



Trichloroethylene and Liver Tumors in Mice

Symposium on New Scientific Research
Related to the Health Effects of TCE

Washington, DC

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RJ Bull

MoBull Consulting



Focus of talk

- Asked to review and discuss:
 - Bull et al. 2002. Contribution of dichloroacetate and trichloroacetate to liver tumor induction by trichloroethylene. *Toxicol. Appl. Pharmacol.* 182:55-65.
 - Merdink et al. 2000. Trapping and identification of the dichloroacetate radical from the reductive dehalogenation of trichloroacetate by mouse and rat liver microsomes. *Free Rad Biol. Med.* 29:125-130.
- Nothing makes sense in isolation!
 - Xu et al., 1995. Metabolism of bromodichloroacetate in B6C3F1 mice. *Drug Metabolism & Disposition* 23:1412-1416.
 - Merdink et al. 1998. The extent of dichloroacetate formation from trichloroethylene, chloral hydrate, trichloroacetate, and trichloroethanol in B6C3F1 mice. *Tox. Sci.* 45:3-41.
 - Merdink et al. 2001. Toxicokinetics of bromodichloroacetate in B6C3F1 mice. *J. Appl. Toxicol.* 21:53-57.
- Interactions between CCl₄, TCA and DCA
 - Bull et al. 2004. Interactions in the tumor promoting activity of carbon tetrachloride, trichloroacetate, and dichloroacetate in the liver of male B6C3F1 mice. *Toxicology*, in press.



Overview

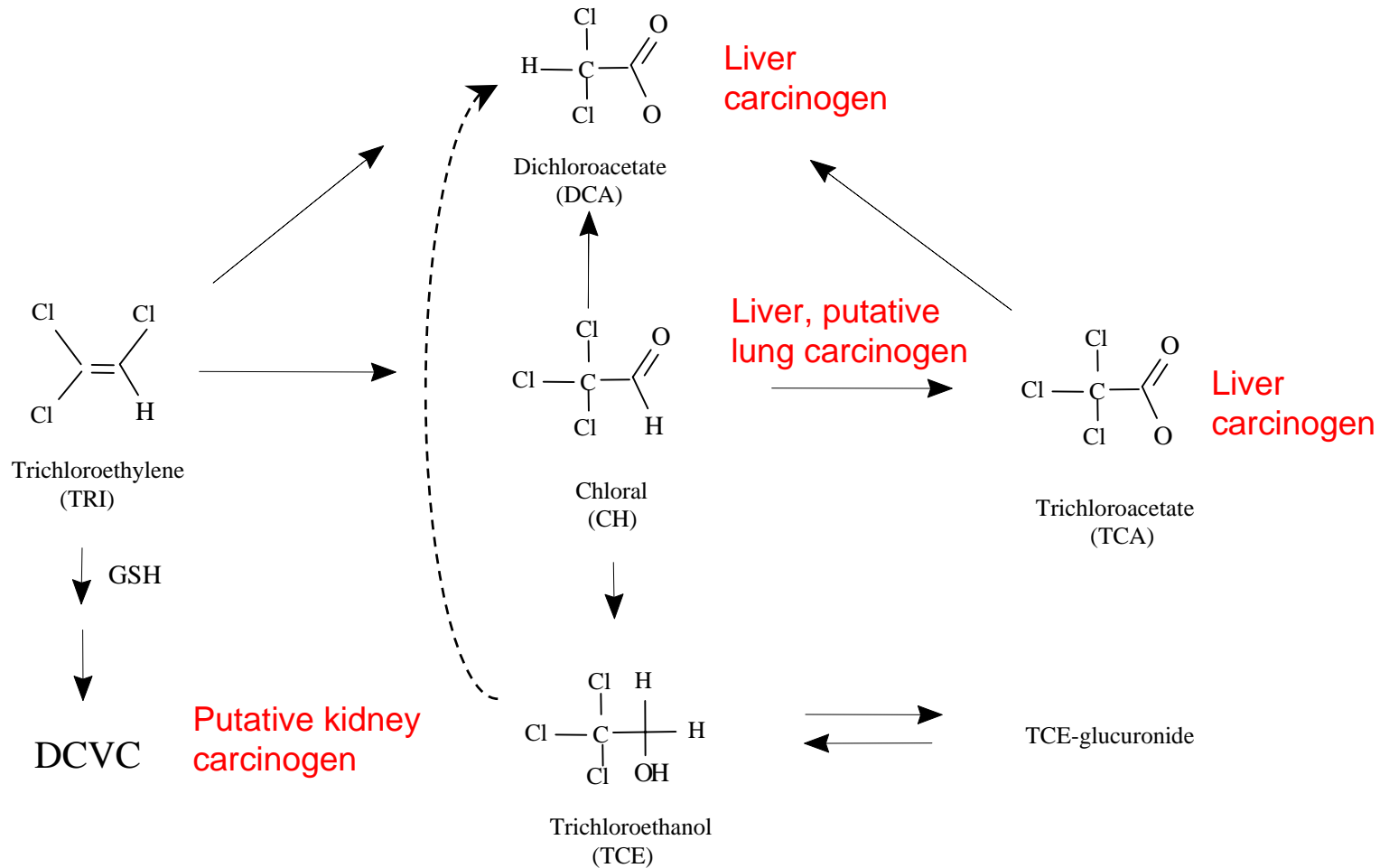
- Contribution of TCA to liver tumor response has been accepted
- Amount of DCA formed and how has become a critical issue
- Characteristics of responses
 - Changes relative to control in tissue and tumors of treated animals
 - Identify differences between TCA & DCA treatments
 - Where data exists, identify these effects in TCE-treated animals
- Identify the dose-region where DCA might contribute to the tumor response

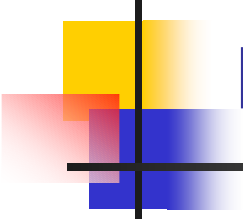


Implications of a DCA contribution to liver tumors produced by TCE treatment

- TCA
 - Peroxisome proliferator
 - Carcinogenic only in mice
- DCA
 - Additional mechanisms are involved
 - Multispecies liver carcinogen
- TCE is not a liver carcinogen in rats
 - May be due to insufficient formation of DCA

TCE Metabolism: Multiple active metabolites





Estimates of maximum AUC of DCA in the blood from bioassay doses of TCE

Treatment	AUCL (mg-h/L)
0.05 g DCA/L of drinking water	0.041
0.5 g DCA/L of drinking water	0.72
1000 mg TCE/kg body weight	0.25
2000 mg TCE/kg body weight	0.31

Barton et al., (1999) Toxicol. Lett. 106:9-21.

Cyclization of PBN adduct of dichloroacetate radical

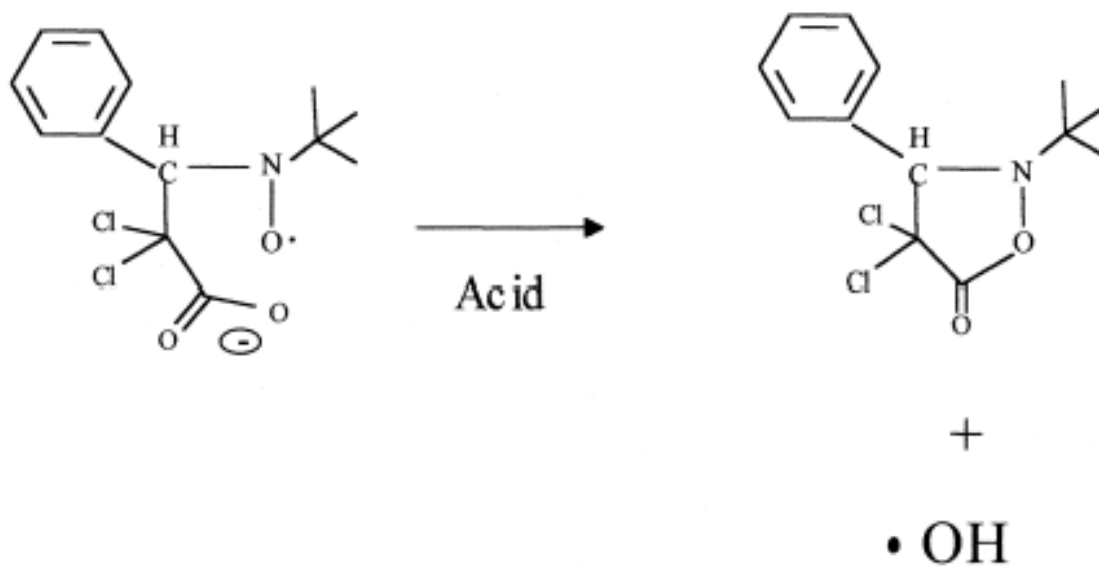


Fig. 4. Postulated formation of the dichloroacetyl radical, its trapping with PBN, and its subsequent cyclization to form the adduct shown in Fig. 2.

