An Overview of the Genotoxicity of Aromatic Hydrocarbons and their Reactive Intermediates

Stephen Nesnow[a] and Andrew Kligerman[b]

[a] Stephen Nesnow, Consulting, Chapel Hill, NC
[b] Integrated Systems Toxicology Division, NHEERL, Research Triangle Park, NC

Introduction

This presentation will briefly review the genetic toxicology of four aromatic hydrocarbons and some of their known reactive intermediates.

The results presented were taken from review documents such as the NTP RoC, IARC, CAL/EPA OEHHA, literature reviews, Leadscope, and some original papers.

Due to the time restraint, this presentation will not be exhaustive but will be illustrative of the types of data available and the data gaps.

Ethylbenzene		*In Vitro*		*In Vivo*	
Effect	**Bacteria**	**Rodents**	**Humans**	**Rodents**	**Humans**
DNA adducts					
Mutation	**NEG ± S9** Multiple strains & studies	**POS - S9** L5178Y cells (w)			
Sister chromatid exchange			**POS + S9** PBLs (w)	**NEG** B6C3F1 mice (IHL) PBLs	
Chromosomal aberrations		**NEG ± S9** CHO **NEG - S9** RL4 cells			
Micronucleus		**POS** SHE cells		**NEG** B6C3F1 mice (IHL) PBLs NMRI mice (IP) bone marrow	
Cell transformation		**POS** SHE cells			
Tumor Sites	**Mice: lung (M) and liver (F); Rats: kidney (M&F)**				

2-Ethylhydroquinone, 4-ethylcatechol		*In Vitro*		*In Vivo*	
Effect	**Other**	**Rodents**	**Humans**	**Rodents**	**Humans**
DNA adducts	**POS + MA** CT-DNA: induce 8-oxo-dG adducts				

CUMENE		*In Vitro*		*In Vivo*	
Effect	**Bacteria**	**Rodents**	**Humans**	**Rodents**	**Humans**
Mutation	**NEG** multiple strains & studies	**NEG** CHO cells			
DNA damage			→	**POS:** F344 Rat (GAV) liver **NEG:** F344 Rat (GAV) blood, lung, kidney **POS:** B6C3F1 Mouse (GAV) lung **NEG:** B6C3F1 Mouse (GAV) blood, liver, kidney	
Chromosomal aberrations		**NEG** CHO cells			
Micronucleus				**NEG:** B6C3F1 Mouse (IHL, GAV) PBLs **POS:** F344 Rat (IP) bone marrow, PCE **NEG:** F344/DuCrl Rat (GAV) PBLs	
Cell Transformation		**NEG** BALB 3T3 cells			
UDS		**NEG** Rat hepatocytes			
Mutations in lung tumors			→ →	**B6C3F1 Mouse lung tumors (IHL)** **POS: K-*ras* mutations** **POS: *p53* mutations**	
Tumor sites	→ **Mice: lung (M&F), liver (F); Rat kidney (M)**				

Cumene → α-methylstyrene → α-methylstyrene oxide

α-Methylstryene		*In Vitro*		*In Vivo*	
Effect	**Bacteria**	**Rodents**	**Humans**	**Rodents**	**Humans**
Mutation	**NEG ± S9** Multiple strains				
Chromosomal aberrations		**NEG ± S9** CHO cells			
Sister chromatid exchange			**POS - S9** PBLs (w) **POS + S9** CHO cells **POS - S9** CHO cells		
Micronucleus				**POS** B6C3F1 female mice (IHL), PBLs **NEG** B6C3F1 male mice (IHL), PBLs	
Tumor Sites	**Mice: liver (F); Rats: kidney (M)**				

α-Methylstryene oxide		*In Vitro*		*In Vivo*	
Effect	**Bacteria**	**Rodents**	**Humans**	**Rodents**	**Humans**
Mutation	**POS - S9** Multiple strains				

Naphthalene		*In Vitro*		*In Vivo*	
Effect	**Bacteria/Insects/Newts**	**Rodents**	**Humans**	**Rodents**	**Humans**
DNA adducts				**POS** SENCAR mouse (dermal) skin	
Mutation	**NEG ± S9** Bacteria, many studies **POS** *D. melanogaster*		**NEG -S9** MCL-5B cells		
DNA damage	**NEG ± S9** *E. coli,* multiple studies	**NEG** Rat hepatocytes, J744a.1 cells		**POS** SD Rat (GAV) liver, brain **POS** C57BL mice (GAV) liver, brain	
Chromosomal aberrations		**POS + S9** CHO cells			
Sister chromatid exchange		**POS ± S9** CHO cells	**NEG + S9** PBLs		
Micronucleus	**POS** *P. walt* larve	**NEG** ICR mice bone marrow	**POS - S9** MCL-5B cells	**NEG** ICR mouse (GAV) bone marrow	
Cell transformation		**NEG** Multiple cell types			
Tumor sites	**Mice: lung (F); Rats nasal (M&F)**				

