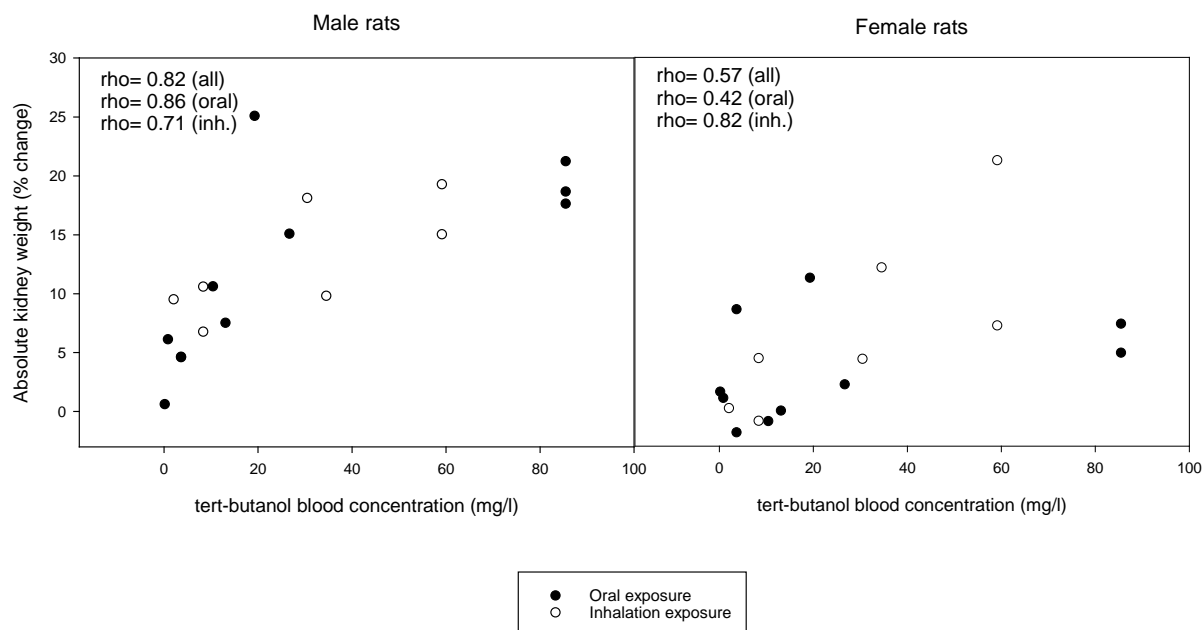
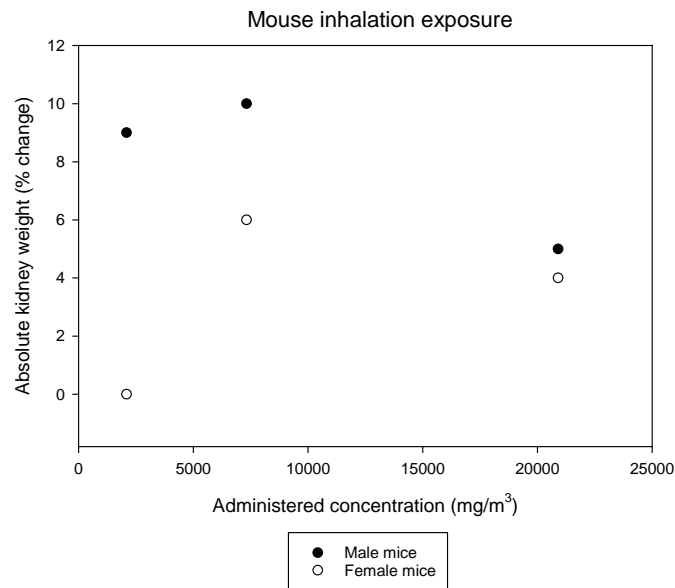


ETBE is metabolized to *tert*-butyl alcohol (*tert*-butanol) and acetaldehyde. Some of the toxicological effects observed following ETBE exposure such as increased kidney weight, increased urothelial hyperplasia (synonymous with transitional epithelial hyperplasia), and increased chronic progressive nephropathy (CPN) are attributed to *tert*-butanol as they were also observed following *tert*-butanol exposure (Salazar et al., 2015); therefore, the rodent bioassays from ETBE could provide supplementary information to the assessment of *tert*-butanol kidney toxicity and carcinogenicity. In addition to these effects, oral or inhalation ETBE exposure also elevated urine and serum biomarkers. No significant increases in renal tumors were observed in 2-year bioassays. Kidney effects were not observed in the one subchronic mouse study of inhalation ETBE exposure.



**Figure 1. Comparison of absolute kidney weight change in male and female rats across oral and inhalation exposure on the basis of internal blood *tert*-butanol concentration. Spearman rank coefficient was calculated to evaluate the direction of a monotonic association and the strength of association.**



**Figure 2. Comparison of absolute kidney weight change in male and female mice following inhalation exposure on the basis of administered ETBE concentration.**

**Table 1. Evidence pertaining to kidney CPN and histopathological effects in animals exposed to ETBE**

Reference and study design	Results (incidence, number/severity, or percent change compared to control)					
<a href="#">Cohen et al. (2011)</a> rat, F344/DuCrCrIj oral - water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d) <sup>a</sup> ; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-d) <sup>a</sup> reanalysis of the histopathology from <a href="#">JPEC (2010a)</a> study where animals were dosed daily for 104 wks	<b>Male</b>			<b>Female</b>		
	<u>Dose</u> (mg/kg-d)	<u>Average severity of CPN</u>	<u>Incidence of CPN</u>	<u>Dose</u> (mg/kg-d)	<u>Average severity of CPN</u>	<u>Incidence of CPN</u>
	0	2.08	49/50	0	1.14	45/50
	28	-	-	46	0.98	41/50
	121	-	-	171	1.2	46/50
542	2.72*	50/50	560	1.36	46/50	
<a href="#">Cohen et al. (2011)</a> rat, F344/DuCrCrIj oral - water male (10/group): 0, 250, 1,600, 4,000, 10,000 ppm (0, 17, 40, 101, 259, 626 mg/kg-d) <sup>a</sup> reanalysis of the histopathology from JPEC 2006 (study No. 0665) study where animals were dosed daily for 13 wks	<b>Male</b>					
	<u>Dose</u> (mg/kg-d)	<u>Number of CPN foci/rat</u>		<u>Number of granular casts/rat</u>		
	0	1.2		0		
	17	-		-		
	40	-		-		
	101	-		-		
	259	-		-		
626	27.2		8.2			
<a href="#">Miyata et al. (2013); JPEC (2008b)</a> rat, CRL:CD(SD) oral - gavage male (15/group): 0, 5, 25, 100, 400 mg/kg-d; female (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 d	<b>Male</b>		<b>Female</b>			
	<u>Dose</u> (mg/kg-d)	<u>Incidence of papillary mineralization</u>	<u>Dose</u> (mg/kg-d)	<u>Incidence of papillary mineralization</u>		
	0	0/15	0	0/15		
	5	0/15	5	-		
	25	0/15	25	-		
	100	1/15	100	-		
	400	0/15	400	0/15		

