

# **Analysis of DNA Adducts and Mutagenic Potency and Specificity in Rats Exposed to Acrylonitrile**

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**\*\***These authors contributed equally to this work, each having designed and directed interfacing projects: Animal exposures and experiments for DNA adduct studies were conducted at the Chemical Industry Institute of Technology and animal exposures and experiments for *Hprt* gene mutation studies were performed at the New York State Department of Health.

**Table S1. Manifestation of *Hprt* Mutant T-cells in Spleen of Female F344 Rats Following 4 Weeks of Drinking Water Exposure to 0 or 500 ppm Acrylonitrile<sup>a</sup>**

Weeks post exposure	Mutant Frequency $\times 10^{-6}$ (Animal number)		<i>P</i> -value
	Controls <sup>b</sup>	Acrylonitrile-exposed	
- 4	2/6		
	2.8		
	2.4		
	Mean $\pm$ SE = 2.6 $\pm$ 0.1		
0	3.32 (ANC10)	7.74 (AN5/13)	
	2.93 (ANC11)	7.28 (AN5/14)	
	2.93 (ANC12)	7.75 (AN5/15)	
	Mean $\pm$ SE = 3.0 $\pm$ 0.2	11.28 (AN5/16)	
		8.53 (AN5/17)	
		Mean $\pm$ SE = 8.5 $\pm$ 0.7	< 0.001*
2	3.15 (ANC13)	9.94 (AN5/18)	
	2.91 (ANC14)	9.22 (AN5/19)	
	2.63 (ANC15)	10.12 (AN5/20)	
	Mean $\pm$ SE = 2.9 $\pm$ 0.2	8.57 (AN5/21)	
		11.25 (AN5/22)	
		Mean $\pm$ SE = 9.8 $\pm$ 0.5	< 0.001*
4	3.24 (ANC18)	8.47 (AN5/23)	
	2.64 (ANC19)	9.06 (AN5/24)	
	1.95 (ANC20)	13.61 (AN5/25)	
	Mean $\pm$ SE = 2.6 $\pm$ 0.4	14.44 (AN5/26)	
		10.08 (AN5/27)	
		Mean $\pm$ SE = 11.1 $\pm$ 1.2	< 0.001*
6	2.23 (ANC21)	2.43 (AN5/28)	
	2.00 (ANC22)	1.86 (AN5/29)	
	1.52 (ANC23)	2.12 (AN5/31)	
	Mean $\pm$ SE = 1.9 $\pm$ 0.2	2.74 (AN5/32)	
		Mean $\pm$ SE = 2.3 $\pm$ 0.2	0.13
8	2.47 (ANC24)	8.20 (AN5/33)	
	2.53 (ANC25)	3.33 (AN5/34)	
	3.17 (ANC26)	5.01 (AN5/36)	
	Mean $\pm$ SE = 2.7 $\pm$ 0.2	2.86 (AN5/37)	
		Mean $\pm$ SE = 4.9 $\pm$ 1.2	0.09

<sup>a</sup> Groups of rats were exposed to 0 or 500 ppm acrylonitrile in drinking water for 4 weeks, and then necropsied at 0, 2, 4, 6, or 8 weeks after the cessation of exposure for isolation of splenic lymphocytes and measurement of *Hprt* mutant frequencies using a T-cell cloning assay.

<sup>b</sup> Mean for all controls ( $n = 18$ ) = 2.6  $\pm$  0.1 (SE)  $\times 10^{-6}$ .

\* Significantly different at  $p < 0.05$  for pairwise tests, Mann-Whitney U-statistic or Student's t-test.

**Table S2. Dose-Response for *Hprt* Mutant T-cells in Spleen of Female F344 rats Following Drinking Water Exposure to Acrylonitrile<sup>a</sup>**

