

---

# How much testing is necessary to call a PREP a PREP?

---

John H. Lauterbach, PhD, DABT  
Lauterbach & Associates, LLC  
Macon, GA 31210-4708

---

# Outline

- Definition of a PREP
- Background
- Objectives for presentation
- Discussion of potency estimation and thresholds
- Is chemistry enough?
- Conclusions

---

## Definition of a PREP

- A product provides "reduced exposure" when there is scientific evidence that the product substantially reduces an average smoker's exposure to one or more tobacco toxicants, with the reduction in exposure being "sufficiently large that independent scientific experts would anticipate finding a measurable reduction in morbidity and/or mortality in subsequent clinical or epidemiological studies."

Source: <http://www.pmusa-science.com/PrepScience/Prep.asp>

---

# Background

- Several schemes have been suggested for evaluating cigarette-type PREPS
  - All involve use of chemistry, *in vitro* and *in vivo* bioassays as well as human biomonitoring
  - Issues of cost, time, and relevance of data
- Apparently no schemes suggested for PREPS based on oral tobacco products
- Most public health experts skeptical of safety and efficacy of PREPS, but a few have suggested reductions in specific compounds to minimize tobacco-related health effects

---

# Objectives

- Review mainstream smoke (MSS) and tobacco components the experts have associated with tobacco-related diseases in terms of potency and dose
- Review relevance of bioassays to assessing changes in product toxicity
- Review alternative testing strategies

# MSS components & cancer endpoints

- Lung cancer
  - Hecht, 1999, weight-of-evidence approach, TSNAs ≈ specific PAHs >> metals, free radicals/oxidative damage, miscellaneous organics
  - Laugesen and Fowles, 2005; Fowles and Dybing, 2003; Haussmann *et al.*, 2001, risk-assessment approaches, 1,3-butadiene, acetaldehyde, acrylonitrile, benzene
  - Pryor, 1997, studies on free radicals (FR), FR derived from reaction of  $\text{NO}_x$  with isoprene and FR from quinone-hydroquinone-semiquinone system
- Bladder cancer
  - IARC, 1986, aromatic amines

---

# MSS components & non-cancer endpoints

- Non-neoplastic lung diseases
  - Haussmann *et al.* 2001 risk-assessment approach acrolein, acetaldehyde
  - Pryor (1990) studies on free radicals (FR), FR derived from reaction of  $\text{NO}_x$  with isoprene and FR from quinone-hydroquinone-semiquinone system
- Other components of potential concern
  - Carbon monoxide (CVD)
  - Diacetyl & other dicarbonyls (ciliastatic agents)
  - Some phenols and pyridines (cytotoxic agents)
  - Heterocyclic aromatic amines (mutagens)

---

# Risk assessment potency values

- Values used by some researchers often come from published risk assessments such as those done by USEPA and California EPA
  - Include both cancer and non-cancer risks
  - “Safe” levels suggested in some cases
  - Values used by non-industry smoking-and-health experts to suggest most (80%) of MSS is in the vapor-phase (v-p) (Health New Zealand)
    - 1,3-butadiene (cancer), HCN (CVD), acrolein (other respiratory diseases) and ten other v-p compounds
    - List similar to one proposed in 1980
- If these researchers are correct, how meaningful are current testing protocols?

---

## Other potency tools – The CPDB

- Carcinogenic Potency Project  $TD_{50}$  values
  - Carcinogenic potency is defined in the CPDB (Carcinogenic Potency Database) in terms of the average daily dose-rate that will halve the probability of remaining tumor-free at the end of a standard lifespan ( $TD_{50}$ )
  - CPDB is a unique and widely used international resource of results from 6153 chronic, long-term animal cancer tests on 1485 chemicals
  - $TD_{50}$  values for the compounds can be used in conjunction with human dosage data to calculate HERP (Human exposure/rodent potency) values

