

2.B. Available Cancer Studies in Humans and Animals

The following text provides an overview of the available studies in humans and animals which characterize the cancer potential for arsenical compounds.

2.B.1. Inorganic Arsenic

Long term oral exposure to iAs has been reported to be associated with several cancers (*e.g.*, skin, lungs, bladder). iAs cancer in the human population may be influenced by a variety of factors including diet (which may influence the extent of methylation of iAs) and by intra-individual and inter-individual variability in arsenic methylation.

The 1999 report by the National Research Council (NRC) of the National Academy of Sciences (NAS) suggests that the bladder and lung cancer human mortality data, particularly from the southwestern Taiwanese studies (Chen *et al.*, 1985, 1988, 1992; Wu *et al.*, 1989) provide the best dose-response data for evaluating the long-term effects of oral exposure to iAs. Issues regarding the revised calculations for the iAs slope factor are described in EPA's draft toxicological review for iAs (which has been provided to the SAB).

Historically, standard chronic bioassays with exposure to iAs in rodents have been negative for increased tumor formation (NRC, 2001). However, it has been suggested that iAs has not been studied adequately in the standard rodent cancer bioassay. Huff *et al.* (2000) points out "as we have stated previously (Huff *et al.*, 1998a,b), arsenic trioxide and other inorganic (and until now organic) arsenicals have in reality never been tested adequately for carcinogenesis, and never by the inhalation route." There are recent studies at relatively high experimental doses, in transgenic animals, and/or following transplacental exposures which have demonstrated cancer potential in rodents following iAs exposure (Simeonova *et al.*, 2000 & 2001; Santra *et al.*, 2000; Waalkes *et al.*, 2000 & 2004). These studies provide qualitative evidence of the cancer potential of iAs.

2.B.2. Monomethylarsonic Acid (MMA^V)

There are no epidemiological studies following chronic exposure to MMA^V. Chronic bioassay studies in rats and mice submitted to EPA for pesticide registration indicate that the large intestine is the primary site of toxicity (Crown *et al.*, 1990; Gur *et al.*, 1991). These studies did not show an increased tumor incidence at any tissue site in either species. Mice were treated at 0, 10, 50, 200 or 400 ppm (approximately 2, 10, 40, 90 mg/kg bw/day). There was no treatment related mortality in mice. Rats were treated with 0, 50, 400 or 1300 ppm (approximately 0, 3, 30, 95 mg/kg bw/day). In the rat study, the highest dose was reduced to 1000

