BIOLOGICAL SIGNIFICANCE OF DNA ADDUCTS PROJECT COMMITTEE

Mission
The mission of the HESI Biological Significance of DNA Adducts Project Committee was to bring basic science to issues around the biological significance of low levels of DNA adducts and derive a scientific consensus on this topic. This mission was fulfilled by providing a unique public forum for sharing experience, knowledge, and applications of risk management methods to this topic, as well as the implications for risk assessment. The objective was to produce a consensus-based, science-driven framework for the application of DNA adduct data to the cancer risk assessment process.

2010-2011 Participating Organizations
AstraZeneca AB
E.I. du Pont de Nemours and Company
Errol Zeiger Consulting
ExxonMobil Biomedical Sciences Inc.
L'Oréal Corporation
New York Medical College
Open University (United Kingdom)
Pfizer Inc.
Procter & Gamble Company
Shell Chemicals Ltd.
The Dow Chemical Company
University of Leicester (United Kingdom)
University of North Carolina
US Environmental Protection Agency
US Food and Drug Administration
US National Institute of Environmental Health Sciences

Committee Publications
Pottenger LH, Andrews LS, Bachman AN, Boogaard PJ, Cadet J, Embry MR, Farmer PB, Himmelstein MW, Jarabek AM, Martin EA, et al: An organizational approach for the assessment of DNA adduct data in risk assessment: case studies for aflatoxin B1, tamoxifen and vinyl chloride*. Critical Reviews in Toxicology, 0:1-44. More details

Himmelstein, M. W., P. J. Boogaard, et al. (2009). "Creating context for the use of DNA adduct data in cancer risk assessment: II. Overview of methods of identification and quantitation of DNA damage." Crit Rev Toxicol 39(8): 679-94. [Abstract]

Jarabek, A. M., L. H. Pottenger, et al. (2009). "Creating context for the use of DNA adduct data in cancer risk assessment: I. Data organization." Crit Rev Toxicol 39(8): 659-78. [Abstract]

Sander, M., et al., Proceedings of a workshop on DNA adducts: biological significance and applications to risk assessment Washington, DC, April 13-14, 2004. Toxicol Appl Pharmacol, 2005. 208(1): p. 1-20. [Abstract]

HESI Project Committee Fact Sheet

Biological Significance of DNA Adducts

2010-2011 Activities and Accomplishments

Chair
Dr. Lynn Pottenger
The Dow Chemical Company

Co-Chair
Dr. Robert Mauthe
Pfizer, Inc.

HESI Manager:
Dr. James Kim

This scientific program is committed to:

- Bringing basic science to issues around the biological significance of low levels of DNA adducts, and derive a scientific consensus on this topic;
- Providing a unique public forum for sharing experience, knowledge, and applications of risk management methods to this topic as well as the implications for risk assessment; and
- Producing a consensus-based, science-driven framework for the application of DNA adduct data to the cancer risk assessment process.

Areas of scientific focus

- Organizational framework for evaluating DNA adduct data for use in risk assessment,
- Reviewing DNA adduct technologies and methodologies, and
- Application of DNA adduct data from case studies to illustrate the use of DNA adduct data in risk assessment.

Why get involved?

- Engage in public and transparent discussions on DNA adducts as biomarkers of exposure versus biomarkers of effect, and
- Address the biological significance of DNA adducts.

Key accomplishments:

- Creating context for the use of DNA adduct data in risk assessment. The Committee published two articles that describe the organizational framework for evaluating DNA adduct data and its use in risk assessment, and the technologies available for assessing and quantifying DNA damage.
- Workshops on the biological significance of DNA adducts. Open workshops have been held at the Environmental Mutagen Society and the European Environmental Mutagen Society to provide a forum for academic, government, and industry scientists to discuss the use of DNA adduct data in risk assessment.
- Case studies to illustrate the organizational framework to evaluate DNA adduct data. The Committee prepared poster presentations of the case study work for the Society of Toxicology (SOT) (March 2011) and the European Societies of Toxicology (EUROTOX) Congress (August 2011). The March poster presentation was awarded "best presentation"

recognition by the SOT Risk Assessment Specialty Section.

What is the Committee's focus for May 2011 - April 2012

- Symposia organized by the Committee will be held at the 2011 conferences of both the Environmental Mutagen Society (October 2011) and the European Environmental Mutagen Society (July 2011).
- The Committee has developed three case studies (vinyl chloride, aflatoxin B1, and tamoxifen) to illustrate the organizational framework developed by the Committee. This will be published in 2011.

Recent publications

Pottenger LH, Carmichael N, Banton MI, Boogaard PJ, Kim JH, Kirkland D, Phillips RD, van Benthem J, Williams GM, Castrovinci A. ECETOC workshop on the biological significance of DNA adducts: Summary of follow-up from an Expert Panel Meeting. Mutat Res, 2009;678, 152-7.

Jarabek AM, Pottenger LH, Andrews LS, Casciano D, Embry MR, Kim JH, Preston RJ, Reddy MV, Schoeny R, Shuker D, Skare J, Swenberg J, Williams GM, Zeiger E. Creating context for the use of DNA adduct data in cancer risk assessment: I. Data organization. Crit Rev Toxicol 2009;39, 659-78.

Himmelstein MW, Boogaard PJ, Cadet J, Farmer PB, Kim JH, Martin EA, Persaud R, Shuker DE. Creating context for the use of DNA adduct data in cancer risk assessment: II. Overview of methods of identification and quantitation of DNA damage. Crit Rev Toxicol 2009;39, 679-94.

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Shell Chemicals Ltd.
The Dow Chemical Company
University of Leicester (United Kingdom)
University of North Carolina
US Environmental Protection Agency
US Food and Drug Administration
US National Institute of Environmental Health Sciences

For more information, contact the committee manager
Dr. James Kim, jkim@hesiglobal.org

Committee Presentations and Data Resources

November 5, 2010: HESI DNA Aducts Committee Presentation.
"HESI Biological Significance of DNA AdductsProject Committee." Presented for the Program Strategy and Stewardship Committee (PSSC). Presentation by Lynn Pottenger, Ph.D., The Dow Chemical Company.

HESI Biological Significance of DNA Adducts Project Committee

Presenter and Chair:
Lynn H. Pottenger, Ph.D., DABT
(The Dow Chemical Company)

Co-Chair:
Robert J. Mauthe, Ph.D.
(Pfizer, Inc.)

Staff:
James Kim, Ph.D., DABT

November 5, 2010 HESI PSSC Review

DNA Adducts Project Committee Mission

- The mission of this Project Committee is to bring basic science and scientific consensus to issues around the biological significance of low levels of DNA adducts by providing a unique public forum for sharing these initiatives and their implications for risk assessment.

- To produce a consensus-based, science-driven framework for the application of DNA adduct data to the cancer risk assessment process.

2010 DNA Adducts Project Committee: Participation

INDUSTRY

AstraZeneca AB

The Dow Chemical Company

DuPont Haskell Laboratory for Toxicology and Environmental Health

ExxonMobil Biomedical Sciences, Inc.

L'Oreal

Pfizer, Inc.

The Procter & Gamble Company

Shell International BV

GOVERNMENT & ACADEMIC

French Atomic Energy Commission

New York Medical College

University of Leicester

University of North Carolina

U.S. Environmental Protection Agency

- National Health and Environmental Effects Laboratory
- National Center for Environmental Assessment
- Office of Water

OTHERS

Errol Zeiger (Errol Zeiger Consulting)

DNA Adducts Project Committee Objectives

- Sponsor workshops and symposia to augment public discussion on the current state of science of DNA adduct detection, measurement, and interpretation.

- Engage a broad-based, multinational and multi-sector expert group of participants in the development of a framework approach to the application of DNA adduct data for risk assessment.

DNA Adducts Project Committee Accomplishments

- Sander, M., Cadet, J., Casciano, D.A., Galloway, S.M., Marnett, L.J., Novak, R.F., Pettit, S.D., Preston, R.J., Skare, J.A., Williams, G.M., Van Houten, B., and Gollapudi, B.B. (2005) Proceedings of a workshop on DNA adducts: biological significance and applications to risk assessment Washington, DC, April 13-14, 2004. *Toxicology and Applied Pharmacology,* 208(1): 1-20.
- Pottenger, L.H., Carmichael, N., Banton, M.I., Boogaard, P.J., Kim, J.H., Kirkland, D., Phillips, R.D., van Benthem, J., Williams, G.M., Castrovinci, A. (2009). ECETOC workshop on the biological significance of DNA adducts: Summary of follow-up from an Expert Panel Meeting. *Mutation Research,* 678: 152-157.
- Jarabek, A.M., Pottenger, L.H., Andrews, L.S., Casciano, D., Embry, M.R., Kim, J.H., Preston, J.R., Reddy, M.V., Schoeny, R., Shuker, D., Skare, J., Swenberg, J., Williams, G.M., Zeiger, E. (2009) Creating context for the use of DNA adduct data in cancer risk assessment: I. Data organization. *Critical Reviews in Toxicology,* 39(8): 659-678.
- Himmelstein, M.W., Boogaard, P.J., Cadet, J., Farmer, P.B., Kim, J.H., Martin, E.A., Persaud, R., Shuker, D. (2009) Creating context for the use of DNA adduct data in cancer risk assessment: II. Overview of methods of identification and quantitation of DNA damage. *Critical Reviews in Toxicology,* 39(8): 679-694.
- Symposium: Use of DNA Adduct Data in Risk Assessment: Context is Everything! (2009) Environmental Mutagenesis Society Annual Meeting (October 28, 2009). St. Louis, MO.

