

**U.S. Environmental Protection Agency Science Advisory  
Board Chemical Assessment Advisory Committee (CAAC)  
Ethylene Oxide Review Public Meeting**

**(Comments related to Charge Question #5)**

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# **“Consider an alternative approach to extrapolation of cancer risk”**

## **Objective of the presentation:**

**To outline the rationale for considering, in addition to the default non-threshold extrapolation now employed, a non-linear extrapolation for determining EO’s cancer risk.**

## Basis for EPA's insistence on **only** a linear non-threshold extrapolation for risk at low dose EO exposures.

### Hypothesis: (by EPA, Evaluation of the Carcinogenicity of Ethylene Oxide, August 2014)

EO carcinogenicity has a mutagenic mode of action (presumably applicable to all tumor types). Based on:

- EO is a direct acting alkylating agent and is mutagenic in several systems.
- “Mutagenicity is a well-established cause of carcinogenicity”.

### Required for a Demonstration: Method using the framework analysis)

- To establish a DNA-reactive mutagenic MOA, it necessary to demonstrate pro-mutagenic DNA adducts in the target tissue for cancer.\*
- However, only the N7HEdG adduct has been found in tissues of animals exposed to low ( $\leq 25$  ppm by inhalation) EO concentrations.

*\*Pottenger et al. 2014*

# EO: Pro-mutagenic adduct(s) in target tissue?

N7-hydroxyethyl-dG (approximately 90% of adducts formed by EO) is **NOT A PROMUTAGENIC ADDUCT\***

- *Tompkins et al. (2009) Mutation Research*  
(pSP189 shuttle vector replicated in human Ad 239 cells)
- *Philippins et al. (2014) DNA repair*  
(N7dG adduct in plasmids transformed into bacteria)

\* Nor do abasic sites accumulate (*Rusyn et al., 2005*)

**Even though mutagenic in several systems, the EPA should consider a non-linear extrapolation of cancer risk for low-dose EO exposures (linear non-threshold is not the necessary default)**

**The Cancer Guidelines state:**

**“A nonlinear approach should be selected when there are sufficient data to ascertain the mode of action and conclude that it is not linear at low doses and the agent does not demonstrate mutagenic or other activity consistent with linearity at low doses. **Special attention is important when the data support a nonlinear mode of action but there is also a suggestion of mutagenicity. Depending on the strength of the suggestion of mutagenicity, the assessment may justify a conclusion that mutagenicity is not operative at low doses and focus on a nonlinear approach, or alternatively, the assessment may use both linear and nonlinear approaches**”. (EPA 2005, at 3-22).**

# Ethylene Oxide Mutagenicity

- Although positive in many systems, EO is a **weak** mutagen.

Genetic Activity Profile shows that average lowest effective exposure concentrations required to give positive results *in vitro* were between 1.0 and 10.0 µg/ml (23 to 230 µM)\*

EO blood levels of this magnitude (~ 23 µM) require inhalation exposures > 150 ppm for four hours in mice\*\*

- Abundant evidence of **non-linearity (thresholds)** at low doses.

Adducts:

Marsden *et al.*, (2009)

Mutations:

Nivard *et al.*, (2003) *Drosophila*

Walker *et al.*, (2000) *Hprt* mammalian *in vivo*

Recio *et al.*, (2004) *Lacl* mammalian *in vivo*

Tompkins *et al.*, (2009) Plasmid *in vitro* human cells

LeBaron *et al.*, (2012) MN mammalian reticulocytes *in vivo*

\* = Waters *et al.*, 1998

\*\* = Brown *et al.*, 1998

# EO DNA ADDUCTS AND CANCER: A threshold in rats

- Two year study in rats exposed to **ethylene gas** by inhalation at concentrations up to **3000 ppm** showed no evidence of cancer. (Hamm *et al.*, 1984)
- N7HEdG DNA adducts increased approximately 20-fold in rats exposed to **ethylene gas at 3000 ppm for 6h/d, 5d/wk for 4 weeks**. (Walker *et al.*, 2000)
- **The significant increase in N7HEdG adducts indicates that EO reaches and reacts with the DNA.**

*(Is this unique to EO – or might thresholds be general, even for DNA-reactive mutagenic carcinogens?)*

# DNA Adducts and Cancer

## Recent results indicate a potential paradigm shift

### Complete Protection against Aflatoxin B1-Induced Liver Cancer, with a Triterpenoid: DNA Adduct Dosimetry, Molecular Signature and Genotoxicity Threshold.

Natalie M. Johnson, Patricia A. Egner, Victoria K. Baxter, et al.

Cancer Prev Res 2014;7:658-665. Published Online First March 24, 2014.

- A life time cancer bioassay was undertaken in F344 rats dosed with AFB1 (200 mg/kg rat/day) for four weeks and receiving either vehicle or CDDO Im (three times weekly), one week before and throughout the exposure period.
- CDDO-Im completely protected (0/20) against AFB1-induced liver cancer compared with a 96% incidence (22/23) observed in the AFB1 group.
- In AFB1-treated rats, the hepatic burden of GST-P–positive foci increased substantially (0%–13.8%) over the four weeks, but was largely absent with CDDO-Im intervention.
- With CDDO-Im treatment, integrated level of urinary AFB1-N7-guanine was significantly reduced (66%).
- The remarkable efficacy of CDDO-Im as an anti-carcinogen is established even in the face of a significant aflatoxin adduct burden.
- **Consequently, the absence of cancer requires a concept of a threshold for DNA damage for cancer development.**

**The EPA is asked to reconsider their insistence on **only** a linear, non-threshold extrapolation for risk assessment for all tumors in recognition of:**

- 1. *Uncertainty in the MOA,***
- 2. Evidence of weak mutagenicity and non-linearity in the dose response (thresholds) for adduct formation and mutation at low exposures,**
- 3. Threshold demonstrated for EO DNA adduct levels and cancer in rat bioassay,**
- 4. Recent evidence for a threshold relationship between DNA adducts and cancer, even for a potent DNA reactive mutagenic carcinogen that does exhibit linear, non-threshold relationships between dose and pro-mutagenic DNA adduct formation/mutation induction.**