Applicable.

8006 mg/kg/d – Females (Probe)

Animal #	Terminal Body Weight (g)	Stomach Wt. (g)	Relative Stomach Wt. (% BW)	Liver Wt. (g)	Relative Liver Wt. (% BW)	Spleen Wt. (g)	Relative Spleen Wt. (% BW)	HCT (%)
116	19.1	0.220	1.152	0.790	4.136	0.230	1.204	NS
117	21.2	0.280	1.321	0.880	4.151	0.180	0.849	NS
118	19.7	0.230	1.168	0.840	4.264	0.180	0.914	NS
119	19.9	0.220	1.106	0.740	3.719	0.110	0.553	NS
120	19.3	0.290	1.503	0.840	4.352	0.090	0.466	NS
Mean:	19.8	0.248	1.250	0.818	4.124	0.158	0.797	N/A
SD:	0.8	0.034	0.163	0.054	0.243	0.057	0.296	N/A

NS=No Sample; NW=Not Weighed; N/A=Not Applicable.

1200 mg/kg/d – Females (Probe)

Animal #	Terminal Body Weight (g)	Stomach Wt. (g)	Relative Stomach Wt. (% BW)	Liver Wt. (g)	Relative Liver Wt. (% BW)	Spleen Wt. (g)	Relative Spleen Wt. (% BW)	HCT (%)
106	18.0	0.220	1.222	0.760	4.222	0.120	0.667	35
107	18.8	0.310	1.649	0.770	4.096	0.100	0.532	25
108	17.5	0.490	2.800	0.760	4.343	0.070	0.400	38
109	21.8	0.260	1.193	0.780	3.578	0.070	0.321	45
110	18.7	0.230	1.230	0.760	4.064	0.100	0.535	NS
Mean:	19.0	0.302	1.619	0.766	4.061	0.092	0.491	35.8
SD:	1.7	0.111	0.687	0.009	0.292	0.022	0.134	8.3

NS=No Sample; NW=Not Weighed; N/A=Not Applicable.

Appendix B-2
Individual Animal Data – Gavage Study, Full
Dosing Regimen, Body Weights, Organ Weights and Hematocrits for Animals at Terminal Sacrifice

Saline Control – Males (Full Study)														
Animal #	Pre-exposure Body Weight (g)	Terminal Body Weight (g)	Days Dosed 1200 mg/kg	Days Dosed 600 mg/kg	Stomach		Liver		Spleen		Kidney		HCT (%)	
					Wt. (g)	Relative Wt. (% BW)								
1	26.1	25.7	2	2	0.165	0.642	1.071	4.167	0.096	0.374	0.198	0.770	55	
3	24.9	25.9	2	2	0.196	0.757	1.105	4.266	0.053	0.205	0.173	0.668	54	
5	26.1	26.7	2	2	0.164	0.614	1.252	4.689	0.066	0.247	0.182	0.682	54	
7	26.8	27.2	2	2	0.166	0.610	1.272	4.676	0.066	0.243	0.187	0.688	47	
9	26.6	27.8	2	2	0.164	0.590	1.520	5.468	0.076	0.273	0.200	0.719	48	
11	27.8	27.5	2	2	0.179	0.651	1.098	3.993	0.079	0.287	0.183	0.665	NS	
13	27.4	27.5	2	2	0.160	0.582	1.207	4.389	0.074	0.269	0.194	0.705	52	
15	28.4	28.9	2	2	0.184	0.637	1.303	4.509	0.068	0.235	0.224	0.775	55	
Mean:	26.76	27.2			0.1723	0.6353	1.2285	4.5197	0.072	0.267	0.1926	0.7091	52.1	
SD:	1.10	1.0			0.0127	0.0548	0.1466	0.4528	0.012	0.050	0.0155	0.0432	3.3	

NS=No Sample; NW=Not Weighed; N/A=Not Applicable.

