Overview of the Draft IRIS Assessment of Trimethylbenzenes

Presentation for the
TMBs Augmented Chemical Assessment Advisory Committee of the Science Advisory Board
June 17th, 2014

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Outline of Presentation

- **Briefly, this presentation will discuss**
  - The key aspects of the Toxicological Review, especially how toxicological similarities between TMB isomers and toxicokinetic modeling were used to fill in data gaps in isomer-specific databases
  - Clarification on science issues that were raised by public commenters at the teleconference held on May 22nd, 2014

Scope of Toxicological Review

- **Critical review of publicly available literature on *individual* isomers of trimethylbenzene (1,2,3-, 1,2,4-, or 1,3,5-TMB)**

- ***Individual* TMB isomers (1,2,4-TMB and 1,3,5-TMB) were nominated to IRIS Program due to their presence at 41 Superfund sites:**
  - 1,2,4-TMB: 35 sites (83%)
  - 1,3,5-TMB: 38 sites (93%)
  - 1,2,3-TMB: 5 sites (12%)
  - Unspecified isomers: 7 sites (17%)
  - 1,2,4-TMB and 1,3,5-TMB were originally nominated for assessment, 1,2,3-TMB was added at the request of multiple Federal agencies

Hazard Identification - Inhalation

Health Outcome Measure	Exposure Duration	1,2,3-TMB	1,2,4-TMB	1,3,5-TMB
Nervous System Effects	acute	✓	✓	✓
Pain Sensitivity	subchronic	✓	✓	
Pain Sensitivity following foot shock challenge	short-term	✓	✓	✓
Neuromuscular Function	acute	✓	✓	✓
	subchronic	✓	✓	
Motor Function / Anxiety	short-term	✓	✓	✓
Sensitization	short-term	✓	✓	
Cognitive Function	short-term	✓	✓	✓
Electrocortical activity	acute	✓	✓	✓
Respiratory Effects	acute	✓	✓	✓
	Subchronic	✓	✓	
Developmental Effects	gestational		✓	✓
Hematological Effects	subchronic	✓	✓	
Carcinogenicity	chronic			

The table denotes the presence or absence of data and does not distinguish between outcomes measures with larger vs. smaller databases

Hazard Identification - Oral

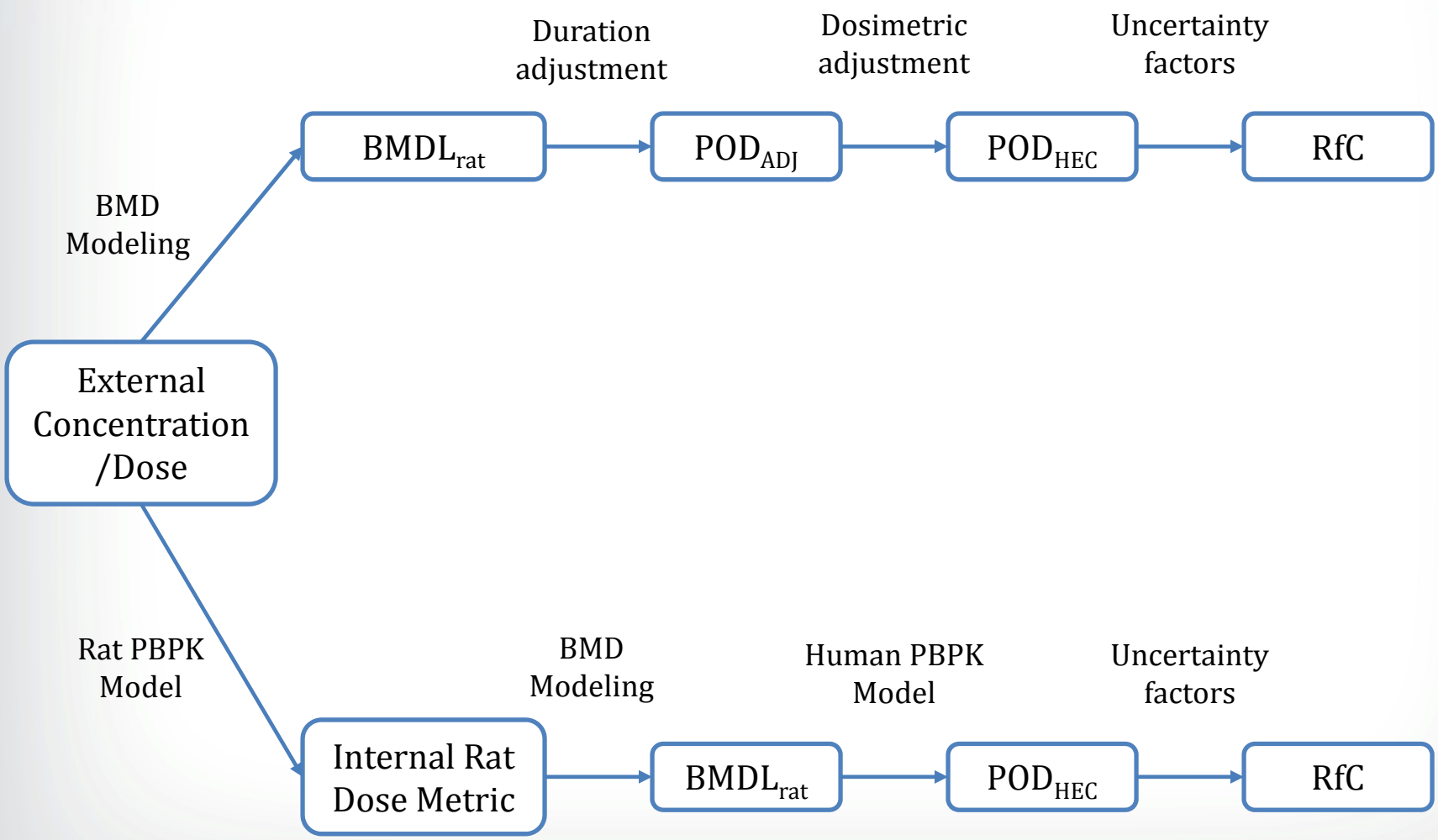
- **No chronic or subchronic studies were identified that investigated the non-cancer toxicity of 1,2,4-TMB or 1,2,3-TMB following oral exposures.**

