ENVIRON

July 29, 2010

Dr. Ellen Mantus
Senior Program Officer for Risk Analysis
Board on Environmental Studies and Toxicology
The National Academies
500 Fifth Street, N.W.
Washington, D.C. 20001

Re: Documents Relevant to Work of the NRC Committee to Review U.S. EPA's Draft IRIS Assessment of Formaldehyde

Dear Dr. Mantus:

On behalf of Hexion Specialty Chemicals, Inc., ENVIRON International Corporation has submitted to the U.S. Environmental Protection Agency the following documents which are relevant to the Agency's development of its "Toxicological Review of Formaldehyde -- Inhalation Assessment: In Support of Summary Information on the Integrated Risk Information System" (the "IRIS Formaldehyde Assessment"):

(1) Letter to EPA, dated July 29, 2010

(2) PowerPoint presentation by James A. Swenberg entitled "Distribution and Molecular Dose of Inhalation-Derived and Endogenous Formaldehyde DNA Adducts Support the Induction of Nasal Carcinoma, but Not Leukemia;

(3) PowerPoint presentation by Philip Cole entitled "Formaldehyde and Lymphohematopoietic Cancers: A Review of Two Recent Studies";

(4) PowerPoint presentation by Kenneth A. Mundt entitled "Occupational formaldehyde exposure and leukemia risks"; and

(5) PowerPoint presentation by Annette M. Shipp entitled "Formaldehyde-Induced LHP: Is There Sufficient Evidence to Conclude a Causal Association?"

These slides represent comments that were presented at the U.S. EPA Listening Session on July 27, 2010. For this reason, we request that you make these documents available to members of the NRC Committee to Review EPA's Draft IRIS Assessment of Formaldehyde.

Please contact me if you have any questions or comments about any of these materials. Thank you.

Sincerely yours,

Annette M. Shipp

Annette Shipp, PhD

attachments (5)

1900 North 18th Street, Suite 804, Monroe, LA 71201
Tel: +1 318.324.8061 Fax: +1 318.325.4889
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ENVIRON

July 29, 2010

Office of Environmental Information (OEI) Docket
Mail Code 2822T
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460

Re: EPA Draft Toxicological Review of Formaldehyde -- Inhalation Assessment
Docket ID No. EPA-HQ-ORD- 2010-0396

Dear Madam/Sir:

On behalf of Hexion Specialty Chemicals, Inc., ENVIRON International Corporation hereby submits to the U.S. Environmental Protection Agency the following documents which are relevant to the Agency's development of its "Toxicological Review of Formaldehyde -- Inhalation Assessment: In Support of Summary Information on the Integrated Risk Information System" (the "IRIS Formaldehyde Assessment'):

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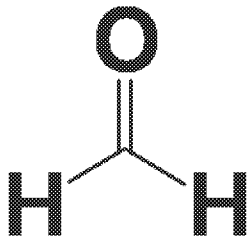
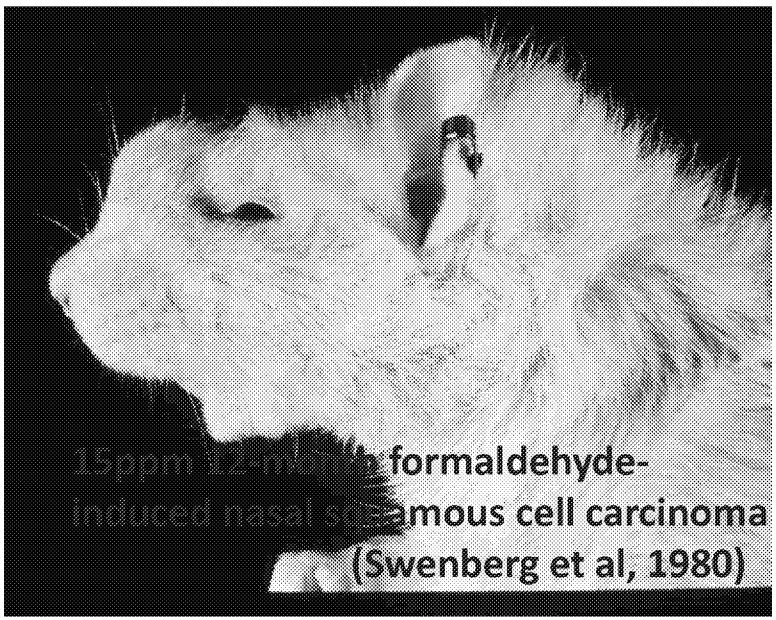
Distribution and Molecular Dose of Inhalation-derived and Endogenous Formaldehyde DNA Adducts Support the Induction of Nasal Carcinoma, but Not Leukemia

James A. Swenberg and Kun Lu
University of North Carolina
Chapel Hill, NC

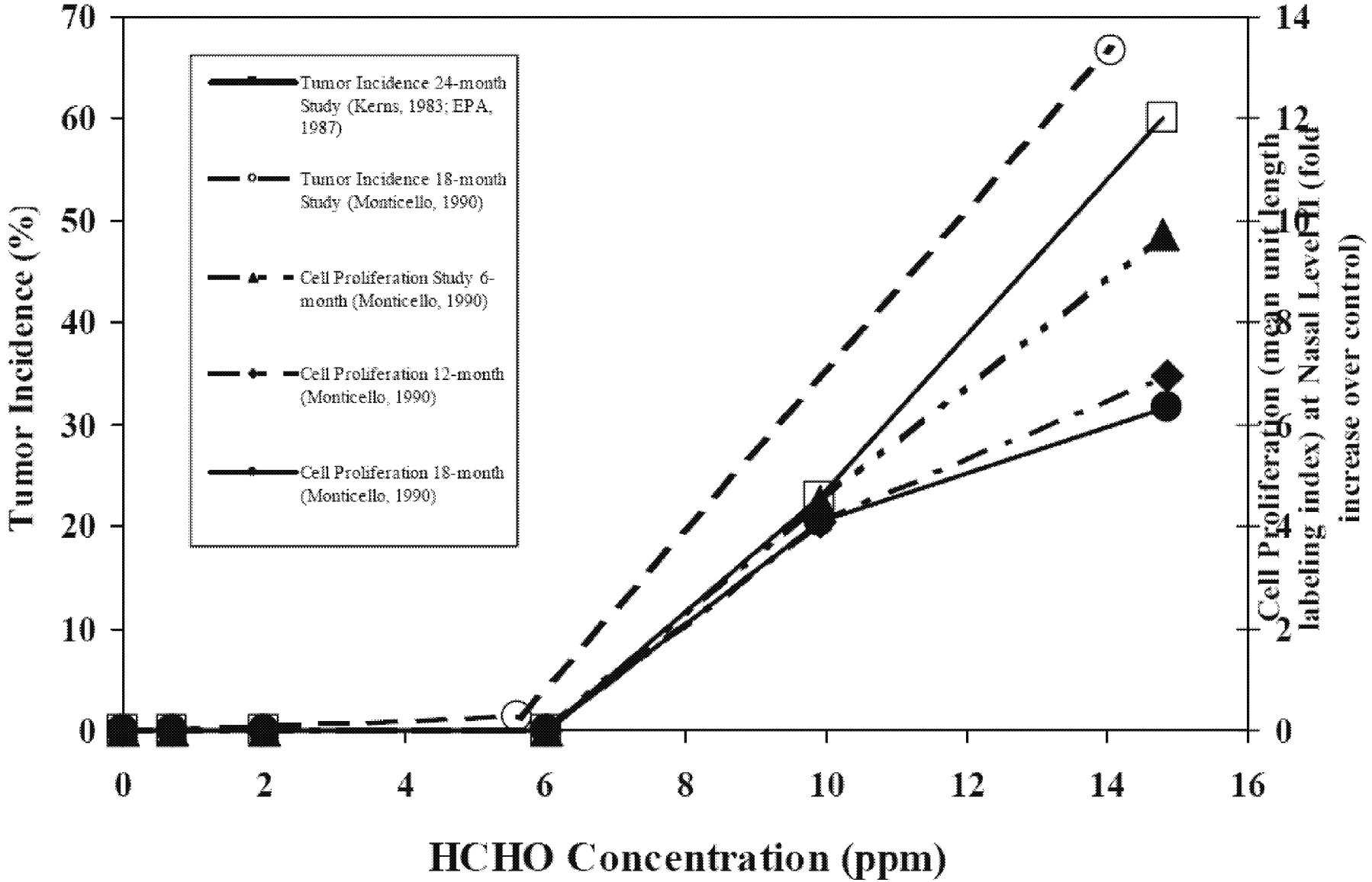
Introduction



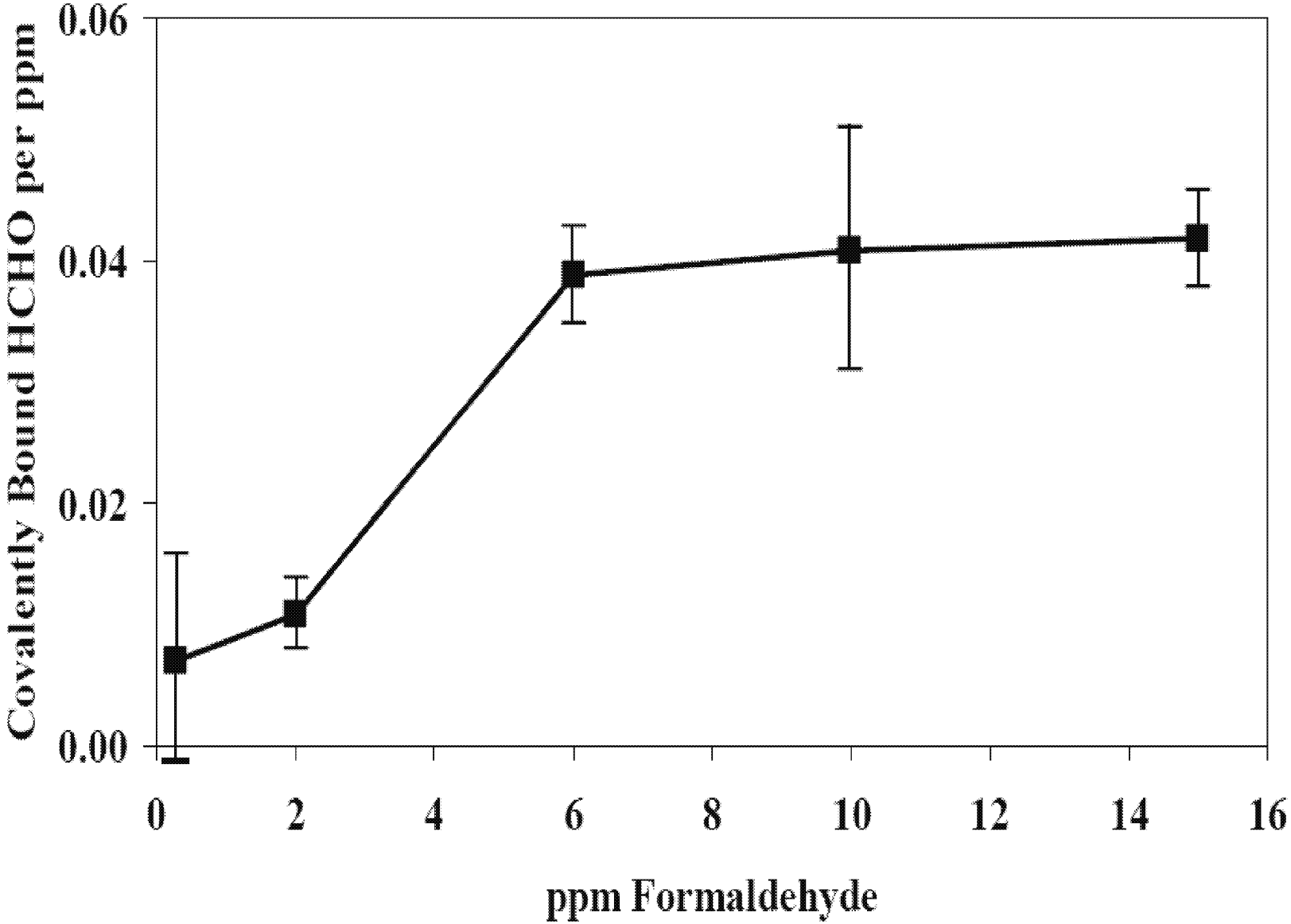
- Formaldehyde is produced worldwide more than 20 million tons/year and used in a wide spectrum of applications. Therefore, formaldehyde exposures from environmental and occupational sources are quite common.
- Formaldehyde is a known animal and human carcinogen, causing nasal cancer.
  - Highly non-linear tumor response
- Limited evidence to support formaldehyde inducing leukemia.
  - No plausible Mode of Action



