

**ETHYLENEAMINES PRODUCT STEWARDSHIP DISCUSSION GROUP  
AEEA TESTING CONSORTIUM**

April 5, 2007

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TSCA Section 8(e) Coordinator  
Document Control Officer  
Office of Pollution Prevention and Toxics  
U.S. Environmental Protection Agency  
Ariel Rios Building; MC: 7407  
1200 Pennsylvania Avenue, N.W.  
Washington, D.C. 20460-0001



CONTAIN NO CBI

Re: Toxic Substances Control Act Section 8(e)

Dear TSCA Section 8(e) Coordinator:

This letter supplements the March 26, 2007, submission of the Ethyleneamines Product Stewardship Discussion Group (EPSDG) Aminoethylethanolamine (AEEA) Testing Consortium's,<sup>1</sup> c/o Mr. Timothy J. Cawley, c/o Bergeson & Campbell, P.C., 1203 Nineteenth Street, N.W., Suite 300, Washington, D.C. 20036-2401, to the U.S. Environmental Protection Agency (EPA), pursuant to Section 8(e) of the Toxic Substances Control Act (TSCA). That letter provided the results of a study with AEEA (CAS No. 111-41-1) entitled *Aminoethylethanolamine: Dermal and Oral Absorption and Limited Pharmacokinetic Study in Wistar Rats*. In our letter, we stated that a copy of the report will be sent under separate cover. Appended is a copy of the report.

If you have any questions, please contact Lynn L. Bergeson at (202) 557-3801 or lbergeson@lawbc.com.

Sincerely,

*Timothy J. Cawley*  
Timothy J. Cawley, Chair  
EPSDG AEEA Testing Consortium



cc: EPSDG AEEA Testing Consortium (via e-mail)

<sup>1</sup> The EPSDG AEEA Testing Consortium is comprised of the following companies: Akzo Nobel Functional Chemicals, LLC, BASF Corporation, The Dow Chemical Company, and Huntsman Corporation. The study was performed by the Dow Chemical Company.

{0237.006 / 6 / 00008990.DOC}

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**STUDY TITLE**

AMINOETHYLETHANOLAMINE: DERMAL AND ORAL ABSORPTION AND  
LIMITED PHARMACOKINETIC STUDY IN WISTAR RATS

**Data Requirement**

N/A

**Author(s)**

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**Study Completion Date**

Original Issue Date: 20 April 2004

Revised Date: 21 March 2007

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**Laboratory Project Study ID**

031053

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**COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS**

Compound: AMINOETHYLETHANOLAMINE

Title: AMINOETHYLETHANOLAMINE: DERMAL AND ORAL  
ABSORPTION AND LIMITED PHARMACOKINETIC STUDY IN  
WISTAR RATS

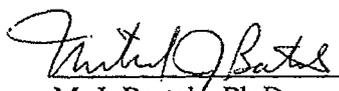
All phases of this study were conducted in compliance with the following Good Laboratory Practice Standards:

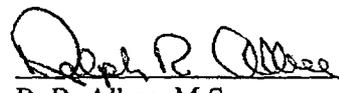
The Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF)  
Good Laboratory Practice Standards, 11 NohSan, Notification No. 6283 - 1 October 1999  
revised by 12 NohSan, Notification No. 8628 - 6 December, 2000

US Environmental Protection Agency - TSCA GLPs  
Title 40 CFR, Part 792 - Toxic Substances Control Act (TSCA); Good Laboratory  
Practice Standards, Final Rule

Organisation for Economic Co-Operation and Development (OECD)  
OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring,  
Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997)  
ENV/MC/CHEM(98)17

European Community (EC)  
EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999)

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### QUALITY ASSURANCE STATEMENT

Compound: AMINOETHYLETHANOLAMINE

Title: AMINOETHYLETHANOLAMINE: DERMAL AND ORAL  
ABSORPTION AND LIMITED PHARMACOKINETIC STUDY IN  
WISTAR RATS

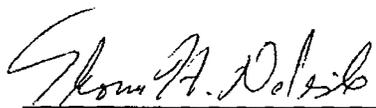
This study was examined for conformance with Good Laboratory Practices as published by the USEPA TSCA, JMAFF, OECD, and EC. The final report was determined to be an accurate reflection of the data obtained. The dates of Quality Assurance activities on this study are listed below.

Study Initiation Date: 16 June 2003

<u>TYPE OF AUDIT:</u>	<u>DATE OF AUDIT:</u>	<u>DATE FINDINGS REPORTED TO STUDY DIRECTOR/MANAGEMENT:</u>
Final protocol	18 June 2003	18 June 2003
Study conduct	18 June 2003	18 June 2003
Protocol, data, and draft report	05 March 2004	15 March 2004
Final Report	06 April 2004	06 April 2004

Report Change Revision      The date of the signature below is the date of the final report change revision audit.

The report change revision accurately reflects the raw data of the study.

 03/21/2007

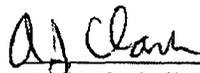
T. H. DeLisle, B.S., Auditor      (Date)  
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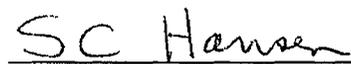
SIGNATURE PAGE

Compound: AMINOETHYLETHANOLAMINE

Title: AMINOETHYLETHANOLAMINE: DERMAL AND ORAL  
ABSORPTION AND LIMITED PHARMACOKINETIC STUDY IN  
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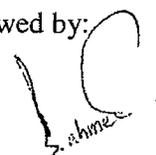
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### SUMMARY

Following a single oral administration,  $^{14}\text{C}$ -AEEA was well absorbed by female Wistar rats, with 85.21-98.18% of the dose recovered in the 0-48 hour urine, 5.16-11.51% in feces, and 0.02-0.03% in expired volatiles and  $^{14}\text{CO}_2$ . Some dose-dependency was seen for oral absorption, where the amount of administered dose recovered in the urine of the 0.5 mg/kg bw dose group was 12% greater than in the 50 mg/kg bw dose group ( $p < 0.05$ ). The low oral dose group also had a higher relative plasma AUC than the high oral dose group ( $p < 0.05$ ). No significant differences were seen in absorption or distribution between non-pregnant and pregnant rats given 50 mg  $^{14}\text{C}$ -AEEA/kg by the oral route. Overall recovery of radioactivity following an 8-hour dermal application of a 25% solution of  $^{14}\text{C}$ -AEEA in water ( $32 \mu\text{l}/\text{cm}^2 \times 12 \text{ cm}^2$ ; 480 mg/kg bw) was 90.97%. Excluding radioactivity recovered from the remote-site skin, the amount of absorbed test material, as measured in excreta, cage wash, tissues and application-site skin was  $7.73 \pm 1.56\%$ . As was seen following oral administration, the major route of elimination was via the urine, comprising  $3.04 \pm 3.54\%$  of the administered dose. The rate of absorption of test material, following oral administration, was quite rapid, with absorption  $t_{1/2}$  values ranging from 0.1-0.2 hour. The plasma elimination of orally administered  $^{14}\text{C}$ -AEEA was biphasic, with alpha and beta elimination  $t_{1/2}$  values of 1.6-1.8 hours and 16.7-17.3 hours, respectively. Only parent compound was found in plasma. Four radiolabeled metabolites were observed in urine from all three oral dose groups. These metabolites comprised approximately 5-10% (Metabolite A), 11-20% (Metabolite B), 5-11% (N-acetyl-AEEA) and 55-65% (AEEA) of the administered dose (Table 1 of Appendix A). No significant differences in metabolic profile were observed between dose level or pregnancy status.

## INTRODUCTION

### Previous Toxicity Information

The acute oral LD<sub>50</sub> of aminoethylethanolamine (AEEA) for female rats was determined to be between 2000-4000 mg/kg body weight (bw) (Lockwood and Borrego, 1980). The NOEL for systemic toxicity following repeated (28 days) dermal administration of AEEA was the maximum dosage tested, 1000 mg/kg/day (Stott *et al.*, 1991). In a previous oral gavage study of AEEA, decreased survival was observed in the offspring of pregnant female rats (Schneider *et al.*, 2003).

### Purpose

The purpose of this study was to provide data on the absorption and metabolism of aminoethylethanolamine (AEEA) following oral gavage administration to pregnant and non-pregnant rats and dermal administration to non-pregnant rats.

### Quality Assurance

The study conduct, data, protocol, protocol changes/revisions, and final report were inspected by the Quality Assurance Unit, Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan.

### Archiving

The data, protocol, protocol changes/revisions, and final report are archived at Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan.

## Test Material Information

### Test Material Name

Aminoethylethanolamine (CAS# 111-41-1)

### Chemical Name

Hydroxyethylethylenediamine

### Synonyms

AEEA, 2-(2-aminoethylamino) ethanol, N-(2-aminoethyl)ethanolamine

### Supplier, City, State (Lot number)

Radiolabeled: Moravek Biochemicals, Inc., Brea, California (160-121-060)

Non-Radiolabeled: BASF, Ludwigshafen, Deutschland (01/0019-2)

**Purity**

Radiolabeled: 98.0% (ACL-2003-57)

Non-Radiolabeled: 99.8% (BASF reference 01L00492)

**Specific Activity of Radiolabeled Test Material**

60 mCi/mmol

**Position of the Label**

Uniformly <sup>14</sup>C-labeled

**Characteristics**

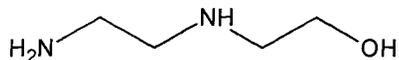
Molecular Formula

C<sub>4</sub>H<sub>12</sub>N<sub>2</sub>O

Molecular Weight

104.15

Chemical Structure



**Test Animals**

**Species and Sex**

Rats, female

Rats, time-mated female

**Strain and Justification**

Wistar rats were selected because of their general acceptance and suitability for toxicity testing, the reliability of the commercial supplier. In a previous oral gavage study of AEEA, decreased survival was observed in the offspring of pregnant female rats (Schneider *et al.*, 2003). It is unknown whether pregnancy has any impact on AEEA absorption and/or metabolism causing these effects in offspring. Therefore, this limited PK/metabolism study was designed to determine oral absorption in both pregnant and non-pregnant Wistar rats. As dermal exposure is the most probable route of exposure to

humans, non-pregnant rats were exposed to the test material via dermal application and absorption also compared between the two routes of exposure.

#### **Supplier and Location**

Hilltop Lab Animals, Inc. (Scottsdale, Pennsylvania)

#### **Age and Weight at Study Start**

At the time of dosing with radiolabeled test material the animals were within the following weight and age ranges:

Females, 249-279 g, 11-12 weeks old

Females, pregnant, 336-397 g, 11-12 weeks old, gestational day 18

#### **Physical and Acclimation**

Upon arrival at the laboratory<sup>1</sup>, each animal was evaluated by a laboratory veterinarian or a trained animal/toxicology technician, under the direct supervision of a lab veterinarian, to determine the general health status and acceptability for study purposes. Upon arrival, jugular vein cannulated (JVC) rats (surgery performed by the supplier) were acclimated in metabolism cages for two days prior to use. The supplier exteriorized the jugular vein cannulae. The rats used for dermal application were equipped with a protective appliance, described below, and fitted with rodent jackets containing dermal inserts (Lomir Biomedical, Malone, New York) to prevent grooming of the application site. Prior to administration of test material, the rats were stage-acclimated to the rodent jackets according to the following scheme: day 1 for 2 hours, day 2 for 4 hours. On day 3, the jackets were put on in the afternoon after the application of the dermal frame, and left on for the remainder of the study. The jackets were used to preserve the integrity of the dermal frame.

#### **Housing**

The rooms where the animals were housed had the relative humidity maintained within a range of 40–70% and the room temperature maintained at  $22 \pm 1^\circ\text{C}$  (with a maximum permissible excursion range of  $\pm 3^\circ\text{C}$ ). An approximate 12-hour light/dark photocycle was maintained for all animal rooms. Room air was exchanged 12–15 times/hour.

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<sup>1</sup> Fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

Following administration of AEEA, the animals were housed one per cage in glass Roth-type metabolism cages, in rooms designed to maintain adequate conditions of temperature, humidity and photocycle as described above. The metabolism cages were designed for the separation and collection of urine, feces, CO<sub>2</sub>, and expired volatiles. Air was drawn through the metabolism cages at ~ 500 ml/minute.

#### **Randomization and Identification**

Animals were identified by a uniquely assigned numbered metal eartag. The selection of animals was based on the integrity of the protective appliance (for dermal administration) and/or the patency of the jugular vein cannulae.

#### **Feed and Water**

Animals were provided LabDiet Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in pelleted form. Feed and municipal water was provided *ad libitum* during the pre-exposure and study periods, except, for animals receiving an oral administration, feed was withdrawn approximately 16-hours prior to the administration of AEEA and was returned about 4-hours post-dosing. Analysis of the feed was performed by PMI Nutrition International to confirm the diet provides adequate nutrition and to quantify the levels of selected contaminants. Drinking water obtained from the municipal water source was periodically analyzed for chemical parameters and biological contaminants by the municipal water department. In addition, specific analyses for chemical contaminants were conducted at periodic intervals by an independent testing facility. Copies of these analyses are maintained at Toxicology & Environmental Research and Consulting. Contamination levels observed in the food and water are not expected to effect the outcome of the study.

