



# *Use of Genotoxicity Data in Mode of Action Analysis for Human Health Risk Assessment*

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October 10, 2008

# *Disclaimer*

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*The views expressed in this presentation  
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# Outline

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- ***Genotoxicity and Risk Assessment***
- Mode of Action (MOA) Frameworks
- Use of Genotoxicity Data in IRIS Assessments
- Case Study – 1,2,3 -Trichloropropane

## *Introduction*

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**IARC**: 2007 - IARC working group on Genetic Toxicology to understand carcinogenic risk to humans

**WHO**: 2007 - Draft Guidance on Mutagenicity Testing for Chemical Risk Assessment

**IWGT**: Understanding of Genotoxic Mechanism of Action for Carcinogen Risk Assessment to Humans: 4<sup>th</sup> International Workshop on Genotoxicity Testing (Mut Res: 627, (1): 1-118, 2007)

## *Introduction (cont'd)*

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**ILSI/HESI:** Initiative on Relevance and Follow-Up of Positive Results in In Vitro Genetic Toxicity Testing (IVGT)

**US EPA:**

- (1) 2005 Guidelines for Carcinogenic Risk Assessment **and** Supplemental Guidance for Assessing Susceptibility from Early-life Exposure to Carcinogens
  
- (2) 2008 Draft Framework for Determining a Mutagenic Mode of Action for Carcinogenicity

## *Use of Genotoxicity Data*

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- To **screen chemicals** for their ability to cause mutations or other types of genetic damage
- Genotoxicity information is used to understand the **MOA of a chemical** and for **hazard identification** to assess an agent for having carcinogenic potential or other effects
- May be included in a **Weight of Evidence (WOE) assessment** on whether the chemical is likely to induce adverse health effect

- *Hazard Identification*: Qualitative assessment of the inherent genetic toxicity of an agent.
- *Mode of Action Analysis*: Understanding the genotoxic mode of action of an agent. e.g. Direct acting vs indirect acting genotoxicant, clastogen vs aneugen.
- *Dose-Response Assessment*: Relationship between the dose of an agent and the induction of an adverse (genotoxic) effect.

- Genotoxicity and Risk Assessment
- ***Mode of Action Frameworks***
- Use of Genotoxicity Data in IRIS Assessments
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## *What is Mode of Action?*

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“*Mode of action* is defined as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation.

Mode of action is contrasted with *mechanism of action*, which implies a more detailed understanding and description of events, often at the molecular level, than is meant by mode of action.”

*EPA Cancer Guidelines, 2005*

## *Key event*

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A “*key event*” is an empirically observable precursor step that is itself a necessary element of the mode of action or is a biologically based marker for such an element. A key event is necessary, but may or may not be sufficient to cause cancer.

EPA Cancer Guidelines, 2005

## *Key Features*

1. Critical analysis of available information
2. Mode of Action
3. Weight of Evidence for data evaluation
4. Dose-response Assessment
5. Susceptible populations and life stages
6. Evaluating risks from childhood exposures
7. Emphasis on risk characterization

# MOA Framework – Cancer Guidelines

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- Hypothesized MOA: Summary description and identification of key events
- Experimental support:
  - ▶ Strength, consistency, specificity of association
  - ▶ Dose-response concordance
  - ▶ Temporal relationship
  - ▶ Biological plausibility and coherence
- Consideration of the possibility of other MOAs
- Relevance to humans

