A Novel Bottom Up Approach to Bounding Potential Human Cancer Risks from Endogenous Chemicals

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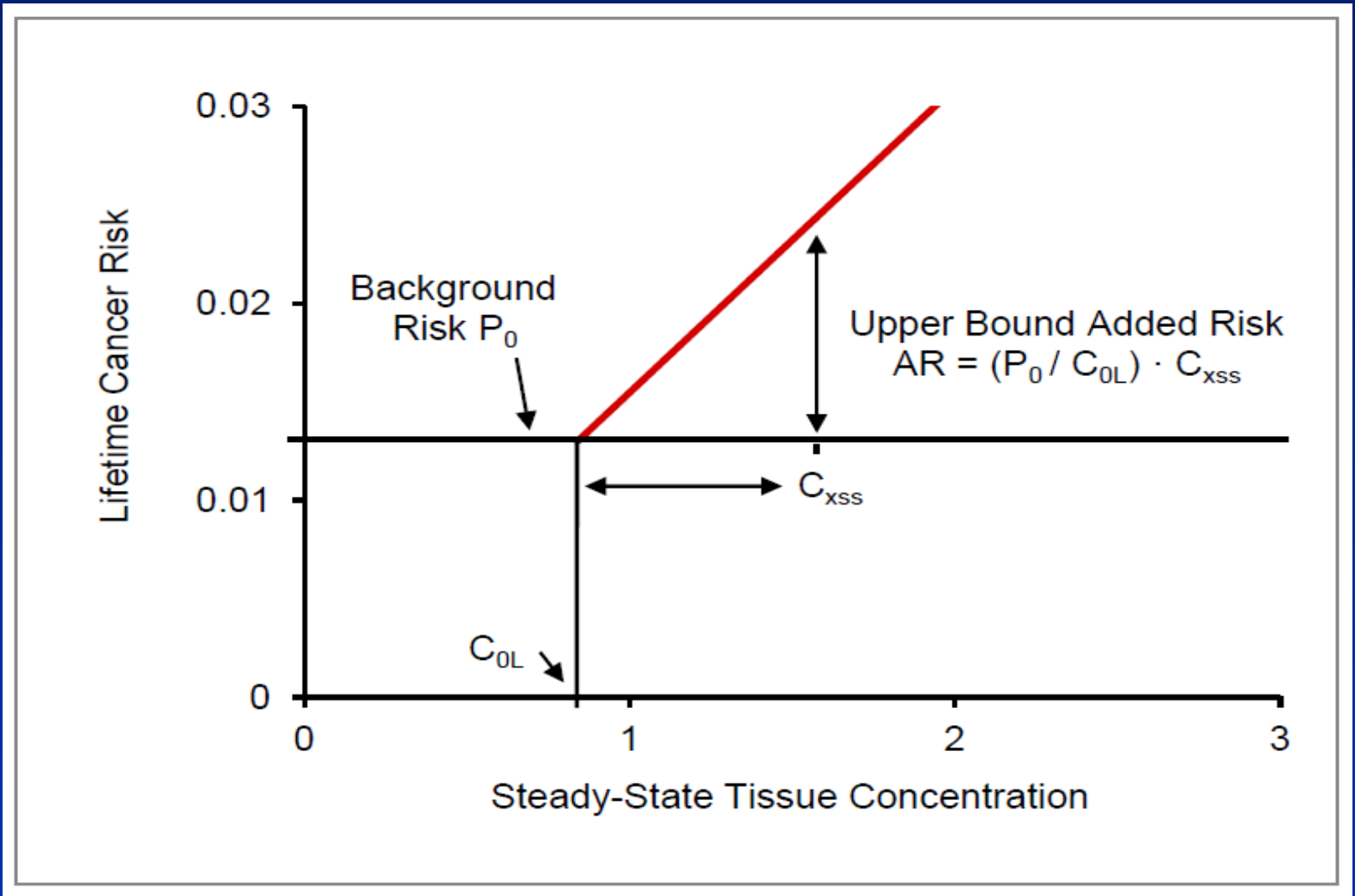
Typical Top Down Approach

- Cancer and exogenous exposure data extracted from epidemiology studies or laboratory animal bioassays
- Empirical or biologically-based dose-response models fit to cancer data vs exogenous exposure, e.g., airborne concentration, cumulative exposure
- Estimated $BMDL_x$ used to calculate unit risk for use in linear extrapolation or, alternatively, to compute MOEs for substances with nonlinear MOAs

The Bottom Up Approach

- Suitable for chemicals present in the body as a result of normal endogenous processes, e.g., metabolism
- Attributes all background risk P_0 to tissue-specific endogenous background exposure C_0
- Assumes linear dose-response for added risk AR vs exogenous exposure C_{xss} with upper 95% confidence bound slope estimate of P_0 / C_{0L}: $AR = (P_0 / C_{0L}) \cdot C_{xss}$
- P_0 data from US SEER cancer statistics
  C_0, C_{0L}, and C_{xss} data from short-term animal studies