#### **Animal Welfare**

In accordance with the U.S. Department of Agriculture's rules on animal welfare, 9 CFR Parts 1-4, the animal care and use activities required for conduct of this study were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The IACUC has determined that the proposed Activities are in full accordance with these Final Rules. The IACUC-approved Animal Care and Use Activities to be used for this study are Metabolism 01, Metabolism 02, Dart 02, and Animal ID 01.

## Animal Preparation

### Oral Administration Groups

#### Breeding Procedure

Sexually mature virgin females were naturally mated with males of the same strain (one male to one female) at Hilltop Lab Animals, Inc. Females were checked for *in situ* copulation plugs the following morning and those found with such a plug were removed from the males' cages. The day on which a vaginal copulatory plug (plug date) observed *in situ* was considered gestation day 0. Rats arrived at the laboratory on day 16 of gestation and were dosed on day 18 of gestation.

### Dermal Application Group

For the dermal absorption phase of the study, approximately 17 hours prior to the application of the test material, the non-pregnant animals were anaesthetized with isoflurane. The hair was then removed from the back by clipping with an Oster (Milwaukee, Wisconsin) small animal electric clipper equipped with a size 40 fine cutting blade. Access to the dose site was restricted by use of a protective appliance described below.

#### Protective Appliance

The protective appliance was made from ~ 1.5-mm thick Teflon (4 cm × 5 cm) with a 3 cm × 4 cm cutout opening and formed into a saddle shape. This frame was positioned intrascapularly and as far anteriorly as possible and attached to the animal using Permabond Industrial Grade 910 adhesive (National Starch and Chemical Co., Englewood, New Jersey).

#### Dose Application

Animals were anaesthetized with isoflurane for dosing. A measured dose of <sup>14</sup>C-AEEA in distilled water was applied topically to an approximately 12 cm<sup>2</sup> area using a round-tipped feeding needle attached to an all-glass syringe. The dose solution was applied evenly to the skin in a volume of 32 μl/cm<sup>2</sup>. The syringe and feeding needle used for application of the test materials was weighed to determine the actual dose applied.

### Site Protection

The application site was semi-occluded by covering with Teflon Spectra/Mesh macroporous filter material (Spectrum Laboratories Inc., Rancho Dominguez, California). The covering was attached to the Teflon frame by Velcro strips (Velcro USA, Inc., Manchester, New Hampshire). In addition, the rats were fitted with rodent jackets containing dermal inserts.

### **Study Design**

This study was designed to determine and compare the dermal and oral absorption of AEEA following a single administration of AEEA. All groups were administered  $^{14}\text{C}$ -AEEA. A plasma  $^{14}\text{C}$  concentration-time course was used to calculate certain PK parameters ( $\text{AUC}$ ,  $C_{\text{max}}$ ,  $T_{\text{max}}$ ) for possible route-dependent comparisons.

All rats were purchased fitted with indwelling jugular vein cannulae. Approximately 0.2 ml blood was collected at indicated times (see below) and plasma separated by centrifugation. The plasma was analyzed for radioactivity by liquid scintillation spectrometry (LSS). Excreta was collected at specified times listed below. The study continued for 2-days post-dosing. Plasma was collected at the determined  $C_{\text{max}}$ , (0.5 hour post-dosing) and total volume of plasma was pooled (per group) and stored at  $-80^{\circ}\text{C}$ . Selected plasma and urine samples from the oral absorption dose group were analyzed via high performance liquid chromatography-mass spectrometry (LC/MS) for quantitation and limited characterization of radiolabeled metabolites.

### **Plasma $^{14}\text{C}$ -Concentration-Time Course**

#### Oral Absorption

3 female Wistar rats, 0.5 mg/kg bw

3 female Wistar rats, 50 mg/kg bw

3 female Wistar pregnant rats, 50 mg/kg bw (dosed on day 18 of gestation)

All rats were sacrificed 48-hours post-dose administration.

Blood was collected at the following intervals: 0.0-, 0.25-, 0.5-, 1-, 2-, 4-, 6-, 8-, 12-, 24-, 36-, and 48-hours post administration, and the plasma separated by centrifugation. A plasma  $^{14}\text{C}$ -concentration-time course was constructed.

Excreta/tissues was collected and analyzed for radioactivity as described below. No blood was collected from the following rats, #03A2454 (50 mg/kg non-pregnant

group) at 12-, 24-, and 36-hours, and #03A2455 (50 mg/kg pregnant group) at 24-, and 36-hours time points, due to lack of cannula patency. Two additional pregnant and two additional non-pregnant rats dosed at 50 mg/kg, were sacrificed at the time of maximum plasma radioactivity ( $C_{max}$ ; 0.5 hour) and plasma samples pooled by total volume for metabolite profiling. Selected urine and  $C_{max}$  plasma samples were pooled and stored for subsequent chemical analysis.

#### Dermal Absorption

8 female Wistar rats, 8-hour application of 25% solution of  $^{14}C$ -AEEA ( $32 \mu\text{l}/\text{cm}^2 \times 12 \text{ cm}^2$  skin; 480 mg/kg bw)

All rats underwent skin-washing at 8-hour post application. Four rats were sacrificed at 8-hours post application, with the remainder sacrificed at 24-hour post-washing.

Blood was collected at the following intervals: 0.0-, 0.5-, 1-, 2-, 4-, 6-, and 8-hour post- application, and the plasma separated by centrifugation. A plasma  $^{14}C$ -concentration-time course was constructed. Excreta/tissues was collected and analyzed for radioactivity as described below. (Only terminal blood was collected from animals #03A2443, 03A2447-48, due to lack of cannula patency).

### **Test Material Administration**

#### Route and Delivery

A single oral administration  $^{14}C$ -AEEA

A single dermal application  $^{14}C$ -AEEA

#### Route Justification

Results of the oral study were used for comparative absorption and/or metabolism of AEEA in pregnant and non-pregnant rats. Dermal exposure is the primary route for human absorption.

#### Dose Justification

The dose levels for this study were selected to provide measurable concentrations of plasma radioactivity after oral administration or dermal application to rats and were based on previous toxicity studies. The dose levels of 0.5 and 50 mg/kg body weight (bw) were chosen to examine oral absorption of AEEA and are several orders of magnitude lower than reported  $LD_{50}$  (Lockwood and Borrego, 1980). In the OECD

421 study, the lowest dose was 50 mg/kg (Schneider *et al.*, 2003). The dermal application amount selected ( $32 \mu\text{l}/\text{cm}^2 \times 12 \text{ cm}^2$  skin; 480 mg/kg) was equivalent, on a mg/cm<sup>2</sup> skin basis, to that applied in a 28 day dermal toxicity study (Stott *et al.*, 1991).

### **Dose Preparation and Analysis**

#### Preparation

The oral dose solution was prepared as a solution in deionized water. Appropriate amounts of <sup>14</sup>C-labeled and/or non-radiolabeled AEEA were added to obtain the target doses of 0.5 or 50 mg AEEA mg/kg bw. The target concentrations of dose solutions were 0.1 and 10 mg/mL. The amount of the dermal dose solution applied was ~ 384  $\mu\text{L}$  (target concentration of dose solution: 250 mg/mL) to a ~12 cm<sup>2</sup> (4 cm x 3 cm) area. Confirmation of the test material concentration in the dose solutions was conducted. Radioactivity in the dose solutions was quantified by liquid scintillation spectrometry (LSS) as described below. The amount of oral dose solution administered was targeted at ~5 g/kg bw. The target radioactivity was ~75  $\mu\text{Ci}/\text{kg}$ .

#### Stability

The AEEA has been reported to be stable in distilled water for 8 days (Stott *et al.*, 1991).

### **SPECIMEN COLLECTION**

#### **Urine**

All urine voided during the study was collected in dry-ice cooled traps.

#### **Oral Absorption**

Urine was collected at 12-, 24-, 36-, and 48-hours post-administration. The cages were rinsed with water after each urine collection and the rinse collected. Each urine specimen and urine/cage rinse was weighed, and a weighed aliquot of each sample was analyzed for radioactivity by LSS as described below. Selected urine samples were also analyzed by high pressure liquid chromatography (HPLC), as described below, to obtain profiles of radiolabeled metabolites. Equal volume aliquots of urine samples from the 0-12 hour and 12-24 hour collection intervals were pooled and stored at -80°C for metabolite identification.

### **Dermal Absorption**

Urine was collected at 8-hours post-application. Urine was also collected at 12-hours and 24-hours post-washing for the four rats sacrificed at 24 hours post-washing. The cages were rinsed with water after each urine collection and the rinse collected. Each urine specimen and urine/cage rinse was weighed, and a weighed aliquot of each sample was analyzed for radioactivity by LSS as described below. Equal volume aliquots of urine samples from the 0-8 hour post-application, and the 0-12 hour and 12-24 hour post-washing collection intervals were pooled by equal volume and stored at -80°C until chemical analysis.

### **Feces**

Feces were collected in dry-ice chilled containers at 24-hour intervals. Feces were also collected at the 8-hour post-application interval following dermal application. An aqueous homogenate (~ 25% w/w) was prepared and weighed aliquots of these homogenates were oxidized (OxiMate 80 Sample Oxidizer, PerkinElmer Life Sciences, Inc., Boston, Massachusetts) and quantitated for radioactivity by LSS. Remaining homogenates were stored at -80°C.

### **Expired Volatiles**

Air was drawn through the cage at approximately 500 ml/minute. Upon exiting the cage, the air was passed through charcoal to trap expired volatiles. The charcoal traps were changed at 24-hour intervals through 48 hours. Radioactivity trapped on the charcoal was desorbed with weighed amounts of toluene. Weighed aliquots of toluene were analyzed for radioactivity. Since < 0.1% of the administered dose was detected in the charcoal trap during the first 24-hour interval, the replacement traps were not analyzed for radioactivity.

### **Expired CO<sub>2</sub>**

Following the charcoal trap (described above) the expired air was passed through a solution of monoethanolamine: 1-methoxy-2-propanol (3:7 v/v) to trap expired CO<sub>2</sub> and analyzed for radioactivity. The CO<sub>2</sub> trap was changed at 12-hour intervals through 24 hours. Since <0.1% of the administered dose was detected in the CO<sub>2</sub> trap during the first 12-hour interval, the replacement traps were not analyzed for radioactivity.

### **Dermal Application Site**

At 8 hours post-application, the macroporous filter material coverings were removed. The skin at the dosed site was washed five times with cotton tip applicators dipped in an aqueous solution of detergent (*i.e.*, ~ 2-4% Ivory dish washing liquid, Proctor and Gamble Co, Cincinnati, Ohio), rinsed several times with gauze soaked with water and the area blotted dry with gauze squares. The skin wash and macroporous covering were collected and analyzed for radioactivity by LSS. Animals providing samples beyond eight hours had a new mesh covering applied over the application site and were returned to their cages.

At the indicated times post-dosing, the animals were anesthetized and sacrificed by exsanguination via cardiac puncture. The Teflon<sup>®</sup> frame was removed and the components analyzed for radioactivity. Stratum corneum was removed by tape stripping from the dose site and analyzed for radioactivity by LSS. The skin at the dosed site was excised, solubilized and an aliquot analyzed for radioactivity by LSS.

### **Terminal Sacrifice**

At the specified post-dosing time, the animals were anaesthetized with CO<sub>2</sub> and O<sub>2</sub> mixture and sacrificed by exsanguination via cardiac puncture. Pregnancy status was verified upon exsanguinations and recorded. The unborn rat fetuses were humanely euthanized with Socumb euthanasia solution (Veterinary Laboratories, Inc., Lenexa, Kansas) and analyzed with the adult carcass. Following sacrifice the Roth cages were washed and the cage wash analyzed for radioactivity.

### **Tissues**

The following tissues were collected at sacrifice:

#### **Dermal Absorption (Phase 1)**

kidney	liver	carcass
blood (terminal)	application-site skin	skin

#### **Oral Absorption (Phase 2)**

kidney	liver	carcass
blood (terminal)	skin	

The carcass, kidney, and liver were collected, homogenized (~ 33% homogenate), oxidized and a weighed aliquot analyzed for radioactivity by LSS. Blood was

centrifuged to obtain plasma and the plasma analyzed for radioactivity by LSS. The skin was removed from the carcass and a representative skin sample was oxidized and analyzed for radioactivity by LSS, except as noted in the dermal application site section above. The carcass was homogenized (~ 33% homogenate) and a weighed aliquot of the carcass homogenate oxidized and analyzed for radioactivity by LSS.