# *EPA's Mutagenic MOA Framework*



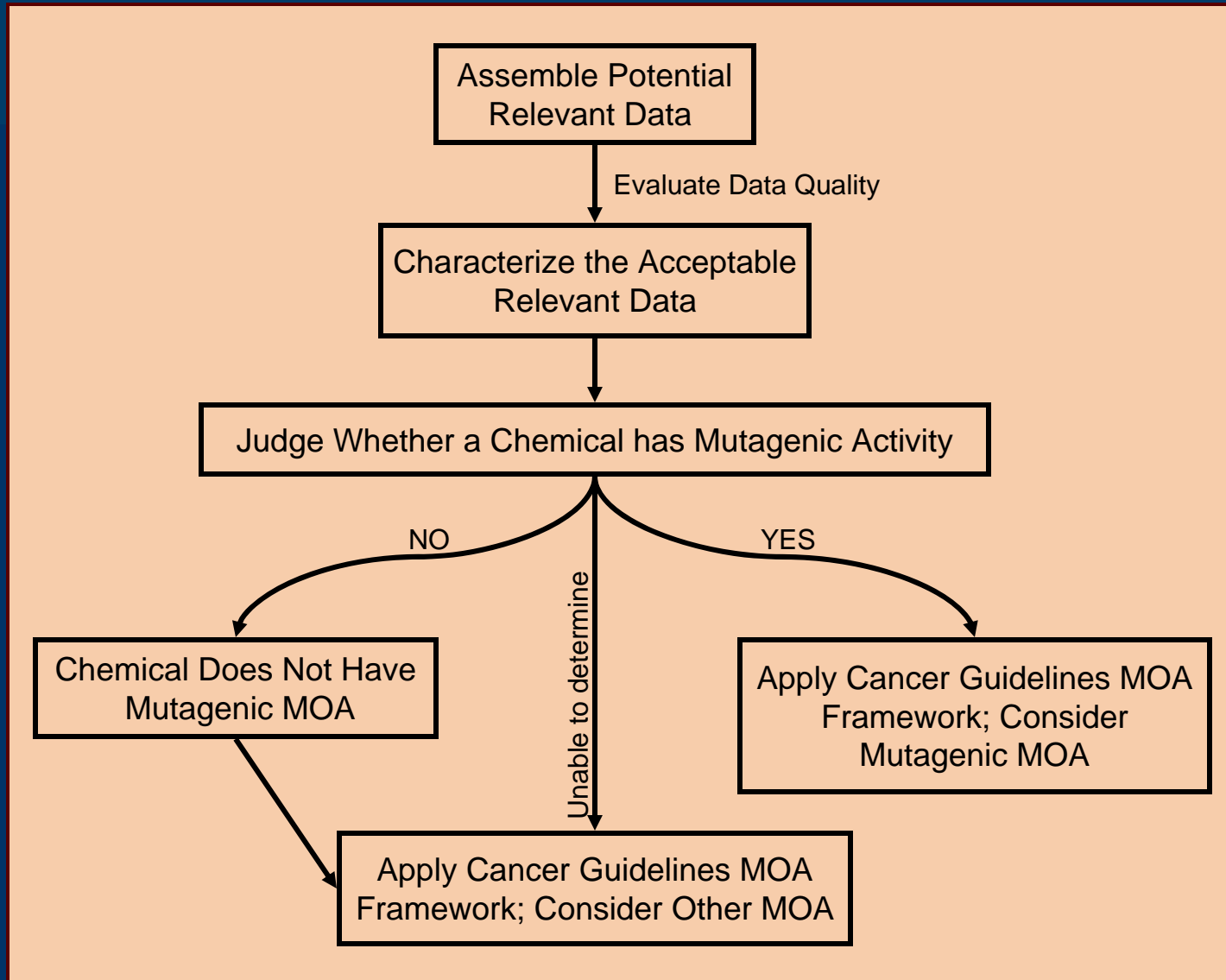
## **Framework for Determining a Mutagenic Mode of Action for Carcinogenicity**

*Using EPA's 2005 Cancer  
Guidelines and Supplemental  
Guidance for Assessing  
Susceptibility from Early-Life  
Exposure to Carcinogens*

- External Peer Review Draft  
September, 2007
- Public Comment completed  
December, 2007
- External peer review  
Completed – April, 2008
- <http://www.epa.gov/osa/mmoa-framework/pdfs/MMOA-ERD-FINAL-83007.pdf>