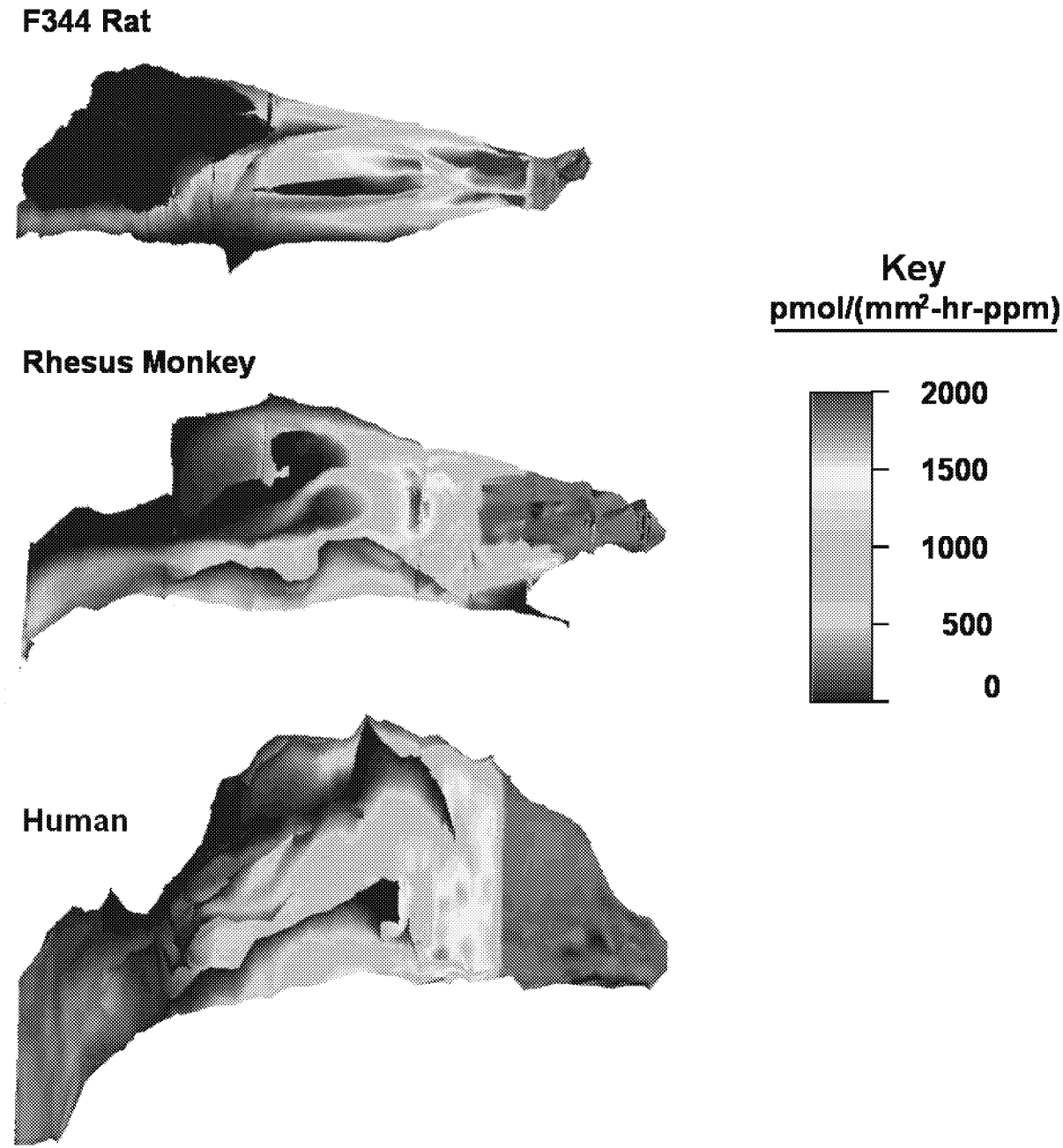
Formaldehyde-induced cell proliferation



DNA-Protein Cross-links versus FA Exposure



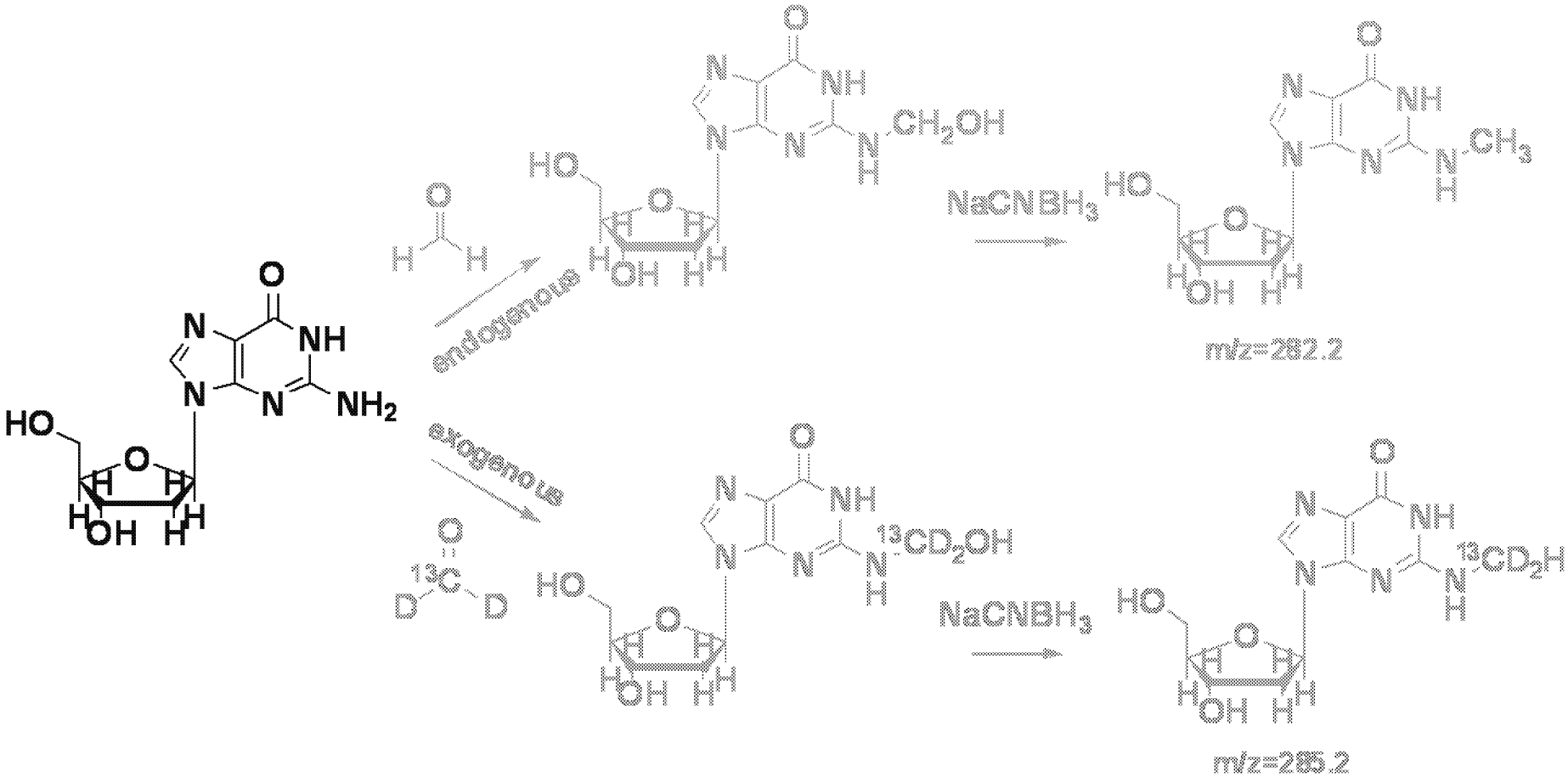
From M. Casanova, T. B. Starr, H. D. Heck, *Toxicol. Appl. Pharmacol.* **76**, 26 (1984).



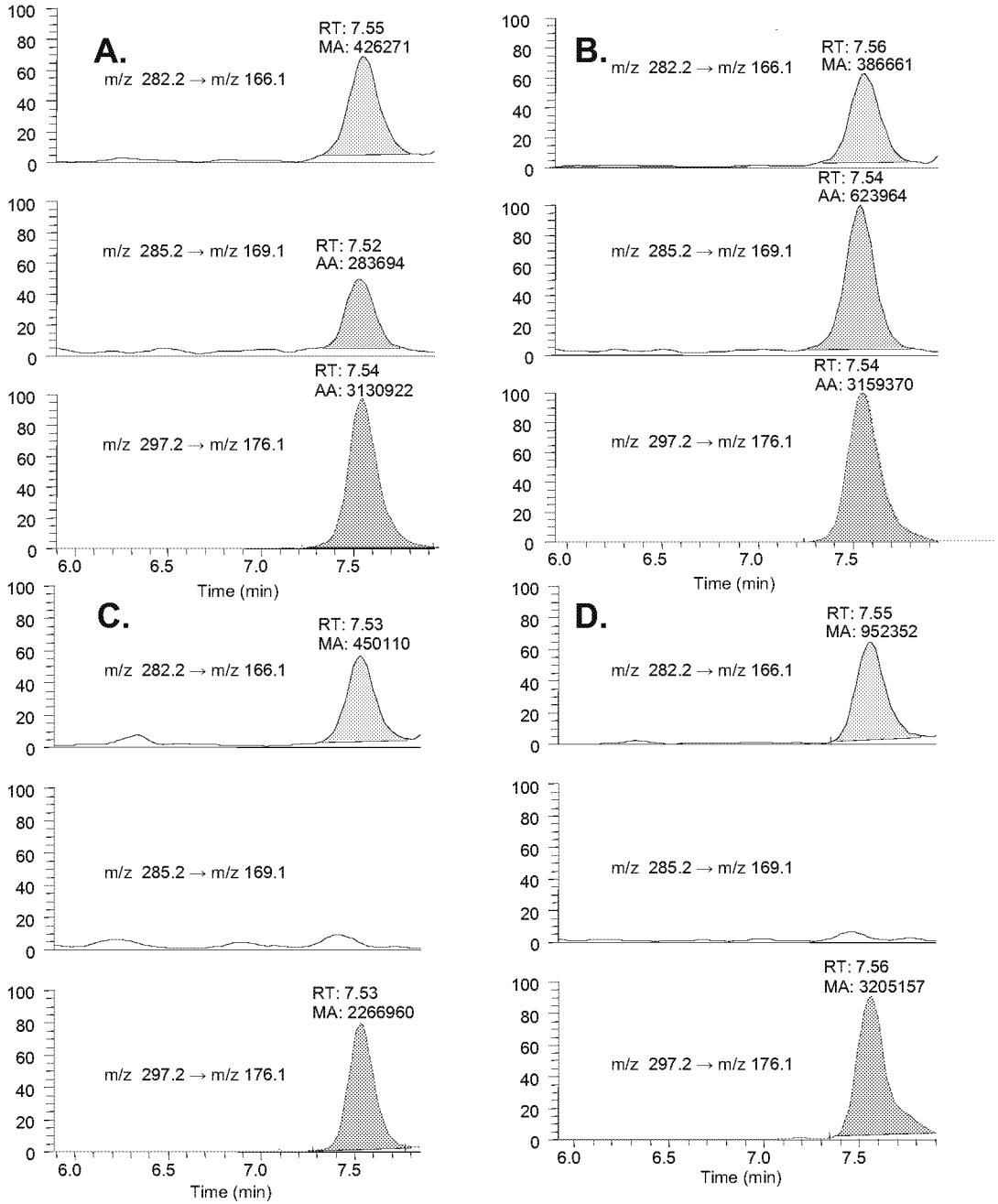
Kimbell et al. Toxicol. Sci. 64, 100-110 (2001) Figure 5.

Experimental Design

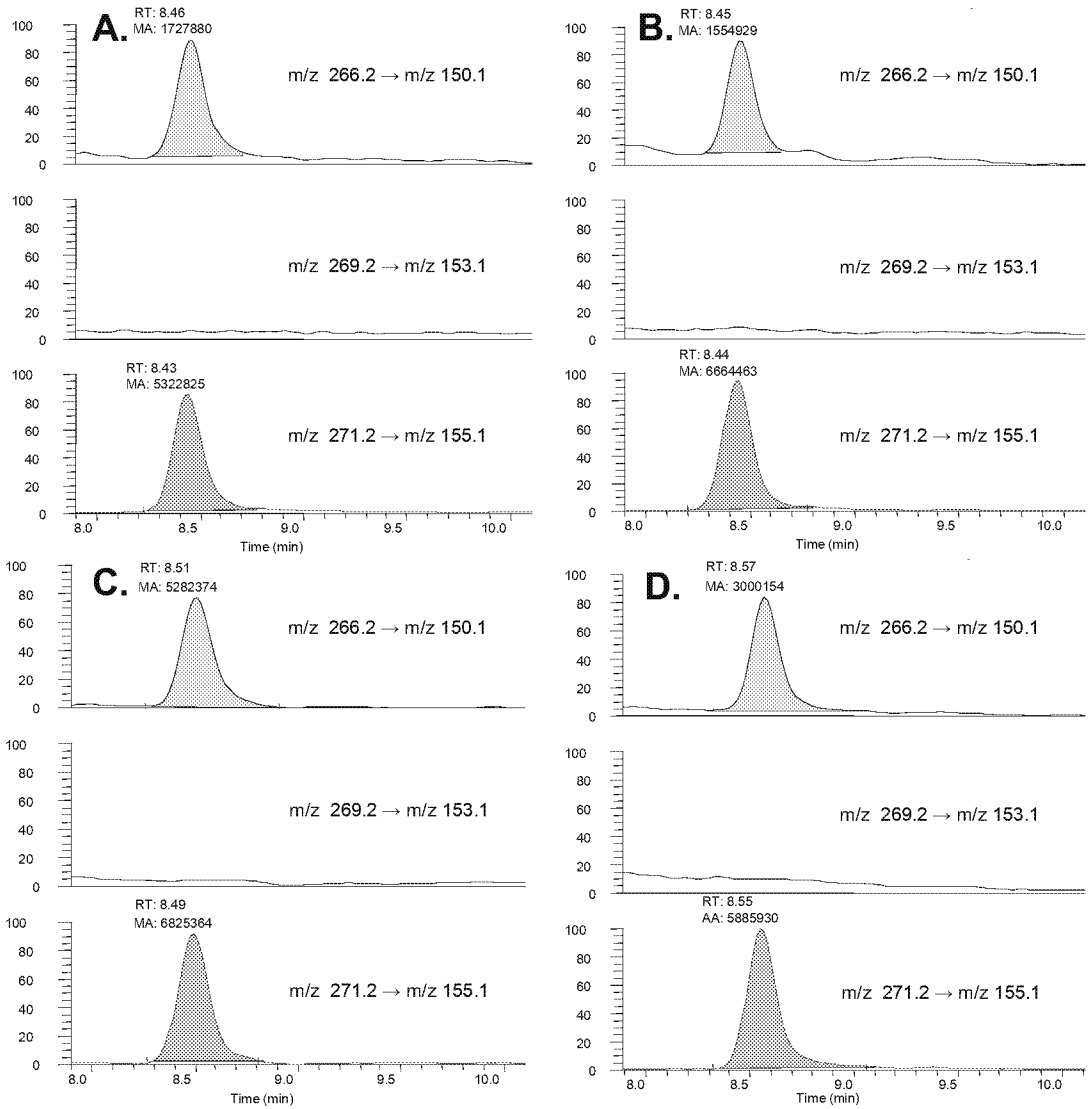
- Rats were exposed to 10 ppm [$^{13}CD_2$]-formaldehyde for 6 hrs/day for 1 or 5 days and sacrificed within 2 hr.
- Nasal mucosa, lung, liver, spleen, thymus and bone marrow were collected for DNA adduct analysis.
- DNA was reduced with $NaCNBH_3$, hydrolyzed to nucleosides and adducts were separated by HPLC and fraction collection. ~40 µg DNA was used for nasal tissue and 200 µg for all others. Thus, 5-fold more DNA was analyzed from distal sites.
- Ultrasensitive UPLC-MS/MS methods were developed for N^2-methyl-dG (detection limit 200 amol) and N^6-CH_3-dA (detection limit 50 amol) monoadducts.
- Nano-UPLC-MS/MS methods were developed for dG-dG cross-links (detection limit 60 amol).
- **Endogenous and [$^{13}CD_2$]-adducts were measured.**



Scheme 1. The formation of *N*2-hydroxymethyl-dG originating from both endogenous and exogenous formaldehyde.