- **Only one subchronic study was identified that examined the effects of oral exposure to 1,3,5-TMB – Koch Industries (1995b).**
  - Minor changes in hematological parameters (increased monocytes) and clinical chemistry parameters (altered sodium, chloride, potassium levels) were noted.
  - Study was unpublished industry report; EPA sought a external peer review which highlighted several deficiencies in the study that precluded using it for RfD derivation.

Isomer-specific RfC and RfD Derivations

Isomer	Reference Concentration 5×10^{-2} mg/m^3	Reference Dose 2×10^{-2} mg/kg-day
1,2,4-TMB	Subchronic toxicity study to support RfC derivation	Route-to-route extrapolation from RfC for 1,2,4-TMB using PBPK model
1,2,3-TMB	Subchronic toxicity study to support RfC derivation	Adopted from 1,2,4-TMB based on sufficient similarity of the isomers
1,3,5-TMB	Adopted from 1,2,4-TMB based on sufficient similarity of the isomers	Adopted from 1,2,4-TMB based on sufficient similarity of the isomers

Use of PBPK Model for 1,2,4-TMB RfC Derivation



Summary of Major Comments

- **Public comments were provided that recommended the inclusion of multiple C9 fraction inhalation studies in the assessment and that these studies should form the basis for RfC derivations**

- **Public commenters disagreed with the selection of decreased pain sensitivity as the critical effect, particularly on the basis that the effect is observed to be reversible following the termination of subchronic exposures**

Use of C9 Fraction Studies in the TMB Assessment – TSCA Testing

- **The C9 fraction studies were conducted as required under the Toxic Substances Control Act, and announced in a1985 Federal Register Notice**

- **In this FRN, EPA agreed that "assessing the toxicity of the C9 mixture as a complete entity should provide a reasonable upper bound for the toxicity of the individual ethyltoluene and TMB [isomers] in the C9 mixture"**
  - The results of multiple peer-reviewed studies conducted after 1985 demonstrate that individual TMB isomers elicit clearly adverse toxicological effects

- **In the same FRN, EPA also agreed that testing the C9 fraction was appropriate under the assumption that TMBs were not released to the environment in substantial quantities nor expected to persist once released**
  - Again, more recent information shows that substantial quantities of TMB isomers are released to the environment (2012 TRI), they persist (detected at Superfund sites) and human exposures do occur

Composition of the C9 Fraction

- **Public commenters agreed with EPA's determination that TMB isomers are toxicologically and toxicokinetically similar to one another,**
  - They further suggested "that if all isomers are equivalent, that studies of mixtures of isomers are also equally relevant"; however, this conclusion is only applicable if the mixtures under consideration are TMB-only mixtures

Constituent	Clark et al. (1990)	Douglas et al. (1993)	Schreiner et al. (1989)	McKee et al. (1990)
TMBs	44.81	55.05	55.05	55.05
ethyltoluenes	30.96	27.59	27.59	27.59
o-xylene	2.27	3.20	3.20	3.20
n-propylbenzene	4.05	3.97	3.97	3.97
Non-aromatics	0.46	n/r	n/r	n/r
cumene	n/r	2.74	2.74	2.74
Benzene	n/a	n/r	n/r	n/r
C10s	8.31*	6.19**	6.19**	6.19**
Unknown	9.15	1.26	1.26	1.26

n/r = not reported; n/a = reported to not be present; * = 1-methyl-3-n-propylbenzene, 1,2-diethylbenzene, and 1-ethyl-3,5-dimethylbenzene; ** specific C10 compounds not reported

Differences in Study Design – C9 vs. TMB Neurotoxicity Studies

- **Uncertainties exist when attempting to discern why less neurotoxicity (in the form of thermal nociception) is observed in the C9 study (Douglas et al, 1993) vs. the individual isomers used in Korsak and Rydzyński (1996)**

Source of Uncertainty	Korsak and Rydzyński, 1996	Douglas et al., 1993
Exposure concentrations	0, 123, 492, 1230 mg/m^3 TMB isomers	0, 273, 1169, 3571 mg/m^3 TMB in the C9 mixture
Strain/Age	Wistar rats/age not given (PND 150 rats used in Nofer short-term studies)	CD rats/PND 52
Hot plate temperature	54.5° C	Unspecified (protocol based on Ankier, 1974 (mouse study) which used separate temperatures: 50°, 55°, or 59° C)
Lag between exposure and testing	Immediately after termination of exposure at end of 90-day exposure*	24-48 hours** after termination of exposure, tested at 0, 5, 9, and 13 weeks
Possible subtle differences in subjective recording of responses	Analgesia indicated by "licking of foot"	Indicated as "vigorous licking of front or hind paws" in Ankier, 1974
Differences in testing design	At 3 months, a single hot plate test and rotarod test (footshock reinforcement may/ may not have been used) were performed	One cohort was tested in hot plate tests, other FOB tests, and motor activity tests at 0, 5, 9, and 13 weeks
Randomization into exposure groups	Not randomized by weight (but weights across groups unchanged at end of study)	Randomized by weights

