ADME NTP Study S0632 alpha-Methylstyrene

The contract laboratory abbreviation for the test article is AMS.
Species: adult male F344 rats.
Vehicles: intravenous, Emulphor EL-620 and phosphate buffered saline (1:20); oral, corn oil; inhalation, conditioned room air.

CASRN 98-83-9
Radiolabeled with carbon-14 in the phenyl ring; alpha-Methylstyrene, [ring-UL-^{14}C]-

 alpha-Methylstyrene Studies Performed:

1. 10 mg/kg single dose intravenous study in male rats with sacrifice at 72 hours postdose.

2. 1000 mg/kg oral gavage study in a single male rat with sacrifice at 48 hours postdose.

3. 300 ppm nose-only inhalation (6 hour exposure) study in male rats with sacrifice at 6, 24, and 72 hours following exposure initiation). Toxicokinetic data was generated with the 24 hour study.

4. 900 ppm nose-only inhalation (6 hour exposure) study in male rats with sacrifice at 6, 24, and 72 hours following exposure initiation. Toxicokinetic data was generated with the 24 hour study.

Tissues collected at 6 hours post exposure initiation were taken immediately after the 6 hour exposure period.

Toxicokinetics:

Extracted whole blood was analyzed for ^{14}C with internal standards by HPLC. Blood AMS concentration vs. time data obtained during and post-exposure in the 300 ppm and 900 ppm inhalation studies were analyzed by noncompartmental and compartmental techniques using WinNonlin software (Version 1.0; Scientific Consulting, Inc., Apex, NC). All pharmacokinetic analyses were conducted on weighted data (1/YHAT, where YHAT is the predicted AMS concentration).

Noncompartmental analysis:
- Model 1: Data from individual animals were analyzed with WinNonlin Model 202 for infusion where the inhaled dose was used as the infused dose.
Compartmental analysis:
- Model 2: Initial fit to a two-compartmental model with zero-order absorption and first order elimination (WinNonlin Model 9). No results available.
Model 3 and Model 4 two-compartmental models were written to simultaneously solve pooled data from all animals within an exposure concentration from each of the two

inhalation experiments. Each of the models permitted zero-order absorption. The dose rate (mg/kg/h, calculated as dose received in mg/kg divided by the duration of exposure in hours) was required as model input.

- Model 3: This model contained a description of first-order elimination.
- Model 4: This model contained a description of nonlinear (Michaelis-Menten) elimination.

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Table 1

Cumulative Excretion of Radioactivity 72 h after Intravenous Administration of [^{14}C]AMS (10 mg/kg) to Male F-344 Rats[a]

End of Collection Period (h)	Percent of Dose Recovered in:				
	Urine	Feces	Breath	CO_2	Total
6 h	34.1 ± 3.9	0.0468± 0.0696	2.01 ± 0.76	0.00666 ± 0.00268	36.1 ± 4.4
12 h	53.0 ± 7.3	—	2.08 ± 0.77	0.0101 ± 0.0037	55.1 ± 8.0
24 h	76.4 ± 2.1	1.20 ± 0.33	2.14 ± 0.78	0.0132 ± 0.0025	79.8 ± 2.7
48 h	84.9 ± 1.6	1.78 ± 0.72	2.15 ± 0.78	0.0200 ± 0.0093	88.9 ± 0.7
72 h	86.0 ± 1.4	1.88 ± 0.73	2.16 ± 0.78	0.0236 ± 0.0078	90.0 ± 0.4
Cagewash	86.2 ± 1.4	1.88 ± 0.73	2.16 ± 0.78	0.0236 ± 0.0078	90.3 ± 0.4

[a] N=4.