Reference and study design	Results (incidence, number/severity, or percent change compared to control)					
<a href="#">Saito et al. (2013); JPEC (2010b)</a> rat, Fischer 344 inhalation - vapor male (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m <sup>3</sup> ) <sup>b</sup> ; female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m <sup>3</sup> ) <sup>b</sup> dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported	<b>Male</b>		<u>Average severity of CPN as calculated by EPA<sup>c</sup></u>	<u>Incidence of CPN</u>	<u>Incidence of papillary mineralization</u>	<u>Incidence of urothelial hyperplasia of the renal pelvis</u>
	<u>Dose (mg/m<sup>3</sup>)</u>					
	0		2.4	49/50	0/50	2/50
	2,090		2.6	50/50	0/50	5/50
	6,270		2.7	49/49	1/49	16/49*
	20,900		3.1*	50/50	6/50*	41/50*
	<b>Female</b>		<u>Average severity of CPN as calculated by EPA<sup>c</sup></u>	<u>Incidence of CPN</u>		
	<u>Dose (mg/m<sup>3</sup>)</u>					
	0		0.9	32/50		
	2,090		1.3	38/50		
	6,270		1.3	41/50		
	20,900		1.6*	40/50		
Atypical tubule hyperplasia not observed in males or females. Papillary mineralization and urothelial hyperplasia of the renal pelvis not observed in females.						
<a href="#">Suzuki et al. (2012); JPEC (2010a)</a> rat, Fischer 344 oral - water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d) <sup>a</sup> ; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-d) <sup>a</sup> daily for 104 wks	<b>Male</b>		<u>Average severity of CPN as calculated by EPA<sup>c</sup></u>	<u>Incidence of atypical tubule hyperplasia</u>	<u>Incidence of CPN</u>	
	<u>Dose (mg/kg-d)</u>	<u>Average severity of CPN</u>				
	0	2.1	2.1	0/50	49/50	
	28	2.0	1.7	0/50	43/50	
	121	2.0	1.8	0/50	45/50	
	542	2.4*	2.3	1/50	48/50	
	<u>Dose (mg/kg-d)</u>	<u>Incidence of papillary necrosis</u>	<u>Incidence of papillary mineralization</u>	<u>Incidence of urothelial hyperplasia of the renal pelvis</u>		
	0	0/50	0/50	0/50		
	28	1/50	0/50	0/50		
	121	0/50	16/50*	10/50*		
	542	2/50	42/50*	25/50*		
	<b>Female</b>		<u>Average severity of CPN as calculated by EPA<sup>c</sup></u>	<u>Incidence of atypical tubule hyperplasia</u>	<u>Incidence of CPN</u>	
<u>Dose (mg/kg-d)</u>	<u>Average severity of CPN</u>					
0	1.2	1.0	0/50	41/50		
46	1.2	0.9	0/50	37/50		
171	1.5	1.1	0/50	37/50		
560	1.5*	1.2	2/50	39/50		

Reference and study design	Results (incidence, number/severity, or percent change compared to control)			
	<u>Dose</u> (mg/kg-d)	<u>Incidence of</u> <u>papillary</u> <u>necrosis</u>	<u>Incidence of</u> <u>papillary</u> <u>mineralization</u>	<u>Incidence of</u> <u>urothelial</u> <u>hyperplasia of</u> <u>the renal pelvis</u>
	0	0/50	0/50	0/50
	46	1/50	0/50	0/50
	171	1/50	1/50	0/50
	560	2/50	3/50	0/50

<sup>a</sup>Conversion performed by study authors.

<sup>b</sup>4.18 mg/m<sup>3</sup> = 1 ppm.

<sup>c</sup>Average severity calculated as (grade × number of affected animals) ÷ total number of animals exposed.

\*: result is statistically significant ( $p < 0.05$ ) based on analysis of data by study authors.

-: for controls, no response relevant; for other doses, no quantitative response reported.

Percent change compared to controls calculated as  $100 \times ((\text{treated value} - \text{control value}) \div \text{control value})$ .

**Table 2. Evidence pertaining to kidney biochemistry effects in animals exposed to ETBE**

Reference and study design	Results (incidence, severity, or percent change compared to control)				
<a href="#">JPEC (2008a)</a> rat, CRL:CD(SD) inhalation - vapor male (10/group): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m <sup>3</sup> ) <sup>a</sup> ; female (10/group): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m <sup>3</sup> ) <sup>a</sup> dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration, and method were reported	<b>Male</b>				
		<u>Blood urea nitrogen</u>			
		<u>Dose (mg/m<sup>3</sup>)</u>	<u>(BUN)</u>	<u>Cholesterol</u>	<u>Creatinine</u>
		0	-	-	-
		627	-9%	8%	-13%
		2,090	-5%	9%	-6%
		6,270	4%	26%	-6%
		20,900	4%	15%	-3%
		<u>Dose (mg/m<sup>3</sup>)</u>	<u>Proteinuria severity<sup>b</sup></u>	<u>Proteinuria incidence</u>	<u>Urinary casts</u>
		0	0.5	3/6	0/6
		627	1.2	5/6	0/6
		2,090	1.2	5/6	0/6
		6,270	1.3	6/6	0/6
		20,900	1.0	4/6	0/6
		<b>Female</b>			
			<u>Blood urea nitrogen</u>		
		<u>Dose (mg/m<sup>3</sup>)</u>	<u>(BUN)</u>	<u>Cholesterol</u>	<u>Creatinine</u>
		0	-	-	-
		627	-5%	7%	0%
		2,090	3%	9%	3%
	6,270	-8%	11%	-9%	
	20,900	-4%	21%	-9%	
	<u>Dose (mg/m<sup>3</sup>)</u>	<u>Proteinuria severity<sup>b</sup></u>	<u>Proteinuria incidence</u>	<u>Urinary casts</u>	
	0	0.2	1/6	0/6	
	627	0.3	1/6	0/6	
	2,090	0.2	1/6	0/6	
	6,270	0.5	2/6	0/6	
	20,900	0.3	2/6	0/6	