#### **Final Cage Wash**

Following the terminal sacrifice of the animals, a final cage wash was performed. The final cage wash and contents were collected and the weight of the sample was determined. A weighed aliquot of the final cage wash was analyzed for radioactivity.

#### **Plasma**

Blood was obtained at sacrifice via cardiac puncture. Blood was centrifuged to obtain plasma and plasma analyzed for radioactivity. Equal-volume aliquots of plasma were pooled (per time, dose, and dose group) and stored at -80 °C for chemical analysis. Selected plasma samples from the oral absorption dose group were also analyzed by HPLC, as described below, to obtain profiles of radiolabeled metabolites.

#### **Control Samples**

Control urine was collected in dry-ice cooled traps from one or more non-pregnant rats(s) not dosed with <sup>14</sup>C-AEEA. The control animal(s) were sacrificed by the same procedure as the dosed animals. At sacrifice, control blood was obtained via cardiac puncture and centrifuged to acquire control plasma from non-pregnant rats(s). No tissues, volatile organics or expired CO<sub>2</sub> were collected. These samples were used to aid in potential chemical analysis of blood and/or excreta samples.

### **Sample Analysis**

#### **Tissue Oxidization**

A weighed aliquot of each of the tissue homogenate (liver, kidney, and carcass) was oxidized using Packard 387 Sample oxidizer (OxiMate 80 Sample Oxidizer, PerkinElmer Life Sciences, Inc., Boston, Massachusetts) and analyzed for radioactivity by LSS as described below.

#### **<sup>14</sup>C Analysis**

Radioactivity was quantified in a liquid scintillation spectrometer (Packard Tri-Carb 2900TR, Packard Bioscience Company, Meriden, Connecticut). Counts per minute

(cpm) were corrected for quench and background, and converted to disintegrations per minute (dpm). The instrument is equipped with sealed  $^{14}\text{C}$ -standards that are counted every 23 hours during the operation to monitor the performance of the LSS. Samples with dpm less than twice the concurrently run background (blanks) were considered to contain insufficient radioactivity to reliably quantify.

#### **Radiochemical Purity**

Radiochemical purity analysis of the  $^{14}\text{C}$ -labeled test material was conducted and the results included in the study file. The results of this analysis were consistent with those supplied by the vendor.

#### **Dose Confirmation and Homogeneity of Dose Solution**

The concentration of AEEA in the dose solution was analyzed in accordance with the standard operating procedures of the Analytical Chemistry Laboratory. LSS analysis of aliquots of the  $^{14}\text{C}$ -labeled dosing solution taken from various locations in the solution container were used to confirm the concentration of radioactivity and the homogeneity of the  $^{14}\text{C}$ -AEEA in the dosing solution.

#### **Metabolite Profiling and Identification**

Pooled samples (plasma and urine) from selected intervals of the oral absorption dose group were analyzed in duplicate via HPLC with in-line radiochemical detection, or singly if fraction collection was required, to obtain profiles of radiolabeled metabolites present. Samples were stored at  $-80^\circ\text{C}$  for metabolite identification. Specific details concerning the analytical methodology employed are included in Appendix A.

#### **Data Analysis**

Descriptive statistics were conducted, (*i.e.*, mean and standard deviation [ $\bar{X} \pm \text{SD}$ ]). Samples with dpm less than twice the concurrently run background (blanks) were considered to contain insufficient radioactivity to reliably quantify. Non-quantifiable excreta samples (urine/rinse/feces/ $\text{CO}_2$ /expired volatiles) were identified in the tables as NQ and a value of '0' used in calculations. For tissues, when a sample was non-quantifiable, that sample was assigned the quantitation limit (QL) for calculations and displayed as NQ with the quantitation limit in parenthesis. The mean was calculated from actual values and calculated QL values and presented as Mean  $\pm$  SD, unless greater than  $\frac{1}{2}$  of the values are presented as NQ, in which case the mean was expressed as NQ(Mean)  $\pm$  SD. If all tissue values were NQ the mean was presented as NQ (QL) with

SD displayed. Quantitation limits (as a fraction of administered dose) were calculated using the following formula:

$$QL = \left( \frac{\text{background dpm}}{\text{aliquot weight}} \right) \times \left( \frac{\text{dilution factor}}{\text{dose dpm}} \right)$$

All calculations in the database were conducted using Microsoft Excel spreadsheets and databases in full precision mode (15 digits of accuracy). Certain pharmacokinetic parameters were estimated from the plasma <sup>14</sup>C concentration-time course including AUC (area-under-curve), C<sub>max</sub>, ½C<sub>max</sub>, elimination rate constants, half-lives, apparent volume of distribution and clearance, using PK Solutions (Summit Research Services, Montrose, Colorado), a pharmacokinetic computer modeling program or Microsoft Excel. Log-linear regression analysis of the interval <sup>14</sup>C-excretion rate data was used to estimate the urinary half-lives of excretion of the radiolabel. Statistical analysis (t-test) of selected datasets was performed with Prism v4.0 (Graphpad Software, Inc., San Diego, California).

### Results and Discussion

The actual test material concentrations and radioactivity levels of the various dose solutions were all within 10% of the target values (Table 1). The mean body weights for non-pregnant females ranged from 260-272 g. The mean body weight of the pregnant female group was 371 g (Table 2; Appendix Table 1, individual animal data). The amount of test material administered to the various groups was also quite consistent, with all administered doses within 10% of targeted levels of 0.5 and 50 mg/kg (Table 2). The dermally applied test material was also within 10% of the targeted application. The mean amount of radioactivity administered to each orally gavaged group was also within 10% of the target amount of 75 µCi/kg body weight and the mean amount of applied radioactivity for the dermal group was also within 10% of the targeted application.

Overall recovery of radioactivity ranged from 98.78-107.32% of orally administered test material (Table 3, Appendix Table 2, individual animal data). The majority of administered radioactivity, 85.21-98.18%, was eliminated via urine. Feces contained 5.16-11.51% of the administered dose (Table 3). Tissues contained 2.30-3.33% of the administered dose (Table 3). Expired volatiles and <sup>14</sup>CO<sub>2</sub> accounted for 0.02-0.03% of the administered dose (Table 3). Some dose-dependency was seen for oral absorption, with a significantly higher amount (12%) of the administered dose recovered in the urine of the 0.5 mg/kg dose group vs. the 50 mg/kg dose group (p < 0.05). No significant

differences were observed in absorption or distribution between non-pregnant and pregnant rats given 50 mg  $^{14}\text{C}$ -AEEA/kg bw. Greater than 85% of the orally administered test material was absorbed in 48 hours, for all dose groups, based on the radioactivity recovered in the urine, tissues, final cage wash, and expired  $\text{CO}_2$ .

Overall recovery of radioactivity of dermally applied  $^{14}\text{C}$ -AEEA was 90.97% (Table 3, Appendix Table 3, individual animal data). As was seen following oral administration, the major route of elimination was via the urine, comprising  $3.04 \pm 3.54\%$  (Tables 3 and 8). Feces contained  $0.34 \pm 0.77\%$  of the administered dose (Table 3). Tissues, including total skin, contained  $15.81 \pm 13.77\%$  of the administered dose (Table 12). Excluding radioactivity recovered from the remote-site skin, the amount of absorbed test material, as measured in excreta, cage wash, tissues, and application-site skin was  $7.73 \pm 1.56\%$  (calculated from data of the four test animals described below).

The volume of test material applied to the skin for this study was  $32 \mu\text{l}/\text{cm}^2$ , to match a prior toxicology study application volume/ $\text{cm}^2$  (Stott *et al.*, 1991). While this volume was visually acceptable, it is approximately 3-fold higher than the suggested dose volume for this study type ( $10 \mu\text{l}/\text{cm}^2$ ; OECD 427 guidelines). Due to this high-dose volume, there was some potential for contamination to the non-application site skin. Based on the four criteria listed below we feel the dermal absorption results from animals 03A2441, 03A2242, 03A2243, and 03A2246 were invalid for the purpose of dermal penetration estimation. First, as shown in Appendix Table 3, significantly higher radioactivity was found in the non-application skin of three rats, 03A2441, 03A2443, and 03A2446, than the remaining five rats in that group and ~10-fold higher than in the oral administration groups (average of 0.6%). These high remote skin values may be due to contamination from the application site, not absorbed dose, and indicate removal of 7-26% of the test material from the application site skin, resulting in invalid exposure. Second, a high spike in plasma concentration from animal 03A2441 and 03A2242 (Table 5) was observed which was in contrast to the relatively flat and low concentrations ( $14 \mu\text{g}$  equiv./g) of other 3 animals with complete plasma time course data (03A2244, 03A2245 and 03A2246) but consistent with the blood time-course of the orally-dosed groups (Table 4). Third, substantial amounts of radioactivity were found in the 0-8 hour urine from 03A2442 and 03A2243 (5-6%), whereas <1% of the dose was found in the 0-8 hour urine from the other 6 animals, indicating that oral ingestion may have occurred in these animals (Appendix Table 3). Fourth, high carcass levels (>4% of dose) were observed in animals 03A2441, 03A2242 and 03A2446 (compared to <2.2% in the 3 oral groups and

<0.6% in the remaining 5 dermal animals) (Appendix Table 3). Based on these criteria, it is felt that the appropriate determination of absorbed test material should be from data of the remaining four animals (03A2444, 03A2445, 03A2447, and 03A2448) (7.73%).

The time course of radioactivity in plasma following a single oral dose of 0.5 or 50 mg <sup>14</sup>C-AEEA/kg is shown in Table 4. Plasma concentrations were quantifiable through 48 hours post-dosing. Maximal plasma concentrations were found to occur at 0.5 hour following oral administration. Approximately 100-fold higher concentrations of radioactivity were observed at the 50 mg/kg dose than at the 0.5 mg/kg dose. In addition the time course of plasma radioactivity following oral administration was similar between pregnant and non-pregnant animals.

The plasma time course following a single dermal application of AEEA is presented in Table 5. Concentrations of radioactivity were usually non-quantifiable at most time points for all animals except animals 03A2441 and 03A2442. The high plasma concentrations at all time points for animal 03A2442, relative to the other animals in this dose group, and the substantial increase in plasma radioactivity between four and six hours for animal 03A2441, may indicate some oral intake of the test material for these animals. Mean plasma concentrations for this dose group were calculated from detectable values as well as quantitation limits for NQ values. The 0-24 hour AUC value was calculated using the mean values reported in Table 5. No elimination rate was calculated nor were dermal plasma concentrations included in Figure 1, since mean levels were quantifiable only at six hours.

The bioavailability of AEEA, as measured by plasma AUC, was fairly linear with dose (Figure 1). The AUC from 0.5 mg/kg dose was slightly higher (~ 30%) than the corresponding 50 mg/kg non-pregnant dose group, when corrected for dose level (Table 6). This difference was statistically significant ( $p < 0.05$ ). However, no statistical differences were observed between the non-pregnant and pregnant rats administered 50 mg/kg <sup>14</sup>C-AEEA, or between the 0.5 mg/kg non-pregnant and the 50 mg/kg pregnant dose groups. The plasma AUC for the 480 mg/kg dermal dose group was calculated to be 13.7  $\mu\text{g}\cdot\text{hr}/\text{ml}$ , which was less than the plasma AUC for the 50 mg/kg oral dose groups (95.0-117.1  $\mu\text{g}\cdot\text{hr}/\text{ml}$ ). Since the dermal dose applied was approximately 10-fold higher than the high oral dose, these plasma data suggest that less than 10% of dermally applied <sup>14</sup>C-AEEA is absorbed, consistent with the measured absorption of 7.73% (Table 3).

The rate of absorption of test material, following oral administration, was quite rapid, with absorption  $t_{1/2}$  values ranging from 0.1-0.2 hour (Table 6). No absorption rate was determined from the plasma time course of the group receiving a dermal application of  $^{14}\text{C}$ -AEEA, since plasma values were non-quantifiable in four of the eight animals and since no sample could be obtained from three of the eight animals.

The elimination of orally administered  $^{14}\text{C}$ -AEEA was biphasic. The alpha elimination phase was quite rapid, ranging from 1.6-1.8 hours. The beta phase was substantially longer for all oral dose groups, with  $t_{1/2}$  values of 16.7-17.3 hours. The urinary elimination rate ranged from 6.0-7.9 hours for the oral dose groups (Table 6). A comparable elimination rate was calculated for the dermal administration route (5.7 hours).

The excretion of administered radioactivity in urine of rats following a single oral gavage administration of  $^{14}\text{C}$ -AEEA is shown in Table 7. The majority of the radioactivity excreted in the urine was excreted in the first 12-hour interval. The total excreted in 48 hours was 98.18, 86.61, and 85.21% for the low dose non-pregnant group, the high-dose non-pregnant group and the high-dose pregnant group, respectively (urine + rinse). Following a single dermal application of  $^{14}\text{C}$ -AEEA, the majority of the radioactivity was excreted in 0-8 hours, 2.15% (Table 8). Following removal of the dermal application by washing at eight hours, 1.31, and 0.47% of the applied radioactivity was recovered in the urine at 8-12 and 12-24 hours.

