

Toxicity  
of Arsenicals  
Clark Lantz

# *Introduction*

- Arsenic is widespread in the environment
- Occupational exposures can occur
  - Smelting industry
  - Coal fired power plants
- Epidemiological studies implicate arsenic as a carcinogen
- Inhalation is a common route of exposure
- Drinking water exposure can also lead to cancer

# *History*

- 50 ppb ( $\mu\text{g/L}$ ) has been the standard for arsenic in drinking water in US since 1942
- Adopted as an interim standard by US EPA in 1974
- SDWA 1986 required EPA to set a final standard
- In 1996, EPA was required by Congress to set a standard by 2000

# *History*

- Based on existing published data, EPA proposed a MCLG (maximum containment level goal) of 0 ppb and an MCL (maximum containment level of 5 ppb)
  - EPA was required to consider cost/benefit analysis in setting the final rule
- EPA requested comments in summer of 2000
  - Could comment on the MCLG, or on an MCL of either 3, 5, 10 or 20 ppb

# *History*

- Just prior to the change in administration, EPA published the final rule as MCLG=0 ppb and MCL=10 ppb
- The new rule has been suspended by the Bush administration and is currently under re-review

# *What data did EPA use to set the new rule?*

- In 1960s, published data from Taiwan indicated that arsenic in drinking water could cause skin cancer
- These data and additional data collected from this region have been the primary sources of establishing arsenic in drinking water as a cause of skin, lung and bladder cancer
- Data have been supported by data from South American studies

## *What data did EPA use to set the new rule?*

- EPA also did a cost estimate of meeting the new rule standards
- Compared cost with the health benefits that would be obtained by lowering to 10 ppb (20-30 lives/year)
- Balance between cost and benefit, EPA arrived at 10 ppb for MCL. No change in MCLG of 0 ppb.

## *Why all the uproar?*

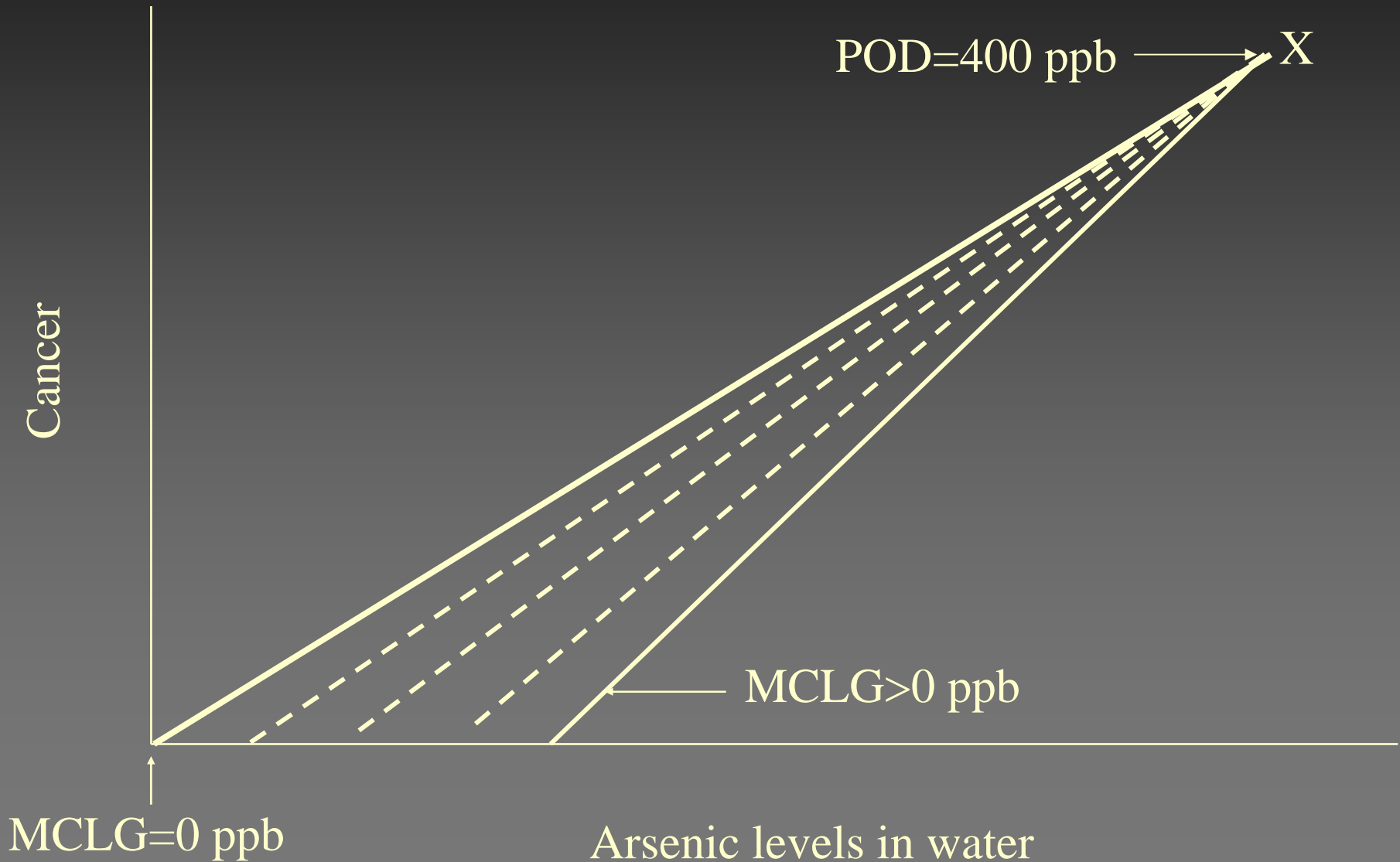
Within the exposure range of 2-20 ppb

- Values are close to background exposure.
- Values are close to toxic events (small safety factor)
- Costs to remediate are greater as the value goes lower