Reference and study design	Results (incidence, severity, or percent change compared to control)			
<a href="#">Miyata et al. (2013); JPEC (2008b)</a> rat, CRL:CD(SD) oral - gavage male (15/group): 0, 5, 25, 100, 400 mg/kg-d; female (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for approximately 26 wks	<b>Male</b>			
	<u>Dose</u> (mg/kg-d)	<u>Blood urea nitrogen</u> (BUN)	<u>Cholesterol</u>	<u>Creatinine</u>
	0	-	-	-
	5	12%	-5%	0%
	25	1%	21%	-10%
	100	4%	12%	-3%
	400	8%	53%*	0%
	<u>Dose</u> (mg/kg-d)	<u>Proteinuria incidence</u>	<u>Proteinuria severity<sup>b</sup></u>	<u>Urinary casts</u>
	0	10/10	1.5	0/10
	5	10/10	1.6	-
	25	10/10	1.6	-
	100	10/10	1.3	-
	400	10/10	1.5	0/10
	<b>Female</b>			
	<u>Dose</u> (mg/kg-d)	<u>Blood urea nitrogen</u> (BUN)	<u>Cholesterol</u>	<u>Creatinine</u>
	0	-	-	-
	5	-5%	-7%	-19%
	25	-7%	-7%	-12%
	100	-1%	-2%	-16%
	400	4%	3%	-16%
	<u>Dose</u> (mg/kg-d)	<u>Proteinuria incidence</u>	<u>Proteinuria severity<sup>b</sup></u>	<u>Urinary casts</u>
0	8/10	1.2	0/10	
5	9/10	1.3	-	
25	7/10	1.0	-	
100	9/10	1.3	-	
400	7/10	1.0	0/10	

Reference and study design	Results (incidence, severity, or percent change compared to control)						
<a href="#">Saito et al. (2013); JPEC (2010b)</a> rat, Fischer 344 inhalation - vapor male (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m <sup>3</sup> ) <sup>a</sup> ; female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m <sup>3</sup> ) <sup>a</sup> dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported	<b>Male</b>						
		<u>Blood urea nitrogen</u>				<u>Proteinuria incidence</u>	<u>Proteinuria severity<sup>b</sup></u>
	<u>Dose (mg/m<sup>3</sup>)</u>	<u>(BUN)</u>	<u>Cholesterol</u>	<u>Creatinine</u>			
	0	-	-	-	44/44	3.7	
	2,090	41%*	10%	14%*	38/38	3.5	
	6,270	45%*	29%*	29%*	40/40	3.6	
	20,900	179%*	52%*	71%*	31/31	3.6	
	<b>Female</b>						
		<u>Blood urea nitrogen</u>				<u>Proteinuria incidence</u>	<u>Proteinuria severity<sup>b</sup></u>
	<u>Dose (mg/m<sup>3</sup>)</u>	<u>(BUN)</u>	<u>Cholesterol</u>	<u>Creatinine</u>			
0	-	-	-	33/38	2.8		
2,090	10%	-3%	0%	39/39	3.1		
6,270	4%	-4%	0%	30/30	3.3		
20,900	30%*	53%*	0%	30/30	3.4*		
<a href="#">Suzuki et al. (2012); JPEC (2010a)</a> rat, Fischer 344 oral - water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d) <sup>c</sup> ; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-d) <sup>c</sup> daily for 104 wks	<b>Male</b>						
		<u>Blood urea nitrogen</u>				<u>Proteinuria incidence</u>	<u>Proteinuria severity<sup>b</sup></u>
	<u>Dose (mg/kg-d)</u>	<u>(BUN)</u>	<u>Cholesterol</u>	<u>Creatinine</u>			
	0	-	-	-	39/39	3.0	
	28	3%	-11%	0%	37/37	3.1	
	121	20%*	10%	17%	34/34	3.1	
	542	43%*	31%*	17%	35/35	3.1	
	<b>Female</b>						
		<u>Blood urea nitrogen</u>				<u>Proteinuria incidence</u>	<u>Proteinuria severity<sup>b</sup></u>
	<u>Dose (mg/kg-d)</u>	<u>(BUN)</u>	<u>Cholesterol</u>	<u>Creatinine</u>			
0	-	-	-	37/37	2.8		
46	-8%	-2%	0%	37/37	3.0		
171	-5%	12%	-17%	38/38	3.0		
560	-5%	8%	0%	38/38	3.1		

<sup>a</sup>4.18 mg/m<sup>3</sup> = 1 ppm.

<sup>b</sup>Severity of proteinuria = (1 × number of animals with “1+”) + (2 × number of animals with “2+”) + (3 × number of animals with “3+”) + (4 × number of animals with “4+”) ÷ total number of animals in group.

<sup>c</sup>Conversion performed by study authors.

\*: result is statistically significant ( $p < 0.05$ ) based on analysis of data by study authors.

-: for controls, no response relevant; for other doses, no quantitative response reported.

Percent change compared to controls calculated as  $100 \times ((\text{treated value} - \text{control value}) \div \text{control value})$ .



**Table 3. Evidence pertaining to kidney tumor effects in animals exposed to ETBE**

Reference and study design	Results (incidence)			
<a href="#">Hagiwara et al. (2011); JPEC (2008c)</a> rat, Fischer 344 oral - gavage male (12/group): 0, 1,000 mg/kg-d daily for 23 wks	<b>Male</b>			
	<u>Dose</u> (mg/kg-d)	<u>Renal transitional</u> <u>cell carcinoma</u>	<u>Renal tubular</u> <u>adenoma or</u> <u>carcinoma</u>	
	0	0/12	0/12	
	1,000	0/12	0/12	
<a href="#">Hagiwara et al. (2011); JPEC (2008c)</a> rat, Fischer 344 oral - gavage male (30/group): 0, 300, 1,000 mg/kg-d daily for 23 wks following a 4 week tumor initiation by DMBDD <sup>a</sup>	<b>Male</b>			
	<u>Dose</u> (mg/kg-d)	<u>Renal tubular</u> <u>adenoma or</u> <u>carcinoma</u>	<u>Renal transitional</u> <u>cell carcinoma</u>	
	0	11/30	1/30	
	300	6/30	0/30	
	1,000	13/30	2/30	
<a href="#">Hagiwara et al. (2015)</a> rat, Wistar oral - gavage male (30/group): 0,100, 300, 500, 1,000 mg/kg-d daily for 23 wks following a 4 week tumor initiation by N-Ethyl-N- hydroxyethylnitrosamine (EHEN)	<b>Male</b>			
	<u>Dose</u> (mg/kg-d)	<u>Renal tubular</u> <u>adenoma or</u> <u>carcinoma</u> <sup>b</sup>		
	0	18/30		
	100	23/30		
	300	25/30		
	500	26/30		
	1,000	26/30		
<a href="#">Saito et al. (2013); JPEC (2010b)</a> rat, Fischer 344 inhalation - vapor male (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m <sup>3</sup> ); female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m <sup>3</sup> ) <sup>c</sup>	<b>Male</b>	<u>Renal cell</u> <u>carcinoma</u>	<b>Female</b>	<u>Renal cell</u> <u>carcinoma</u>
	<u>Dose</u> (mg/m <sup>3</sup> )		<u>Dose</u> (mg/m <sup>3</sup> )	
	0	0/50	0	0/50
	2,090	1/50	2,090	0/50
	6,270	0/49	6,270	0/50
	20,900	0/50	20,900	0/50
<a href="#">Suzuki et al. (2012); JPEC (2010a)</a> rat, Fischer 344 oral - water male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d) <sup>d</sup> ; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-d) <sup>d</sup> daily for 104 wks	<b>Male</b>		<b>Female</b>	
	<u>Dose</u> (mg/kg-d)	<u>Renal cell</u> <u>carcinoma</u>	<u>Dose</u> (mg/kg-d)	<u>Renal cell</u> <u>carcinoma</u>
	0	0/50	0	0/50
	28	0/50	46	0/50
	121	0/50	171	0/50
	542	1/50	560	1/50