*Note: short-term TMB isomer studies evaluated effects weeks to months after end of exposure

**Timing of testing after exposure was not reported, but was indicated as either 24 or 48 hours in public comments

Differences in Neurotoxicity Outcomes – C9 vs. TMB

- **One study (McKee et al., 2010) investigated the neurotoxicity of the C9 fraction and 1,2,4-TMB alone in the same study following exposures of 1 or 3 days**

	C9 Fraction		1,2,4-TMB	
	1 day	**3 days**	**1 day**	**3 days**
Touch Response	5000* mg/m^3: -27% (NS) 1010 mg/m^3: -14% (NS)	5000 mg/m^3: -26%** 1010 mg/m^3: -5% (NS)	No Effect at 5000 or 1250 mg/m^3	
Systemic Toxicity				
Body Weight	No Effect	5000 mg/m^3: -10%**	No Effect	5000 mg/m^3: -5%**
Body Temperature	5000 mg/m^3: -1.5°**		No Effect	5000 mg/m^3: -0.5°**
Respiratory Toxicity	5000 mg/m^3: Overt toxicity (red-colored exudate around mouth and nose)		No Effects	

*5000 and 1010 mg^3 C9 fraction ~ 2727 and 556 mg^3 1,2,4-TMB; effects seen at 1010 mg/m^3 C9 are very close to LOAELs reported in Nofer Institute studies (492 mg/m^3)
** $p < 0.05$

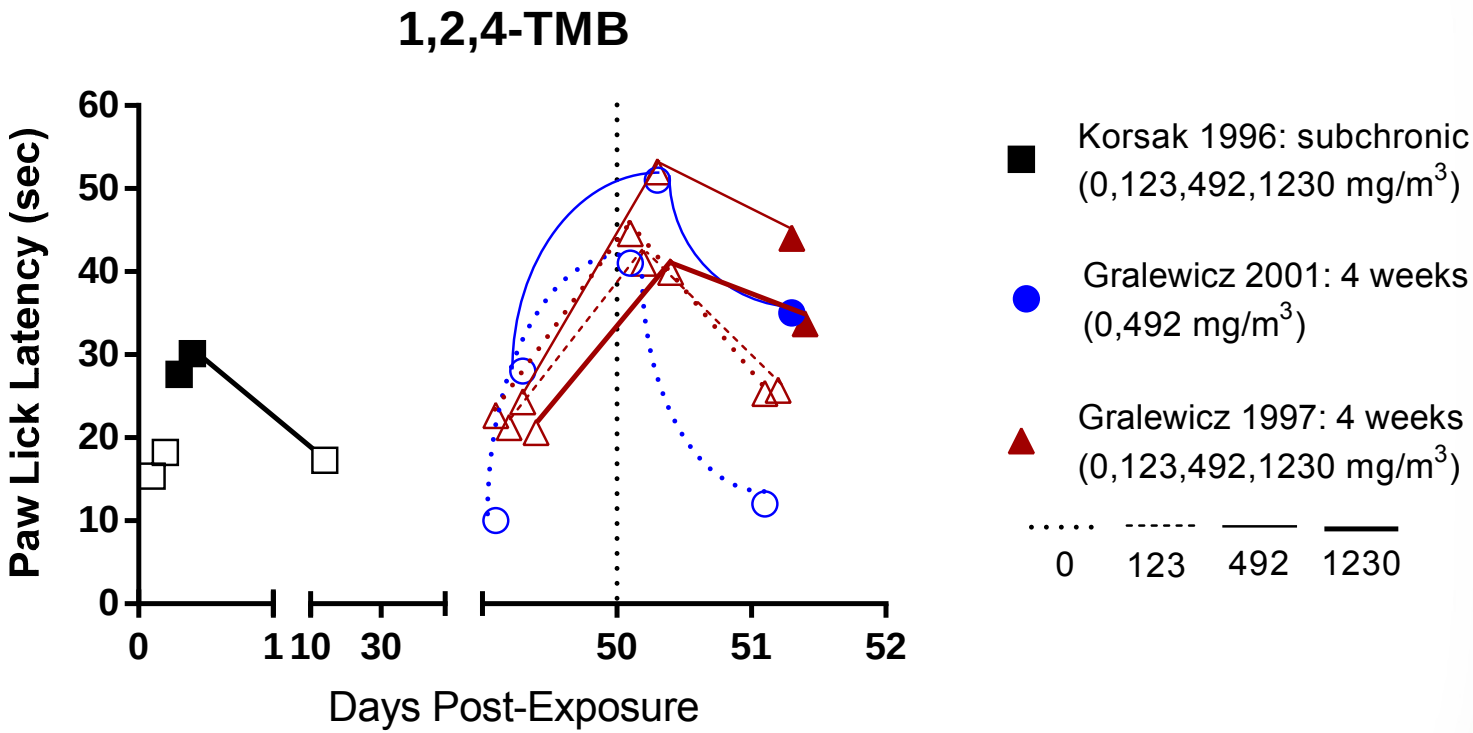
- **Although it remains unclear why, differences in neurotoxicity and systemic toxicity are observed for the C9 fraction and 1,2,4-TMB. These differences support the conclusion to exclude C9 fraction studies from the TMB assessment**