DNA Adducts Project Committee Accomplishments

- Assembled and maintained the engagement of well-known, highly experienced scientists from the government, industry, and academic sectors
- Strong, enthusiastic participation from all sectors, including U.S. EPA scientists

DNA Adducts Project Committee: Major Accomplishments "Risk Assessment Manuscript"

- Biomarker vs. Bioindicator
  - Biomarker of Exposure
    - DNA adducts (and other types of DNA damage)
    - DNA adducts can provide a quantitative measure of internal/target dose of an electrophilic chemical or metabolite
  - Biomarker of Early Biological Effect
    - For example, mutations in reporter genes, chromosome aberrations, micronuclei, and aneuploidy
    - Mutations demonstrate the potential and dose-response for a chemical to cause heritable genetic alterations
  - Bioindicator
    - Key event for tumor formation for any given MOA
    - *e.g.*, Mutations (gene and chromosomal) in a cancer gene that directly affect a pathway known to be involved in specific carcinogenesis

DNA Adducts Project Committee: Major Accomplishments "Risk Assessment Manuscript"

- Biomarker vs. Bioindicator (cont'd)
  - Biomarkers of early biological effect and bioindicators can be used quantitatively to describe a dose-response curve
  - Bioindicators are specifically in the causal pathway and would be considered the most informative
- DNA adducts
  - biomarkers of exposure
  - biologically effective dose

DNA Adducts Project Committee: Major Accomplishments "Risk Assessment Manuscript"

Organizational Approach

se of DNA adduct data in cancer risk assessment.

Dosimetry

- Phys… e chemical
- Toxi…
- Targ… tissue distribution

•Dosimetry

Adduct characterization

- General adduct profile (Type of adducts formed, Endogenous or background adduct levels, Data quality and reliability)
- Phyl…
- Dose…
- Pers…
- Mutagenic Efficiency
- Number of adducts relative to endogenous or background damage

•Adduct Characterization

Genetic Alterations ***(Note: Evaluation of these data needs to differentiate data in critical genes versus reporter genes)***

- Gen… mutations
- Chro…
- Aneu…
- Micronuclei
- Dose-response of genetic alterations

•Genetic Alterations

Other ada…

•Other Adaptive Changes

Tumor dat…

- Labo… Tumor type(s), Dose-response)
- Epid… mor type(s), Dose-response)

•Tumor Data

Evaluation of Coherence and Extrapolation Premises

- Coherence across species, sex, and target tissue sites
- Mult…
- Coh…
- Rele…
  - mammalian vs non-mammalian
  - *in vitro* to *in vivo*

•Evaluation of Coherence and Extrapolation Premises

DNA Adducts Project Committee: Major Accomplishments "Risk Assessment Manuscript"

Summary

- DNA is not pristine; background levels of adducts and damage are always present.
- Not all adducts are mutagenic or lead to a heritable effect; the biological consequences of DNA adducts must be understood.
- Identification of DNA adducts in the absence of tumors is suggestive of potential cancer risk, but such adduct data alone cannot be used in quantitative estimation of cancer risk.
- It is essential to relate adduct levels to a therapeutic dose or to an environmental exposure through steady state toxicokinetic and DNA adduct data
- Data obtained from *in vitro* experiments at concentrations not achievable in experimental animals or humans are not useful for quantitative risk assessment.

DNA Adducts Project Committee: Major Accomplishments "Risk Assessment Manuscript"

Conclusion: DNA adduct data by themselves are informative but not sufficient for assigning a MOA for tumor development

- Context is key. Need to evaluate relevance and utility of all data including adducts.
- Some DNA adducts may represent a key event in the carcinogenic MOA
- Not all DNA adducts result in mutation and not all mutations are in critical genes for carcinogenesis

DNA Adducts Project Committee: Major Accomplishments "Measurement Manuscript"

Goal: Summarize the methodologies used to measure DNA adducts and suggest future direction for more in-depth assessment and evaluation

- Review and critique current methodologies
- Discuss factors that can influence the measurement of DNA adducts

DNA Adducts Project Committee: Major Accomplishments "Measurement Manuscript"

Method: HPLC-MS/MS

Sensitivity: Good (sub-fmole range) ≈1 adduct/10^9 bases

Amount of DNA: 10-100µg

Advantages: Structural identification; potential for high accuracy

Limitations: Requires specialist equipment; may require internal standards

	Sensitivity	Amount DNA Required	Advantages	Limitations
	Highest available (attomole range)	1-1000µg	High sensitivity	$^{14}C/^{3}h$-labeled compound required; no specificity; interference with contaminants including protein (requires good purification of DNA); requires specialist equipment; not widely available; complex sample preparation
GC-ECD			High sensitivity	Requires (laborious) derivatization; requires internal standards; requires specialist equipment
^{32}P-Post-labeling			Low amount DNA required; sensitive and versatile	High levels of radioactivity; no specificity
HPLC-MS/MS	Good (sub-fmole ran ≈1 adduct/10^9 nucs		entification; high IRM mode)	Requires specialist equipment; may require internal standards
GC-MS	Good ≈1 adduct/10^9 nucs		entification	Requires derivatization; requires internal standards; requires specialist equipment; some GC-MS techniques have a high risk of introducing artefactual oxidative DNA damage
Radiolabeled [^{14}C]-DNA binding assay	Moderate 1-10 adducts/10^8 nucs	0.5 - 3mg		eled compound required; no specificity; interference with inants including protein (requires good purification of
HPLC- fluorescence or ECD	Moderate ≈1-10 adduct/10^8 nucs	20-100µg		pplicable for fluorescent / electrochemically active adducts; ds required
Immunoassay	Moderate ≈1 adduct/10^8 nucs	1-200µg		dies required; risk of cross-reactivity with other adducts
Histochemistry	Variable (pico–fmole range)	-	Robust and easy	
LMPCR	Fair ≈2.5 adduct/10^4 nucs	> 1µg	Detection at the DNA sequence level	
Modified alkaline elution	Very high (1000 cells)	< 1 µg	Single cell level, detection of different classes of damage	
Enzyme modified comet assay	Very high (1000 cells)	< 1µg	Single cell level; lesion specific	Poor quantitation (lack of calibration); no specificity

DNA Adducts Project Committee: Major Accomplishments "Measurement Manuscript"

Conclusions:

- Adduct analysis methods should be validated appropriately for the intended application and include consideration of analytical and biological measurement conditions.
- While *in vitro* systems can be very useful for adduct identification, quantitation *in vivo* is important to help establish relevance for risk assessment.
- Continuing advancements in analytical instrumentation and methodologies may enable low-dose exposures using a combination of *in vitro* systems and *in vivo* laboratory animal experiments.
- Enhanced methodologies may be needed to establish an adduct as a biomarker on the continuum of exposure to effect.

DNA Adducts Project Committee Conclusions from Publications

Risk Assessment Manuscript:

- DNA adducts are not equivalent to mutations;
- DNA adduct data by themselves are informative but not sufficient for assigning a MOA for tumor development.

Measurement Manuscript:

- Enhancements in specificity, sensitivity, method validation
- Bridging between *in vitro* and *in vivo* studies
- Needed to advance the use of DNA adduct data in quantitative risk assessment.

DNA Adducts Project Committee: Recent Progress – Case Studies

Goal: apply the concepts of the risk assessment framework and DNA adducts measurements to case studies to clarify these concepts

- Case studies on data-rich compounds (both animal & human info on MOA, DNA adducts, tumor induction, *etc.*)

DNA Adducts Project Committee: Recent Progres - Case Studies

Aflatoxin | Tamoxifen | Vinyl Chloride | Ethylene Oxide | Propylene Oxide | Butadiene | 2-AAF | BaP | Formaldehyde | Acrylamide

Data Type	Aflatoxin	Tamoxifen	Vinyl Chloride	Ethylene Oxide	Propylene Oxide	Butadiene	2-AAF	BaP	Formaldehyde	Acrylamide
Phys. Chem. Properties										
MW		564	62	44	42/58	54	224	252	30	71
log Kow	1.23	6.3	1.58	-0.3	x/0.03	1.99	3.22	6.13	0.35	-0.67
reactivity	metab req.	metab req.	metab. req.	rxt epoxide	met req/rxt.oxi	metab. Req	metab req.	metab req.	Direct	met. Important
Route of Exposure	Oral	Oral	Inhalation	Inhalation	Inhalation	Inhalation	Oral	Oral	Inhalation	Oral
Adduct type										
Chem-specific	+	+	+	+	+/+	+	+	+		+
Non-Chem-specific	+		+	+						
bulky	+	+					+	+		
rapidly repaired					+/+					
[illegible]	+		+			+				
Mode of Action										
DNA reactive – direct	+	+	+	+	-/+	+	+	+		+
DNA reactive – indirect					-/+	+				
non-DNA reactive					-/+					
funct. Interference	Aflatoxin	Tamoxifen								
cytotox		+			-/+		+			
sat metabolism			+	+	+/-	+				
receptor-mediated		+								
horm'l-mediated		+								+
multiple		+		+	-/+	+				

DNA Adducts Project Committee: Recent Progress – Case Studies

Aflatoxin | Tamoxifen | Vinyl Chloride | Ethylene Oxide | Propylene Oxide | Butadiene | 2-AAF | BaP | Formaldehyde | Acrylamide