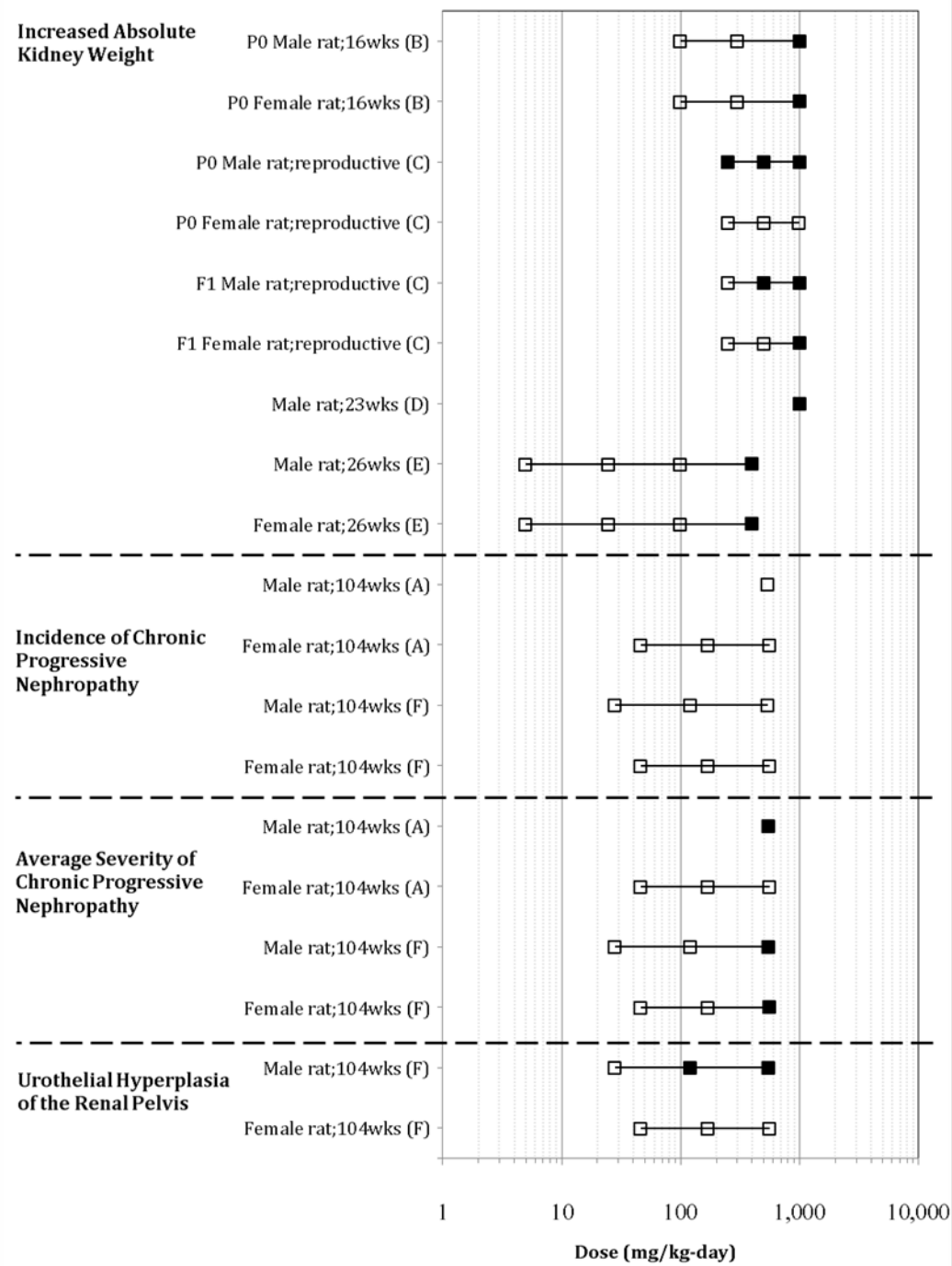
<sup>a</sup>Diethylnitrosamine (DEN), N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN), N-methyl-N-nitrosourea (MNU), 1,2-dimethylhydrazine dihydrochloride (DMH), and N-bis(2-hydroxypropyl)nitrosamine (DHPN)

<sup>b</sup>Authors report significant trend.

<sup>c</sup>4.18 mg/m<sup>3</sup> = 1 ppm.