Selection of Decreased Pain Sensitivity as Critical Effect

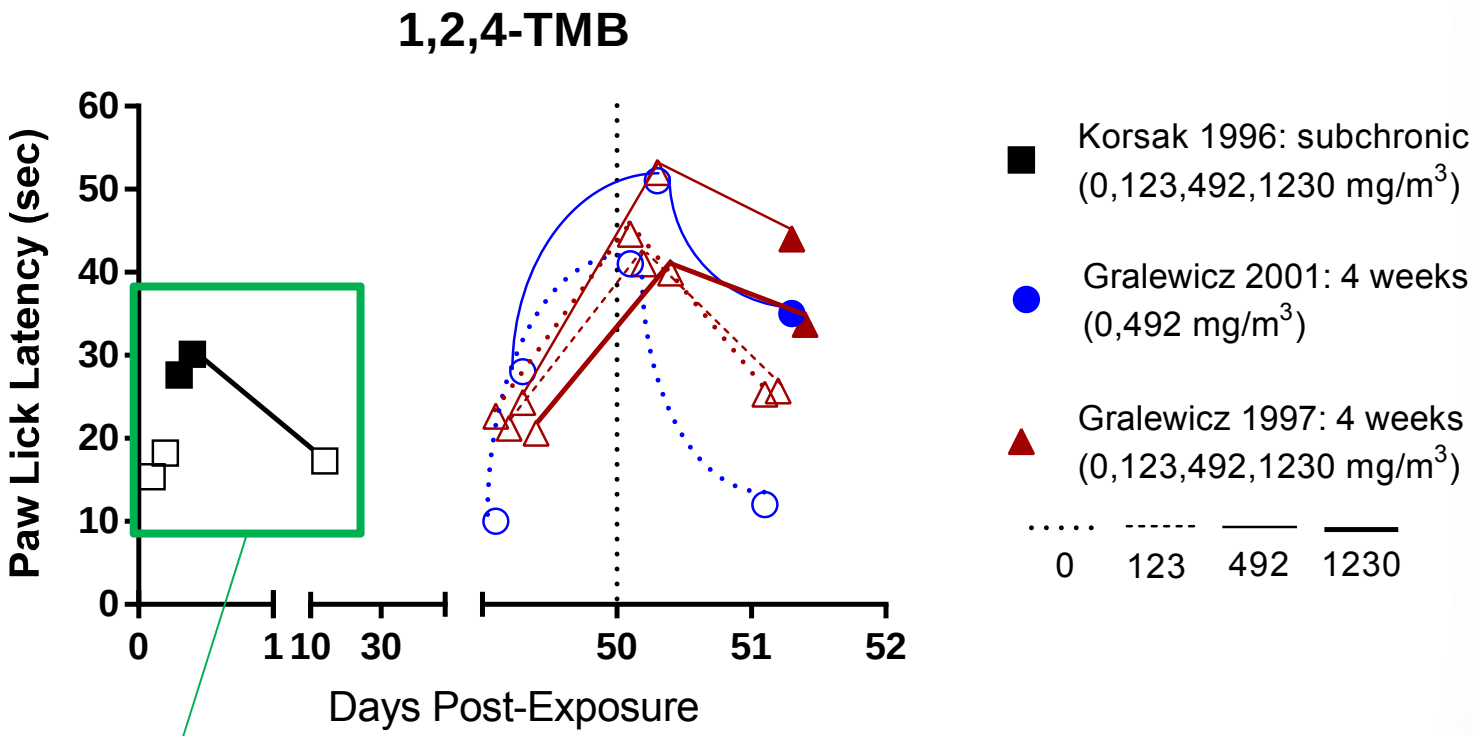
- **Public commenters disagreed with the selection of decreased pain sensitivity as the critical effect, particularly on the basis that the effect is observed to be reversible following the termination of subchronic exposures**

- **EPA's Guidelines for Neurotoxicity Risk Assessment state that rapidly reversible effects that appear to be associated with the pharmacokinetics of the causal agent and its presence in the body are of lesser concern**
  - However, the Guidelines also also state that reversible effects that occur in settings where these effects could pose a danger (e.g., occupational settings) may be of high concern
  - Further, several neurotoxicity studies provide evidence of sustained effects not related to the presence of TMB isomers in the body:
    - Extension of footshock-induced decreases in pain sensitivity and decreased step-down latency in passive avoidance tests several weeks after short term exposures
    - Impaired rotarod performance 14 days after subchronic exposure