Bottom Up Approach Elements



Bottom Up Approach Features

- Bounds low-dose cancer risk without using high dose cancer data from epidemiology studies or animal bioassays

- Provides a completely independent “reality check” on extrapolations from high-dose data

- Conservative:
  - All background risk attributed to endogenous background exposure
  - Assumes linearity at low doses
  - Upper bound estimates of lifetime risk

Estimating Steady-State Exogenous Adducts from Time Point-Specific Data

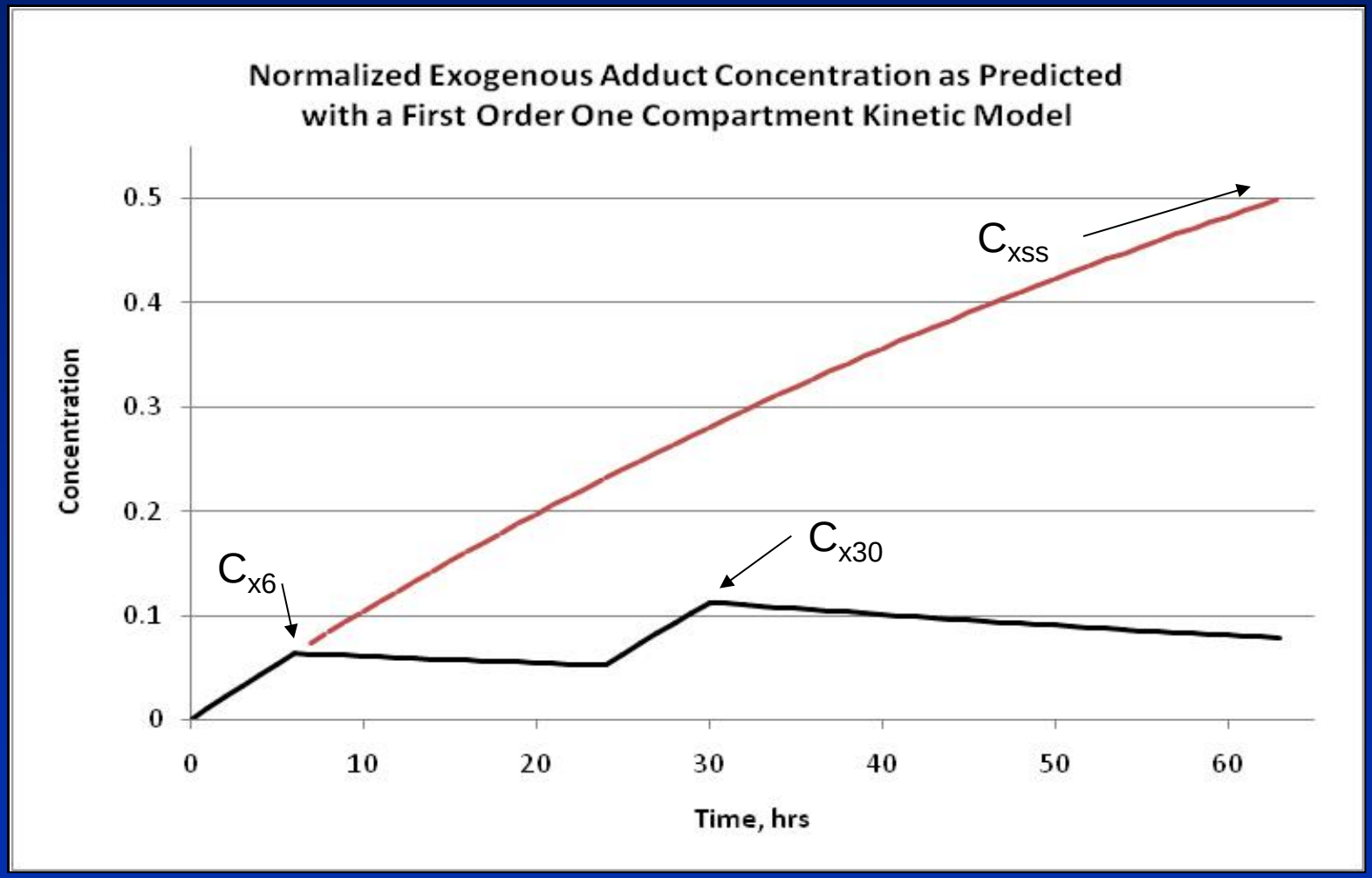
- Used one compartment model with constant forcing and first order elimination with half-life $T_{1/2} = T \cdot \ln(2)$

- For N^2-hydroxymethyl-dG adducts in rats (10 ppm for 6 hrs)
  $T_{1/2}$ = 63 hrs , T = 90.9 hrs (Swenberg 2012)