LC-ESI-MS/MS SRM chromatograms of *N*2-Me-dG in typical tissues: 1 day-exposed nasal epithelium (**A**), 5 day-exposed nasal epithelium (**B**), bone marrow (**C**) and spleen (**D**).



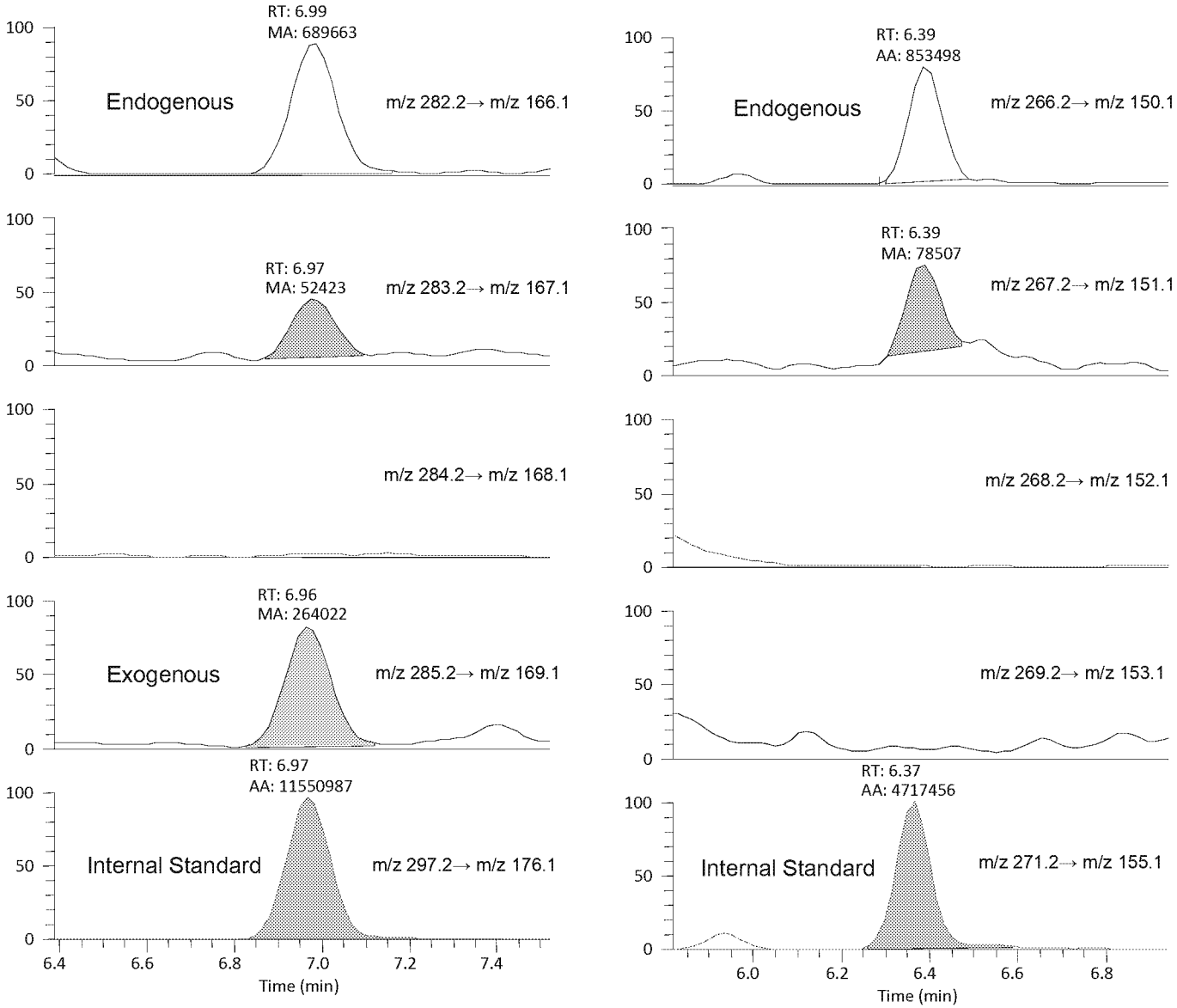
LC-ESI-MS/MS SRM chromatograms of *N*6-Me-dA of typical tissues of rats: nasal epithelium of a 1 day-exposed rat (**A**); nasal epithelium of a 5 day-exposed rat (**B**) bone marrow of a 5 day-exposed rat (**C**); spleen of a 5 day-exposed rat (**D**).

Formaldehyde-induced monoadduct amounts in rats exposed to 10 ppm formaldehyde for 1 day and 5 days

Exposure period	Tissues	N^2-$HOCH_2$-dG		N^6-$HOCH_2$-dA	
		Exogenous (adduct/10^7 dG)	**Endogenous** (adduct/10^7 dG)	**Exogenous** (adduct/10^7 dA)	**Endogenous** (adduct/10^7 dA)
1 day	Nose*	1.28±0.49+	2.63±0.73	n.d.	3.95±0.26
	Lung‡	n.d. §	2.39±0.16	n.d.	2.62±0.24
	Liver	n.d.	2.66±0.53	n.d.	2.62±0.46
	Spleen	n.d.	2.35±0.31	n.d.	1.85±0.19
	Bone marrow	n.d.	1.05±0.14	n.d.	2.95±1.32
	Thymus	n.d.	2.19±0.36	n.d.	2.98±1.11
5 day	Nose	2.43±0.78	2.84±1.13	n.d.	3.61±0.95
	Lung	n.d.	2.61±0.35	n.d.	2.47±0.55
	Liver	n.d.	3.24±0.42	n.d.	2.87±0.65
	Spleen	n.d.	2.35±0.59	n.d.	2.23±0.89
	Bone marrow	n.d.	1.17±0.35	n.d.	2.99±0.08
	Thymus	n.d.	1.99±0.30	n.d.	2.48±0.11

**n=5-8 for nose samples; +Mean ± SD; ‡n=4-5 for distant tissues; § not detectable*

1 day exposed nose sample



dG adduct

dA adduct

Conclusions

- Exogenous formaldehyde DNA monoadducts were **only** formed in nasal DNA.
- Identical endogenous DNA monoadducts were present in all tissues. The average number of endogenous formaldehyde adducts is ~1000 per cell.
- The number of exogenous and endogenous formaldehyde dG monoadducts formed from 10 ppm exposures 6 hr/day for 5 days is about equal, but in addition, numerous endogenous dA monoadducts are present.
- There is **no evidence** that [$^{13}CD_2$]-formaldehyde reaches distant sites in an active form.
- There is **no evidence** that methanediol transports [$^{13}CD_2$]-formaldehyde to distant sites.
- Formaldehyde monoadducts are **labile** with a $T_{1/2}$ of hours.
- **These data do not support a causal role of inhaled formaldehyde in the induction of leukemia.**
- **They do support both a cytotoxic and genotoxic MOA for nasal cancer.**

TOXICOLOGICAL SCIENCES 116(2), 361–363 (2010)
doi:10.1093/toxsci/kfq164
Advance Access publication June 28, 2010

TOXICOLOGICAL HIGHLIGHT

Paracelsus and Formaldehyde 2010: The Dose to the Target Organ Makes the Poison

Lois Lehman-McKeeman[1]

Discovery Toxicology, Bristol-Myers Squibb Co., Princeton, New Jersey 08543

[1]To whom correspondence should be addressed. Fax: (609) 252-7346. E-mail: lois.lehman-mckeeman@bms.com.

Received May 25, 2010; accepted May 25, 2010

"In their present work, Lu et al. have provided the first definitive evaluation of DNA adducts arising from endogenous and exogenous formaldehyde, a novel and important contribution to the scientific literature. Moreover, they have generated the type of data that should be given strong consideration by toxicologists and risk assessors as the data and their interpretations are used to improve the scientific basis of human health risk assessment."

J|A|C|S
ARTICLES

Published on Web 02/23/2010

Structural Characterization of Formaldehyde-Induced Cross-Links Between Amino Acids and Deoxynucleosides and Their Oligomers

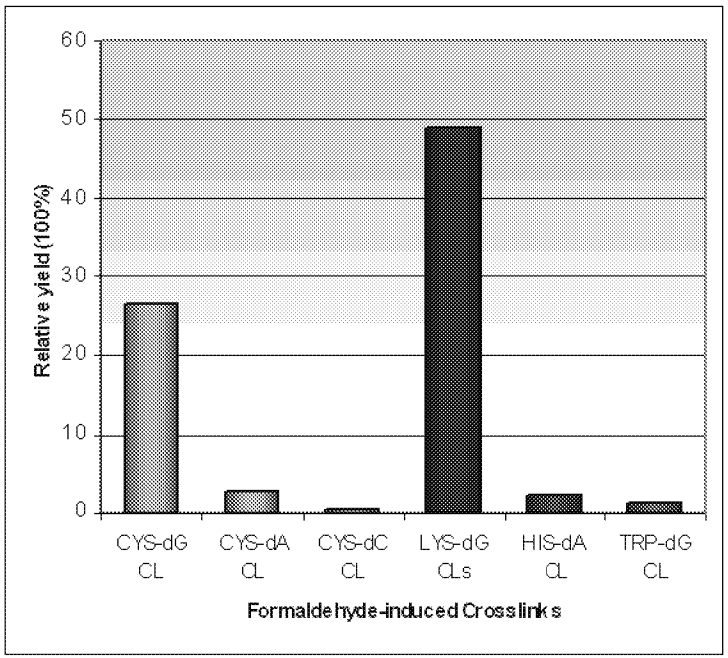
Kun Lu,[†] Wenjie Ye,[†] Li Zhou,[‡] Leonard B. Collins,[†] Xian Chen,[‡] Avram Gold,[*,†] Louise M Ball,[*,†] and James A. Swenberg[*,†]

Department of Environmental Sciences and Engineering and Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599

Received October 6, 2009; E-mail: golda@email.unc.edu; lmball@email.unc.edu; jswenber@email.unc.edu

Abstract: Exposure to formaldehyde results in the formation of DNA−protein cross-links (DPCs) as a primary genotoxic effect. Although DPCs are biologically important and eight amino acids have been reported to form stable adducts with formaldehyde, the structures of these cross-links have not yet been elucidated. We have characterized formaldehyde-induced cross-links of Lys, Cys, His, and Trp with dG, dA, and dC. dT formed no cross-links, nor did Arg, Gln, Tyr, or Asn. Reaction of formaldehyde with Lys and dG gave the highest yield of cross-linked products, followed by reaction with Cys and dG. Yields from the other coupling reactions were lower by a factor of 10 or more. Detailed structural examination by NMR and mass spectrometry established that the cross-links between amino acids and single nucleosides involve a formaldehyde-derived methylene bridge. Lys yielded two additional products with dG in which the linking structure is a 1,N^2-fused triazino ring. The Lys cross-linked products were unstable at ambient temperature. Reactions between the reactive N^{α}-Boc-protected amino acids and the trinucleotides d($T_1B_2T_3$) where B_2 is the target base G, A, or C and reactions between dG, dA and dC and 8-mer peptides containing a single reactive target residue at position 5 yielded cross-linked products with structures inferred from high resolution mass spectrometry and fragmentation patterns that are consistent with those between N^{α}-Boc-protected amino acids and single nucleotides rigorously determined by NMR studies. These structures will provide a basis for investigation of the characteristics and properties of DPCs formed in vivo and will be helpful in identifying biomarkers for the evaluation of formaldehyde exposure both at the site of contact and at distant sites.

No exogenous dA adducts, Why?

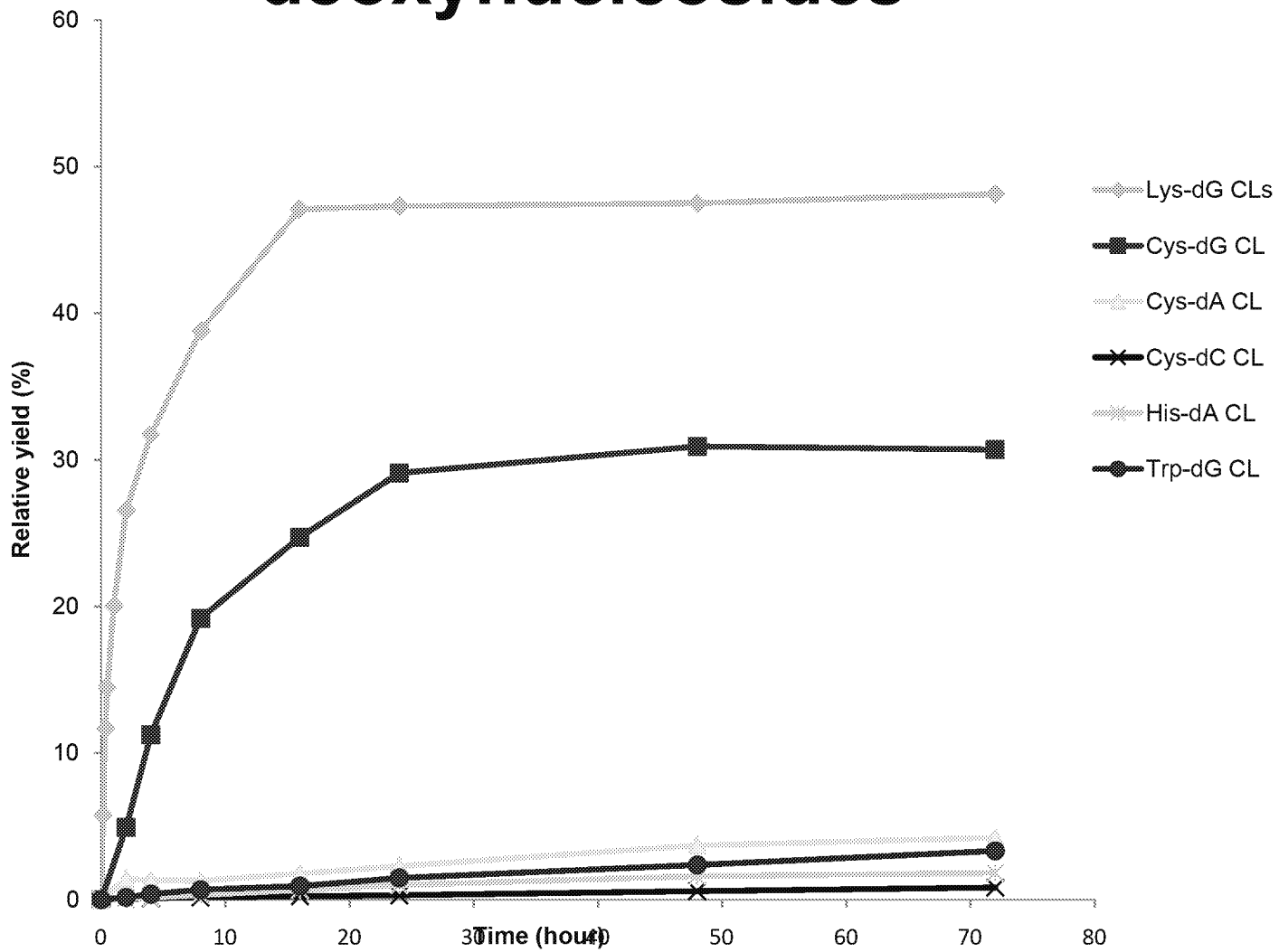


Formaldehyde-induced cross-links (relative yield[a]) observed for the reactions between 5 mM amino acid, 50 mM formaldehyde during 48 h.