Time Course of Decreased Pain Sensitivity – 1,2,4-TMB

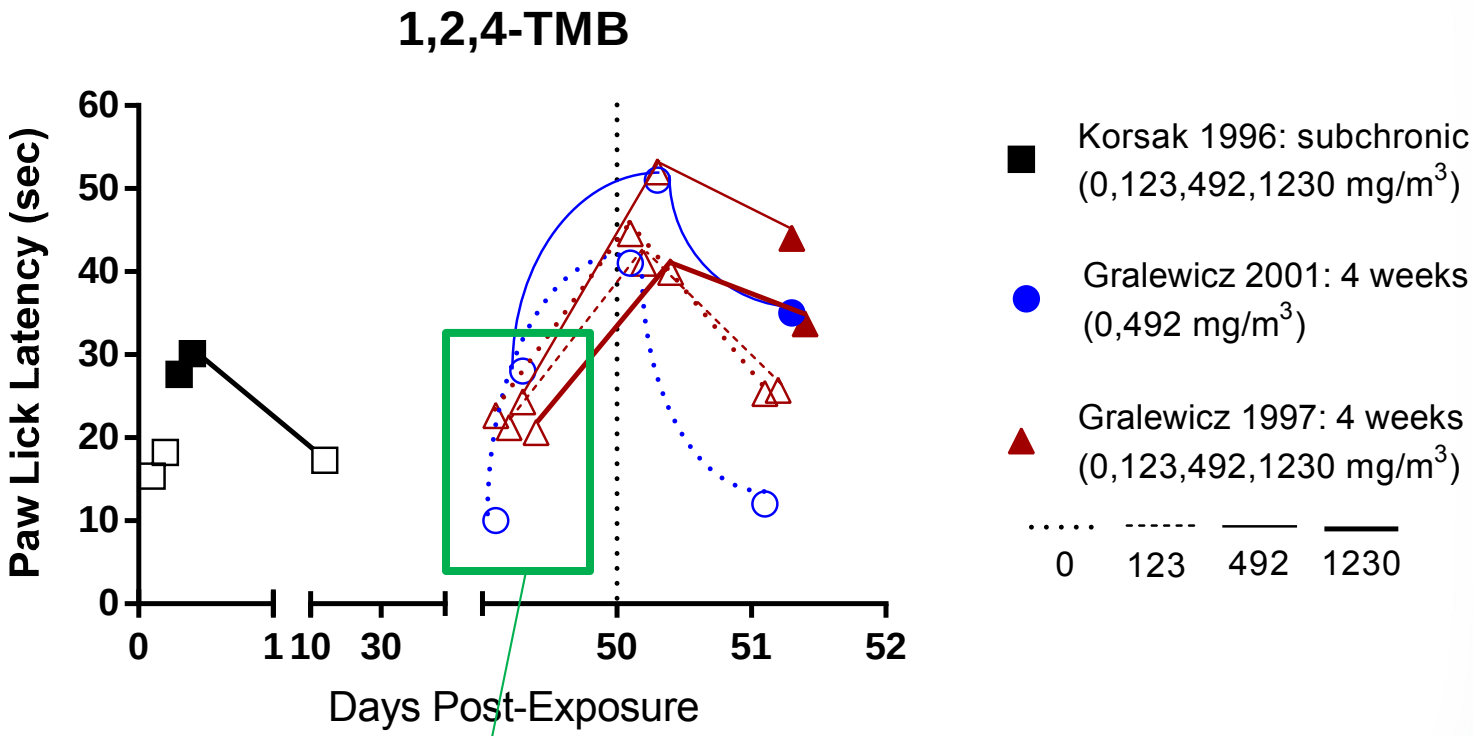


Time Course of Decreased Pain Sensitivity – 1,2,4-TMB



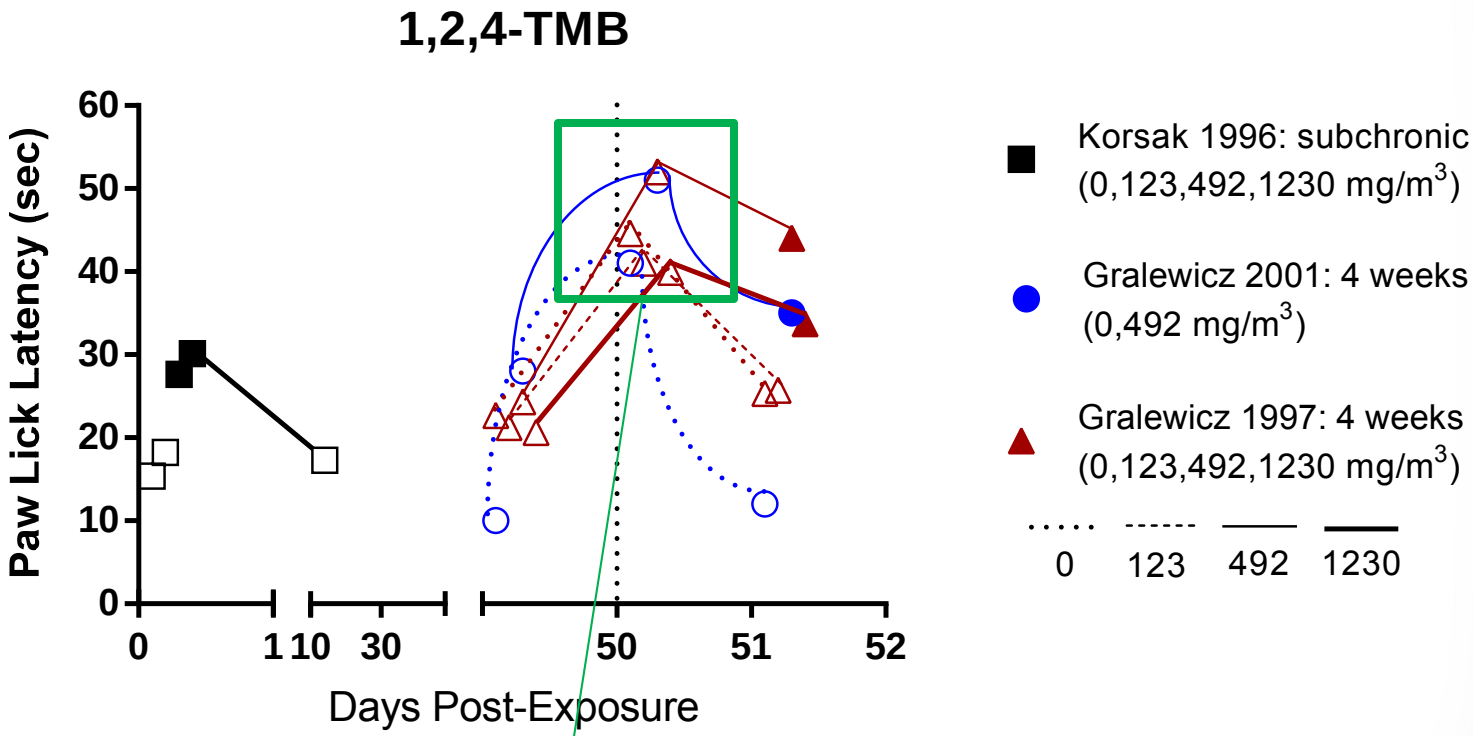
Effects at ≥ 492mg/m^3 measured immediately after subchronic exposure appear to resolve within 2 weeks