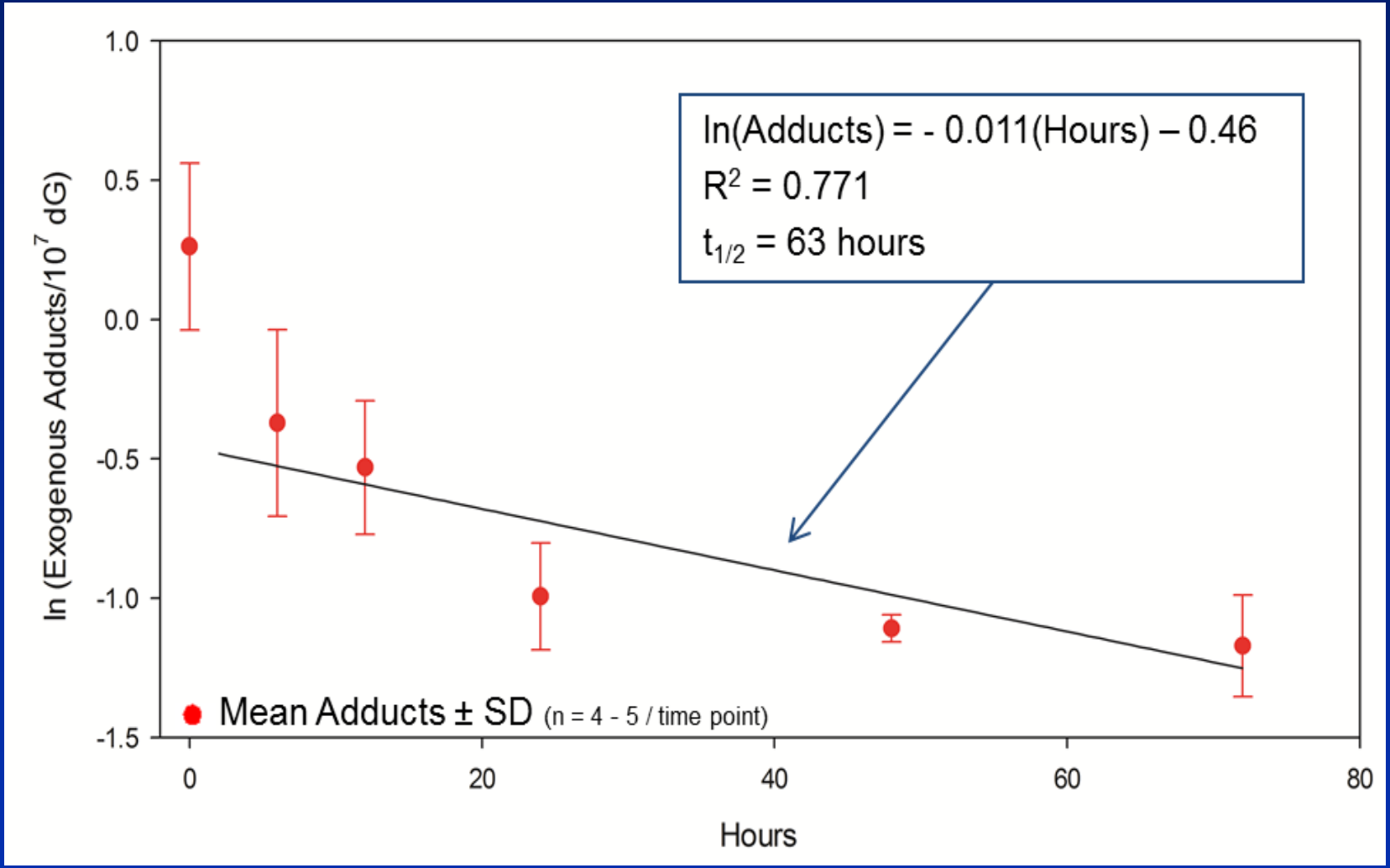
- At the end of one 6 hour exposure:
  $C_{xss} = C_{x6} /(1 – \exp(-6/T)) = 15.65 \cdot C_{x6}$

- After two 6 hour exposures on consecutive days:
  $C_{xss} = C_{x30} /\{[1 – \exp(-6/T)] \cdot [1 + \exp(-24/T)]\} = 8.85 \cdot C_{x30}$