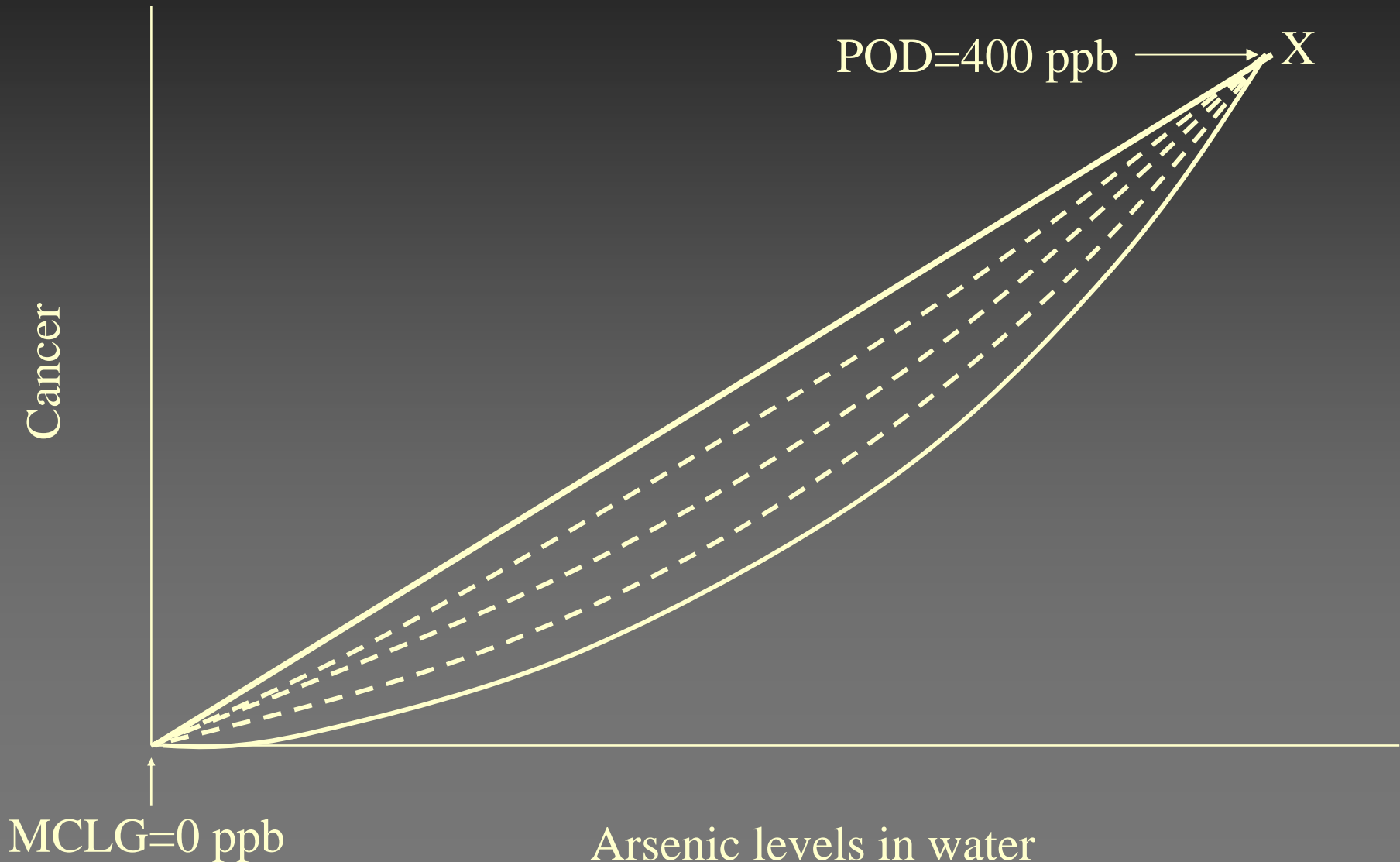
## *Why all the uproar?*

- Data showing cancer in Taiwan population was obtained at high doses (100 - 2000 ppb).
  - Actual analysis showed that exposure above 400 ppb resulted in cancer
  - EPA extrapolated from this point to low doses
- The shape of the dose-response curve may be sublinear
  - EPA assumed a linear dose-response curve extrapolated to zero
  - Large percentage of investigators believe the dose-response curve to be sublinear

# *Setting the MCL (Effect of MCLG)*



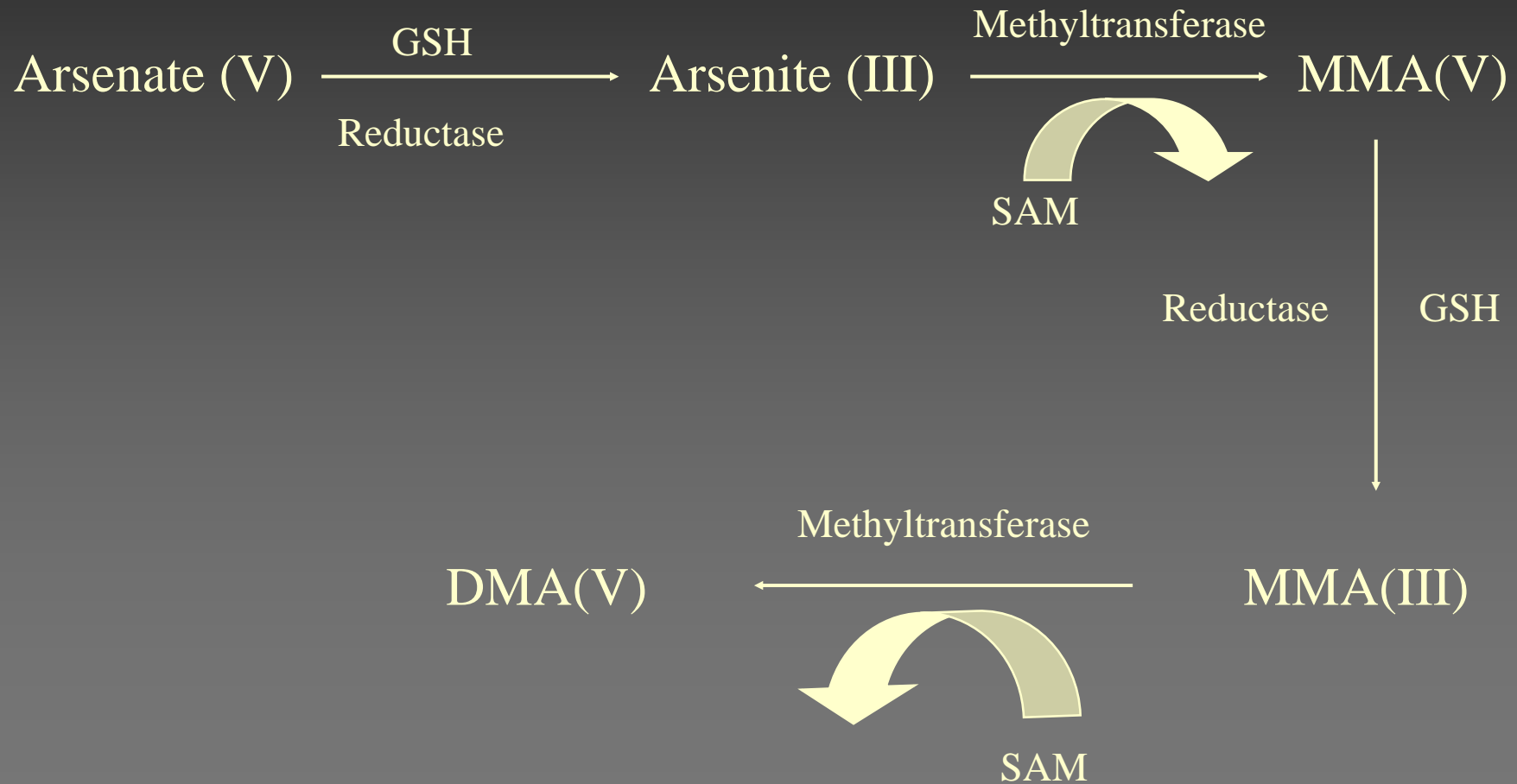
# *Setting the MCL (Sublinear Dose-Response)*



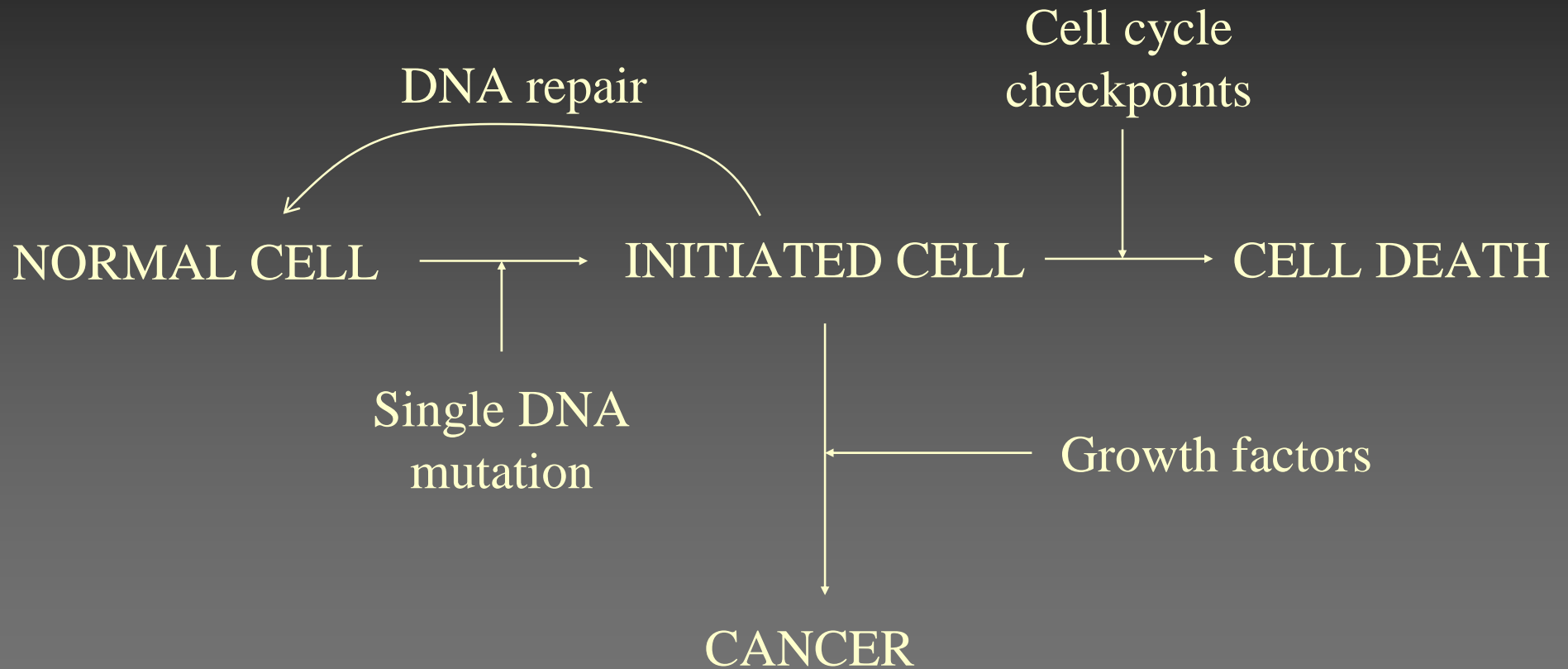
## *Determining the mechanism of action*