# Example of CPDB and HERP

	Rats	Hamsters	Mice	KY1R4F	KY1R4F	KY1R4F	HERP	HERP	HERP
	TD <sub>50</sub>	TD <sub>50</sub>	TD <sub>50</sub>	MA	MA	MA	Rats	Hamsters	Mice
Compound	mg/kg/day	mg/kg/day	mg/kg/day	µg/cig	µg/day	mg/kg/day	(%)	(%)	(%)
1,3-Butadiene	261	NA	13.9	79	3160	0.0451	0.017	NA	0.325
Acetaldehyde	153	565	NA	1054	42160	0.602	0.394	0.107	NA
Acrylonitrile	16.9	NA	NA	21	840	0.012	0.071	NA	NA
Benzene	169	NA	77.5	66	2640	0.038	0.022	NA	0.0487
EO	21.3	NA	63.7	35	1400	0.020	0.094	NA	0.0314
Catechol	84.7	NA	244	81	3240	0.046	0.055	NA	0.0190
Hydroquinone	82.8	NA	225	82	3280	0.047	0.057	NA	0.0208
Formaldehyde	1.35	NA	43.9	36.8	1472	0.021	1.56	NA	0.0479
Toluidine	32.8	NA	633	0.103	4.12	0.0001	0.00018	NA	0.00001
PhiP	1.45	NA	28.6	0.0042	0.168	0.000002	0.00017	NA	0.00001
IQ	0.812	NA	19.6	0.0046	0.184	0.000003	0.00032	NA	0.00001
B[a]P	0.956	NA	11	0.0107	0.428	0.00001	0.00064	NA	0.00006
NNK	0.0999	NA	NA	0.197	7.88	0.0001	0.113	NA	NA
NNN	NA	10.8	NA	0.173	6.92	0.0001	NA	0.00092	NA

TD50 values taken from <http://potency.berkeley.edu> ; HERP values estimated from procedure given at <http://potency.berkeley.edu/pdfs/herp.pdf>

Most smoke deliveries taken from M. E. Counts *et al.*, *Regul. Toxicol. Pharmacol.* 2005 41:185-227; smoke delivery for toluidine taken from E. Roemer *et al.*, *Toxicol.* 2004 195:31-52; smoke delivery for EO estimated from value determined under FTC conditions and reported by B. M Gordon and W. M Coleman, 2003 TSRC Paper #63; smoke deliveries for PhiP and IQ estimated from values determined under FTC conditions and reported by T. A. Sasaki *et al.*, *Analytical Letters* 2001 34:1749-1761.