Time Course of Decreased Pain Sensitivity – 1,2,4-TMB



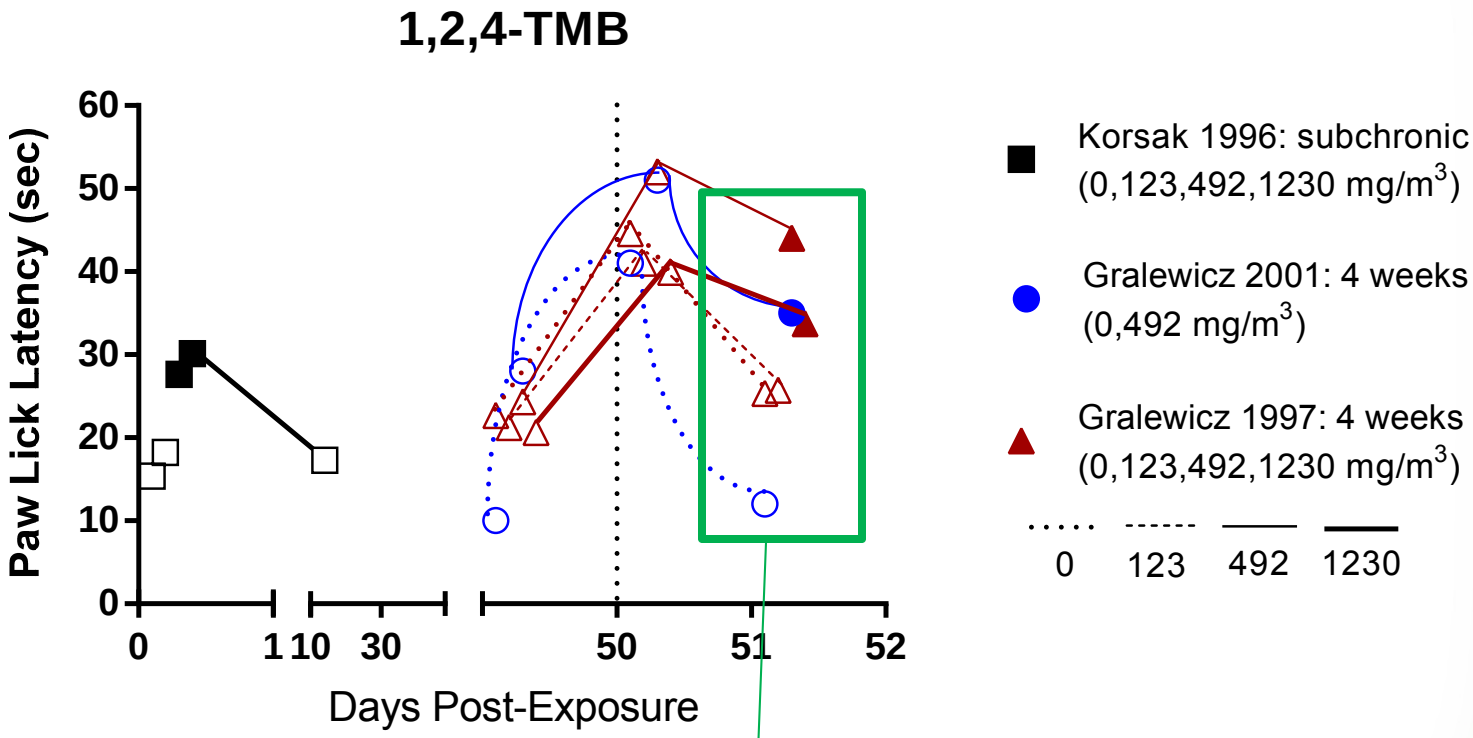
No statistically significant effects several weeks after short-term exposure