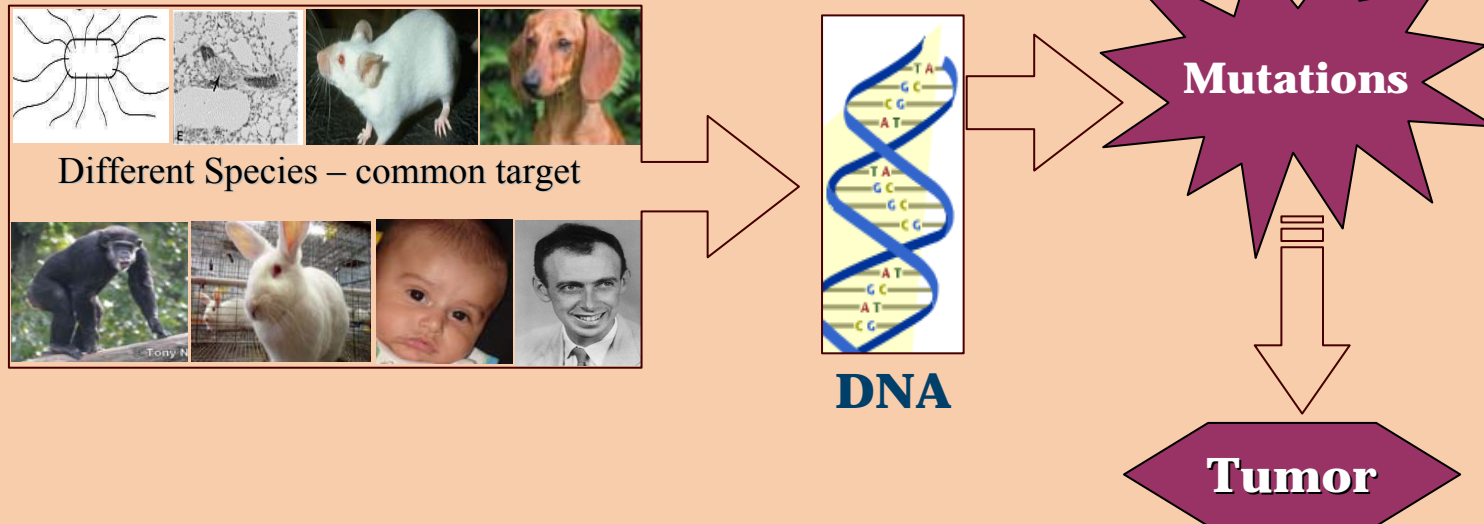
- Genotoxicity and Risk Assessment
- Mode of Action Frameworks
- ***Use of Genotoxicity Data in IRIS Assessments***
- Case Study – 1,2,3 -Trichloropropane