Data Type	Aflatoxin	Tamoxifen	Vinyl Chloride	Ethylene Oxide	Prop Ox	Butadiene	2-AAF	BaP	Formaldehyde	Acrylamide
Target tissues/Tumor types										
Mult target tissues	+	+	+	+	-/+	+	+	+		+
liver	+	+	+				+			
lung	+		+	+		+		+		+
mammary	+		+			+		+		+
blood/leukemia				+		+				
brain				+						
uterine		+		+		+		+		
thyroid										+
colon	+									
site-of-contact			+		-/+					
Database										
Mult target tissues	+	+	+	+	-/+	+	+	+		+
minimum only										
DNA adduct data + neg cancer					+/-					
anti-neoplastic										
human cancer data	+	+	+	+		+				
Good phylogenetic representation				+	+/+	+	+			+
				+	+/+	+	+			+

DNA Adducts Project Committee: Organizational Framework Aflatoxin B1

Organizational framework for information and data analysis for the use of adduct data in cancer risk assessment			
Types of Available Data		**Information – Data Summary**	**References**
1. I	*…operties of*	Aflatoxins are slightly soluble in water, soluble in moderately polar organic	
	Target tissues versus non-target tissue distribution	corresponding low rates of tumors are found.	
2. Ch		…the DNA with the exo version reacting via …position of guanine bases to form 8,9-…oxin B1 (AFB1-N7-Gua)	Essigman et al. (1977)
	Dose-response for adduct levels	…dose and DNA adduct levels in multiple other studies in rat liver	Appleton et al., 1982
	Stability, repair and persistence		
	Mutagenic Efficiency		
	Phylogenic representation		
3. Al			
	Aneuploidy		
	Micronuclei		
	Dose-response of genetic alterations		
	Sequence analysis of mu		

DOSIMETRY

CYP3A4 forms exo- version of the epoxide leading to DNA adducts formation

ADDUCT CHARACTERIZATION

AFB1-N7-Gua adduct is unstable in the liver of male Fischer rats (7.5 h half life) and 20% is converted into AFB1-FAPyr within 24 h

GENETIC ALTERATIONS

Rats and mice treated with AFB1 showed increased frequency of chromosomal aberrations and micronuclei in the bone marrow with rats showing a greater effect at lower doses

DNA Adducts Project Committee: Organizational Framework Aflatoxin B1

Organizational framework for information and data analysis for the use of adduct data in cancer risk assessment			
Types of Available Data		**Information – Data Summary**	**References**
Toxic Effects			
6. Evaluation of Coherence and	*Coherence across species, sex, and target tissue sites*	rats is consistent with low carcinogenic potential of AFB1 in mice	Monroe & Eaton (1987)
		several strains of mice and After oral umors were observed in all species except mice. There is sufficient epidemiologic evidence for	IARC (1993)
	Coherence across scales of observation: genome to who tissue		
	Relevance of observations to target context	AFB1 adduct formation directly implicates the AFB1 adduct(s) in liver carcinogenesis.	(1999)

EPIGENETICS

Statistically significant correlation when comparing AFB1-albumin adducts, p16 plasma DNA hypermethylation, and p53 mutations

TUMOR DATA

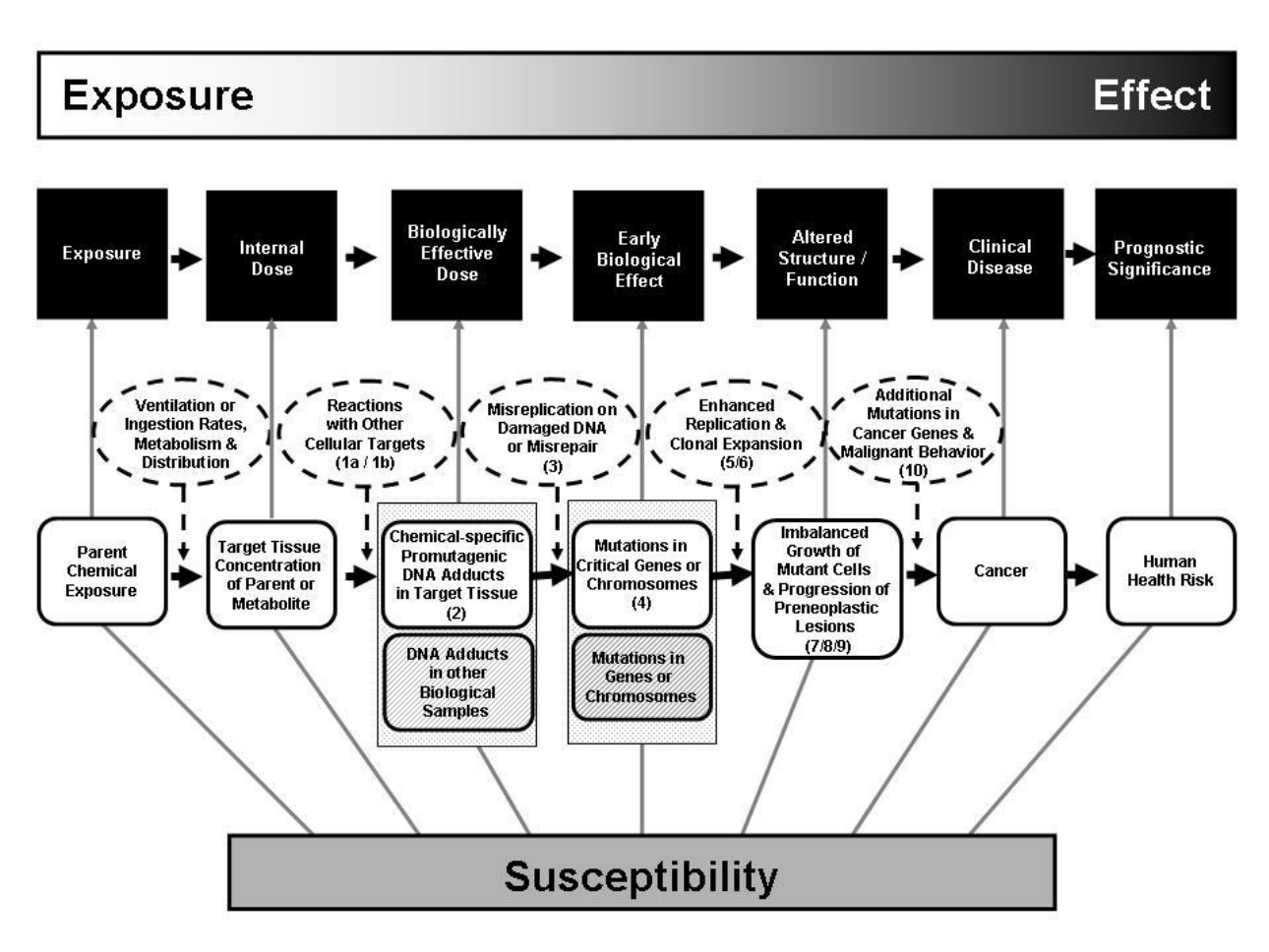
In Shanghai, highly significant increase in relative risk for those liver cancer cases where urinary aflatoxin biomarkers (AFB1-N7-gua adduct) were detected

EVALUATION OF COHERENCE

Carcinogenic across multiple species and in both sexes. Susceptibility of animals to the carcinogenic effects of AFB1 appear to be consistent with ability to metabolically activate and detoxify the genotoxic metabolite

DNA Adducts Project Committee: Recent Progress – Case Studies

- Evaluate data for each case study according to the process model described in "risk assessment" manuscript
- Discuss quality, relevance and utility of data on each step



DNA Adducts Project Committee: Recent Progress – Case Studies

Key Event:
2. Reaction with DNA in target cells to produce promutagenic damage.

Biomarker/Bioindicator of Key Event:
[Increase in] Chemical-specific promutagenic DNA adducts in target tissue

Example: VCl is metabolized by CYP-450 enzymes to form chloroethylene oxide (CEO)

CEO reacts with DNA *in vitro* to form the major DNA adduct, N7-OEG, and minor adducts: εdA, εG and εdC in hepatocytes & NPC. N7-OEG adduct is not promutagenic; the ε-adducts demonstrate mutagenicity in site-specific mutagenicity experiments.

Events for Carcinogenesis (modified from Preston & Williams, 2005).

	indicator nt	Examples	Reference
species. *N.B.*: this requires m	of parent etabolites	Butadiene & metabolites quantified in rodent blood; Ethylene Oxide (EO): N7 - oxyethylguanine in target tissue; aldehyde: DNA protein crosslink (DPX) spiratory epithelium	Bechtold *et al.*, 1995; Himmelstein *et al.*, 1994; Beranek 1990; Walker *et al.* 1992,1993; Swenberg *et al.*, 1990; Casanova *et al.* 1994
1b. Reactions DNA cellular ta impact on addu depletion of de pathways critic		AFB1; lene Oxide: GSH depletion; chemical- fic GSH conjugates in target tissue	Stressor *et al.*, 1994; Rothman *et al.*, 1995 Lee *et al.* 2005
2. Reaction wit cells to produc damage.		adducts in target tissue	
3. Misreplication on damaged DNA template or misrepair of DNA damage.		Chromosomal alterations (structural and numerical changes) and gene mutations	
4. Mutations in critical genes in replicating target cell.		Mutations in oncogenes or tumor suppressor genes	
5. Mutations in critical genes result in enhanced DNA/cell replication.		Mitotic figures, increase in organ weight	bromodeoxyuridine [BrdU] or proliferating cell nuclear antigen [PCNA], Ki67 immunohistochemistry Rios-Blanco *et al.* 2005

DNA Adducts Project Committee: Recent Progress – Case Studies

Key Event:
7. *DNA replication leads to further mutations in critical genes.*

As: Key Events for Carcinogenesis (continued).