Table 2

Tissue Distribution of Radioactivity 72 h after Intravenous Administration of [^{14}C]AMS (10 mg/kg) to Male F-344 Rats[a]

Tissue	ng-eq AMS per g Tissue			Tissue/Blood Ratio			% Dose in Total Tissue	
Adipose	25.3	±	2.7	1.75	±	0.74	0.0170	± 0.0020
Bladder	95.3	±	96.6	7.47	±	9.69	0.000630±	0.000597
Blood	16.4	±	6.7	unity			0.00811	± 0.00315
Brain	4.53	±	0.94	0.319	±	0.160	0.000298±	0.000050
Heart	73.2	±	21.9	4.74	±	1.51	0.00203	± 0.00060
Kidney	160	±	52	11.6	±	6.1	0.0113	± 0.0039
Liver	72.8	±	14.2	4.85	±	1.78	0.0304	± 0.0039
Lung	87.7	±	36.7	6.11	±	3.24	0.00608	± 0.00272
Muscle	4.50	±	1.05	0.310	±	0.150	0.0205	± 0.0041
Skin	55.4	±	26.4	4.09	±	3.15	0.0892	± 0.0406
Spleen	233	±	81	16.6	±	8.1	0.00616	± 0.00243
Testis	4.49	±	0.56	0.310	±	0.134	0.000454±	0.00006
Stomach[b]							0.00234	± 0.00104
Small intestine[b]							0.0479	± 0.0145
Cecum[b]							0.0219	± 0.0092
Large intestine[b]							0.00843	± 0.00318
						Total in Tissue	0.276	± 0.074

[a] N=4.

[b] Includes contents.

Table 3

Cumulative Excretion of Radioactivity 48 h after Oral Administration of [^{14}C]AMS (1000 mg/kg) to a Male F-344 Rat

End of Collection Period (h)	Percent of Dose Recovered in:		
	Urine	Feces	Total
6 h	3.28	0.00	3.28
24 h	57.8	1.51	59.3
48 h	74.6	2.96	77.6
Cagewash	78.1		81.1

Table 4

Radioactivity Excreted Over Time from Male Rats Exposed to 300 ppm [^{14}C]AMS for 6 h

(All Values Expressed as % of the Recovered Dose)[a]

End of Collection[b] (h)	Volatile Breath	Urine	Cage Rinse	Feces	Carcass & Tissues	Total Recovered Dose
6	c	1.3 ± 1.3	c	0.3 ± 0.3	c	1.6 + 1.6
12	3.0 ± 0.8	30.1 ± 6.4 (31.3)	c	c	c	33.0 ± 5.6 (34.6)
24	0.2 ± 0.1	42.1 ± 5.0 (73.5)	c	1.0 ± 0.2 (1.4)	c	43.3 ± 4.9 (77.9)
48	c	12.4 ± 3.8 (85.9)	c	0.7 ± 0.0[d](2.1)	c	13.2 ± 3.4 (91.4)
72	c	2.3 ± 0.7 (88.2)	0.6 ± 0.2	0.1 ± 0.0[d](2.2)	5.9 ± 3.8	3.1 ± 0.7 (100[e])
Overall Mean Recovery	3.1 ± 0.9	88.2 ± 3.9	0.6 ± 0.2	2.2 ± 0.3	5.9 ± 3.8	100[e]

[a] All values expressed as Mean ± S.D. (N=4). Values in parentheses are cumulative percent of radiolabeled dose excreted.
[b] These times represent the interval between initiation of exposure and collection.
[c] No samples collected.
[d] Value less than 0.05.
[e] Administered dose is calculated as the absorbed dose (total radioactivity in the residual carcass, tissues, and all excreta).

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Table 5

Radioactivity Excreted Over Time from Male Rats Exposed to 900 ppm [^{14}C]AMS for 6 h

(All Values Expressed as % of the Recovered Dose)[a]

End of Collection[b] (h)	Volatile Breath	Urine	Cage Rinse	Feces	Carcass & Tissues	Total Recovered Dose
6	c	3.6 ± 3.6	c	0.0^d ± 0.0^d	c	3.7 + 3.6
12	2.1 ± 0.4	27.0 ± 4.5 (27.0)	c	c	c	29.1 ± 4.3 (32.8)
24	1.1 ± 0.2 (3.2)	43.5 ± 5.1 (70.5)	c	1.1 ± 0.2 (1.4)	c	44.8 ± 5.0 (77.5)
48	0.2 ± 0.0^d (3.4)	15.8 ± 2.9 (86.3)	c	1.3 ± 0.2^d (2.1)	c	17.2 ± 3.1 (94.7)
72	0.0d+ 0.0d (2.5)	2.5 + 0.6 (92.4)	0.9± 0.6	0.2 ± 0.1 (2.6)	1.6 ± 0.6	3.7 ± 0.8 (100^e)
Overall Mean Recovery	2.5 ± 0.4	92.4 ± 1.0	0.9± 0.6	2.6 ± 0.2	1.6 ± 0.6	100^e