# Evaluation of Mutagenicity Data

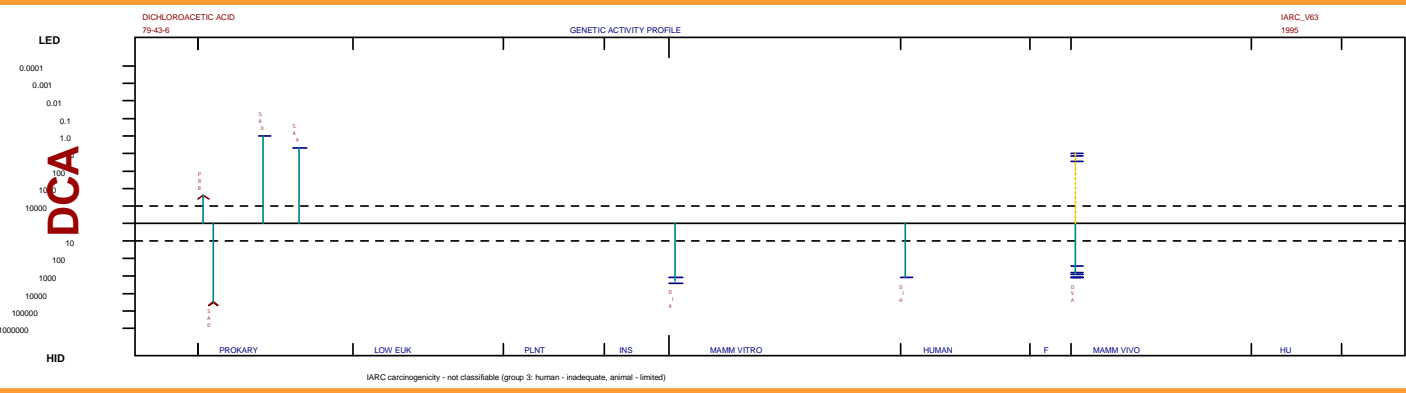
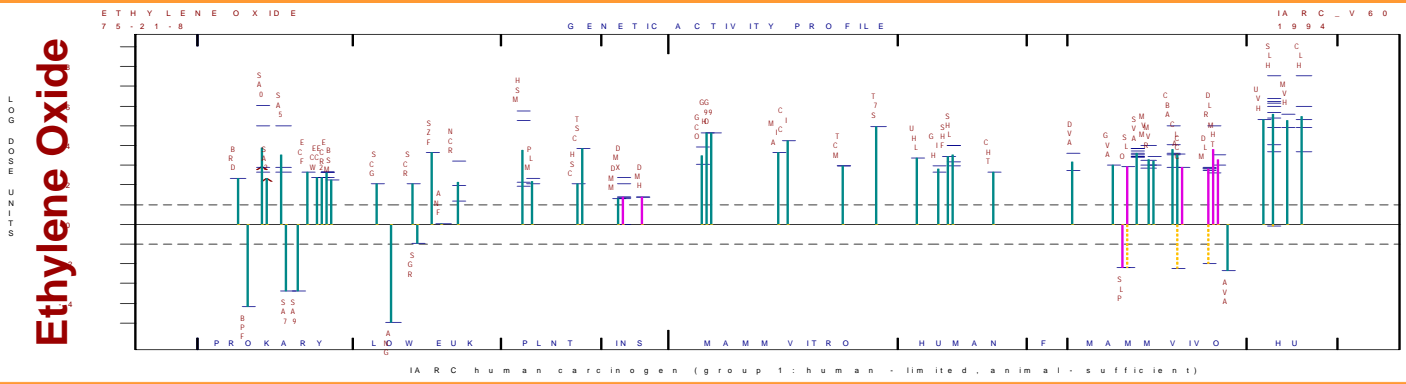
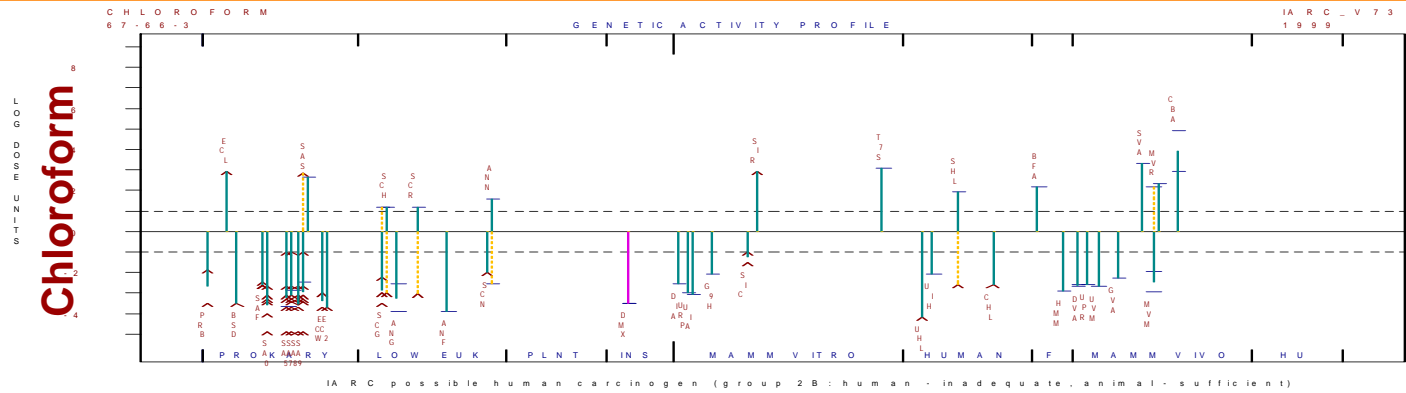


