



# IMPROVEMENTS IN UNDERSTANDING EXPOSURE AND TOXICITY ISSUES ASSOCIATED WITH RDX: RECENT UPDATES

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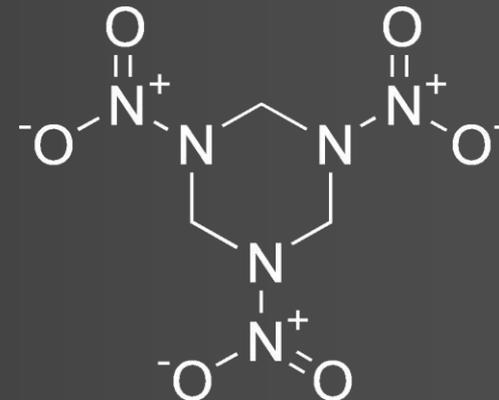
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# Outline

- Background
- The regulatory issues
- Recent advances
  - Cancer reassessment
  - Non-cancer reassessment
  - Mode of action in the development of neurotoxic effects
  - Physiologically-based, pharmacokinetic (PBPK) models
  - Reassessment of the relative source contribution in groundwater

# Background



- RDX , 1,3,5-trinitrohexahydro-1,3,5-triazine is an explosive with widespread application as a component in propellants, detonators, grenades, bombs and a variety of other military ordnance.
- RDX has become a contaminant at military bases in the U.S. Inadvertent accumulation of low order/incomplete detonations on ranges has resulted in environmental contamination in soil with concentrations detected in groundwater.
- Issues – Oral exposures from RDX-contaminated groundwater. Log  $K_{ow}$  = 0.87, low affinity for carbon; water solubility = 60 mg/L

# Current USEPA Criteria

- Possible human carcinogen - hepatocellular carcinomas in female (not male) B6C3F1 mice (Lish et al., 1984). Oral Slope Factor = 0.11 (mg/kg)/d.
- Oral Referenced Dose (RfD) of 3  $\mu\text{g}/\text{kg}/\text{d}$  is based on incidence of prostatic inflammation in a 24 month rat study (Levine et al., 1983; NOAEL = 0.3 mg/kg/day).
- Longer-Term Health Advisory (LTHA) for RDX of 0.35 mg/L is based on the No-Observed-Adverse-Effect-Level (NOAEL) in seizing monkeys (Martin and Hart, 1974). Lifetime HA = 2  $\mu\text{g}/\text{L}/\text{d}$  based on prostate inflammation in geriatric rats.
- Acute oral Minimum Risk Level (MRL) of 0.06 mg/kg/d is based on the 20 mg/kg/day dose of RDX-induced convulsions in animals (Angerhofer et al., 1986).
- Included in Contaminant Candidate Lists (CCL) 1,2 &3 but not selected for regulatory determination. RDX is not regulated by Safe Drinking Water Act. DWEL = 100  $\mu\text{g}/\text{L}$  Lifetime HA = 2  $\mu\text{g}/\text{L}$

# Regulatory criteria - summary

- Cancer – based on single study/single sex
- Non-cancer effects
  - Blood effects
  - Convulsions (neurological)
  - Prostate inflammation
- Federal and State drinking water values are variable.

# Uncertainty Factors - RfD

- Animal to Human (Interspecies) - 10
- Sensitive Sub-population (Intraspecies) - 10
- Relative Source Contribution – 0.2
- Developmental Effects - 3
- Subchronic to Chronic Uncertainty – 10
- LOAEL to NOAEL - 10

Lowest Adverse Observed Effect Level (LOAEL) = 100 mg/kg/d

LOAEL to NOAEL = 10

Interspecies = 10

Intraspecies = 10

Subchronic to chronic = 10

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RfD = 0.01 mg/kg/d

# USEPA Re-evaluation of RDX

- The EPA encourages information which would refine estimates of the HA and RfD enabling a more predictive approach to determine UFs rather than a conservative protective approach using classic defaults.
- Recent, scientific information regarding toxicodynamics, toxicokinetics and mechanisms will provide a more accurate reassessment and determination of safe levels of exposure.
  - If these values remain low or become lower, training and testing activities will be adversely affected, adversely affecting military readiness.
  - If these values are artificially low, significant resources will be spent clean up costs associated with unnecessary remediation costs.

# Cancer Reassessment

- Weak evidence for carcinogenicity
  - Negative results for:
    - Ames assays (bacterial mutagenicity)
    - Mammalian *in vitro* assays (mouse lymphoma & CHO)
    - Mammalian *in vivo* assays (mouse micronucleus).
    - Not teratogenic in rats or rabbits (Cholakis et al., 1980; Reddy et al., 2005).
  - Positive in the non-standard TA97a strain of *Salmonella tryphimurium* following s9 activation (Pan et al., 2007).
- Negative for cancer in chronic rat study (Levine et al. 1983).
- Positive for liver neoplasms found in female mice (Lish et al. 1984)
  - Reassessed by a team of pathologists to determine incidence of cancer using current criteria (Parker et al., 2006).
    - Lish did not define diagnostic criteria; several neoplasm were reclassified as non-neoplastic lesions using modern diagnostic criteria.
    - Used military grade (~89% RDX); changed dose midway through study.
    - Incidence of neoplasm for all groups within range of spontaneous neoplasms of female mice (incidence in Lish controls inordinately low) significance only in 35 mg/kg/day, i.e., only equivocal evidence of carcinogenicity.