One Compartment Model: Adduct Time Profile

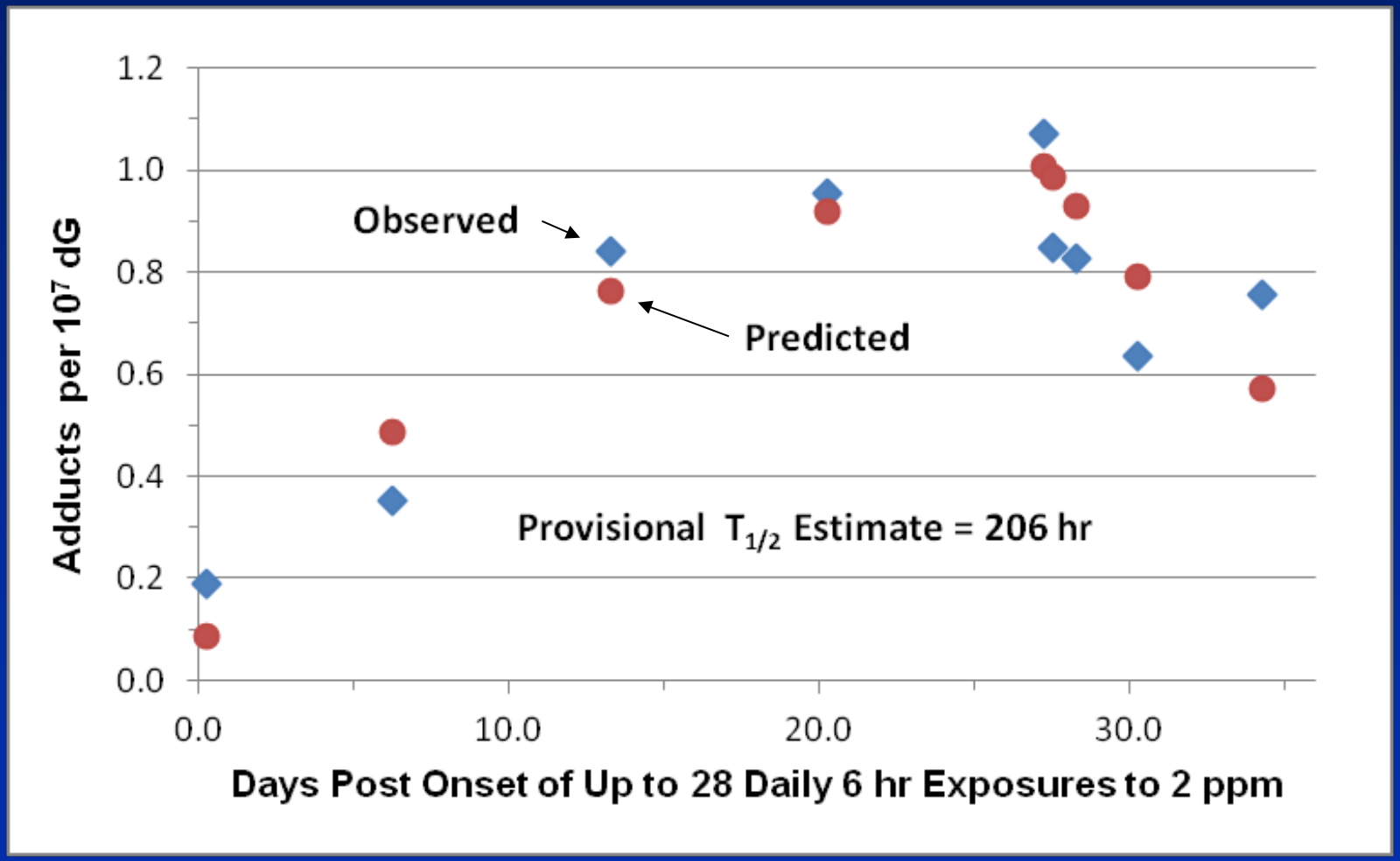


N^2-Hydroxymethyl-dG Elimination Half-Life Data



One 6 hr exposure of rats to 10 ppm, Swenberg et al., 2012

New N^2-Hydroxymethyl-dG Elimination Half-Life Data



Swenberg (unpublished data)

N^2-hydroxymethyl-dG Adducts in Monkeys Exposed Twice for 6 hrs to 2 ppm $^{13}CD_2O$

Tissue	Endogenous Adducts at 30 hrs	Exogenous Adducts at 30 hrs	Exogenous Adducts at Steady-State
Nasal Epithelium Mean ± se Lower 95% Bound	2.49 ± 0.23 2.11 C_{0L}	0.25 ± 0.020 C_{x30}	2.21 ± 0.18 C_{xss}
Bone Marrow Mean ± se Lower 95% Bound	17.5 ± 1.31 15.34 C_{0L}	< 0.00103[a] C_{x30}	<0.00912[a] C_{xss}

a: no exogenous adducts were detected in bone marrow; upper limit estimate based on the detection limit reported in Moeller et al. (2011).

