

Historical Performance of Weak Sensitizers in Different Animal Models

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ABSTRACT

In the mid-1990s, government regulators moved from allowing the use of strong potency positive control agents in animal studies for dermal sensitization to requiring the use of weak to moderate sensitizers. This shift arose from the desire to prove that the performing laboratories could demonstrably identify weak to moderate sensitizers under their specific testing conditions, thereby reducing concerns regarding false negatives. Twenty years later, the recommended replacement agents, including those used in the authors' laboratory (mercaptobenzothiazole [MBT] and hexylcinnamaldehyde [HCA]), are in widespread use and indeed demonstrate the test methods' capacity to identify weak to moderate sensitizers. In the authors' laboratory, the Magnusson and Kligman Guinea Pig Maximization Test (GPMT) returns 30-45% positive responders with MBT, while the Buehler Method returns 20-65% positive responders with HCA. In the Murine Local Lymph Node Assay, a concentration of 25% HCA returns 40-100% positive responders using both the flow cytometric method and the more recent BrdU-ELISA method. Factors potentially affecting positive response rates, including induction and/or challenge concentrations, vehicle effects, and positive control batch are compared.

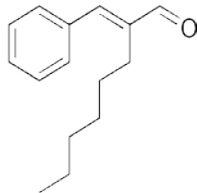
MATERIALS AND METHODS

	GP-Buehler	GPMT	LLNA-ELISA	LLNA-FC
Species	Guinea Pig	Guinea Pig	Mouse	Mouse
Strain	Hartley	Hartley	CBA/J or CBA/Ca	CBA/J or CBA/Ca
Agent	HCA	MBT	HCA	HCA
Vehicle	Ethanol/Acetone	Acetone	Various	Various
Guideline	OECD 406 OCSP 870.2600	OECD 406 OCSP 870.2600	OECD 442B	Non-Guideline
Type	Induction/Elicitation	Induction/Elicitation	Induction Only	Induction Only
Endpoint	Dermatitis	Dermatitis	Lymph Node Cell Proliferation	Lymph Node Cell Proliferation
Assessed	Visually	Visually	ELISA	Flow Cytometry

CONCLUSIONS

- Using weak-to-moderate sensitizers in lieu of strong/extreme sensitizers in predictive toxicology assures that laboratory methodology is accurate and precise, such that chemical hazards with lower-potency sensitizing potential are able to be identified.
- Sensitization assays with larger sample size (guinea pig) are amenable to individual positive response analysis (% responding). Sensitization assays with smaller sample size (LLNA) are less amenable to frequency-of-response analysis (% responding) and are more appropriately interpreted by the average strength of positive response, v.s. control (vehicle or sham).

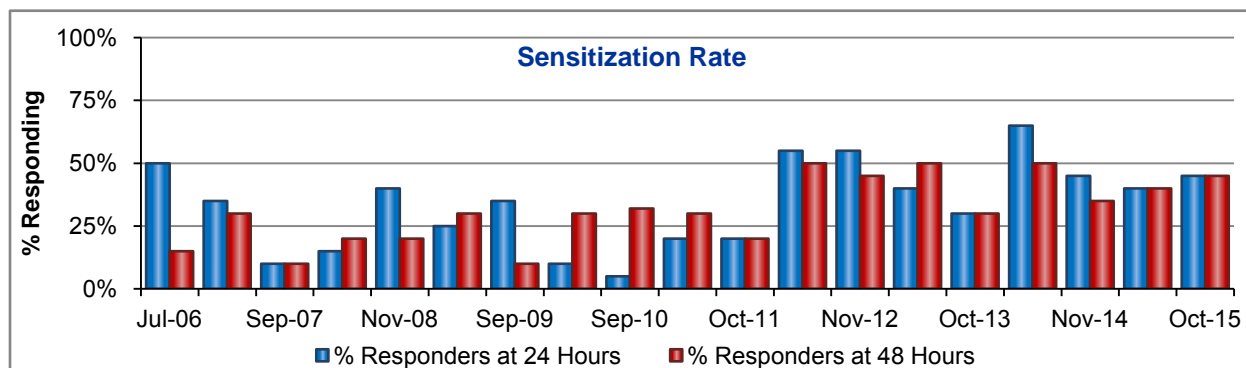
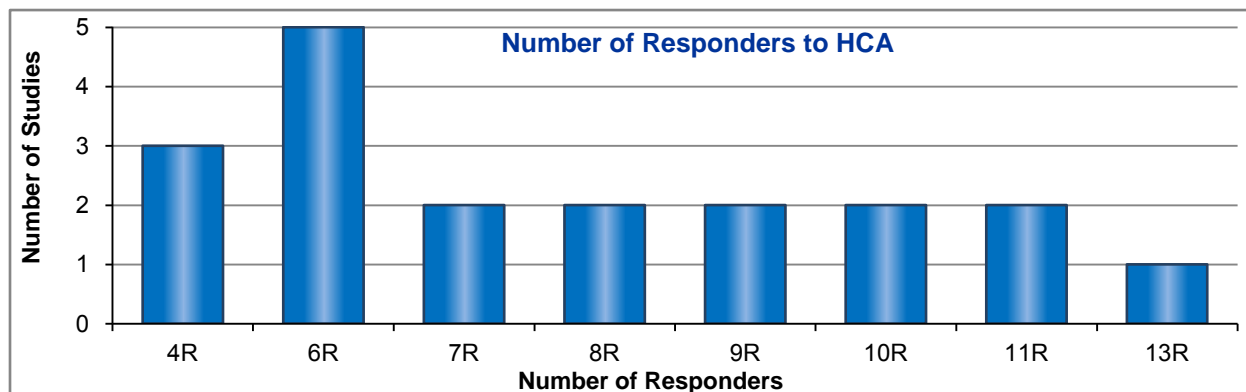
GUINEA PIG BUEHLER ASSAY