[a] All values expressed as Mean ± S.D. (N=4). Values in parentheses are cumulative percent of radiolabeled dose excreted.
[b] These times represent the interval between initiation of exposure and collection.
[c] No samples collected.
[d] Value less than 0.05.
[e] Administered dose is calculated as the absorbed dose (total radioactivity in the residual carcass, tissues, and all excreta).

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Table 6

Tissue Distribution of Radioactivity in Male F-344 Rats Immediately following a 6-h Nose-Only Inhalation Exposure to 300 ppm [^{14}C]AMS[a]

Tissue	μg-eq AMS per g Tissue			Tissue/Blood Ratio			% Dose in Total Tissue		
Adipose	412.3	±	58.74	7.89	±	1.07	27.85421	±	8.67878
Bladder	211.5	±	70.74	4.04	±	1.32	0.07259	±	0.03346
Blood	52.09	±	1.555		unity		2.33139	±	0.16343
Brain	36.51	±	1.276	1.039	±	0.351	0.24563	±	0.04178
Heart	43.58	±	0.412	0.834	±	0.012	0.12430	±	0.02716
Kidney	114.9	±	6.141	2.20	±	0.082	0.82861	±	0.11538
Liver	142.8	±	20.62	2.73	±	0.345	5.13890	±	1.42606
Lung	51.03	±	7.700	0.975	±	0.132	0.25580	±	0.10187
Muscle	40.84	±	3.677	0.781	±	0.058	18.71510	±	4.38545
Skin	85.40	±	7.349	1.64	±	0.138	13.92857	±	3.60203
Spleen	41.65	±	5.144	0.797	±	0.089	0.07538	±	0.00447
Testis	39.20	±	1.239	0.751	±	0.034	0.40964	±	0.07401
Stomach[b]	9.193	±	4.624	0.175	±	0.085	1.99003	±	0.74909
Small intestine[b]	122.8	±	4.970	2.35	±	0.141	29.48797	±	3.79862
Cecum[b]	27.71	±	0.591	0.530	±	0.107	6.39434	±	2.08418
Large intestine[b]	2.889	±	1.205	0.055	±	0.022	0.63875	±	0.23418

[a] N=3.

[b] Includes contents.

Table 7

Tissue Distribution of Radioactivity in Male F-344 Rats 24 h Post Initiation of a 6-h Nose-Only Inhalation Exposure to 300 ppm [^{14}C]AMS[a]

Tissue	μg-eq AMS per g Tissue		Tissue/Blood Ratio		% Dose in Total Tissue
Adipose	59.98 ±	82.35	18.8 ±	1.48	2.90159 ± 0.41629
Bladder	52.44 ±	28.41	17.2 ±	10.8	0.01599 ± 0.01019
Blood	3.079 ±	0.635	unity		0.10988 ± 0.02476
Brain	1.841 ±	0.351	0.577 ±	0.086	0.00938 ± 0.00167
Heart	2.832 ±	0.314	0.894 ±	0.100	0.00600 ± 0.00063
Kidney	12.79 ±	2.006	4.00 ±	0.240	0.06785 ± 0.01439
Liver	11.98 ±	3.868	3.74 ±	0.928	0.31899 ± 0.09041
Lung	3.288 ±	0.492	1.03 ±	0.082	0.01267 ± 0.00486
Muscle	2.228 ±	0.665	0.686 ±	0.106	0.74002 ± 0.22493
Skin	6.430 ±	7.043	1.85 ±	1.72	0.75241 ± 0.81271
Spleen	3.010 ±	0.839	0.929 ±	0.152	0 00437 ± 0.00114
Testis	4.149 ±	1.492	1.32 ±	0.523	0.03046 ± 0.01229
Stomach[b]	0.648 ±	0.522	0.203 ±	0.172	0.10994 ± 0.08874
Small intestine[b]	14.52 ±	1.414	4.62 ±	0.848	2.46813 ± 0.42324
Cecum[b]	6.601 ±	0.573	2.09 ±	0.264	1.14698 ± 0.10379
Large intestine[b]	2.789 ±	0.976	0.918 ±	0.468	0.47356 ± 0.17072