- Animal studies have been negative
- Not sure what form of arsenic is the carcinogenic species

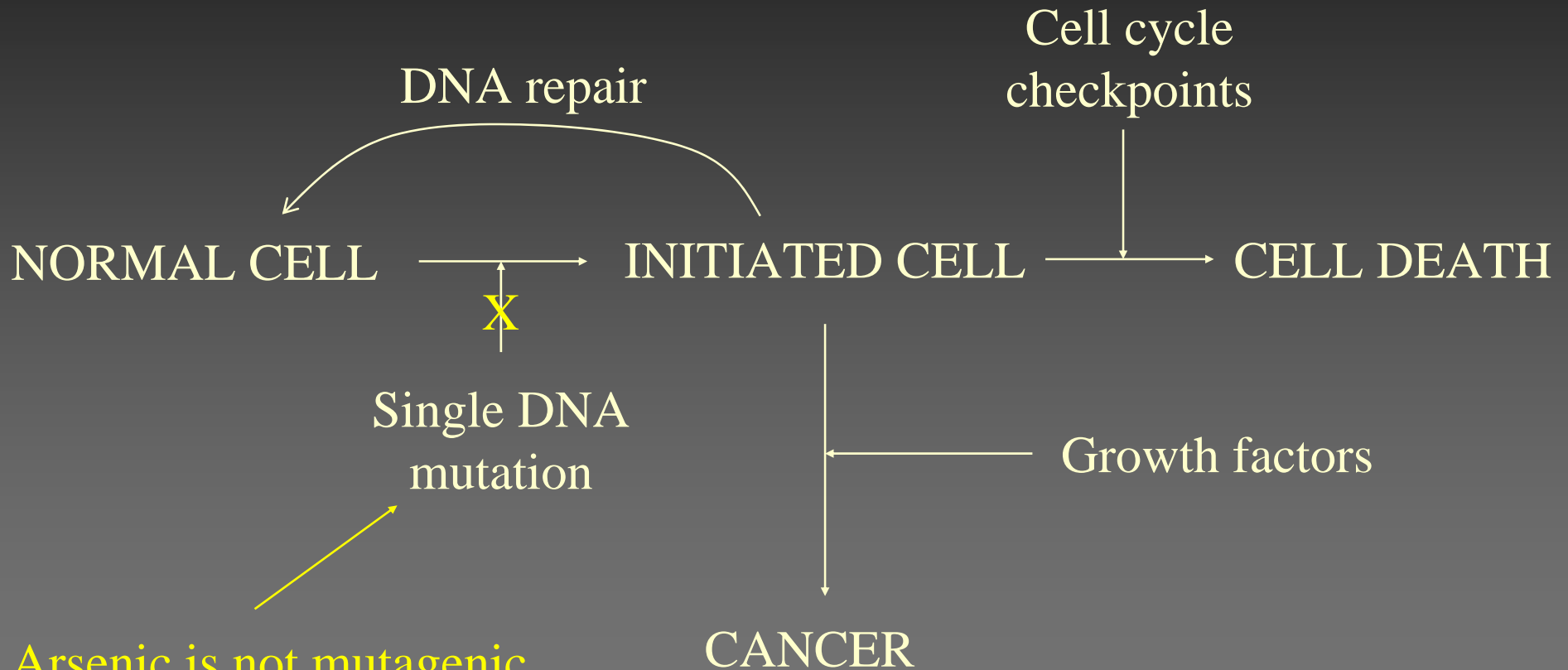
# Arsenic Metabolism



# *Multistage Carcinogenesis*



# Multistage Carcinogenesis



Arsenic is not mutagenic

Arsenic is a co-carcinogen

# *Proposed modes of action*

- Alteration in DNA repair
- Changes in DNA methylation
- Suppression of cell cycle check point proteins (p53)
- Altered expression of growth factors
- Oxidative stress

## *Arsenic-induced oxidative stress*

- Arsenic may cycle between oxidative states, producing radicals
- Arsenic may interact with and reduce intracellular levels of antioxidants
- Arsenic may increase inflammation, leading to chronic presence of cells that produce radicals and/or growth factors

# *OVERALL OBJECTIVES*

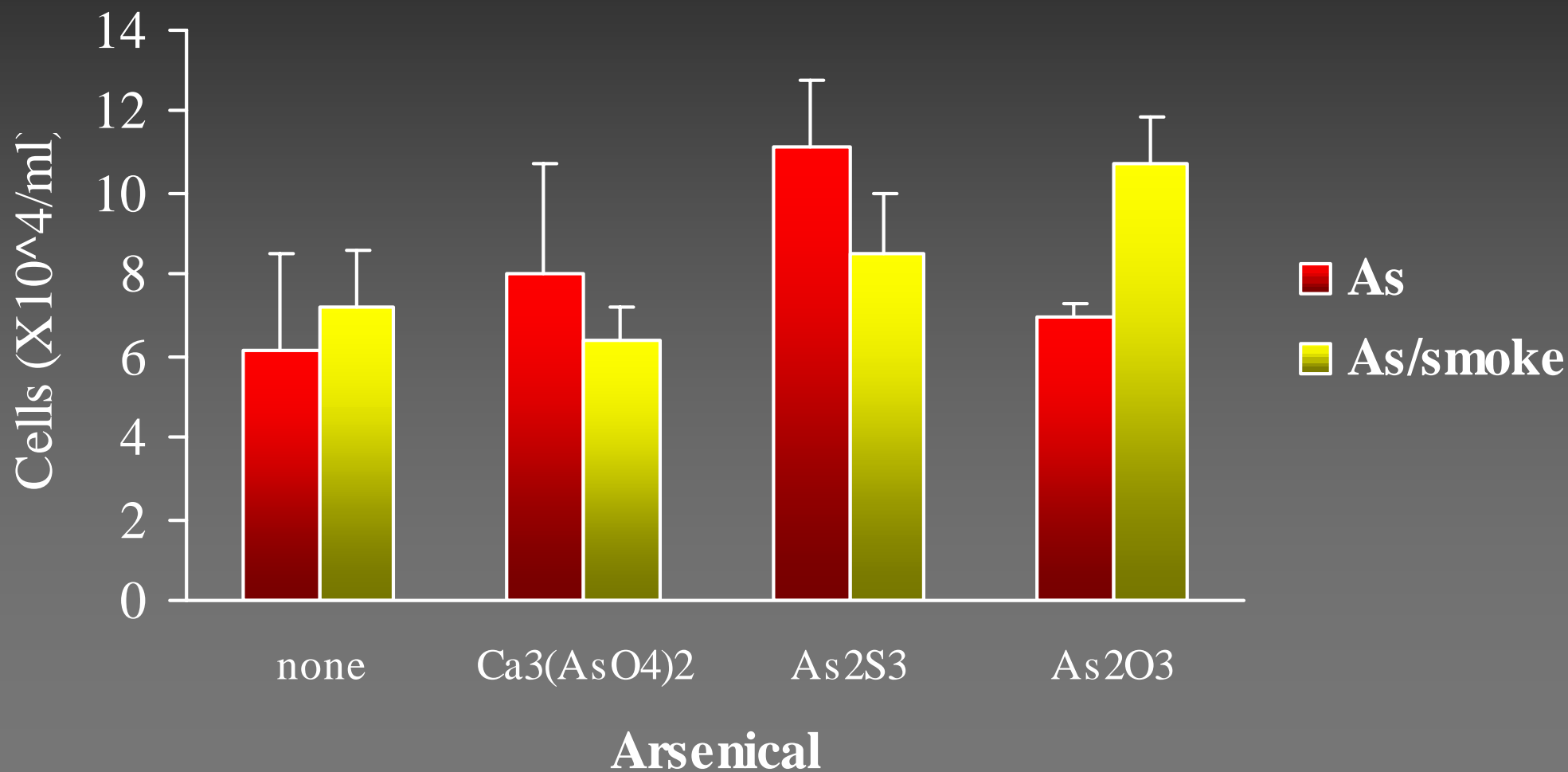
- To determine if arsenicals will lead to oxidative stress
- To determine if exposure to arsenicals can lead to oxidative damage by initiating pulmonary inflammation.