Merdink et al., 2000

MS of trapped dichloroacetate radical produced from TCA

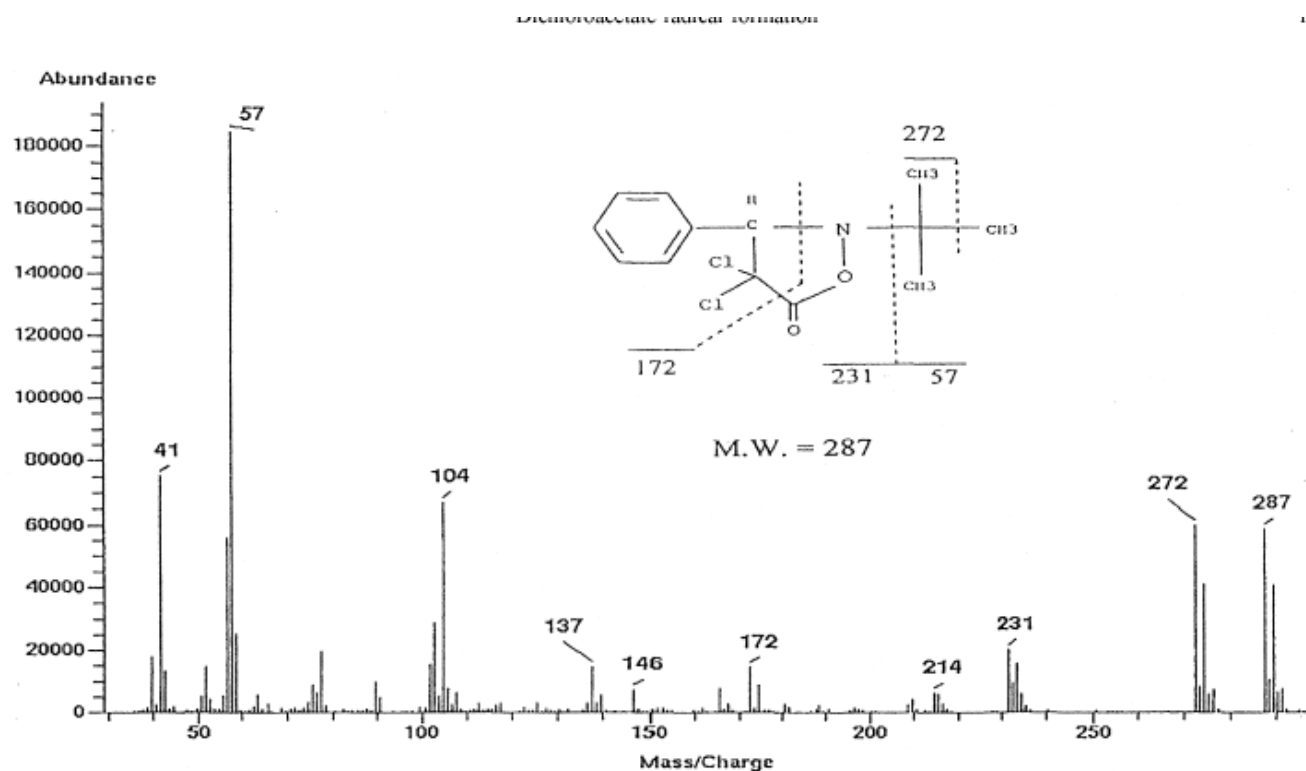


Fig. 2. The mass spectrum and the putative structure of the cyclized, PBN/dichloroacetate radical adduct. This adduct has a molecular weight of 287 dalton. The characteristic isotope cluster due to the presence of two chlorines is apparent at the molecular ion.

Formation of dichloroacetyl radical from TCA in microsomes of mice vs. rats

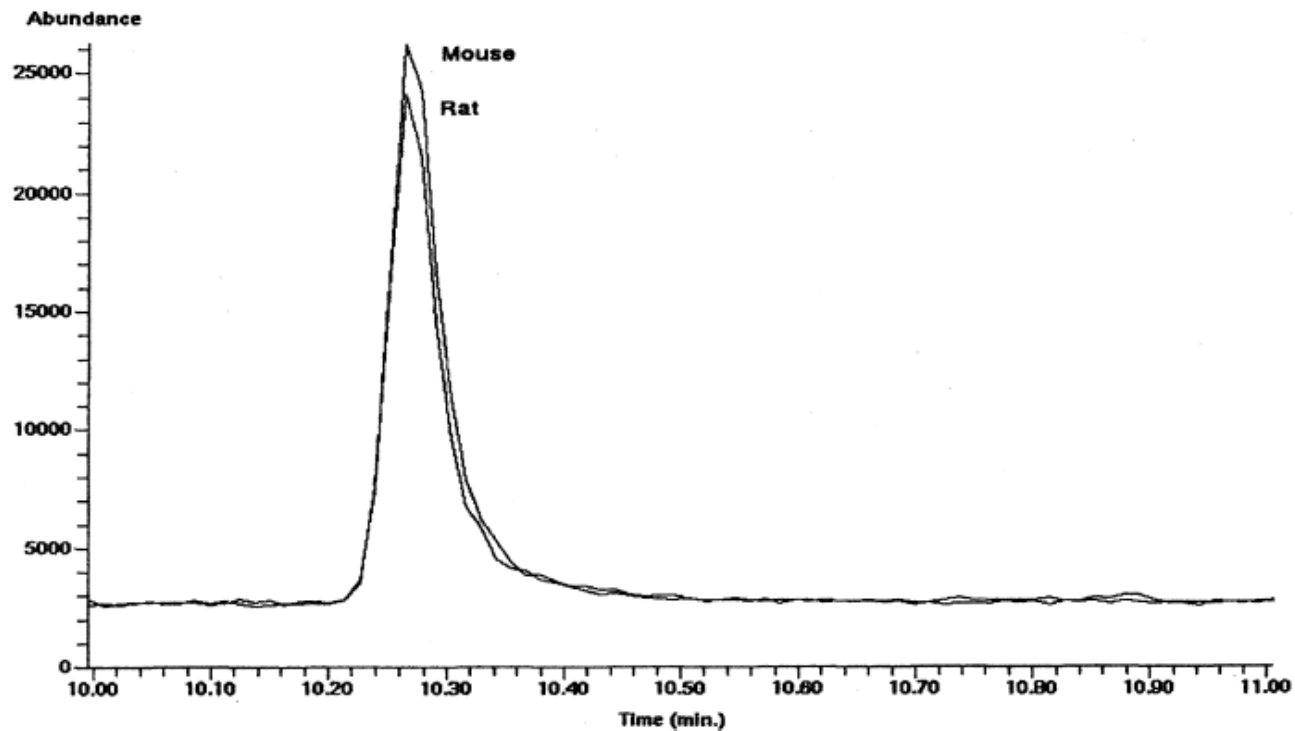
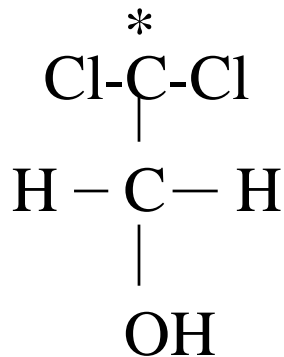
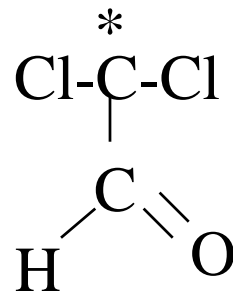


Fig. 3. The cyclized PBN/dichloroacetyl adduct was detected in extracts from incubations of TCA with mouse and rat microsomes. The SIM chromatograms of the 287 m/z molecular ion show similar formation of the dichloroacetate radical by both species.

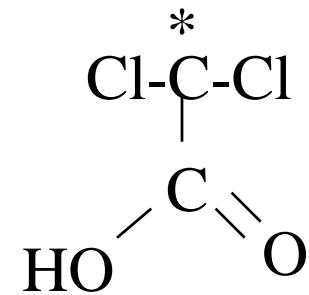
Free radical intermediates formed with all three trihalo metabolites