Comparison of Bottom Up and Top Down Upper Bound Added Risk Estimates

Cancer	Background Risk P_0	Bottom-Up Slope P_0/C_{0L} [a]	C_{xss} at 2 ppm	Bottom-Up Risk at 1 ppm [b]	USEPA Risk at 1 ppm
NPC	7.25×10^{-4}	3.44×10^{-4}	2.21 ± 0.18	0.038×10^{-2}	1.1×10^{-2}
LEU	1.30×10^{-2}	8.50×10^{-4}	< 0.00912	$< 3.9 \times 10^{-6}$	5.7×10^{-2}

For NPC, $AR_{BU} = (3.44 \times 10^{-4} \cdot 2.21) / 2 = 0.038 \times 10^{-2}$

$= 29.8$-fold lower than AR_{EPA}

For LEU, $AR_{BU} = (< 8.5 \times 10^{-4} \cdot 0.00912) / 2 = < 3.9 \times 10^{-6}$

$= > 14{,}615$-fold lower than AR_{EPA}

Bottom Up Uncertainties (Human Analysis)

- P_0 very precise due to large number of cases in US population of more than 300,000,000:
  Annually, > 2,550 NPC, > 45,880 LEU

  NPC $P_0 = 7.2500 \times 10^{-4}$, $P_{0U} = 7.2656 \times 10^{-4}$
  LEU $P_0 = 1.3000 \times 10^{-2}$, $P_{0U} = 1.3011 \times 10^{-2}$

- C_0 uncertain due to small monkey sample sizes:
  Nasal $C_0 = 2.49 \pm 0.23$, $C_{0L} = 2.11$
  Bone Marrow $C_0 = 17.5 \pm 1.31$, $C_{0L} = 15.34$

- $T_{1/2}$ and C_{xss} uncertain due to small rat sample sizes

Top Down Uncertainties (Human Analysis)

NPC: - VERY small number of deaths: 2 UnExp, 7 Exp
- coarsely stratified cumulative exposure metric
- marginally significant trend due to excess in highest exposure category (3 deaths)
- non-monotonic dose-response

LEU: - small number of deaths: 7 UnExp, 116 Exp
- coarsely stratified cumulative exposure metric
- non-significant positive trend due largely to ~ 47% deficit in Unexposed group relative to the Exposed groups
- no dose-response in Exposed groups

Advantages of the Bottom Up Approach

- Uses background cancer risk in humans
- Uses background (endogenous) adduct concentrations in humans, if available, or short-term animal data and equivalence assumptions
- Conservative:
  - Linear at low doses (consistent with additivity)
  - All background risk attributed to endogenous adducts
  - Provides an upper bound on low-dose slope
- Produces a completely independent “reality” check on risk extrapolations from high-dose data