# Cancer slope factor

- Data for quantitative evaluation of cancer risk from RDX exposure are weak at best.
- The RDX cancer reevaluation is in process:  
Data are consistent with the descriptor:

“Suggestive of Cancer”

- Quantitative risk assessments may soon have to be done using only the non-cancer RfD

\*Pending outcome of USEPA reevaluation

# Non-cancer Reevaluation

- Evaluate endpoints
  - Neurotoxic – brain
  - Hemotoxic – blood
  - Immunotoxic – prostate
- Reduce uncertainty
  - Determine dose to target in animals
    - Extrapolate more accurately to humans
  - Determine if humans are as sensitive as animals
    - Understand mechanism
    - Understand physiological differences
- Reduce uncertainty factors
  - Reduce interspecies UF – 3
  - Provide data to support changing RSC to 0.5

# SUBCHRONIC ORAL TOXICITY OF RDX IN RATS

(Crouse et al. 2006)

## ■ Results

- Maximum Tolerated Dose (MTD) was 20 mg/kg/d; loss of body weight, convulsions and death observed.
- No blood effects seen at any dose; a result inconsistent with anemia reported by Cholakis et al., 1980 (90 d) and Levine et al., 1983 (2 yr; NOAEL = 0.3 mg/kg/day).
- No evidence of immunosuppression.
- No treatment-related prostate effects.
- LOAEL based on neurological effects (convulsions) at 8 mg/kg/d; NOAEL = 1 mg/kg/day.

# Neurotoxicity is consistent endpoint

- Convulsions consistently observed with increased salivation in mammals
  - Human - Barsotti and Crofti, 1949; Kucukardalr, 2003
  - Primates – Monkey (Martin and Hart 1974)
  - Rat - Burdette et al., 1988; Crouse et al., 2006
  - Pig – Bannon (in prep).
  - Northern Bobwhite – Quinn et al. 2008
  - Western fence lizard – McFarland et al. 2009

Mechanism of Seizure Unknown

# Interspecies variation in LOAEL/NOAEL values

	Western fence lizard (mg/kg-d)	Northern bobwhite (mg/kg-d)	Mammals (mg/kg-d)
TNT	25/15	70/20	8/2
DNT (2,4/2,6)	25/15	15/5 40/10	1.5/0.2 7/nf
RDX	<b>5/2.5</b>	<b>8/3*</b>	<b>8/4</b>
A-DNT	15/5	14/3	In progress
HMX	~5000/na	~5000/na	10/5

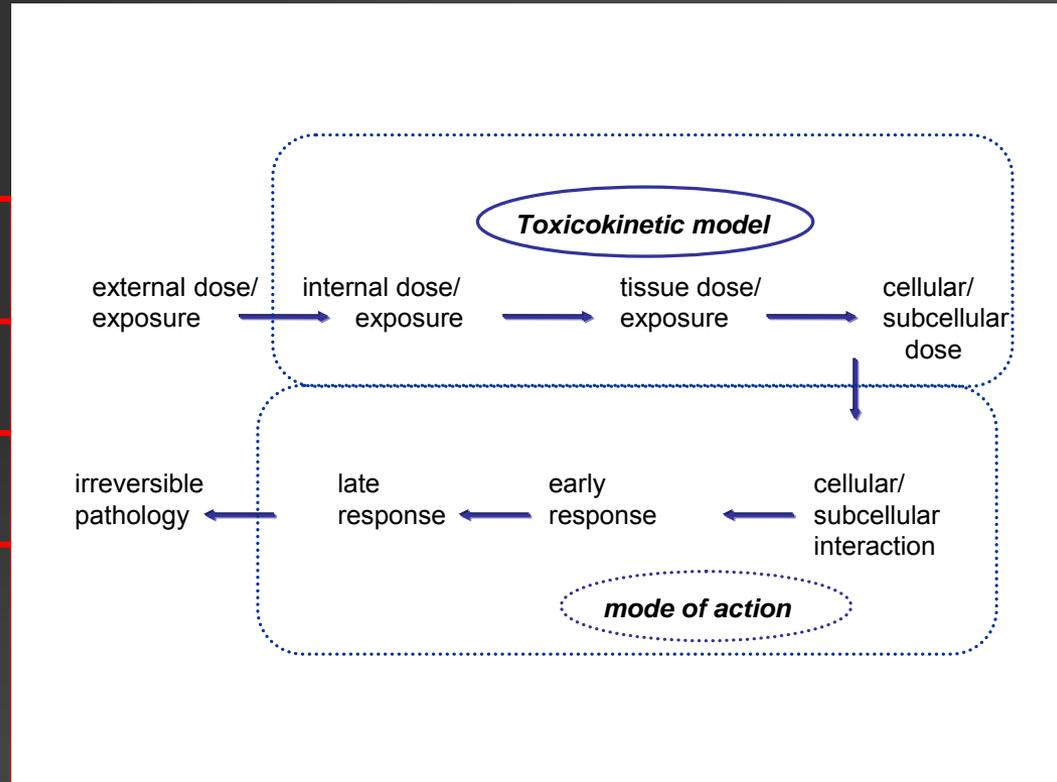
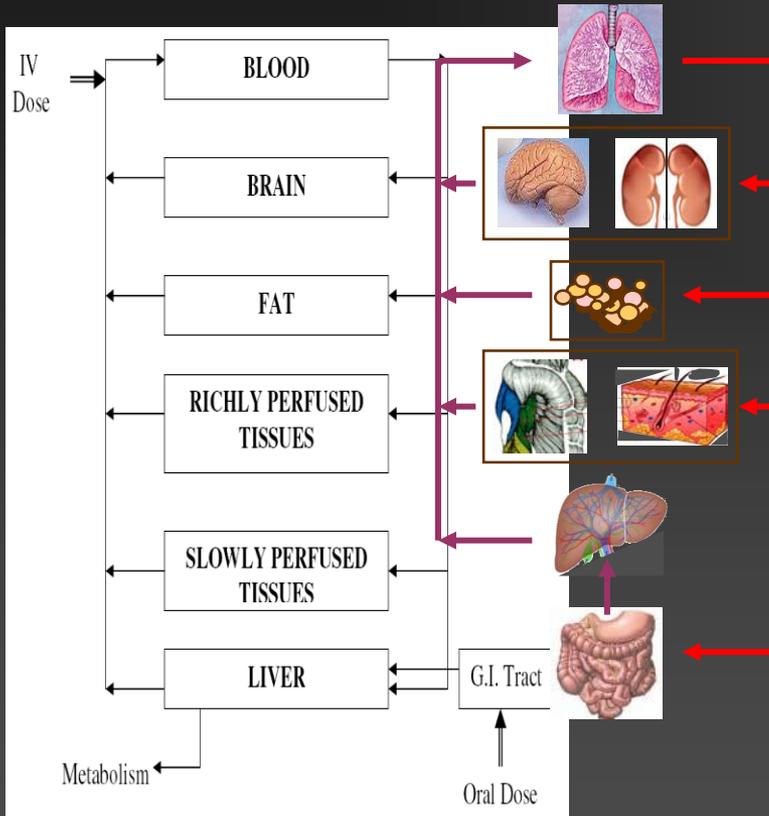
\* - 14-d gavage; all others 60-d exposures.