Figure 2.2: Challenger's Scheme for the Methylation of As

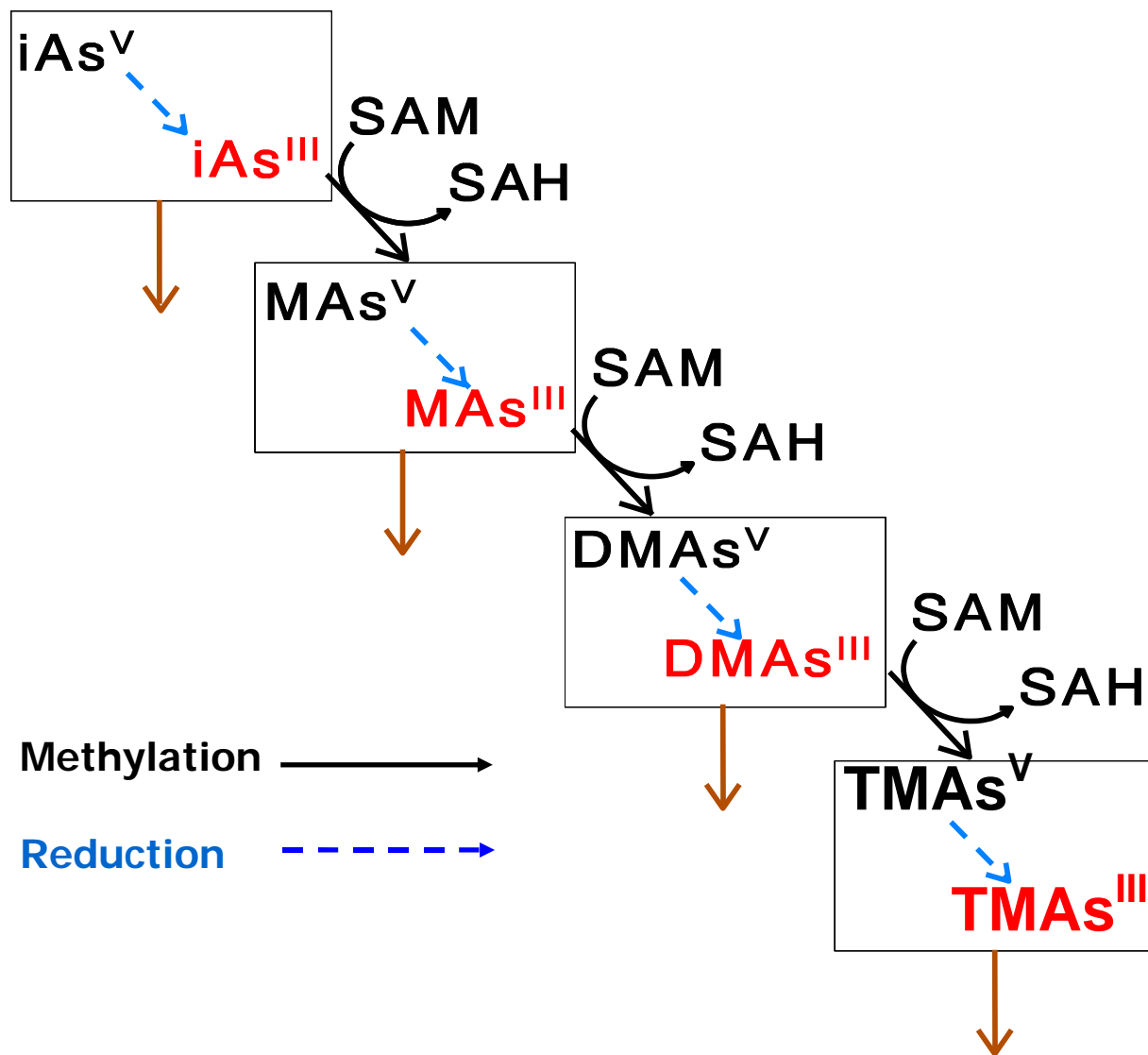


Figure 2.4: Summary of toxicities observed with arsenical compounds (References provided in Appendix A)