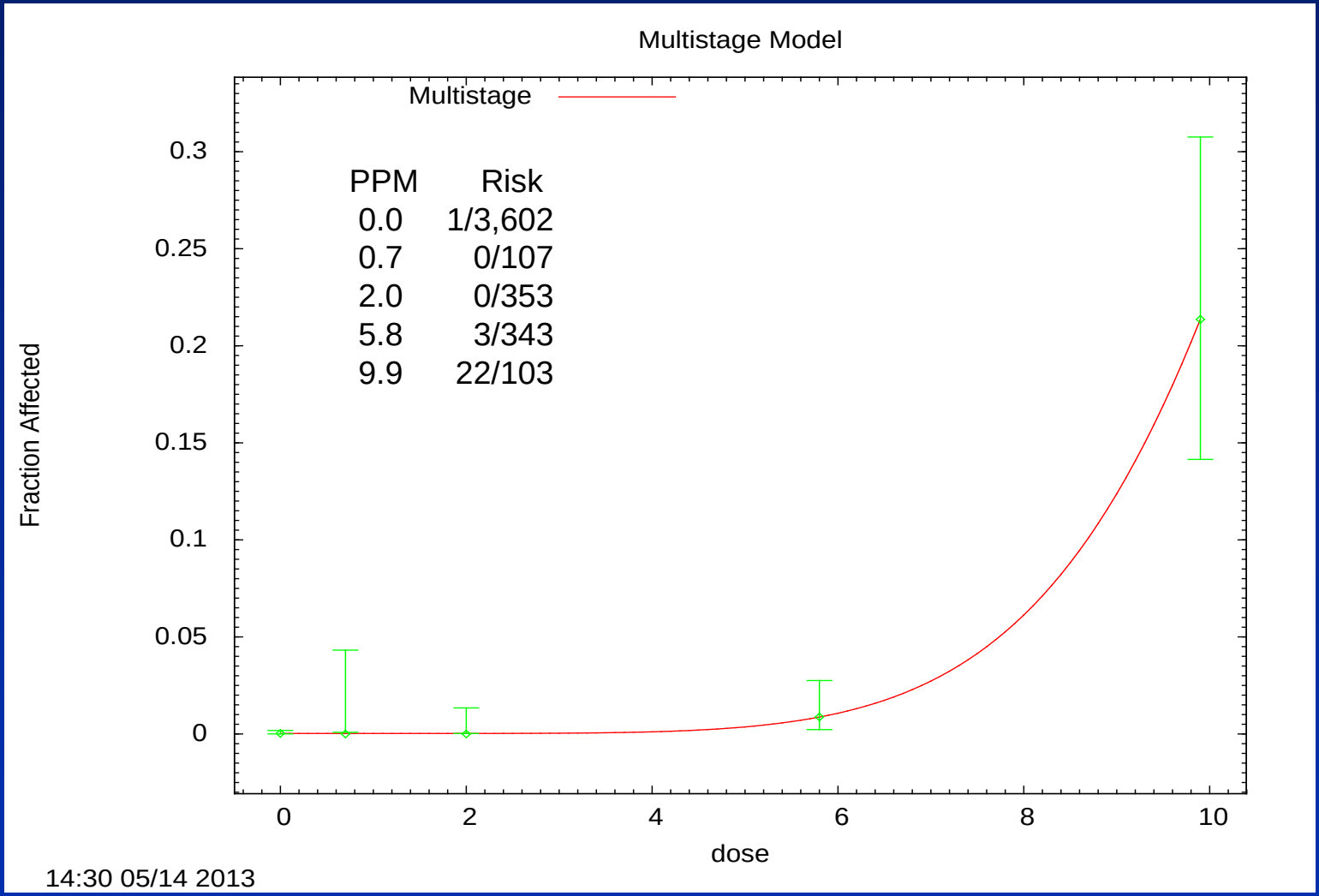
Analyses of Rat Tumor Data

- Fit the multi-stage model to rat nasal tumor data vs air concentration to obtain a Top Down unit risk estimate

- Apply Bottom Up approach to the same rat tumor data to determine corresponding unit risk estimate

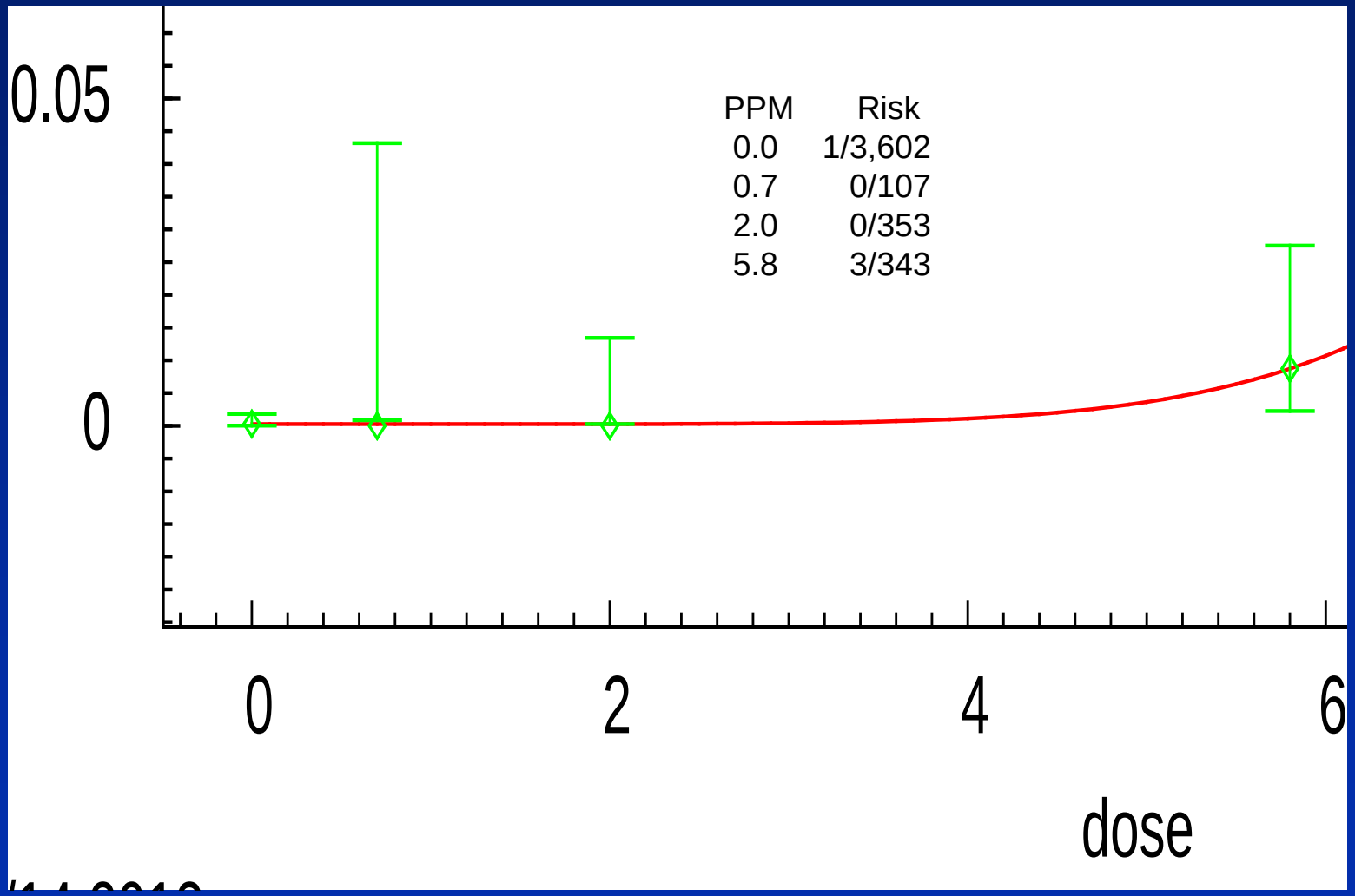
- Compare estimates and identify uncertainties in the two approaches as implemented with rat tumor data