TCEtOH

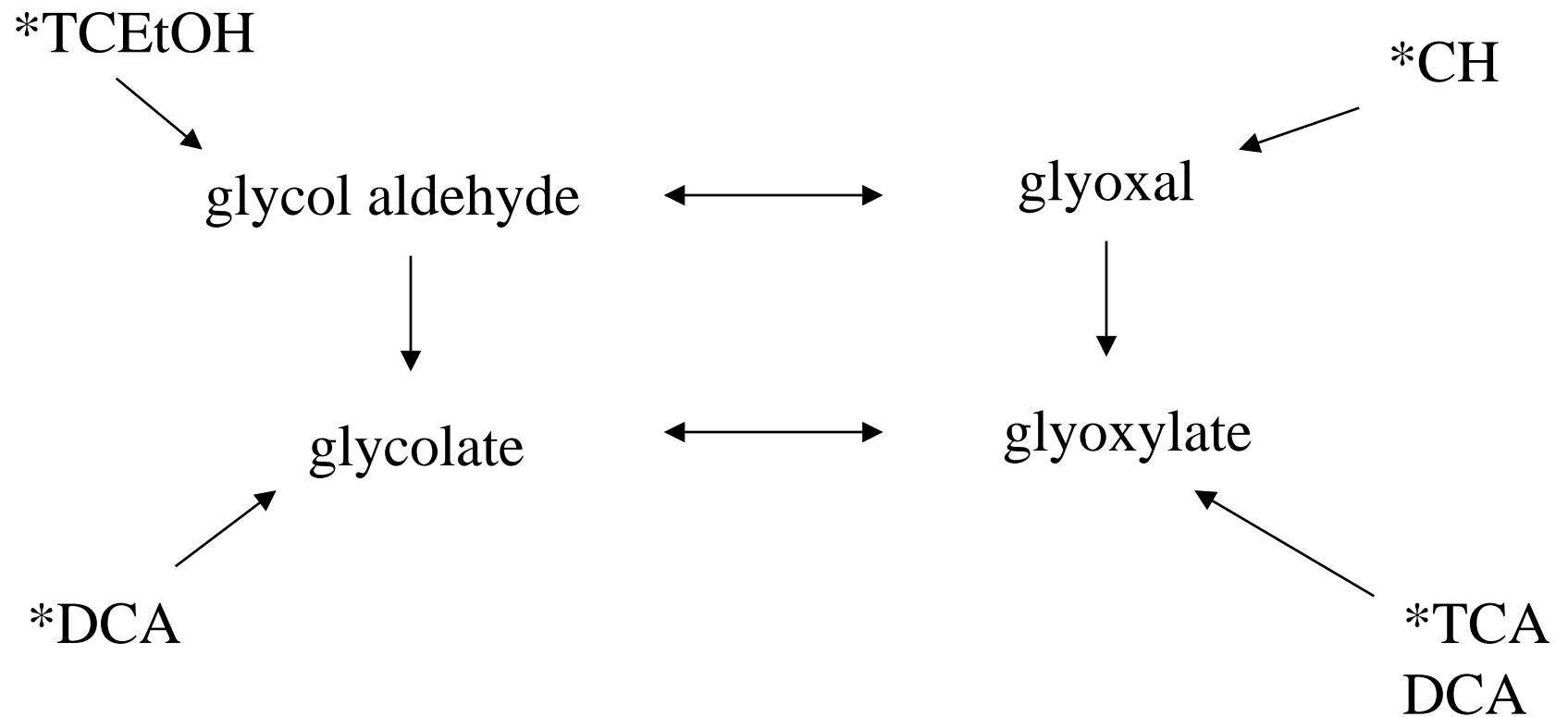


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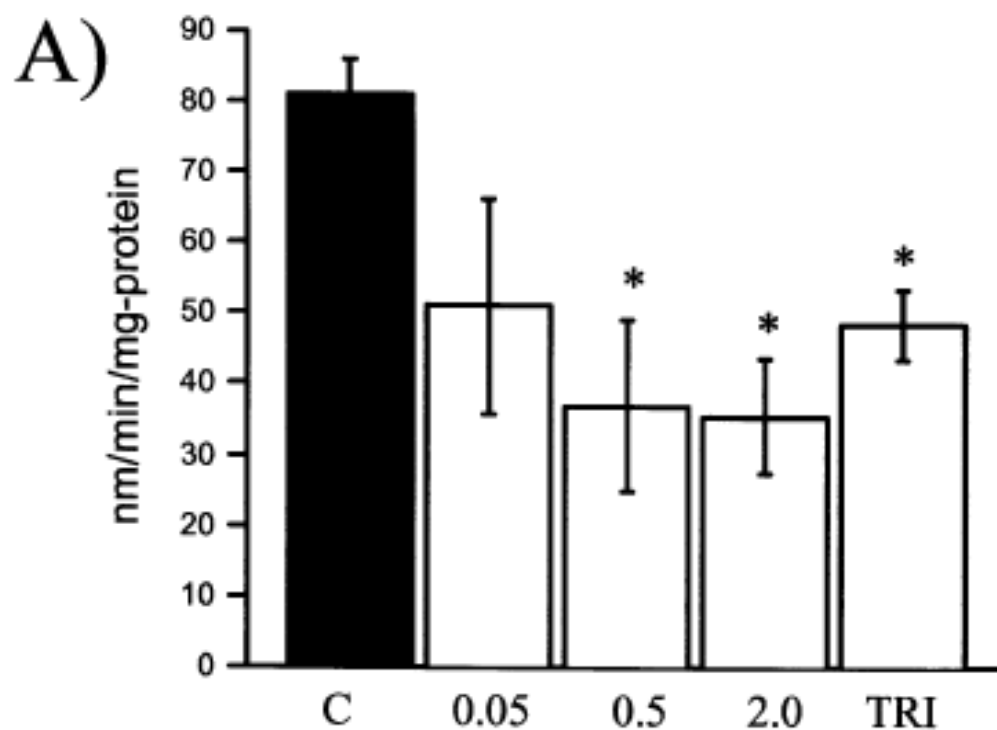


TCA

End products of oxidative metabolites are the same



Indirect evidence of DCA formation from TCE



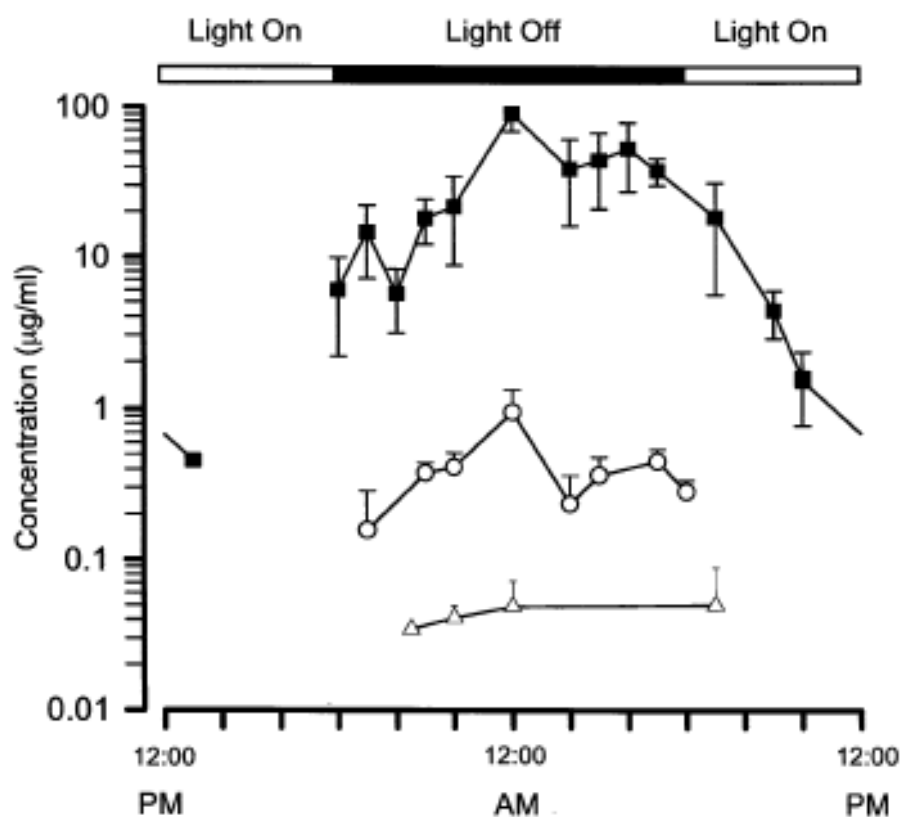
Effect of pretreatment with DCA or TRI on activity of MAAI (GSTz)
Schultz et al. 2002 Toxicology 173:229-247



TCA can be converted to DCA but:

- Alternative pathways to DCA from TCE still have not been quantified
 - TCA metabolism, itself, does not account for DCA that is formed
 - Others have shown that TCEtOH forms free radicals more readily than TCA
 - Adducts seen by Pumford et al suggest that it can form directly from TCE
- Indirect evidence of DCA formation from TCE documented by the inhibition of GST-zeta in mice pre-treated with TCE (Schultz et al. 2002)
 - Does not occur with TCA
- TCA produces a distinct phenotype of liver tumor. If significant conversion of TCA to DCA tumor phenotype should be observed TCA treatment, it is not.
- **Therefore, formation by reductive dehalogenation of TCA does not contribute significantly to liver tumor responses to TCE or TCA.**