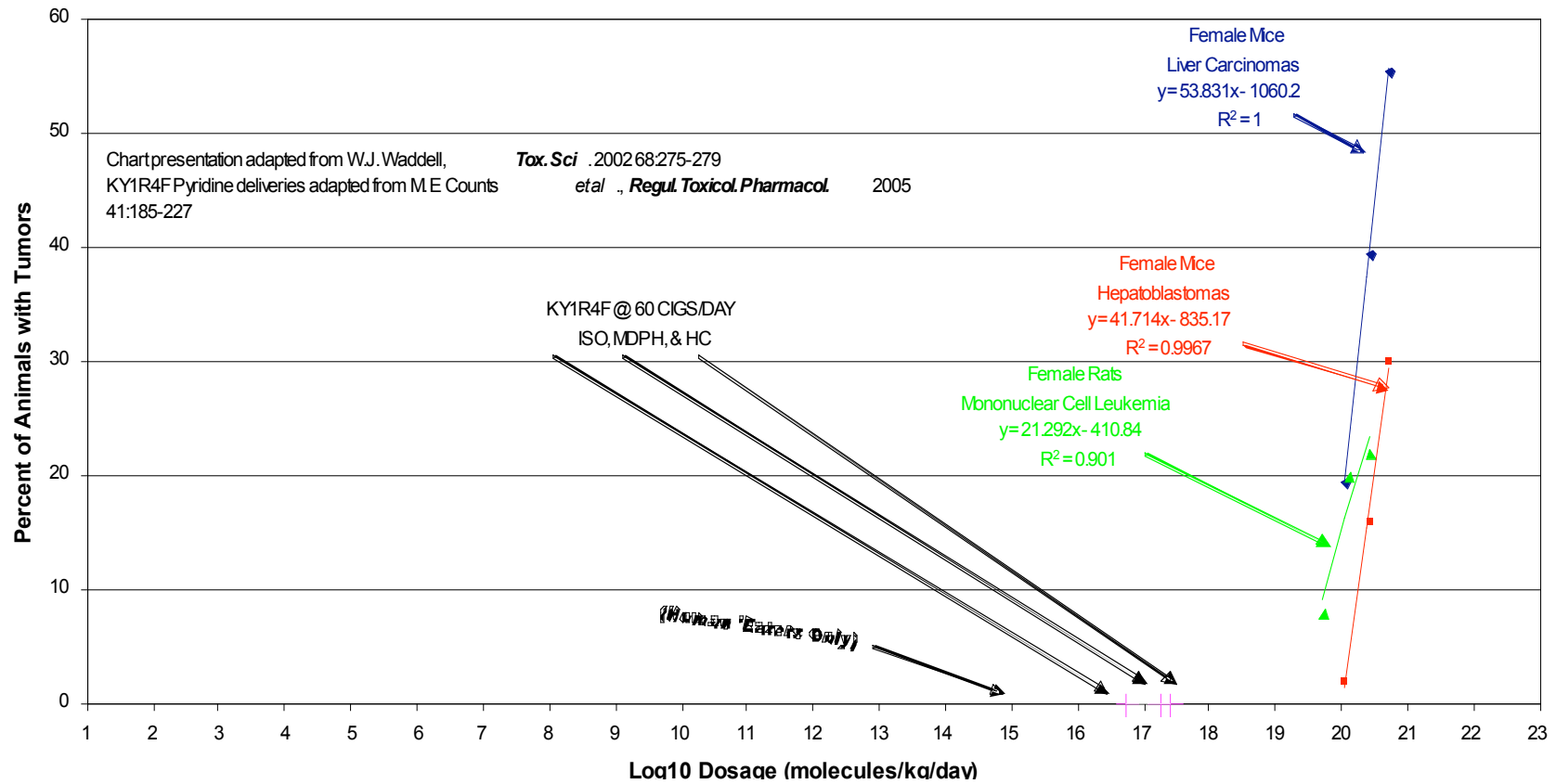
---

## Comments on HERP values

- Values presented are based on two-packs per day and 70 kg body weight
- Volatile components appear to dominate the estimations
  - Acetaldehyde and formaldehyde dominate values for rats as rats reportedly do not give high response with 1,3-butadiene
  - Other than the response in rats to NNK, HERP values for to other particulate-phase compounds are low because of very low levels in smoke
  - Is this approach relevant for particulate-phase compounds in MSS?

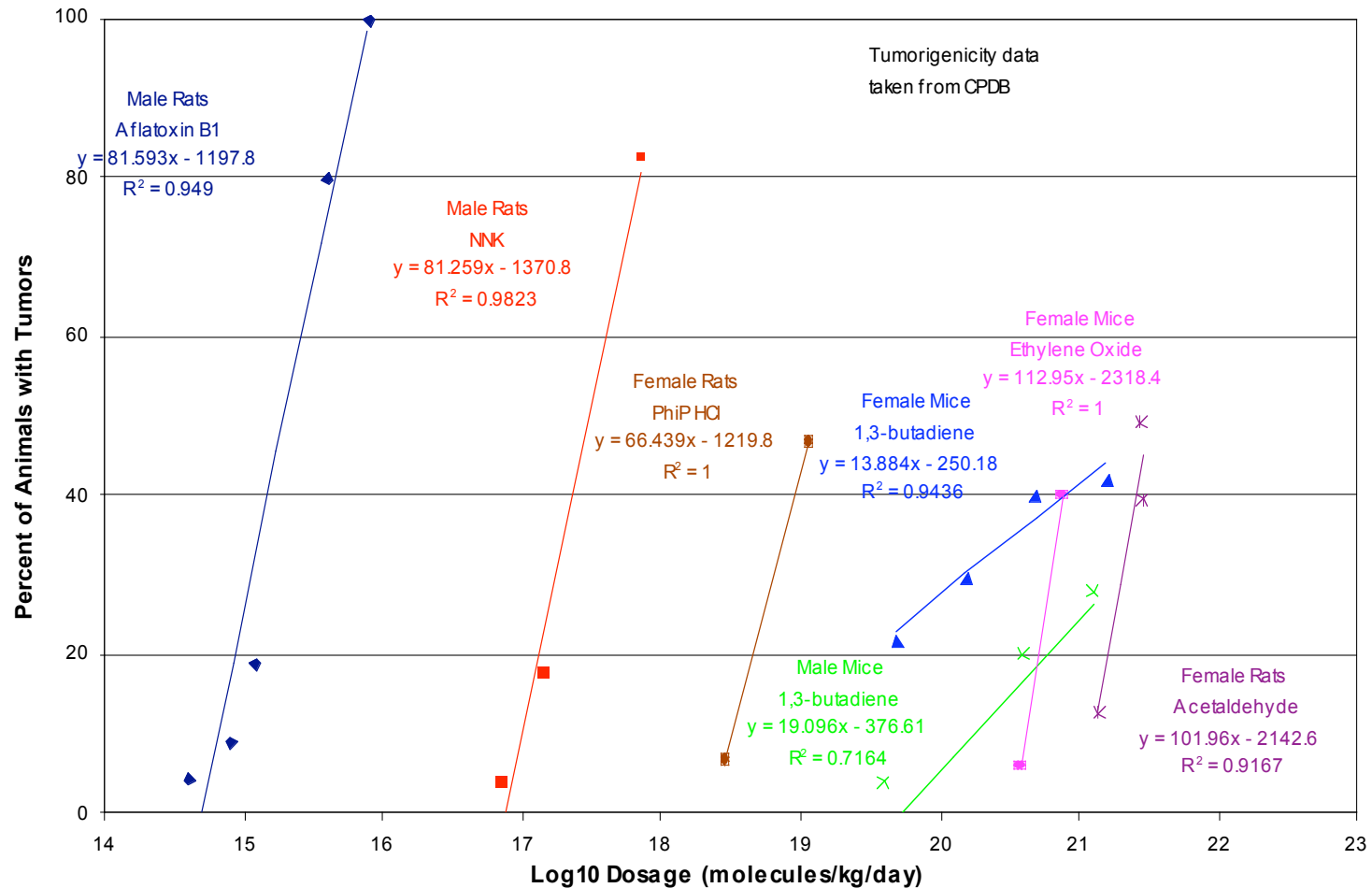
# Is there a threshold for tumorigenicity?

