Polycyclic aromatic hydrocarbons (PAHs), including benz[a]pyrene (BP), are environmental pollutants linked to increased disease susceptibilities. Alpha-Tocopherol (α-T) supplementation decreases BP-DNA adducts in smokers, particularly women; but the mechanism is unknown. To test the hypothesis that α-T protection from BP exposure is gender-dependent, male and female rats received 7 daily subcutaneous (SQ) injections of α-T (100 mg α-T/kg body wt) or vehicle, followed by a single injection of BP (20 mg/kg, spiked with N,N′-BP) on day 9. Urine and bile were collected pre- and post-BP. Plasma and tissues were collected 6 or 24 h post-BP. α-T supplementation increased α-T levels in females greater than males. Compared to vehicle, α-T supplementation increased total urinary and biliary excretion of BP and/or BP metabolites more than 2.5-fold in females, but decreased total BP and/or BP metabolite excretion in males (p < 0.05). SQ α-T prevented BP-induced increases in urine 8-isoprostanes (males) and urinary and biliary 8-isoprostanes (males) and decreased tissue malondialdehyde levels in a tissue- and gender-dependent manner. These data are the first to suggest gender-dependent increases in BP-DNA adducts in smokers, but the mechanism is unknown. To test the hypothesis that α-T protection from BP exposure is gender-dependent and occurs by both antioxidant and non-antioxidant mechanisms. Further elucidation of the mechanism(s) of α-T protection against PAHs may lead to the development of novel protective strategies for occupational PAH exposures.

### Introduction

- PAHs are environmental toxins produced by incomplete combustion processes.
- High occupational exposures occur: road paving, roofing, second-hand smoke (bars, casinos), houses.
- High level PAH exposure is linked to increased risk of several cancers: lung, skin, and scrotal cancer.
- Benz[a]pyrene (BP) is often used as a model compound for PAH exposure studies as it is present in almost all PAH mixtures.
- α-T supplementation decreases BP-DNA adduct levels in smokers.
- Effects were greater in women than men (Mooney et al., 2005).
- SQ α-T increases expression of enzymes and transport proteins involved in BP detoxification and excretion (Mustacich et al., 2006).

### Hypothesis

We hypothesized that:

1. Elevated levels of α-T decrease BP-induced damage by two synergistic mechanisms:
   - (1) increased antioxidant protection against oxidative stress-induced damage
   - (2) modulation of BP metabolism and/or excretion.
2. In addition, we hypothesized that this protection would be greater in female rats compared to males.

### Methods & Study Design

**Animal Studies.** Male and female Sprague Dawley rats (n=5-8/gender/treatment) were given 7 daily subcutaneous (SQ) injections of either RRV-α-T (100 mg α-T/kg body wt) or vehicle (vehicle). On day 9, rats received an intraperitoneal injection of BP (20 mg BP/kg body wt, spiked with N,N′-BP) dissolved in tocopherol-stripped corn oil. Urine and bile were collected pre- and post-BP exposure (only collected from 5th rats), 5 or 24 h post BP injections, rats were euthanized with sodium pentobarbital (80 mg/kg) and tissue and blood were collected. Plasma was obtained by centrifugation, and tissue and plasma were stored at -80°C until analysis.

**Measurement of α-T.** Plasma and tissue α-T concentrations were determined by a modification of the method described by Podila et al. (1996) and measured using HPLC with fluorescence detection and quantification by comparison to standard curves generated with authentic compounds.

**Measurement of Total Radiocactivity.** Urine and bile total radiocactivity were measured by liquid scintillation counting (LSC).

**Measurement of 8-IsoPGF2α and Creatinine.** Urine 8-isoPGF2α was extracted using the method described by Taylor et al. (2006), measured by enzyme immunoassay (Cayman Chemical), and normalized to creatinine (Jaffe reaction, Assay Designs).

**Measurement of Malondialdehyde.** Tissue malondialdehyde concentrations were determined by a modification of the Lykkesfeldt (2001) and measured using HPLC fluorescence.

**Measurement of Reduced and Oxidized Glutathione.** Liver reduced and oxidized glutathione concentrations were determined as described by Farris and Reed (1997).

### Conclusions

- **α-T supplementation:**
  - Alters BP exposure outcomes in both antioxidant and non-antioxidant mechanisms
  - Alters BP exposure outcomes in a gender-dependent manner

Elucidation of mechanisms of α-T protection against PAHs may lead to development of novel protective strategies for occupational PAH exposures.