Following oral gavage administration the majority of the radioactivity excreted in the feces was eliminated in the 0-24 hour interval (Table 9). Fecal excretion was approximately two-fold higher for the 50 mg/kg oral groups, vs. the 0.5 mg/kg oral group. Pregnant and non-pregnant animals had comparable percentages of recovered radioactivity in the feces. Following dermal application of  $^{14}\text{C}$ -AEEA, little of the applied dose was recovered in 0-8 hours (0.18%) and post-washing of the application site only 0.33% of the applied dose was recovered in the feces (Table 10).

The distribution of radioactivity in tissues following oral administration with  $^{14}\text{C}$ -AEEA is presented in Table 11 (see Appendix Table 4 for individual animal data) as the percentage of the administered dose and as  $\mu\text{g}$  equivalents ( $\mu\text{g}$  eq) AEEA/g tissue. The greatest percentage of the administered dose was recovered in the carcass as compared to kidneys, liver, and skin. Approximately 3% of the administered dose was recovered in tissues from all three oral dose groups. The greatest percentage of the administered dose

was found in the non-dosed skin following an eight-hour dermal application and a 16 hour post-washing period (Table 12; Appendix Table 5, individual animal data). As discussed above, the applied dose may have contaminated other skin than at the application site. The percentage of radioactivity in the carcass was 2.76% and less than 0.3% of the applied  $^{14}\text{C}$ -AEEA was found in the liver and kidneys.

The plasma samples at  $T_{\text{max}}$  for the 50 mg/kg non-pregnant and pregnant dose groups were found to contain only parent compound (Metabolite D; Figure 2 of Appendix A). Analysis of selected urine samples from the three oral-dose groups afforded four radiolabeled components in all samples analyzed (Figure 1 of Appendix A). These metabolites, labeled A, B, C, and D, were found to comprise approximately 5-10%, 11-20%, 5-11%, and 55-65% of the administered dose respectively (Table 1 of Appendix A). No substantial differences in metabolic profile were observed between dose level or pregnancy status.

Urinary metabolite D was shown to coelute with parent compound (data not shown). Analysis of the 0-12 hour urine samples from the two 50 mg/kg dose groups was performed via HPLC-electrospray-mass spectrometry (LC/ESI-MS) to obtain limited identification of these four metabolites. This limited identification consisted of comparison with six synthetic standards: AEEA, ethylenediamine, ethanolamine, N-acetyl-AEEA, N-acetyl-ethylenediamine and N-acetyl-ethanolamine. Both parent AEEA and the corresponding N-acetyl-AEEA were identified as metabolites C and D, respectively (Figures 3 and 4 of Appendix A). Metabolites A and B were determined not to be ethylenediamine, ethanolamine, or the corresponding acetyl analogs.

The acetylation of AEEA is consistent with the metabolic fate of other amines. Yang *et al.*, (1982) report that the major urinary metabolite of ethylenediamine is the N-acetylated parent compound. The lack of ethylenediamine or ethanolamine as metabolites of AEEA in this study is consistent with the extremely low level of expired radioactivity from rats given this test material orally. Previous metabolism studies with both ethylenediamine and ethanolamine have shown that 8-11%, respectively, of these test materials are converted to  $^{14}\text{CO}_2$  in the rat (Yang *et al.*, 1982, Taylor and Richardson, 1967). Since less than 0.05% of the administered  $^{14}\text{C}$ -AEEA is converted to radiolabeled  $\text{CO}_2$ , these results indicate that neither of these compounds were significant (> 0.5%) metabolites of AEEA.

### Conclusion

Following a single oral administration,  $^{14}\text{C}$ -AEEA was well absorbed by female Wistar rats, with 85.21-98.18% of the dose recovered in the 0-48 hour urine, 5.16-11.51% in feces and 0.02-0.03% in expired volatiles and  $^{14}\text{CO}_2$ . Some dose-dependency was seen for oral absorption, where the amount of administered dose recovered in the urine of the 0.5 mg/kg bw dose group was 12% greater than in the 50 mg/kg bw dose group ( $p < 0.05$ ). The low oral dose group also had a higher relative plasma AUC than the high oral dose group ( $p < 0.05$ ). No significant differences were seen in absorption or distribution between non-pregnant and pregnant rats given 50 mg  $^{14}\text{C}$ -AEEA/kg by the oral route. Overall recovery of radioactivity following an 8-hour dermal application of a 25% solution of  $^{14}\text{C}$ -AEEA in water ( $32 \mu\text{l}/\text{cm}^2 \times 12 \text{ cm}^2$ ; 480 mg/kg bw) was 90.97%. Excluding radioactivity recovered from the remote-site skin, the amount of absorbed test material, as measured in excreta, cage wash, tissues and application-site skin was  $7.73 \pm 1.56\%$ . As was seen following oral administration, the major route of elimination was via the urine, comprising  $3.04 \pm 3.54\%$  of the administered dose. The rate of absorption of test material, following oral administration, was quite rapid, with absorption  $t_{1/2}$  values ranging from 0.1-0.2 hour. The plasma elimination of orally administered  $^{14}\text{C}$ -AEEA was biphasic, with alpha and beta elimination  $t_{1/2}$  values of 1.6-1.8 hours and 16.7-17.3 hours, respectively. Only parent compound was found in plasma. Four radiolabeled metabolites were observed in urine from all three oral dose groups. These metabolites comprised approximately 5-10% (Metabolite A), 11-20% (Metabolite B), 5-11% (N-acetyl-AEEA), and 55-65% (AEEA) of the administered dose (Table 1 of Appendix A). No significant differences in metabolic profile were observed between dose level or pregnancy status.

### Acknowledgements

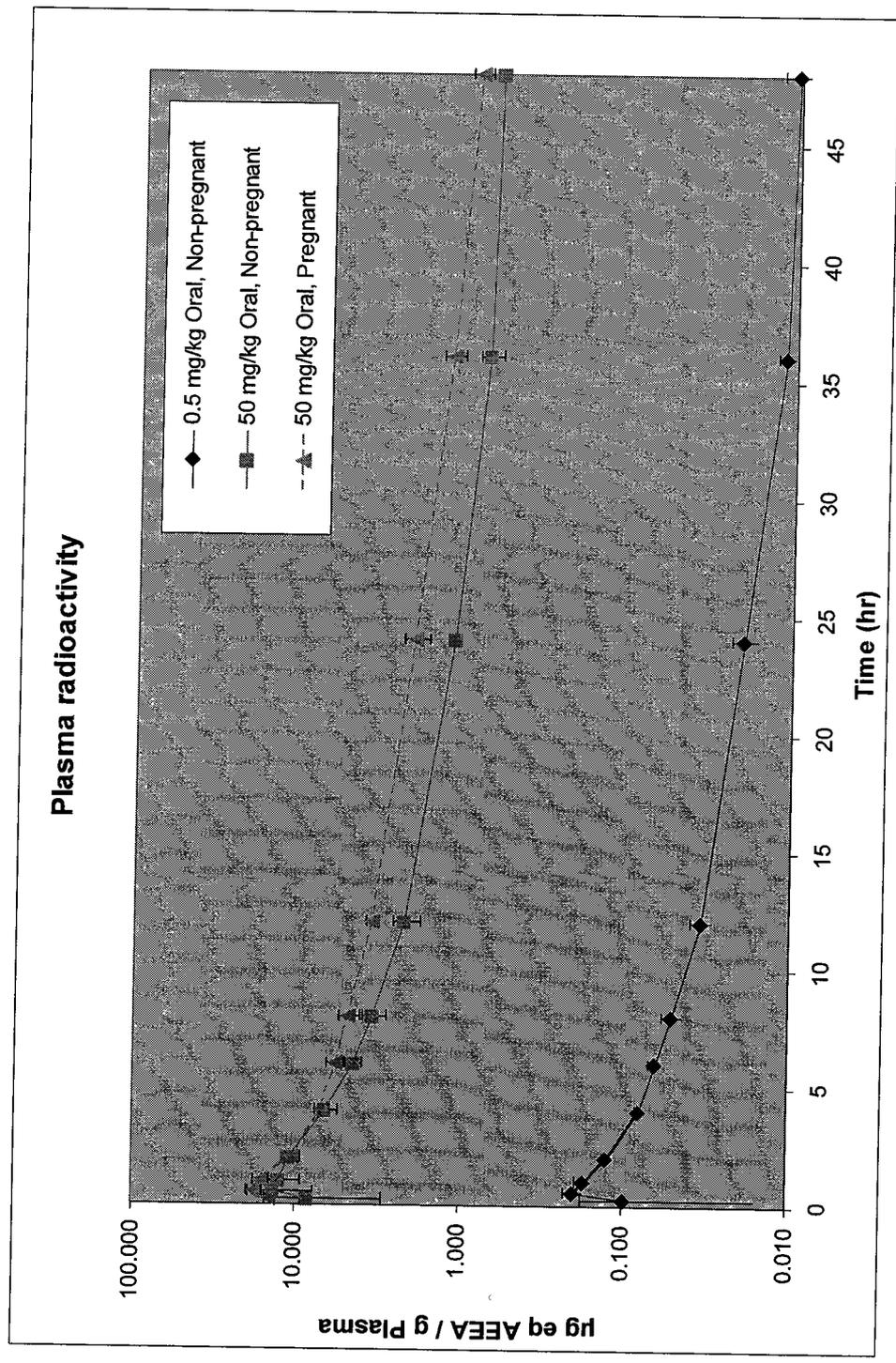
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AMINOETHYLETHANOLAMINE: DERMAL AND ORAL ABSORPTION AND LIMITED PHARMACOKINETIC STUDY IN WISTAR RATS

Figure 1. Time-Course of Plasma Radioactivity following Oral Administration of <sup>14</sup>C-AEEA



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Table 1. Concentration of Radioactivity and AEEA in the Oral and Dermal Dose  
 Solutions

Dose Solution		Target Radioactivity ( $\mu\text{Ci/g}$ )	Actual Radioactivity ( $\mu\text{Ci/g}$ )	Target AEEA (mg/g)	Actual AEEA (mg/g)
Oral dose	0.5 mg/kg bw				
	Non-pregnant females	30.0	30.1	0.2	0.214
Oral dose	50 mg/kg bw				
	Non-pregnant females	30.0	29.5	20.0	20.1
	Pregnant females	30.0	29.5	20.0	20.1
Dermal application	480 mg/kg				
	Non-pregnant females	57.7	60.8	369	357

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Table 2. Mean Body Weights (bw), Amounts of Dose Solution and Radioactivity Administered

Group/Sex	Body Weight (kg)		Dose Solution (g)		AEEA (mg)		AEEA (mg/kg bw)		Radioactivity (μCi)		Radioactivity (mg/kg bw)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Oral dose 0.5 mg/kg bw												
Non-pregnant females	0.272	0.005	0.6911	0.0130	0.15	0.01	0.54	0.01	20.80	0.39	76.40	1.22
Oral dose 50 mg/kg bw												
Non-pregnant females	0.269	0.009	0.6796	0.0290	13.66	0.58	50.85	0.54	20.05	0.85	74.61	0.78
Pregnant females	0.371	0.032	0.9335	0.0611	18.77	1.23	50.60	1.07	27.54	1.80	74.25	1.58
Dermal application 480 mg/kg												
Non-pregnant females	0.260	0.009	0.3400	0.0212	121.33	7.42	466.08	23.10	20.64	1.26	79.31	3.90

SD = Standard Deviation  
 Values represent mean and SD (N=3) in each Oral group and (N=8) in Dermal group.