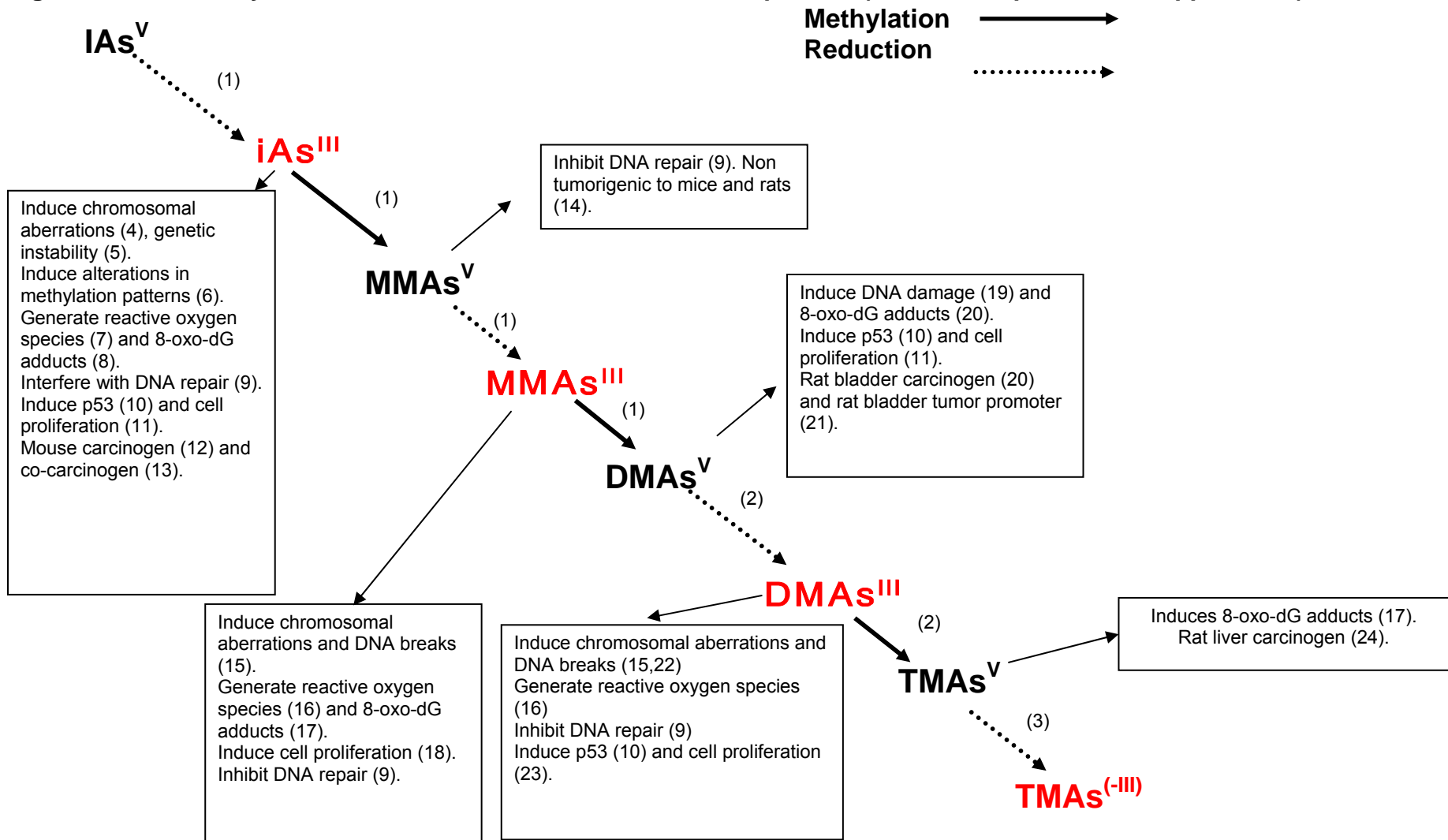


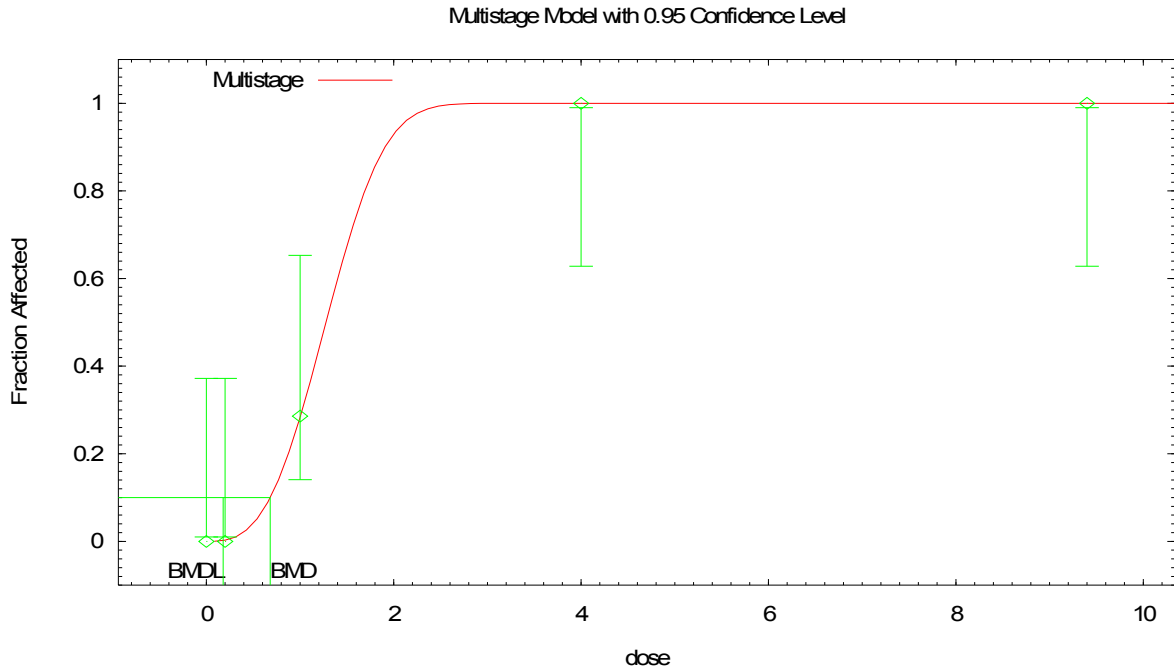
Table 3.7: Reversibility of Three Key Precursor Events in F344 Female Rats Administered DMA^V in the Diet (Arnold et al., 1999).