Dosimetric limits to use of DCA data to effects of TCE



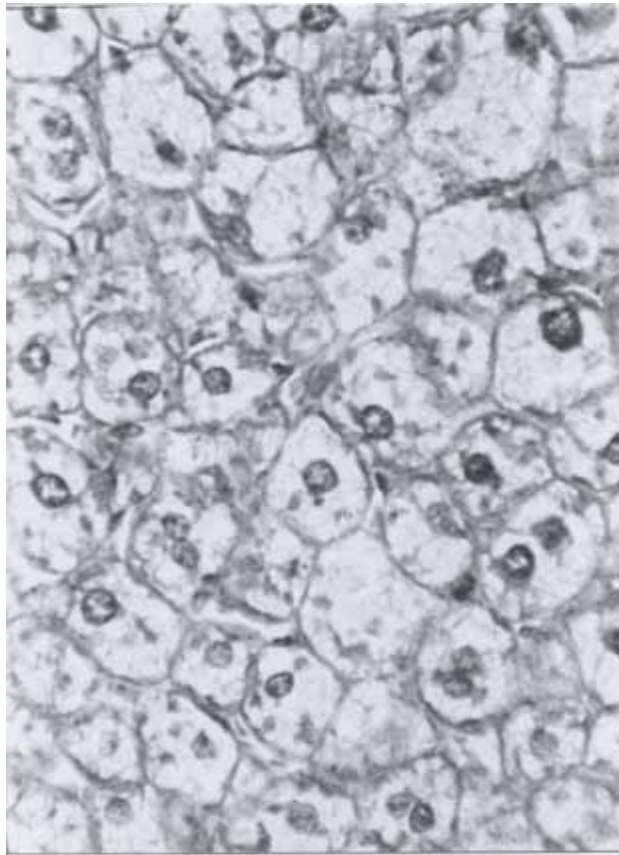
2 g/L all animals, multiple tumors

0.5 g/L high incidence

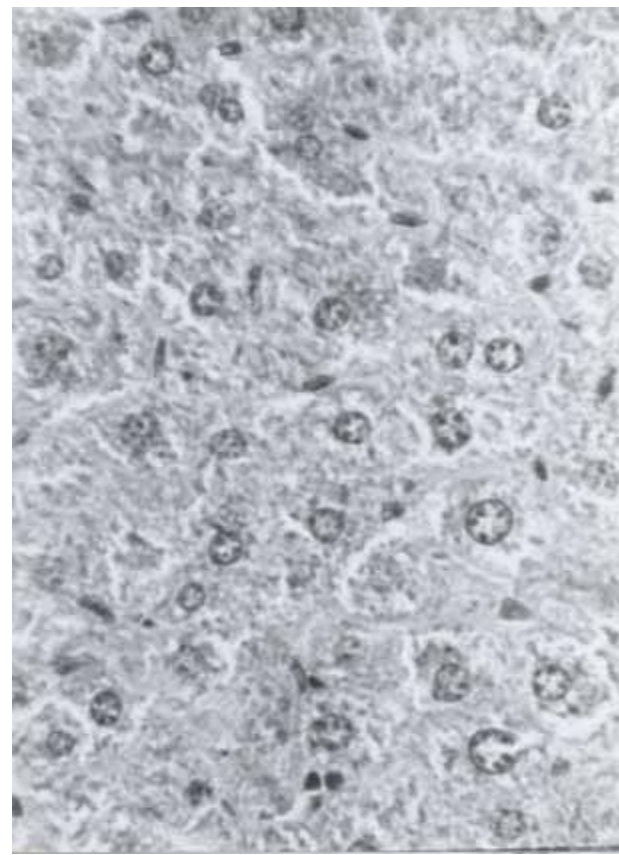
0.05 g/L no excess tumors

From: Schultz et al. 2002 Toxicology 173:229-247

Mouse Liver Treated with DCA or TCA

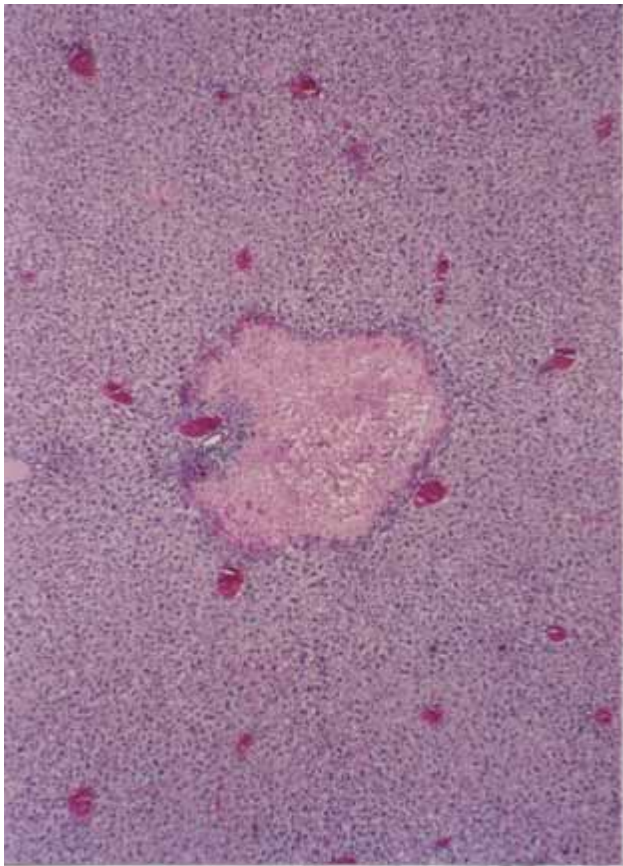


DCA



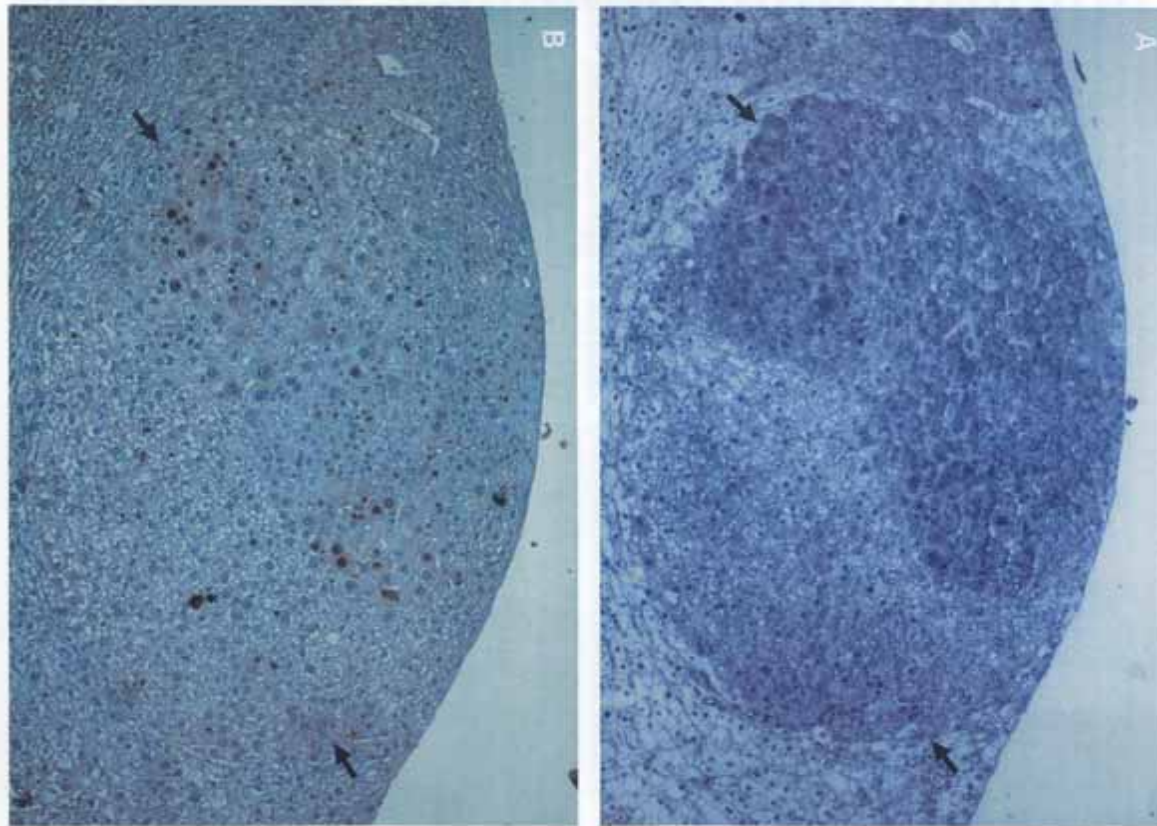
TCA

Acinar Necrosis at high doses of DCA

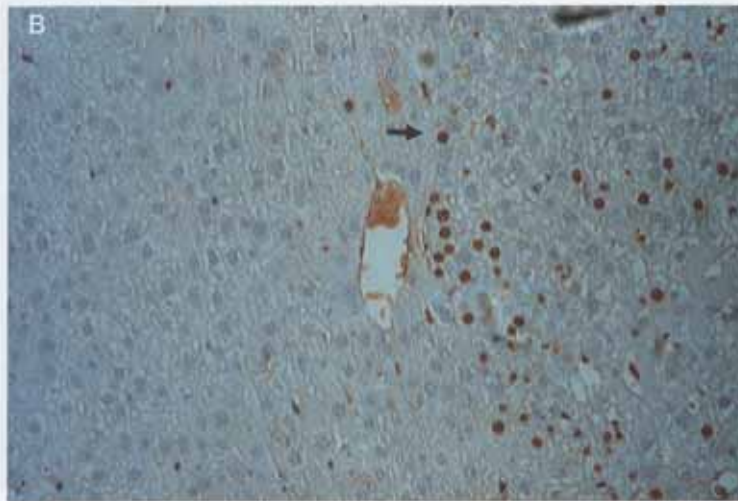


- Occurs at a high dose (2 g/L and above)
- Sporadic
- Not seen at lower doses
- Could affect tumor response at high dose
- Unlikely contributor to TCE-induced tumors
- Raises serious concerns about relevance of high dose DCA experiments