1,2-Naphthoquinone		*In Vitro*		*In Vivo*	
Effect	**Bacteria/other**	**Rodents**	**Humans**	**Rodents**	**Humans**
DNA adducts	**POS** Aldehydic lesions in CT-DNA			**POS** SENCAR mice (dermal) skin	
Mutation	**POS ± S9** Multiple strains				
DNA damage			**POS - S9** MCF-7 cells		
Sister chromatid exchange			**POS - S9** PBLs		

1,4-Naphthoquinone		*In Vitro*		*In Vivo*	
Effect	**Bacteria**	**Rodents**	**Humans**	**Rodents**	**Humans**
DNA adducts					
Mutation		**NEG - S9 V79 cells**			
DNA damage			**NEG - S9** MCF-7 cells		
Sister chromatid exchange		**NEG - S9 V79 cells**	**POS - S9** PBLs		
Micronucleus		**POS - S9 V79 cells**			

STYRENE		*In Vitro*		*In Vivo*	
Effect	**Bacteria Other**	**Rodents**	**Humans**	**Rodents**	**Humans**
DNA adducts			→	**POS** NMRI mice (IP) SO adducts in lung, liver spleen → NMRI mice (IHL) SO adducts lung → CD-1 mice (IHL) SO adducts liver CD-1 mice (IP) 8-oxodG adducts lung → CD rats (IHL) SO adducts lung (w), liver (w) SD rats (IHL) SO adducts lung (w), liver (w) → **NEG** CD-1 mice (IHL) 8-oxodG adducts lung → CD rats (IHL) 8-oxodG adducts lung	**POS** SO N7 & O6 DNA adducts in PBLs of hand-lamination workers (all studies). 8-oxo-dG adducts in workers PBLs (1 study)
Mutation	**NEG ± S9** Bacteria, multiple strains, studies **POS** *D. melanogaster* *S. cerevisiae* **NEG** *S. pombe*	**POS + S9** V79 cells **NEG ± S9** L5178Y cells			**POS** Workers RBC, Glycophorin A (W) **NEG** Workers (HRPT) PBLs, other studies
DNA damage		**POS - S9** Rat hepatocytes	→	**POS** NMRI mice (IP) lung, PBLs, other organs C57BL mice (IP) PBLs, other organs NMRI mice (IHL) liver **NEG** F344 Rat (IHL) PBLs	**POS/NEG** PBLs. Many studies. POS in most studies
Tumor sites	→	**Mice: lung (M&F); Humans: lymphohematopoietic**			

STYRENE		*In Vitro*		*In Vivo*	
Effect	**Bacteria Other**	**Rodents**	**Humans**	**Rodents**	**Humans**
Chromosomal aberrations	**POS** *Allium cepa*	**POS + S9** CHL cells (w)	**POS** Whole blood cultures, PBLs	**POS** Wistar rat (IHL) bone marrow CD-1 mice (GAV) bone marrow **NEG** C57BL6 mice (IP) bone marrow B6C3F1 mice (IHL) PBLs, lung SD rat (IHL) bone marrow F344 rat (IHL) bone marrow, PBLs Chinese hamster (IHL) bone marrow	**POS/NEG** Meta analysis found 22 studies with positive association
Sister Chromatid Exchange		**POS** Rat lymphocytes (whole blood) **POS** CHO cells	**POS** Whole blood cultures, PBLs	**POS** B6C3F1 mice (IHL) PBLs, lung BDF mice (IHL) bone marrow, liver, macrophages **NEG** F344 rat (IHL) PBLs	**POS/NEG** Conflicting results from many studies of workers
Micronucleus	**POS** *Allium cepa*		**POS** PBLs (whole blood)	**POS** C57BL (IP) mice bone marrow **NEG** B6C3F1 mice (IHL) NCEs, spleen (binucl.) F344 rat (IHL) PBLs (PCEs) Chinese hamsters (IP) PCEs, NCEs	**POS/NEG** Conflicting results from many studies of workers
UDS			**NEG ± S10** Heteroploid EUE cells	**NEG** CD-1 mice (IHL) liver	
Cell Transformation		**NEG** **C3H10T1/2 cells**			
Tumor sites	**Mice: lung (M&F); Humans: lymphohematopoietic**				