nf – NOAEL not determined

# Toxicokinetics:

- Oral absorption is fast
  - Onset of seizures in rats is ~11 min at 75 mg/kg
  - Plasma concentration at seizures is unknown
  - Brain concentration in rats at seizure is unknown (~20 ppm in quail)
  - Blood/plasma coeff = 1:1
- Metabolism and excretion – Oxidative enzymes cleave ring, produce (Jackson et al., 2007; Major et al., 2007):
  - 4-nitro-2,4, diazabutanal (NDAB)
  - methylenedinitramine (MEDINA)
  - 4-nitro-2,4,-dianza-butanamide in urine

# PBPK Modeling



ADME

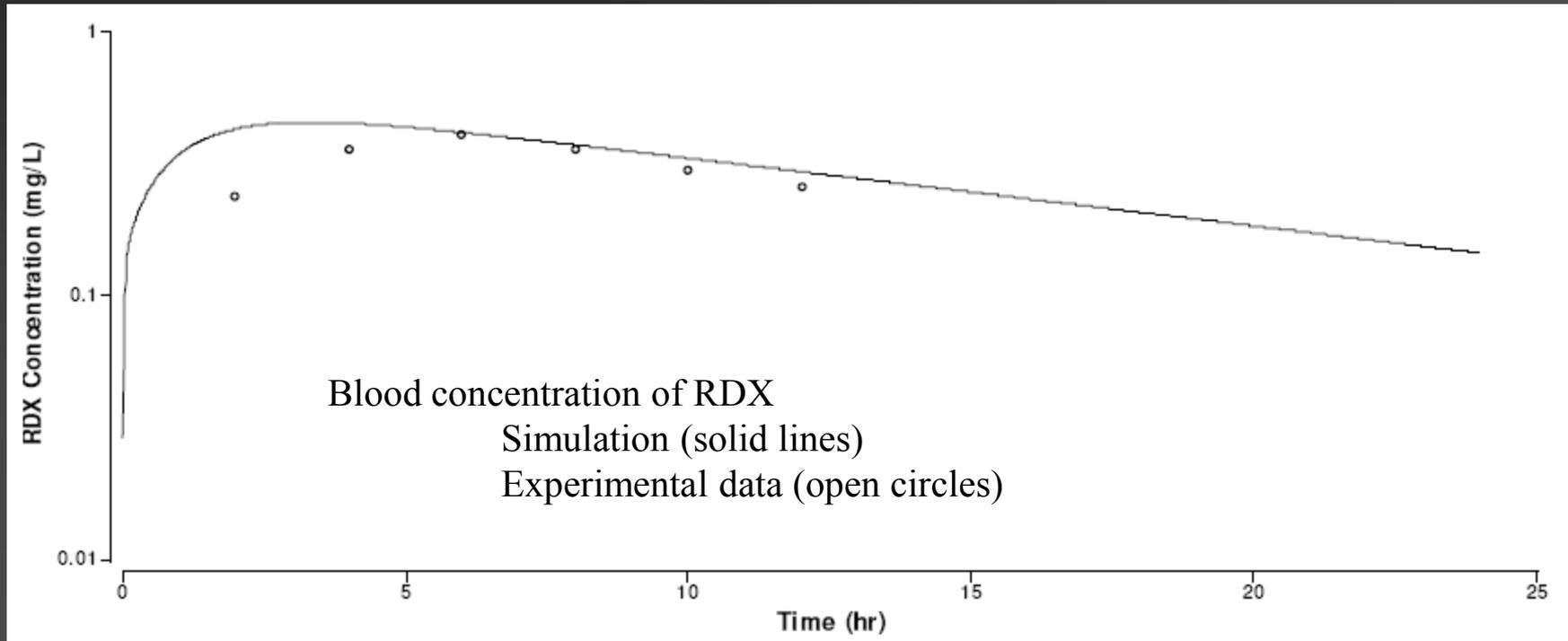
Absorption  
Distribution  
Metabolism  
Elimination

