Epigenetic alterations induced by genotoxic occupational and environmental human chemical carcinogens:
A systematic literature review

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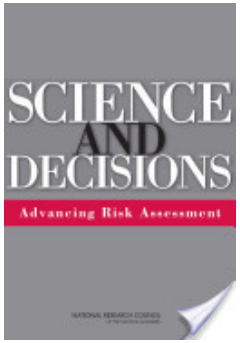
Disclaimers:

- Research is funded by grants from NIH and US EPA
- Currently serve on several National Research Council committees that consider issues relevant to this workshop
- Health Assessments Workspace Collaborative (hawcproject.org) online tool was developed and supported, in part, by funding from Texas A&M University and NIH grants

MECHANISTIC DATA:

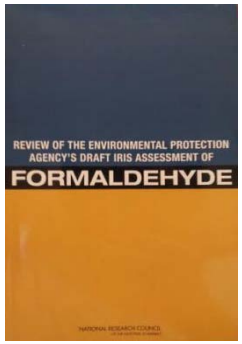
The Third (Rail?) Data Stream in Risk Assessment

A typical statement found in many human health assessment documents:
"***The mode of action of [insert your chemical's name here] is complex and has not been fully elucidated yet***"



- *"Decisions to protect public health and the environment cannot await "perfection" in scientific knowledge […]. It is important that risk assessments incorporate* ***the best available scientific information in scientifically rigorous ways and that they capture and describe the uncertainties*** *in the information in ways that are useful for decision-makers"*

NRC (2009): "Science and Decisions: Advancing Risk Assessment"



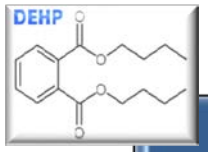
- *"Clear concise statements of* ***criteria used to exclude, include, and advance studies*** *[as part of IRIS process]"*
- *"****Standardized evidence tables*** *that provide the methods and results of each study are needed for all health outcomes; if appropriate tables were used, long descriptions of the studies could be moved to an appendix or deleted"*

NRC (2011): "Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde"

Opportunity: Improved evidence capture and reporting for risk assessments through systematic literature review

Identifying and Presenting "Mechanism Data":
DEHP Case Study [pre-historic years: 3 B.K.C.]

Deliverables:	
IRIS Tox Review	• Evidence tables • Short narrative summaries
Appendix to the IRIS Tox Review	• Search terms (*e.g.*, MeSH) • Literature trees • Inclusion/exclusion criteria • Literature tags (for HERO)
HERO Database	• Primary literature (PDF+tags) • EndNote reference libraries



1. Select a chemical and target tissue

2. Identify relevant mechanisms of action

3. Devise overall search strategy

4. Define assessment factors (i.e., exclusion criteria)

5. Conduct literature searches

6. Construct literature trees for each mechanism

7. Construct evidence tables

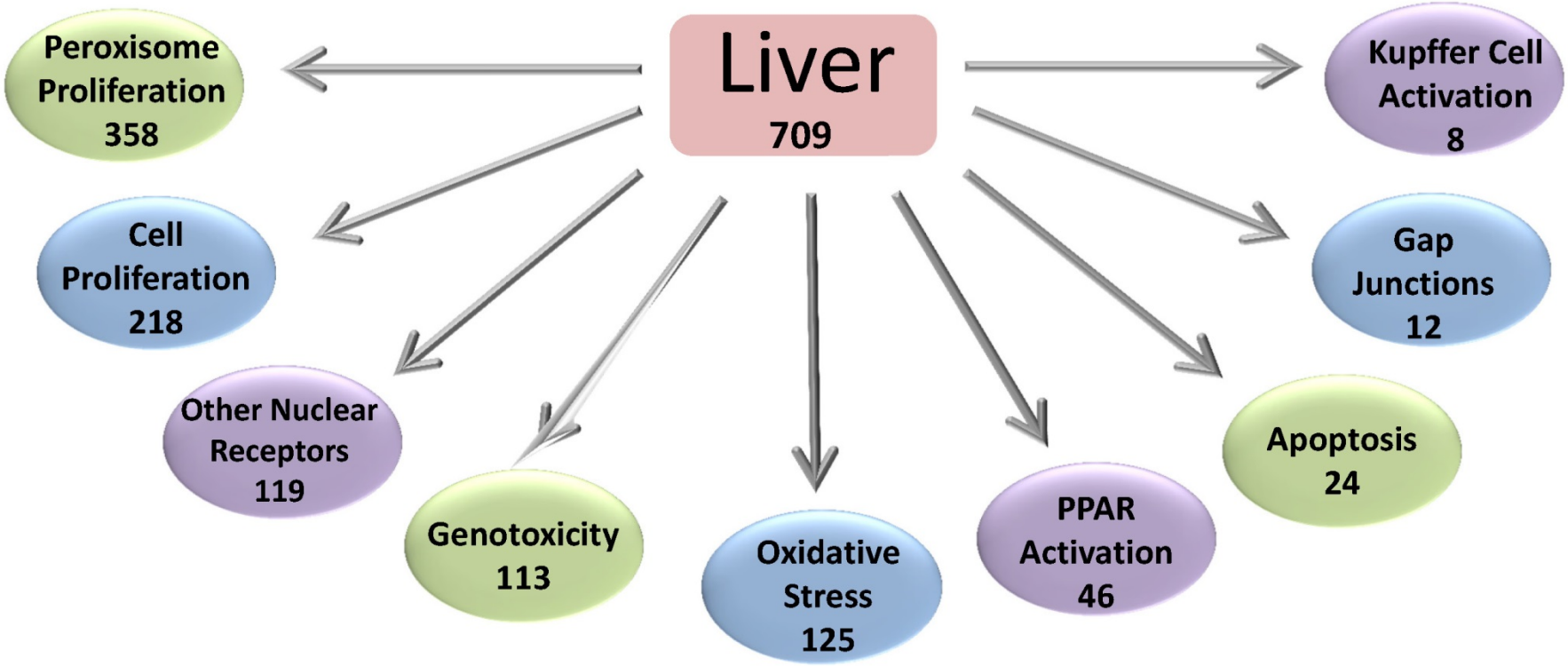
8. Organize and tag evidence

Kushman et al., ***Regul Toxicol Pharmacol*** (2013)

Specify study question:

Effects of **DEHP** and its **metabolites** on the **liver**

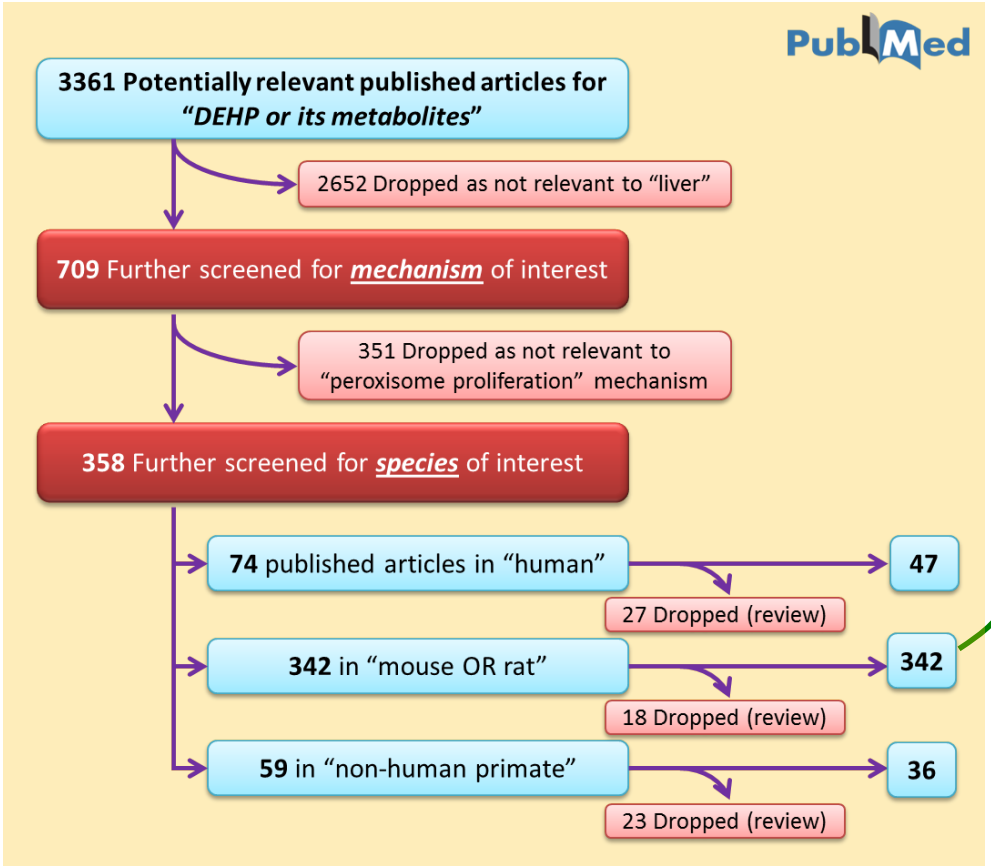
Identify relevant mechanistic events (**in liver**) from diverse and authoritative literature reviews



Kushman et al., ***Regul Toxicol Pharmacol*** (2013)

Construct Literature Trees for Each Mechanism

Large database example: "*Peroxisome Proliferation*"



Evaluation of the evidence by experts:

342 Publications selected for manual evaluation and inclusion into evidence tables

262 Excluded based on manual evaluation of the study's ***abstract*** (not DEHP/metabolites, tissue, species, or a mechanism of interest; or it was a review)

19 Excluded based on manual evaluation of the study's ***full text*** (not DEHP/metabolites, tissue, species, or a mechanism of interest)

61 Publications screened as ***full text*** for possible exclusion using Tier 2 assessment factors

43 Excluded based on detailed evaluation:
- **31** were single dose/concentration studies
- **8** reported no variability measures for quantitative data
- **4** studies had unclear description of the methodology

18 Unique publications selected for inclusion in the evidence table for these species

Kushman et al., ***Regul Toxicol Pharmacol*** (2013)

Constructing evidence tables for each mechanistic event

Provide sufficient details about:
- the study,
- study design,
- model system,
- endpoint,
- dose & concentration,
- fold change & significance

Study Design and Reference	Endpoint and Assay	Results (% Change from Control)						
Humans								
In Vivo								
No primary literature evidence identified								
In Vitro								
(Kamendulis et al., 2002)				mM MEHP				
Human 1° hepatocytes (3 males)			0	0.05	0.1	0.2	0.3	0.5
HLEC-04 cells (SV-40 transf. hum.heps)	GJIC disruption, *in situ* dye transfer (ISDT)	1° Heps, 4 hrs	0	-1	-3	nd	-2	nd
Duration: 4 or 24 hrs		24 hrs	0	-8	-6	nd	-7	nd
N = 4 cultures/group		HLEC-04, 4 hrs	0	-8	-6	nd	-7	nd
		24 hrs	0	-7	-6	nd	-7	-2
Non-human primates								
In Vivo								
(Pugh et al., 2000)			mg/kg/day DEHP					
Cynomolgus monkeys (2 yrs of age)	GJIC disruption, ISDT		0	500				
Duration: 2 wks			0	8				
N = 4 males/group								
In Vitro								
(Kamendulis et al., 2002)				mM MEHP				
Cynomolgus monkey 1° heps (5 males)	GJIC disruption, ISDT		0	0.05	0.1	0.2	0.3	0.5
Duration: 4 or 24 hours		4 hrs	0	-1	0	nd	-11	nd
N = 4 cultures/group		24 hrs	0	-3	-2	nd	-5	nd

Group studies by species:
- humans,
- primates,
- rodents

Group studies by model system:
- *in vivo*
- *in vitro*

Highlight studies that have challenged the hypothesized mode of action using knockout and transgenic (*e.g.*, humanized) mouse strains

Study Design and Reference	Endpoint and Assay			Results (% Change from Control[¶])		
***In Vivo* Chronic Cancer Bioassays**						
(Ito et al., 2007)				DEHP (mg/kg/day) [1]		
Mice				0	13	65
Sv/129 (wild type) and PPARa-null	Neoplastic changes in liver	Adenoma	Wild type	0	2	2
Males (6 wks old)			PPARa-null	0	1	6
Dietary feeding: 0, 0.01, 0.05%		Carcinoma	Wild type	0	0	0
Duration: 22 months			PPARa-null	1	0	1
N = 20-31		Cholangiocarcinoma	Wild type	0	0	0
			PPARa-null	0	0	1
		Total liver tumors	Wild type	0 (0%)	2 (8.7%)	2 (10%)
			PPARa-null	1 (4%)	1 (4%)	8* (26%)

Systematic Review of "Mechanism Data":
Styrene genotoxicity/mutagenicity (NRC 2014)

BOX D-4 Exclusion Criteria and Search Strategy for Studies of Genotoxicity and Related Mechanisms of Styrene

Exclusion Criteria

- The publication did not evaluate health effects of styrene or its metabolites known to be formed in humans.
- The study evaluated cellular, biochemical, or molecular effects not relevant to the carcinogenesis or the mechanistic event under consideration.
- The publication did not contain primary data.
- The publication did not include information sufficient to determine what species were studied or what experimental methods were used.