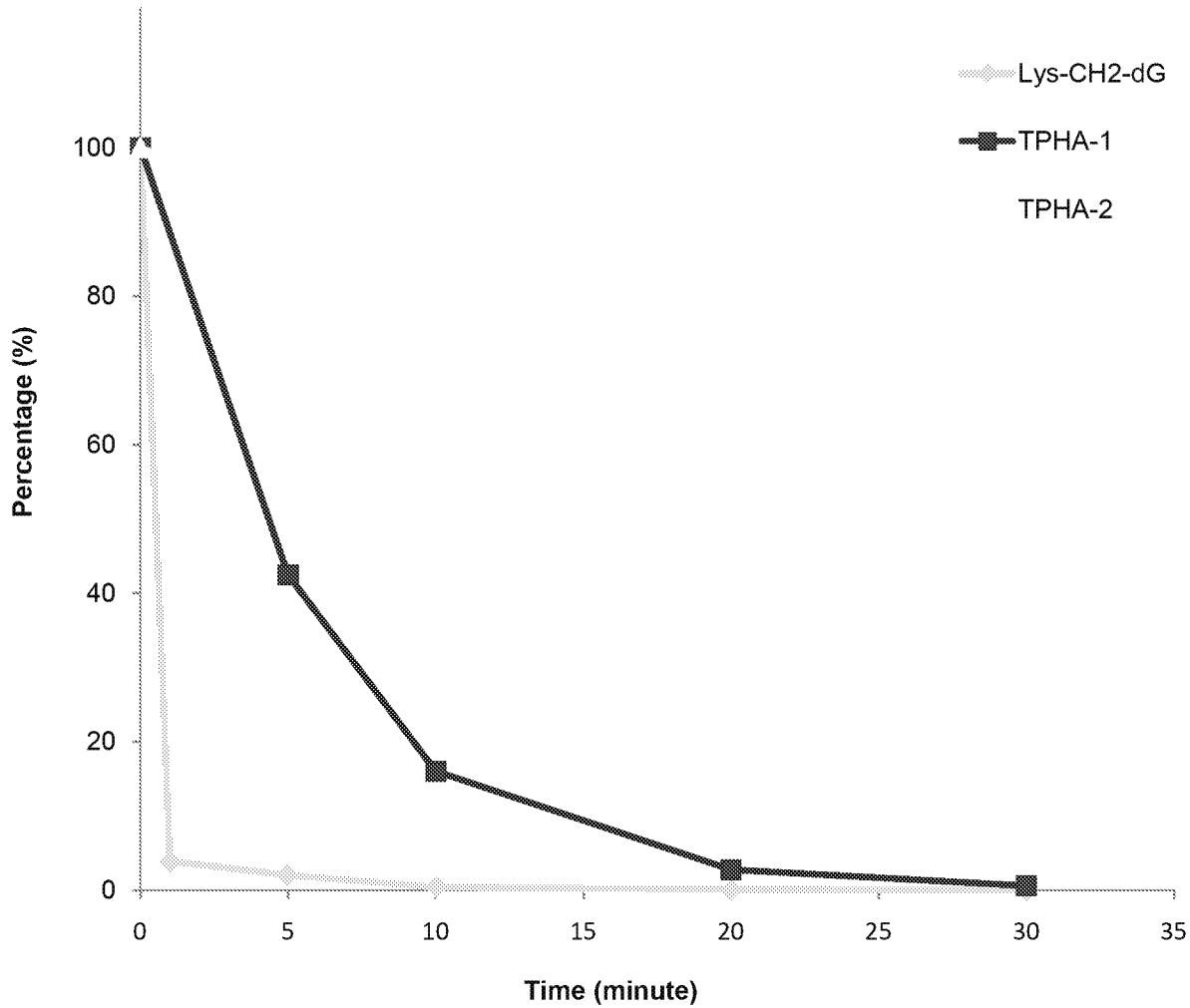
	dA	dT	dC	dG
Lys	-	-	-	**100[b]**
Cys	**5**	-	**2**	**53**
His	**4**	-	-	-
Trp	-	-	-	**3**

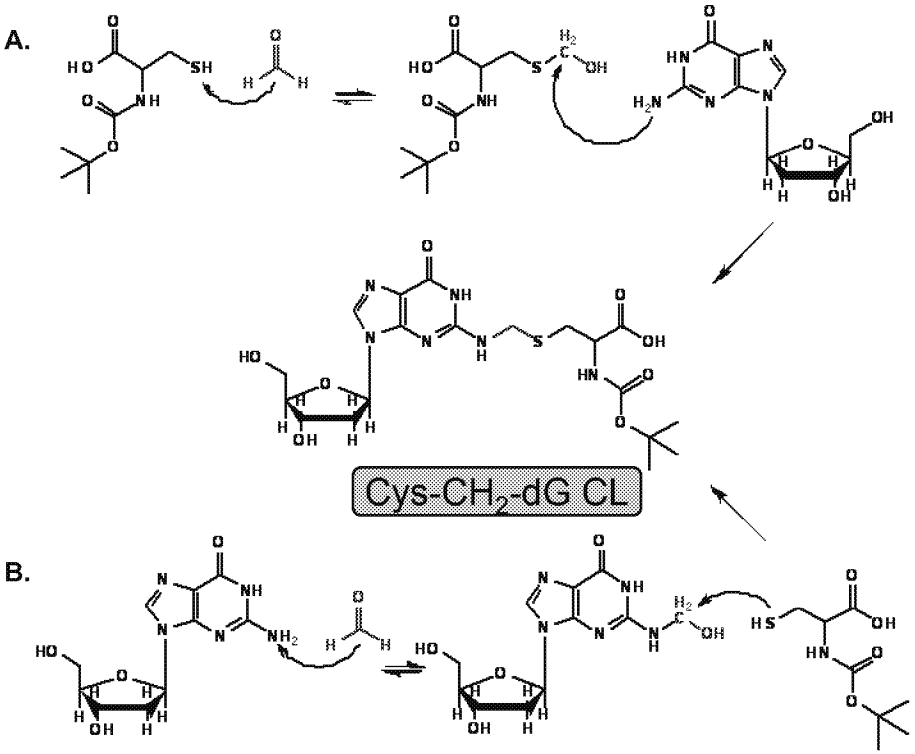
[a]*Relative yield based on integration of HPLC peaks.*
[b]*Sum of three cross-linked products.*

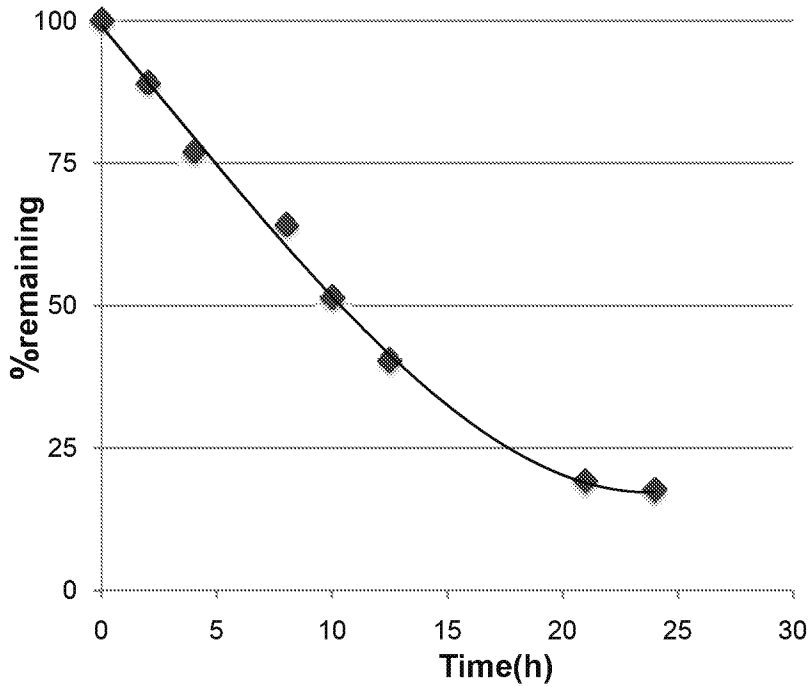
Kinetics of formation of cross-links between amino acids and deoxynucleosides



Kinetics of degradation of lysine-dG cross-links







Route A: *S*-hydroxymethyl group could further conjugate with DNA base
Route B: N^2-hydroxymethyl group could further react with cysteine residue

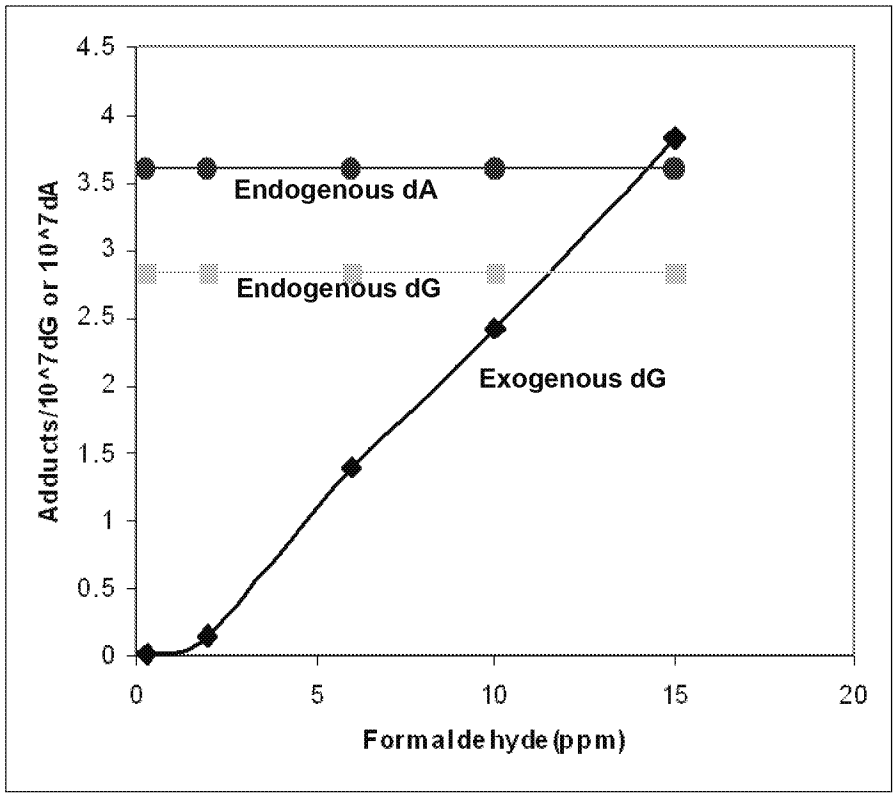
Leukemia

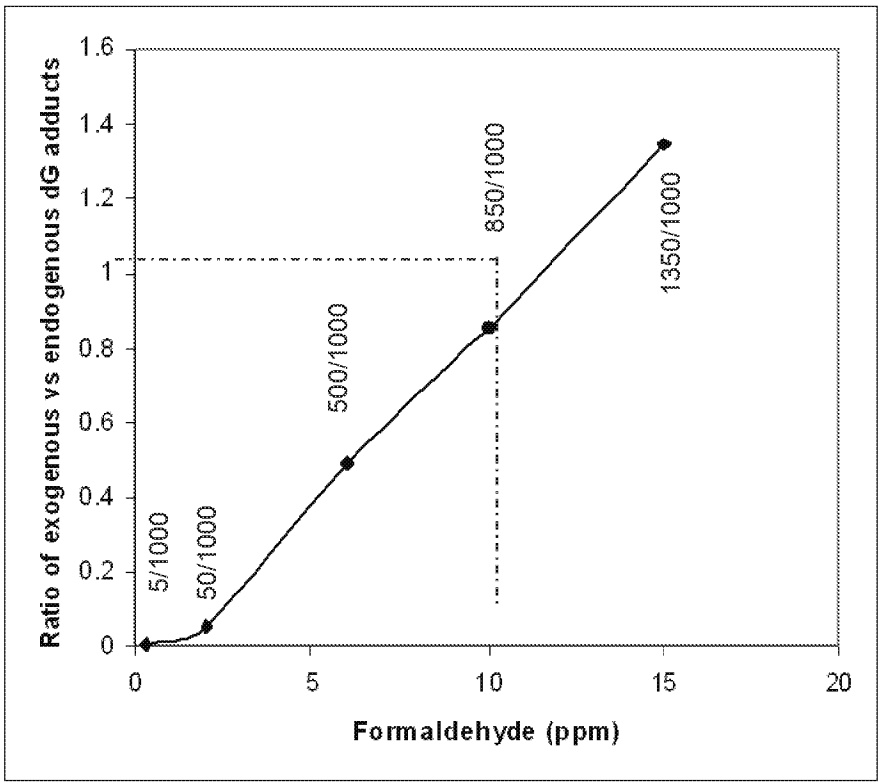
- **Three mechanisms for the induction of leukemia were proposed by Zhang et al.**
  - Targeting bone marrow hematopoietic stem cells.
  - Targeting blood stem cells and progenitors .
  - Targeting pluripotent nasal/oral stem cells .
- **Our results cast serious questions on the biological plausibility of formaldehyde inducing leukemia**
  - No exogenous formaldehyde-induced DNA adducts formed in any distant tissue.
  - The formaldehyde-DNA adducts are labile.
  - High number of endogenous formaldehyde-induced DNA adducts are always present.

Ongoing Studies

- Rats are obligatory nose breathers, but primates and humans do oral and nasal breathing. Similar studies will be done in primates this quarter.
- The *in vivo* half-life of formaldehyde DNA adducts will be established using sequential sacrifice [$^{13}CD_2$]-adduct evaluations. Exposures occur on August 1, 2010.
- Exposure-response studies will be completed this August to define the expected nonlinear relationship between inhalation-derived and endogenous adducts at 15, 10, 6, 2 and 0.7 ppm [$^{13}CD_2$]-formaldehyde.
- The source of formaldehyde (inhalation versus intracellular metabolism) is important, but must be examined with stable isotope exposures.
- A new method is under development for the measurement of exposure-specific dG-cystein cross-links. This method will be applied to our DNA samples to obtain similar information on DNA-protein cross-links.