# Rat PBPK Model Construction – Krishnan et al., 2009

- Each tissue compartment in the RDX PBPK model was described with a mass balance differential equation (MBDE) that consisted of a series of clearance terms.
- The perfusion-limited tissue uptake of RDX in blood was described according to Fick's law of simple diffusion.
- Liver metabolism and oral absorption were described as a first order process
- Tissue:blood partition coefficients for RDX

# PBPK Model Simulation - Krishnan et al., 2009

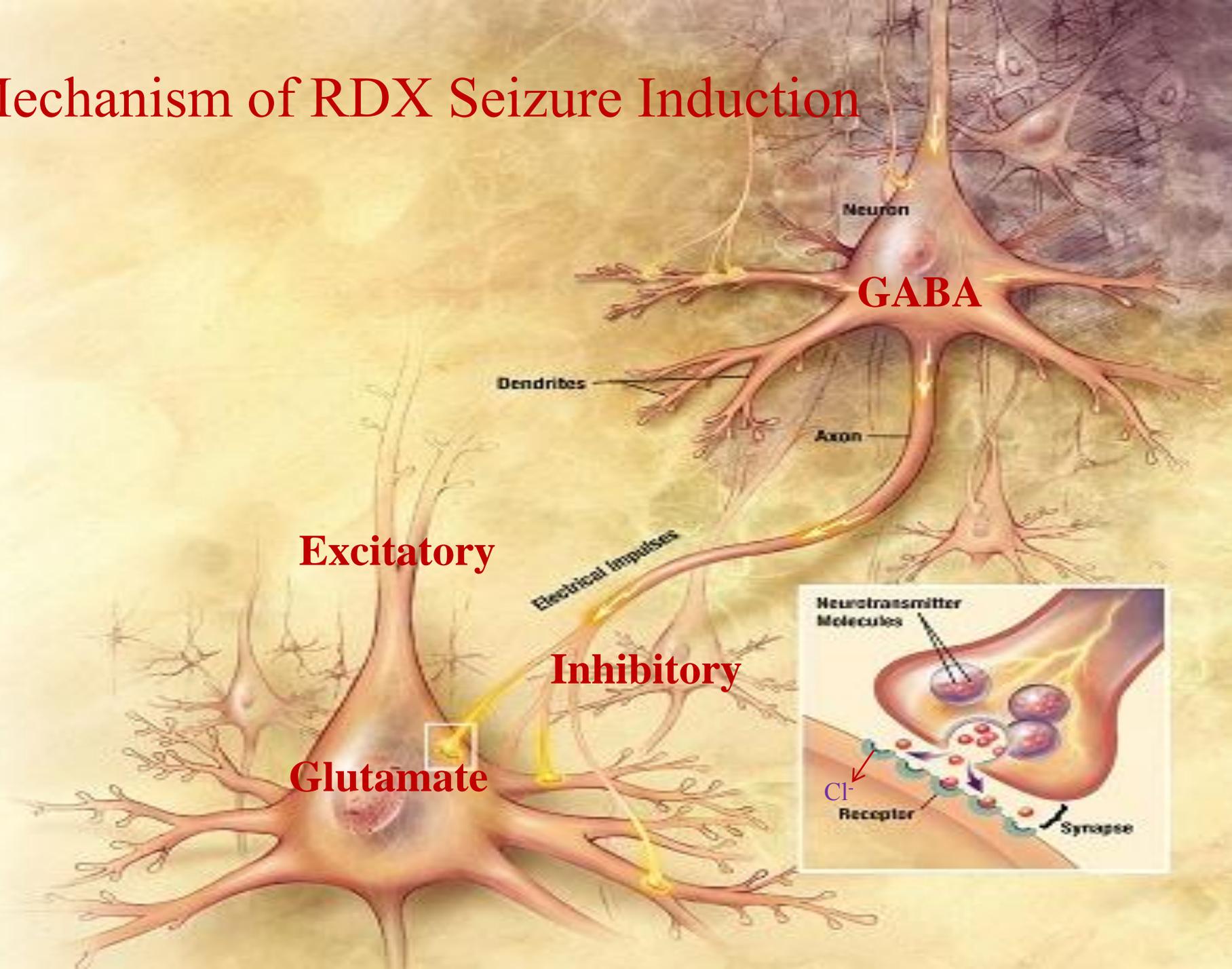
first order metabolic rate constant  $K_{fc} = 2.2 \text{ hr}^{-1} \text{ kg}^{-1}$