- Is there a correlation between inflammation and arsenic-induced genotoxic events?
- Does inflammatory cell oxygen radical production produce genotoxicity and cell proliferation?
- Will inhalation of environmental levels of arsenicals produce similar inflammation and genotoxicity?
- Is there synergism between arsenic and other pulmonary carcinogens?
- Does exposure to arsenic alter inflammatory mediator expression?

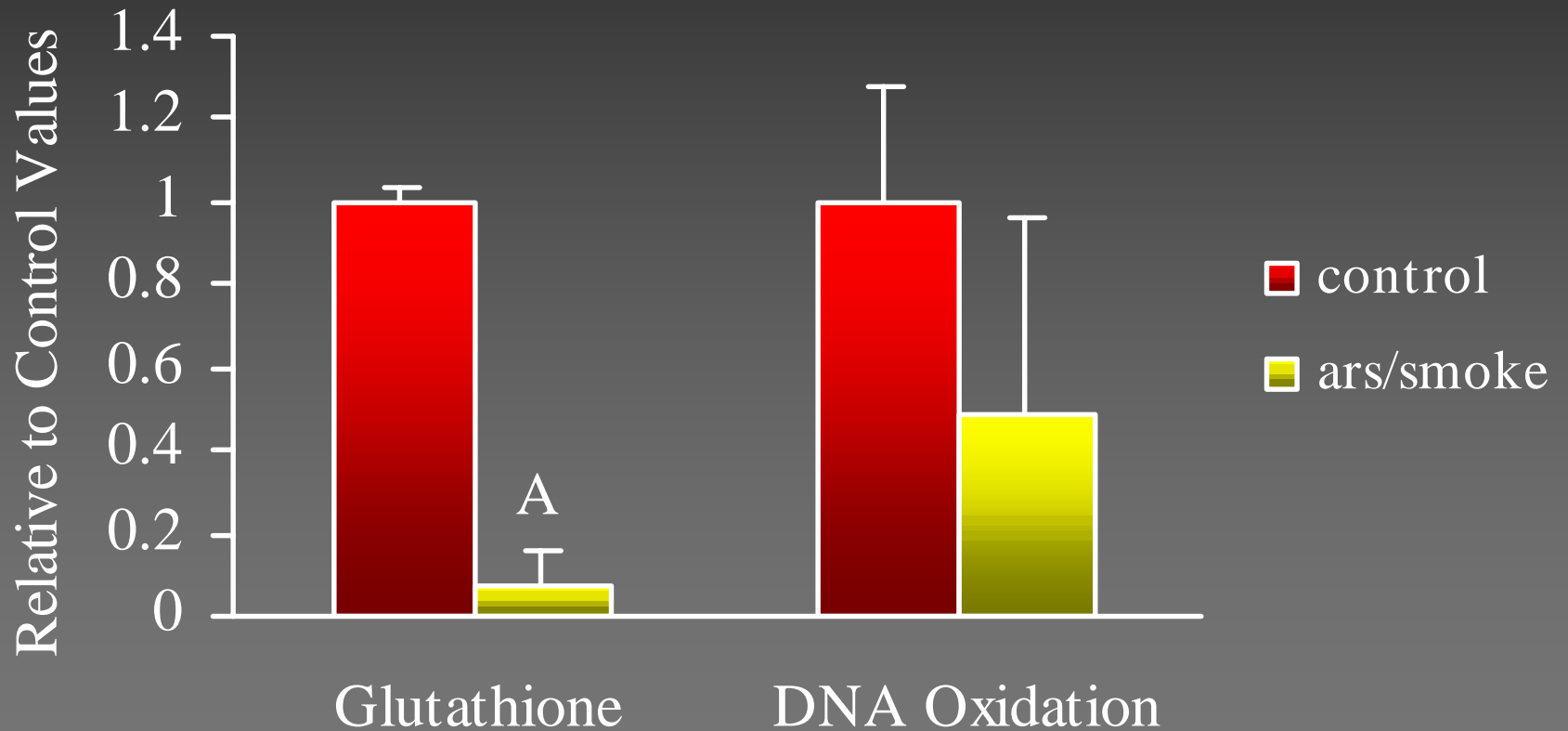
## *Will inhalation of arsenicals lead to increased inflammation and oxidative stress?*

- Male Syrian golden hamsters were exposed for 5 days by nose-only inhalation to 200  $\mu\text{g}/\text{m}^3$  TWA of either calcium arsenate, arsenic trisulfide or arsenic trioxide and/or primary cigarette smoke.
- BALF was analyzed for indicators of inflammation by determining total cell counts and TNF concentrations.
- PAM were analyzed for their ability to produce superoxide and TNF.
- Whole lung GSH levels and DNA oxidation were used to evaluate oxidative stress

## *BALF Cell Counts after 5 day inhalation exposure*



*Glutathione and DNA oxidation following 5 days inhalation exposure*



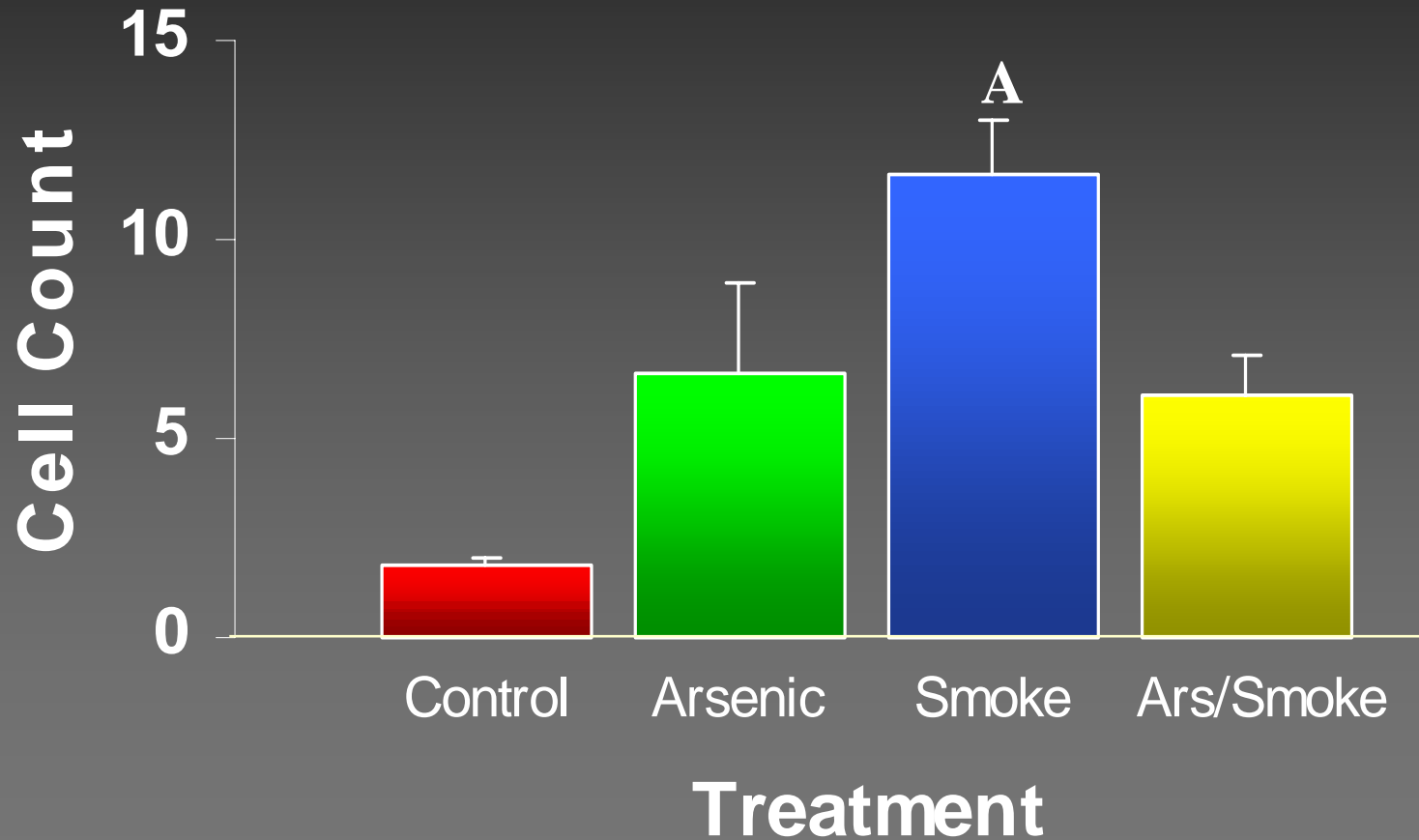
## *Will inhalation of arsenicals lead to increased inflammation and oxidative stress?*

- Inhalation of arsenicals at realistic environmental exposure levels does not lead to inflammation
- PAM lavages from animals after 5 day exposure do not show any differences in superoxide or TNF production compared to controls.
- Combined exposure to arsenic and cigarette does not lead to increased inflammatory response. However, it does lead to decreased GSH levels.