[a] N=5. Tissues were obtained from rats used for serial blood sampling.

[b] Includes contents.

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Table 8

Tissue Distribution of Radioactivity in Male F-344 Rats 72 h Post Initiation of a 6-h Nose-Only Inhalation Exposure to 300 ppm [^{14}C]AMS[a]

Tissue	μg-eq AMS per g Tissue		Tissue/Blood Ratio		% Dose in Total Tissue
Adipose	1.203 ±	0.604	7.88 ±	3.96	0.06092 ± 0.02965
Bladder	1.064 ±	0.660	7.63 ±	5.64	0.00032 ± 0.00012
Blood	0.156 ±	0.056	unity		0.00578 ± 0.00168
Brain	0.159 ±	0.064	1.039 ±	0.351	0.00079 ± 0.00028
Heart	0.386 ±	0.472	2.03 ±	1.70	0.00080 ± 0.00091
Kidney	0.928 ±	0.228	6.18 ±	1.39	0.00504 ± 0.00089
Liver	0.876 ±	0.209	5.79 ±	0.692	0.02413 ± 0.00419
Lung	0.427 ±	0.472	2.31 ±	1.63	0.00231 ± 0.00284
Muscle	0.138 ±	0.039	0.899 ±	0.078	0.04742 ± 0.01063
Skin	1.060 ±	0.424	6.77 ±	0.897	0.12834 ± 0.04199
Spleen	0.440 ±	0.540	2.30 ±	1.95	0.00085 ± 0.00098
Testis	0.339 ±	0.347	1.871 ±	1.189	0.00252 ± 0.00241
Stomach[b]	0.025 ±	0.018	0.179 ±	0.149	0.00434 ± 0.00316
Small intestine[b]	0.441 ±	0.142	2.89 ±	0.584	0.07811 ± 0.01804
Cecum[b]	0.278 ±	0.142	1.74 ±	0.331	0.04796 ± 0.02062
Large intestine[b]	0.090 ±	0.038	0.571 ±	0.073	0.01485 ± 0.00520

[a] N=4.

[b] Includes contents.

Table 9

Tissue Distribution of Radioactivity in Male F-344 Rats Immediately following a 6-h Nose-Only Inhalation Exposure to 900 ppm [^{14}C]AMS[a]

Tissue	µg-eq AMS per g Tissue			Tissue/Blood Ratio			% Dose in Total Tissue		
Adipose	1710.0	±	73.7	12.2	±	0.999	35.0	±	1.76
Bladder	897.0	±	377.0	6.40	±	2.88	0.142	±	0.064
Blood	141.0	±	7.3		unity		2.14	±	0.238
Brain	124.0	±	1.540	0.881	±	0.0398	0.251	±	0.0369
Heart	138.0	±	2.660	0.983	±	0.0627	0.120	±	0.0159
Kidney	282.0	±	10.8	2.00	±	0.103	0.606	±	0.0652
Liver	321.0	±	11.4	2.28	±	0.144	3.53	±	0.203
Lung	159.0	±	12.7	1.13	±	0.111	0.174	±	0.0121
Muscle	129.0	±	7.140	0.916	±	0.0660	18.1	±	2.02
Skin	33.7	±	82.3	2.42	±	0.690	16 6	±	3.32
Spleen	124.0	±	8.40	0.876	±	0.0413	0.0676	±	0 00576
Testis	112.0	±	6.390	0.794	±	0.0338	0.377	±	0.0474
Stomach[b]	13.8	±	1.310	0.0980	±	0.00476	0.958	±	0.133
Small intestine[b]	219.0	±	33.5	1.56	±	0.251	15.7	±	2.41
Cecum[b]	75.60	±	21.1	0.542	±	0.172	5.12	±	1.14
Large intestine[b]	10.8	±	4.690	0.0769	±	0.0358	0.716	±	0 309

[a] N=3.