# Interspecies extrapolation – next steps

- Determine dose to brain
- Extrapolate rat PBPK model to human
- Peer-reviewed

# Mechanism of RDX Seizure Induction



# GABA<sub>A</sub> Receptor

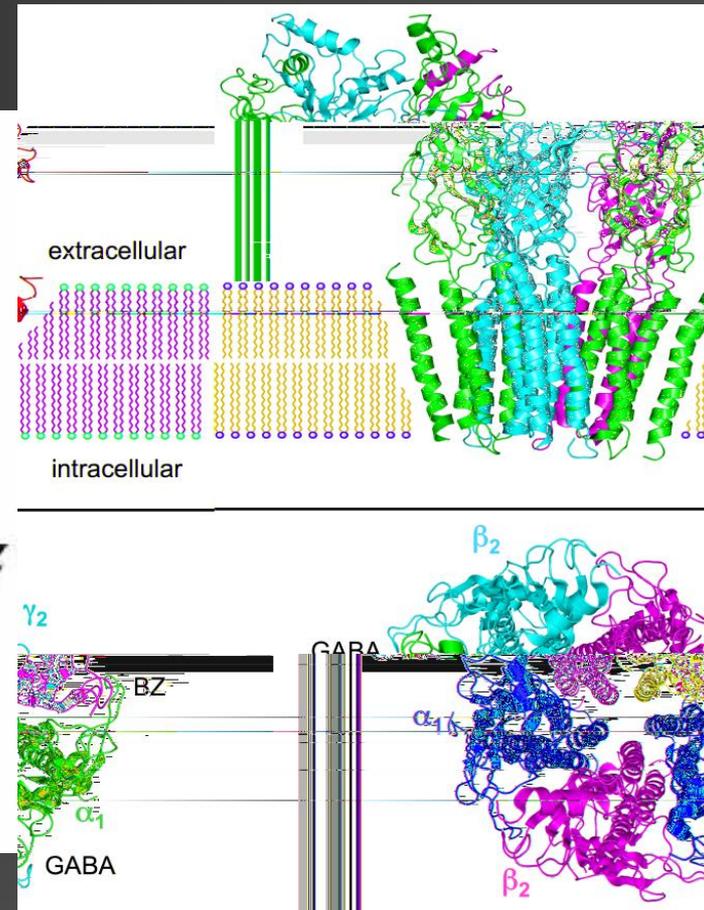
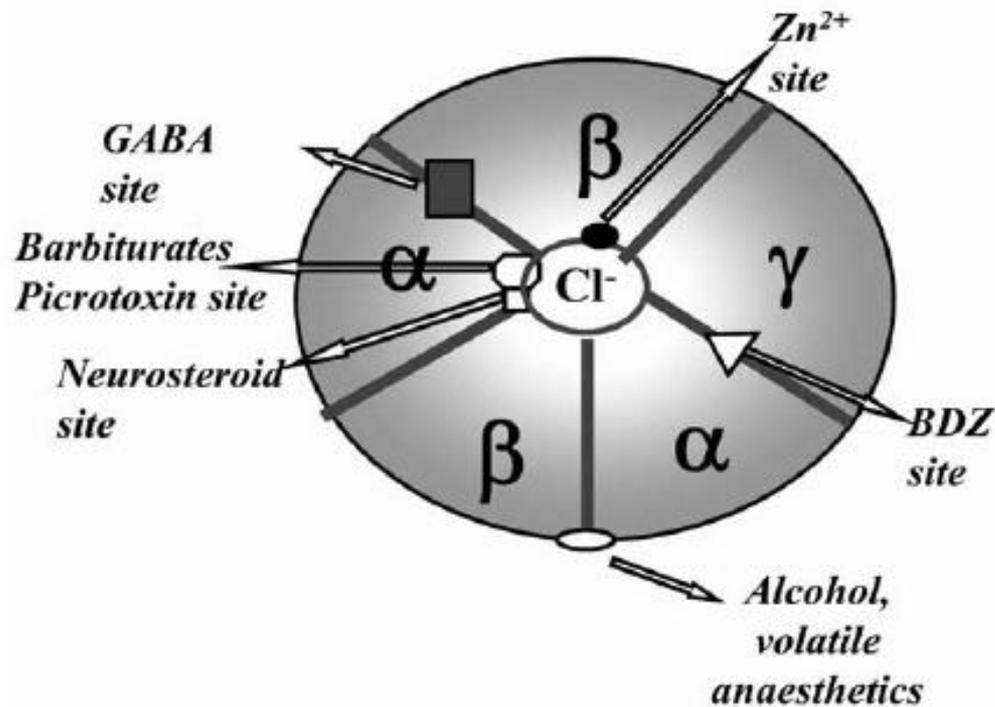
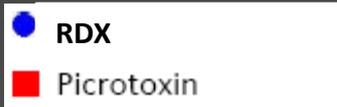
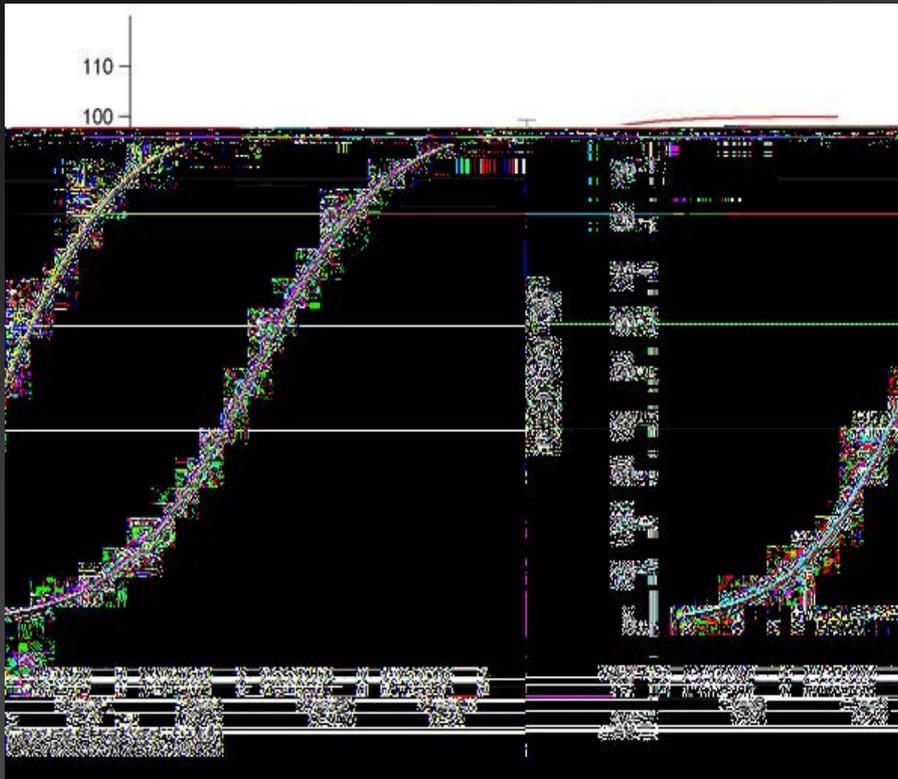