	Biomarker or Bioindicator of Key Event	Examples	Reference
7. DNA replication leads to further mutations in critical genes.	Histological identification of	Identical mutations i	
8. Imbalanced and uncontrolled clonal growth of mutant cells leads to preneoplastic lesions.	foci), genomic instability	cells	
9. Progression of preneoplastic cells results in emergence of overt neoplasms, solid tumors (which require neoangiogenesis) or leukemia.	*Angiogenesis markers Nuclear/cytoplasmic ratios; Leukemia-specific chromosomal changes, tumor-specific histopathology/clinpath*	*Vascular endothelia (VEGF) expression; genetic alterations*	
10. Additional mutations in critical genes in subpopulation of cells as a result of clonal expansion and additional mutations result in malignant behavior.	*Gross observation of abnormal mass with heterogeneous morphology*	*Tumor-specific mark genomic instability, histopathology/clinp*	

Biomarker/Bioindicator
Histological identification of growth of abnormal cells of heterogeneous phenotype

Examples:
No VCl-specific data are available to support this, but it is plausible based on the fact that mutations appear to be induced in likely critical tumor suppressor genes and oncogenes (p53 and K-ras) in hepatic angiosarcoma tissue from workers exposed to VCl.

DNA Adducts Project Committee: Recent Progress – Case Studies

Conclusions: 10 General Principles

Dosimetry Considerations

1. DNA is not pristine – there is a background level of DNA modifications, mutations, and cancer.
2. Target tissue and adduct type depend on exposure concentration, exposure duration, and determinants of internal dose such as physico-chemical properties, anatomical and physiological factors, and ADME processes.
3. The adduct profile (relative proportion of different adducts) can change with exposure duration, or dose, due to differences in repair/persistence of specific adducts.
4. Structural identification and characterization of DNA adducts are necessary for their use in MOA assessment.
5. Quantitative data for DNA adducts should be related to experimental dose, environmental exposure, and/or therapeutic dose (e.g., via PK/TK) in order to inform quantitative risk assessment.
6. To establish a DNA-reactive MOA, it is necessary to demonstrate DNA adducts in the target tissues for carcinogenicity.

DNA Adducts Project Committee: Recent Progress – Case Studies

Conclusions: 10 General Principles

Response Considerations

7. Some mutations in critical genes (*e.g.*, p53, ras) in tumors should be attributable to chemical-specific DNA adducts detected in the target tissue.
8. While DNA adducts may lead to mutations, an adduct is not equivalent to a mutation. For a DNA adduct to result in a mutation, it must be mis-repaired followed by cell replication, or it must cause an error in replication of DNA.
9. DNA adducts serve as biomarkers of exposure, and can also represent a key event, but they are not bioindicators of cancer and cannot be used directly to predict cancer risk.

Overall Characterization

10. Overall confidence in causality requires confidence in both the dosimetry and response characterizations.

DNA Adducts Project Committee: Next Steps

- The Committee has published 2 of its manuscripts, realizing its objectives.

- The Case Studies manuscript will be submitted early in 2011.

- Outreach plans:
  - Abstract submissions for 2011 SOT (done)
  - 2011 Eurotox;
  - 2011 symposium proposals being prepared for
    - Environmental Mutagen Society (EMS) (done),
    - European EMS (in progress),
    - Society for Risk Analysis (under discussion).

- Remaining budget allocated to support these symposia in 2011

DNA Adducts Project Committee: Next Steps

2011 EMS Symposium Proposal

- Lynn Pottenger (The Dow Chemical Company): Introduction and Overview & Objectives of the DNA Adducts Project Committee
- Bob Mauthe (Pfizer Inc.): Review of Case Study Outcomes: Tamoxifen, AFB1, and VCl
- Annie Jarabek (U.S. EPA): Application of Decision Analytic Approach to Case Studies
- Robert Fuchs (Centre National de la Recherche Scientifique): Site-Specific Mutagenicity of O^6- and N7-alkylguanine Adducts
- Rita Schoeny (U.S. EPA): Relevance of These Approaches to Risk Assessment

DNA Adducts Project Committee: Moving Forward

Recommendation to PSSC:

It is recommended that the DNA Adducts Project Committee be sunset in 2010, with the use of its remaining funds to conduct international outreach activities in 2011.

January 19, 2009: HESI DNA Adducts Committee Presentation.
"Biological Significance of DNA Adducts." Presented at the 2009 HESI Annual Meeting. Tucson, Arizona. Presentation by Dr. Gary Williams, New York Medical College.

HESI Biological Significance of DNA Adducts Project Committee

Presenter:
Gary Williams, M.D.
(New York Medical College)

Chair:
Lynn H. Pottenger, Ph.D., DABT
(The Dow Chemical Company)

Vice-Chair:
Robert J. Mauthe, Ph.D.
(Pfizer, Inc.)

Staff:
James Kim, Ph.D., DABT

January 2009 HESI Annual Meeting

2008 DNA Adducts Project Committee: Participation

H E S I

INDUSTRY

AstraZeneca AB

The Dow Chemical Company

DuPont Haskell Global Centers for Health and Environmental Sciences

ExxonMobil Biomedical Sciences, Inc.

L'Oreal

LyondellBasell Industries

Pfizer, Inc.

The Procter & Gamble Company

Rohm and Haas Company

Shell International BV

OTHERS

Dan Casciano (Casciano & Associates)

Errol Zeiger (Errol Zeiger Consulting)

GOVERNMENT & ACADEMIC

French Atomic Energy Commission

New York Medical College

Open University

University of Leicester

University of North Carolina

U.S. Environmental Protection Agency
- National Health and Environmental Effects Lab
- National Center for Environmental Assessment
- Office of Water

U.S. Food and Drug Administration
- National Center for Toxicological Research
- Center for Drug Evaluation and Research

U.S. National Institute of Environmental Health Sciences

DNA Adducts Project Committee: Mission

- Bring basic science and scientific consensus to issues regarding the biological significance of low levels of DNA adducts
- Provide a unique public forum to discuss these issues and their implications for risk assessment.
- Develop a consensus-based, science-driven framework for the application of DNA adduct data to the cancer risk assessment process.

DNA Adducts Project Committee: Objectives

- Sponsor workshops and symposia to augment public discussion on the current state-of-the-science of DNA adduct detection, measurement, and interpretation

- Engage a broad-based, multi-national group to work on the development of a framework approach for the application of DNA adduct data to risk assessment

DNA Adducts Project Committee: 2008 Accomplishments

Manuscripts

Creating context for the use of DNA adduct data in cancer risk assessment: I. Data organization

- Annie Jarabek (USEPA)
- Lynn Pottenger (Dow Chemical)
- Larry Andrews (Rohm & Haas)
- Daniel Casciano (Consultant)
- Michelle Embry (HESI)
- James Kim (HESI)
- Julian Preston (USEPA)
- Vijay Reddy (Merck)
- Rita Schoeny (USEPA)
- David Shuker (Open University)
- Julie Skare (Procter & Gamble)
- James Swenberg (University of North Carolina)
- Gary Williams (New York Medical College)
- Errol Zeiger (Consultant)

DNA Adducts Project Committee: "Risk Assessment Manuscript"

Conclusion

DNA adduct data by themselves are informative but not sufficient for assigning a MOA for tumor development

- Some DNA adducts may represent a key event in the carcinogenic MOA
- Not all DNA adducts result in mutation and not all mutations are in critical genes for carcinogenesis

DNA Adducts Project Committee: 2008 Accomplishments

Manuscripts

Creating context for the use of DNA adduct data in cancer risk assessment: **II.** Overview of methods of identification and quantitation of DNA damage

- Matthew Himmelstein (DuPont, USA)
- Peter J. Boogaard (Shell, NL)
- Jean Cadet (CEA/Grenoble, FR)
- Peter B. Farmer (University of Leicester, UK)
- James H. Kim (HESI, USA)
- Elizabeth A. Martin (AstraZenaca, UK)
- David E.G. Shuker (The Open University, UK)
- Ravi Persaud (L'Oreal, USA)

DNA Adducts Project Committee: "Measurement Manuscript"

Conclusions

- Enhancements in specificity, sensitivity, method validation, and bridging between in vitro and in vivo studies are needed to advance the use of DNA adduct data in quantitative risk assessment.

- Framework can be used to improve interpretation of existing data and help plan future work
  - *e.g.*, case study for specific chemical data

DNA Adducts Project Committee: Moving Forward – Case Studies

H E S I

Draft Conclusions: General Principles (subset)

- DNA is not pristine.
- Structural identification & characterization of DNA adducts is necessary for their use in MOA assessment.
- To establish a DNA-reactive MOA, it is necessary to demonstrate DNA adducts in the target tissues for carcinogenicity.
- DNA adducts may lead to mutations, but are not equivalent to mutations.
- For DNA adducts to lead to mutations, erroneous cellular DNA synthesis is required.
- DNA adducts are biomarkers of exposure; a subset might also be key events, but they are not biomarkers of effect and cannot be used to predict cancer risk.

DNA Adducts Project Committee: Activities

H E S I

2008:

- Submitted the Risk Assessment & the DNA Adduct Measurements manuscripts to Critical Review in Toxicology for publication.