Search Strategy

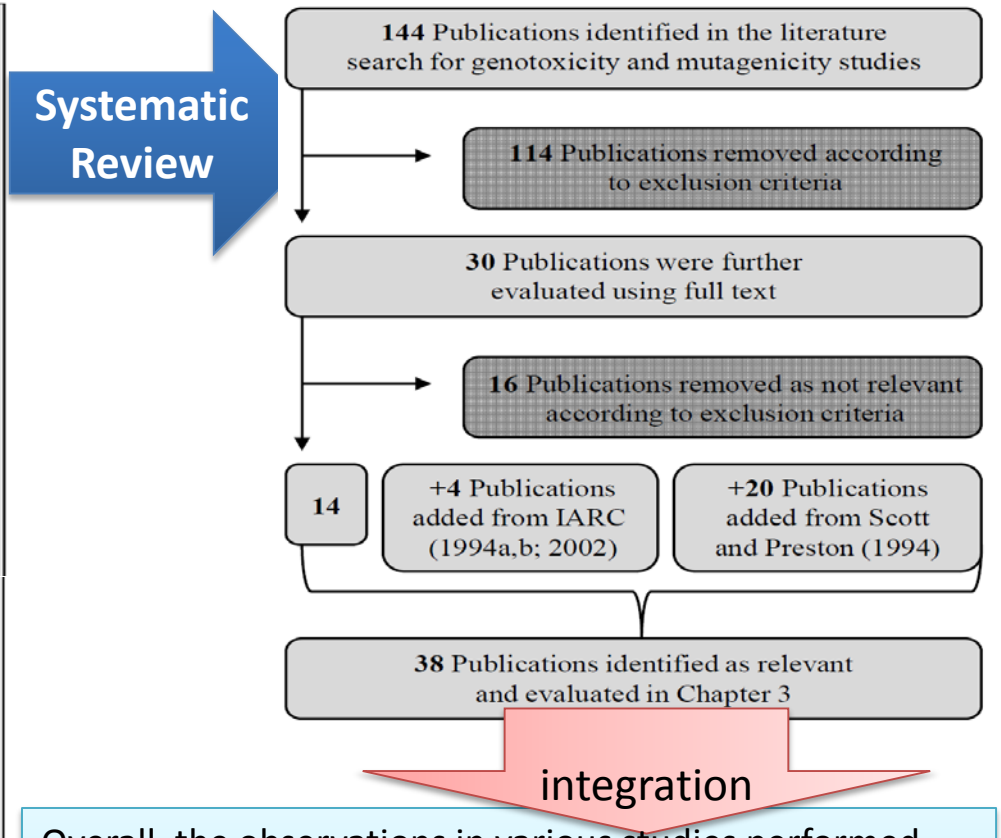
PubMed: [("Styrene"[Title/Abstract]) AND ("Mutation"[Mesh] OR "Cell Transformation, Neoplastic"[Mesh] OR "Cytogenetic Analysis"[Mesh] OR "Mutagens"[Mesh] OR "Oncogenes"[Mesh] OR "Genetic Processes"[Mesh] OR chromosom* OR clastogen* OR "genetic toxicology" OR "strand break" OR "unscheduled DNA synthesis" OR "DNA damage" OR "DNA adducts")]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

Medline and Embase: [(styrene.ab. or styrene.ti.) and (1 or 2 or 3 or 4 or 5 or 6 or chromosom*.mp. or clastogen*.mp. or genetic toxicology.mp. or strand break.mp. or unscheduled DNA synthesis.mp. or DNA damage.mp. or DNA adducts.mp.), where the following keywords are: 1) Mutation, 2) Cell Transformation, Neoplastic, 3) Cytogenetic Analysis, 4) Mutagens, 5) Oncogenes, 6) Genetic Processes]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

Scopus: [(("Styrene") AND ("mutation" OR "cell transformation, neoplastic" OR "cytogenetic analysis" OR "mutagens" OR "oncogenes" OR "genetic processes" OR "chromosom*" OR "clastogen* OR "genetic toxicology" OR "strand break" OR "unscheduled DNA synthesis" OR "DNA damage" OR "DNA adducts")]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

Web of Science: [(("Styrene") AND ("mutation" OR "cell transformation, neoplastic" OR "cytogenetic analysis" OR "mutagens" OR "oncogenes" OR "genetic processes" OR "chromosom*" OR "clastogen*" OR "genetic toxicology" OR "strand break" OR "unscheduled DNA synthesis" OR "DNA damage" OR "DNA adducts")]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

ISBN: 978-0-309-30178-7 (free from: www.nas.edu)



Overall, the observations in various studies performed over the last 3 decades have been consistent. Temporal and exposure–response relationships have been established. Not only is the experimental evidence extensive, it is likely to be relevant to all target tissues that have been associated with cancer after exposure to styrene. Causality is strengthened by the large amount of evidence obtained **from studies of exposed humans**.

HAWC: Web-based content management system

"How to be as consistent as possible (framework)?"
"How to document everything (transparency)?"
"How to increase efficiency (time considerations)?"

Information
- PubMed
- EPA HERO
- Journal Articles
- NTP, ATSDR, etc.
- DRAGON

Approaches
- EPA Guidance
- Systematic Review

Tools
- BMDS
- NTP Meta Data Viewer
- PBPK Modeling
- Dosimetry

HAWC
- Staff
- Contractors
- Internal Reviewers

External Stakeholders

End Users (risk managers, public)

Create a HAWC account or view public-assessments:

https://hawcproject.org

Actively under development; feedback is appreciated.

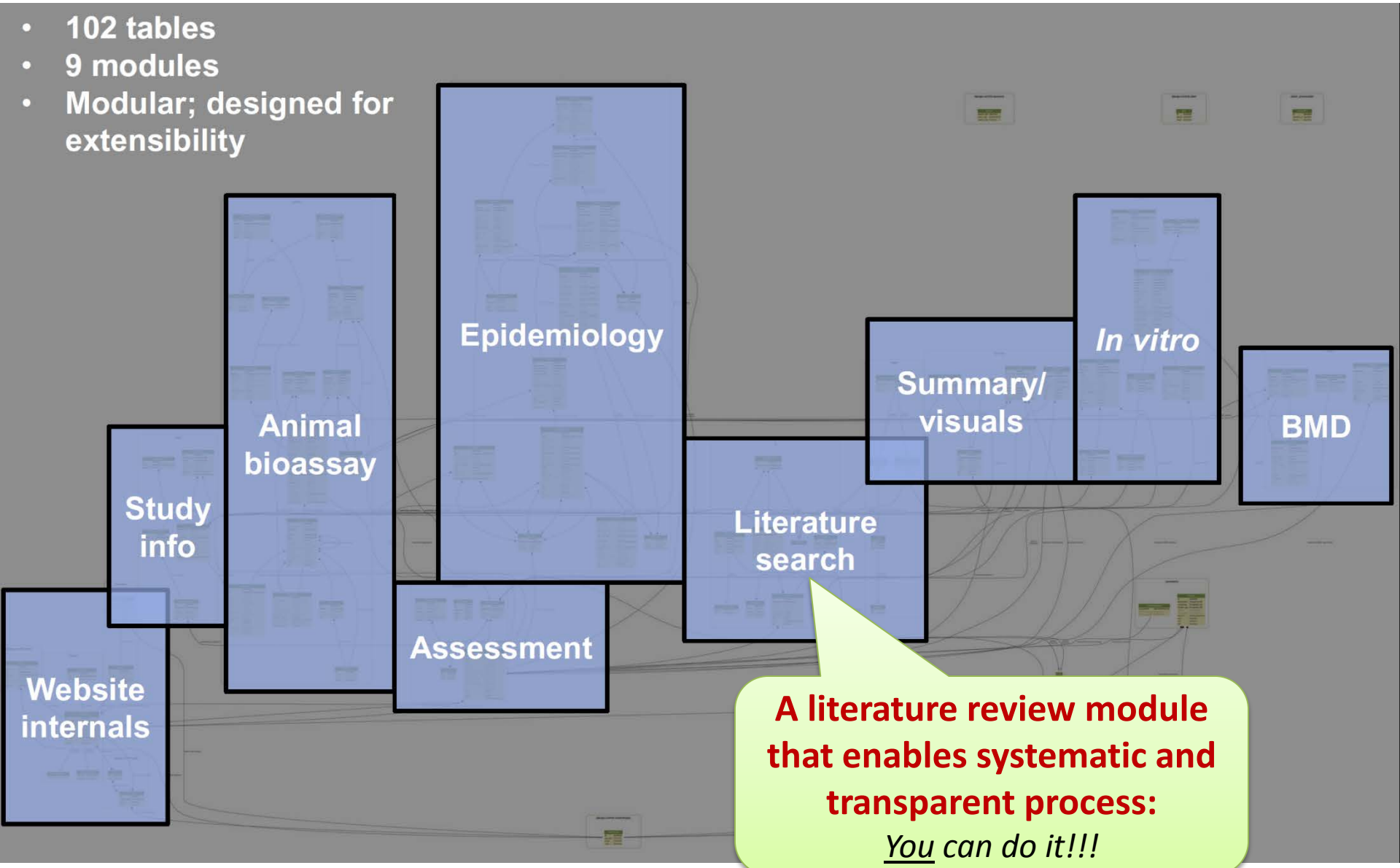
Compatible browsers:

Chrome* IE 9+

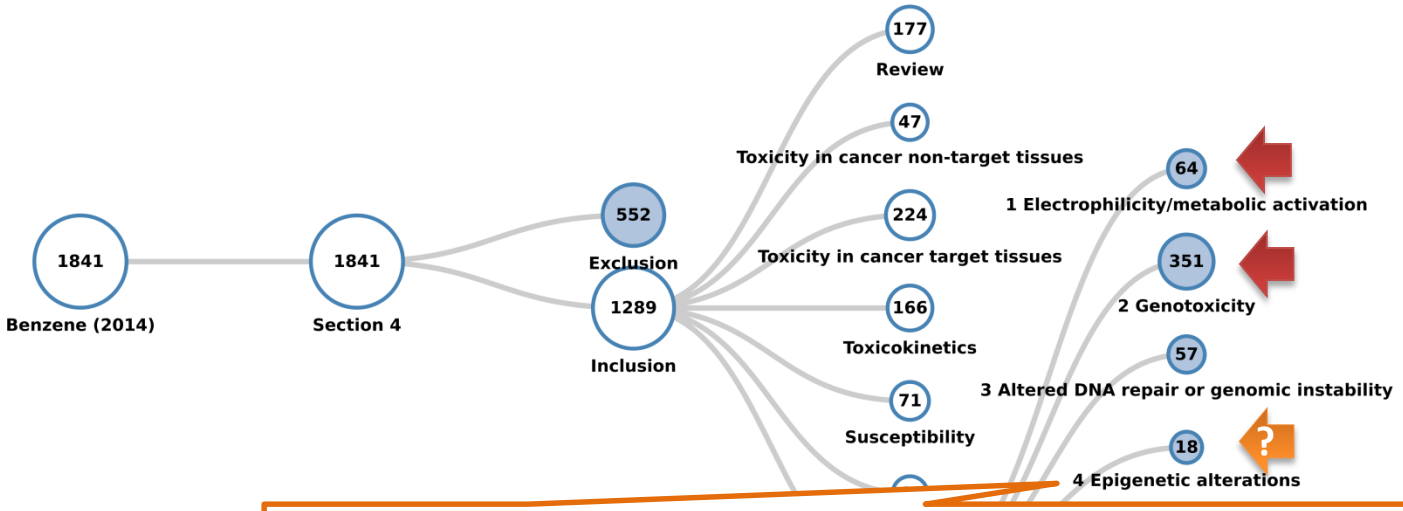
Safari Firefox

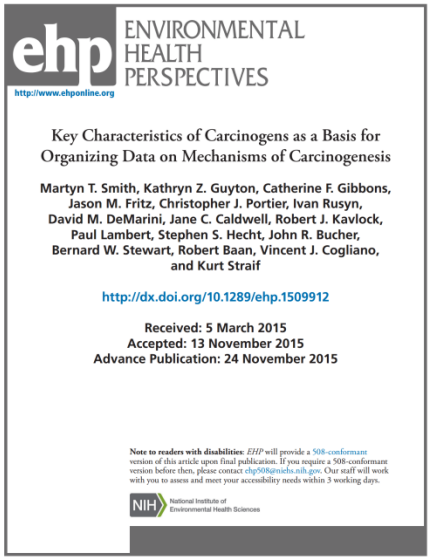
*Recommended browser

HAWC: Web-based content management system



Benzene (2014): Literature Tree




ehp ENVIRONMENTAL HEALTH PERSPECTIVES
http://www.ehponline.org

Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis

Martyn T. Smith, Kathryn Z. Guyton, Catherine F. Gibbons, Jason M. Fritz, Christopher J. Portier, Ivan Rusyn, David M. DeMarini, Jane C. Caldwell, Robert J. Kavlock, Paul Lambert, Stephen S. Hecht, John R. Bucher, Bernard W. Stewart, Robert Baan, Vincent J. Cogliano, and Kurt Straif

http://dx.doi.org/10.1289/ehp.1509912

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NIH National Institute of Environmental Health Sciences

(New) Epigenetics (#4)

Actions

Description	Epigenetics
Search Type	Search
Search Database	PubMed
Search Text	Benzene[Mesh] AND ("rna"[MeSH] OR "epigenesis, genetic"[MesH] OR rna OR "rna, messenger"[MeSH] OR "rna" OR "messenger rna" OR mrna OR "histones"[MeSH] OR histones OR epigenetic OR miRNA OR methylation)
Created	Dec. 1, 2014, 1:38 p.m.
Last Updated	Dec. 1, 2014, 1:38 p.m.