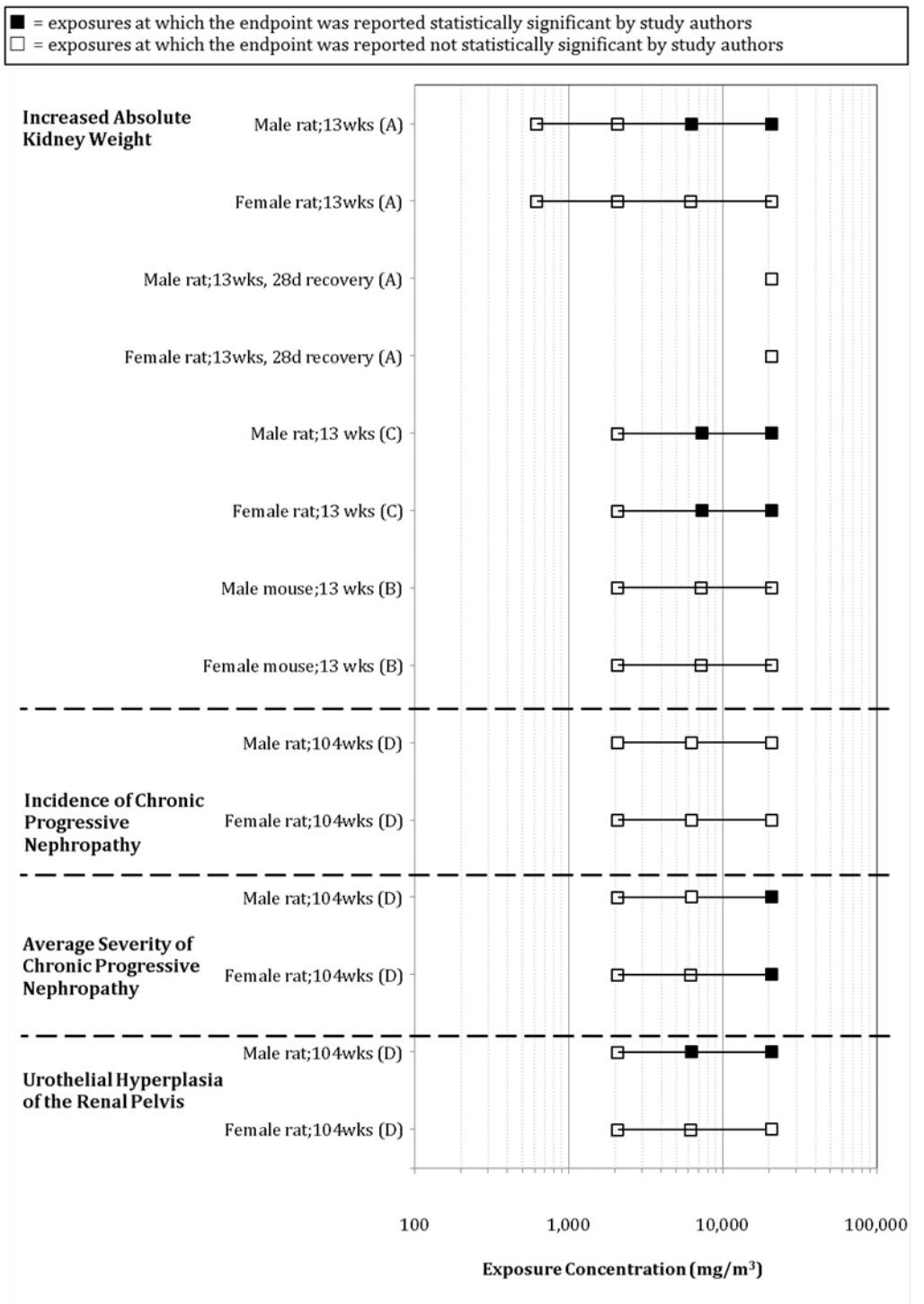
<sup>d</sup>Conversion performed by study authors.

■ = exposures at which the endpoint was reported statistically significant by study authors  
 □ = exposures at which the endpoint was reported not statistically significant by study authors



Sources: (A) Cohen et al., 2011 reanalysis of JPEC, 2010a; (B) Fujii et al., 2010; JPEC, 2008e; (C) Gaoua, 2004b; (D) Hagiwara et al., 2011; (E) Miyata et al., 2013; JPEC, 2008c; (F) Suzuki et al., 2012; JPEC, 2010a

**Figure 3. Exposure-response array of kidney effects following oral exposure to ETBE.**



Sources: (A) JPEC, 2008b; (B) Medinsky et al., 1999; Bond et al., 1996a (C) Medinsky et al., 1999; Bond et al., 1996b (D) Saito et al., 2013; JPEC, 2010b

**Figure 4. Exposure-response array of kidney effects following inhalation exposure to ETBE.**

**Table 4. Additional kidney effects potentially relevant to mode of action in animals exposed to ETBE**

Reference and study design	Results (incidence or severity)																																	
<p><a href="#">JPEC (2008a)</a>  rat, CRL:CD(SD)  inhalation - vapor  male (10/group): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m<sup>3</sup>)<sup>a</sup>;  female (10/group): 0, 150, 500, 1,500, 5,000 ppm (0, 627, 2,090, 6,270, 20,900 mg/m<sup>3</sup>)<sup>a</sup>  dynamic whole body chamber; 6 hrs/d, 5 d/wk for 13 wks; generation method, analytical concentration and method were reported</p>	<p><b>Male</b></p> <table border="1"> <thead> <tr> <th><u>Dose (mg/m<sup>3</sup>)</u></th> <th><u>Incidence of hyaline droplets in the proximal tube epithelium</u></th> </tr> </thead> <tbody> <tr> <td>0</td> <td>0/10</td> </tr> <tr> <td>627</td> <td>3/10</td> </tr> <tr> <td>2,090</td> <td>8/10*</td> </tr> <tr> <td>6,270</td> <td>8/10*</td> </tr> <tr> <td>20,900</td> <td>8/10*</td> </tr> </tbody> </table> <p>Unspecified representative samples reported as "weakly positive" for <math>\alpha_{2u}</math>-globulin in males; no hyaline droplets observed in proximal tubule of females; hyaline droplets positive for <math>\alpha_{2u}</math>-globulin not examined in females.</p>				<u>Dose (mg/m<sup>3</sup>)</u>	<u>Incidence of hyaline droplets in the proximal tube epithelium</u>	0	0/10	627	3/10	2,090	8/10*	6,270	8/10*	20,900	8/10*																		
<u>Dose (mg/m<sup>3</sup>)</u>	<u>Incidence of hyaline droplets in the proximal tube epithelium</u>																																	
0	0/10																																	
627	3/10																																	
2,090	8/10*																																	
6,270	8/10*																																	
20,900	8/10*																																	
<p><a href="#">JPEC (2008b)</a>; <a href="#">Miyata et al. (2013)</a>  rat, CRL:CD(SD)  oral - gavage  male (15/group): 0, 5, 25, 100, 400 mg/kg-d;  female (15/group): 0, 5, 25, 100, 400 mg/kg-d daily for 180 d</p>	<p><b>Male</b></p> <table border="1"> <thead> <tr> <th><u>Dose (mg/kg-d)</u></th> <th><u>Incidence of hyaline droplets</u></th> <th><u>Incidence of positive for <math>\alpha_{2u}</math>-globulin</u></th> </tr> </thead> <tbody> <tr> <td>0</td> <td>0/15</td> <td>0/1</td> </tr> <tr> <td>5</td> <td>0/15</td> <td>-</td> </tr> <tr> <td>25</td> <td>0/15</td> <td>-</td> </tr> <tr> <td>100</td> <td>4/15</td> <td>2/2</td> </tr> <tr> <td>400</td> <td>10/15*</td> <td>1/1</td> </tr> </tbody> </table>		<u>Dose (mg/kg-d)</u>	<u>Incidence of hyaline droplets</u>	<u>Incidence of positive for <math>\alpha_{2u}</math>-globulin</u>	0	0/15	0/1	5	0/15	-	25	0/15	-	100	4/15	2/2	400	10/15*	1/1	<p><b>Female</b></p> <table border="1"> <thead> <tr> <th><u>Dose (mg/kg-d)</u></th> <th><u>Incidence of hyaline droplets</u></th> </tr> </thead> <tbody> <tr> <td>0</td> <td>0/15</td> </tr> <tr> <td>5</td> <td>-</td> </tr> <tr> <td>25</td> <td>-</td> </tr> <tr> <td>100</td> <td>-</td> </tr> <tr> <td>400</td> <td>0/15</td> </tr> </tbody> </table>		<u>Dose (mg/kg-d)</u>	<u>Incidence of hyaline droplets</u>	0	0/15	5	-	25	-	100	-	400	0/15
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Reference and study design	Results (incidence or severity)
<p><a href="#">Saito et al. (2013)</a>; <a href="#">JPEC (2010b)</a>  rat, Fischer 344  inhalation - vapor  male (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m<sup>3</sup>)<sup>a</sup>; female (50/group): 0, 500, 1,500, 5,000 ppm (0, 2,090, 6,270, 20,900 mg/m<sup>3</sup>)<sup>a</sup>  dynamic whole body inhalation; 6 hrs/d, 5 d/wk for 104 wks; generation method, analytical concentration and method were reported</p>	<p><b>Male</b>  No hyaline droplets observed.</p> <p><b>Female</b>  No hyaline droplets observed.</p>
<p><a href="#">Suzuki et al. (2012)</a>; <a href="#">JPEC (2010a)</a>  rat, Fischer 344  oral - water  male (50/group): 0, 625, 2,500, 10,000 ppm (0, 28, 121, 542 mg/kg-d)<sup>b</sup>; female (50/group): 0, 625, 2,500, 10,000 ppm (0, 46, 171, 560 mg/kg-d)<sup>b</sup>  daily for 104 wks</p>	<p><b>Male</b>  No hyaline droplets observed.</p> <p><b>Female</b>  No hyaline droplets observed.</p>