Estimated relationships between endogenous and exogenous formaldehyde DNA adducts





Predicted dose-response of DNA adducts generated based on previous DPC results

Predicted contribution of exogenous and endogenous dG adducts based on previous DPC results

Steady-state Amounts of Endogenous DNA Damage

Endogenous DNA Lesions	Number per Cell
Abasic sites	30,000
OHEtG	3,000
8-oxodG	2,400
7-(2-Oxoethyl)guanine	1,500
Formaldehyde	960
AcrdG	120
M_1dG	60
N^2,3-Ethenoguanine	36
1N^2-Etheno dG	30
1N^6-Etheno dA	12
Total	**38,118**

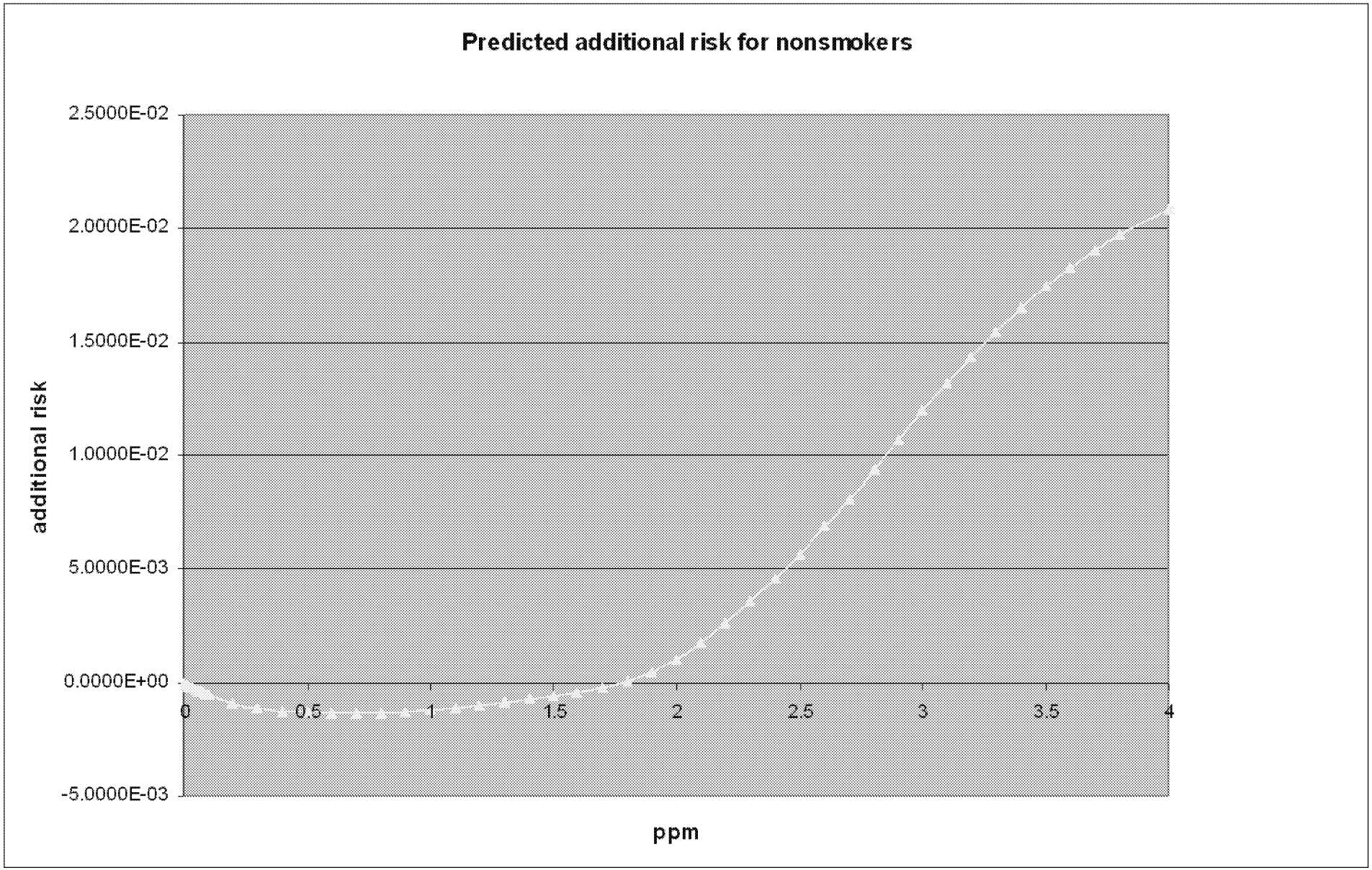
Formaldehyde-induced point mutations in *p53* gene

Table 38. DNA sequence analysis of *p53* cDNA (PCR fragment D) from squamous-cell carcinomas of nasal passages induced in rats by formaldehyde

DNA sequence[a]	Mutation (codon)[b]		Equivalent human *p53* codon no.	Location in conserved region
$_{396}$C→A	TT<u>C</u>→TT<u>A</u> (132)	phe→leu	134	II
$_{398}$G→T	T<u>G</u>C→T<u>T</u>C (133)	cys→phe	135	II
$_{638}$G→T	A<u>G</u>C→A<u>T</u>C (213)	ser→ile	215	
$_{812}$G→A	C<u>G</u>T→C<u>A</u>T (271)	arg→his	273	V
$_{842}$G→C	C<u>G</u>G→C<u>C</u>G (281)	arg→pro	283	V

- Exogenous dG and dG-dG cross-links were only detected in nasal epithelium of rats. This result is consistent with the finding of point mutations at guanines in the *p53* gene in formaldehyde-induced nasal carcinomas.

From L. Recio *et al.*, *Cancer Res.* **52**, 6113 (1992).


Predicted additional risk for nonsmokers
2.5000E-02
2.0000E-02
1.5000E-02
1.0000E-02
5.0000E-03
0.0000E+00
-5.0000E-03
additional risk
0
0.5
1
1.5
2
2.5
3
3.5
4
ppm

Conclusions to date

- Exposure-induced DNA monoadducts and cross-links only occur in nasal epithelial DNA in rats.
- Only dG monoadducts and cross-links are formed following inhalation and *in vitro* exposures.
- dA monoadducts form from intracellular formation of formaldehyde secondary to intracellular metabolism.
- Endogenous DNA monoadducts (dG and dA) are present in all cells and tissues.
- Endogenous adducts are present in 2.5-3-fold greater amounts than exogenous adducts following 10 ppm exposures to [$^{13}CD_2$]-formaldehyde.

Conclusions to date

- Both cytotoxicity and genotoxicity are key events for the induction of nasal carcinoma.
- The sustained increase in cell proliferation that results from formaldehyde cytotoxicity “fixes” **both** endogenous and exogenous DNA adducts into heritable mutations.
- Formaldehyde DNA adducts are very labile, so they have short half lives.
- Such short half-lives make it unlikely that the adducts will persist long enough for a stem cell to travel to bone marrow, divide and possibly mutate.

Collaborators and Sponsors

- Kun Lu
- Patricia Upton
- Jun Nakamura
- Ed Bermudez

- Formaldehyde Council
- Hamner Institutes for Health Sciences
- Superfund Basic Research Program (P42-ES 5948)
- NIEHS (P30 ES 10126)

Formaldehyde and Lymphohematopoietic Cancers: A Review of Two Recent Studies

Philip Cole, Hans-Olov Adami, Dimitrios Trichopoulos, Jack Mandel

Presented at the

EPA Listening Session
July 27, 2010

U.S. EPA. Draft IRIS Toxicological Review of Formaldehyde-Inhalation Assessment
Docket EPA-HQ-ORD-2010-0396

This presentation is supported by the Hexion Specialty Chemicals Co. Inc. It is based on the paper listed above which was supported in part by a grant from the Formaldehyde Council, Inc.

ED_014350_00001003-00030

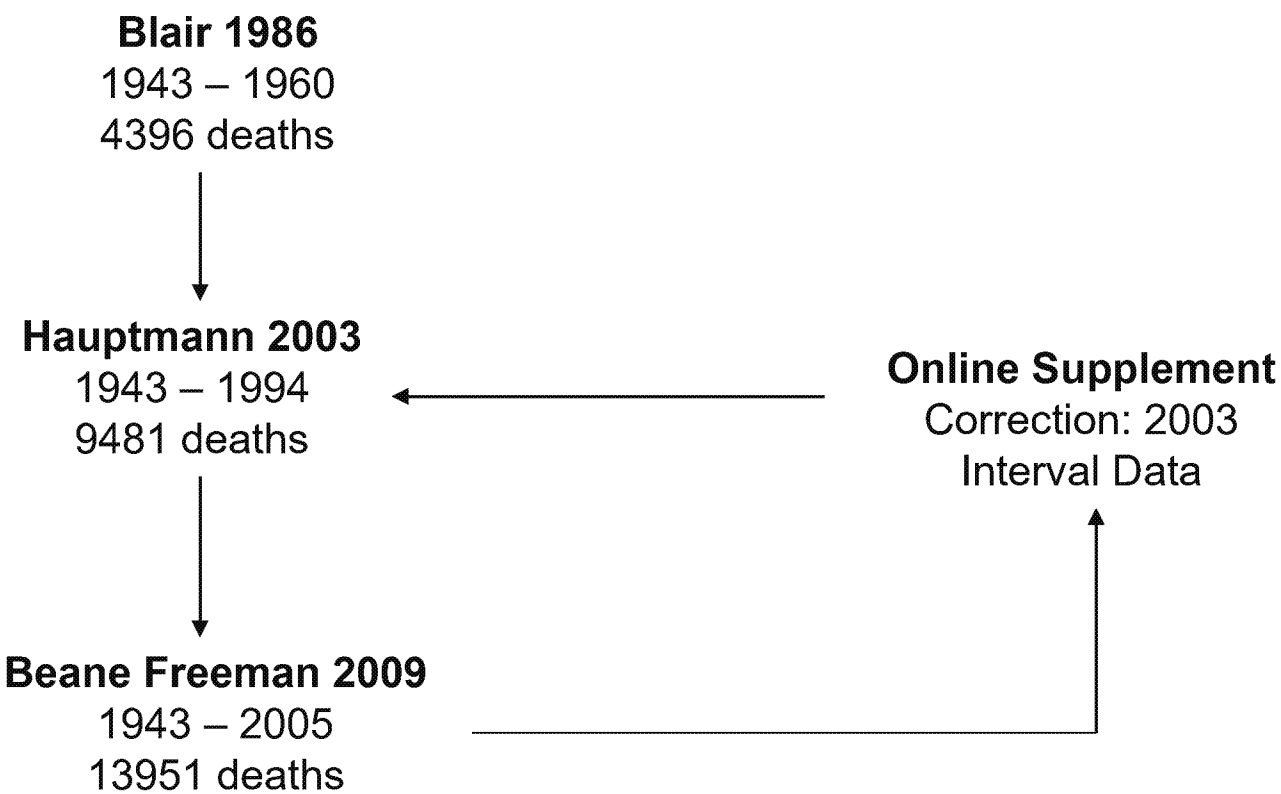
Cohort Studies of Occupational Groups with Exposure to Formaldehyde

Affil / Origin	MRC/UK	NIOSH/USA	NCI/USA
Published	2003	2004	1986, 2003, 2009
Facilities	6	3	10
Subjects	14,014	11,039	25,615
Decedents	5,185	2,206	13,951
Processes	Produce/Use	Garments:Fold/Press	Produce/Use
Avg. Exposure (ppb)	>2,000	150	450
Avg. follow-up (yrs)	>40	31	40
		SMRs	
All LHC cancer	90	97	94
All leukemia	91	109	102
The myeloid leukemias	89*	144	90

*Estimated by us.

ED_014350_00001003-00031

Progression of the NCI Cohort Study



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SMRs for Formaldehyde and the LHCs Reports from the NCI Cohort Study

	Last Year of Follow-up		
Disease Group	1979	1994	2004
All LHCs	86	93	94
NHLs	-	73*	85
Hodgkin lymphomas	142	132	142
Multiple myeloma	-	111	94
Leukemias	74	98	102
lymphoid	-	-	115
myeloid	-	95**	90

* The only statistically significant value: 95% CI: 56-96.
** Estimated by us.

ED_014350_00001003-00033

Five Indices of Exposure

1. Peak exposure (single peak, ppm).
2. Average intensity (ppm).
3. Cumulative (ppm-yrs).
4. Cumulative number of peaks.*
5. Duration of exposure (yrs).**

* Not evaluated by Hauptmann, 2003. No association in Beane Freeman, 2009.

** No association in either report.

ED_014350_00001003-00034

The Myeloid Leukemias
Tests of Statistical Significance

Hauptmann, 2003:	4 indices x 2 exposure strata	=	8 tests
	4 indices x 2 subject groupings	=	8 tests
Beane Freeman, 2009:	5 indices x 2 exposure strata	=	10 tests
	5 indices x 2 subject groupings	=	10 tests
	Total		36 tests
	$p<0.05$		3 (8.3%)

ED_014350_00001003-00035

The Myeloid Leukemias
Relative Risks According to Peak Exposure

	Parts per Million				p-Trend	
	0	>0 – 1.9	2.0 – 3.9	4.0+	All	Exp.
Hauptmann, 2003	0.60	1.0	1.98	**2.79**	**0.01**	**0.02**
Beane Freeman, 2009	0.82	1.0	1.30	1.78	0.13	0.07

ED_014350_00001003-00036

Peak Exposure and the Myeloid Leukemias: The Interval Data *

	Blair <1981		Hauptmann 1981 – 1994		Beane Freeman 1995 – 2004	
Peak Exposure	N	RR	N	RR**	N	RR
0	**2**	2.5	0	0	**2**	1.5
0.1 – 1.9	2	1.0	5	1.0	7	1.0
2.0 – 3.9	5	3.7	3	1.2	3	0.7
4.0 - +	6	3.9	9	2.7	4	0.7
Total	15	-	17	-	16	-

* Adapted from Beane Freeman et al., 2009. Table S5.
** p-trend: 0.04 (all subjects); 0.21 among exposed.

ED_014350_00001003-00037

Relative Risk of Death for the Myeloid Leukemias According to Time Since First Exposure*

	Time Since First Exposure (Yrs.)					
	0	<16	16 - 25	26 - 35	36+	Total
Deaths	2	3	11	8	24	48
Relative Risk	0.3	1.0	2.4**	0.8	0.7	-

* Adapted from Beane Freeman, et al., 2009. Table S6.