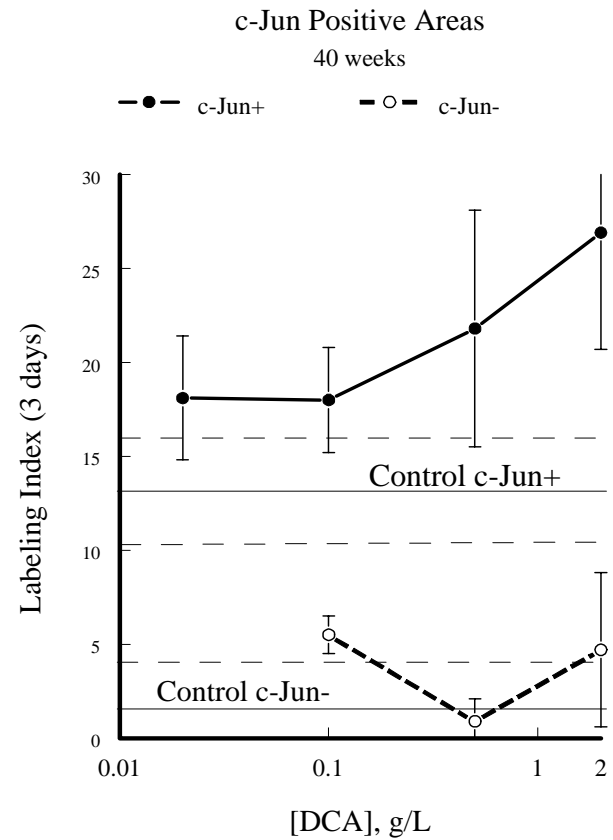
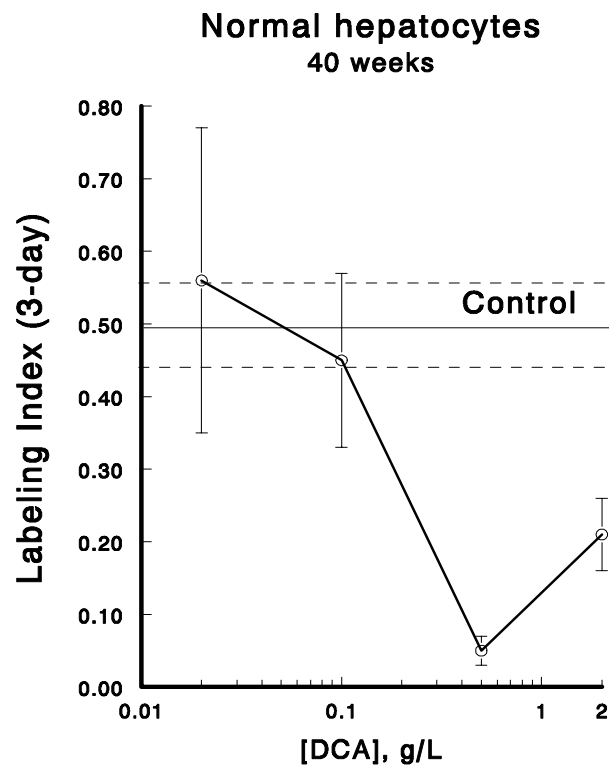
DCA and c-Jun+ lesions



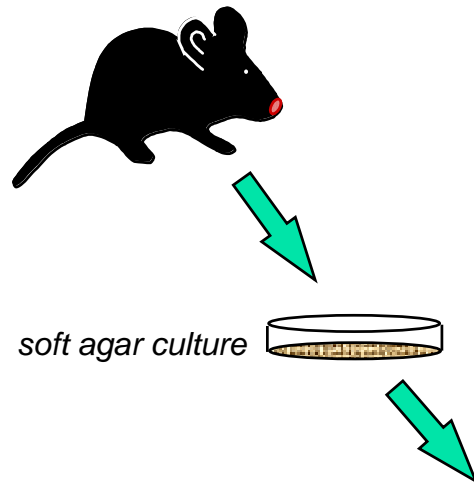
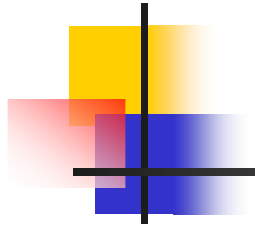
TCA and c-Jun⁻ lesions



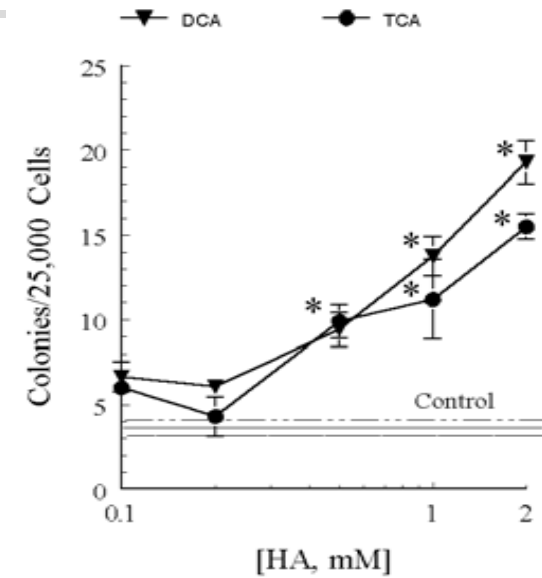
Selective Effects of DCA on Growth



Selection appropriate tumor phenotypes *In Vitro*



cellular/molecular
analysis of phenotype
and genotype



Phenotypic characteristics of initiated hepatocyte colonies promoted to survive by *in vitro* treatment with DCA or TCA. The reactivity with *c-Jun* antibody reflect the characteristics of tumors induced in the intact animal with these chemicals.



Other phenotypic differences exist in tumors produced by DCA and TCA

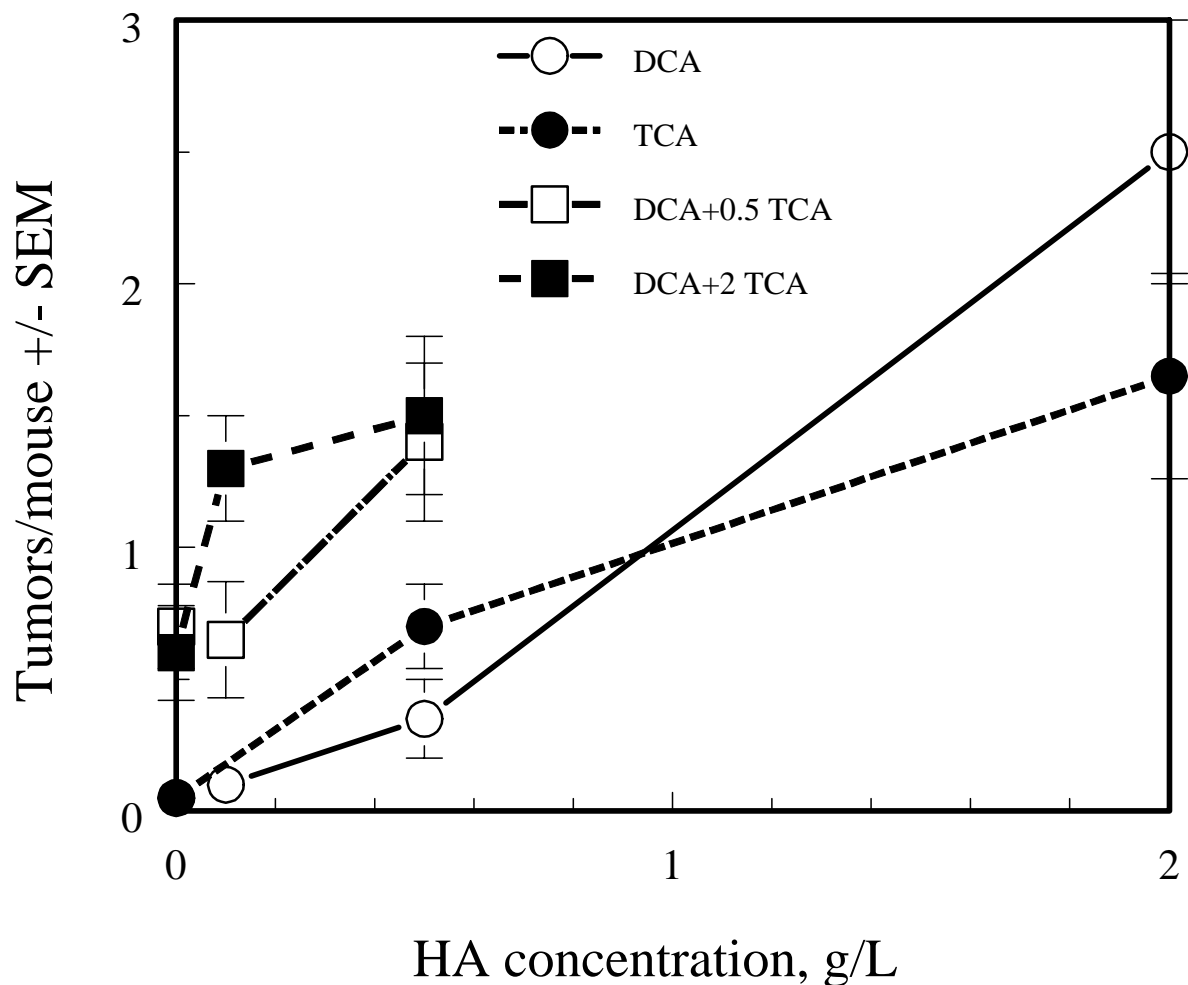
- Pereira and Phelps, 1996
- Latendresse and Pereira, 1997
- Tao et al. 1996; 1998
- Ge et al. 2001

c-Jun+ lesions with DCA, TCA, mixtures of DCA-TCA, and TCE

Treatment	c-Jun+	c-Jun-	Mixed	Number of tumors examined
TCA 0.5 g/L	0 (0)	14 (1.0) ^a	0 (0)	14
2.0 g/L	0 (0)	12 (1.0)	0 (0)	12
DCA 0.1 g/L	1 (0.5)	1 (0.5)	0 (0)	2
0.5 g/L	3 (0.43)	4 (0.57)	0 (0)	7
2 g/L	14 (0.45)	17 (0.55)	0 (0)	31
TCA 0.5 + DCA 0.1 g/L	0 (0)	12 (0.86)	2 (0.14)	14
DCA 0.5 g/L	0 (0)	13 (0.81)	3 (0.19)	16
TCA 2 g/L + DCA 0.1 g/L	1 (0.04)	25 (0.92)	1 (0.04)	27
DCA 0.5 g/L	1 (0.04)	12 (0.44)	14 (0.52)	27
TRI 1.0 g/kg bw	16 (0.42)	13 (0.34)	9 (0.24)	38