Dose (ppm)	Urothelial toxicity					Regenerative proliferation response	Urothelial simple hyperplasia
	SEM classification					BrdU labeling index (%)	Incidence
	1	2	3	4	5		
0 (20 weeks)	6	4	0	0	0	0.25 ± 0.03	1/10
100 (20 weeks)	0	0	3	6	1	0.97 ± 0.11 ^a	4/10
0 (10 weeks)	5	5	0	0	0	0.22 ± 0.05	1/10
100 (10 weeks) followed by 10 week recovery phase	0	0	6	4	0	0.21 ± 0.04 ^b	0/10

^aP<0.05 when compared to respective controls

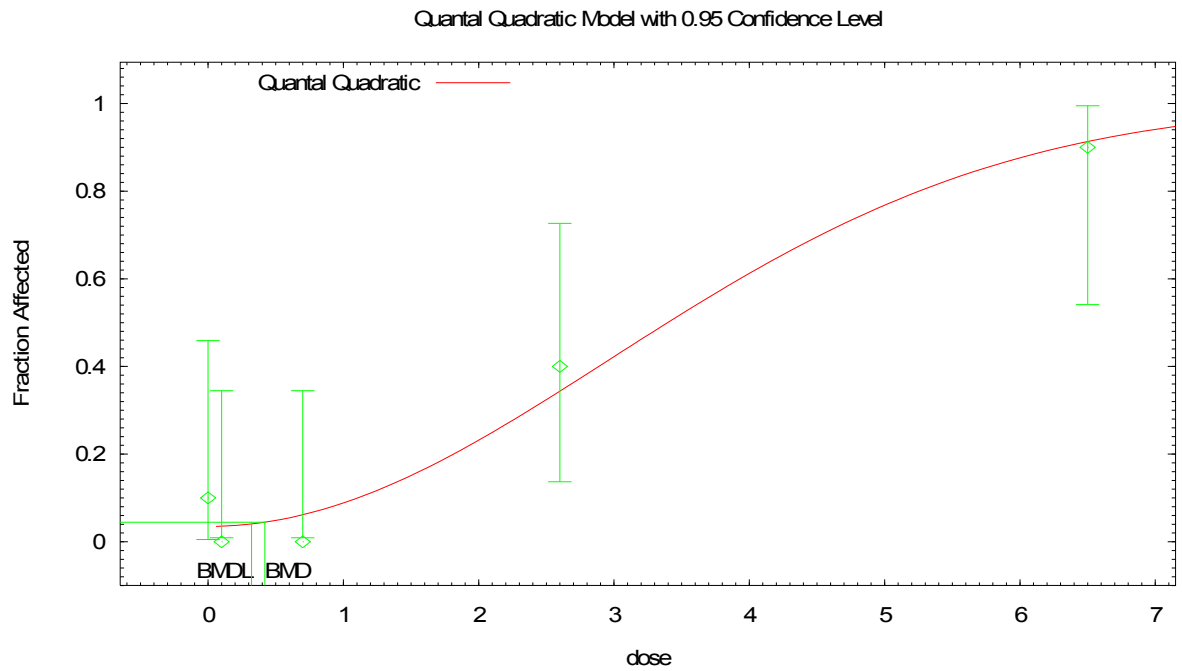
^bP<0.05 when compared to 100 ppm at 20 weeks