2009 Plan:

- Complete the first Case Studies Manuscript on Aflatoxin B1, Tamoxifen, and Vinyl Chloride.
- Conduct additional case studies on compounds relevant to current DNA adduct issues in cancer risk assessment.
- SOT 2009 Platform Presentation
- EMS 2009 Workshop

2010+ Plan:

- Additional manuscripts…
- Outreach plans: Poster(s), Workshops, Symposia at relevant professional society mtgs, *e.g.*, SOT, EMS, European EMS, SRA…

July 14, 2008: HESI DNA Aducts Committee Presentation.
"HESI Biological Significance of DNA Adducts Project Committee." Presented at the Board Program Strategy and Stewardship Committee (PSSC) Meeting and Board dinner. Reston, VA.
Presentation by Robert J. Mauthe, Ph.D. Pfizer, Inc.

HESI Biological Significance of DNA Adducts Project Committee

Presenter and Vice-Chair:

Robert J. Mauthe, Ph.D.

(Pfizer, Inc.)

Chair:

Lynn H. Pottenger, Ph.D., DABT

(The Dow Chemical Company)

Staff:

James Kim, Ph.D., DABT

July 14, 2008 HESI PSSC Review

DNA Adducts Project Committee: Mission

- To bring basic science and scientific consensus to issues around the biological significance of low levels of DNA adducts by providing a unique public forum for discussing these issues and their implications for risk assessment.

- To produce a consensus-based, science-driven framework for the application of DNA adduct data to the cancer risk assessment process.

2008 DNA Adducts Project Committee: Participation

INDUSTRY

AstraZeneca AB

The Dow Chemical Company

DuPont Haskell Laboratory for Toxicology and Environmental Health

ExxonMobil Biomedical Sciences, Inc.

L'Oreal

LyondellBasell Industries

Pfizer, Inc.

The Procter & Gamble Company

Rohm and Haas Company

Schering-Plough Research Institute

Shell International BV

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- National Center for Toxicological Research
- Center for Drug Evaluation and Research

U.S. National Institute of Environmental Health Sciences

DNA Adducts Project Committee: Objectives

- Sponsor workshops and symposia to augment public discussion on the current state of the science of DNA adduct detection, measurement, and interpretation

- Engage a broad-based, multinational participant base in the development of a framework approach to the application of DNA adduct data for risk assessment

DNA Adducts Project Committee: History of Events

- April 2004: Workshop, co-sponsored by NIEHS held in Washington DC entitled, “DNA Adducts: Biological Consequences and Application to Risk Assessment” [TAAP 208(2005): 1-20]
- Fall 2004: Steering team initiated the development of a consensus-based framework for the use an interpretation of DNA adduct data in risk assessment
- 2005: Four committee subteams formed to develop manuscripts outlining key issues in adduct interpretation and measurement
  - Risk Assessment of Adducts
  - Measurement of Adducts
  - Thresholds for Adducts
  - Mode of Action for Adducts
- Jan. 2006: Subcommittee elevated to Project Committee Status

DNA Adducts Project Committee: History of Events

- 2007-2008 Preparation of two companion manuscripts for submission to Critical Reviews in Toxicology:
  - Creating Context for the Use of DNA Adduct Data in Cancer Risk Assessment: I. Proposed Framework for Data Organization ("Risk Assessment Manuscript")
  - Creating Context for the Use of DNA Adduct Data in Cancer Risk Assessment: II. Overview of Methods for Identification and Quantification ("Measurement Manuscript")

- Currently developing 3 case studies and resulting principles for application of DNA adduct data, that will be prepared as a single manuscript: aflatoxin, tamoxifen, and vinyl chloride

DNA Adducts Project Committee: Major Accomplishments

- Creation, assembly, and engagement of committee members
- Progress towards consensus view on how to utilize DNA adduct information for risk assessment
- Late stage draft of Risk Assessment manuscript
- Late stage draft of DNA Adducts measurements manuscript
- Development of available information on chemicals and associated data for application to the initial framework

DNA Adducts Project Committee: Major Accomplishments

- Assembled and maintained the engagement of well-known, highly experienced scientists from the government, industry, and academic sectors
- Strong, enthusiastic participation from all sectors including U.S. EPA scientists

DNA Adducts Project Committee: Major Accomplishments "Risk Assessment Manuscript"

- Based on discussions from the 2004 workshop
- EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (or "*Cancer Guidelines*") stressed MOA as the key decision in cancer risk assessment
  - DNA adduct data are among those quantitative measurements that can be used in the cancer risk assessment process
- Goal: develop an organizational approach for incorporation of a variety of data, specifically on DNA adducts (type of adduct, frequency, *etc.*), in concert with other typically available and more traditional data (toxicokinetics, mutagenicity, genotoxicity, tumor incidence, *etc.*), to inform characterization of the mode-of-action of carcinogenic chemicals

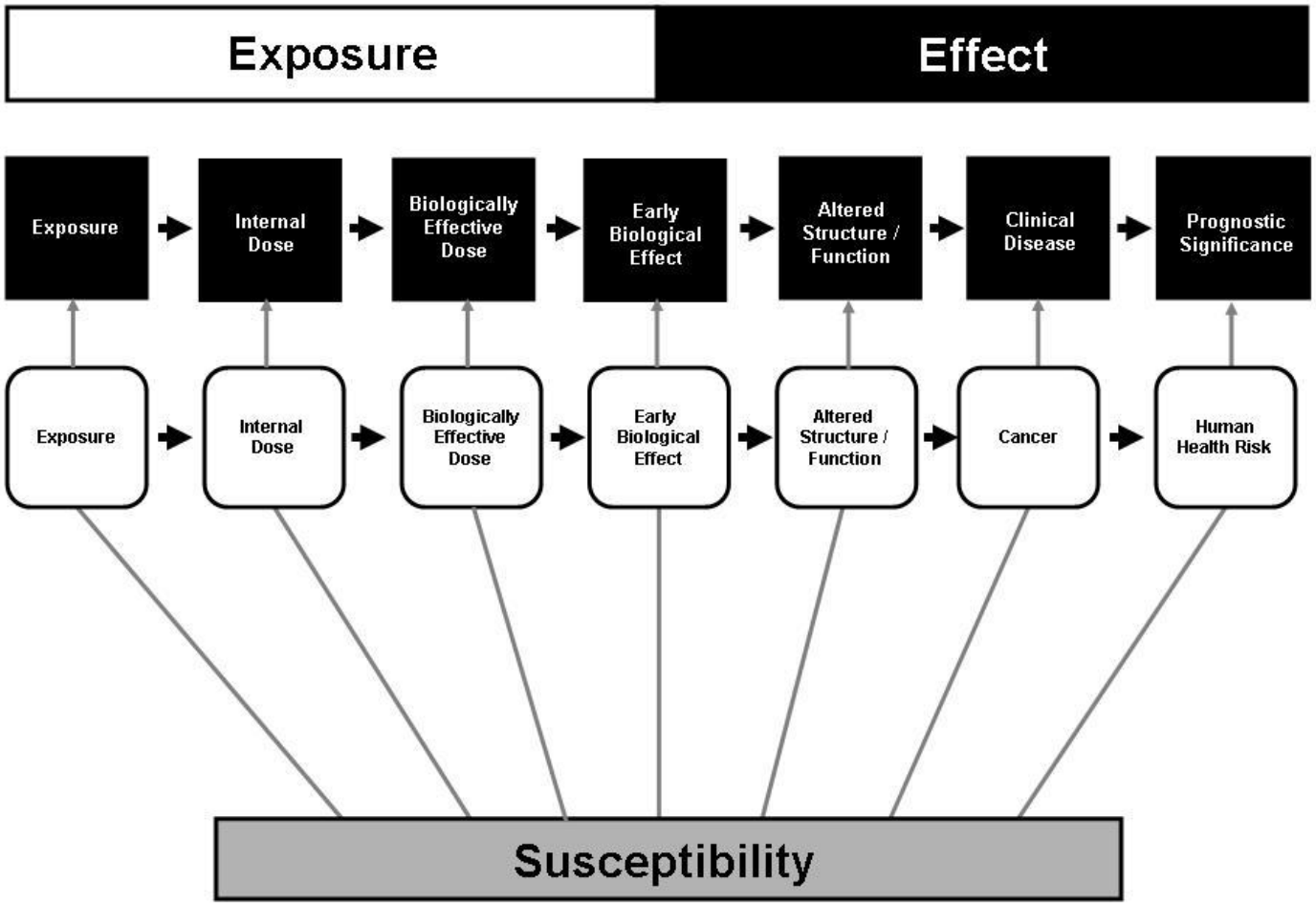
DNA Adducts Project Committee: Major Accomplishments "Risk Assessment Manuscript"

- Biomarker vs. Bioindicator
  - Biomarker of Exposure
    - DNA adducts (and other types of DNA damage)
    - DNA adducts can provide a quantitative measure of internal/target dose of an electrophilic chemical or metabolite
  - Biomarker of Early Biological Effect
    - For example, mutations in reporter genes, chromosome aberrations, micronuclei, and aneuploidy
    - Mutations demonstrate the potential and dose-response for a chemical to cause heritable genetic alterations
  - Bioindicator
    - Key event for tumor formation for any given MOA
    - *e.g.*, Mutations (gene and chromosomal) in a cancer gene that directly affect a pathway known to be involved in specific carcinogenesis