Bull et al., 2002 Toxicol & Appl Pharmacol. 182:55-65

Interactions of DCA and TCA in uninitiated male mice

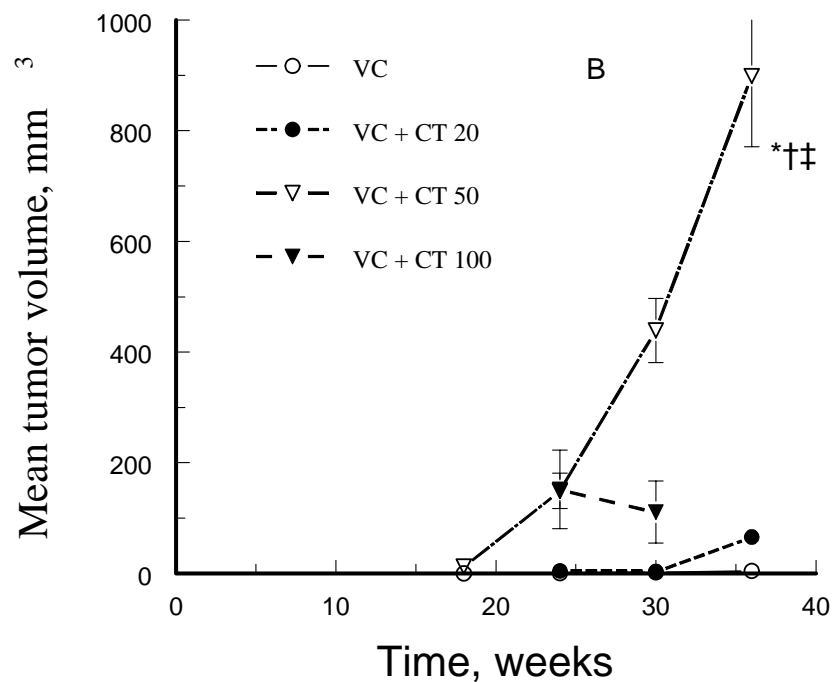
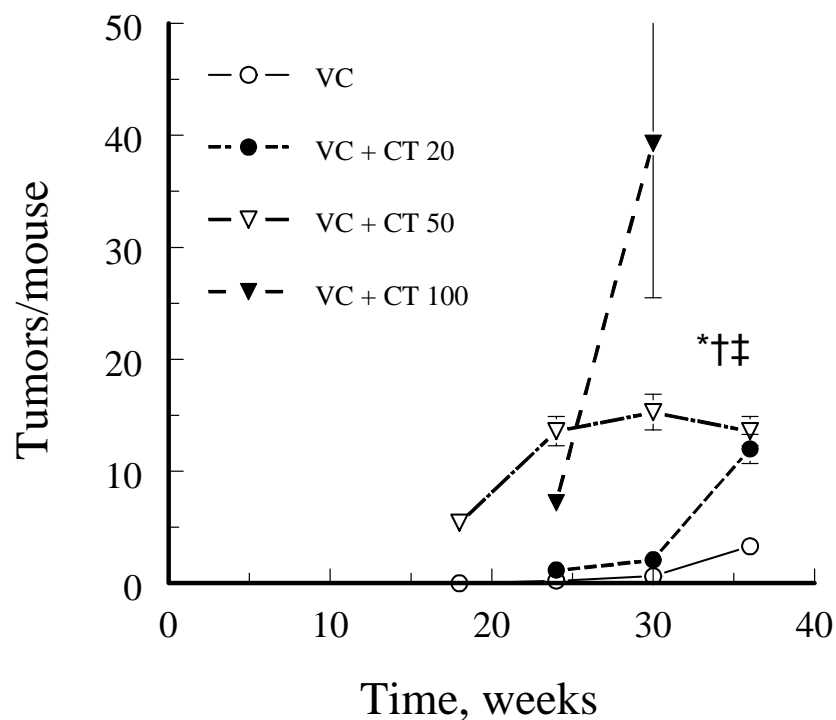




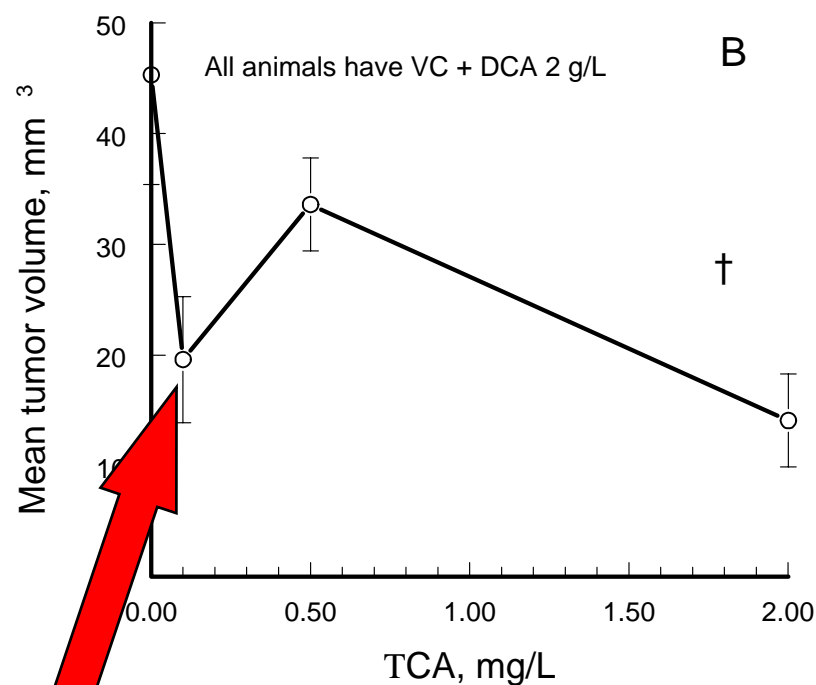
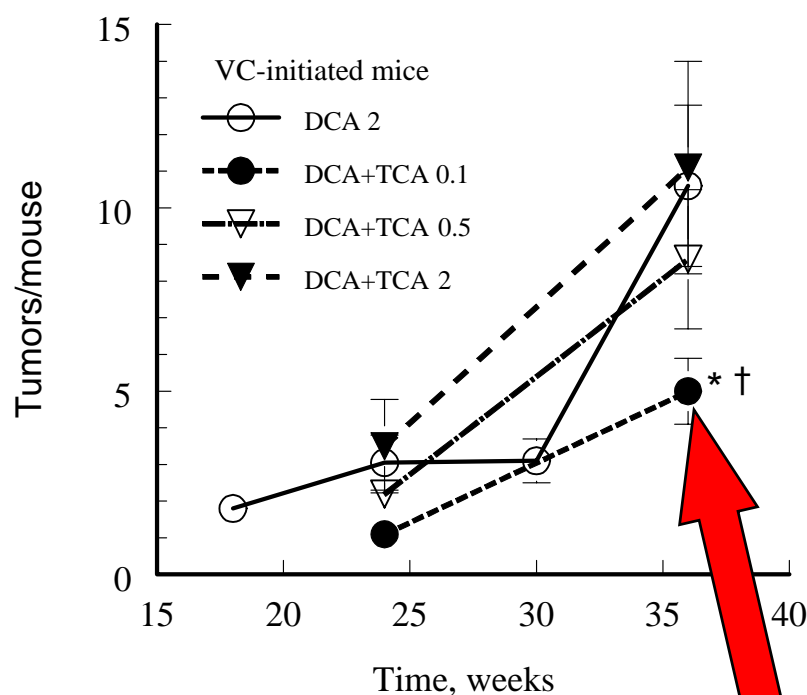
Interactions in the tumor promoting activity of TCA, DCA, and CCl₄

- Utilized an initiation-promotion protocol to produce multiple tumors/mouse
- Initiator - vinyl carbamate 3 mg/kg, 14 days of age
- Varying doses of CCl₄, DCA, and TCA administered alone and in combination beginning at 21 days of age
- Ten animals per time period per group, total of 70 groups of ten
- More than 8000 tumors scored
- Sacrificed at 18, 24, 30 and 36 weeks
- Monitored effects of treatment on tumor numbers vs. tumor size
- Accepted for publication in Toxicology