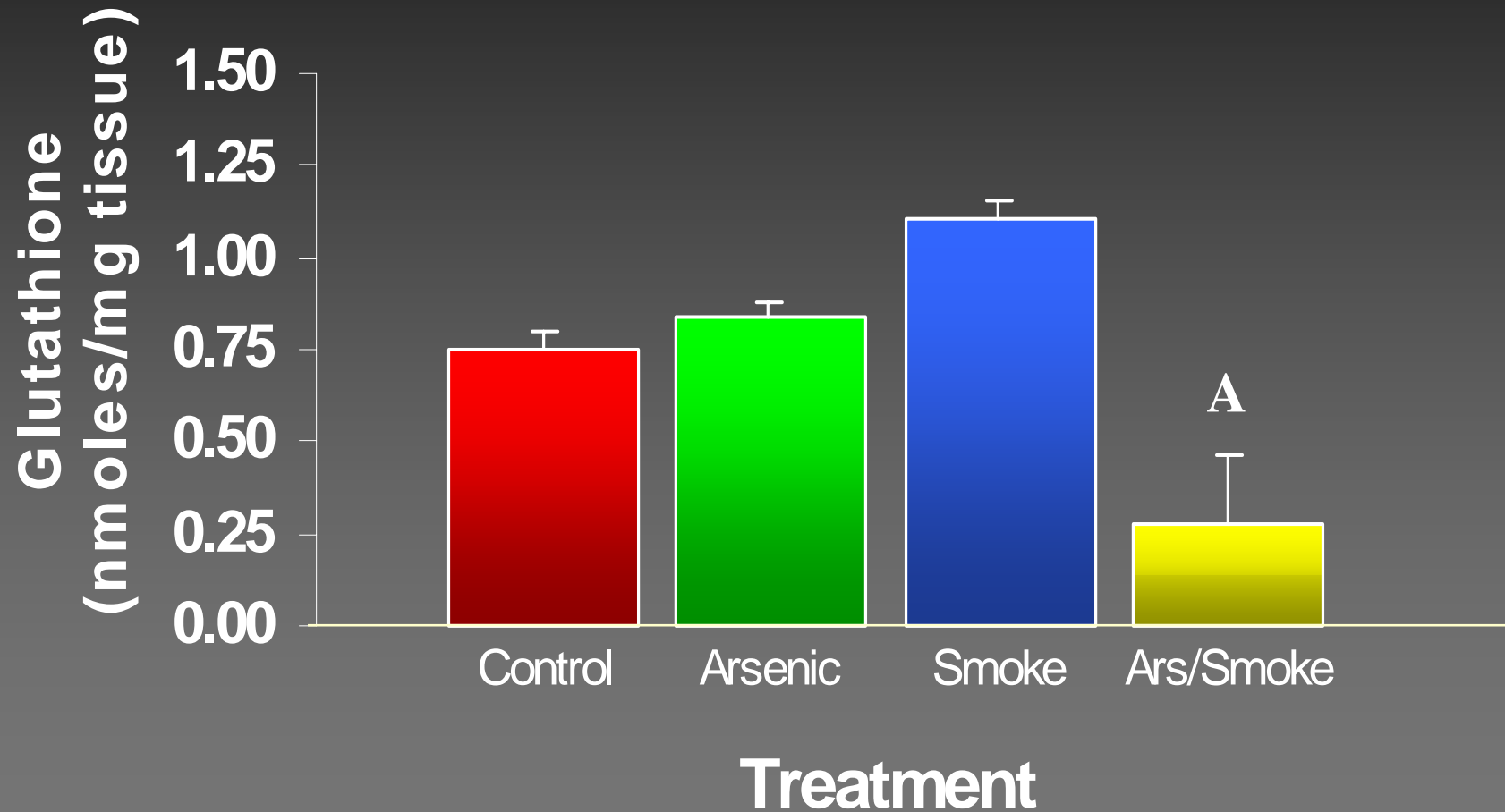
## *Will inhalation of arsenicals lead to increased inflammation and oxidative stress?*

- Male Syrian golden hamsters were exposed for 28 days by nose-only inhalation to 200  $\mu\text{g}/\text{m}^3$  TWA of arsenic trioxide and/or primary cigarette smoke.
- BALF was analyzed for indicators of inflammation by determining total cell counts. Histological tissue sections were also examined for signs of inflammation.
- Levels of total, reduced and oxidized glutathione were determined.
- DNA oxidation was determined as an indicator of genotoxicity.

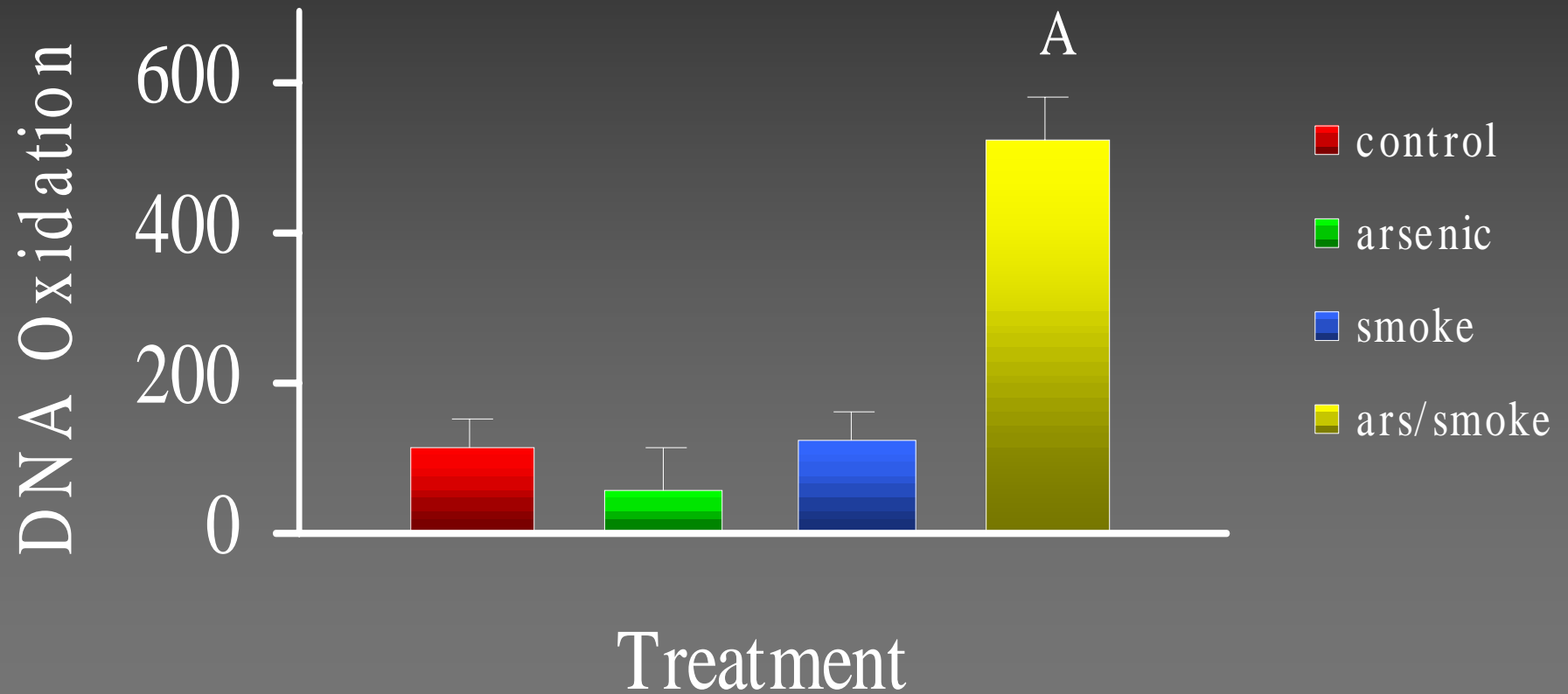
# Cell Counts after 28 day inhalation exposure