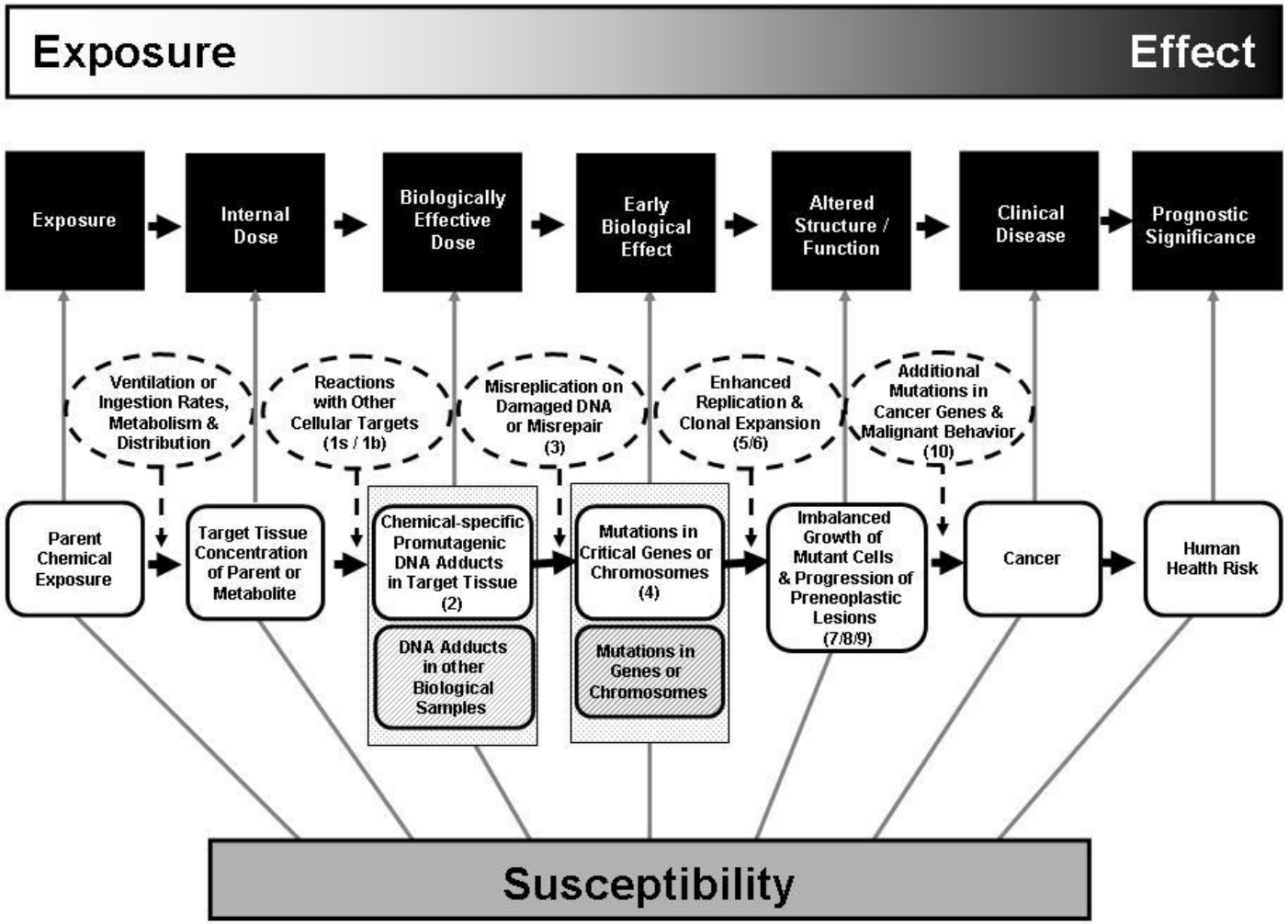
DNA Adducts Project Committee: Major Accomplishments "Risk Assessment Manuscript"

- Biomarker vs. Bioindicator (cont'd)
  - Biomarkers of early biological effect and bioindicators can be used quantitatively to describe a dose-response curve
  - Bioindicators are specifically in the causal pathway and would be considered the most informative
- DNA adducts
  - biomarkers of exposure
  - biologically effective dose

DNA Adducts Project Committee: Major Accomplishments "Risk Assessment Manuscript"



DNA Adducts Project Committee: Major Accomplishments "Risk Assessment Manuscript"



DNA Adducts Project Committee: Major Accomplishments "Risk Assessment Manuscript"

Key Event:
2. Reaction with DNA in target cells to produce promutagenic damage.

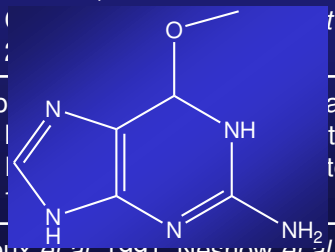
Biomarker/Bioindicator of Key Event:
[Increase in] Chemical-specific promutagenic DNA adducts in target tissue

Examples:
MMS or MNU: O^6methylguanine [O^6-MeG]

C8 deoxyguanine; acetylaminofluorene [C8-dG-AAF]

Events for Carcinogenesis (modified from Preston & Williams, 2005).

		indicator nt	Examples	Reference
species. *N.B.*: in some case this requires metabolism.		of parent etabolites	Butadiene & metabolites quantified in rodent blood; Ethylene Oxide (EO): N7 - e in target tissue; NA protein crosslink (DPX) elium	Bechtold *et al.*, 1995; Himmelstein *et al.*, 1994; Beranek 1990; Walker *et al.* 1992,1993; Swenberg *et al.*, 1990; Casanova *et al.* 1994
1b. Reactions with other nor DNA cellular targets that hav impact on adduct fate (*e.g.*, depletion of detoxication pathways critical to clearanc			GSH depletion; chemical- gates in target tissue	Stressor *et al.*, 1994; Rothman *et al.*, 1995 Lee *et al.*, 2005
2. Reaction with DNA in targ cells to produce promutagenic damage.	specific promutagenic DNA adducts in target tissue		methylguanine [O^6-MeG],	Beranek 1990; Beland and Kadlubar, 90; 00, *et al.*
3. Misreplication on damaged DNA template or misrepair of DNA damage.	Chromosomal alterations (structural and numerical changes) and gene mutations		esto 98; 98; *al.*,	ang t al. telstadt
4. Mutations in critical genes in replicating target cell.	Mutations in oncogenes or tumor suppressor genes		heterozygosity; ACR-PCR; K-ras; Er	isieux *et al.* 1991; Neshow *et al.* 1998; Groopman and Kensler 2005; McKinzie
5. Mutations in critical genes result in enhanced DNA/cell replication.	Mitotic figures, increase in organ weight		Fo de br nu im	



DNA Adducts Project Committee: Major Accomplishments "Risk Assessment Manuscript"

Key Event:
7. *DNA replication leads to further mutations in critical genes.*

As: Key Events for Carcinogenesis (continued).

	Biomarker or Bioindicator of Key Event	Examples	Reference
7. DNA replication leads to further mutations in critical genes.	Histological identification of	Identical mutations in critical genes l amplification as ace markers 0 in lung	Kocks and Rajewsky, 1989; Yang *et al.*, 2004
		mutations in	(Loeb *et al.* 2003; Guo *et al.*
8. *Imbalanced and uncontrolled clonal growth of mutant cells leads to preneoplastic lesions.*	foci), genomic instability	cells	
9. *Progression of preneoplastic cells results in emergence of overt neoplasms, solid tumors (which require neoangiogenesis) or leukemia.*	*Angiogenesis markers Nuclear/cytoplasmic ratios; Leukemia-specific chromosomal changes, tumor-specific histopathology/clinpath*	*Vascular endothelia (VEGF) expression; genetic alterations*	
10. *Additional mutations in critical genes in subpopulation of cells as a result of clonal expansion and additional mutations result in malignant behavior.*	*Gross observation of abnormal mass with heterogeneous morphology*	*Tumor-specific markers for malignancy; genomic instability, tumor histopathology/clinpath, metastasis*	Nowell, 1976; Poste *et al.*, 1982 ; Gupta *et al.* 2005

Biomarker/Bioindicator
Histological identification of growth of abnormal cells of heterogeneous phenotype

Examples:
Genomic instability, mutations in polymerases, additional oncogenes or tumor suppressor genes, mutator gene mutations

DNA Adducts Project Committee: Major Accomplishments "Risk Assessment Manuscript"

Organizational Framework

se of DNA adduct data in cancer risk assessment.

Dosimetry

- Phys… e chemical
- Toxi…
- Targ… tissue distribution

•Dosimetry

Adduct characterization

- General adduct profile (Type of adducts formed, Endogenous or background adduct levels, Data quality and reliability)
- Phyl…
- Dose…
- Pers…
- Mutagenic Efficiency
- Number of adducts relative to endogenous or background damage

•Adduct Characterization

Genetic Alterations ***(Note: Evaluation of these data needs to differentiate data in critical genes versus reporter genes)***

- Gen… mutations
- Chro…
- Aneu…
- Micronuclei
- Dose-response of genetic alterations

•Gene Alterations

Other ada…

•Other Adaptive Changes

Tumor dat…

- Labo… Tumor type(s), Dose-response)
- Epid… umor type(s), Dose-response)

•Tumor Data

Evaluation of Coherence and Extrapolation Premises

- Coherence across species, sex, and target tissue sites
- Mult…
- Coh…
- Rele…
  - mammalian vs non-mammalian
  - *in vitro* to *in vivo*

•Evaluation of Coherence and Extrapolation Premises

DNA Adducts Project Committee: Major Accomplishments "Risk Assessment Manuscript"

Summary

- DNA is not pristine; background levels of adducts and damage are always present.
- Not all adducts are mutagenic or lead to a heritable effect; the biological consequence of DNA adducts must be understood.
- Identification of DNA adducts in the absence of tumors is suggestive of potential cancer risk, but such adduct data alone cannot be used in quantitative estimation of cancer risk.
- It is essential to relate adduct levels to a therapeutic dose or to an environmental exposure through steady state toxicokinetic and DNA adduct data
- Data obtained from *in vitro* experiments at concentrations not achievable in experimental animals or humans are not useful for quantitative risk assessment.