STYRENE OXIDE	*In Vitro*			*In Vivo*	
Effect	**Bacteria, other**	**Rodents**	**Humans**	**Rodents**	**Humans**
DNA Adducts	**POS** SO adducts w CT DNA & dG, dA, dC, dT	→	**POS** Whole blood, PBLs, embryonic lung fibroblasts, keratinocytes →	**POS** NMRI mice (IP, 2hr) binding: lung, brain liver (N7-Gua) CD-1 mice (IP) 8-oxo-dG adducts lung **NEG** CD rats (GAV) binding forestomach B6C3F1 mice (GAV) binding liver	←
Mutation	**POS** Bacteria, multiple strains, studies; *D. melanogaster, S. pombe*	**POS - S9** V79, L5178Y cells	**POS - S9** T & B lymphocytes, PBLs		
DNA damage	→	**POS** V79 cells, Rat hepatocytes, PC12 cells, testicular cells	**POS - S9** Embryonic lung fibrobasts, testicular cells, PBLs and HL60 cells	**POS** CD-1 mice (IP) lung, liver other organs ddY mice (IP) lung, liver, multiple organs C57BL6 mice (IP) liver, multiple organs; **NEG** F344 rats (INH) PBLs	← ←
Tumor sites	**Mice: forestomach (M&F) liver (M); Rats: forestomach (M&F)**				

STYRENE OXIDE	*In Vitro*			*In Vivo*	
Effect	**Bacteria, other**	**Rodents**	**Humans**	**Rodents**	**Humans**
Chromosomal aberrations	**POS** *Allium cepa*	**POS - S9** V79, CHL cells	**POS - S9** PBLs	**POS** CD-1 mice (GAV) bone marrow **NEG** Chinese hamster (IHL) bone marrow **NEG** BALB/c mice (IP) dominant lethal mutations or translocations in meiotic germ cells	
Sister Chromatid Exchange	→	**POS - S9** CHO, V79 cells	**POS - S9** PBLs	**POS** CD-1 (IP) bone marrow **NEG** Chinese hamster (IHL) bone marrow BDF mice (IHL) bone marrow	
Micronucleus	**POS** *Allium cepa*	**POS - S9** V79 cells	**POS - S9** PBLs	**NEG** BALB/c mouse (IP) bone marrow Chinese hamster (IP) bone marrow F344 rats (IHL) bone marrow	
UDS			**POS - S9** PBLs		
Cell Transformation		**NEG** C3H10T1/2 cells			
Tumor sites	**Mice: forestomach (M&F) liver (M); Rats: forestomach (M&F)**				

STYRENE		*In Vitro*		*In Vivo*	
Effect	**Bacteria, other**	**Rodents**	**Humans**	**Rodents**	**Humans**
DNA adducts				POS NEG	POS
Mutation	POS NEG	POS NEG			POS NEG
DNA damage		POS		POS NEG	POS NEG
Chromosomal aberrations	POS	POS	POS	POS NEG	POS NEG
Sister Chromatid Exchange		POS	POS	POS NEG	POS NEG
Micronucleus	POS		POS	POS NEG	POS NEG
UDS			NEG	NEG	
Cell transformation		NEG			
Tumor sites	Mice: lung (M&F); Humans: lymphohematopoietic				

STYRENE OXIDE	*In Vitro*			*In Vivo*	
Effect	**Bacteria, other**	**Rodents**	**Humans**	**Rodents**	**Humans**
DNA Adducts	POS		POS	POS NEG	
Mutation	POS	POS	POS		
DNA damage		POS	POS	POS NEG	
Chromosomal aberrations	POS	POS	POS	POS NEG	
Sister Chromatid Exchange		POS	POS	POS NEG	
Micronucleus	POS	POS	POS	NEG	
UDS			POS		
Cell Transformation		NEG			
Tumor sites	Mice: forestomach (M&F) liver (M); Rats: forestomach (M&F)				

Styrene and Styrene Oxide similar results box legend:

RED = lung

Purple = any system

Conclusions

Unlike some strong genotoxins, these aromatic hydrocarbons give a mixed pattern of responses seemingly dependent on many factors (e.g. metabolic capability, cell type, species, strain, gender, tissue, route of administration).

In some cases they are only partially active across the breadth of bioassays for DNA adducts, DNA damage, mutation, chromosomal effects and related endpoints.

For genotoxic activity, they may require specific groups of enzymes that are only induced by the parent chemical for their genotoxic responses (e.g. AMS).

The lack of substantial data on some of these agents hinders a full evaluation of their genotoxic potential.

There is some evidence that ROS can contribute to the genotoxicity of several of these agents (e.g. ethylbenzene, naphthalene, styrene).

In mouse lung, styrene induced styrene oxide-DNA adducts, 8-oxo-dG DNA adducts, DNA damage and SCE. In mouse lung styrene oxide bound to DNA, induced 8-oxo-dG DNA adducts, and DNA damage.

Thus, there is evidence that styrene possesses genotoxic activity in mouse lung that could contribute to its MOA of tumor formation.