<sup>a</sup>4.18 mg/m<sup>3</sup> = 1 ppm.

<sup>b</sup>Conversion performed by study authors.

\*: result is statistically significant ( $p < 0.05$ ) based on analysis of data by study authors.

-: for controls, no response relevant; for other doses, no quantitative response reported.

**Table 5. Summary of data informing whether the  $\alpha_{2u}$ -globulin process is occurring in male rats exposed to ETBE**

Criterion	Duration	Results	Reference
(1) Hyaline droplets are increased in size and number	1 wk	(+) <sup>a</sup>	<a href="#">Medinsky et al. (1999)</a>
	4 wks	(+) <sup>a</sup>	<a href="#">Medinsky et al. (1999)</a>
	13 wks	(+) <sup>a</sup>	<a href="#">Medinsky et al. (1999)</a>
	13 wks	+	<a href="#">JPEC (2008a)</a>
	26 wks	+	<a href="#">Miyata et al. (2013); JPEC (2008b)</a>
	104 wks	–	<a href="#">Suzuki et al. (2012)</a>
	104 wks	–	<a href="#">Saito et al. (2013); JPEC (2010b)</a>
(2) The protein in the hyaline droplets is $\alpha_{2u}$ -globulin	1 wk	(+) <sup>b</sup>	<a href="#">JPEC (2008a)</a>
	4 wks	(+) <sup>b</sup>	<a href="#">Medinsky et al. (1999)</a>
	13 wks	(+) <sup>b</sup>	<a href="#">Medinsky et al. (1999)</a>
	13 wks	(+) <sup>b</sup>	<a href="#">JPEC (2008a)</a>
	26 wks	(+) <sup>c</sup>	<a href="#">Miyata et al. (2013); JPEC (2008b)</a>
(3) Several (but not necessarily all) additional steps in the pathological sequence are present in male rats, such as:			
(a) single-cell necrosis	13 wks	–	<a href="#">JPEC (2008a)</a>
	13 wks	–	<a href="#">Medinsky et al. (1999)</a>
	26 wks	–	<a href="#">Miyata et al. (2013); JPEC (2008b)</a>
	104 wks	–	<a href="#">Suzuki et al. (2012); JPEC (2010a)</a>
	104 wks	–	<a href="#">Saito et al. (2013); JPEC (2010b)</a>
(b) exfoliation of epithelial cells into the tubular lumen	13 wks	–	<a href="#">JPEC (2008a)</a>
	13 wks	–	<a href="#">Medinsky et al. (1999)</a>
	26 wks	–	<a href="#">Miyata et al. (2013); JPEC (2008b)</a>
	104 wks	–	<a href="#">Suzuki et al. (2012); JPEC (2010a)</a>
	104 wks	–	<a href="#">Saito et al. (2013); JPEC (2010b)</a>
(c) granular casts	13 wks	–	<a href="#">JPEC (2008a)</a>
	13 wks	(+)	<a href="#">Cohen et al. (2011)</a>
	13 wks	–	<a href="#">Medinsky et al. (1999)</a>
	26 wks	–	<a href="#">Miyata et al. (2013); JPEC (2008b)</a>
	104 wks	–	<a href="#">Suzuki et al. (2012); JPEC (2010a)</a>
	104 wks	–	<a href="#">Saito et al. (2013); JPEC (2010b)</a>
(d) linear mineralization of tubules in the renal papilla	13 wks	–	<a href="#">JPEC (2008a)</a>
	13 wks	–	<a href="#">Medinsky et al. (1999)</a>
	26 wks	–	<a href="#">Miyata et al. (2013); JPEC (2008b)</a>
	104 wks	+	<a href="#">Suzuki et al. (2012); JPEC (2010a); Cohen et al. (2011)</a>
	104 wks	+	<a href="#">Saito et al. (2013); JPEC (2010b)</a>
(e) foci of tubular hyperplasia	13 wks	–	<a href="#">JPEC (2008a)</a>
	13 wks	+/- <sup>d</sup>	<a href="#">Medinsky et al. (1999)</a>
	26 wks	–	<a href="#">Miyata et al. (2013); JPEC (2008b)</a>
	104 wks	–	<a href="#">Suzuki et al. (2012); JPEC (2010a)</a>
	104 wks	–	<a href="#">Saito et al. (2013); JPEC (2010b)</a>

<sup>a</sup>Droplet severity.

<sup>b</sup>Unspecified “representative samples” examined.

<sup>c</sup>Three samples from highest two dose groups examined.

<sup>d</sup>Labeling index statistically significantly increased, but no hyperplasia reported.

+ = Statistically significant change reported in one or more treated groups.

(+) = Effect was reported in one or more treated groups, but statistics not reported.

– = No statistically significant change reported in any of the treated groups.