Time Course of Decreased Pain Sensitivity – 1,2,4-TMB



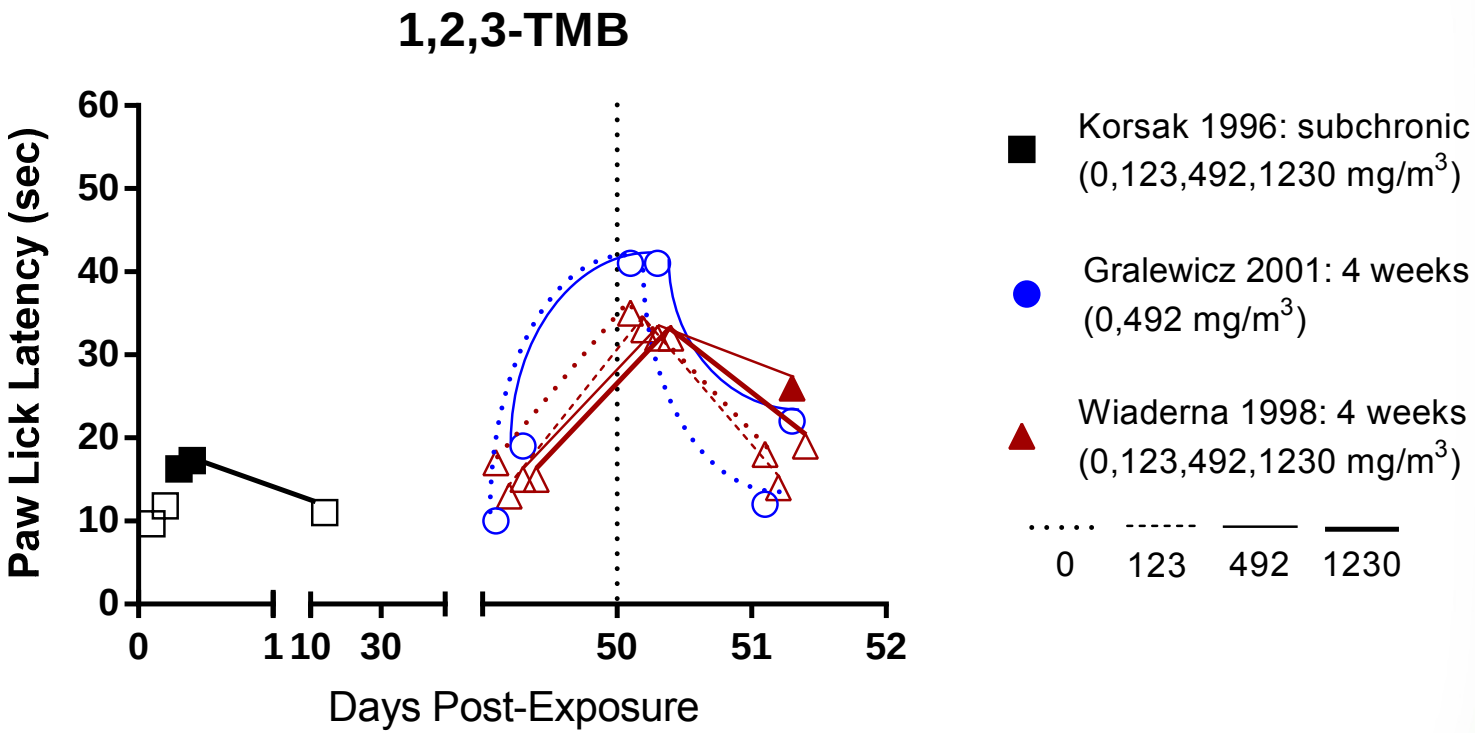
No statistically significant amplification of footshock-induced decreases in pain sensitivity several weeks after exposure