Top Down MultiStage with Rat Tumor Data (1)



Maximum Likelihood fit using BMDS

Top Down MultiStage with Rat Tumor Data (2)



Maximum Likelihood fit using BMDS

Top Down Multistage with Rat Tumor Data (3)

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   THE CHI-SQUARE GOODNESS OF FIT STATISTIC IS .132132

   MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

     Q( 0)= .246282488309E-03
     Q( 1)= .000000000000
     Q( 2)= .000000000000
     Q( 3)= .000000000000
     Q( 4)= .000000000000
     Q( 5)= .000000000000
     Q( 6)= .178971172177E-06
     Q( 7)= .768126937566E-08

   THE MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -79.9416846141

   CALCULATIONS ARE BASED UPON ADDITIONAL RISK

   GLOBAL 82 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE
*********************************************************************

     CONFIDENCE LIMITS FOR A DOSE OF 1.00.

   THE MLE ESTIMATE OF ADDITIONAL RISK IS    .186606E-06
   THE 95 PERCENT UPPER CONFIDENCE LIMIT IS .134698E-02

   THE RATIO OF THE 95% UCL ESTIMATE TO THE MLE ESTIMATE IS 7,218.

   THE COEFFICIENTS CORRESPONDING TO THE 95.0 PERCENT UPPER BOUND ARE:

     Q( 0)= .239464193777E-03
     Q( 1)= .134818337981E-02
     Q( 2)= .000000000000
     Q( 3)= .000000000000
     Q( 4)= .000000000000
     Q( 5)= .000000000000
     Q( 6)= .000000000000
     Q( 7)= .235701733574E-07

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MLE $q_1 = 0$

MLE AR < 10^{-6}

UCL AR = 0.135 x 10^{-2}

UCL95 q_1 = 0.1348 x 10^{-2} / ppm

Bottom Up Approach with Rat Tumor Data

$P_0 = 1 / 3{,}602 = 2.78 \times 10^{-4}$ $P_{0U} = 13.16 \times 10^{-4} = 4.73 \cdot P_0$

$C_0 = 6.09 \pm 1.52$ $C_{0L} = 3.60$

$P_{0U} / C_{0L} = 3.656 \times 10^{-4}$

For 2 ppm:

$C_{x6} = 0.19 \pm 0.04$ $C_{xss} = 0.19 \cdot 15.65 \cdot (6/24) \cdot (5/7) = 0.531$

$AR = (P_{0U}/C_{0L}) \cdot C_{xss} = (3.656 \times 10^{-4} \cdot 0.531) = 1.941 \times 10^{-4}$