Agent	HCA	
CAS	101-86-0	
Purity	85% Tech Grade	
Studies	19	
Induction	Neat	
Challenge	50%	
Animals/Group	20	
Positive Response	≥15%	

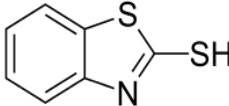
Hexylcinnamaldehyde (HCA)

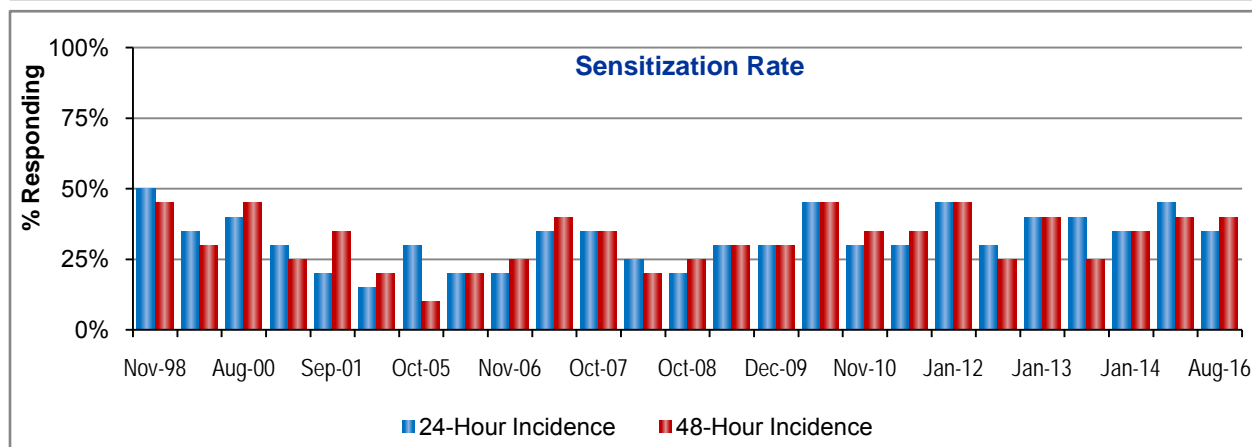
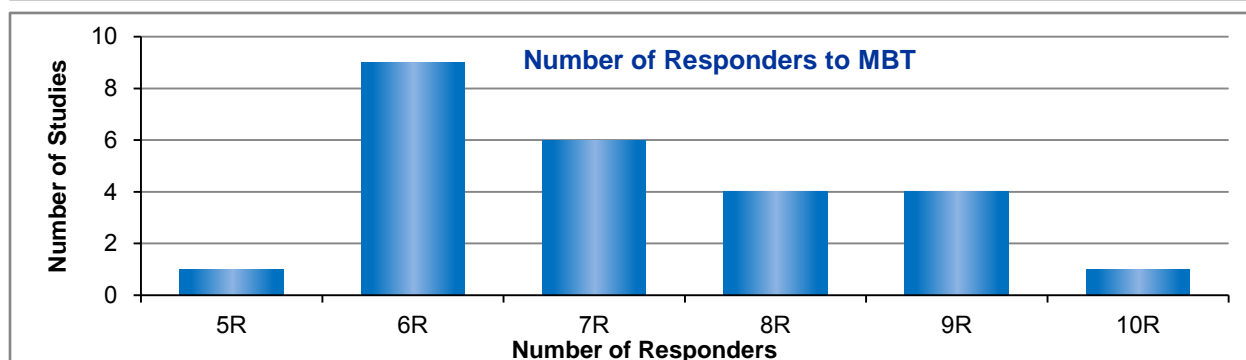
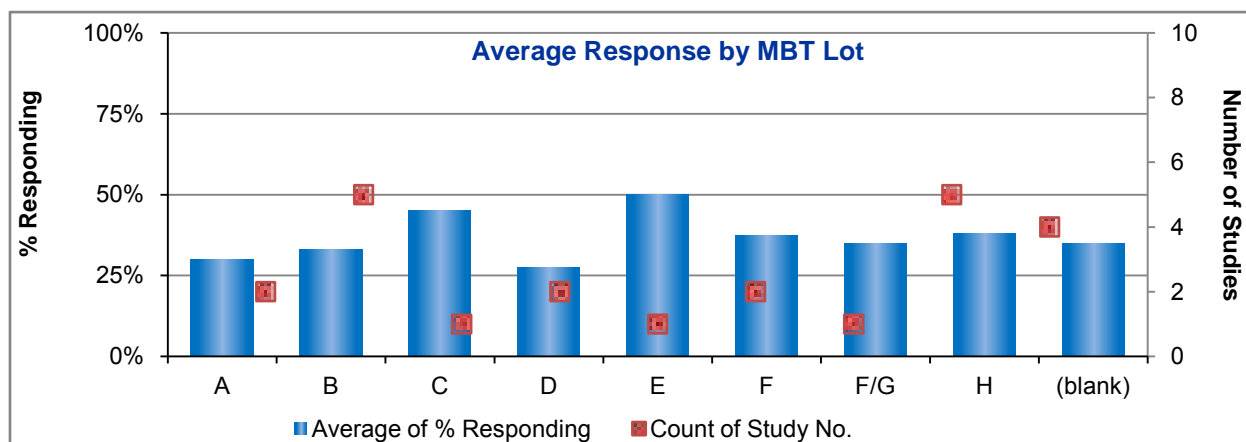
Individual Study Data