# Total Glutathione after 28 day inhalation exposure



# DNA Oxidation after 28 day inhalation exposure



## *Will inhalation of arsenicals lead to increased inflammation and oxidative stress?*

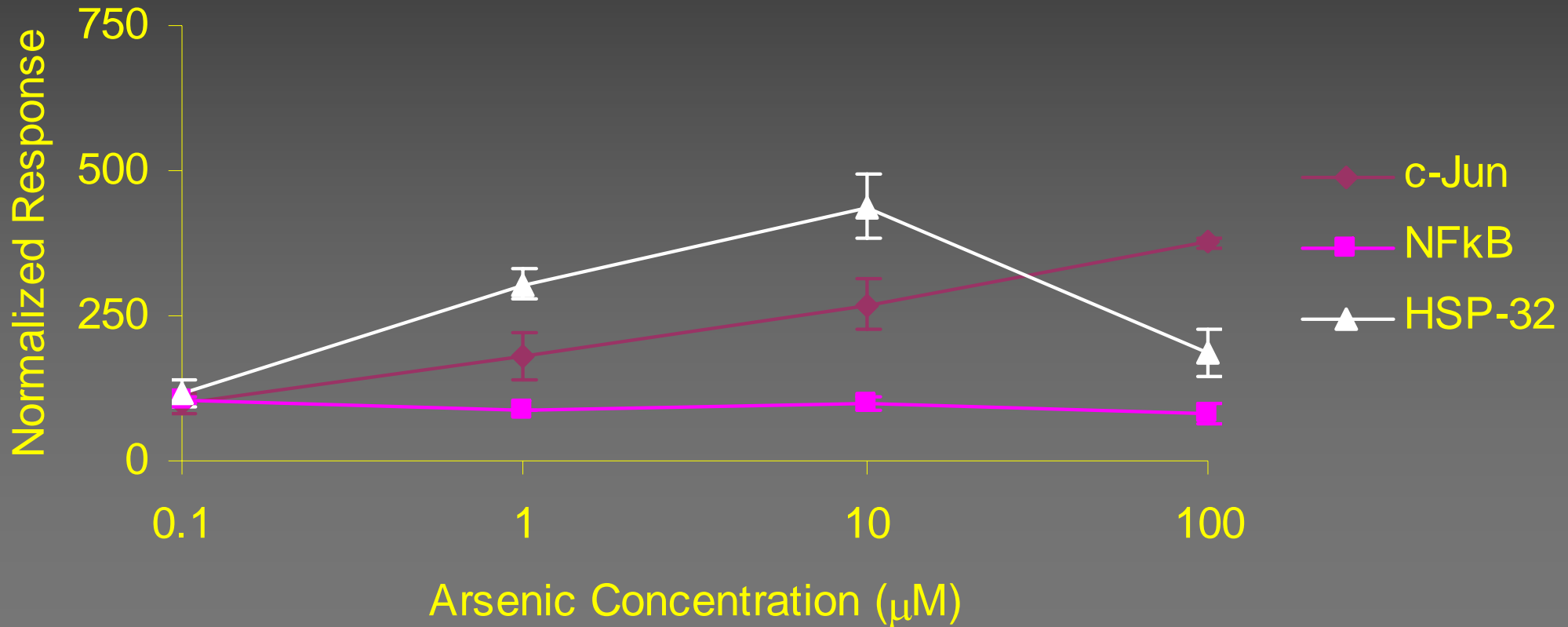
- Inhalation of arsenicals at realistic environmental exposure levels does not lead to inflammation
- Inhalation of arsenicals or cigarette smoke alone at the exposures studied, does not lead to alterations in GSH or in DNA oxidation.
- Combined exposure to arsenicals and cigarette smoke significantly reduces GSH as early as 5 days after beginning exposure.
- Significant increases in DNA oxidation are seen after 28 day exposures.
- Effects of combined exposure to arsenic and cigarette smoke does not appear to be due to increased inflammatory response.

## *Can arsenic activate oxidative stress sensitive transcription factors?*

- Rat lung slices were exposed to arsenite (0.1 to 100  $\mu$ M) for up to 24 hr.
- Nuclear proteins were isolated and analyzed transcription factors c-Jun/AP-1 and p65 NF $\kappa$ B by Western and EMSA
- HSP protein expression was also analyzed (HSP-32, 60, 72 and 90)
- Immunohistochemistry was used to identify the cells affected by the arsenic exposure

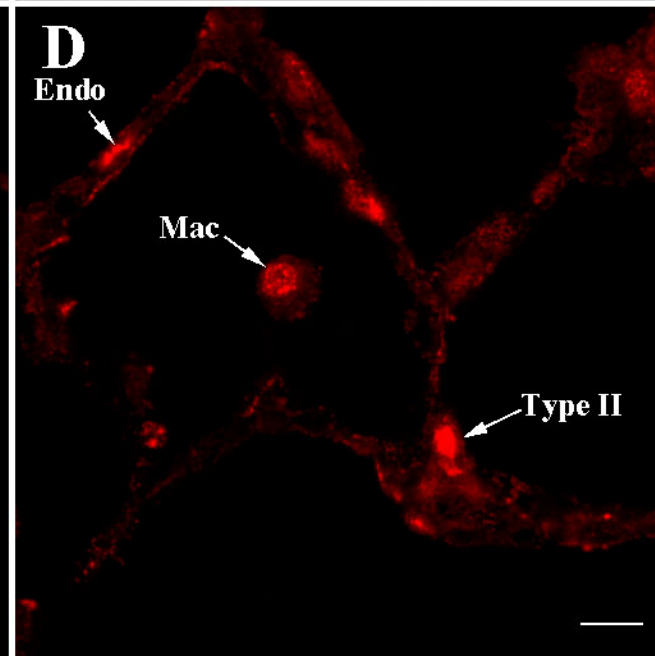
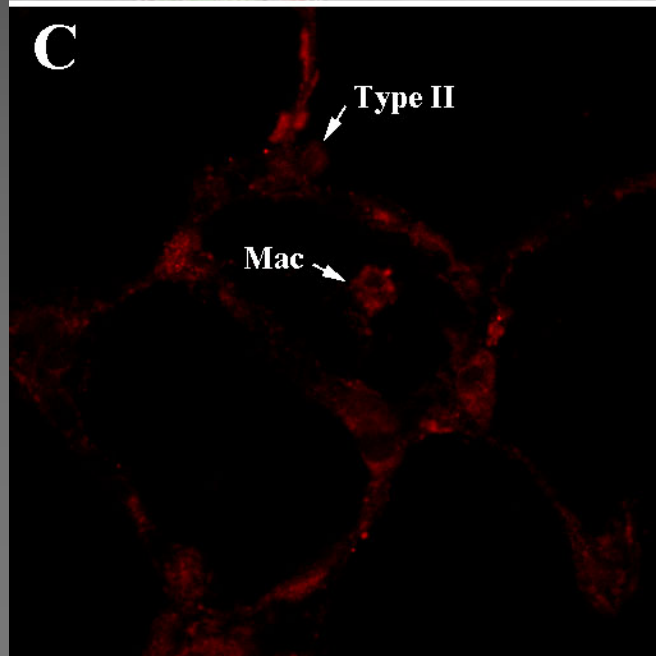
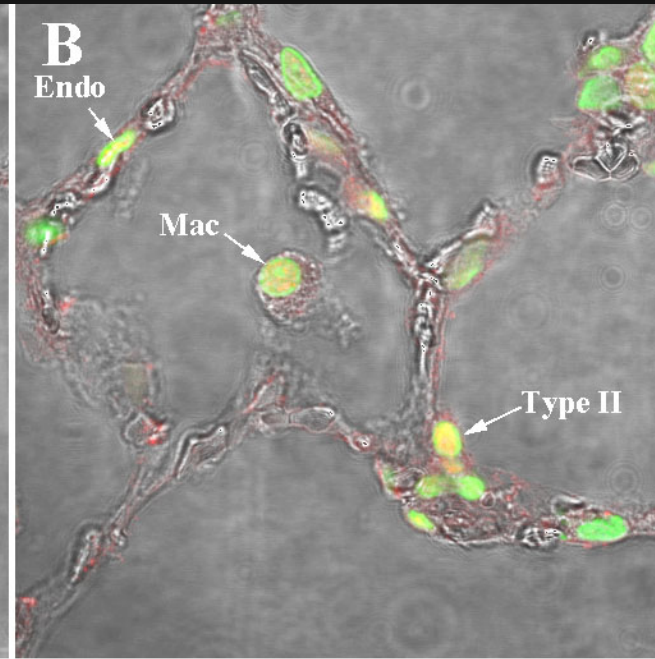
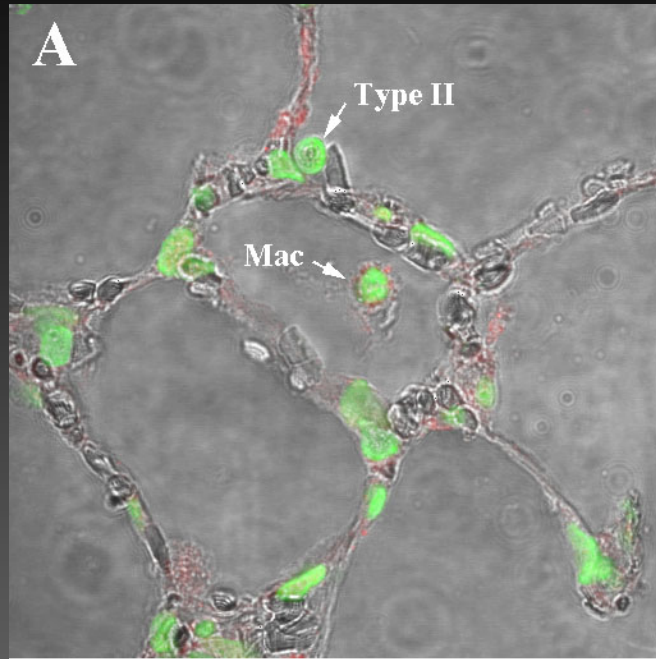
# *Levels of expression*