Rozman-scale Plots for Pyridine



# Tumorigenicity thresholds

Rozman-scale Plots for Several Tumorigenic Agents



# Threshold versus estimated dosage

Compound	Species	Sex	Strain	Log10 Threshold	KY1R4F HC Avg	Intake	Log10 Dosage
				(molecules/kg/day)	µg/cig	µg/day	molecules/kg/day
NNK	Rat	M	F344	16.9	0.199	12.0	14.7
1,3 Butadiene	Mouse	F	B6C3F1	18.0	99.5	5967	18.0
PhiP HCl	Rat	F	F344/DuCrj	18.4	0.0062	0.370	13.1
1,3 Butadiene	Mouse	M	B6C3F1	19.7	99.5	5967	18.0
Ethylene oxide	Mouse	F	B6C3F1	20.5	50	3000	17.8
Acetaldehyde	Rat	F	Wistar	21.0	1404	84210	19.2

- Assumptions for estimations
  - Cigarette is KY1R4F
  - Smoker weighs 70 kg and smokes 60 cigarettes per day with intake approximating Health Canada protocol
  - FTC delivery of ethylene oxide for KY1R4F similar to that of KY2R4F

---

## Potential issues with thresholds

- Approaches consider each compound without consideration of other compounds
  - No accounting for possible additivity, synergism, potentiation, antagonism
  - No consideration for reactivity of MSS constituents or partitioning in MSS aerosol
- Apparently no chronic rodent studies done that provide data needed to estimate threshold values and potency for free-radicals found in MSS

---

# Issues with current testing schemes

## ■ Smoke chemistry

- ❑ Are efforts aimed at the right compounds?
- ❑ Should a new list of analytes be drafted based upon today's understanding of bioactivity and analytical chemistry?
- ❑ Can free-radicals continue to be ignored?

## ■ Smoke bioassays

- ❑ Are *in vitro* assays of genotoxicity meaningful if they exclude genotoxic agents not in the TPM?
- ❑ Are *in vitro* assays that show predictable responses to conventional blend and design changes giving value-added information?

---

## Moving forward with MSS testing

- Refocus smoke analyses on compounds that are most relevant at doses incurred over the range of human smoking behaviors
  - Use QSTR information to help chose analytes
  - Use the St. Charles filter analysis method to estimate nicotine delivered to smoker and number of cigarettes consumed and butt length
- If smoke analyses show reductions that are toxicologically significant, what else is needed to move the product to PREP status?