Effect of CCl₄ on tumor numbers and size

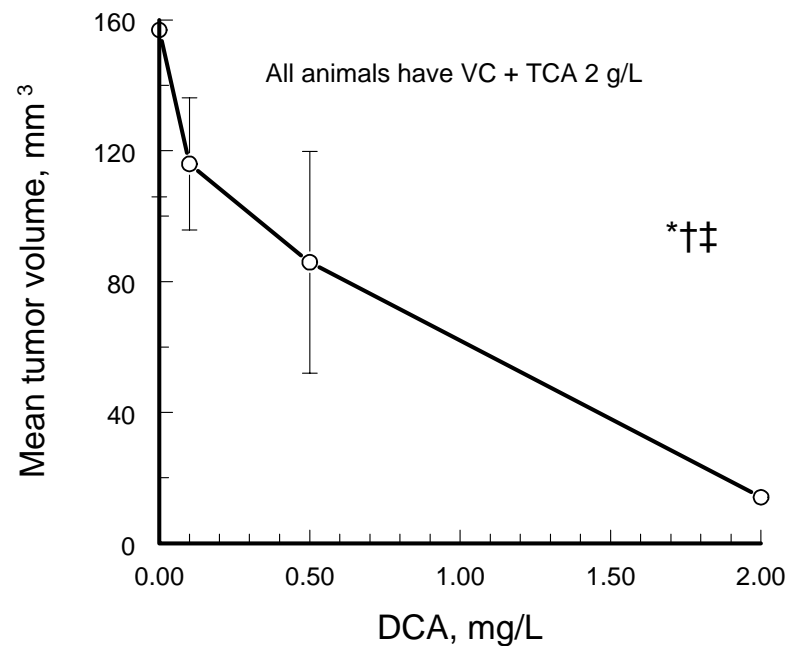
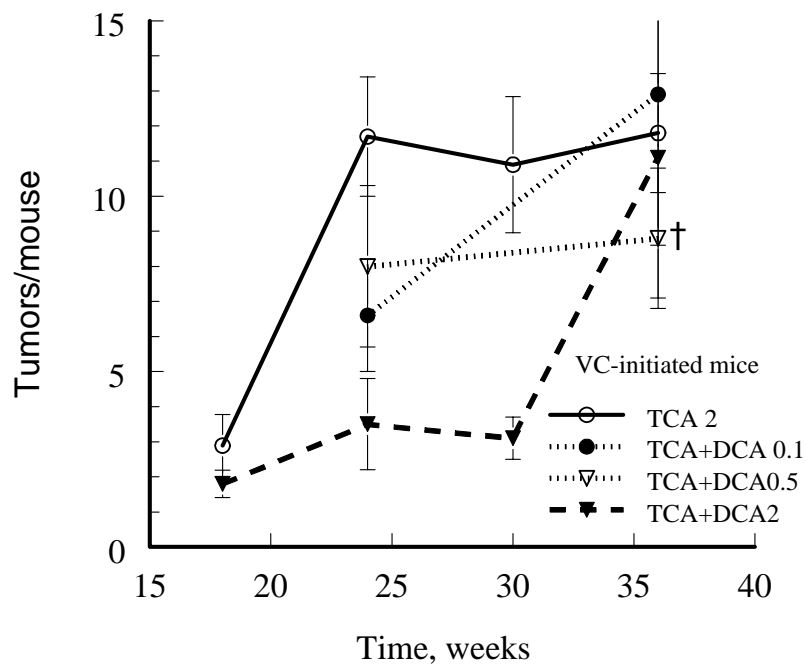


Modification of DCA promotion of liver tumors in mice by TCA

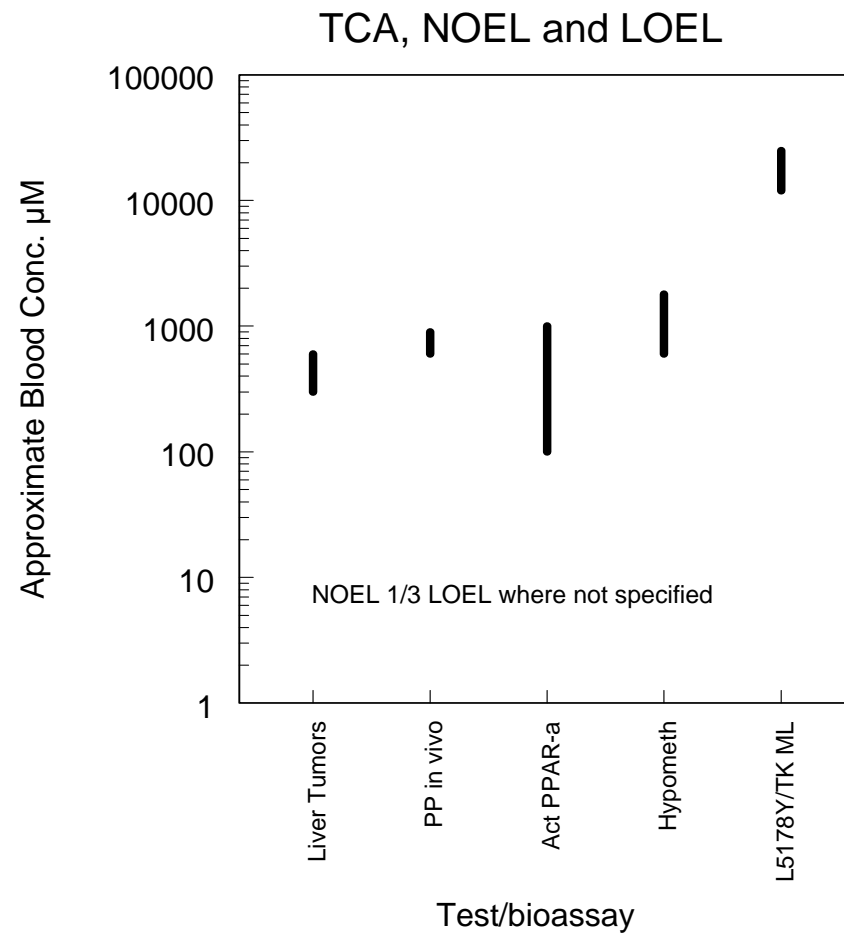


Always have one point in a data set that you don't understand!

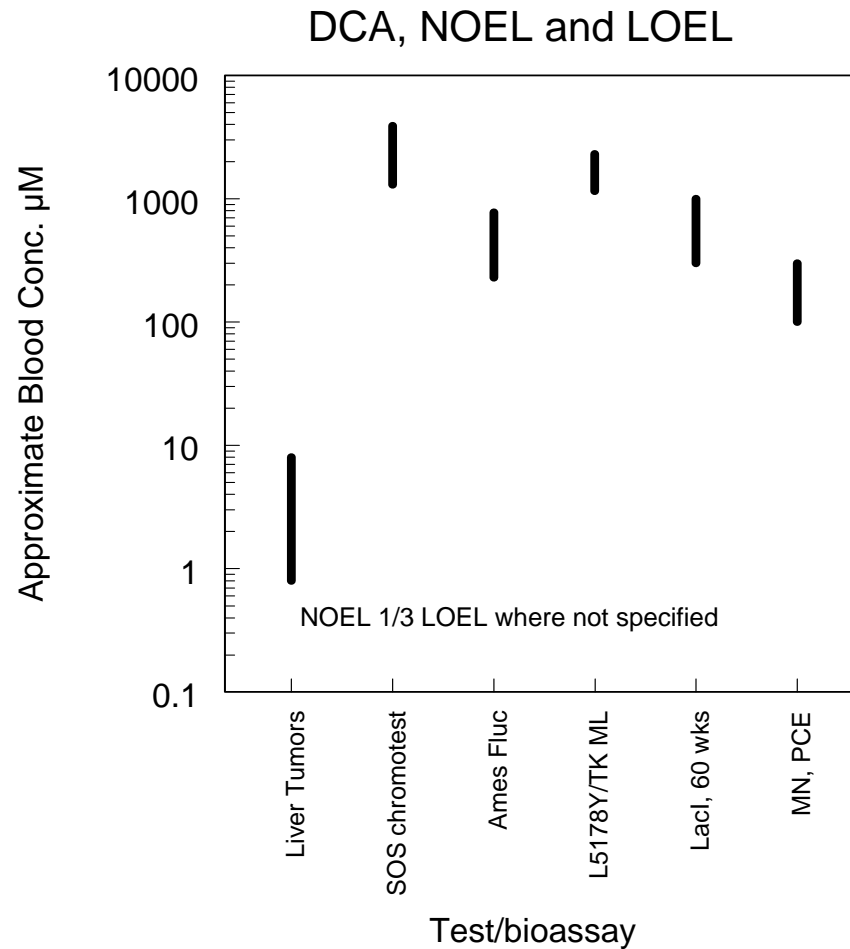
Modification of TCA promotion of liver tumors in mice by DCA