400/200 mg/kg/d – Males (Full Study)

Animal #	Pre-exposure Body Weight (g)	Terminal Body Weight (g)	Days Dosed 400 mg/kg	Days Dosed 200 mg/kg	Stomach h Wt. (g)	Relative Stomach h Wt. (% BW)		Liver Wt. (g)	Relative Liver Wt. (% BW)		Spleen Wt. (g)	Relative Spleen Wt. (% BW)		Kidney Wt. (g)	Relative Kidney Wt. (% BW)		HCT (%)
						Stomach h Wt. (g)	(% BW)		Liver Wt. (g)	(% BW)		Spleen Wt. (g)	(% BW)		Kidney Wt. (g)	(% BW)	
203	25.8	26.2	2	2	0.191	0.729	1.392	5.313	0.125	0.477	0.205	0.782	53				
205	25.2	26.3	2	2	0.199	0.757	1.255	4.772	0.097	0.369	0.186	0.707	52				
207	26.4	27.3	2	2	0.173	0.634	1.707	6.253	0.069	0.253	0.185	0.678	48				
211	27.9	27.6	2	2	0.151	0.547	1.440	5.217	0.103	0.373	0.182	0.659	NS				
Mean:	26.33	26.85			0.1785	0.6666	1.4485	5.3887	0.0985	0.3680	0.1895	0.7067	51.0				
SD:	1.16	0.70			0.0213	0.0955	0.1893	0.6224	0.0231	0.0917	0.0105	0.0542	2.6				

NS=No Sample; NW=Not Weighed; N/A=Not Applicable.

Appendix B-2
Individual Animal Data - Gavage Study, Full

800/400 mg/kg/d - Males (Full Study)

Animal #	Pre-exposure Body Weight (g)	Terminal Body Weight (g)	Days Dosed 800 mg/kg	Days Dosed 400 mg/kg	Stomach h	Stomach		Liver		Spleen		Kidney		Relative Kidney Wt. (% BW)	Relative Kidney Wt. (% BW)	HCT (%)
						Wt. (g)	(% BW)									
402	25.2	22.9	2	2	0.209	0.913	1.126	4.917	0.096	0.419	0.162	0.707	46			
404	26.4	22.2	2	2	0.176	0.793	1.030	4.640	0.091	0.410	0.181	0.815	42			
408	26.8	25.9	2	2	0.223	0.861	1.340	5.174	0.156	0.602	0.210	0.811	48			
410	26.9	27.0	2	2	0.219	0.811	1.547	5.730	0.094	0.348	0.182	0.674	52			
411	27.4	25.1	2	2	0.183	0.729	1.068	4.255	0.088	0.351	0.164	0.653	48			
412	27.5	27.5	2	2	0.176	0.640	1.589	5.778	0.188	0.684	0.222	0.807	44			
414	28.3	24.0	2	2	0.231	0.963	1.202	5.008	0.083	0.346	0.186	0.775	NS			
415	28.5	29.5	2	2	0.243	0.824	1.463	4.959	0.130	0.441	0.209	0.708	46			
Mean:	27.13	25.51			0.2075	0.8166	1.2956	5.0576	0.1158	0.4500	0.1895	0.7440	46.6			
SD:	1.06	2.46			0.0261	0.1014	0.2201	0.5120	0.0385	0.1262	0.0221	0.0657	3.2			

NS=No Sample, NW=Not Weighed, N/A=Not Applicable.