---

# Oral tobacco products as PREPS

- Oral tobacco products should be a simpler situation for PREP status
  - No combustion products
  - No issues related to smoking protocols
  - Can they be treated as any other product taken orally in terms of potential health risks?
- Only components of concern to receive significant attention are the TSNAs
- Epidemiological studies on users of Swedish snus do not show it to be a cause of cancer; only some effect on circulatory system

# Swedish snus and limits

- Swedish snus reportedly made by GothiaTek® process

Component	Limit	Content 2004	Component	Limit	Content 2004
<a href="#">Nitrite (mg/kg)</a>	3.5	1.0 (<0.5 - 2.0)	<a href="#">Cadmium (mg/kg)</a>	0.5	0.2 (0.1 - 0.3)
<a href="#">TSNA (mg/kg)</a>	5	0.8 (0.6 - 1.0)	<a href="#">Lead (mg/kg)</a>	1	0.2 (0.1 - 0.3)
<a href="#">NDMA (µg/kg)</a>	5	0.6 (<0.5 - 1.2)	<a href="#">Arsenic (mg/kg)</a>	0.25	0.07 (<0.03 - 0.13)
<a href="#">BaP (µg/kg)</a>	10	1 (<0.5 - 2.0)	<a href="#">Nickel (mg/kg)</a>	2.25	0.7 (0.2 - 1.3)
Pesticides	According to the Swedish Match pesticide policy		<a href="#">Chromium (mg/kg)</a>	1.5	0.5 (0.3 - 0.8)

*mg/kg = thousandth gram per kilogram product (based on Snus with 50% water content)*

*µg/kg = millionth gram per kilogram product (based on Snus with 50% water content )*

Source: [https://www.gothiatek.com/templates/start.aspx?page\\_id=84](https://www.gothiatek.com/templates/start.aspx?page_id=84)

# Wet snuff, TD50, and HERP values

Component	Assumed	Rats	Hamsters	Mice	Intake	Intake	Dosage	Dosage	HERP-30	HERP-30	HERP-30	HERP-60
	Concentration at 50% Moisture	TD <sub>50</sub> mg/kg/day	TD <sub>50</sub> mg/kg/day	TD <sub>50</sub> mg/kg/day	Wet Snuff @ 30 g/day	Wet Snuff @ 60 g/day	Wet Snuff-30 mg/kg/day	Wet Snuff-60 mg/kg/day	Rats (%)	Hamsters (%)	Mice (%)	Rats (%)
NNK (mg/kg)	5*	0.0999	NA	11	0.15 (mg/day)	0.30 (mg/day)	2.143E-03	4.286E-03	2.1	NA	0.02	4.3
NNK (mg/kg)	0.25**	0.0999	NA	11	0.0075 (mg/day)	0.015 (mg/day)	1.071E-04	2.143E-04	0.11	NA	0.001	0.21
NNN (mg/kg)	5*	NA	10.8	NA	0.15 (mg/day)	0.15 (mg/day)	2.143E-03	8.571E-03	NA	0.02	NA	NA
NNN (mg/kg)	0.55**	NA	10.8	NA	0.0165 (mg/day)	0.033 (mg/day)	2.357E-04	4.714E-04	NA	0.002	NA	NA
NDMA (µg/kg)	5	0.0959	NA	0.189	0.00015 (mg/day)	0.0003 (mg/day)	2.143E-06	4.286E-06	0.002	NA	0.001	0.004
BaP (µg/kg)	10	0.956	NA	NA	0.0003 (mg/day)	0.0006 (mg/day)	4.286E-06	8.571E-06	0.0004	NA	NA	0.001

\* GothiaTek limit on all TSNA's combined is 5 mg/kg

\*\* Estimated amount in several brands of Swedish snus - adapted from Rodu and Jansson, *Crit. Rev. Oral Biol. Med.* . 2004 15:252-263

- Dosage and HERP values based on 30 g per day or 60 g per day and 70 kg body weight
- NNK appears to dominate the estimations
- Data may imply a threshold for NNK effects

---

# Conclusions - 1

- Experts on health effects of smoking continue to point to a few sets of compounds as being responsible for smoking-related diseases
  - They are not saying everything in smoke
  - They are not saying we do not know what compounds should be reduced to make a PREP
- Evidence still points to toxicants in MSS vapor-phase as the likely cause of most smoking-related diseases
  - This puts a high emphasis on chemical analyses
  - It also calls into question bioassays that only use TPM as the matrix and disregard the vapor-phase

---

## Conclusions -2

- Until disproved, free radicals must be viewed as a cause of smoking-related disease
- Chemical data must be obtained under conditions which represent product usage
- If an oral tobacco product has an NNK concentration of less than 0.5 PPM (DWB), and meets other parts of the Gothiatex® standard, available evidence shows that further testing should not be needed for the product to be classified as a PREP