## Arsenic Dose-Response



CONTROL

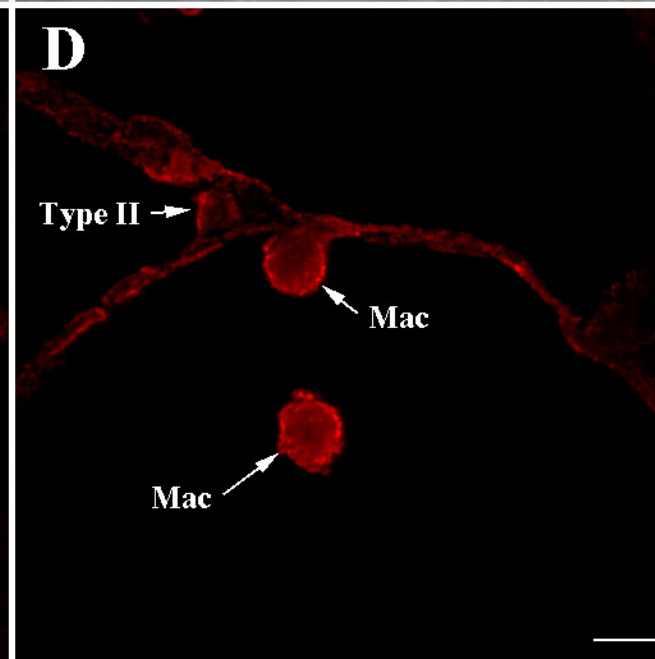
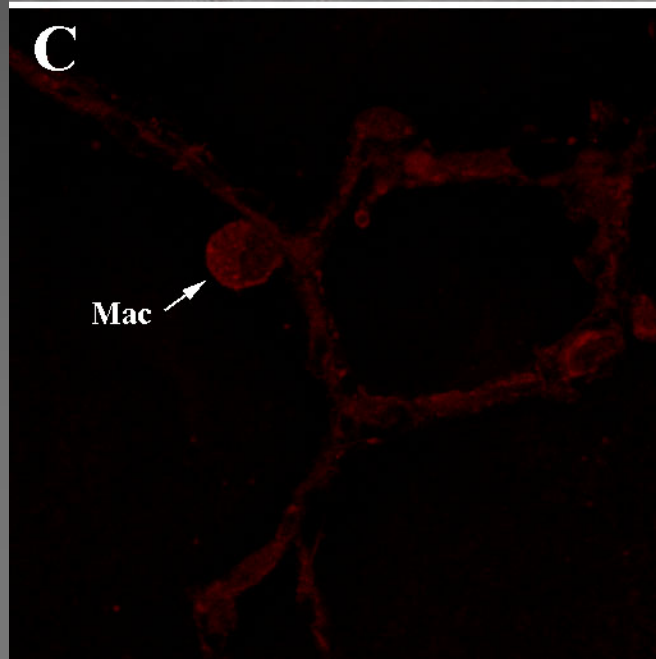
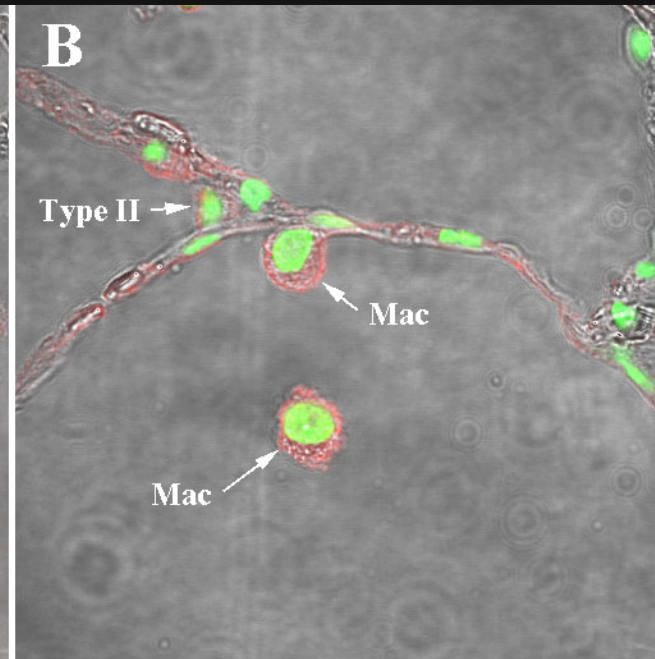
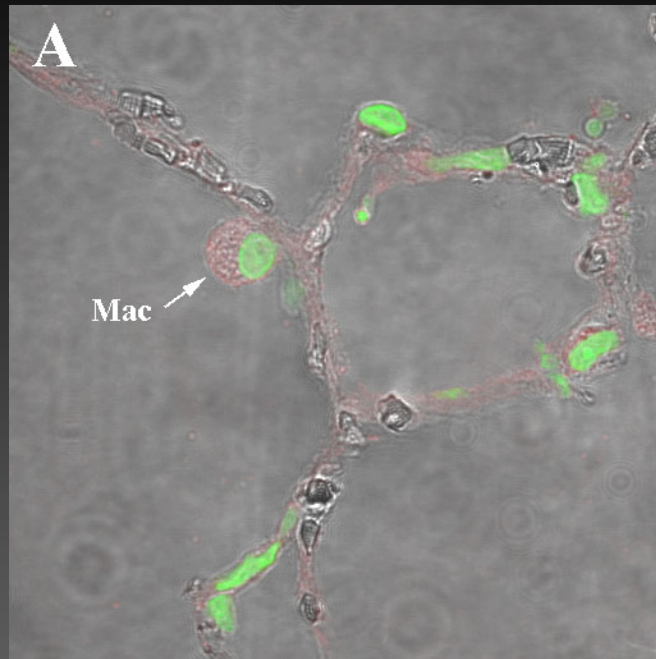
ARSENIC