1200/600 mg/kg/d - Males (Full Study)

Animal #	Pre-exposure Body Weight (g)	Terminal Body Weight (g)	Days Dosed 1200 mg/kg	Days Dosed 600 mg/kg	Stomach h	Stomach Wt. (g)	Relative Stomach		Stomach h	Stomach Wt. (g)	Relative Liver		Liver Wt. (g)	Liver Wt. (% BW)	Relative Spleen		Spleen Wt. (g)	Spleen Wt. (% BW)	Relative Kidney		Kidney Wt. (g)	Kidney Wt. (% BW)	HCT (%)
							h	(% BW)			Wt. (g)	(% BW)			Wt. (g)	(% BW)			Wt. (g)	(% BW)			
602	24.8	20.0	2	2	0.142	0.710	1.027	5.135	0.125	0.625	0.160	0.800	38										
603	26.1	21.1	2	2	0.155	0.735	NW	NW	0.077	0.365	0.148	0.701	NS										
608	29.1	22.7	2	2	0.243	1.070	1.029	4.533	0.082	0.361	0.167	0.736	NS										
610	28.1	27.3	2	2	0.233	0.853	1.508	5.524	0.201	0.736	0.189	0.692	43										
616	26.6	24.8	2	2	0.245	0.988	1.303	5.254	0.092	0.371	0.192	0.774	39										
Mean:	26.94	23.18			0.2036	0.8713	1.2168	5.1115	0.1154	0.4917	0.1712	0.7407	40.0										
SD:	1.69	2.93			0.0507	0.1568	0.2335	0.4185	0.0514	0.1770	0.0189	0.0462	2.6										

NS=No Sample; NW=Not Weighed; N/A=Not Applicable.

Saline Control - Females (Full Study)

Animal #	Pre-exposure Body Weight (g)	Terminal Body Weight (g)	Days Dosed	Days Dosed	Days Dosed	Relative			Relative			Relative		
						Stomach Wt. (g)	Stomach Wt. (% BW)	Liver Wt. (g)	Liver Wt. (% BW)	Spleen Wt. (g)	Spleen Wt. (% BW)	Kidney Wt. (g)	Kidney Wt. (% BW)	HCT (%)
101	21.7	20.3	2	2	0.115	0.567	0.951	4.685	0.063	0.310	0.124	0.611	53	
103	20.4	20.3	2	2	0.175	0.862	0.930	4.581	0.086	0.424	0.116	0.571	48	
105	21.2	20.2	2	2	0.170	0.842	0.836	4.139	0.074	0.366	0.114	0.564	53	
107	20.8	20.7	2	2	0.138	0.667	0.949	4.585	0.075	0.362	0.129	0.623	49	
109	21.1	21.0	2	2	0.149	0.710	0.935	4.452	0.088	0.419	0.136	0.648	52	
111	21.1	22.1	2	2	0.145	0.656	1.050	4.751	0.078	0.353	0.129	0.584	53	
113	21.7	21.6	2	2	0.140	0.648	0.971	4.495	0.075	0.347	0.108	0.500	52	
115	16.2	20.2	2	2	0.222	1.099	0.966	4.782	0.064	0.317	0.147	0.728	47	
Mean:	20.53	20.8			0.1568	0.7562	0.9485	4.5588	0.075	0.3623	0.1254	0.6036	50.9	
SD:	1.80	0.7			0.0324	0.1707	0.0589	0.2056	0.009	0.0415	0.0127	0.0671	2.5	

400/200 mg/kg/d - Females (Full Study)

Animal #	Pre-exposure Body Weight (g)	Terminal Body Weight (g)	Days Dosed	Days Dosed	Days Dosed	Relative			Relative			Relative		
						Stomach Wt. (g)	Stomach Wt. (% BW)	Liver Wt. (g)	Liver Wt. (% BW)	Spleen Wt. (g)	Spleen Wt. (% BW)	Kidney Wt. (g)	Kidney Wt. (% BW)	HCT (%)
301	18.2	12.8	2	2	0.103	0.805	0.378	2.953	0.021	0.164	0.08	0.625	NS	
305	21.6	19.2	2	2	0.178	0.927	0.821	4.276	0.078	0.406	0.132	0.688	NS	
311	16.7	15.4	2	2	0.126	0.818	0.706	4.584	0.043	0.279	0.12	0.779	NS	
313	21.6	22.6	2	2	0.170	0.752	NW	NW	0.124	0.549	0.134	0.593	47	
Mean:	19.53	17.50			0.1443	0.8255	0.6350	3.9379	0.0665	0.3496	0.1165	0.6712	47	
SD:	2.47	4.30			0.0358	0.0734	0.2299	0.8666	0.0449	0.1655	0.0251	0.0820	N/A	

NS=No Sample; NW=Not Weighed; N/A=Not Applicable.