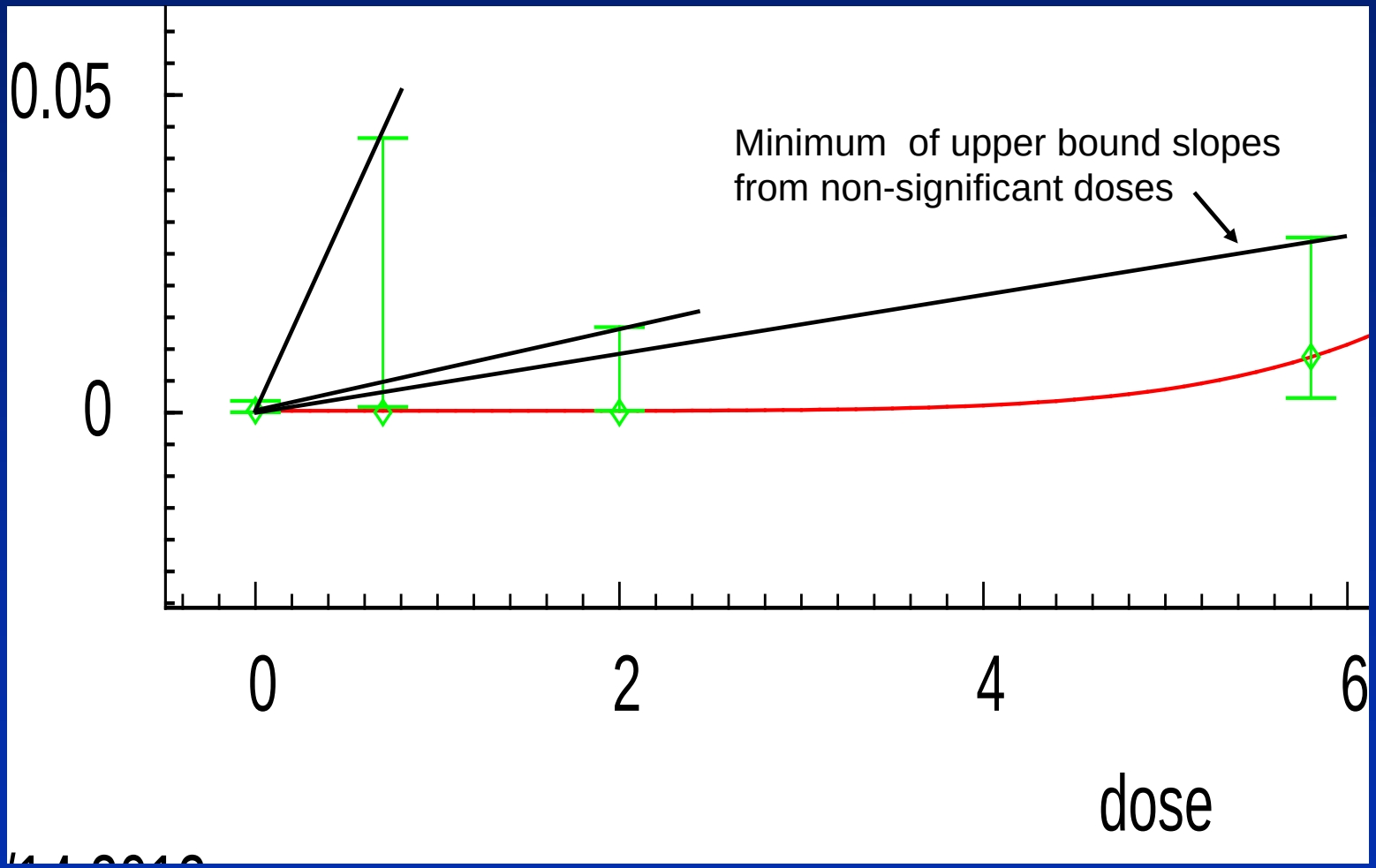
For 1 ppm:

AR = AR at 2ppm / 2 = $1.941 \times 10^{-4} / 2 = 0.971 \times 10^{-4}$

This is 520-fold GREATER than the Top Down MLE estimate of 0.1866×10^{-6}, and 13.9-fold SMALLER than the Top Down UCL95 estimate of 0.1347×10^{-2}

Connection to Model-Free Extrapolation

Krewski, Gaylor, Szyszkowicz. 1991. *EHP* 90:279-285



Bottom Up Uncertainties (Rat Analysis)

- P_0 uncertain due to only 1 case in 3,602 NTP historical controls (inhalation studies only)

  Nasal SCC $P_0 = 2.78 \times 10^{-4}$, $P_{0U} = 13.16 \times 10^{-4}$

- C_0 uncertain due to small rat sample sizes:

  Nasal $C_0 = 6.09 \pm 1.52$, $C_{0L} = 3.60$

- $T_{1/2}$ and C_{xss} uncertain due to small rat sample sizes

Top Down Uncertainties (Rat Analysis)

- VERY small exposed group sample sizes: 103 - 353
- Highly nonlinear dose-response
  MLE risk < 0.2×10^{-6} at 1 ppm, > 20% at 10 ppm
- No dose-response below 6 ppm
- High dose data are assumed, but not proven,
  to be relevant to risk at low doses
- MOA not known, could be all due to cell proliferation

Advantages of the Bottom Up Approach

- Uses background cancer risk in humans
- Uses background (endogenous) adduct concentrations in humans, if available, or short-term animal data and equivalence assumptions
- Conservative:
  - Linear at low doses (consistent with additivity)
  - All background risk attributed to endogenous adducts
  - Provides an upper bound on low-dose slope
- Produces a completely independent “reality” check on risk extrapolations from high-dose data

Generalizing to Other Chemicals

- Methanol (metabolized to formaldehyde)
- Acetaldehyde (N^2-hydroxyethyl-dG adducts
- Vinyl Acetate (metabolized to acetaldehyde)
- Vinyl Chloride (metabolized to chloroethylene oxide, producing 1 oxoethyl and 3 exocyclic etheno adducts)
- Ethylene Oxide (4 hydroxy-ethyl adducts)

Some Criteria for Use in Risk Assessment

- Specific target sites in humans (epidemiology studies)
- Valid biomarkers of target site exposure that are plausibly correlated with the apical endpoint
- High precision/accuracy measurements that distinguish between endogenous / exogenous sources at low exogenous exposure levels
- Use conservative assumptions to fill data gaps
- Use to “reality check” and, when appropriate, replace top down analyses

Acknowledgments

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