DNA Adducts Project Committee: Major Accomplishments "Risk Assessment Manuscript"

Conclusion: DNA adduct data by themselves are informative but not sufficient for assigning a MOA for tumor development

- Some DNA adducts may represent a key event in the carcinogenic MOA
- Not all DNA adducts result in mutation and not all mutations are in critical genes for carcinogenesis

DNA Adducts Project Committee: Major Accomplishments "Measurement Manuscript"

Goal: Summarize the methodologies used to measure DNA adducts and suggest future direction for more in-depth assessment and evaluation

- Review and critique current methodologies
- Discuss factors that can influence the measurement of DNA adducts

DNA Adducts Project Committee: Major Accomplishments "Measurement Manuscript"

Method: HPLC-MS/MS

Sensitivity: Good (sub-fmole range) ≈1 adduct/10^9 bases

Amount of DNA: 10-100µg

Advantages: Structural identification; potential for high accuracy

Limitations: Requires specialist equipment; may require internal standards

	Sensitivity	Amount DNA Required	Advantages	Limitations
	Highest available (attomole range)	1-1000µg	High sensitivity	$^{14}C/^{3}h$-labeled compound required; no specificity; interference with contaminants including protein (requires good purification of DNA); requires specialist equipment; not widely available; complex sample preparation
GC-ECD			High sensitivity	Requires (laborious) derivatization; requires internal standards; requires specialist equipment
^{32}P-Post-labeling			Low amount DNA required; sensitive and versatile	High levels of radioactivity; no specificity
HPLC-MS/MS	Good (sub-fmole ran ≈1 adduct/10^9 nucs		entification; high IRM mode)	Requires specialist equipment; may require internal standards
GC-MS	Good ≈1 adduct/10^9 nucs		entification	Requires derivatization; requires internal standards; requires specialist equipment; some GC-MS techniques have a high risk of introducing artefactual oxidative DNA damage
Radiolabeled [^{14}C]-DNA binding assay	Moderate 1-10 adducts/10^8 nucs	0.5 - 3mg		eled compound required; no specificity; interference with inants including protein (requires good purification of
HPLC- fluorescence or ECD	Moderate ≈1-10 adduct/10^8 nucs	20-100µg		plicable for fluorescent / electrochemically active adducts; ds required
Immunoassay	Moderate ≈1 adduct/10^8 nucs	1-200µg		lies required; risk of cross-reactivity with other adducts
Histochemistry	Variable (pico–fmole range)	-	Robust and easy	
LMPCR	Fair ≈2.5 adduct/10^4 nucs	> 1µg	Detection at the DNA sequence level	
Modified alkaline elution	Very high (1000 cells)	< 1 µg	Single cell level, detection of different classes of damage	
Enzyme modified comet assay	Very high (1000 cells)	< 1µg	Single cell level; lesion specific	Poor quantitation (lack of calibration); no specificity

Major Accomplishments
"Measurement Manuscript"

Conclusions:

- Important to have appropriate validation that includes analytical and biological conditions considerations.
- While *in vitro* systems can be very useful for adduct identification, quantitation *in vivo* is important to help establish relevance.
- Continuing advancements in analytical instrumentation and methodologies may enable low-dose exposures using a combination of *in vitro* systems and *in vivo* laboratory animal experiments.
- Enhanced methodologies may be needed to establish an adduct as a biomarker on the continuum of exposure to effect.

DNA Adducts Project Committee: Moving Forward

- Completion of risk assessment and DNA adducts measurement manuscript
- Application of organizational framework from first 2 manuscripts on 3 “data-rich” compounds
  - Aflatoxin B1, tamoxifen, and vinyl chloride
- Extension of framework to additional set of compounds with more limited data
- Develop guidance on when adduct information should be generated and what associated information is needed to be informative for inclusion for risk assessment
- Consideration of framework with data-poor compounds

DNA Adducts Project Committee: Moving Forward – Case Studies

Goal: apply the concepts of the risk assessment framework and DNA adducts measurements to case studies to clarify these concepts

- First round of case studies on data-rich compounds (info on MOA, DNA adducts, tumor induction, *etc.*)
- Second round of case studies on compounds that have less DNA adduct information and less data-rich

DNA Adducts Project Committee: Moving Forwar[illegible] Case Studies

Aflatoxin | Tamoxifen | Vinyl Chloride | Ethylene Ocide | Propylene Oxide | Butadiene | 2-AAF | BaP | Formaldehyde | Acrylamid

Data Type	Aflatoxin	Tamoxifen	Vinyl Chloride	Ethylene Oxide	Propylene Oxide	Butadiene	2-AAF	BaP	Formaldehyde	Acrylamide
Phys. Chem. Properties										
MW		564	62	44	42/58	54	224	252	30	71
log Kow	1.23	6.3	1.58	-0.3	x/0.03	1.99	3.22	6.13	0.35	-0.67
reactivity	metab req.	metab req.	metab. req.	rxt epoxide	met req/rxt.oxi	metab. Req	metab req.	metab req.	Direct	met. Important
Route of Exposure	Oral	Oral	Inhalation	Inhalation	Inhalation	Inhalation	Oral	Oral	Inhalation	Oral
Adduct type										
[illegible]-specific	+	+	+	+	+/+	+	+	+		+
Non-Chem-specific	+		+	+						
bulky	+	+					+	+		
rapidly repaired					+/+					
[illegible]	+		+			+				
Mode of Action										
DNA reactive – direct	+	+	+	+	-/+	+	+	+		+
DNA reactive – indirect					-/+	+				
non-DNA reactive					-/+					
funct. Interference	Aflatoxin	Tamoxifen								
cytotox		+			-/+		+			
sat metabolism			+	+	+/-	+				
receptor-mediated		+								
horm'l-mediated		+								+
multiple		+		+	-/+	+				

DNA Adducts Project Committee: Moving Forward – Case Studies

Aflatoxin | Tamoxifen | Vinyl Chloride | Ethylene Ocide | Propylene Oxide | Butadiene | 2-AAF | BaP | Formaldehyde | Acrylamide

Data Type	Aflatoxin	Tamoxifen	Vinyl Chloride	Ethylene Oxide	Prop Ox	Butadiene	2-AAF	BaP	Formaldehyde	Acrylamide
Target tissues/Tumor types										
Mult target tissues	+	+	+	+	-/+	+	+	+		+
liver	+	+	+				+			
lung	+		+	+		+		+		+
mammary	+		+			+		+		+
blood/leukemia				+		+				
brain				+						
uterine		+		+		+		+		
thyroid										+
colon	+									
site-of-contact			+		-/+					
Database	+	+	+	+	-/+	+	+	+		+
minimum only										
DNA adduct data + neg cancer					+/-					
anti-neoplastic										
human cancer data	+	+	+	+		+				
Good phylogenetic representation				+	+/+	+	+			+
				+	+/+	+	+			+

DNA Adducts Project Committee: Moving Forward – Case Studies

Conclusions: 10 General Principles

- DNA is not pristine.
- DNA adducts are biomarkers of exposure where a subset might also be key events, but they are not biomarkers of effect.
- DNA adducts may lead to mutations, but are not equivalent to mutations.
- For DNA adducts to lead to mutations, cellular DNA synthesis is required.
- Structural identification & characterization of DNA adducts is necessary for their use in MOA assessment.
- Reduction of DNA adducts, as demonstrated by chemoprevention studies, results in reduction of tumors.