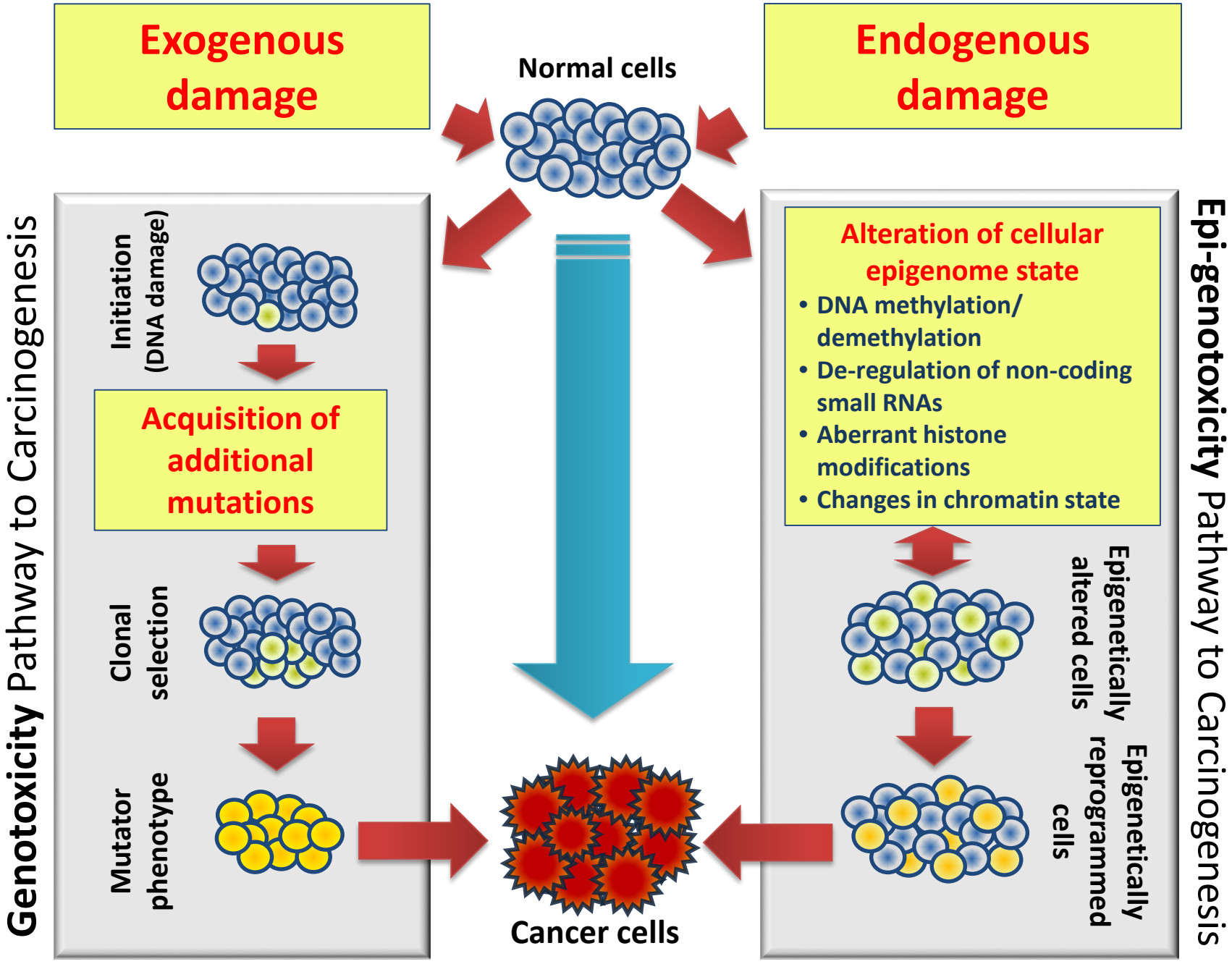
Literature Tagging Statistics

Total References	249
Total Tagged	240
Total Untagged	9
Reference details	View by tag Visualization

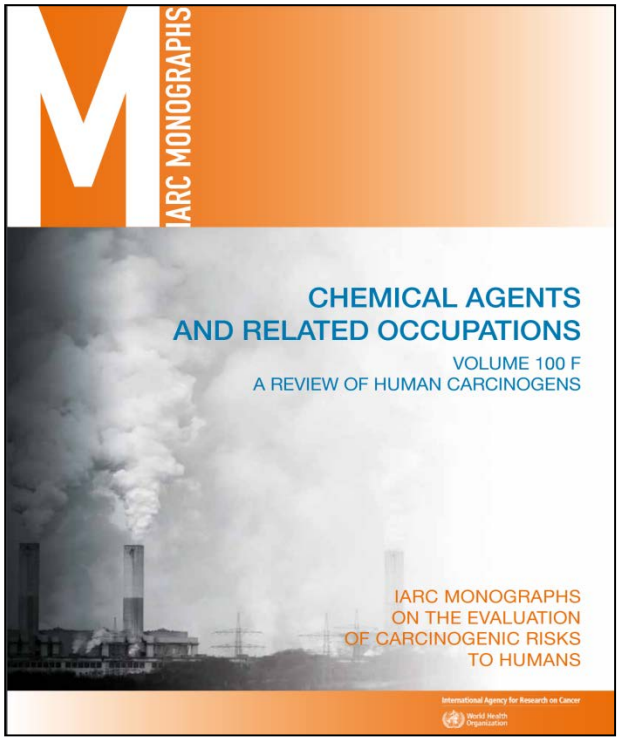
Results from queries

Date last executed	Total references found	References added	References removed
Dec. 12, 2015, 1:43 p.m.	249	9	0
Dec. 1, 2014, 1:38 p.m.	240	240	0

Tag Literature Rerun search


Exogenous damage
Endogenous damage
Normal cells
Genotoxicity Pathway to Carcinogenesis
Initiation (DNA damage)
Acquisition of additional mutations
Clonal selection
Mutator phenotype
Alteration of cellular epigenome state
• DNA methylation/ demethylation
• De-regulation of non-coding small RNAs
• Aberrant histone modifications
• Changes in chromatin state
Epigenetically altered cells
Epigenetically reprogrammed cells
Cancer cells
Epi-genotoxicity Pathway to Carcinogenesis

- Epigenetic alterations play an important role in chemical carcinogenesis
- Although changes to the epigenome may be as important in carcinogenicity, the genotoxicity of chemicals has been studied more thoroughly
- "**Most carcinogens were evaluated by IARC before new data on their epigenetic effects became available**" (Herceg *et al*., 2013)



Agents and Related Occupations (Volume 100F)

- ***4-Aminobiphenyl***
- ***Benzidine***
- ***Dyes Metabolized to Benzidine***
- ***4,4'-Methylenebis(2-chloroaniline)***
- ***2-Naphthylamine***
- ***ortho-Toluidine***
- Auramine and Auramine Production
- Magenta and Magenta Production
- ***Benzo[a]pyrene***
- ***Coal gasification***
- ***Occupational Exposures during Coal-tar Distillation***
- ***Coal-tar pitch***
- ***Coke Production***
- ***Mineral Oils, Untreated or Mildly Treated***
- ***Shale Oils***
- ***Soot, as found in occupational exposure of chimney-sweeps***
- ***Occupational Exposures during Aluminium Production***
- ***Aflatoxins***
- ***Benzene***
- ***Bis(chloromethyl)ether and Chloromethyl Methyl Ether***
- ***1,3-Butadiene***
- 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF, and PCB 126
- ***Ethylene Oxide***
- ***Formaldehyde***
- ***Sulfur Mustard***
- ***Vinyl Chloride***
- ***Isopropyl Alcohol Manufacture by the Strong-acid Process***
- ***Mists from Strong Inorganic Acids***
- ***Occupational Exposures during Iron and Steel Founding***
- ***Occupational Exposure as a Painter***
- ***Occupational Exposures in the Rubber Manufacturing Industry***