** 95% CI : 0.5 -13.3.

ED_014350_00001003-00038

Conclusion A*

In the current follow-up, the overall risk of myeloid leukemia has declined from our previous report, but remains somewhat elevated. Although that time trend may suggest that the previous excess risk estimates were due to chance, the pattern is consistent with a possible causal association, with the largest risks occurring closer in time to relevant exposure.

* From Beane Freeman et al., 2009. Discussion.

ED_014350_00001003-00039

Conclusion B

- No excess of myeloid leukemia deaths ever occurred among this cohort.

- A weak and transient exposure-response relationship of formaldehyde with the myeloid leukemias was seen for only one of five indices of exposure.

ED_014350_00001003-00040

Embalmers Study: Overview I

Purpose: To evaluate LHC mortality in relation to work practices and formaldehyde exposure levels among embalmers and funeral directors.

Type: Exceptional – A case-control study nested within a series of proportional mortality data sets.

ED_014350_00001003-00041

Embalmers Study: Overview II

Subjects:

1. 6808 decedents from three PMR studies previously reported by NCI staff.
2. Update or additional source?
3. Employed 1902 – 1985.
4. Deaths occurred from 1960 – 1986.

Exposure:

1. Interviews with next of kin and co-workers of decedents were conducted 1990 - 1992.
2. Previous experiment / modeling.

ED_014350_00001003-00042

Embalmers Study: Overview III
Exposure Assessment

Interviews

- Method (telephone, in person) not stated.
- At least one next-of-kin for 95% of subjects
- No information on co-worker interviews.
- Interviews occurred 4 to 33 years after subjects' deaths and possibly up to 55 years after start of relevant exposure.

Experiment / Modeling

- Average levels assessed validly.
- Peak levels not validated.

Embalmers Study: PMRs

Disease Group	Estimated* Number	PMR	95% Conf. Int.
All LHCs	143	90	(76-106)
Myeloid leukemias	29	108**	(72-156)
Nasopharyngeal cancer	4	128	(35-328)
Brain tumors	44	120	(87-161)

* Underlying cause of death.
** Embalmers: 29/6808 = 4.3 / 1000. USA, males aged 25+ in 1979: 3424 / 852,003 = 4.0 / 1000. PMR: 108 (72-156).

ED_014350_00001003-00044

The Myeloid Leukemias: First Analysis *

Characteristic	No. of control subjects	Myeloid leukemia: No. of case subjects	Myeloid leukemia: OR (95% CI)
Embalming			
Never	55	1	1.0 (Ref.)
Ever	210	33	11.2 (1.3 to 95.6)
Duration of working in jobs with embalming, y			
0	55	1	1.0 (Ref.)
>0 to 20	71	6	5.0 (0.5 to 51.6)
>20 to 34	69	13	12.9 (1.4 to 117.1)
>34	70	14	13.6 (1.6 to 119.7)
P for trend†			.020 (.588)
Peak formaldehyde exposure, ppm			
0	55	1	1.0 (Ref.)
>0 to 7.0	67	12	15.2 (1.6 to 141.6)
>7.0 to 9.3	72	9	8.0 (0.9 to 74.0)
>9.3	71	12	13.0 (1.4 to 116.9)
P for trend†			.036 (–.778)

* From Table 3 of Hauptmann et al., 2009.

The Myeloid Leukemias: Two Analyses

Because there was only one case subject with myeloid leukemia in the reference group of nonembalmers, we also evaluated risks for this condition by considering subjects who performed fewer than 500 lifetime embalmings (to include five case subjects) as the reference group

These represent more conservative but probably more reliable risk estimates for high-level exposure than those shown in Table 3.

ED_014350_00001003-00046

Embalming and the Myeloid Leukemias: Second Analysis*

Characteristic	No. of control subjects	LHPM of nonlymphoid origin: No. of case subjects	LHPM of nonlymphoid origin: OR† (95% CI)	Myeloid leukemia: No. of case subjects	Myeloid leukemia: OR† (95% CI)	Acute myeloid leukemia: No. of case subjects	Acute myeloid leukemia: OR† (95% CI)
<500 embalmings	83	9	1.0 (Ref.)	5	1.0 (Ref.)	3	1.0 (Ref.)
>500 embalmings, questionnaire-based metrics							
Duration of working in jobs with embalming, y							
<20	47	2	0.3 (0.1 to 1.7)	2	0.5 (0.1 to 2.9)	1	0.4 (0.04 to 4.9)
>20–34	67	16	2.0 (0.8 to 5.0)	13	3.2 (1.0 to 10.1)	8	2.9 (0.7 to 12.2)
>34	68	21	2.6 (1.0 to 6.4)	14	3.9 (1.2 to 12.5)	8	3.1 (0.7 to 13.7)
P for trend‡			.046 (.348)		.020 (.588)		.063 (.612)
No. of embalmings							
≥500 to 1422	42	3	0.6 (0.2 to 2.6)	3	1.2 (0.3–5.5)	0	0 (0–1.8)
>1422 to 3068	70	15	1.8 (0.7 to 4.6)	12	2.9 (0.9 to 9.1)	8	2.9 (0.7 to 12.0)
>3068	70	21	2.3 (1.0 to 5.7)	14	3.0 (1.0 to 9.2)	9	2.9 (0.7 to 11.6)
P for trend‡			.247 (.662)		.314 (–.991)		.492 (0.698)
Questionnaire- and model-based exposure metrics							
Cumulative formaldehyde exposure, ppm-h§							
≤4058	43	5	1.1 (0.3 to 3.8)	5	2.1 (0.5 to 8.1)	2	1.3 (0.2 to 9.4)
>4058 to 9253	69	12	1.4 (0.5 to 3.7)	10	2.2 (0.7 to 7.1)	6	1.9 (0.4 to 8.2)
>9253	70	22	2.4 (1.0 to 5.8)	14	3.1 (1.0 to 9.6)	9	3.2 (0.8 to 13.1)
P for trend‡			.14 (.523)		.192 (.966)		.294 (.940)
Average formaldehyde intensity while embalming, ppm							
≤1.4	63	13	1.7 (0.7 to 4.5)	10	2.6 (0.8 to 8.7)	6	2.5 (0.6 to 10.9)
>1.4 to 1.9	50	12	1.7 (0.7 to 4.6)	10	2.0 (0.6 to 6.1)	5	2.0 (0.4 to 9.4)
>1.9	60	14	1.8 (0.7 to 4.7)	9	2.3 (0.7 to 7.5)	6	2.3 (0.5 to 10.3)
P for trend‡			.096 (–.997)		.058 (–.722)		.068 (.869)
TWA8 formaldehyde intensity, ppm							
≤0.10	56	9	1.3 (0.5 to 3.6)	8	2.4 (0.7 to 8.2)	3	1.4 (0.3 to 7.8)
>0.10 to .18	61	16	2.1 (0.8 to 5.3)	10	2.6 (0.8 to 8.7)	7	2.6 (0.6 to 11.4)
>0.18	65	14	1.9 (0.7 to 4.8)	11	2.6 (0.8 to 8.3)	7	2.6 (0.6 to 11.3)
P for trend‡			.256 (.961)		.396 (–.642)		.441 (–.672)
Peak formaldehyde exposure, ppm							
≤7.0	54	10	1.6 (0.6 to 4.5)	9	2.9 (0.9 to 9.8)	4	1.8 (0.4 to 9.3)
>7.0 to 9.3	66	12	1.4 (0.5 to 3.7)	9	2.0 (0.6 to 6.6)	5	2.1 (0.5 to 9.2)
>9.3	62	17	2.3 (0.9 to 5.6)	11	2.9 (0.9 to 9.5)	7	2.9 (0.7 to 12.5)
P for trend‡			.089 (–.944)		.036 (–.778)		.035 (.636)

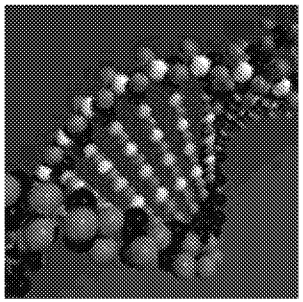
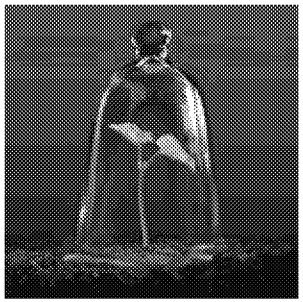
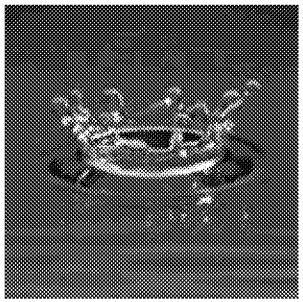
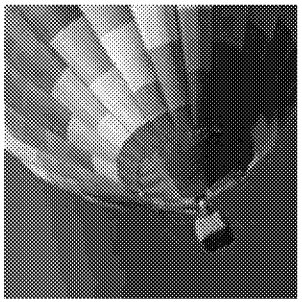
‡ *P* trend (Wald test) among exposed only (ie, subjects who embalmed) in parentheses. Trend tests for LHPM of nonlymphoid origin and myeloid leukemia are the same as those presented in Table 3.

* Table 4 of Hauptmann et al., 2009.

Embalmers Study: Conclusions

- With a PMR of 108 (72-156), there is no evidence of an absolute increase in deaths due to the myeloid leukemias among embalmers.
- Although many analyses are presented, the most reliable data have not been tested for statistical significance.

Occupational formaldehyde exposure and leukemia risks

Kenneth A. Mundt, Ph.D., Diane J. Mundt, Ph.D. and Annette Bachand, Ph.D.

EPA Listening Session, July 27, 2010

U.S. EPA. Draft IRIS Toxicological Review of

Formaldehyde-Inhalation Assessment

Docket EPA-HQ-ORD-2010-0396

Supported by the Hexion Specialty Chemicals Co. Inc., and based on a review and published meta-analysis supported by the Formaldehyde Council, Inc.

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EPA – IRIS Conclusion: All leukemia

"While the epidemiologic evidence for a causal association between formaldehyde and all leukemia as a group is not at strong as for all LHP as a group, the repeated identification of an association in multiple meta-analyses taken together with the clear causal association between myeloid leukemia demonstrated by Hauptmann et al. (2009) and the consistent evidence reported by Beane Freeman et al. (2009) are sufficient to conclude that there is a causal association between formaldehyde exposure and mortality from all leukemia as a group."

Draft IRIS Review – page 4-182

Epidemiological basis for causal conclusion?

- Validity of aggregating disease outcomes
- Meta-analysis and causal inference
- Study overlap
- Are exposures validly measured and comparable across studies?

- Is there consistency, strength of association, biological plausibility and other support for a causal conclusion?

Validity of aggregating diseases

- Validity of aggregating disease outcomes:
  - All lymphohematopoietic
  - All leukemias (lymphoid and myeloid)
  - Myeloid leukemia (acute and chronic)
- Heterogeneity in disease process and risk factors across diseases.
- Stronger association between formaldehyde and all LHPs vs. all leukemias in IRIS draft argues ***against*** *a* causal association with leukemias.

Meta-analyses and Causal Inference

"The primary role of meta-analysis in occupational epidemiology in general, is not causal inference per se. . . Causal inference is another matter altogether. As Greenland notes, one should be aware of the limitations of meta-analysis; causal explanations are outside its realm."[1]

Nevertheless, EPA relies on meta-analyses, but fails to consider the most recent (Bachand, et al., 2010)

1 Weed, Ann Epidemiol 2010;20:348

Formaldehyde Meta-Analyses

Leukemias	Collins 2004	Bosetti 2008	Zhang 2009	Bachand 2010
Overall meta-estimate	NS		Sig ↑	NS
Cohort studies	NS			NS
Case-control studies	NS			**
Industrial	NS	NS*		NS
Embalmers	Sig ↑			
Pathologists/ anatomists	NS			
"Professional" workers		Sig ↑		NS

NS – not statistically significant: i.e., confidence interval includes 1.0

* Meta RR for lymphatic and hemopoietic combined is ***statistically significantly decreased*** .

** Summary estimate for case-control not calculated, only 2 studies

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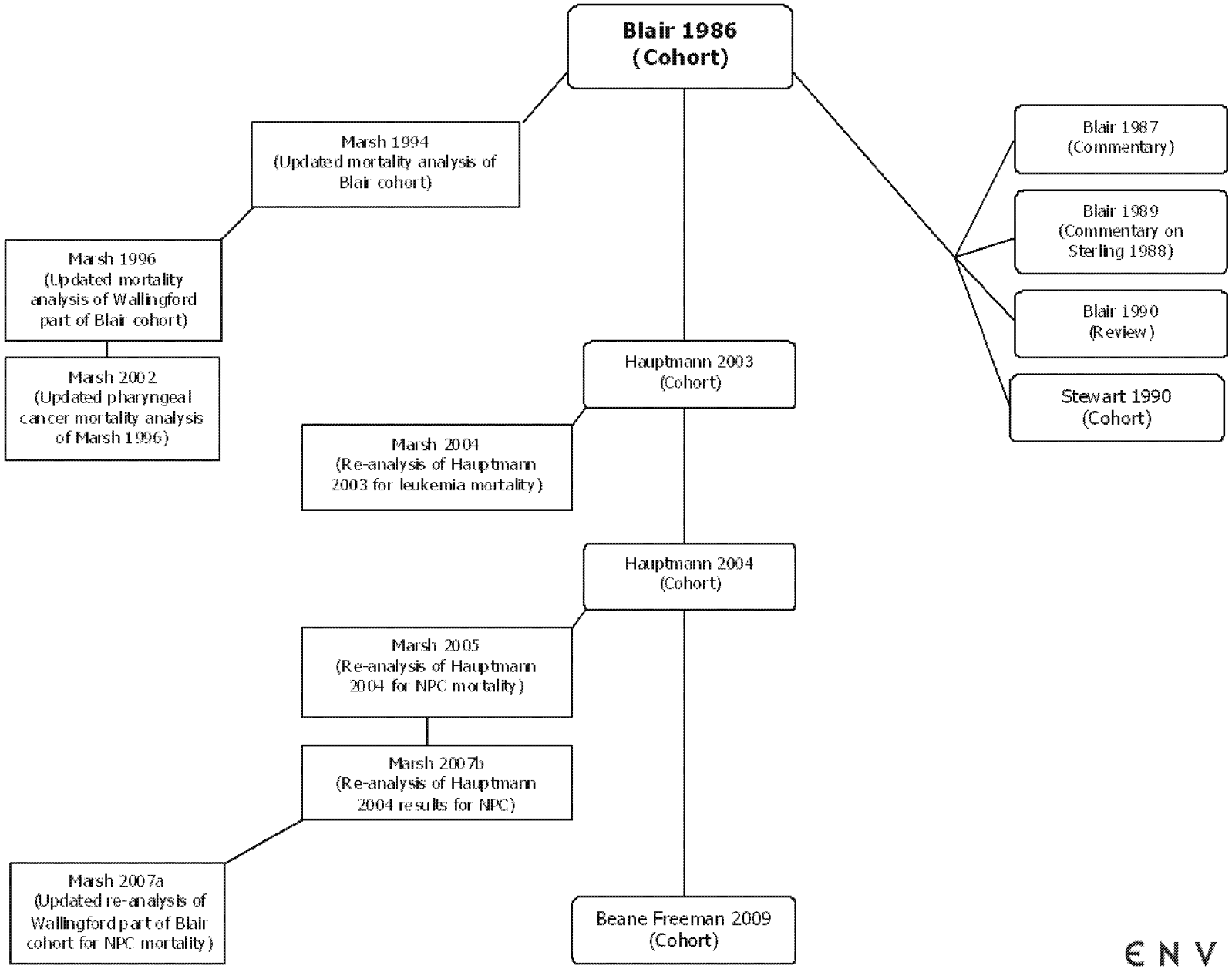
Study overlap

- NCI Industrial cohort – studied by many investigators
  - Multiple follow-ups
  - Corrections
  - Reanalysis of findings (internal and external comparisons)
- Citing multiple studies of essentially the same cohort gives the false impression of consistency vs. what would be seen by limiting synthesis to independent observations.