Figure 5.2: Plot of cytotoxicity data from 3 weeks of exposure to DMA^V. (Doses in mg/kg/day)



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Figure 5.5: Plot of incidence of hyperplasia data from 10 weeks of exposure to DMA^V in the feed (Arnold *et al.*, 1999). (Doses in mg/kg/day)



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Table 5.1: Summary of benchmark dose estimates and lower 95% confidence limits for cytotoxicity, BrdU labeling index, hyperplasia and tumor data. (Doses in mg/kg/day)

Biological Event	Duration	Feeding				Drinking water				
		10%		1%		10%		1%		
		BMD (mg/kg/day)	BMDL (mg/kg/day)	BMD (mg/kg/day)	BMDL (mg/kg/day)	BMD (mg/kg/day)	BMDL (mg/kg/day)	BMD (mg/kg/day)	BMDL (mg/kg/day)	
Tumor	104 weeks	7.74	5.96	6.80	2.22	104 weeks	1.92	1.21	0.88	0.14
Hyperplasia	10 weeks	1.36	1.04	0.42	0.32	104 weeks	1.63	1.04	0.74	0.14
	104 weeks	1.97	1.61	0.93	0.66					
BrdU labeling (proliferation)	10 weeks	0.65	0.29	0.54	0.07	Not determined. Available data not suitable for modeling.				
Cytotoxicity	3 weeks	0.68	0.18	0.31	0.02	No reliable dose-response data available				
	10 weeks	0.02	0.008	0.002	0.0007					

Appendix B Detailed tables for MOA analysis

Table B1. Dose-response relationships across time: Cytotoxicity^a

DMA ^V (ppm)	Toxicity (SEM)																			
	Hour 6					Hour 24					Day 3					Week 1				
	SEM classification					SEM classification					SEM classification					SEM classification				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
0	---	---	---	---	---	5	2	0	0	0	5	2	0	0	0	2	5	0	0	0
2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
10	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
40	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
100	0	1	6	0	0	0	3	2	2	0	0	4	3	0	0	0	7	0	0	0

Cohen et al. 2001

et al. 2001

et al. 2001

Cohen et al. 2001

DMA ^V (ppm)	Toxicity (SEM)																								
	Week 2					Week 2 <i>Cohen</i>					Week 2 <i>Cohen</i>					Week 2					Week 3				
	SEM classification					SEM classification					SEM classification					SEM classification					SEM classification				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
0	4	3	0	0	0	7	3	0	0	0	6	4	0	0	0	6	4	0	0	0	6	1	0	0	0
2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	4	3	0	0	0
10	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	0	5	2	0	0
40	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	0	0	7	0	0
100	0	4	0	3	0	0	2	2	0	6 ^b	0	0	5	3	2 ^b	0	0	1	9 ^b	0	0	0	7	0	0

Cohen et al. 2001

et al. 2002

et al. 2001

Wei et al. 2004

Arnold et al. 2004

DMA ^V (ppm)	Toxicity (SEM)																													
	Week 8					Week 10					Week 10					Week 10					Week 20					Week 26				
	SEM classification					SEM classification					SEM classification					SEM classification (males)					SEM classification					SEM classification				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
0	0	7	3	0	0	5	5	0	0	0	3	7	0	0	0	0	3	7	0	0	6	4	0	0	0	6	4	0	0	0
2	---	---	---	---	---	0	4	5	1	0	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
10	---	---	---	---	---	0	2	5	3	0	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
40	---	---	---	---	---	0	5	3	2	0	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
100	0	0	0	2	8 ^b	0	0	0	4	6 ^b	0	0	0	1	9 ^b	0	1	4	5	0	0	0	3	6	1	3	6	0	0	0