# Weight of Evidence

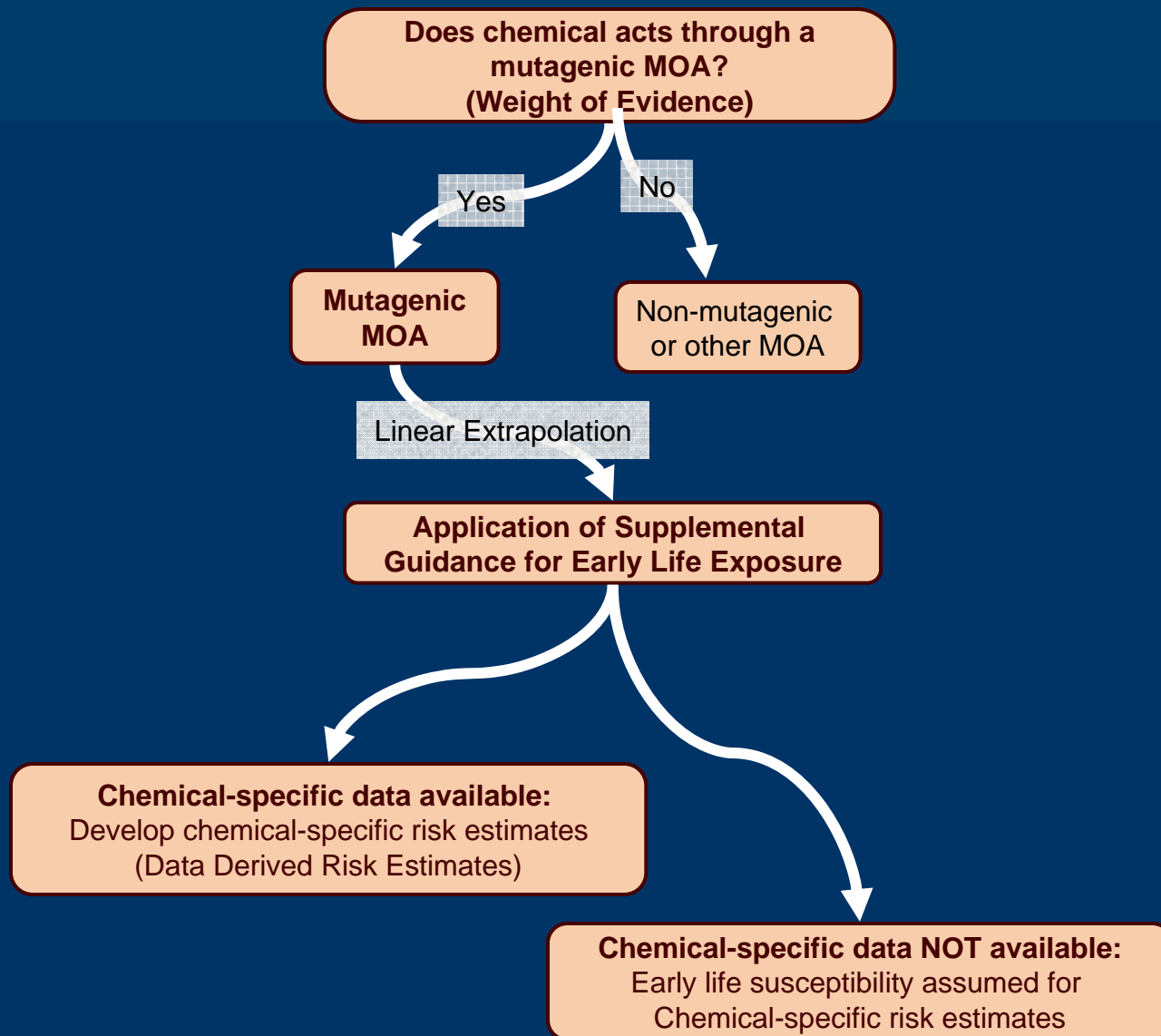
## DNA as Common Target for Environmental Exposures and Cancer – Basis for Mutagenic Mode of Action



# Genetic Activity Profile



## Implication of Mutagenic MOA and Application of ADAFs in IRIS Assessments



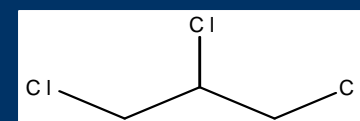
- Genotoxicity and Risk Assessment
- Mode of Action Frameworks
- Use of Genotoxicity Data in IRIS Assessments
- ***Case Study – 1,2,3 –Trichloropropane (TCP)***

## *Challenges*

- Availability of chemical specific data
- Quality and quantity of data
- Mechanistic understanding and MOA analysis
- Extrapolation of in vitro data to in vivo systems
- Analysis is based on WOE - Not a check list
- Extensive review process

## 1,2,3-Trichloropropane

- 1,2,3-Trichloropropane is a three-carbon alkane with a single chlorine atom per carbon atom



- Used in the chemical industry as a solvent for oils and fats, waxes, and resins. It has also been used as paint thinner and varnish remover, and as a degreasing agent
- TCP is also a frequent contaminant at hazardous waste sites and in the environment as a drinking water contaminant
- EPA's IRIS Program is currently evaluating the hazard associated with exposure to TCP including an analysis of the potential MOA