NCI Industrial Cohort Pedigree



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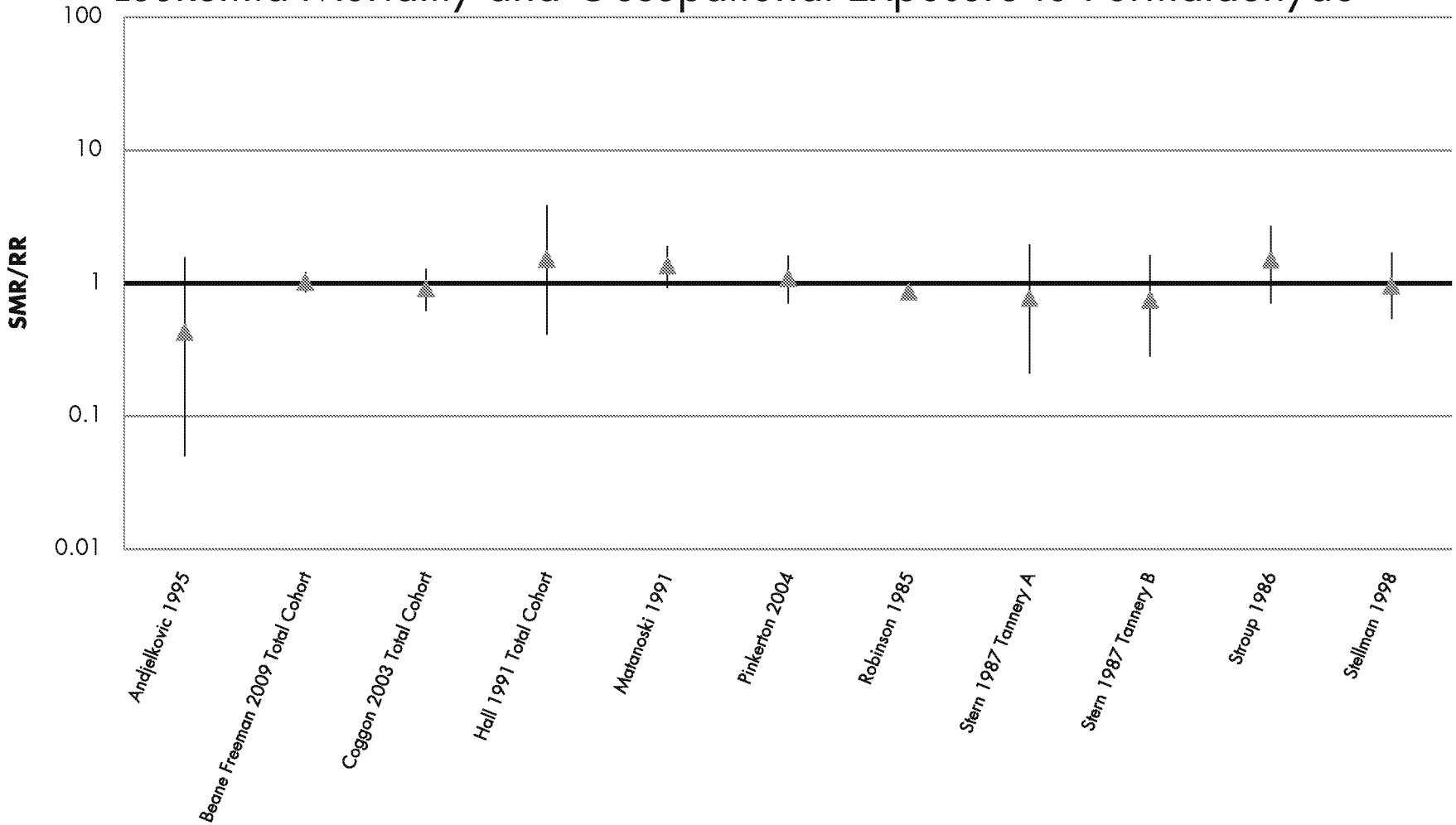
Cohort studies

- Three large formaldehyde exposed industrial worker cohorts:
  - British chemical workers: Coggon 2003
  - US garment workers: Pinkerton 2004
  - US formaldehyde manufacturers: Beane Freeman 2009
- Several smaller cohorts of industrial and professional workers
- Body of cohort studies does not support a causal conclusion between formaldehyde and leukemia

Leukemia Cohort Studies:
Leukemia Mortality and Occupational Exposure to Formaldehyde



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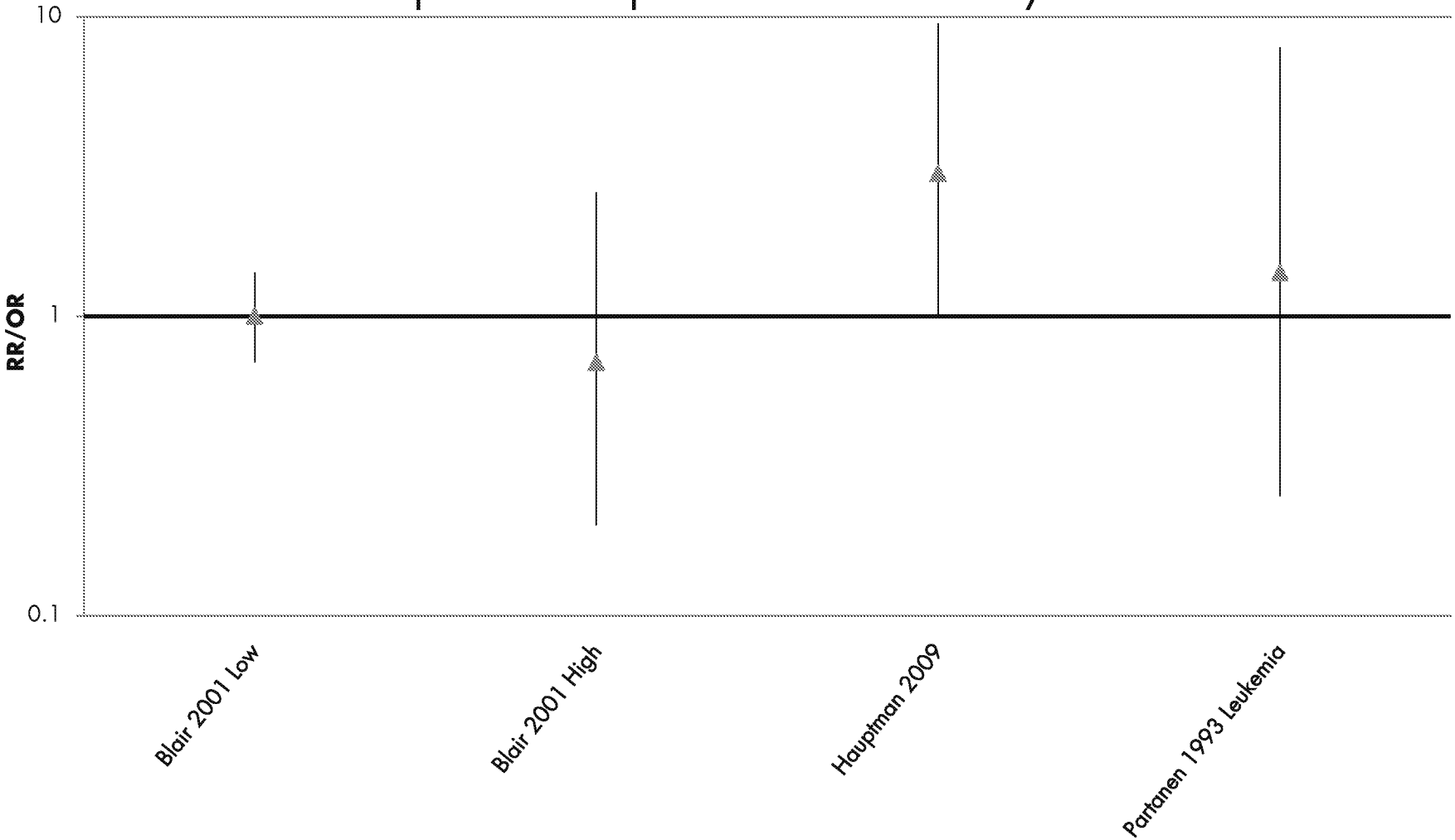
Case-control studies

- There are few case-control studies:
- Blair, et al., 2001
  - Cases from Iowa and Minnesota cancer registries
- Partanen, et al., 1993
  - Identified from Finnish cancer registry
- Hauptmann, et al., 2009
  - Deaths from previous studies of embalmers and funeral directors
  - Resembles a case-control study "nested" in a proportionate mortality (PMR) study

Leukemia Case-Control Studies: Occupational Exposure to Formaldehyde



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PMR Studies

"PMR studies have the attractive feature of providing results relatively quickly. . .

However, the validity of a PMR study depends on whether the deaths included are generally representative of all deaths that would be identified if follow-up of the full cohort had been conducted."[1]

[1] Checkoway 2004. *Research Methods in Occupational Epidemiology*

Embalmers and funeral PMR

- Walrath and Fraumeni 1983,1984 and Hayes 1990 are PMR studies, and the source of Hauptmann 2009 cases
- Walrath and Fraumeni 1983 stated: "State health departments were surveyed to determine whether sufficient licensure records existed to establish a cohort of embalmers. No state contacted retained the records necessary for a retrospective cohort study. The Bureau of Funeral Directing and Embalming in the New York State Department of Health, however, maintained a file on deceased embalmers, so it was possible to examine the proportions of deaths due to specific causes."

Lack of exposure measurements

- Most studies to date have not attempted to estimate individual exposure
- Only NCI industrial workers study quantitatively estimated formaldehyde exposure
- NCI embalmers study relied on next-of-kin and co-worker interviews for exposure surrogates, combined with some experimental exposure estimates.

EPA – IRIS Conclusion: Myeloid leukemia

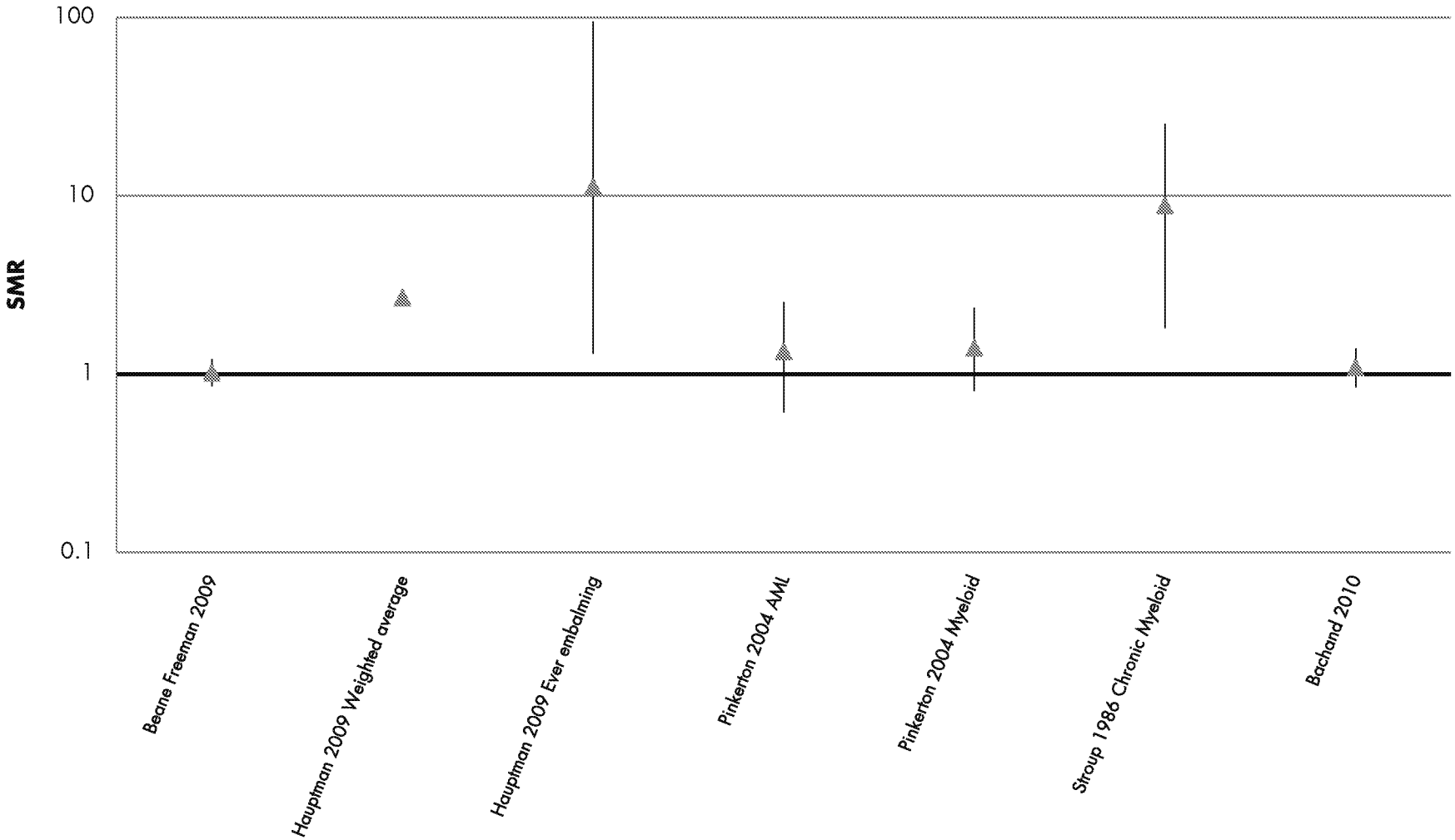
"Given the consistency of the positive associations for formaldehyde with myeloid leukemia cancer mortality across five of the six studies (Hauptmann et al., 2009; Pinkerton at al., 2003; Hayes et al., 1990; Stroup et al., 1986; Walrath and Fraumeni, 1984, Walrath and Fraumeni, 1983; but not Beane Freeman et al., 2009), the statistically significant meta analysis by Zhang et al. (2009) and the convincing results from Hauptmann et al. (2009), the human epidemiologic evidence is sufficient to conclude that there is a causal association between formaldehyde exposure and mortality from myeloid leukemia."