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Table 3. Percentage of Orally or Dermally Administered <sup>14</sup>C-AEEA Recovered in Wistar Rats

	Single Oral Dose of 0.50 mg AEEA/kg bw Non-pregnant females		Single Oral Dose of 50 mg AEEA/kg bw Non-pregnant females		Single Oral Dose of 50 mg AEEA/kg bw Pregnant females	
	Mean	SD	Mean	SD	Mean	SD
Urine & Rinse	98.18	2.51	86.61	2.58	85.21	5.58
Feces	5.16	0.89	8.88	2.40	11.51	5.72
Tissues	3.09	0.67	2.30	0.09	3.33	0.49
Final Cage Wash	0.88	0.55	0.97	0.26	1.01	0.77
CO <sub>2</sub> , Charcoal	0.02	0.01	0.02	0.02	0.03	0.01
Mean Recovery	107.32	2.46	98.78	2.32	101.09	1.91

	Single Dermal Application of 480 mg AEEA/kg Non-pregnant females	
	Mean	SD
Urine & Rinse	3.04	3.54
Feces	0.34	0.77
Tissues	3.01	5.93
Skin, remote from application site	7.91	9.32
Skin, at application site	4.89	2.12
Final Cage Wash	2.05	1.57
CO <sub>2</sub> , Charcoal	NS	NS
Mean Unabsorbed <sup>1</sup>	69.73	11.69
Mean Recovery	90.97	7.93

	Mean	SD
Mean Absorbed (excluding remote-site skin) <sup>2</sup>	7.73	1.56

SD = Standard Deviation

NS = No Sample

<sup>1</sup>Values represent mean and SD (N=3) in each Oral group and (N=8) in Dermal group

<sup>2</sup>Includes covering, frame, skin wash and tape stripping

<sup>3</sup>This value only represents animals 03A2444, 03A2445, 03A2447 and 03A2448

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Table 4. Time-Course of Radioactivity in Plasma Following a Single Oral Dose of 0.5 or 50 mg <sup>14</sup>C-AEEA/kg bw

Time(hour)	0.5 mg/kg Non-pregnant						50 mg/kg Non-pregnant						50 mg/kg Pregnant						
	03A2449		03A2450		03A2451		0A2452		0A2453		03A2454		03A2455		03A2456		03A2457		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
0.25	0.003	0.137	0.152	0.097	0.0817	0.097	0.0817	13.574	8.814	8.814	2.718	8.368	5.4416	13.620	14.144	15.280	14.348	0.8485	
0.5	0.227	0.183	0.187	0.199	0.0243	0.199	0.0243	19.479	13.128	7.966	13.524	5.7672	16.704	16.501	19.799	17.668	17.668	1.8480	
1	0.164	0.161	0.195	0.173	0.0192	0.173	0.0192	15.732	12.638	9.123	12.498	3.3065	17.831	14.397	15.648	15.958	15.958	1.7380	
2	0.125	0.125	0.133	0.128	0.0047	0.128	0.0047	9.666	10.486	12.179	10.777	1.2812	11.628	9.313	10.444	10.461	10.461	1.1576	
4	0.081	0.078	0.083	0.081	0.0024	0.081	0.0024	5.495	6.676	7.554	6.575	1.0333	7.070	6.693	7.262	7.008	7.008	0.2892	
6	0.061	0.060	0.071	0.064	0.0058	0.064	0.0058	3.907	4.538	4.976	4.474	0.5375	5.371	5.381	6.566	5.773	5.773	0.6871	
8	0.049	0.047	0.059	0.051	0.0068	0.051	0.0068	2.771	3.533	3.881	3.395	0.5679	4.523	4.304	5.625	4.817	4.817	0.7078	
12	0.033	0.031	0.041	0.035	0.0053	0.035	0.0053	1.911	2.471	NS	2.191	0.3962	3.406	3.266	3.813	3.495	3.495	0.2841	
24	0.022	0.016	0.022	0.020	0.0036	0.020	0.0036	1.090	1.230	NS	1.160	0.0989	NS	NS	1.736	2.250	1.993	1.993	0.3633
36	0.012	0.010	0.013	0.012	0.0012	0.012	0.0012	0.660	0.825	NS	0.743	0.1167	NS	NS	1.114	1.370	1.242	1.242	0.1809
48	0.010	0.008	0.013	0.010	0.0022	0.010	0.0022	0.598	0.700	0.694	0.664	0.0575	0.766	0.904	1.018	0.896	0.896	0.1259	

NS = No Sample

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Table 5. Time-Course of Radioactivity in Plasma Following a Single Dermal Application of 480 mg <sup>14</sup>C-AEEA/kg bw

Time(hr)	µg-eq <sup>14</sup> C-AEEA/g Plasma										Mean	SD
	03A2441	03A2442	03A2445	03A2446	03A2443	03A2444	03A2447	03A2448	03A2449	03A2450		
0.5	NQ(0.576)	35.630	NQ(0.688)	NQ(0.690)	NS	NQ(0.972)	NS	NS	NS	NS	NQ(7.711)	15.608
1	NQ(0.594)	36.692	NQ(1.823)	NQ(0.551)	NS	0.756	NS	NS	NS	NS	NQ(8.083)	16.001
2	NQ(0.913)	2.986	NQ(0.706)	NQ(0.730)	NS	NQ(0.780)	NS	NS	NS	NS	NQ(1.223)	0.989
4	NQ(1.144)	4.953	NQ(0.997)	NQ(1.053)	NS	NQ(0.963)	NS	NS	NS	NS	NQ(1.822)	1.752
6	55.406	8.885	1.011	NQ(0.720)	NS	NQ(0.759)	NS	NS	NS	NS	13.356	23.764
8	10.148	4.613	NQ(0.383)	NQ(0.386)	NS	NQ(0.734)	NS	NS	NS	NS	NQ(3.253)	4.248
24	NS	NS	NS	NS	0.968	NQ(0.276)	NQ(0.301)	NQ(0.552)	NQ(0.524)	NQ(0.524)	NQ(0.524)	0.321

SD = Standard Deviation  
 NQ = Non-Quantifiable (limit of quantitation)  
 NS = No Sample

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Table 6. Summary of Pharmacokinetic Parameters

Experimental group	Plasma AUC (µg-hr/ml)		Plasma absorption		Plasma elimination		Urine t <sub>1/2</sub> (hr) <sup>d</sup>
	(0-24 hr)	(0-5 hr)	t <sub>1/2</sub> (hr) <sup>a</sup>	t <sub>1/2α</sub> (hr) <sup>b</sup>	t <sub>1/2β</sub> (hr) <sup>c</sup>	t <sub>1/2</sub> (hr) <sup>e</sup>	
0.5 mg/kg oral, non-pregnant	1.3 ± 0.1	0.1	0.1 ± 0.1	1.8 ± 0.1	17.3 ± 0.7	6.0 ± 0.3	
50 mg/kg oral, non-pregnant	95.0 ± 12.9	12.9	0.2 ± 0.1	1.8 ± 0.1	17.1 <sup>e</sup> ± 0.9	6.1 ± 0.5	
50 mg/kg oral, pregnant	117.1 ± 9.7	9.7	0.2 ± 0.1	1.6 ± 0.3	16.7 ± 0.7	7.9 ± 0.3	
480 mg/kg dermal, non-pregnant	13.7 ± 0.8 <sup>f</sup>	0.8 <sup>f</sup>	na	na	na	5.7 <sup>g</sup>	

<sup>a</sup> calculated from 0-5 hr values

<sup>b</sup> calculated from 1-6 hr values

<sup>c</sup> calculated from 8-48 hr values

<sup>d</sup> calculated from 0-48 hr values

<sup>e</sup> n=2

<sup>f</sup> calculated from actual and quantitation limit values of animals 03A2444, 03A2445, 03A2446

<sup>g</sup> calculated from mean 8-12 and 12-24 hr values

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Table 7. Excretion of Radioactivity in Urine of Rats Following a Single Oral Dose of 0.5 or 50 mg <sup>14</sup>C-AEEA/kg bw

Time (hr)	Percentage of Administered Dose					
	Single Oral Dose of 0.50 mg AEEA/kg bw Non-pregnant females		Single Oral Dose of 50 mg AEEA/kg bw Non-pregnant females		Single Oral Dose of 50 mg AEEA/kg bw Pregnant females	
	Mean	SD	Mean	SD	Mean	SD
0-12	81.76	3.85	72.47	3.54	41.41 *	34.70
12-24	12.22	0.75	10.38	0.32	36.72	29.45
24-36	2.94	0.48	2.14	0.58	1.83	1.67
36-48	1.26	0.11	1.63	0.40	5.25	3.43

SD = Standard Deviation

Values represent mean and SD (N=3) in each Oral group.

\* One rat did not excrete urine at the 0-12 hr time point. A zero value was used for that animal.

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Table 8. Excretion of Radioactivity in Urine of Rat Following a Single Dermal Application of 480 mg <sup>14</sup>C-AEEA/kg bw

Time (hr)	Percentage of Administered Dose	
	Mean	SD
0-8 <sup>1</sup>	2.15	2.70
8-12 <sup>2</sup>	1.31	1.44
12-24 <sup>2</sup>	0.47	0.26

SD = Standard Deviation

<sup>1</sup>Values represent mean and SD (N=8)

<sup>2</sup>Values represent mean and SD (N=4)

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Table 9. Excretion of Radioactivity in Feces of Rat Following a Single Oral Dose of 0.5 or 50 mg <sup>14</sup>C-AEEA/kg bw

Time (hr)	Percentage of Administered Dose					
	Single Oral Dose of 0.50 mg AEEA/kg bw Non-pregnant females		Single Oral Dose of 50 mg AEEA/kg bw Non-pregnant females		Single Oral Dose of 50 mg AEEA/kg bw Pregnant females	
	Mean	SD	Mean	SD	Mean	SD
0-24	4.42	0.99	8.36	2.26	10.25	5.34
24-48	0.74	0.12	0.52	0.14	1.26	0.39

SD = Standard Deviation  
 Values represent mean and SD (N=3) in each Oral group.

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Table 10. Excretion of Radioactivity in Feces of Rat Following a Single Dermal Application of 480 mg <sup>14</sup>C-AEEA/kg bw

Time (hr)	Percentage of Administered Dose	
	Mean	SD
0-8 <sup>1</sup>	0.18	0.37
8-24 <sup>2</sup>	0.33	0.55

SD = Standard Deviation

NQ = Non-Quantifiable

<sup>1</sup>Values represent mean and SD (N=8)

<sup>2</sup>Values represent mean and SD (N=4)

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Table 11. Disposition of Radioactivity in Tissues of Rat Following a Single Oral Dose of 0.5 or 50 mg <sup>14</sup>C-AEEA/kg bw

Tissue	Percentage of Administered Dose					
	Single Oral Dose of 0.50 mg AEEA/kg bw		Single Oral Dose of 50 mg AEEA/kg bw		Single Oral Dose of 50 mg AEEA/kg bw	
	Non-pregnant females Mean	SD	Non-pregnant females Mean	SD	Pregnant females Mean	SD
Carcass	1.68	0.39	1.19	0.07	2.20	0.34
Kidneys	0.29	0.18	0.14	0.06	0.13	0.01
Liver	0.47	0.07	0.33	0.02	0.58	0.09
Skin	0.65	0.29	0.64	0.15	0.42	0.07
Total Tissues	3.09	0.67	2.30	0.09	3.33	0.49

Tissue	µg-eq AEEA/g					
	Single Oral Dose of 0.50 mg AEEA/kg bw		Single Oral Dose of 50 mg AEEA/kg bw		Single Oral Dose of 50 mg AEEA/kg bw	
	Non-pregnant females Mean	SD	Non-pregnant females Mean	SD	Pregnant females Mean	SD
Carcass	0.012	0.003	0.834	0.026	1.399	0.192
Kidneys	0.204	0.117	9.793	4.917	12.139	0.388
Liver	0.057	0.009	4.140	0.425	8.030	1.585
Skin	0.015	0.007	1.395	0.340	1.078	0.232

SD = Standard Deviation  
 Values represent mean and SD (N=3) in each Oral group.

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Table 12. Disposition of Radioactivity in Tissues of Rat Following a Single Dermal  
Administration of 480 mg <sup>14</sup>C-AEEA/kg bw

Percentage of Administered Dose

Single Dermal Application of 480 mg AEEA/kg bw  
Non-pregnant females

Tissue	Mean	SD
Carcass	2.76	3.88
Kidneys	0.10	0.16
Liver	0.15	0.24
Skin	7.91	9.32
Skin, Application-site	4.89	2.12
Total Tissues	15.81	13.77

µg-eq AEEA/g tissue

Single Dermal Application of 480 mg AEEA/kg  
Non-pregnant females

Tissue	Mean	SD
Carcass	18.807	26.847
Kidneys	60.018	97.689
Liver	18.635	30.715
Skin	175.022	202.631
Skin, Application-site	1539.587	883.295

SD = Standard Deviation

Values are mean and SD of 8 animals in Dermal group

AMINOETHYLETHANOLAMINE: DERMAL AND ORAL ABSORPTION AND LIMITED PHARMACOKINETIC STUDY IN WISTAR RATS

Appendix Table 1. Body Weight and Amount of Dose Solution, Radioactivity, and AEEA Administered to Wistar Rats