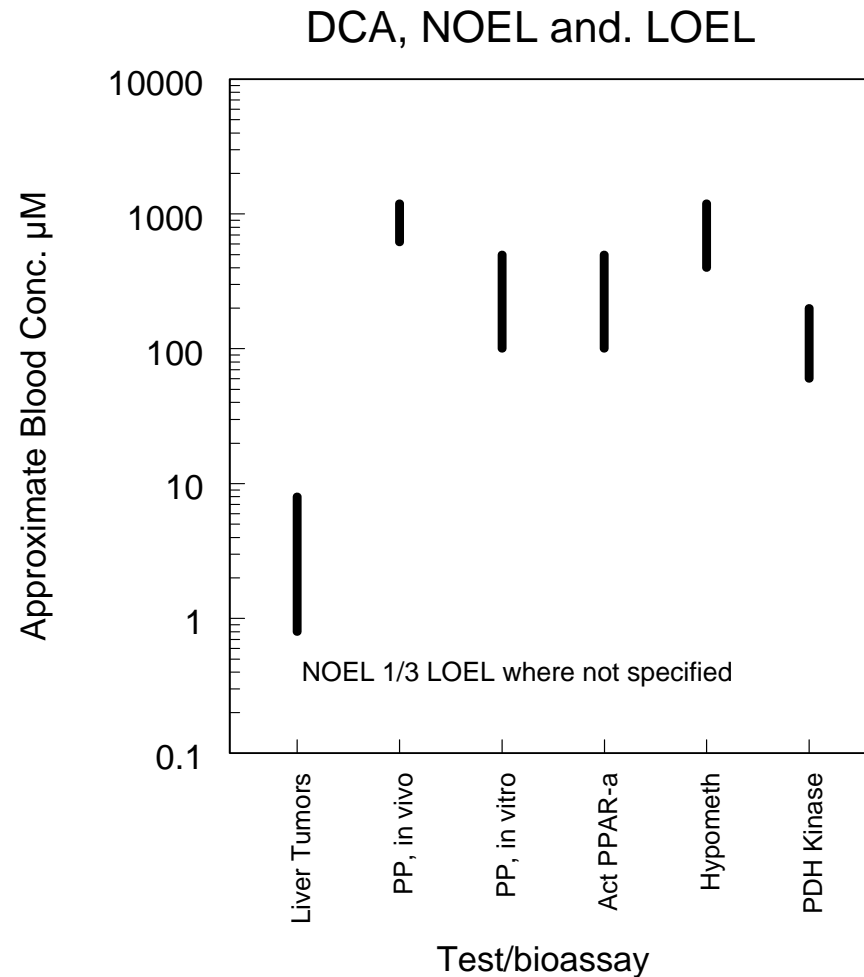
Mode of Action of TCA



DCA and Genotoxicity

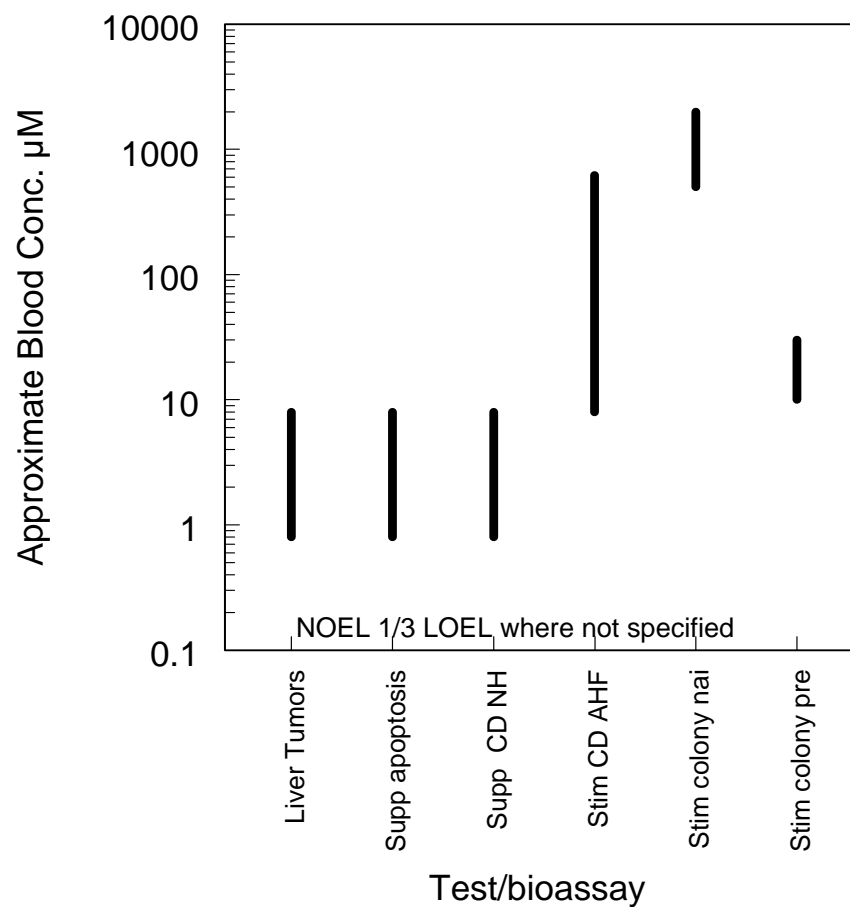


DCA, peroxisome proliferation, and miscellaneous endpoints

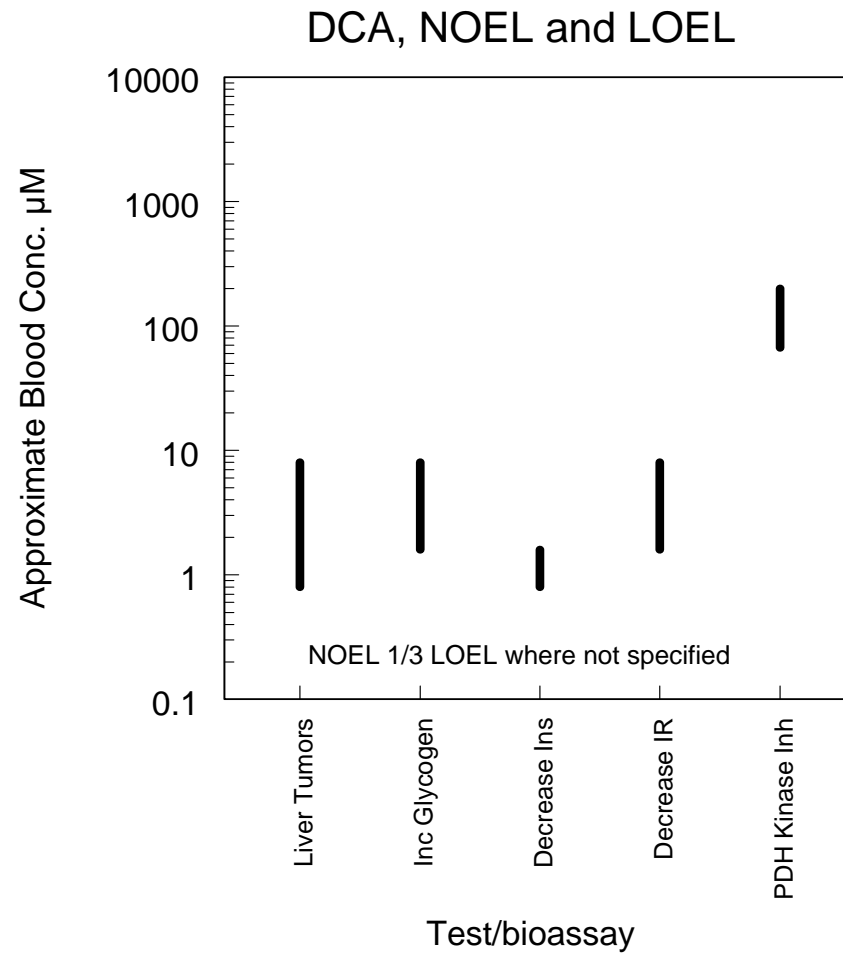


DCA, Cell Division, and Apoptosis

DCA, NOEL and LOEL



DCA and Carbohydrate Metabolism





Conclusions

- The mixed phenotype of tumors induced by TCE indicates that both DCA and TCA contribute
- DCA's contribution is likely to be a combined action:
 - Inhibition of the TCA-dependent phenotype
 - Stimulation/creation of DCA-dependent phenotype
 - At low doses achieved from metabolism of TCE, suppressed apoptosis more important than stimulation of cell division
- Raises the question of why the rat was negative for liver cancer
 - TCA was negative
 - Not enough DCA formed from TCE to independently induce tumors



Contributors

- Co-Investigators
 - Irvin Schultz, PNNL
 - Brian Thrall, PNNL
 - Lyle Sasser, PNNL
 - Kevin Minard, PNNL
 - Robert Wind, PNNL
 - John Miller, PNNL
- Post-doctoral Fellows
 - Douglas Stevens, WSU
 - Gayle Orner, PNNL
- Graduate Students
 - Anja Stauber, WSU
 - Melissa Lingohr, WSU
 - Junko Kato-Weinstein, WSU
 - James Merdink, WSU
 - Eric Austin, WSU
 - Michael Templin, WSU

- Resources
 - Department of Energy
 - Department of Defense – SERDP
 - U.S. Environmental Protection Agency