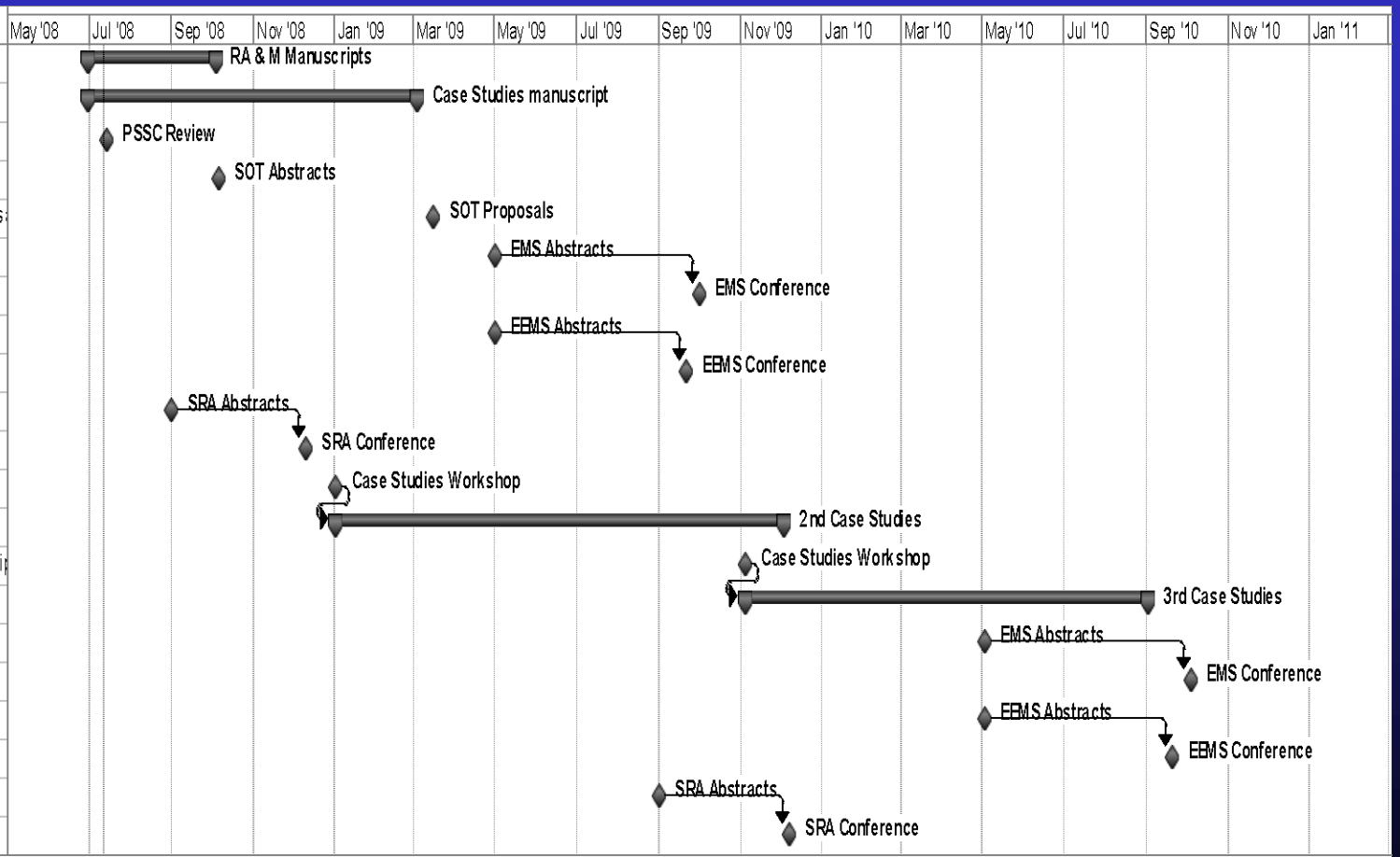
DNA Adducts Project Committee: Moving Forward – Case Studies

Conclusions: 10 General Principles

- To establish a DNA-reactive MOA, it is necessary to demonstrate DNA adducts in the target tissues for carcinogenicity.
- The adduct profile (relative proportion of different adducts) can change with duration or dose, due to differences in repair/persistence of specific adducts.
- Mutations in the critical genes in tumors associated with the identified chemical-specific DNA adducts add significantly to the WOE for MOA.
- Quantitative adduct data should be able to be related to experimental dose, environmental exposure, or therapeutic dose (*e.g.*, *via* PK/TK) in order to inform quantitative risk assessment.

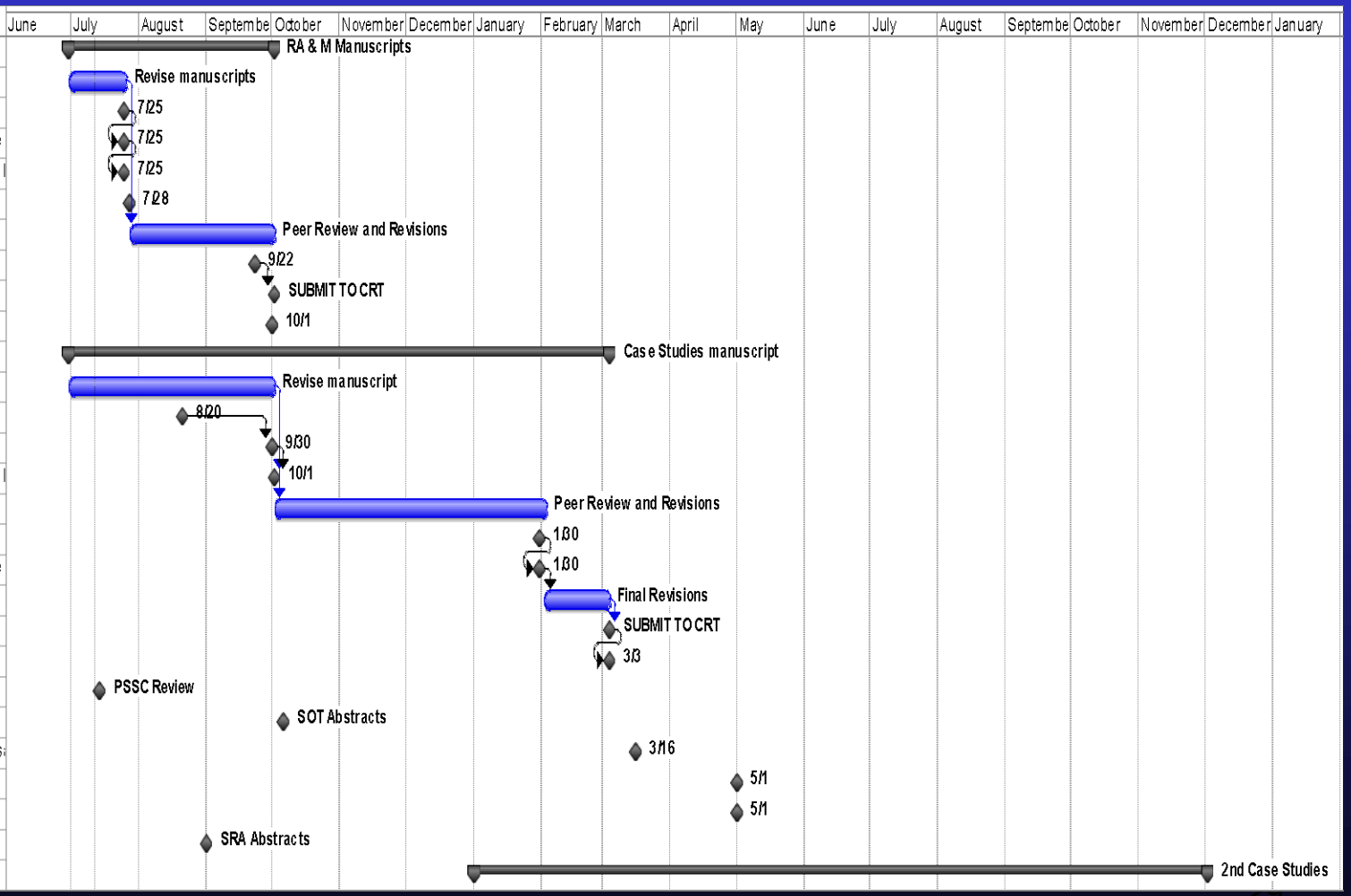
DNA Adducts Project Committee: Moving Forward – Gantt Chart

ID	Task Name
1	Prepare RA & M Manuscripts
2	Prepare CS Manuscript
3	HESI PSSC Review
4	SOT 2009 Abstract Submission
5	SOT 2010 Workshop/Symposium Propos
6	EMS 2009 Abstracts
7	EMS 2009 Conference
8	EEMS 2009 Abstracts
9	EEMS 2009 Conference
10	SRA 2008 Abstracts
11	SRA 2008 Conference
12	Workshop for 2nd Case Studies
13	Prepare 2nd Case Studies Manuscript
14	Workshop for 3rd Case Studies Manuscri
15	Prepare 3rd Case Studies Manuscript
16	EMS 2010 Abstracts
17	EMS 2010 Conference
18	EEMS 2010 Abstracts
19	EEMS 2010 Conference
20	SRA 2009 Abstracts
21	SRA 2009 Conference



DNA Adducts Project Committee: Moving Forward – Gantt Chart

ID	Task Name
1	**Prepare RA & M Manuscripts**
2	Revise RA & M Manuscripts
3	Conference Call - Authors
4	Conference Call - Project Committee
5	Submit RA & M Manuscripts for HESI
6	Conference Call
7	Revise RA & M Manuscripts
8	Conference Call - Authors
9	Submit RA & M Manuscripts to CRT
10	Conference Call - RA & M Outreach
11	**Prepare CS Manuscript**
12	Revise CS Manuscript
13	Conference Calls - Authors
14	Conference Calls - Authors
15	Submit CS Manuscript for HESI Peer
16	Revise CS Manuscript
17	Conference Call - Authors
18	Conference Call - Project Committee
19	Final Revisions
20	Submit CS Manuscript to CRT
21	Conference Call - CS Outreach
22	HESI PSSC Review
23	SOT 2009 Abstract Submission
24	SOT 2010 Workshop/Symposium Propos
25	EMS 2009 Abstracts
26	EEMS 2009 Abstracts
27	SRA 2008 Abstracts
28	Prepare 2nd Case Studies Manuscript



DNA Adducts Project Committee: Moving Forward

Recommendation to PSSC:

It is recommended that the DNA Adducts Project Committee be re-chartered for a two-year period to allow for the following planned activities:

- Submit the Risk Assessment & the DNA Adduct Measurements manuscripts, and the first Case Studies Manuscript.
- Conduct additional case studies on compounds relevant to current DNA adduct issues in cancer risk assessment and prepare manuscripts.
- Outreach plans: Poster(s), Workshops, Symposia at relevant professional society mtgs, *e.g.*, Society of Toxicology, Environmental Mutagenesis Society, European Environmental Mutagenesis Society, Society of Risk Assessment