[b] Includes contents.

Table 10

Tissue Distribution of Radioactivity in Male F-344 Rats 72 h Post Initiation of a 6-h Nose-Only Inhalation Exposure to 900 ppm [^{14}C]AMS[a]

Tissue	µg-eq AMS per g Tissue		Tissue/Blood Ratio		% Dose in Total Tissue	
Adipose	1.770 ±	1.590	3.84 ±	3.37	0.0329 ±	0.0300
Bladder	1.060 ±	0.622	2.29 ±	0.999	0.000118±	0.0000690
Blood	0.447 ±	0.0599	unity		0.00623 ±	0.000235
Brain	0.336 ±	0.0727	0.753 ±	0.139	0.000665±	0.000116
Heart	0.490 ±	0.0712	1.10	±0630	0.000481±	0.0000420
Kidney	2.020 ±	0.221	4.56 ±	0.716	0.00407 ±	0.000551
Liver	2.330 ±	0.277	5.25 ±	0.531	0.0221 ±	0 00133
Lung	0.522 ±	0.0622	1.18 ±	0.141	0 000833±	0.000183
Muscle	0.326 ±	0.0798	0.723 ±	0.0951	0.0417 ±	0.00687
Skin	1.670 ±	0.614	3.83 ±	1.64	0.0778 ±	0.0329
Spleen	0.503 ±	0.151	1.15 ±	0.420	0.000373±	0.000178
Testis	0.422 ±	0.119	0.934 ±	0.170	0 00139 ±	0.000306
Stomach[b]	0.0535±	0.0261	0.118 ±	0.0507	0.00349 ±	0.00161
Small intestine[b]	0.898 ±	0.193	2.02 ±	0.431	0.0626 ±	0.0143
Cecum[b]	0.921 ±	0.203	2.11 ±	0.653	0.0630 ±	0.0193
Large intestine[b]	0.179 ±	0.159	0.389 ±	0.302	0.0114 ±	0.00906

[a] N=4.

[b] Includes contents.

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Table 11

Concentration of AMS (µg/mL) in Blood during and Post a Single 6-h 300 or 900 ppm [^{14}C]AMS Nose-Only Inhalation Exposure[a]

300 ppm Study

Timepoint (hh:mm)	Animal				
	M1	M2	M3	M4	M5
5:00	-	-	-	6.18	6.32
5:30	7.30	5.76	5.01	-	-
6:05	4.08	2.26	2.70	-	-
6:10	2.07	2.31	2.30	-	-
6:15	-	-	-	1.81	1.95
6:20	2.46	1.26	1.35	-	-
6:25	-	-	-	1.16	b
6:30	2.16	1.15	1.23	-	-
6:35	-	-	-	b	b
6:45	-	-	-	0.98	0.447
7:00	1.27	0.65	0.98	-	-
7:30	-	-	-	0.69	0.508
8:00	0.96	0.51	0.53	-	-
9:00	-	-	-	0.47	0.50
10:00	0.64	0.38	0.25	-	-
12:00	-	-	-	0.14	0.28
24:00	0.09	0.61	0.07	0.05	0.05

900 ppm Study

Timepoint (hh:mm)	Animal				
	M1	M2	M3	M4	M5
5:00	-	-	-	24.75	24.84
5:30	18.27	26.72	29.16	-	-
6:05	10.80	21.92	22.58	-	-
6:10	14.94	17.07	23.42	-	-
6:15	-	-	-	19.73	14.91
6:20	12.97	14.01	15.47	-	-
6:25	-	-	-	16.98	12.38
6:30	11.23	9.13	20.52	-	-
6:35	-	-	-	18.19	8.99
6:45	-	-	-	14.94	8.68
7:00	6.64	8.11	15.33	-	-
7:30	-	-	-	11.00	4.87
8:00	4.27	4.30	11.11	-	-
9:00	-	-	-	6.19	2.55
10:00	2.11	2.53	5.54	-	-
12:00	-	-	-	2.18	1.26
24:00	0.13	4.28	0.17	0.14	0.09

[a] All values are expressed as µg/mL of blood. Blood sampling timepoints (hh:mm) were as follows for two groups of rats:

M1, M2, M3: 5:30, 6:05, 6:10, 6:20, 6:30, 7:00, 8:00, 10:00, 24:00.