# Tumor Incidence and DNA Adduct Data

Organ	Dose	Tumor Incidence <sup>a</sup>	Adduct level ( $\mu$ mol/mol guanine) <sup>b</sup>
<b>Male rats</b>			
Forestomach <sup>c</sup>	3	33/50	3.7
	30	43/52	14.6
Kidney <sup>c</sup>	3	2/50	6.6 $\pm$ 1.4
	30	21/52	38.9 $\pm$ 5.0
Pancreas <sup>c</sup>	3	21/50	5.3 $\pm$ 1.0
	30	29/52	37.8 $\pm$ 12.8
Preputial gland	3	6/47	ND <sup>d</sup>
	30	16/50	ND <sup>d</sup>
Oral <sup>c</sup>	3	2/50	4.0
	30	37/52	20.4
Glandular stomach	3	0/50	3.8
	30	0/52	20.4
Liver	3	1/50	5.4 $\pm$ 0.7
	30	3/52	47.6 $\pm$ 21.0

# Tumor Incidence and DNA Adduct Data (cont'd)

Male mice			
Forestomach <sup>c</sup>	6	50/51	19.8
	60	55/56	41.0
Liver <sup>c</sup>	6	24/51	12.1 ± 4.6
	60	31/56	59.3 ± 21.7
Lung	6	11/51	0.77 ± 0.16
	60	6/56	5.3 ± 0.2
Glandular stomach	6	0/51	28.1
	60	0/56	208.1
Kidney	6	0/51	4.4 ± 2.9
	60	0/56	32.5 ± 11.3

<sup>a</sup> from NTP, 1993 and tallied in La et al., 1993.

<sup>b</sup> from La et al., 1995; expressed as mean ± standard deviation from four animals with statistical significance not analyzed.

<sup>c</sup> statistically significant increase in tumor formation from NTP, 1993.

<sup>d</sup> not detected.

Source: La et al., 1995

# Genotoxicity Data

## In Vitro Gene Mutation Assays

Test System	Cells/strain	Positive concentrations	Results		Reference
			-S9	+S9	
<i>S. typhimurium</i> (Ames test)	TA100, A1535	10, 33, 100, 333 $\mu$ g/plate	-	+	Haworth et al., 1983
	TA1537, TA98		-	-	
	TA98,100,1535,1537	20, 200, 2000 $\mu$ g/plate	-	+	Shell Oil Co., 1979
	TA97, TA100, TA1535	10, 33, 100, 333 $\mu$ g/plate	-	+	NTP, 1993
	TA98	100, 333 $\mu$ g/plate	-	+	
	TA1537		-	NP	
	TA100	0. 1, 1 $\mu$ mol/plate	-	+	Stolzenberg&Hine, 1980
	TA100	0.01, 0.02, 0.04, 0.1	-	+	Lag et al., 1994
	TA1535, A100	5, 10, 50, 100 $\mu$ g/plate	-	+	Ratpan and Plaumann, 1988
	TA98, TA1538, TA1537	N/A	-	-	
	TA98, TA100, TA1535	0.02-1.0 mg/plate	-	+	Kier, 1982
	TA1537, TA1538	N/A	-	-	
<i>E. coli</i> (SOS chromotest)	PQ37		-	-	von der Hude et al., 1988
<i>E. coli</i> (DNA-repair deficient strain)	WP2 <i>uvrA</i>	2000 $\mu$ g/plate	-	+	Shell Oil Co., 1979
<i>E. coli</i> (DNA- repair-proficient)	WP2	N/A	-	-	

# Genotoxicity Data

## In Vitro Mammalian Cell Assays

Chromosomal Aberrations	CHO cells	59.5, 69.4, 79.2 $\mu$ g/mL	–	+	NTP, 1993
	Rat liver epithelial	N/A	–	–	Shell Oil Co., 1979
Micronucleus	Human lymphocytes	N/A	–	–	Tafazoli and Kirsch-Volders, 1996
Micronucleus:	AHH-1	0.01, 1, 2, 5 mM	+	NP	Doherty et al., 1996
	MCL-5	1, 2, 5 mM	+	NP	
	H2E1	0.01, 1, 2, 5 mM	+	NP	
Unscheduled DNA synthesis (UDS)	Male rat hepatocytes (F344/N)	N/A	–	NP	Williams et al., 1989
DNA strand breaks (Comet assay)	Human lymphocytes	2, 4 mM	+	+	Tafazoli and Kirsch-Volders, 1996
	Wistar rat hepatocytes	N/A	–	NP	Holme et al., 1991
DNA Fragmentation	V79	4, 5 mM		+ <sup>a</sup>	Eriksson et al., 1991
Sister chromatid exchanges	CHO	14.2, 39.7, 49.6, 59.5 $\mu$ g/ml	–	+	NTP, 1993
	V79	0.3, 1.0 mM	–	+	von der Hude et al., 1987