	Animal ID	kg Body Weight	g Dose Solution Administered	mg AEEA administered or applied	mg AEEA administered or applied/kg bw	µCi AEEA administered or applied	µCi AEEA administered or applied/kg bw
Oral dose 0.5 mg/kg bw Non-pregnant	03A2449	0.270	0.6766	0.14	0.54	20.37	75.4
	03A2450	0.278	0.7016	0.15	0.54	21.12	76.0
	03A2451	0.269	0.6950	0.15	0.55	20.92	77.8
	Mean ± SD	0.272 ± 0.005	0.6911 ± 0.0130	0.15 ± 0.01	0.54 ± 0.01	20.80 ± 0.39	76.40 ± 1.22
Oral dose 50 mg/kg bw Non-pregnant	03A2452	0.264	0.6606	13.28	50.31	19.49	73.8
	03A2453	0.279	0.7130	14.33	51.38	21.03	75.4
	03A2454	0.263	0.6653	13.38	50.86	19.63	74.6
	Mean ± SD	0.269 ± 0.009	0.6796 ± 0.0290	13.66 ± 0.58	50.85 ± 0.54	20.05 ± 0.85	74.61 ± 0.78
Pregnant	03A2455	0.381	0.9486	19.07	50.06	27.98	73.4
	03A2456	0.397	0.9856	19.82	49.91	29.08	73.2
	03A2457	0.336	0.8663	17.42	51.84	25.56	76.1
	Mean ± SD	0.371 ± 0.032	0.9335 ± 0.0611	18.77 ± 1.23	50.60 ± 1.07	27.54 ± 1.80	74.25 ± 1.58
Dermal application 480 mg/kg Non-pregnant	03A2441	0.265	0.3604	128.79	486.45	21.91	82.7
	03A2442	0.271	0.3435	122.75	451.74	20.88	77.0
	03A2443	0.249	0.3049	108.96	437.55	18.54	74.5
	03A2444	0.252	0.3122	111.56	442.78	18.98	75.3
	03A2445	0.267	0.3513	125.54	470.77	21.36	80.0
	03A2446	0.272	0.3499	125.04	458.99	21.27	78.2
	03A2447	0.253	0.3361	119.92	473.99	20.40	80.6
	03A2448	0.253	0.3619	128.11	506.37	21.80	86.2
Mean ± SD	0.260 ± 0.009	0.3400 ± 0.0212	121.33 ± 7.42	466.08 ± 23.10	20.64 ± 1.26	79.31 ± 3.90	

SD = Standard Deviation

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Appendix Table 2. Distribution of Radioactivity after Oral Administration of AEEA to  
Wistar Rats

Ear Tag #		Percentage of Applied Radioactivity			Mean	SD
		Oral 0.5 mg/kg bw (non-pregnant females)				
		03A2449	03A2450	03A2451		
Carcass		1.35	1.59	2.11	1.68	0.39
Kidneys		0.49	0.16	0.21	0.29	0.18
Liver		0.43	0.42	0.55	0.47	0.07
Skin		0.57	0.41	0.98	0.65	0.29
Total Tissues		2.84	2.58	3.85	3.09	0.67
Plasma	0.25 hr	0.01	0.01	0.01	0.01	0.00
	0.50 hr	0.02	0.01	0.01	0.01	0.00
	1 hr	0.01	0.01	0.01	0.01	0.00
	2 hr	0.01	0.01	0.01	0.01	0.00
	4 hr	0.00	0.00	0.01	0.01	0.00
	6 hr	0.00	0.00	0.01	0.00	0.00
	8 hr	0.00	0.00	0.00	0.00	0.00
	12 hr	0.00	0.00	0.00	0.00	0.00
	24 hr	0.00	0.00	0.00	0.00	0.00
	36 hr	0.00	0.00	0.00	0.00	0.00
	48 hr	0.00	0.00	0.00	0.00	0.00
Urine	0-12 hr	76.48	77.52	68.49	74.16	4.94
	12-24 hr	10.48	10.94	12.15	11.19	0.86
	24-36 hr	2.51	2.38	3.22	2.70	0.45
	36-48 hr	1.13	1.06	1.28	1.16	0.11
Rinse	0-12 hr	7.15	6.79	8.85	7.60	1.10
	12-24 hr	1.43	0.73	0.92	1.03	0.36
	24-36 hr	0.19	0.24	0.27	0.23	0.04
	36-48 hr	0.07	0.13	0.12	0.10	0.03
Total Urine, Rinse		99.45	99.80	95.29	98.18	2.51
Feces	0-24 hr	3.38	5.36	4.52	4.42	0.99
	24-48 hr	0.83	0.61	0.77	0.74	0.12
Total Feces		4.21	5.97	5.29	5.16	0.89
Final Cage Wash		0.57	1.52	0.54	0.88	0.55
Charcoal Trap	0-24 hr	NQ	NQ	NQ	0.00	0.00
	24-48 hr	NS	NS	NS	NS	--
CO <sub>2</sub>	0-12 hr	0.02	0.02	0.01	0.02	0.01
	12-24 hr	NQ	NQ	NQ	0.00	0.00
	24-36 hr	NS	NS	NS	NS	--
	36-48 hr	NS	NS	NS	NS	--
Total CO <sub>2</sub> , charcoal trap		0.02	0.02	0.01	0.02	0.01
Total Recovery		107.10	109.88	104.98	107.32	2.46

SD = Standard Deviation

NQ = Non-Quantifiable

NS = No Sample

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Appendix Table 2. Distribution of Radioactivity after Oral Administration of AEEA to Wistar Rats  
(continued)

Ear Tag #		Percentage of Applied Radioactivity			Mean	SD
		Oral 50 mg/kg bw (non-pregnant females)				
		03A2452	03A2453	03A2454		
Carcass		1.27	1.13	1.17	1.19	0.07
Kidneys		0.12	0.08	0.21	0.14	0.06
Liver		0.33	0.35	0.30	0.33	0.02
Skin		0.59	0.81	0.52	0.64	0.15
Total Tissues		2.32	2.38	2.20	2.30	0.09
Plasma	0.25 hr	0.01	0.00	0.00	0.01	0.00
	0.50 hr	0.02	0.01	0.01	0.01	0.01
	1 hr	0.01	0.01	0.01	0.01	0.00
	2 hr	0.01	0.01	0.01	0.01	0.00
	4 hr	0.00	0.01	0.01	0.01	0.00
	6 hr	0.00	0.00	0.00	0.00	0.00
	8 hr	0.00	0.00	0.00	0.00	0.00
	12 hr	0.00	0.00	NS	0.00	--
	24 hr	0.00	0.00	NS	0.00	--
	36 hr	0.00	0.00	NS	0.00	--
	48 hr	0.00	0.00	0.00	0.00	0.00
Urine	0-12 hr	66.50	70.87	70.82	69.39	2.51
	12-24 hr	9.23	9.62	8.49	9.11	0.57
	24-36 hr	1.85	1.22	1.97	1.68	0.40
	36-48 hr	1.82	1.35	0.94	1.37	0.44
Rinse	0-12 hr	1.91	4.04	3.26	3.07	1.08
	12-24 hr	1.49	0.71	1.60	1.27	0.49
	24-36 hr	0.57	0.24	0.55	0.45	0.18
	36-48 hr	0.26	0.13	0.39	0.26	0.13
Total Urine, Rinse		83.64	88.18	88.01	86.61	2.58
Feces	0-24 hr	9.73	5.75	9.59	8.36	2.26
	24-48 hr	0.65	0.36	0.55	0.52	0.14
Total Feces		10.38	6.11	10.14	8.88	2.40
Final Cage Wash		0.68	1.19	1.05	0.97	0.26
Charcoal Trap	0-24 hr	NQ	NQ	NQ	0.00	0.00
	24-48 hr	NS	NS	NS	NS	--
CO <sub>2</sub>	0-12 hr	NQ	0.02	NQ	0.01	0.01
	12-24 hr	NQ	0.03	0.00	0.01	0.01
	24-36 hr	NS	NS	NS	NS	--
	36-48 hr	NS	NS	NS	NS	--
Total CO <sub>2</sub> , charcoal trap		0.00	0.04	0.00	0.02	0.02
Total Recovery		97.02	97.91	101.40	98.78	2.32

SD = Standard Deviation

NQ = Non-Quantifiable

NS = No Sample

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 PHARMACOKINETIC STUDY IN WISTAR RATS

Appendix Table 2. Distribution of Radioactivity after Oral Administration of AEEA to Wistar Rats  
 (continued)

Ear Tag #		Percentage of Applied Radioactivity				
		Oral 50 mg/kg bw (pregnant females)			Mean	SD
		03A2455	03A2456	03A2457		
Carcass		1.82	2.29	2.49	2.20	0.34
Kidneys		0.13	0.12	0.12	0.13	0.01
Liver		0.49	0.58	0.67	0.58	0.09
Skin		0.34	0.46	0.47	0.42	0.07
Total Tissues		2.79	3.45	3.76	3.33	0.49
Plasma	0.25 hr	0.00	0.01	0.01	0.01	0.00
	0.50 hr	0.01	0.01	0.01	0.01	0.00
	1 hr	0.01	0.01	0.00	0.01	0.00
	2 hr	0.00	0.00	0.01	0.00	0.00
	4 hr	0.00	0.00	0.00	0.00	0.00
	6 hr	0.00	0.00	0.00	0.00	0.00
	8 hr	0.00	0.00	0.00	0.00	0.00
	12 hr	0.00	0.00	0.00	0.00	0.00
	24 hr	NS	0.00	0.00	0.00	--
	36 hr	NS	0.00	0.00	0.00	--
	48 hr	0.00	0.00	0.00	0.00	0.00
Urine	0-12 hr	0.00	51.95	53.76	35.24	30.53
	12-24 hr	69.87	19.46	17.58	35.63	29.66
	24-36 hr	0.04	2.97	1.81	1.61	1.48
	36-48 hr	9.01	2.44	3.61	5.02	3.50
Rinse	0-12 hr	1.91	3.35	13.24	6.17	6.17
	12-24 hr	0.84	1.38	1.05	1.09	0.27
	24-36 hr	0.04	0.44	0.19	0.22	0.20
	36-48 hr	0.12	0.16	0.40	0.23	0.16
Total Urine, Rinse		81.83	82.15	91.65	85.21	5.58
Feces	0-24 hr	12.50	14.09	4.15	10.25	5.34
	24-48 hr	1.39	1.57	0.83	1.26	0.39
Total Feces		13.89	15.66	4.98	11.51	5.72
Final Cage Wash		0.36	0.81	1.86	1.01	0.77
Charcoal Trap	0-24 hr	NQ	0.00	NQ	0.00	0.00
	24-48 hr	NS	NS	NS	NS	--
CO <sub>2</sub>	0-12 hr	0.01	0.02	0.01	0.01	0.01
	12-24 hr	0.01	0.01	0.02	0.01	0.01
	24-36 hr	NS	NS	NS	NS	--
	36-48 hr	NS	NS	NS	NS	--
Total CO <sub>2</sub> , charcoal trap		0.02	0.03	0.03	0.03	0.01
Total Recovery		98.88	102.11	102.27	101.09	1.91

SD = Standard Deviation  
 NQ = Non-Quantifiable  
 NS = No Sample

AMINOETHYLETHANOLAMINE: DERMAL AND ORAL ABSORPTION AND LIMITED  
PHARMACOKINETIC STUDY IN WISTAR RATS

Appendix Table 3. Distribution of Radioactivity after Dermal Administration of AEEA  
to Wistar Rats

Ear Tag #	Dermal 480 mg/kg (non-pregnant females)								Mean	SD	
	03A2441	03A2442	03A2445	03A2446	03A2443	03A2444	03A2447	03A2448			
Carcass	10.98	4.96	0.45	4.50	0.54	NQ (0.00)	0.42	0.23	2.76	3.88	
Kidneys	0.33	0.38	0.01	0.00	0.04	0.01	0.02	0.01	0.10	0.16	
Liver	0.57	0.51	NQ (0.00)	NQ (0.00)	0.06	0.01	0.03	0.01	0.15	0.24	
Skin <sup>1</sup>	17.92	1.01	1.65	26.45	7.12	1.21	3.34	4.58	7.91	9.32	
Skin, application-site	9.43	3.27	4.05	5.24	3.98	2.57	5.90	4.66	4.89	2.12	
Total Tissues	39.23	10.12	6.16	36.20	11.73	3.81	9.72	9.49	15.81	13.77	
Total Tissues w/o remote-site skin	21.31	9.11	4.50	9.75	4.62	2.60	6.38	4.91	7.90	5.93	
Plasma	0.50 hr	NQ (0.00)	0.00	NQ (0.00)	NQ (0.00)	NS	NQ (0.00)	NS	NS	NQ (0.00)	0.00
	1 hr	NQ (0.00)	0.00	NQ (0.00)	NQ (0.00)	NS	0.00	NS	NS	NQ (0.00)	0.00
	2 hr	NQ (0.00)	0.00	NQ (0.00)	NQ (0.00)	NS	NQ (0.00)	NS	NS	NQ (0.00)	0.00
	4 hr	NQ (0.00)	0.00	NQ (0.00)	NQ (0.00)	NS	NQ (0.00)	NS	NS	NQ (0.00)	0.00
	6 hr	0.00	0.00	0.00	NQ (0.00)	NS	NQ (0.00)	NS	NS	0.00	0.00
	8 hr	0.00	0.00	NQ (0.00)	NQ (0.00)	NS	NQ (0.00)	NS	NS	NQ (0.00)	0.00
	24 hr	NS	NS	NS	NS	0.00	NQ (0.00)	NQ (0.00)	NQ (0.00)	NQ (0.00)	0.00
Urine	0-8 hr	0.03	6.48	0.05	0.04	5.08	NQ	0.30	0.01	1.50	2.67
	8-12 hr	NS	NS	NS	NS	2.55	0.49	0.84	0.13	1.01	1.07
	12-24 hr	NS	NS	NS	NS	0.49	0.25	0.65	0.14	0.38	0.23
Rinse	0-8 hr	0.87	0.46	0.88	NQ	0.69	2.23	0.06	0.01	0.65	0.69
	8-12 hr	NS	NS	NS	NS	0.87	0.27	0.06	0.02	0.31	0.39
	12-24 hr	NS	NS	NS	NS	0.15	0.08	0.09	0.03	0.09	0.05
Total Urine, Rinse		0.91	6.94	0.93	0.04	9.83	3.33	2.01	0.35	3.04	3.54
Feces	0-8 hr	0.14	NQ	NQ	NQ	1.06	0.25	NQ	NQ	0.18	0.37
	8-24 hr	NS	NS	NS	NS	1.16	0.11	0.04	NQ	0.33	0.55
Total Feces		0.14	0.00	0.00	0.00	2.22	0.36	0.04	0.00	0.34	0.77
Final Cage Wash		4.44	0.82	3.30	3.44	2.15	1.71	0.31	0.19	2.05	1.57
Covering <sup>2</sup>		12.77	28.44	20.58	5.66	8.98	27.31	35.86	36.66	22.03	11.96
Frame		6.16	5.17	3.74	7.40	4.38	4.79	4.88	5.26	5.22	1.12
Skin wash <sup>3</sup>		35.48	35.21	44.26	36.81	65.44	45.54	38.54	37.83	42.39	10.08
Tape Stripping		0.22	0.11	0.07	0.15	0.04	0.01	0.03	0.03	0.08	0.07
Total Unabsorbed		54.63	68.94	68.65	50.01	78.83	77.64	79.32	79.78	69.73	11.69
Total Recovery		99.35	86.82	79.04	89.69	104.77	86.86	91.39	89.81	90.97	7.93
Percent Absorbed <sup>4</sup>		26.80	16.87	8.73	13.23	18.82	8.00	8.74	5.45	7.73	1.56