Time Course of Decreased Pain Sensitivity – 1,2,4-TMB

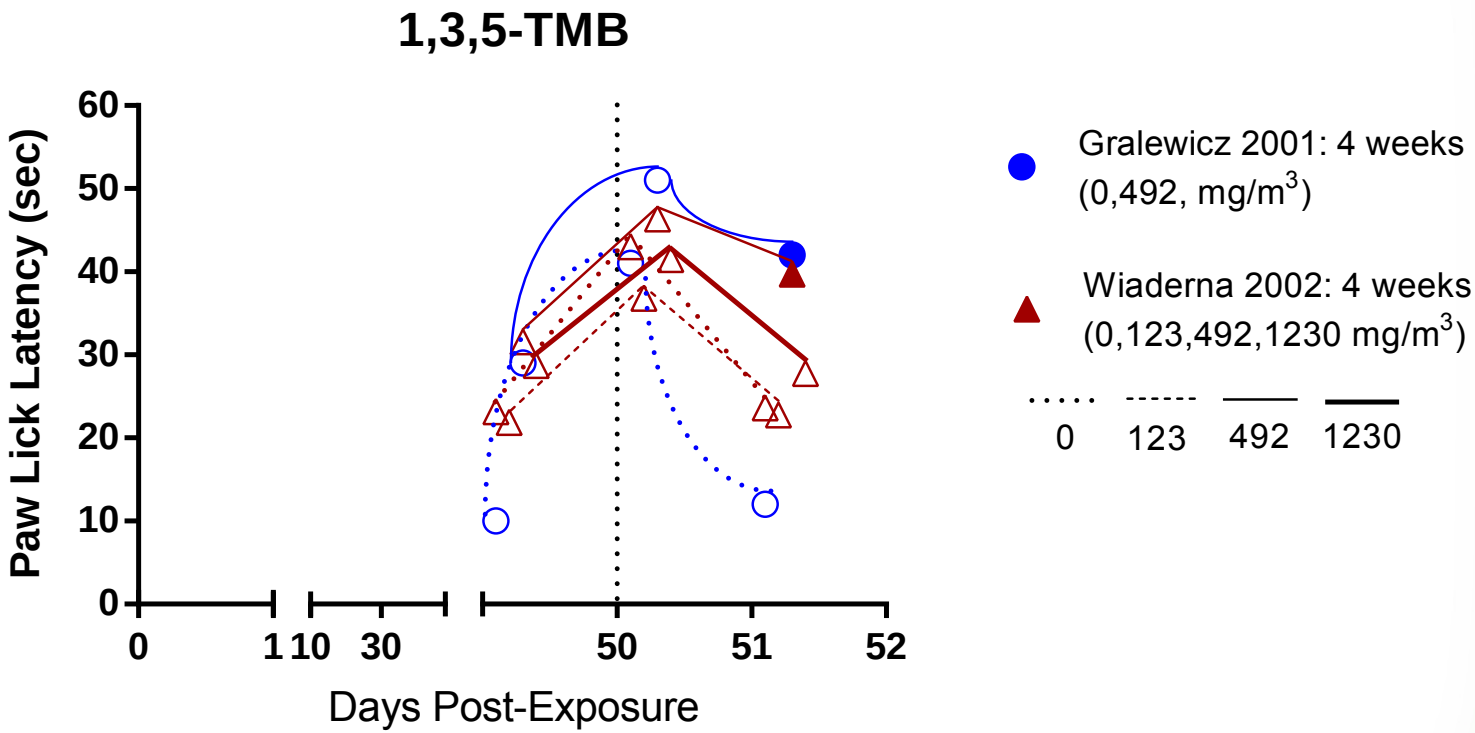


Consistently, across isomers, footshock-induced decreases in pain sensitivity appear to persist/ are elongated at ≥ 492mg/m^3 when tested 1 day post-footshock several weeks after exposure

Time Course of Decreased Pain Sensitivity – 1,2,3-TMB



Time Course of Decreased Pain Sensitivity – 1,3,5-TMB



EPA Guidelines Support Selection of Critical Effect

- **EPA's Guidelines for Neurotoxicity Risk Assessment support the identification of decreased pain sensitivity as adverse and neurotoxic, despite its apparent reversibility in the Korsak and Rydzyński (1996) subchronic study, as follows:**
  - Portions of the nervous system have a high capacity for adaptation; however, once damaged, neurons are thought to have a limited capacity for regeneration. Thus, as stated in the Guidelines (p. 9): "Reversibility of effects resulting from cell death or from the destruction of cell processes may represent an activation of repair capacity, *decreasing future potential adaptability. Therefore, even reversible neurotoxic changes should be of concern*."
  - Regarding the short-term Nofer studies, "delayed-onset effects (those that occur at a time distant from the last [exposure]) or latent effects (those that become evident only after an environmental challenge or aging) *have a high level of concern*."
  - In addition, the specific context of the RfC assumes a constant, lifetime exposure.
  - Finally, the apparently-reversible effects on pain sensitivity are supported by multiple other neurotoxic effects that persist well after exposure is ended, and at levels equivalent to or below those identified for decreased pain sensitivity. These supportive data indicate that at least some effects on the CNS are irreversible.

C9 vs. TMB Neurotoxicity Studies

- **Public comments suggest that Douglas et al. (1993) provides complementary information that, in animals tested 24-48 hours after termination of exposure to the C9 fraction, no effects on the hot plate test were observed after 5, 9, or 13 weeks of exposure**
  - This forms the basis of the public's conclusion that latency to response in the hot plate test is due to acute, reversible effects that are not influenced by repeated exposures
  - However, this lack of effects 24-48 hours after exposure observed in Douglas et al. (1993) does not necessarily contradict the results from the Nofer Institute studies, which observed effects immediately following termination of exposure, but not several weeks after exposure without the addition of an exogenous challenge
  - Further, the public's conclusion does not account for extending footshock-induced decreases in pain sensitivity observed 51 days following the termination of short-term exposures

Reversibility of Decreased Pain Sensitivity – Subchronic UF

- **The observed reversibility of decreased pain sensitivity in the subchronic Korsak and Rydzyński (1996) study has been taken into account in the RfC and RfD derivations**
  - The subchronic-to-chronic uncertainty factor was lowered from 10 to 3 given the evidence of reversibility for the chosen neurotoxic effect as tested in the particular protocol employed by Korsak and Rydzyński (1996) study
  - A value of 3 was used instead of 1 given the possibility of cumulative damage occurring over the duration of a chronic study, or the possibility that adaptive capabilities would be overwhelmed in a chronic exposure scenario

Summary of Major Comments

- **Public comments were provided that recommended the inclusion of multiple C9 fraction inhalation studies in the assessment and that these studies should form the basis for RfC derivations**
  - The C9 fraction is complex mixture containing only ~45-55% TMB isomers
  - The C9 fraction data has been evaluated by IRIS and determined not to be applicable to the TMB IRIS assessment

- **Public commenters disagreed with the selection of decreased pain sensitivity as the critical effect, particularly on the basis that the effect is observed to be reversible following the termination of subchronic exposures**
  - EPA's Guidelines for Neurotoxicity Risk Assessment support the selection of decreased pain sensitivity as the critical effect based on the observance of persistent neurological effects (characterized as both delayed-onset and latent effects) and that reversible effects may indicate activation of repair capacity and potential decreases in future adaptability