Arnold et al. 1999

Arnold et al. 1999

Cohen et al. 2001

Arnold et al. 1999

Arnold et al. 1999

Cohen et al. 2002

^aAll results in female rats, except where noted; ^b P<0.05 when compared to respective controls

SEM classification key for bladder toxicity used in Cohen et al. (2001; 2002):

- 1 = flat, polygonal superficial urothelial cells
 - 2 = occasional small foci of urothelial necrosis
 - 3 = numerous small foci of superficial urothelial necrosis
 - 4 = extensive superficial urothelial necrosis, especially in the dome of bladder
 - 5 = necrosis and piling up of rounded urothelial cells
- Normal bladders are usually Class 1 or 2, but occasionally Class 3.

Table B2. Dose-response and temporal relationships: Compensatory Regenerationc

DMA ^V (ppm)	Regenerative response (BrdU labeling index)														
	6	Hour 24	Day 3	Week 1	Week 2	Week 2	Week 2	Week 2	Week 8 (males) ^b	Week 10	Week 10 (males)	Week 10	Week 20	Week 26	Week 104 (males)
0	---	0.42 ± 0.05	0.23 ± 0.04	0.44 ± 0.09	0.22 ± 0.03	0.19 ± 0.04	0.16 ± 0.02	0.10 ± 0.02	~0.09 ± 0.06	0.22 ± 0.05	0.23 ± 0.03	0.18 ± 0.03	0.25 ± 0.03	0.13 ± 0.02	~0.16 ± 0.1
2	---	---	---	---	---	---	---	---	---	0.20 ± 0.03	---	---	---	---	---
10	---	---	---	---	---	---	---	---	~0.75 ± 0.25 ^a	0.33 ± 0.08	---	---	---	---	---
12.5	---	---	---	---	---	---	---	---	---	---	---	---	---	---	~0.22 ± 0.1
25	---	---	---	---	---	---	---	---	~0.21 ± 0.02 ^a	---	---	---	---	---	---
40	---	---	---	---	---	---	---	---	---	0.95 ± 0.15 ^a	---	---	---	---	---
50	---	---	---	---	---	---	---	---	---	---	---	---	---	---	~0.5 ± 0.25 ^a
100	0.22 ± 0.04	0.24 ± 0.04 ^a	0.33 ± 0.11	0.96 ± 0.14 ^a	1.36 ± 0.13 ^a	0.94 ± 0.20 ^a	0.63 ± 0.10 ^a	1.61 ± 0.22 ^a	---	0.93 ± 0.11 ^a	0.95 ± 0.05	0.61 ± 0.10 ^a	0.97 ± 0.11	0.21 ± 0.03 ^a	---
200	---	---	---	---	---	---	---	---	---	---	---	---	---	---	~0.65 ± 0.3a

Cohen et al. 2001; Cohen et al. 2001; Cohen et al. 2001; Cohen et al. 2001; Cohen et al. 2001; Cohen et al. 2001; Cohen et al. 2002; Wei et al. 2004; Wanibuchi et al. 1996; Arnold et al. 1999; Arnold et al. 1999; Cohen et al. 2001; Arnold et al. 1999; Cohen et al. 2002; Wei et al. 2002

Uncertainty expressed as ± S.E. of the mean in all studies, except in Wanibuchi et al. (1996), which used ± 1 S.D. of the mean

^a P<0.05 when compared to respective controls

^b Results are difficult to interpret, because indices for 0, 10 and 25 ppm are based on 10, 5, and 5 animals, respectively, and all rats treated with 100 ppm died from DMA toxicity after 4 weeks of treatment