Exposure level (ppm)	Average daily dose (mg/kg bw/day) <sup>b</sup>	Observed mutant frequency $\times 10^{-6}$ (Animal number)	Average induced mutant frequency ( $\times 10^{-6}$ ) <sup>c</sup>	<i>P</i> -value
0	0	3.24 (ANC18) 2.64 (ANC19) 1.95 (ANC20) Mean $\pm$ SE = 2.6 $\pm$ 0.4	---	
33	8	2.52 (AN33/1) 4.06 (AN33/2) 3.56 (AN33/3) 3.36 (AN33/4) 3.55 (AN33/5) Mean $\pm$ SE = 3.4 $\pm$ 0.3	0.8 $\pm$ 0.3	0.057
100	21	6.93 (AN1/5) 4.64 (AN1/6) 5.90 (AN1/7) 4.92 (AN1/8) 3.10 (AN1/9) Mean $\pm$ SE = 5.1 $\pm$ 0.6	2.5 $\pm$ 0.6	0.036*
500	76	8.47 (AN5/23) 9.06 (AN5/24) 13.61 (AN5/25) 14.44 (AN5/26) 10.08 (AN5/27) Mean $\pm$ SE = 11.1 $\pm$ 1.2 <sup>d</sup>	8.5 $\pm$ 1.2	0.018*

<sup>a</sup> Groups of rats (4-week-old) were exposed to 0, 33, 100 or 500 ppm acrylonitrile in drinking water for 4 weeks, and then necropsied 4 weeks after the cessation of exposure for isolation of splenic lymphocytes and measurement of *Hprt* mutant frequencies using a cloning assay.

<sup>b</sup> Water consumption and body weight data were used to estimate the maximal average daily dose of acrylonitrile as described in the Materials and Methods (see Figure 1).

<sup>c</sup> The average induced mutant frequency equals the average experimentally observed mutant frequency minus the average background mutant frequency in control rats.

<sup>e</sup> Significantly elevated at 500 ppm acrylonitrile compared to mutant frequency values at 33 or 100 ppm acrylonitrile (*p*-value < 0.05), Dunn's method.

\* Significantly different at *P* < 0.05 for pairwise tests, Mann-Whitney U-statistic or Student's *t*-test.

**Table S3. Base Alterations in *Hprt* Exon 3 of Splenic T-Cells from F344 Rats Exposed to Acrylonitrile<sup>a</sup>**

Base <sup>b</sup> and mutation <sup>c</sup>	Animal Number								Total
	AN5/9	AN5/10	AN5/18	AN5/19	AN5/20	AN5/21	AN5/22	AN5/23	
135 G→T							1		1
137 C→T	2								2
140 A→T								1	1
143 G→A							1		1
148 G→C <sup>e</sup>		2							2
154 G→A <sup>d,e</sup>			1		1			2	4
157 G→T	1								1
164 A→T <sup>d,e,f</sup>		1							1
168 A→G	1								1
173 G→A <sup>g</sup>	1								1
179 A→G						1			1
182 A→T						1			1
204 G→C						1			1
205 A→G						1			1
205 A→T <sup>e</sup>					1				1
206 A→G <sup>f</sup>			1						1
206 A→C				1					1
206 A→T <sup>g</sup>						1			1
207 G→C <sup>f</sup>	1								1
208 G→A <sup>d,e,g</sup>							1		1
209 G→A				2					2
215 A→C <sup>d</sup>				1			1		2
218 A→T			1						1
221 T→C <sup>d,f,g</sup>						1			1
221 T→G	1								1
223 T→C <sup>f,g</sup>	1					1		2	4
233 T→C				1					1
252 G→A						1			1
140 +T <sup>d</sup>								1	1
145-146 CTT→TGT		1							1
181-183 CAC→CTG						1			1
196 +A					1				1
207-212 +G <sup>d,f</sup>		1				1			2
207-212 -G <sup>e,f,g</sup>								1	1
219 -G <sup>g</sup>						1			1
Total	8	5	3	5	3	11	4	7	46

<sup>a</sup> Splenic T-cells were collected for the *Hprt* cloning and selection assay at 2 or 4 weeks after cessation of drinking water exposure of female F344 rats for 4 weeks to 500 ppm acrylonitrile.

<sup>b</sup> Numbering of the rat *Hprt* gene according to<sup>51</sup>.