M4, M5: 5:00, 6:15, 6:25, 6 35, 6 45, 7:30, 9:00, 12:00, 24:00.

[b] Sample not analyzed.

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Table 12

Derived Pharmacokinetic Parameters from Noncompartmental Analyses[a]

Parameter	Formula
AUC_{last} (h x mg/L)	$= \sum (t_i - t_{i-1})(C_i + C_{i-1}) / 2$; i=1 to n (last time point)
β (h^{-1})	Estimated via linear regression of time vs. log concentration
$t_{1/2}$ (h)	$= - \ln(2) / \beta$
AUC_{INF} (h x mg/L)	$= AUC_{last} + C_n / \beta$; C_n = last measurable concentration
V_z (L/kg)	$= Dose / (\beta \times C_{INF})$
Cl (L/h/kg)	$= Dose / AUC_{INF}$

[a] AUC_{last} is area under the blood concentration-time curve from time zero to the last measurable concentration; β is the terminal elimination rate constant; $t_{1/2}$ is the terminal elimination rate constant; AUC_{INF} is the area under the blood concentration-time curve extrapolated to time infinity; V_z is the volume of distribution based on the terminal phase; Cl is clearance.

Table 13

Noncompartmental Analysis of Blood AMS Concentration versus Time Data from Individual Rats in 300 and 900 ppm Inhalation Exposure Studies[a]

	300 ppm Exposure			900 ppm Exposure		
Parameter[b]	Mean	±	SD	Mean	±	SD
Dose (mg/kg)	137.6	±	7.0	345.3	±	12.9
AUC_{last} (h x mg/L)	25.9	±	5.3	130.8	±	34.3
β (h^{-1})	0.144	±	0.028	0.256	±	0.060
$t_{1/2}$ (h)	4.99	±	1.14	2.81	±	0.54
AUC_{INF}(h x mg/L)	26.8	±	4.9	132.6	±	33.5
V_z (L/kg)	38.6	±	15.1	11.2	±	4.1
Cl (L/h/kg)	5.3	±	0.9	2.7	±	0.7

[a] Data are mean ± standard deviation from 5 rats in each inhalation exposure study.

[b] Parameters as defined in Table 2.

Table 14

Summary of Compartmental Analyses of Pooled Blood AMS Concentration (mg/L) versus Time Data from Rats in the 300 and 900 ppm Inhalation Exposures[a]

First-Order Elimination[b]			*Michaelis-Menten Elimination*[c]		
Parameter	Estimate	± SE	Parameter	Estimate	± SE
Cl (L/h/kg)	2.25	± 0.20	K_{12} (h^{-1})	1.96	± 0.43
K_{12} (h^{-1})	0.723	± 0.321	K_{21} (h^{-1})	0.250	± 0.065
K_{21} (h^{-1})	0.321	± 0.123	V (L/kg)	1.31	± 0.27
V (L/kg)	1.68	± 0.51	K_M (mg/L)	3.58	± 1.42
			V_{MAX} (mg/h/kg)	31.9	± 7.0

[a] Values are parameter estimate ± associated standard error as determined in the optimization routine.

[b] A two-compartment model was written to simultaneously solve data from the 300 and 900 ppm inhalation exposures with zero-order absorption and first-order elimination. See Figure 1 for model structure.

[c] A two-compartment model was written to simultaneously solve data from the 300 and 900 ppm inhalation exposures with zero-order absorption and saturable (Michaelis-Menten) elimination. See Figure 1 for model structure.