Appendix B-2
Individual Animal Data - Gavage Study, Full

800/400 mg/kg/d - Females (Full Study)

Pre-exposure		Terminal	Days Dosed	Days Dosed	Stomach	Relative	Liver	Relative	Spleen	Relative	Kidney	Relative	Kidney	HCT
Animal #	Weight (g)	Body Weight (g)	800 mg/kg	400 mg/kg	Wt. (g)	(% BW)	Wt. (g)	(%)						
502	17.9	17.5	2	2	0.162	0.926	0.996	5.691	0.151	0.863	0.118	0.674	0.674	39
505	21.7	22.1	2	2	0.204	0.923	1.270	5.747	0.125	0.566	0.173	0.783	0.783	38
510	19.7	18.2	2	2	0.179	0.984	0.879	4.830	0.239	1.313	0.126	0.692	0.692	36
513	22.3	21.0	2	2	0.176	0.838	1.228	5.848	0.157	0.748	0.138	0.657	0.657	37
Mean:	20.40	19.70			0.1803	0.9176	1.0933	5.5288	0.1680	0.8723	0.1388	0.7016	0.7016	37.5
SD:	2.0	2.20			0.0175	0.0599	0.1869	0.4706	0.0493	0.3184	0.0243	0.0560	0.0560	1.29

1200/600 mg/kg/d - Females (Full Study)

Pre-exposure		Terminal	Days Dosed	Days Dosed	Stomach	Relative	Liver	Relative	Spleen	Relative	Kidney	Relative	Kidney	HCT
Animal #	Weight (g)	Body Weight (g)	1200 mg/kg	600 mg/kg	Wt. (g)	(% BW)	Wt. (g)	(%)						
701	21.2	14.8	2	2	0.131	0.885	0.686	4.635	0.081	0.547	0.106	0.716	0.716	35
702	17.8	17.4	2	2	0.214	1.230	1.096	6.299	0.194	1.115	0.122	0.701	0.701	40
703	21.1	19.8	2	2	0.208	1.051	NW		0.227	1.146	0.128	0.646	0.646	36
707	21.0	19.8	2	2	0.218	1.101	1.013	5.116	0.203	1.025	0.130	0.657	0.657	38
709	21.1	16.5	2	2	0.169	1.024	0.842	5.103	0.133	0.806	0.126	0.764	0.764	35
715	22.5	21.5	2	2	0.193	0.898	1.173	5.456	0.181	0.842	0.14	0.651	0.651	NS
Mean:	20.7833	19.4000			0.1970	1.0184	1.0093	5.2250	0.1860	0.9549	0.1310	0.6795	0.6795	36.3
SD:	1.5664	2.0928			0.0213	0.0865	0.1655	0.2000	0.0400	0.1598	0.0062	0.0563	0.0563	1.5

NS=No Sample; NW=Not Weighed; N/A=Not Applicable.

Appendix B-3
Individual Animal Data -- Gavage Study, Full
Histopathological (H&E) Observations of Forestomach and Liver