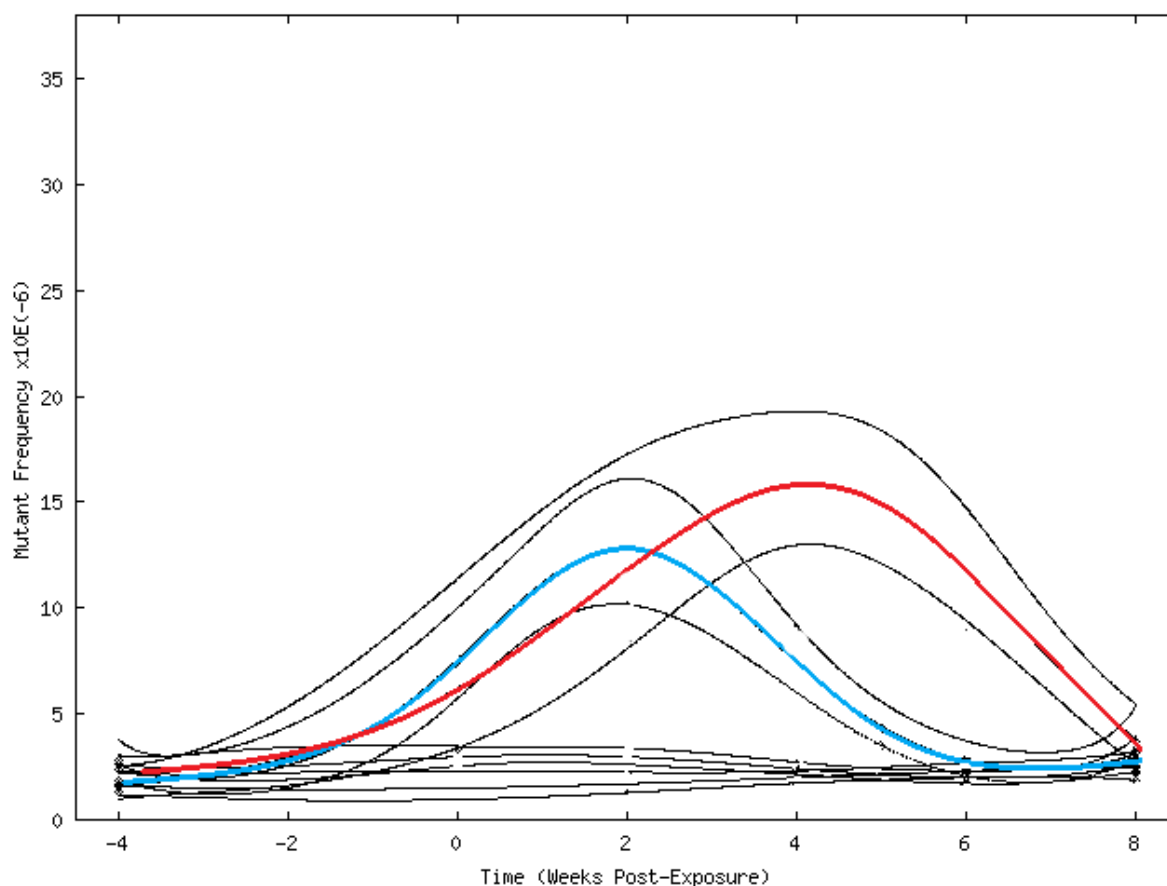
<sup>c</sup> The mutation in the non-transcribed strand is reported.

<sup>d</sup> The same mutation was found in F344 rats exposed to 1,3-butadiene (Table 8 in<sup>43</sup>).

<sup>e</sup> The same mutation was found in F344 rats exposed to butadiene diepoxide (Table 10 in<sup>43</sup>).

<sup>f</sup> The same mutation was found in B6C3F1 mice exposed to 1,3-butadiene (Table 7 in<sup>43</sup>).

<sup>g</sup> The same mutation was found in mice exposed to butadiene diepoxide (Table 9 in<sup>43</sup>).



**Figure S1.** Relationships between the time elapsed since exposure and *Hprt* mutant frequencies in splenic T-cells of female F344 rats (4 weeks old) exposed for 4 weeks to 500 ppm acrylonitrile (blue curve) versus female B6C3F1 mice (4-5 weeks old) exposed for 4 weeks (6 h/day, 5 days/week) to 0 or 625 ppm 1,3-butadiene (red curve). Animals were necropsied before initiation of exposure (-4 week timepoint), after 2 weeks of exposure (-2 week timepoint), and up to 8 weeks after the last day of exposure. Curves were fit to the *Hprt* mutant frequencies of individual rats exposed to acrylonitrile or mice exposed to 1,3-butadiene, 95% confidence limits were determined (black curves), and the mutagenic potency estimate for each exposure scenario was obtained as described in the Materials and Methods.