# Genotoxicity Data

## In Vivo Mammalian Assays

Test System	Cells/organs	Positive Doses	Results	Reference
Micronucleus	CD-1 mice, bone marrow erythrocytes	N/A	–	Crebelli et al., 1999
DNA strand breaks (Comet assay)	F344/N male rat hepatocytes	30, 100, 300 mg/kg	+	Weber and Sipes, 1991
	Wistar male rat Kidney	≥ 375 μ mol/kg	+	Lag et al., 1991
DNA adducts	F/344/N male rat (multiple organs)	3 or 30 mg/kg	+	La et al., 1995
	B6C3F1 male mice (multiple organs)	6 or 60 mg/kg	+	

# Genotoxicity Data

## Other In Vivo Assays

Dominant lethal mutation	SD male rats, Implants and embryos	N/A	–	Saito-Suzuki et al., 1982
Wing spot test	Drosophila melanogaster	4.51 $\mu$ g/L (inhalation)	+	Chroust et al., 2007
Polyploidy	Albino male rat hepatocytes	0.8 mg/L (inhalation)	+	Belyaeva et al., 1974
		0.8, 2.16 mg/L (inhalation)	+	Belyaeva et al., 1977

N/A: Either chemical had no effect or information is not available (abstracts only)

NP: Assay is not performed

<sup>a</sup> Metabolic enzyme induction not specified

## *IRIS Summary/Conclusion Based on Available Data*

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- The proposed mode of action for 1,2,3-TCP tumorigenicity involves mutagenicity via reactive metabolites.
  - The available in vitro and in vivo data also indicate that metabolites of TCP have an affinity for certain nucleic acids and a capacity to form DNA adducts, *although*, in vivo assays which directly measure mutagenicity are unavailable.
  - Given the weight of the available evidence, TCP *may be acting through a mutagenic MOA; however, the database is lacking in vivo evidence that mutagenic events* occur following TCP exposure. For these reasons, the application of ADAFs when assessing risks associated with early-life exposure is not recommended.

# External Peer Review Charge Question

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## *In Vivo Mammalian Assays*

*Evidence indicating the MOA for carcinogenicity of 1,2,3-TCP was considered. A conclusion was reached that it is possible that this chemical is operating through a mutagenic mode of action, but the database contains limited evidence of in vivo mutagenic events that could lead to the observed cancer. Please comment on whether the weight of the scientific evidence supports this conclusion.*

## External Peer Reviewer's Opinion

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*.....Even though there is no in vivo evidence for mutations after exposure to TCP, there is sufficient converging scientific evidence for mutagenic mode of action.....*

*....I concur that the weight of evidence supports mutagenesis, as the primary mode of action of TCP.*

*.....given the available evidence, the statement that the compound “may be acting through a mutagenic MOA” understates the overall weight of the evidence. It would be more concise to report that the compound “is likely to act by a mutagenic MOA”.*

*.....there are sufficient data to indicate that TCP should be considered a genotoxic carcinogen based upon criteria established under the current risk assessment guidelines. However, alternative modes of action have not been sufficiently considered.*

*.....the data in support of a genotoxic mechanism of action are limited. Most significant is the lack of in vivo mutagenicity data..... together, these lines of evidence would seem to make a strong case for a genotoxicity. However, establishing etiology on the basis of mutation spectra in oncogenes or tumors induced by the compound is not justified.....*

## *Acknowledgements*

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*Lynn Flowers, Ph.D., DABT*

Assistant Center Director, NCEA, EPA

• *Martin Gehlhaus, M.Ph.*

Health Scientist, IRIS, EPA

• *Abdel Kadry, Ph.D., DABT*

Director, IRIS EPA

EPA Risk Assessment Forum - MMOA Technical Panel