<sup>1</sup> Skin remote from application site

<sup>2</sup> Covering and Jacket with dermal insert

<sup>3</sup> Skin Wash at 8-hrs post-application

<sup>4</sup> Percentage absorbed based on radioactivity found in tissues, application-site skin, feces, urine, rinse, final cage wash (FCW)

Note: bolded values only used in calculation of mean percent absorbed value.

SD = Standard Deviation

NQ = Non-Quantifiable

NS = No Sample

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 PHARMACOKINETIC STUDY IN WISTAR RATS

Appendix Table 4. Distribution of Radioactivity after Oral Administration of AEEA to  
 Wistar Rats

µg-eq AEEA/g Tissue					
Ear Tag #	Oral 0.5 mg/kg bw (non-pregnant females)			Mean	SD
	03A2449	03A2450	03A2451		
Carcass	0.010	0.011	0.016	0.012	0.003
Kidneys	0.338	0.119	0.156	0.204	0.117
Liver	0.053	0.052	0.067	0.057	0.009
Skin	0.012	0.010	0.023	0.015	0.007
Ear Tag #	Oral 50 mg/kg bw (non-pregnant females)			Mean	SD
	03A2452	03A2453	03A2454		
Carcass	0.858	0.806	0.838	0.834	0.026
Kidneys	7.836	6.156	15.388	9.793	4.917
Liver	3.702	4.551	4.166	4.140	0.425
Skin	1.266	1.781	1.139	1.395	0.340
Ear Tag #	Oral 50 mg/kg bw (pregnant females)			Mean	SD
	03A2452	03A2453	03A2454		
Carcass	1.178	1.529	1.489	1.399	0.192
Kidneys	11.737	12.169	12.512	12.139	0.388
Liver	6.203	9.041	8.846	8.030	1.585
Skin	0.819	1.146	1.269	1.078	0.232

SD = Standard Deviation

AMINOETHYLETHANOLAMINE: DERMAL AND ORAL ABSORPTION AND LIMITED PHARMACOKINETIC STUDY IN WISTAR RATS

Appendix Table 5. Distribution of Radioactivity after Dermal Administration of AEEA to Wistar Rats

Ear Tag #	Dermal 480 mg/kg (non-pregnant females)										Mean	SD
	03A2441	03A2442	03A2443	03A2444	03A2445	03A2446	03A2447	03A2448	03A2449	03A2450		
Carcass	76.402	31.748	2.999	NQ (0.006)	3.081	31.620	2.893	1.719	18.807	26.847		
Kidneys	206.628	229.016	16.984	6.275	3.674	2.671	10.327	4.566	60.018	97.689		
Liver	70.532	65.961	6.264	1.829	NQ (0.121)	NQ (0.108)	3.487	1.237	18.635	30.715		
Skin <sup>1</sup>	392.609	22.588	151.106	26.090	41.322	578.199	77.279	110.987	175.022	202.631		
Skin, application-site	3227.399	902.864	1010.211	601.765	1162.494	1568.369	2475.148	1368.449	1539.587	883.295		

µg-eq. AEEA/g

<sup>1</sup> Skin remote from application site.  
 SD = Standard Deviation  
 NQ = Non-Quantifiable

AMINOETHYLETHANOLAMINE: DERMAL AND ORAL ABSORPTION AND LIMITED  
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Appendix A. Analytical Report Number 2003-88

TERC ANALYTICAL CHEMISTRY LABORATORY

Metabolite Quantitation  
ANALYTICAL REPORT NUMBER: 2003-88

Study number:	031053		
Study Title:	Aminoethylethanolamine: Dermal and Oral Absorption and Limited PK Study in Wistar Han Rats		
File number:	K-004409-015	Project #:	60165 Task/rep: 278-1
Project / Sample Description (optional):			
Test material(s):	14C-AEEA		
Test material lot #:	160-121-060,01/0019-2		
Report recipient(s):	Clark, Amy; Bartels, Michael		
Analysis request date:	06/04/2003		
Date submittor prepared samples:			
Number of samples or estimate:	8		
Date samples prepared for analysis:	08/02/2003		
Analysis date:	8-2-03 through 8-6-03		
Number of samples analyzed:	8		
Analysis method:	HPLC with radioactivity flow detection, LC/MSD		
Method reference:	see raw data files		

Results.

Amy Clark submitted urine and plasma samples for strong cation exchange high-performance liquid chromatography analysis (SCX-HPLC) from female rats orally dosed with <sup>14</sup>C-aminoethylethanolamine (AEEA). The pooled urine and plasma samples were stored frozen at -80 °C until analyzed. The urine (0-12 and 12-24 hr) samples were from non-pregnant rats dosed with either 0.5 or 50 mg <sup>14</sup>C-AEEA/kg bw, and pregnant rats dosed at 50 mg <sup>14</sup>C-AEEA/kg. Plasma samples (C<sub>max</sub>, 0.5-hr post-dosing) were from non-pregnant and pregnant rats orally dosed with 50 mg <sup>14</sup>C-AEEA/kg bw.

The preparation of the urine and plasma samples, prior to analysis, is listed beginning on Page 2. The HPLC, LC/MSD systems and conditions are listed on Page 3.

There were 4 radioactive peaks detected in the urines. Figure 1 (page 4) is a radioactive profile of the 0-12 hr pooled urine from pregnant females orally dosed with 50 mg <sup>14</sup>C-AEEA/kg bw and is typical of all the urines analyzed. The radioactivity distribution (as percentage of administered dose) is listed in Table 1 (page 7) for all dose levels and pregnancy status. Peak D accounted for 53, 57, and 31% of the administered dose in the 0-12 hr post-dosing urines for non-pregnant females dosed with 0.5 and 50 mg/kg bw and pregnant females dosed with 50

Analyst SC Hansen 2-4-04

QAU

work conducted in accordance with applicable GLP's. QAU signature required if study run outside of TERC

Approval Amy Clark 2/4/04

AMINOETHYLETHANOLAMINE: DERMAL AND ORAL ABSORPTION AND LIMITED  
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## Appendix A. Analytical Report Number 2003-88 (continued)

mg/kg bw, respectively. Peak D accounted for 5, 6, and 20% of the administered dose in the 12-24 hr post-dosing urines for non-pregnant females dosed with 0.5, and 50 mg AEEA/kg bw and pregnant females dosed with 50 mg/kg bw, respectively. Peak D was the largest peak in all the urine samples and had an HPLC retention-time match with an authentic standard of AEEA.

A typical radiochromatogram of a pooled-plasma sample from pregnant females is presented in Figure 2. The radioactivity distribution (as  $\mu\text{g eq }^{14}\text{C-AEEA/g plasma}$ ) is listed in Table 2 (page 8). Peak D was the only peak detected and had a retention-time match with an authentic standard of AEEA.

Urinary Metabolite/Peak Identification

The pooled urine (0-12 hr) from pregnant females dosed with 50 mg  $^{14}\text{C-AEEA/kg bw}$  and synthetic standards were analyzed via positive-ion electrospray (PESI) LC/MS analysis. The purpose of this analysis was to determine if the parent AEEA, ethylenediamine (EDA), monoethanolamine (MEA) or the analogous three monoacetylated compounds (Ac-AEEA, Ac-EDA, AC-MEA) corresponded to any of the four previously characterized radiolabeled urinary metabolites of AEEA.

A standard solution of N N-acetyethanolamine (N-Ac-AEEA) afforded a positive-ion electrospray mass spectrum containing an  $\text{M}+\text{H}^+$  parent ion at  $m/z$  147 and a fragment ion at 86  $m/z$  (Figure 3a). Metabolite C afforded an LC/MS peak with a comparable retention time and spectrum (Figure 3b). This peak is therefore identified as N-Ac-AEEA.

A standard solution of AEEA afforded a positive-ion electrospray mass spectrum containing an  $\text{M}+\text{H}^+$  parent ion at  $m/z$  105 and a fragment ion at 88  $m/z$  (Figure 4a). Metabolite D afforded an LC/MS peak with a comparable retention time and spectrum (Figure 4b). This peak is therefore identified as AEEA.

Authentic standards of ethanolamine (EA), N-acetyethanolamine (N-Ac-EA), ethylenediamine (EDA), and N-acetyethylenediamine (N-Ac-EDA), were analyzed via positive-ion electrospray LC/MS but did not have a retention-time match with either Peaks A or B (data not shown).

## SAMPLE PREPARATION:

Urine

Pooled urines were removed from the  $-80\text{ }^\circ\text{C}$  freezer and allowed to thaw to laboratory temperatures. The urines were centrifuged at  $\sim 600\text{G}$  for approximately 10 minutes. Twenty  $\mu\text{L}$  of the supernatant was injected on the HPLC system. The HPLC system recovery for a selected urine sample was 93% (data not shown).

The centrifuged vs. un-centrifuged recovery combined with 7-week freezer stability was  $100 \pm 1\%$  (data not shown).

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Appendix A. Analytical Report Number 2003-88 (continued)

Plasma

Pooled C<sub>max</sub> plasma (50 mg/kg, pregnant and non-pregnant) were removed from the -80 °C freezer and allowed to thaw to laboratory temperatures. Fifty µL of glacial acetic acid was added to ~ 1 g of respective plasma samples and vortex mixed for ~2 minutes, then centrifuged at ~600G for ~10 minutes prior to HPLC analysis.

Analysis Conditions

Injection Volume: varied (20-50 µL)

Analytical Pump: HP 1100, s/n US70600796, 1 mL/min flow

HPLC Eluent: 250 mmol ammonium formate, pH ~3.5 with formic acid, 1 mL/min

Analytical Column: Whatman Partisphere, SCX, 4.6 x 235 mm, 5µm, s/n 1SHO2E63

Autosampler: HP 1100, s/n US72101984

Mass Selective Detector: HP 1100, s/n US07070096

Scan 50-350, fragmentor 45, Gain 10, threshold 5, gas temperature 350 °C, drying gas  
9.0 L/min, neb. pressure 30 psi, VCap 4000

Radiochemical Detector Components:

Berthold LB 509 (s/n 126633-1028), 500-µL liquid cell (Z-500-4)

Hitachi pump L6200A (ELC-0358), 1.4 mL/min

Scintillation cocktail: Ultima Flo-M (Packard Bioscience)

Standards

N-(2-aminoethyl) ethanolamine (AEEA) – TAG # 0535 (Lot # 01/0019-2)

N- Acetyl-AEEA – TAG # 0564

N-Acetylenethylenediamine (N-Ac-EDA) – Aldrich, Lot #02101CO

Ethylenediamine (EDA) – Aldrich, Lot #13307MS

N-Acetyethanolamine (N-Ac-EA) ) – Aldrich, Lot #02002HN

Ethanolamine (EA) ) – Aldrich, Lot #09605TO

AMINOETHYLETHANOLAMINE: DERMAL AND ORAL ABSORPTION AND LIMITED  
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Appendix A. Analytical Report Number 2003-88 (continued)

TERC ANALYTICAL CHEMISTRY LABORATORY

Figure 1. Typical 0-12 hr pooled urinary radiochemical profile represented by pregnant female rats dosed with 50 mg  $^{14}\text{C}$ -AEEA/kg bw.