^c All results in female rats, except where noted

Table B3. Dose-response relationships across time: Hyperplasiac

DMA ^v (ppm)	Non-neoplastic changes (Simple hyperplasia)															
Hour	6	Hour 24	Day 3	Week 1	Week 2	Week 2	Week 2	Week 2	Week 3	Week 8	Week 10	Week 10 (males)	Week 10	Week 20	Week 26	Week 104 (males and females)
0	---	2/7	3/7	0/7	1/10	0/7	0/10	0/10	0/7	0/10	1/10	0/10	0/10	1/10	0/10	0/120
2	---	---	---	---	---	---	---	---	0/7	---	0/10	---	---	---	---	1/118
10	---	---	---	---	---	---	---	---	0/7	---	0/10	---	---	---	---	0/120
40	---	---	---	---	---	---	---	---	0/7	---	4/10	---	---	---	---	35/117 ^b
100	1/7	0/7	1/7	2/7	0/10	0/7	1/10	0/10	2/7	7/10 ^a	9/10 ^a	2/10	6/10 ^a	4/10 ^d	4/9 ^a	88/119a,b

Cohen et al. 2001 Cohen et al. 2001 Cohen et al. 2001 Cohen et al. 2001 Cohen et al. 2001 Cohen et al. 2001 Cohen et al. 2002; Wei et al. 2004; Arnold et al. 2004; Arnold et al. 1999 Arnold et al. 1999; Arnold et al. 1999; Cohen et al. 2001; Arnold et al. 1999; Cohen et al. 2002 Gur et al. 1989a

^a P<0.05 when compared to respective controls

^bmales: 6/58 and 40/59 at 40 and 100 ppm, resp.; females: 29/59 and 48/60 at 40 and 100 ppm, resp.

^cAll results in female rats, except where noted

^dstatistical significance not measured

Dose-response results in bold

Endpoint	Study	Result	Reference
Micronucleated reticulocytes	Mouse (ICR) (single IP injection) study	DMA (10.6 mg/kg bw) only induced micronucleated reticulocytes after co-administration of glutathione	Kato et al., 2003
DNA strand breakage	Rat gavage study using alkaline elution	Positive at 387 mg/kg bw in lung cells	Kitchin and Ahmad, 2003
DNA strand breakage	Mouse (B6C3F1) gavage study using alkaline elution	Weakly positive in liver but not lung tissue at 720 mg/kg bw. Decreases in GSH content also found.	Ahmad et al., 1999
DNA strand breakage	Mouse gavage study	Positive in lung tissue at 1500 mg/kg bw	Yamanaka and Okada, 1994
Oxidative DNA adducts	Rat (oral-0, .5, 10, and 20 mg/kg)	Positive in kidney at 10 mg/kg bw	Vijayanahavan et al., 2001
Oxidative DNA adducts	Rat drinking water study	8-OHdG adducts found in bladder at 200 ppm (1.21 versus 1.76 /10 ⁵ dG) Wei could not replicate this finding in a repeat study in the laboratory of S. Cohen (personal communication, S. Cohen)	Wei et al., 2002
Oxidative DNA adducts	mouse drinking water study	8-OHdG adducts in lung (1.23 versus 1.79 /10 ⁵ dG) and liver tissue (1.17 versus 2.22 /10 ⁵ dG) at 400 ppm	Yamanaka et al., 2004

ROS = reactive oxygen species; 8-OHdG = 8-hydroxy deoxyguanosine.

Table App.C-3 Chemical Specific Parameters DMAV Model

Parameter	Symbol	Units	Mouse	Rat	Human	Comments
liver:plasma PC	p9l	none - ratio	1.0	1.0	1.0	fitted ¹
skin:plasma PC	p9i	none - ratio	0.87	0.87	0.87	area method, initial liver ²
resid:plasma PC	p9r	none - ratio	0.83	0.83	0.83	area method, initial lung ²
lung:plasma PC	p9n	none - ratio	5.0	5.0	5.0	fitted ¹
kidney:plasma PC	p9k	none - ratio	10	10	10	fitted ¹
bladder:plasma PC	p9d	none - ratio	0.87	0.87	0.87	area method, initial liver ²
liver diffusion constant	palc	none - ratio	0.3	0.3	0.3	fitted ¹
kidney diffusion constant	pakc	none -ratio	1.0	1.0	1.0	fitted ¹
lung diffusion constant	pafc	none - ratio	0.107	0.107	0.107	fitted ¹
1st Order GI Absorption Rate	ka9	hr-1	0.5	0.5	0.5	fitted ³
Renal Excretion Rate for DMA	RE9C	μg/hr/kg ^{0.75}	0.1	0.1	0.1	fitted ⁴ ,
1st Order metabolism to TMAO	ktma	L/hr	0.00073	0.00178	0.00049	calculated, see text
hematocrit (proportion)	hemcrt	none - ratio	0.415	0.45	0.45	literature
RBC diffusion coefficient	pabc	none-ratio	51.05	0.0133	51.05	Fitted ⁵
RBC binding/partition coeff	prbc	none-ratio	0.50	11530	0.50	Fitted ⁵