Test Date	% Responders at 24 Hours	% Responders at 48 Hours	Test Date	% Responders at 24 Hours	% Responders at 48 Hours
07/07/06	50%	15%	10/28/11	20%	20%
03/01/07	35%	30%	04/27/12	55%	50%
09/28/07	10%	10%	11/30/12	55%	45%
03/20/08	15%	20%	05/09/13	40%	50%
11/07/08	40%	20%	10/31/13	30%	30%
04/03/09	25%	30%	04/10/14	65%	50%
09/17/09	35%	10%	11/14/14	45%	35%
03/18/10	10%	30%	04/23/15	40%	40%
09/16/10	5%	32%	10/14/15	45%	45%
04/14/11	20%	30%	Average	34%	31%

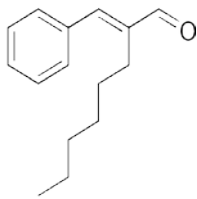


GUINEA PIG MAXIMIZATION TEST (GPMT)

Agent	MBT	 <p>Mercaptobenzothiazole (MBT)</p>
CAS	149-30-4	
Purity	97%-98%	
Studies	25	
Induction A/B	5% / 50%	
Challenge	25%	
Animals/Group	20	
Positive Response	≥30%	

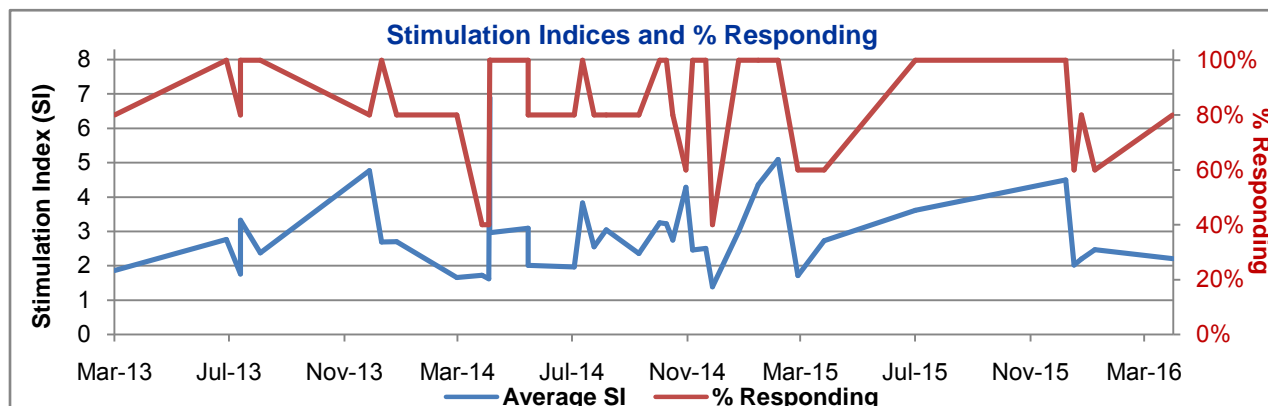
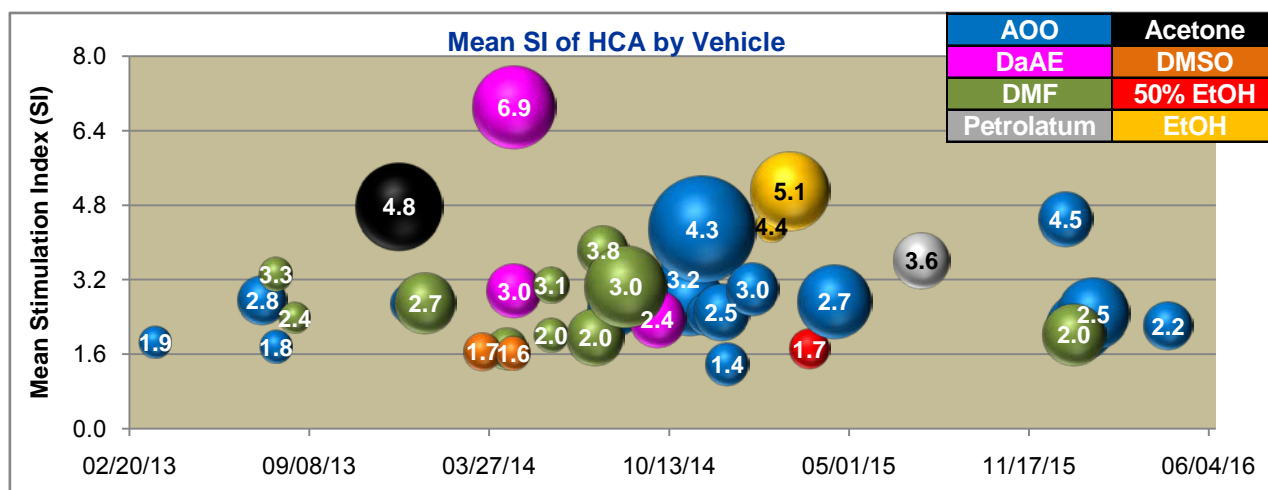


LOCAL LYMPH NODE ASSAY – BrdU-ELISA (LLNA-ELISA)