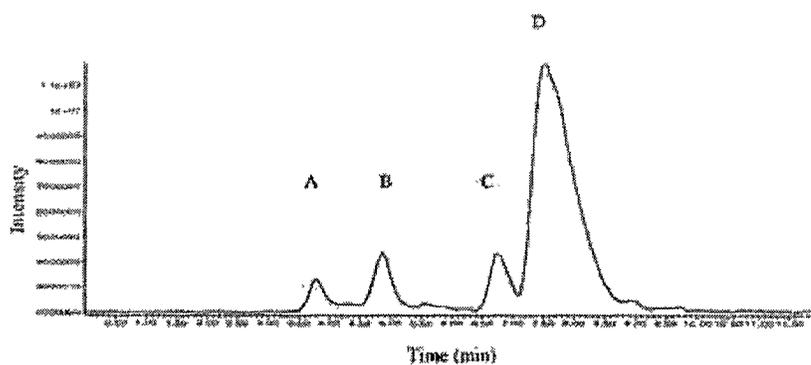
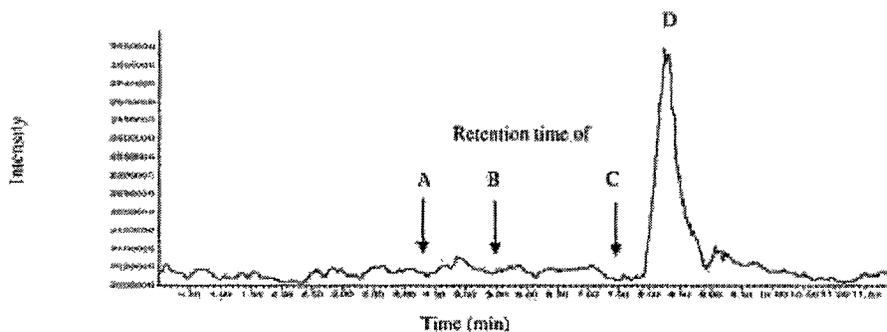


Figure 2. Typical radiochemical profile of  $C_{\text{max}}$  (0.5 hr) plasma from non-pregnant female rats dosed with 50 mg  $^{14}\text{C}$ -AEEA/kg bw.



AMINOETHYLETHANOLAMINE: DERMAL AND ORAL ABSORPTION AND LIMITED  
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Appendix A. Analytical Report Number 2003-88 (continued)

TERC ANALYTICAL CHEMISTRY LABORATORY

Figure 3a. Positive-ion electrospray (PESI) LC/MS analysis of an authentic standard of N-Ac-AEEA standard spiked in control female rat urine.

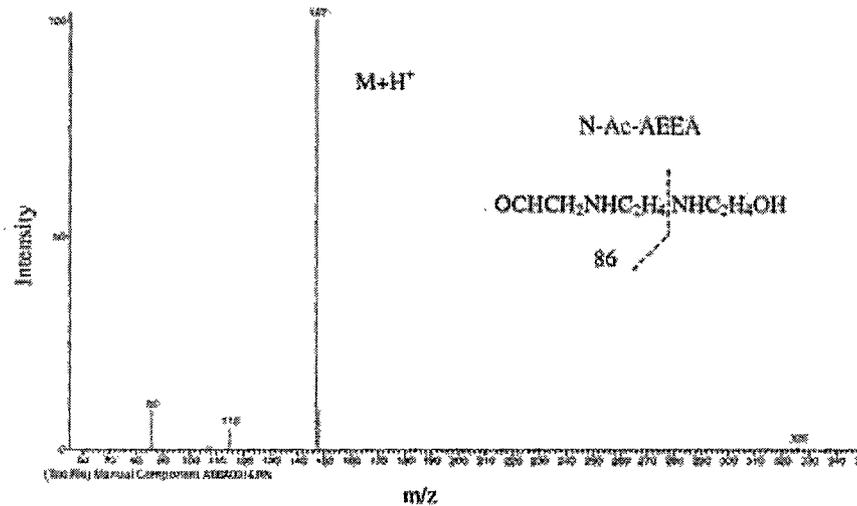
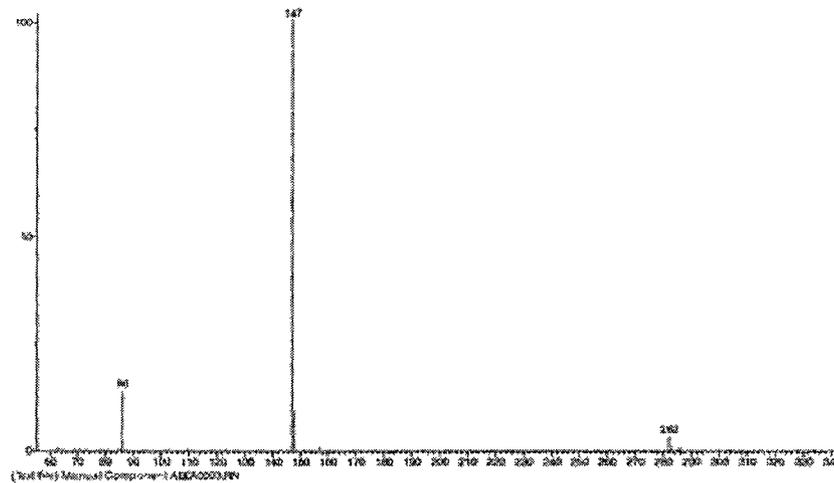


Figure 3b. Positive-ion electrospray (PESI) LC/MS analysis of Peak C from 0-12 hr pooled urine from pregnant females dosed with 50 mg <sup>14</sup>C-AEEA/kg bw.



AMINOETHYLETHANOLAMINE: DERMAL AND ORAL ABSORPTION AND LIMITED  
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Appendix A. Analytical Report Number 2003-88 (continued)

TERC ANALYTICAL CHEMISTRY LABORATORY

Figure 4a. Positive-ion electrospray (PESI) LC/MS analysis of an authentic standard of AEEA standard spiked in control female rat urine.

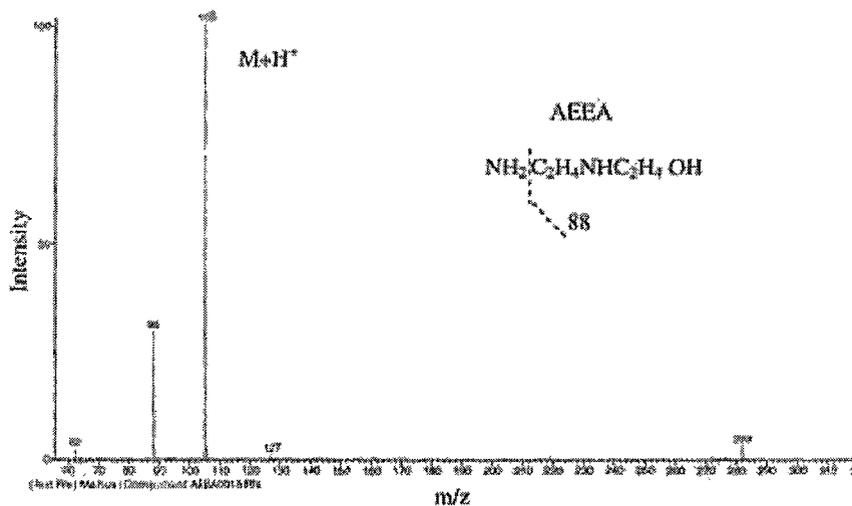
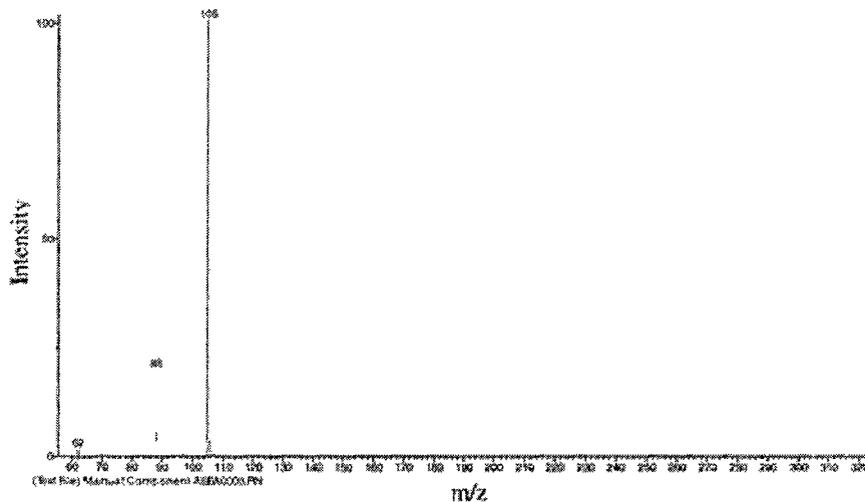


Figure 4b. Positive-ion electrospray (PESI) LC/MS analysis of Peak D from 0-12 hr pooled urine from pregnant females dosed with 50 mg <sup>14</sup>C-AEEA/kg bw.



AMINOETHYLETHANOLAMINE: DERMAL AND ORAL ABSORPTION AND LIMITED  
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Appendix A. Analytical Report Number 2003-88 (continued)

TERC ANALYTICAL CHEMISTRY LABORATORY

Table 1. Urinary metabolite distribution from female rats dosed with either 0.5 or 50 mg <sup>14</sup>C-AEEA/kg bw and pregnant females dosed with 50 mg/kg bw.

time (hr)	Matrix	mg/kg	Status	as Percentage of Administered Dose				Totals
				Metabolite A	Metabolite B	Metabolite C <sup>1</sup>	Metabolite D <sup>2</sup>	
0-12	urine	0.5	non-pregnant	6.77	16.92	5.39	52.95	82.04
12-24 <sup>3</sup>	urine	0.5	non-pregnant	2.27	2.50	2.00	5.48	12.26
24-48 <sup>3</sup>	urine	0.5	non-pregnant	0.54	0.60	0.46	1.31	2.94
48-72 <sup>3</sup>	urine	0.5	non-pregnant	0.23	0.25	0.21	0.56	1.25
			0-72 hr Totals	9.83	20.28	8.07	60.31	98.49
0-12	urine	50	non-pregnant	3.68	8.76	2.74	56.69	71.26
12-24	urine	50	non-pregnant	1.30	1.56	1.38	5.97	10.21
24-48 <sup>1</sup>	urine	50	non-pregnant	0.27	0.33	0.29	1.25	2.14
48-72 <sup>3</sup>	urine	50	non-pregnant	0.21	0.25	0.22	0.95	1.63
			0-72 hr Totals	4.86	10.89	4.62	64.86	85.23
0-12	urine	50	pregnant	1.76	4.27	3.46	51.22	40.72
12-24	urine	50	pregnant	3.93	5.57	6.31	20.29	36.11
24-48 <sup>3</sup>	urine	50	pregnant	0.20	0.28	0.32	1.03	1.83
48-72 <sup>3</sup>	urine	50	pregnant	0.57	0.81	0.92	2.95	5.25
			0-72 hr Totals	6.46	10.94	11.01	55.49	83.91

<sup>1</sup> - Ac-AEEA

<sup>2</sup> - parent AEEA

<sup>3</sup> - The 24-48 and 48-72 hour urines were not analyzed. Radioactivity distributions were based on the percentages of injected activity of the respective 12-24 hr urine and multiplied by the percentage of administered dose in the respective interval.

AMINOETHYLETHANOLAMINE: DERMAL AND ORAL ABSORPTION AND LIMITED  
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Appendix A. Analytical Report Number 2003-88 (continued)

TERC ANALYTICAL CHEMISTRY LABORATORY

Table 2.  $C_{max}$  plasma metabolite distribution from non-pregnant and pregnant females dosed with 50 mg  $^{14}C$ -AEEA/kg.bw.

Time (hr)	Matrix	mg/kg	Status	as $\mu g$ eq $^{14}C$ -AEEA/g plasma			
				Metabolite A	Metabolite B	Metabolite C <sup>1</sup>	Metabolite D <sup>2</sup>
0.5	plasma	50	non-pregnant	ND <sup>3</sup>	ND	ND	13.299
0.5	plasma	50	pregnant	ND <sup>4</sup>	ND	ND	17.374

<sup>1</sup> - N-Ac-AEEA

<sup>2</sup> - parent AEEA

<sup>3</sup> - Not detected at or below 2.955  $\mu g$  eq  $^{14}C$ -AEEA/g plasma

<sup>4</sup> - Not detected at or below 3.861  $\mu g$  eq  $^{14}C$ -AEEA/g plasma