AP-1

CONTROL

ARSENIC



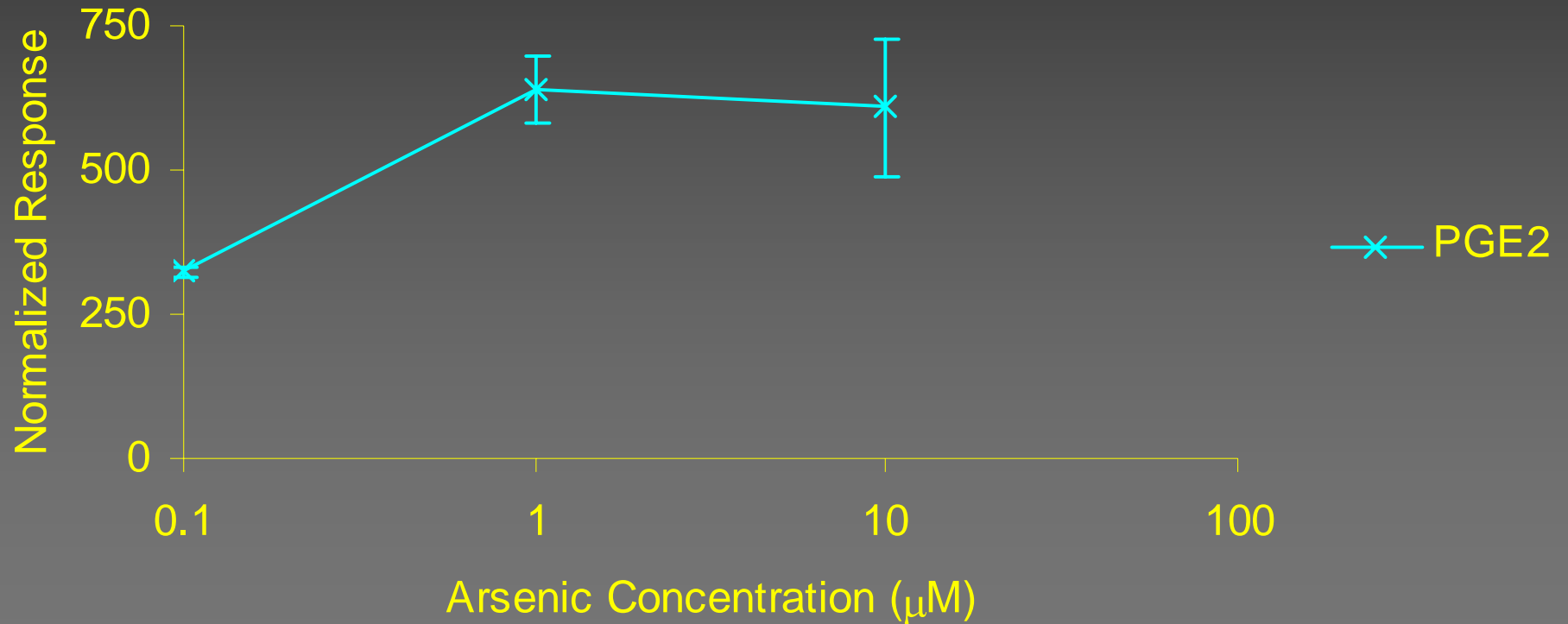
NF $\kappa$ B

## *Can arsenic activate oxidative stress sensitive transcription factors?*

- c-Jun/AP-1 was activated at 1  $\mu$ M.
- p65 NF $\kappa$ B was not translocated to the nucleus
- HSP-32 (HOX-1) was upregulated
- Other HSPs were either not affected or only increased at high arsenic concentrations
- Up regulation of AP-1 and HSP-32 were seen in PAM, EPII and endothelial cells.

# *Effect of arsenic exposure in vitro on PGE<sub>2</sub> production*

Arsenic Dose-Response

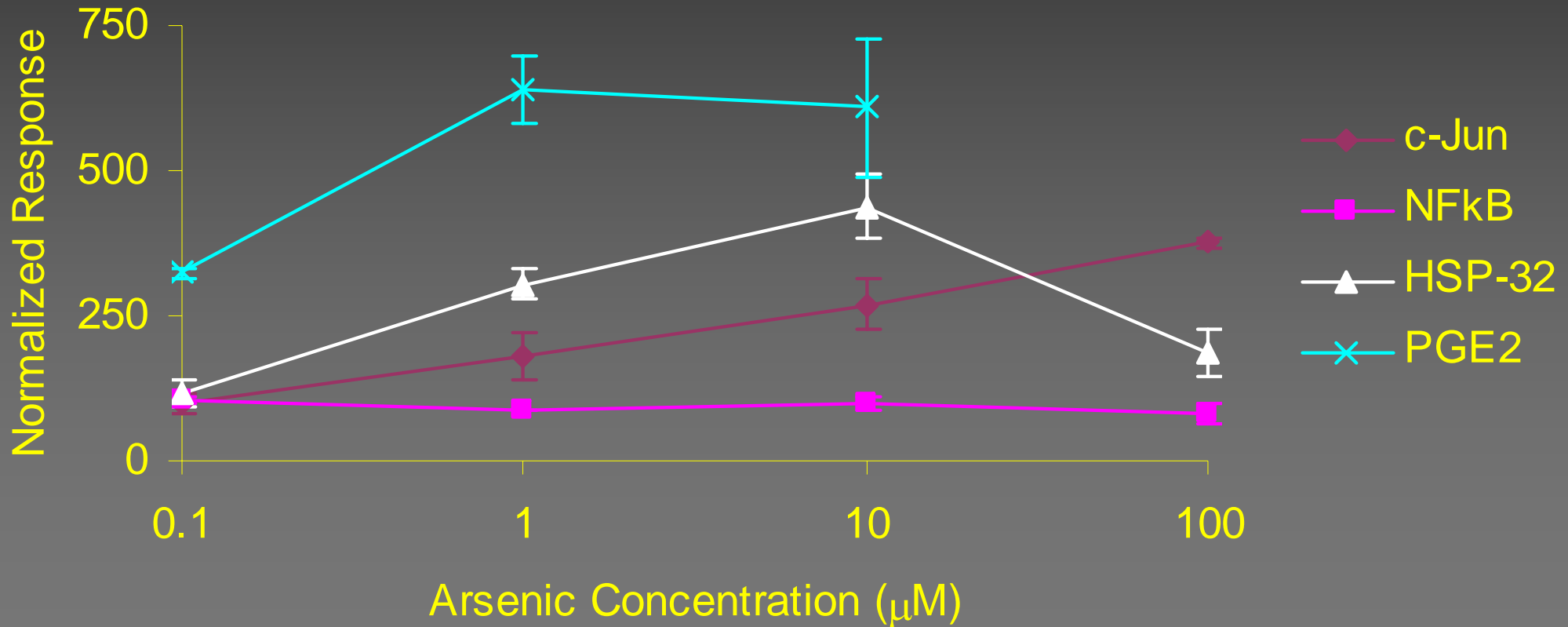


## *Overall Conclusions*

- Arsenic at environmentally relevant levels does cause oxidative stress in the lung
  - Changes in GSH
  - Activation of oxidative stress sensitive transcription factors
  - Expression of HOX-1
  - Is not due to release of oxygen radicals by inflammatory cells
- Exposure to arsenic can potentiate the response following exposure to a second toxicant.

# *Levels of expression*

## Arsenic Dose-Response



## *Future Questions*

- What is the cellular site(s) of action that lead to synergistic effects?
- Does the response depend on the chemical form of arsenic?
- What is the time course for development of the responses?

## *Other concerns with EPA rule*

- Taiwan data used “ecological” values for the dose
- US based studies (Utah study) have not demonstrated increased cancer associated with arsenic in the water (0-100 ppb)
- EPA underestimated costs associated with treatment, particularly in the Southwest
- EPA proposed best treatment will probably not work for Southwestern US water treatment

# *Issues for Arizona water companies*

- The economic effect will depend on the location within the state.
  - Only about 10% of Tucson Water wells exceed 10 ppb
  - However, some companies in the Phoenix area are already having problems meeting 50 ppb
  - Cost of treatment would be prohibitive for small companies
  - Small companies may be abandoned

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