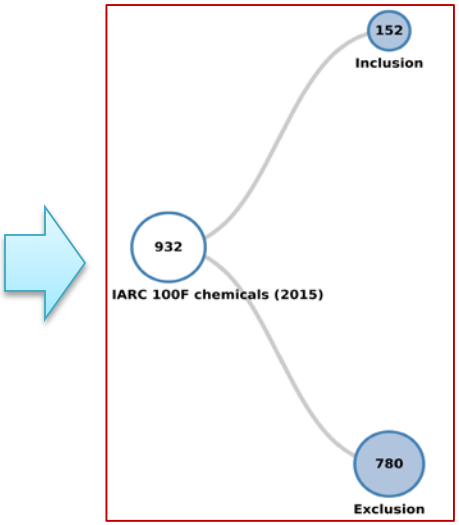
“Epigenotoxic” Literature Search Terms

IARC (vol 112 and 113) search string:

"rna"[MeSH] OR "epigenesis, genetic"[MesH] OR rna OR "rna, messenger“ [MeSH] OR "rna" OR "messenger rna" OR mrna OR "histones"[MeSH] OR histones OR epigenetic OR miRNA OR methylation

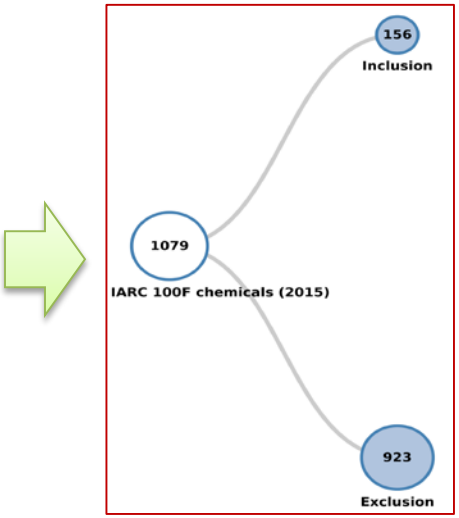
TAMU initial search string of [All Fields] terms:

"epigenetic"[All Fields] OR "microRNA"[All Fields] OR "miRNA"[All Fields] OR "lncRNA"[All Fields] OR "non-coding RNA"[All Fields] OR "non coding RNA"[All Fields] OR "ncRNA"[All Fields] OR "small RNA"[All Fields] OR "smallRNA"[All Fields] OR "DNA methylation"[All Fields] OR "methylated DNA"[All Fields] OR "promoter methylation"[All Fields] OR "chromatin modification"[All Fields] OR "open chromatin"[All Fields] OR "histone"[All Fields] OR "histone positioning"[All Fields] OR "histone methylation"[All Fields] OR "histone acetylation"[All Fields] OR “histone mark”[All Fields] OR “histone modification”[All Fields]

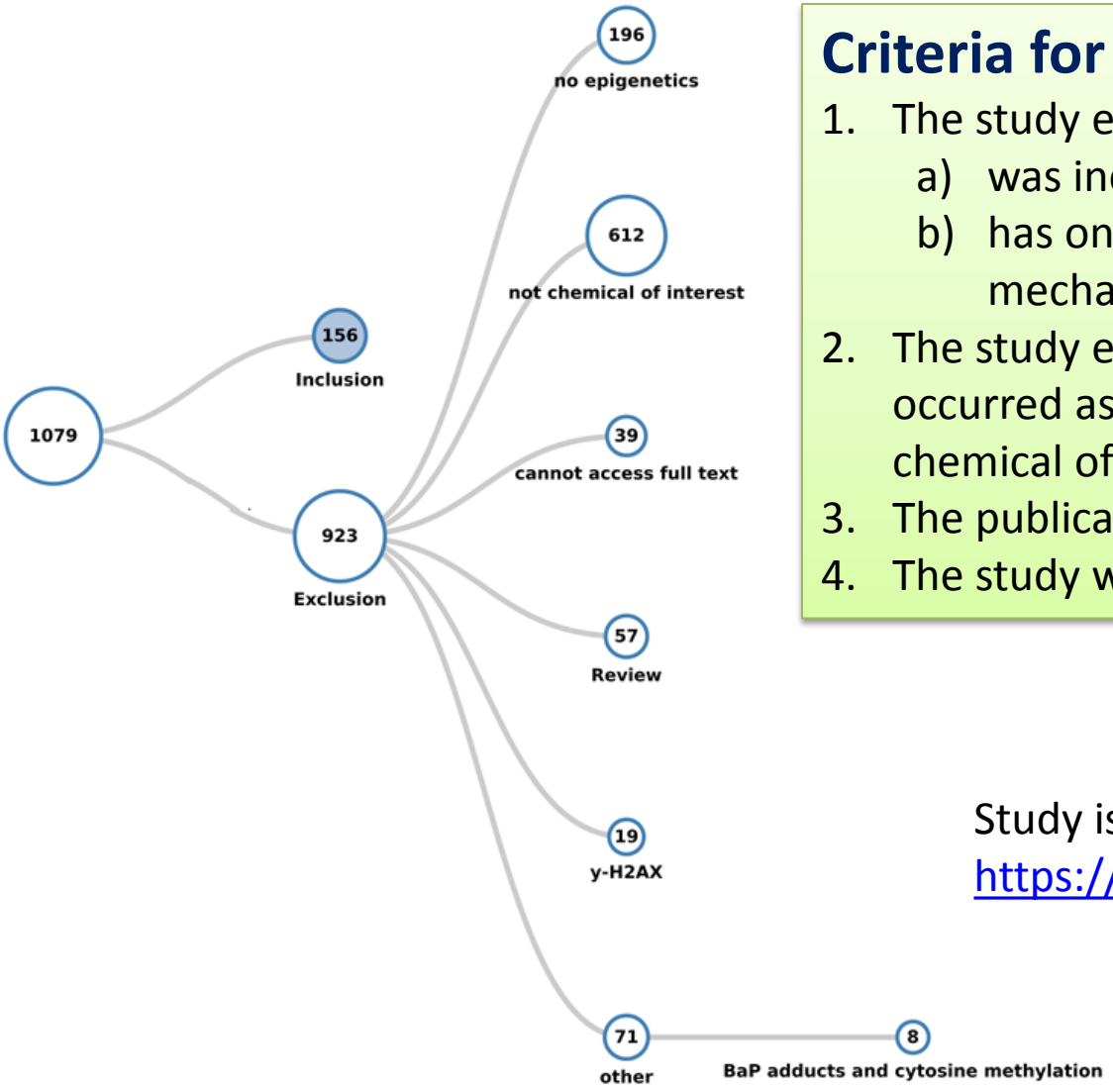


TAMU final search string of a refined set of [All Fields] and [MeSH]:

"epigenomics"[MeSH Terms] OR "epigenomics"[All Fields] OR "epigenetic“ [All Fields] OR "microrna"[All Fields] OR "micrornas"[All Fields] OR "miRNAs"[All Fields] OR "miRNA"[All Fields] OR "lncRNA"[All Fields] OR "rna, long noncoding"[MeSH Terms] OR "non-coding RNA"[All Fields] OR "non coding RNA"[All Fields] OR "ncRNA"[All Fields] OR "small RNA"[All Fields] OR "smallRNA"[All Fields] OR "dna methylation"[All Fields] OR "promoter methylation"[All Fields] OR "methylated DNA"[All Fields] OR "chromatin modification"[All Fields] OR "open chromatin"[All Fields] OR "histones"[MeSH Terms] OR "histones"[All Fields] OR "histone"[All Fields]



Epigenetic alterations induced by genotoxic occupational and environmental human chemical carcinogens

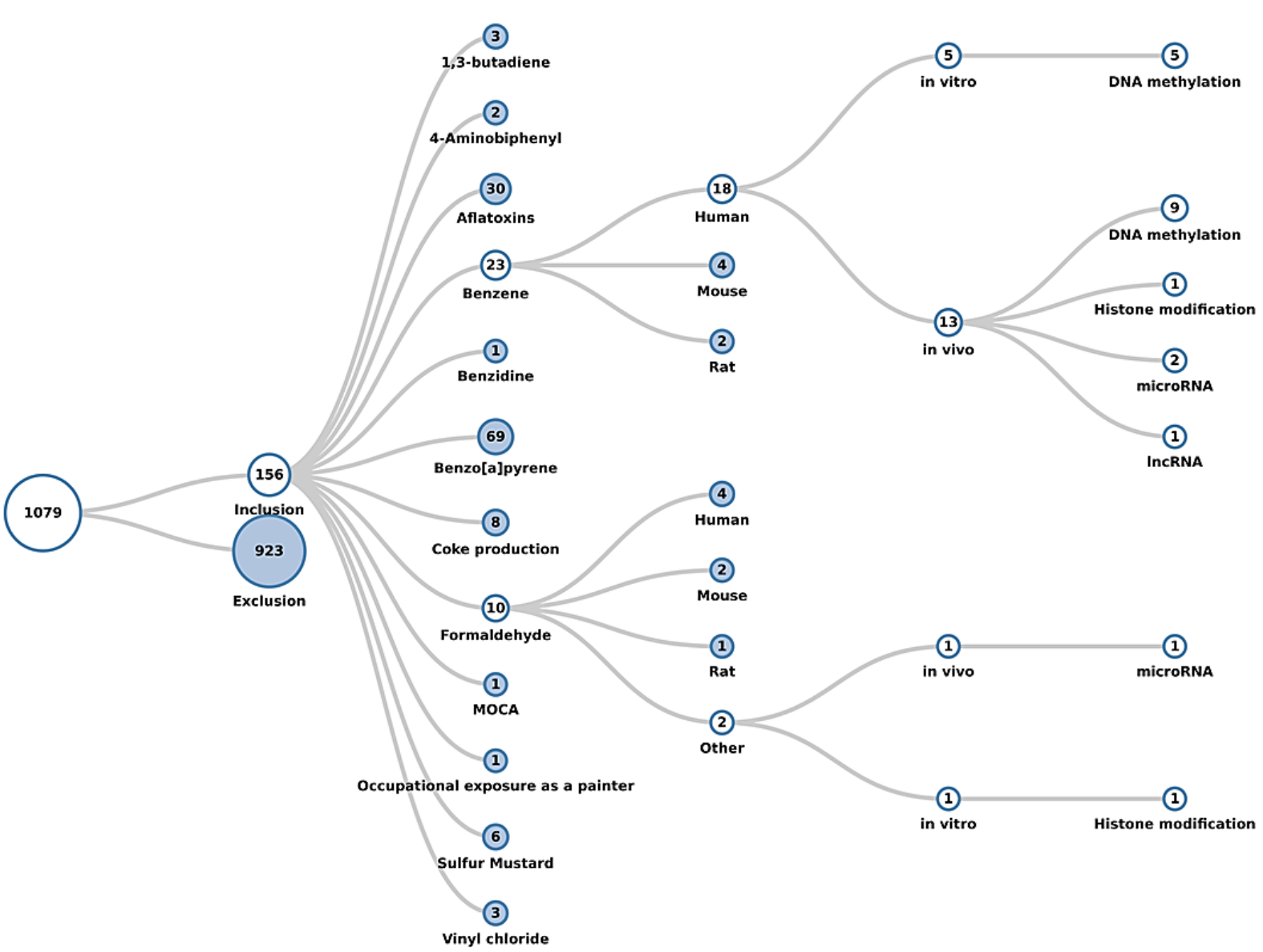


Criteria for inclusion in the review:

1. The study evaluated a chemical that:
   a) was included in IARC Monograph vol.100F
   b) has one or more demonstrated genotoxic mechanism(s) of carcinogenesis
2. The study evaluated epigenetic alterations that occurred as a consequence of exposure to the chemical of interest
3. The publication included original data
4. The study was published in English

Study is publicly available in HAWC:
https://hawcproject.org/assessment/185/

Epigenetic alterations induced by genotoxic occupational and environmental human chemical carcinogens



Epigenetic alterations induced by genotoxic occupational and environmental human chemical carcinogens

in vitro
in vivo
min max

		DNA methylation				Histone modification				Non-coding RNA				
		Human	Mouse	Rat	Other	Human	Mouse	Rat	Other	Human	Mouse	Rat	Other	total:
Benzo[a]pyrene	in vitro	11	6		3	6	2		1	16	3			48
	in vivo	5	4		5		1	1		1	7	1		25
Aflatoxins	in vitro	1			2				1	7	1			12
	in vivo	10	2	1	1		1		1	3		2		21
Benzene	in vitro	5	1	1				1						8
	in vivo	9	1	1		1	1			3	2			18
Formaldehyde	in vitro	1				2			1	1				5
	in vivo										2	1	1	4
Coke production	in vitro													0
	in vivo	6								2				8
1,3-butadiene	in vitro													0
	in vivo		3				3							6
Sulfur mustard	in vitro	1								1				2
	in vivo									3	1			4
Vinyl chloride	in vitro													0
	in vivo	3												3
4-aminobiphenyl	in vitro					1				1				2
	in vivo													0
Benzidine	in vitro													0
	in vivo		1											1
MOCA	in vitro							1						1
	in vivo													0
Occupational exp. as a painter	in vitro													0
	in vivo	1												1