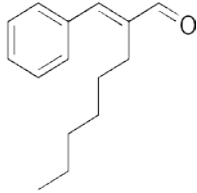
Agent	HCA	
CAS	101-86-0	
Purity	85% Tech Grade	
Studies	40	
Exposure	25%	
Animals/Group	5	
Positive Response	SI \geq 1.6	

Hexylcinnamaldehyde (HCA)

Vehicle	0	1	2	3	4	5	No. of Studies
50% EtOH				1		1	2
Acetone					1		1
AOO			1	3	7	7	18
DaAE					1	2	3
DMF			1	1	4	4	10
DMSO			1		1	1	3
EtOH						2	2
Petrolatum						1	1
No. of Studies	0	0	3	5	14	18	40



LOCAL LYMPH NODE ASSAY – FLOW CYTOMETRY (LLNA-FC)

Agent	HCA	 <p>Hexylcinnamaldehyde (HCA)</p>
CAS	101-86-0	
Purity	85% Tech Grade	
Studies	145	
Exposure	25%	
Animals/Group	5	
Positive Response	SI \geq 3.0	

Vehicle	0	1	2	3	4	5	No. of Studies
50% EtOH						4	4
Acetone				2	11	19	32
AOO						3	3
DaAE					2	2	4
DMF			1	5	4	21	31
DMSO			1	5	13	39	58
EtOH					2	2	4
Petrolatum			1		2	6	9
No. of Studies	0	0	3	12	43	96	145