Group	Animal #	Observations
Control Male	1	forestomach and glandular stomach normal, liver normal
	3	(no stomach present), liver normal
	5	forestomach normal, liver normal
	7	forestomach normal, liver normal
	9	forestomach normal, liver normal
Low Dose Male 400/200 mg/kg	11	forestomach and glandular stomach normal, liver not present
	13	forestomach and glandular stomach normal, liver normal
	15	(forestomach not present), glandular stomach normal, liver normal
	203	mild epithelial hyperplasia of the forestomach, liver normal
	205	forestomach normal, liver normal
Mid Dose Male 800/400 mg/kg	207	minimal forestomach epithelial hyperplasia, liver cells seem highly glycogenated
	211	mild forestomach epithelial hyperplasia, liver normal
	402	mild forestomach epithelial hyperplasia, liver normal
	404	glandular stomach normal, forestomach normal, liver normal
	408	minimal forestomach epithelial hyperplasia, liver normal
	410	mild forestomach epithelial hyperplasia, liver normal
	411	(no stomach present), liver normal
	412	glandular stomach normal, moderate forestomach epithelial hyperplasia with inflammatory cells in submucosa and muscularis, liver normal
	414	moderate forestomach epithelial hyperplasia, liver normal
	415	moderate forestomach epithelial hyperplasia with inflammatory cells in the submucosa and muscularis, liver normal

Appendix B-3 (continued)
Individual Animal Data – Gavage Study, Full
Histopathological (H&E) Observations of Forestomach and Liver

Group	Animal #	Observations
High Dose Male 1200/600 mg/kg	602	mild forestomach epithelial hyperplasia, liver normal
	603	minimal ulcer of forestomach, minimal forestomach epithelial hyperplasia, liver normal
	608	glandular stomach normal, mild forestomach epithelial hyperplasia with a small abscess in the lamina propria, liver normal
	610	mild forestomach epithelial hyperplasia with inflammation in the muscularis. There is a small island of epithelium in the inflamed area and evidence of extension of the inflammation into the peritoneal cavity. Liver normal
	616	marked forestomach epithelial hyperplasia with epithelial downgrowth through the lamina propria, muscularis mucosa and into the outer muscular layers. This is associated with prominent inflammation of the muscularis. Liver normal

Appendix B-3 (continued)
Individual Animal Data -- Gavage Study, Full
Histopathological (H&E) Observations of Forestomach and Liver

Group	Animal #	Observations
Control Female	101	forestomach normal, liver = minimal centrilobular necrosis
	103	forestomach = minimal focal ulcer and minimal epithelial hyperplasia, liver normal
	105	forestomach normal, liver normal
	107	(forestomach not present), glandular stomach normal, liver normal
	109	forestomach normal, liver normal
	111	forestomach normal, liver normal
	113	minimal focal necrosis and epithelial hyperplasia at glandular/forestomach junction, liver normal
	115	forestomach normal, liver normal
		forestomach normal, liver normal
Low Dose Female	301	forestomach normal, liver normal
	305	mild forestomach epithelial hyperplasia, liver normal
	311	glandular stomach normal, minimal forestomach epithelial hyperplasia, minimal focal forestomach inflammation, liver normal
	313	glandular stomach normal, forestomach normal, liver normal
Mid Dose Female	502	moderate forestomach epithelial hyperplasia with inflammatory cells in the muscularis, liver appears to be highly glycogenated
	505	minimal forestomach ulcer, moderate forestomach epithelial hyperplasia with inflammatory cells in the muscularis, liver normal
	510	moderate forestomach epithelial hyperplasia with inflammatory cells in the muscularis, liver normal
	513	mild forestomach epithelial hyperplasia, liver appears to be highly glycogenated

Appendix B-3 (continued)
Individual Animal Data - Gavage Study, Full
Histopathological (H&E) Observations of Forestomach and Liver

Group	Animal #	Observations
High Dose Female	701	mild forestomach epithelial hyperplasia with some inflammation in the lamina propria. Liver normal.
	702	mild forestomach epithelial hyperplasia, liver appears to be highly glycogenated.
	703	mild forestomach epithelial hyperplasia, liver normal
	707	glandular stomach has minimal suppurative inflammation, moderate forestomach epithelial hyperplasia with inflammation in the muscularis, liver not present; spleen is within normal limits
	709	glandular stomach normal, mild forestomach epithelial hyperplasia and minimal inflammatory cell infiltrate in lamina propria, liver normal
	715	(stomach not present), liver normal