85 **25** **59**

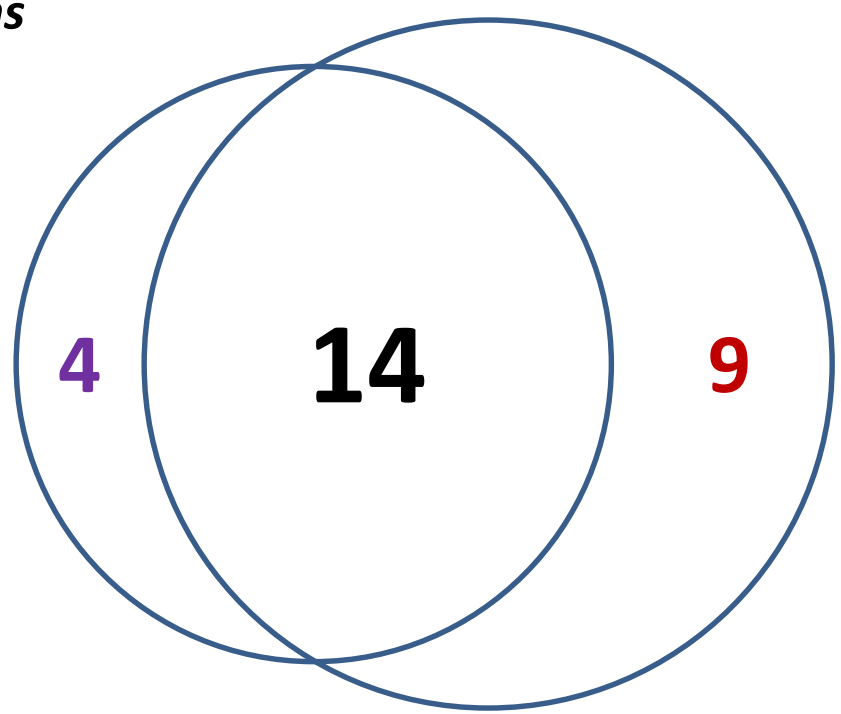
"Epigenotoxic" literature search terms: Splitting hairs?

"Benzene" AND "Epigenetic alterations"

IARC search terms (in 2014):
18 publications

TAMU search terms (in 2015):
23 publications

- A review
- Two non-English publications
- One study of chemical reactivity with proteins (histones)



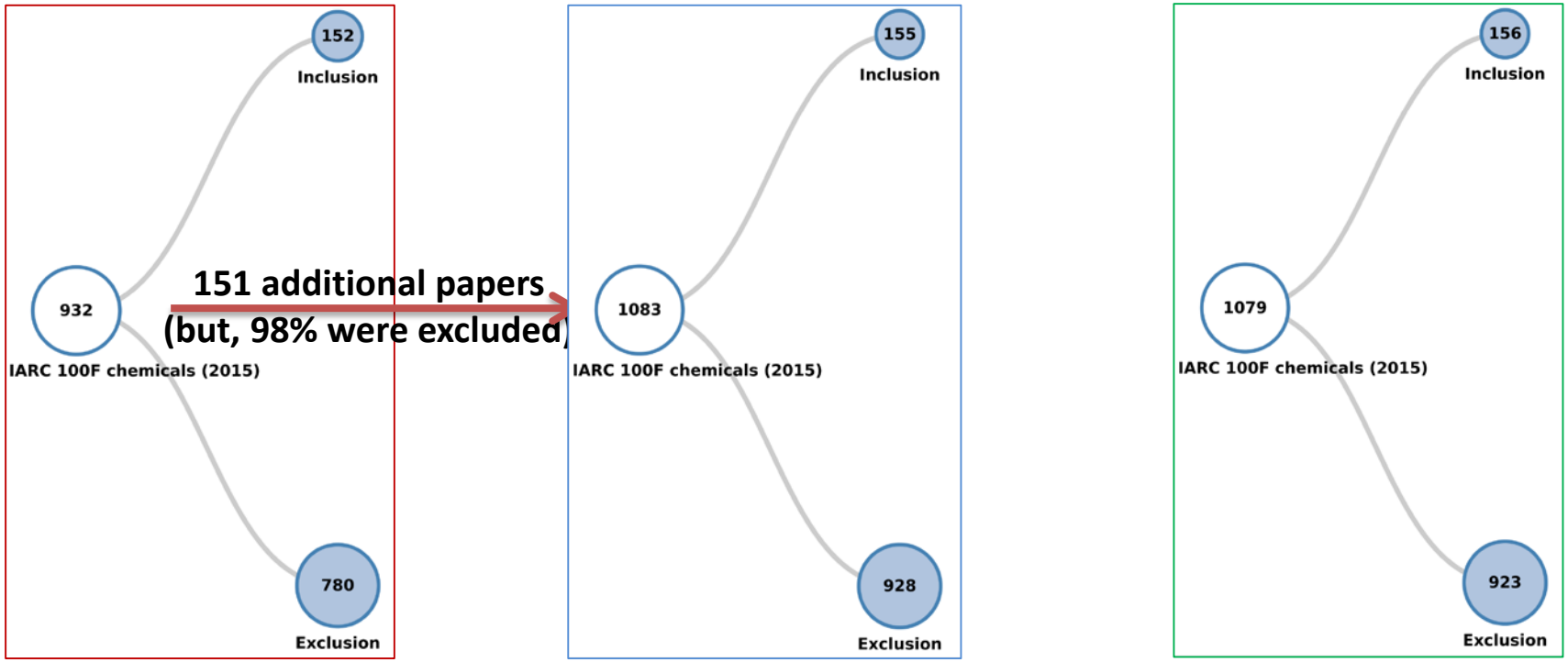
- Five studies published in 2014 or after (more recent than the review)
- Four studies are relevant and published pre-2014 (omitted?)

A refined literature search compendium of terms is more "specific"

"Epigenotoxic" literature search terms: Splitting hairs?

[All Fields] *versus* [MeSH Terms]

[All Fields] terms:	[All Fields] and [MeSH Terms]:	Removing "redundant" terms:
932 total	**1083 total**	**1079 total**
152 Included	155 Included	156 Included
780 Excluded	928 Excluded	923 Excluded



Conclusions

1. While the number of studies devoted to understanding the epigenetic alterations caused by exposure to chemical carcinogens is rapidly increasing, there remains a dearth of well-designed comprehensive studies that identify epigenetic alterations that are associated with the carcinogenesis:

- Only 1/3 (4 out of 12) of the chemicals (and occupational hazard) included in the review had a maximum of two published reports of epigenetic alterations

2. While there is extensive information about the fundamental role of epigenetic alterations in cancer development and progression, the understanding of the mechanistic significance and specificity of carcinogen-induced epigenetic abnormalities in the carcinogenic process is insufficient:

- Delineation between normal epigenetic processes in cells and the epigenetic alterations that have a causal relationship to cancer is needed for all epigenetic marks, not only for DNA methylation

3. To fully understand the importance of epigenetic and epigenomic responses to environmental stressors, studies that investigate and compare both epigenetic data with functional measures (such as gene and protein expression) and within the same study and controlled exposure scenario are needed

4. Epigenetic marks represent a class of biomarkers with great potential in the identification of exposure status, damage response, and/or disease state; however, few studies looked beyond epigenetic effects immediately after exposure

Acknowledgements

Literature reviews:

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Matt Martin

David Reif

Weihsueh Chiu

Kate Guyton

Chris Portier

IARC staff and Monographers