¹Parameters estimated using tissue-time course i.v. data in mice administered DMA^V (Hughes et al., 2000) and assumed to be the same across species. PC is partition coefficient.

²Initial estimate for partition coefficient (PC) using area method of Gallo et al. (1987).

³Estimated using blood time course data in mice after a single oral dose of DMA^V (Hughes et al, 2005) and assumed same across species.

⁴Estimated using data for time course of DMA excretion in urine after i.v. administration to mice (Hughes and Kenyon, 1998) and assumed same across species.

⁵Estimated using plasma and red blood cell time course data in mice after i.v. administration of DMA^V (Hughes et al, 2000) and assumed to be the same in humans. Rat parameter values were optimized based upon time course whole body retention data of Vahter et al. (1984).

Figure App.C-8A.

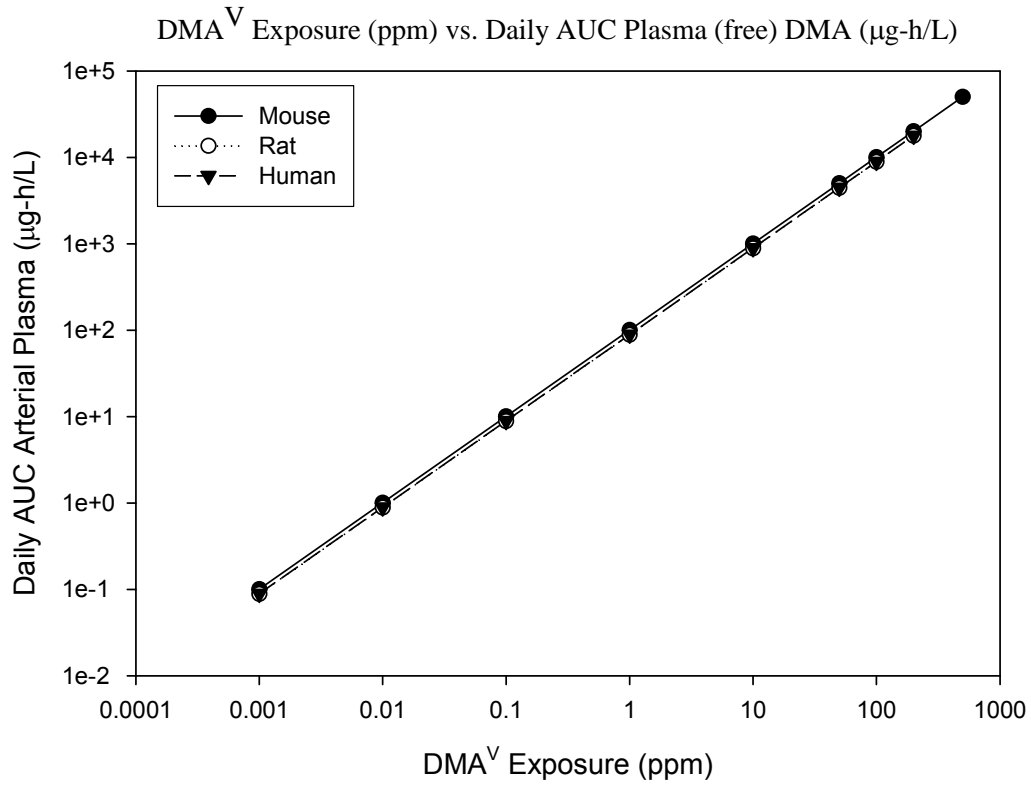


Figure App.C-8B.