Appendix C-1
Individual Animal Data - IP vs. SC Injection, Full Study
Pre-Exposure Body Weights and Terminal Body and Liver Weights

Group	Animal #	Pre-exp. BW (g)	Terminal BW (g)	Liver Wt. (g)	Rel. Liver Wt. (%)
SQ Saline	101	24.6	24.9	1.08	4.34
	102	22.5	21.6	0.89	4.12
	103	21.7	20.6	0.79	3.84
	104	22.4	21.5	0.92	4.28
	105	23.2	23.4	0.91	3.89
	106	22.1	—	0.84	—
	Mean:	22.8	22.4	0.905	4.09
SD:	1.0	1.7	0.099	0.23	
IP Saline	111	22.0	21.4	0.90	4.21
	112	23.4	24.1	1.02	4.23
	113	22.6	21.8	0.88	4.04
	114	21.7	21.4	0.87	4.07
	115	22.0	21.4	0.94	4.40
	116	22.4	22.1	0.77	3.48
	Mean:	22.4	22.0	0.897	4.07
SD:	0.6	1.1	0.083	0.31	
SQ 400 mg/kg	201	22.1	22.4	1.03	4.59
	202	22.2	21.7	0.92	4.24
	203	23.4	23.5	1.10	4.68
	204	22.5	22.6	1.02	4.51
	205	23.2	23.7	1.20	5.06
	206	21.4	22.6	1.14	5.04
	Mean:	22.5	22.8	1.068	4.69
SD:	0.7	0.7	0.099	0.32	
IP 400 mg/kg	211	23.7	23.9	1.10	4.60
	212	24.6	24.1	1.02	4.23
	213	21.6	23.4	0.88	3.76
	214	22.1	21.8	1.00	4.59
	215	22.2	21.7	0.96	4.42
	216	24.3	24.5	0.97	3.96
	Mean:	23.1	23.2	0.988	4.26
SD:	1.3	1.2	0.073	0.34	

Appendix C-2
Individual Animal Data - IP vs. SC Injection, Probe Study
Histopathological Observations of the Forestomach

Group	Animal #	Observations
IP 400 mg/kg	24	hyperplasia, epithelium, forestomach +1
	16	normal
	7	normal
IP 600 mg/kg	2	hyperplasia, epithelium, forestomach +2; inflammation +2; necrosis +1
	10	hyperplasia, epithelium, forestomach +2; inflammation +1; ulcer +1
	23	hyperplasia, epithelium, forestomach +2; inflammation +1
SC 400 mg/kg	18	hyperplasia, epithelium, forestomach
	11	normal
	8	normal
SC 600 mg/kg	31	hyperplasia, epithelium, forestomach
	20	normal
	21	normal

Appendix C-3
Individual Animal Data - IP vs. SC Injection, Full Study
Histopathological Observations of the Forestomach

Group	Animal #	Observations
IP Control	111	Normal
	112	Normal
	113	Normal
	114	Normal
	115	Normal
	116	Normal
IP 400 mg/kg	211	Normal
	212	Normal
	213	Normal
	214	Normal
	215	Hyperplasia, epithelium, forestomach +1 (focal); (also identified by blinded evaluation)
	216	Normal
SC Control	101	Normal
	102	Normal
	103	Normal
	104	Normal
	105	Normal
	106	Normal
SC 400 mg/kg	201	Normal
	202	Normal
	203	Normal
	204	Hyperplasia, epithelium, forestomach +1; inflammation +1; very minimal diffuse epithelial thickening with submucosal mononuclear inflammatory cells (also identified by blinded evaluation)
	205	Normal
	206	Hyperplasia, epithelium, forestomach +1; inflammation +1; very minimal diffuse epithelial thickening with submucosal mononuclear inflammatory cells (also identified by blinded evaluation)