■ = exposures at which the endpoint was reported statistically significant by study authors  
 □ = exposures at which the endpoint was reported not statistically significant by study authors  
 ● = effect was observed but statistics not reported  
 + = unspecified representative samples reported positive for  $\alpha_{2u}$ -globulin

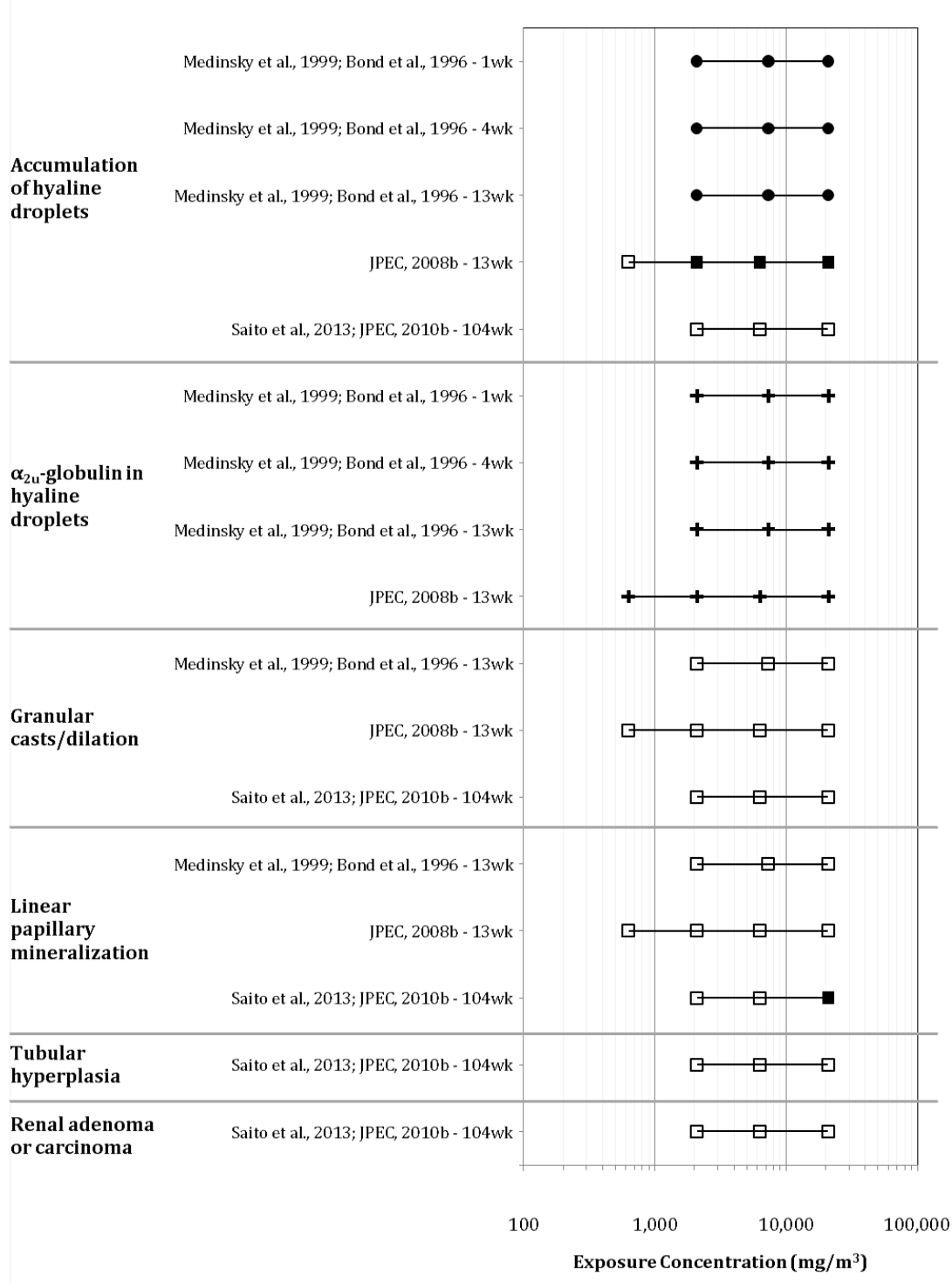


Figure 5. ETBE inhalation exposure array of  $\alpha_{2u}$ -globulin data in male rats

■ = exposures at which the endpoint was reported statistically significant by study authors  
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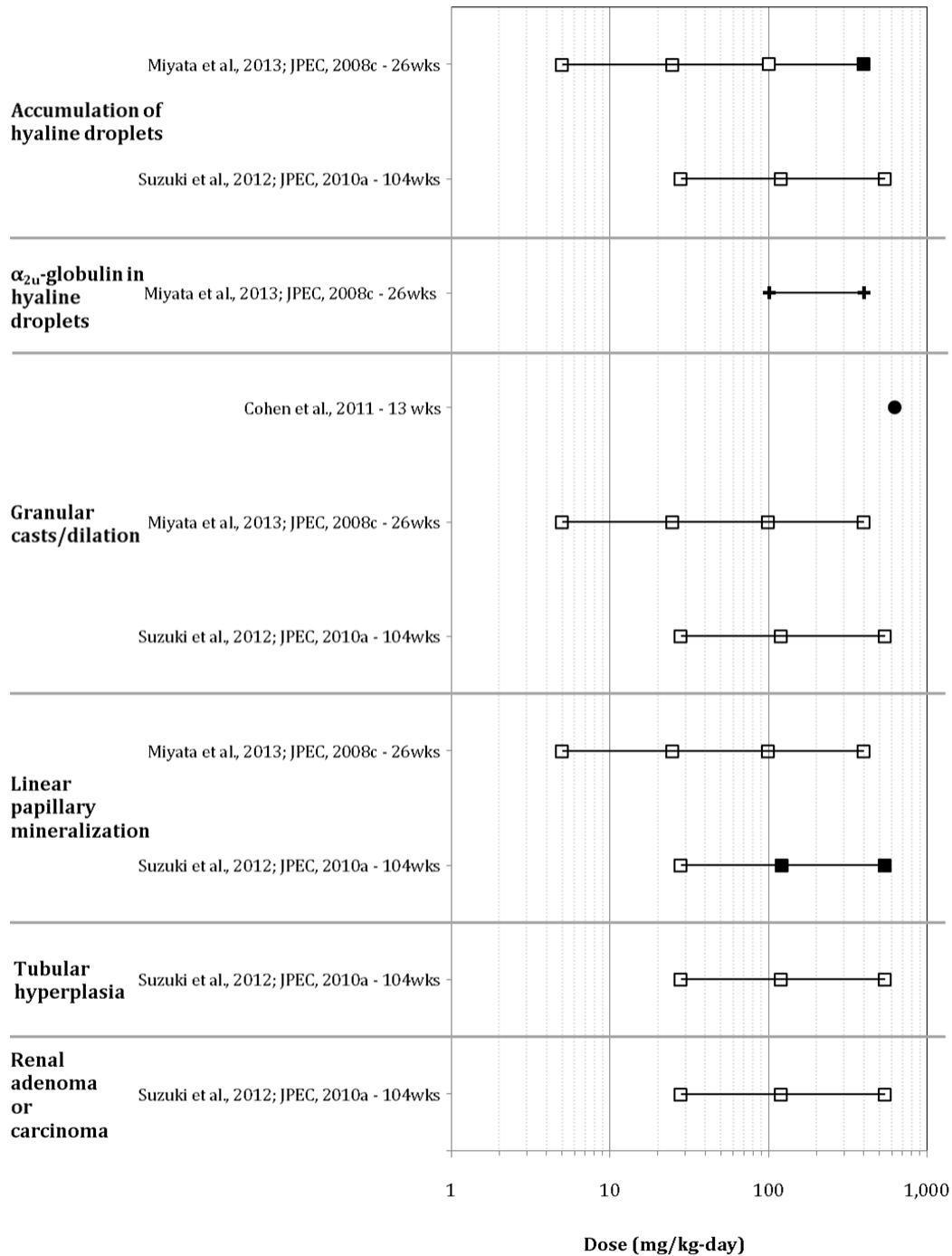