Draft IRIS Review – page 4-184/5

Overlapping studies

- Note that results from Hauptmann et al., 2009; Hayes et al., 1990; Walrath and Fraumeni, 1984, Walrath and Fraumeni, and Zhang 2009 are largely based on or influenced by the embalmers death certificate (i.e., PMR) study.
- PMR studies do not carry the same weight as studies based on well-enumerated cohorts followed over time to determine and compare disease risks.

Myeloid Results



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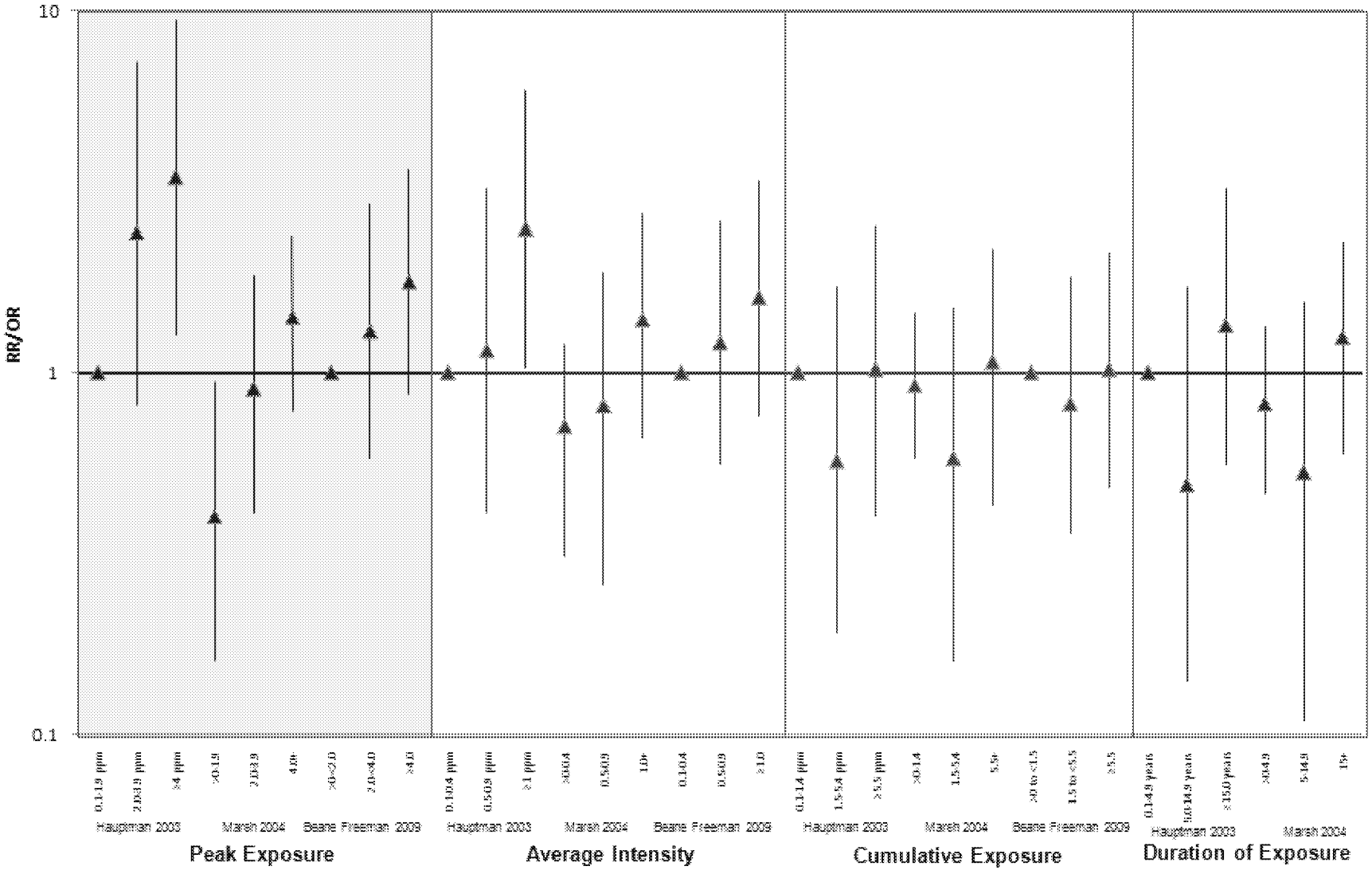
NCI Cohort and Myeloid Leukemia



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Additional Exposure Metrics

"There was no evidence that risks increased with cumulative number of peaks ≥ 4.0 ppm or for duration of exposure for any cause of death evaluated (data not shown)."[1]

[1] Beane-Freeman et al., 2009

Epidemiological basis for causal conclusion?

- With few exceptions, the epidemiological literature on formaldehyde exposure and leukemia risks consistently demonstrates
  - No statistically significant risks among exposed workers;
  - No consistent associations with various exposure metrics
- The EPA draft document does not properly synthesize the epidemiological evidence on formaldehyde and leukemia, including myeloid, risks; therefore, the causal conclusions remain unsubstantiated.

Formaldehyde-Induced LHP: Is There Sufficient Evidence to Conclude a Causal Association?

Annette M. Shipp, Ph.D.
ENVIRON International Corporation

USEPA Listening Sentence, July 27, 2010
USEPA Draft IRIS Toxicological Review of Formaldehyde-Inhalation Assessment
Docket EPA-HQ-ORD-2010-0396

Supported by Hexion Specialty Chemicals Co. Inc.

Overview of Evidence

Weight of Evidence Approach consistent with USEPA (2005)

- Evidence must be considered in it's totality in an integrated manner – compare and contrast
  - Epidemiological Studies in Industrial and Professional Workers
  - Animal Bioassays
  - Evaluation of Biological Plausibility
    - Toxicokinetic Studies
    - Hematological and Cytogenic Studies
  - Consideration of Mode of Action
- Preparation of the weight of evidence analysis

Animal Studies

- USEPA states that the animal studies "provide limited evidence for leukemia and lymphoma". Three lifetime studies have been conducted:
  - Oral Study:
    - Til et al. (1989) did not show an association with leukemia or other LHP cancers at doses up to 1900 mg/L
  - Inhalation Studies:
    - Battelle et al. (1981) (unpublished):
      - The original analysis did not show a statistical significant increase in ieukemia or lymphoma at exposures up to 15 ppm
      - Re-analysis of rat data to consider early deaths showed a significant increase in leukemia in female rats but not male rats
      - Two published papers (Swenberg et al. 1980; Kerns et al. 1983) did not discuss these results.
    - Sellakumar et al. (1985):
      - No significant increase in leukemia or lymphoma at exposures up to 10 ppm but despite survival issues, USEPA stated, "Nonetheless, this study did not indicate formaldehyde-induced lymphoma or leukemia.
    - Questions:
      - Relevance of findings at an exposure concentration of 15 ppm
      - Relevance of responses in rats/mice with regard to both dosimetry and tumor type

Animal Studies (cont)

- A review of all of the chemicals identified in the NTP 11th RoC (NTP 2009) identified 45 agents classified as "known" human carcinogens, with 38 of these having concordant animal data.
  - Seven of these were identified as known human leukemogens: benzene, ionizing radiation, MeCCNu, 1,3-butadiene, chlorambucil, thiotepa, and cyclophosphamide.
  - For all of these agents, the production of leukemia or other hematopoietic cancers (benzene and 1,3-butadiene) was reported in mice and/or rats, and, in the case of ionizing radiation, positive results for leukemia in dogs, monkeys and rabbits were also reported.
- For most of the known human leukemogens human and animal data appear to be concordant.

Toxicokinetics

- USEPA states that, “Inhalation studies in several species indicate that exposure to formaldehyde does not result in elevated levels in blood.”
  - Rats - Heck et al. (1982, 1985) at 14 ppm for 2 hours
  - Humans - Heck and Casanova-Schmidt et al. (1984) at 1.9 ppm for 40 minutes
  - Rhesus monkeys – Casanova et al. (1988) at 6 ppm for 6 hours per day for 4 weeks
- More recent, unpublished results (Andersen et al.) in agreement
- Toxicokinetics have been evaluated by Dr. Swenberg who reached the same conclusions.
- Questions:
  - Ability of GC-MS to distinguish bound and unbound
  - Physiological differences in rats regarding breathing type and resulting dosimetry
  - Other unidentified sources of exogenous formaldehyde contributing to the “endogenous” pool

Zhang et al. - Hematological Effects

- Reported a significant decrease in all blood cells evaluated and stated that the results suggested pancytopenia – and indicator of bone marrow toxicity
- All hematological parameters were within normal reference ranges raising the following questions (Note some information based on FOIA request for original data)
  - When Thallassemia considered (one case in exposed and 4 cases in unexposed), were the differences in RBC and MCV changes between unexposed and exposed worker still statistically significant?
  - How were the difference in recent respiratory infections that would affect WBC counts not considered?
  - What other possible differences in these populations (age, smoking, alcohol, medications, general physical condition, etc.) could have affected the outcomes?
- No evidence of an effect on hematological systems, e.g., pancytopenia, was not noted in animal studies that evaluated such effects.
- Is the conclusion of pancytopenia as an indicator of effects on bone marrow justified for formaldehyde?
- The significance of the changes in blood counts reported by Zhang *et al.* (2009, 2010) is of questionable clinical relevance.

Zhang et al. - Cytogenic Effect CFU-GM

- Effects on specific on a biological marker of an effect on stem cells – the number of granulocyte-macrophage colony forming units (CFU-GM) were not statistically significantly different between exposed and non-exposed workers
  - The paper mentions an “outlier” but no mention is made of any statistical test to determine if it is a “true” outlier.
  - Was this outlier included in the analysis of the change in CFU-GM in the exposed group and if not, would the amount of change be different?
- Zhang et al. stated that:
  - Decreases in blood cells result in deficiencies in the production of CFU-GM units
  - Human leukomogens lower the ability of stem or progenitor cells to replicate in colony-forming units
- These results are internally inconsistent again raising the question of the clinical relevance of these data

Zhang et al. (2009): *In vitro* Cytogenic Effects

Significant decreases with increasing formaldehyde concentrations added to the culture media for three biomarkers

- Concerns:
  - Data are the average of 6 replicates from one individual
  - While amount applied is around that found naturally, but when directly applied thereby, by-passing all normal metabolic and kinetic processes that prevent formaldehyde from reaching the target tissue, the bone marrow.
  - Biomarkers are not specific only for leukemia and other chemicals may show similar results

Zhang et al. - Aneuploidy

- The clinical relevance of the changes in the levels of monosomy of chromosome 7 and trisomy of chromosome 8 reported is unclear.
- Lack of evidence that these are leukemia-specific
  - The reference cited (Rowley 2000) does not mention these
  - These changes are not predictive of the development of myeloid leukemia and are not commonly observed in individuals diagnosed with either acute (AML) or chronic myeloid leukemia (CML).
    - For example, In 200 patients with AML, only a limited number of patients had monosomy 7 (2.2%) and trisomy 8 (5.6%) (Ahmad *et al.* 2008). In addition, in 122 AML patients in China, monosomy 7 was not reported, and only approximately 3% had trisomy 8 (Zheng *et al.* 2007). For chronic myeloid leukemia (CML), translocation of chromosomes 9 and 22 of the Philadelphia chromosome is the chromosomal change most commonly found (Bonassi *et al.* 2008).
    - Recent studies report other biomarkers to provide more relevant association with leukemia(Li et al. 2010; Albertini (2004).
  - These are a heterogenous group of diseases – it would be surprising if one set of biomarkers was relevant to leukemia in general

Aneuploidy (cont)

- Biologically significant methodological issues
  - Text says that a minimum of 150 cells per person were evaluated in the FISH assay
    - For monosomy 7, only 1 of 10 individuals in exposed group and only 4 of unexposed group has at least 150 cells evaluated
    - 7 of 10 exposed and 7 of 12 non-exposed had counts at or below 100
    - When combined, only 4 of 10 and 6 of 12 were above 150 cells
    - Would the results still be statistically significant if 150 cells were counted, i.e., 2 of 20 is 10% but 19 of 288 is 6.6%

Aneuploidy (cont)

- Methodological limitations: From FOIA
  - Unknown if participants were from different plants
  - Exposure for each individual unknown
  - Apparently, one or more “clones” were combined to get the number of cells evaluated
  - No other chromosome changes were evaluated – especially the mirrow-image: trisomey 7 and monosomy 8
  - No consistent relationship in an individual with monosomy and trisomy
- **Critical point: If it is assumed that all or almost all of the cells in the colony were derived from a stem cell, the data do not support such an assumption or the idea/conclusion regarding clonal expansion.**

Proposed Modes of Action

- A reliance on those proposed by Zhang et al. to include:
  - Genetically damaged circulating stem or progenitorcells return to bone marrow where they initiate leukemia or other LHP cancers
    - Evidence that circulating hematopoietic or progenitor stem cells do not return to the bone marrow during homeostasis (McKinney-Freeman and Goodell (2004).
  - Production of specific aneuploidy – monosomy 7 and trisomy 8
    - Discussed previously
  - Transport of metabolite methanediol to bone marrow where formaldehyde is generated
    - Discussed by Dr. Swenberg – not a plausible MOA
  - Direct genetic damage to bone marrow
    - Discussed by Dr. Swenberg – not a plausible MOA

Does all the Evidence Support a Causal Association

- Epidemiological Data – The overall weight of evidence concludes:
  - No statistically significant risks amount exposed workers
  - No consistent associations with various exposure metrics
  - The EPA draft document does not adequately synthesize this evidence on formaldehyde and leukemia and, therefore, the causal conclusions remain unsubstantiated.
- Animal Data –
  - Unlike known leukemogens, formaldehyde data is not concordant and does not support the conclusion regarding a causal association
- Biological Plausibility and MOA does not support a causal association
  - Biologically significant methodological limitations make the conclusions invalid
- CONCLUSION: These data do not support a plausible causal association between formaldehyde exposure and leukemia or other LHP cancers.