



Is it Mutagenic?

The Curious Case of Chemical X

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Colonic mucosa highlighted by Picro-Mallory trichrome special stain, from an exploratory study for Pulmonary Arterial Hypertension (PAH)

Credit: Vini Carreira, Pathology, Preclinical Sciences & Translational Safety

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Background on Chemical X

- Chemical X can be used in drug synthesis
 - Thus, could be a drug substance or drug product impurity
- Chemical X is also an API* for a rare disease
 - Doses can be as high as 1-2 g per day in patients 12 years of age or older
- Drug labels in US and EMA describe Chemical X as non-mutagenic
 - One label says “not mutagenic in Ames test”
 - A more detailed summary from EMA shows a sponsor tested TA98, TA100, TA1535, TA1537 and WP2 uvrA with and without rat induced S9 in plate incorporation method up to 5 mg/plate and results were negative (no toxicity or precipitate)
- CCRIS* has an entry for the same study conditions in WP2 uvrA pKM101 without S9 called both negative and positive
- EChA shows the same strain from the same study source as negative

*Chemical Carcinogenesis Research Information System

Data from WP2 uvrA pKM101 from referenced CCRIS study

Test 1 WP2 uvrA pKM101						
Dose	w/o S9	mean	fold change	w/S9	mean	fold change
0		64			102	
1.22	61;69	65	1.02	86;102	94	0.92
4.88	78;70	74	1.16	121;102	112	1.10
19.5	70;62	66	1.03	101;79	90	0.88
78.1	55;72	64	1.00	104;93	99	0.97
313	68;60	64	1.00	84;75	80	0.78
1250	61;69	65	1.02	70;72	71	0.70
5000	104;128	116	1.81	96;82	89	0.87

Test 2 WP2 uvrA pKM101						
Dose	w/o S9	mean	fold change	w/S9	mean	fold change
0		69			97	
156	75;64	70	1.01	96;98	97	1.00
313	75;54	65	0.94	84;112	98	1.01
625	62;59	61	0.88	84;82	83	0.86
1250	57;69	63	0.91	74;91	83	0.86
2500	75;54	65	0.94	78;84	81	0.84
5000	146;153	150	2.17	54;66	60	0.62

Test 3 WP2 uvrA pKM101						
Dose	w/o S9	mean	fold change	w/S9	mean	fold change
0		57			100	
156	49;60	55	0.96	84;61	73	0.75
313	72;59	66	1.16	86;71	79	0.81
625	49;64	57	1.00	75;72	74	0.76
1250	61;57	59	1.04	76;68	72	0.74
2500	56;56	56	0.98	57;67	62	0.64
5000	126;126	126	2.21	70;71	71	0.73

Dose in µg/plate

What did we do? New Ames test Testing (differences)

- Purity
 - Janssen study: Purity 99.2% w/w
 - CCRIS: Purity: 98% w/w
- Study design
 - Janssen: 10 doses in triplicate up to 5 mg/plate (GLP, single assay)
 - CCRIS: 6 or 7 doses in duplicate up to 5 mg/plate (Non-GLP assumed based on replicates, three assays)
- Vehicle selection
 - Janssen: DMSO, based on Cameo Chemicals software for predicting reactivity [May generate hydrogen sulfide gas (H₂S) with **water** as a vehicle]
 - CCRIS: Water was used as vehicle

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Janssen-sponsored test

Janssen	WP2 uvra PKM101					
Dose	w/o S9	mean	fold change	w/S9	mean	fold change
0	198;190;169	186		211;229;165	202	
10	185;167;135	162	0.87	199;203;200	201	1.00
25	176;179;155	170	0.91	190;183;175	183	0.91
50	174;164;160	166	0.89	205;186;160	184	0.91
75	183;186;179	183	0.98	185;197;176	186	0.92
150	162;175;132	156	0.84	167;168;173	169	0.84
300	152;185;140	159	0.85	178;190;153;	174	0.86
600	176;167;184	176	0.95	162;159;170	164	0.81
1200	178;152;138	156	0.84	179;150;176	168	0.83
2500	176;152;163	164	0.88	164;185;154	168	0.83
5000	179;196;180	185	0.99	169;189;162	173	0.86

Questions for Panel

- Thoughts on these data?
- Would you describe this chemical as Ames positive with these data?
- How would you follow-up?
 - In vitro
 - In vivo

BACKUP

Chemical X Testing

- Chemical Sources
 - Janssen study: EMD Millipore Corporation, 400 Summit Drive, Burlington MA 01803
 - CCRIS: Tokyo Kasei Kogyo Co. Ltd.
- Standard Plate Incorporation GLP Ames Study (Janssen test)
 - Strains: Salmonella typhimurium TA98, TA100, TA1535 and TA1537; E. coli strain WP2 uvrA/pKM101 with and without rat induced S9, 10 doses in triplicate up to 5 mg/plate. No cytotox or precipitation observed
 - Results: Negative for all strains in the absence and presence of rat S9 mix
- Standard Plate Incorporation Ames Study (CCRIS, tested 3x)
 - Strains: Salmonella typhimurium TA98, TA100, TA1535 and TA1537; E. coli strain WP2 uvrA/pKM101 with and without rat induced S9, 6 or 7 doses in duplicate up to 5 mg/plate. No cytotox or precipitation observed.
 - Results: Negative/Positive for WP2 uvrA/pKM101 in the absence of rat S9 mix (negative/positive/positive). All other strains were negative.

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Follow-on testing: WP2 uvrA pKM101 –S9 only

Dose (mcg/plate)	Janssen	CCRIS, 1 st test	CCRIS, 2 nd test	CCRIS, 3 rd test
Vehicle ^A (100 uL/plate)	186	61	69	57
1.22		65		
4.88		74		
10	162			
19.5		66		
25	170			
50	166			
75	183			
78.1		64		
150	156			
156			70	55
300	159			
313		64	65	66
600	176			
625			61	57
1200	156			
1250		65	63	59
2500	164		65	56
5000	185	116 ^B	150 ^C	126 ^C

A= Vehicle, Janssen DMSO. CCRIS distilled water. Volume of vehicle for CCIS is unknown.

B= Negative (1.9 fold increase)

C= Positive (2.2 fold increase, 2nd and 3rd test)