Figure 6. ETBE oral exposure array of  $\alpha_{2u}$ -globulin data in male rats



- [Bond, JA; Medinsky, MA; Wolf, DC; Dorman, DC; Cattley, R; Farris, G; Wong, B; Morgan, K; Janszen, D; Turner, MJ; Sumner, SCJ.](#) (1996). Ethyl tertiary butyl ether (ETBE): ninety-day vapor inhalation toxicity study with neurotoxicity evaluations in Fischer 344 rats [TSCA Submission] (pp. 1-90). (89970000047). Research Triangle Park, NC: Chemical Industry Institute of Toxicology under contract to ARCO Chemical Company.  
[http://yosemite.epa.gov/oppts/epatscat8.nsf/by+Service/1332F4B209355DC785256F9E006B7EA0/\\$File/89970000047.pdf](http://yosemite.epa.gov/oppts/epatscat8.nsf/by+Service/1332F4B209355DC785256F9E006B7EA0/$File/89970000047.pdf).
- [Cohen, SM; Hard, GC; Regan, KS; Seely, JC; Bruner, RH.](#) (2011). Pathology working group review of selected histopathologic changes in the kidneys of rats assigned to toxicology and carcinogenicity studies of ethyl tertiary butyl ether (ETBE): Japan Bioassay Research Center studies no.: 0065 and 0691 [Unpublished report] (pp. 1-30). Research Triangle Park, NC: Research Pathology Associates under contract to Lyondell Chemical Company.
- [Hagiwara, A; Doi, Y; Imai, N; Nakashima, H; Ono, T; Kawabe, M; Furukawa, F; Tamano, S; Nagano, K; Fukushima, S.](#) (2011). Medium-term multi-organ carcinogenesis bioassay of ethyl tertiary-butyl ether in rats. *Toxicology*. 289: 160-166.  
<http://dx.doi.org/10.1016/j.tox.2011.08.007>.
- [Hagiwara, A; Doi, Y; Imai, N; Suguro, M; Kawabe, M; Furukawa, F; Tamano, S; Nagano, K; Fukushima, S.](#) (2015). Promotion of liver and kidney carcinogenesis by ethyl tertiary-butyl ether (ETBE) in male Wistar rats. *J Toxicol Pathol*. 28: 189-195.  
<http://dx.doi.org/10.1293/tox.JTP-2015-0023>.
- [JPEC](#) (Japan Petroleum Energy Center). (2008a). A 90-day repeated dose toxicity study of ETBE by whole-body inhalation exposure in rats. (Study Number: B061829). Mitsubishi Chemical Safety Institute Ltd.
- [JPEC](#) (Japan Petroleum Energy Center). (2008b). A 180-Day repeated dose oral toxicity study of ETBE in rats. (Study Number: D19-0002). Japan: Hita Laboratory, Chemicals Evaluation and Research Institute (CERI).
- [JPEC](#) (Japan Petroleum Energy Center). (2008c). Medium-term multi-organ carcinogenesis bioassay of 2-ethoxy-2-methylpropane (ETBE) in rats. (Study Number: 0635). Ichinomiya, Japan: DIMS Institute of Medical Science.
- [JPEC](#) (Japan Petroleum Energy Center). (2010a). Carcinogenicity test of 2-Ethoxy-2-methylpropane in rats (Drinking water study). (Study No: 0691). Japan Industrial Safety and Health Association, Japan Bioassay Research Center.
- [JPEC](#) (Japan Petroleum Energy Center). (2010b). Carcinogenicity test of 2-Ethoxy-2-methylpropane in rats (Inhalation study). (Study No: 0686). Japan: Japan Industrial Safety and Health Association.
- [Medinsky, MA; Wolf, DC; Cattley, RC; Wong, B; Janszen, DB; Farris, GM; Wright, GA; Bond, JA.](#) (1999). Effects of a thirteen-week inhalation exposure to ethyl tertiary butyl ether on Fischer-344 rats and CD-1 mice. *Toxicol Sci*. 51: 108-118.  
<http://dx.doi.org/10.1093/toxsci/51.1.108>.
- [Miyata, K; Koga, T; Aso, S; Hoshuyama, S; Ajimi, S; Furukawa, K.](#) (2013). A subchronic (180-day) oral toxicity study of ethyl tertiary-butyl ether, a bioethanol, in rats. *Drug Chem Toxicol*.  
<http://dx.doi.org/10.3109/01480545.2013.851690>.

- Saito, A; Sasaki, T; Kasai, T; Katagiri, T; Nishizawa, T; Noguchi, T; Aiso, S; Nagano, K; Fukushima, S. (2013). Hepatotumorigenicity of ethyl tertiary-butyl ether with 2-year inhalation exposure in F344 rats. Arch Toxicol. 87: 905-914. <http://dx.doi.org/10.1007/s00204-012-0997-x>.
- Salazar, KD; Brinkerhoff, CJ; Lee, JS; Chiu, WA. (2015). Development and application of a rat PBPK model to elucidate kidney and liver effects induced by ETBE and tert-butanol. Toxicol Appl Pharmacol. 288: 439-452. <http://dx.doi.org/10.1016/j.taap.2015.08.015>.
- Suzuki, M; Yamazaki, K; Kano, H; Aiso, S; Nagano, K; Fukushima, S. (2012). No carcinogenicity of ethyl tertiary-butyl ether by 2-year oral administration in rats. J Toxicol Sci. 37: 1239-1246.