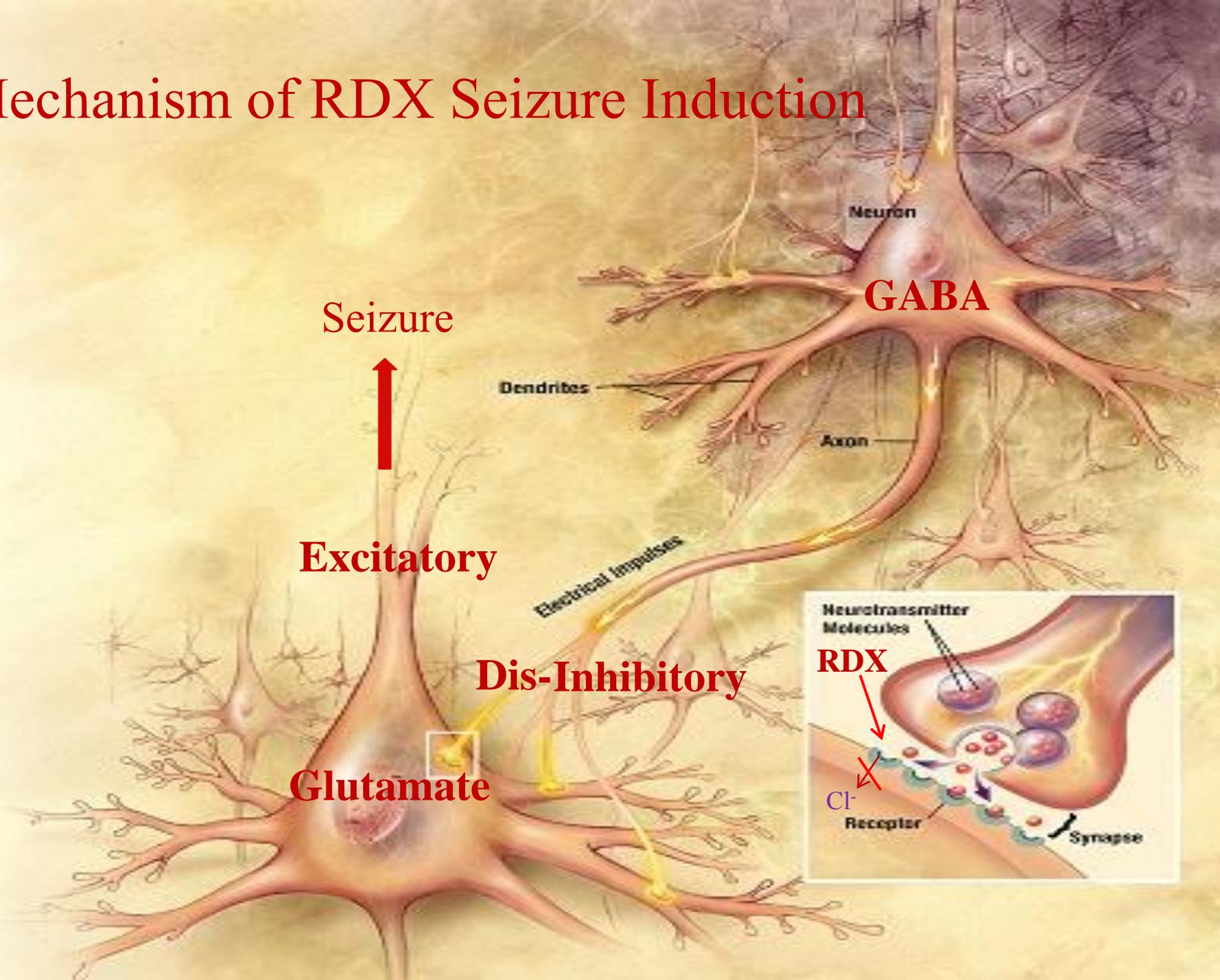
Fig. (1). Overview of the hypothetical pentameric GABA<sub>A</sub> receptor. A) Schematic representation of the GABA receptor, showing the arrangement and stoichiometry of the GABA receptor and the location of some drug binding sites. B) Representation of the GABA<sub>A</sub> receptor sitting in a cell membrane.

## Effect of RDX on [<sup>35</sup>S]TBPS (Convulsant Site) Binding

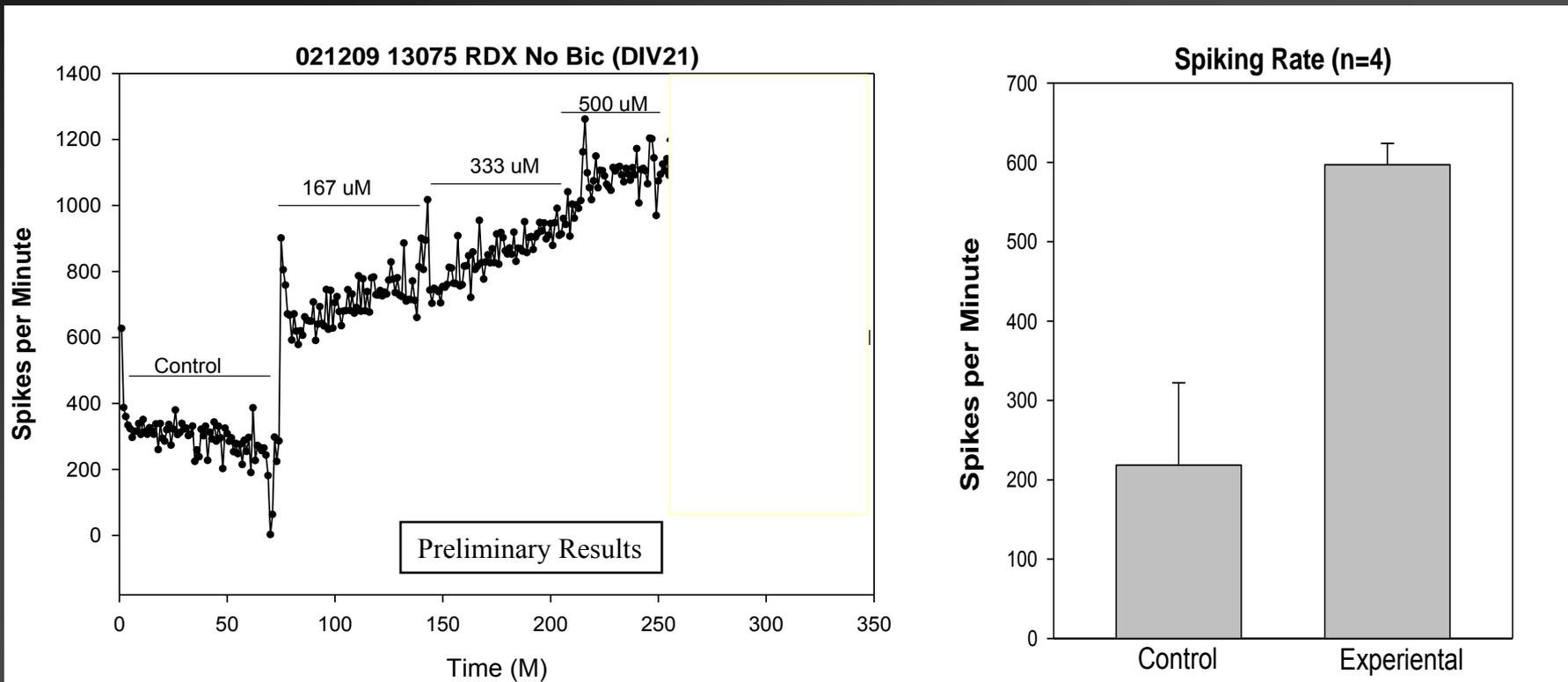


	PT	RDX
IC <sub>50</sub>	0.20 μM	21.6 μM
K <sub>i</sub>	0.20 μM	21.1 μM

# Mechanism of RDX Seizure Induction

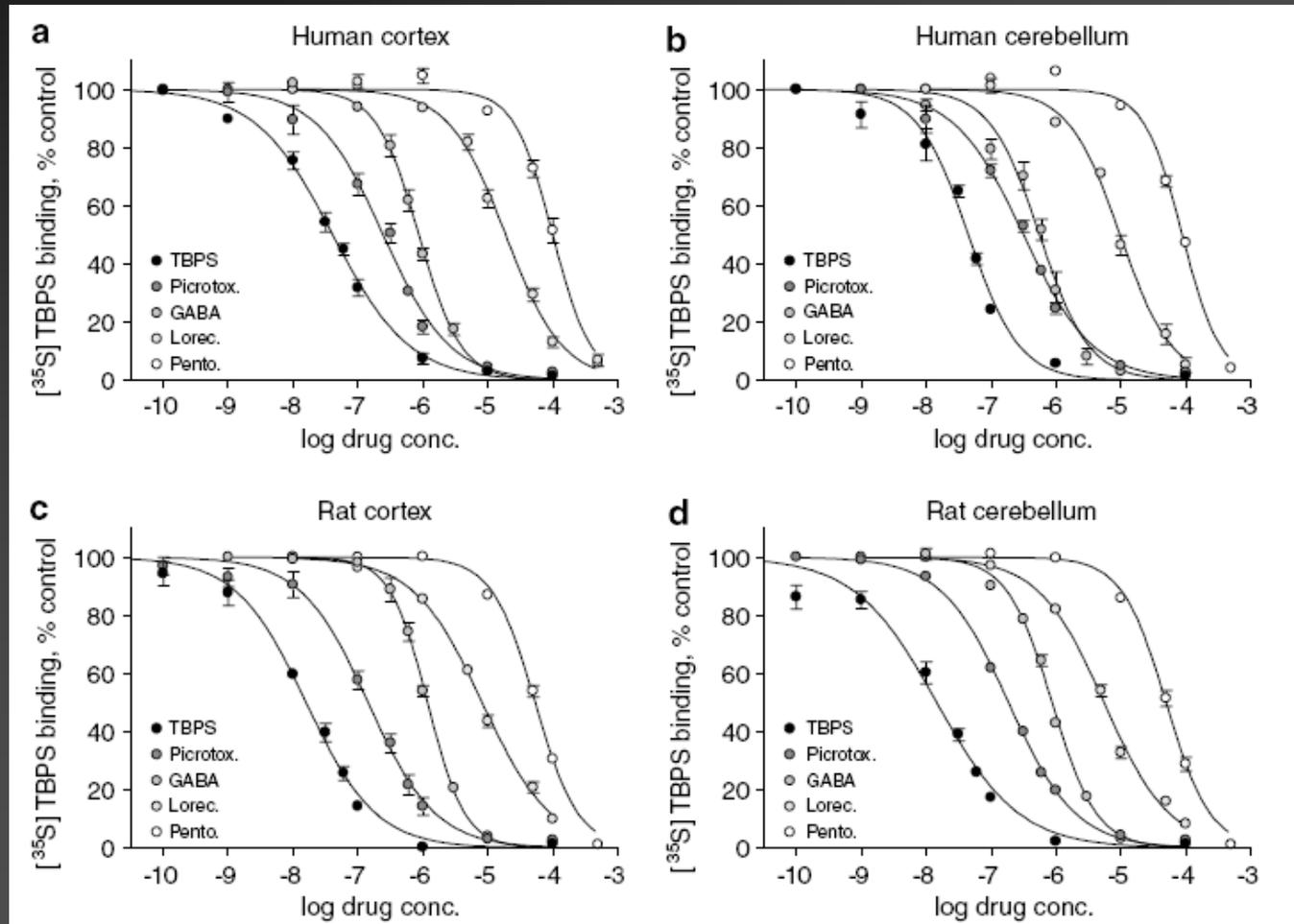


# Effect of RDX on Cortical Spike Activity *in vitro*



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Research Triangle Park, NC 27711

# Pharmacology of Human GABA<sub>A</sub> Receptor is very similar to rat – Atack et al., 2007



## If - Interspecies LOAEL/NOAEL Similar

	Western fence lizard (mg/kg-d)	Northern bobwhite (mg/kg-d)	White-footed mouse (mg/kg-d)	Fisher 344 Rat (mg/kg-d)
RDX	<b>5/2.5</b>	<b>8/3</b>	<b>8/4</b>	<b>8/1</b>

If - Interspecies GABA<sub>A</sub> Receptor Affinities Similar

Then – Interspecies UF can be reduced

# Metabolism & Excretion

- Metabolism of RDX is a first order process reported to be mediated by *CYP2B4*, a phenobarbital-inducible enzyme (Bhushan et al. 2003).
- Less than 5% parent RDX eliminated in urine; majority is metabolized (Levine et al., 1977, 1978).
- Primary metabolites are NDAB & MEDINA

# Completed Data

- Sub-chronic or chronic study using appropriate dose, and delivery method using at least 4 dose groups.
- Reasonable understanding of the compounds mode of action
- Metabolite study *in vivo*
- Pharmacokinetic studies and PBPK Model with 2 species validation
- Toxicodynamic assessment
- Toxicogenomic assessment
- Developmental toxicity assessment
- Reevaluation of RSC - 50% from water

# Uncertainty Factors after Re-Assessment

- Animal to Human (Interspecies)  
Toxicokinetics/Toxicodynamics - 3
- Sensitive Sub-population (Intraspecies) – 10
- Level of Carcinogenicity - <10 to 0
- Relative Source Contribution – 0.5

# Summary & Conclusions

- USEPA IRIS website suggests that RDX draft cancer reassessment is under agency review.
- New data that support refinement of Non-cancer Reference Dose (RfD) through the reduction of uncertainty:
  - Understanding target of toxicity.
  - Understanding absorption, distribution, metabolism, excretion and mechanism in test animals.
  - Greater confidence in understanding dose at target site and extrapolation to humans.
- Use new data to support a refined relative source contribution value for drinking water numbers.
  - Should result in raising of the Health Advisory from 2 ppb to ~5-6 ppb,  $\mu\text{g/L}$ .