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GENETIC AND ENVIRONMENTAL EFFECTS OF SIDESTREAM SMOKE ON PUP SURVIVORSHIP OF THREE INBRED STRAINS OF MICE

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ABSTRACT: Crosses and backcrosses of three inbred mouse strains, BALB/c, C57BL, and C3H, were made to study the effects of pup genetic composition and sidestream smoke exposure on *in utero* pup survivorship. Pups that varied in genetic composition were produced by making the appropriate crosses and backcrosses of these inbred strains. A Model I ANOVA showed that pup survivorship for all three strain crosses and backcrosses was significantly affected both by the genetic composition of the pups and by sidestream smoke exposure ($P < 0.001$). Analysis of variance also indicated that significant interaction between genetic composition and smoke exposure occurred only in the BALB/c x C57BL crosses and backcrosses. Variation in the survivorship for pups of different genetic composition was identified using Tukey's multiple comparison test. The only comparison of pups differing in genetic composition that consistently resulted in significant differences in survivorship was between 0% and 75% genetic composition. Several significant differences in mean percent survivorship between the control and smoke-exposed pups were also found. For example, control pups with 75% of their genes derived from strain C3H (produced by C3H x BALB/c crosses and backcrosses) had a mean percent survivorship that was significantly greater than that of sidestream smoke-exposed pups (63.8% for the controls in contrast to 46.0% for the experimentals; $P < 0.001$). Our findings suggest that both sidestream smoke and genetic composition significantly affect pup survivorship.

KEYWORDS: Genetic composition, passive smoking, sidestream smoke, survivorship, tobacco smoke exposure.

INTRODUCTION

Tobacco smoking is a major cause of morbidity and mortality. Smoking is responsible for an estimated 434,000 premature deaths each year in the United States (Center for Disease Control, 1988) and up to 3 million each year worldwide (Schwarz and Schmeiser-Rieder, 1996). Many of the deleterious substances found in mainstream smoke are also present in sidestream smoke. These substances include ammonia, carbon monoxide, aromatic amines, nitrosamines, and polycyclic hydrocarbons (U.S. Department of Health and Human Services, 1986). The concentrations of tar, nicotine, carbon monoxide, and carbon dioxide as well as benzo(a)pyrene, a known carcinogen, are 2 to 12 times higher in sidestream smoke than in mainstream smoke (Adams, *et al.*, 1987; Eatough, *et al.*, 1990; Guerin, *et al.*, 1992; Morris, 1995). Aqueous extracts from cigarette tar can bind to and nick DNA (Stone, *et al.*, 1995).

Because of these disturbing facts, concern is growing that involuntary exposure to sidestream smoke (passive smoking) produces risks for many of the same health hazards as does active smoking. Sidestream smoke exposure decreases pulmonary function (Sun, *et al.*, 1995), is associated with asthma and other respiratory illness in the children of active smokers (Chilmonczyk, *et al.*, 1993; Cunningham, *et al.*, 1996; Halken, *et al.*, 1995), and increases adult non-neoplastic lung disease (Tredaniel, *et al.*, 1994). Passive smoking also increases the risk of lung cancer (Brownson, *et al.*, 1992; Liu, *et al.*, 1993; Morris, 1995; Stockwell, *et al.*, 1992) and heart disease (Glantz and Parmley, 1995; Kawachi, *et al.*, 1997; Steenland, 1992). Overall estimates of the number of annual passive smoking deaths range from 39,000 to 53,000 (Wells, 1988).

Ample evidence exists to show that maternal smoking during pregnancy constitutes a hazard to the fetus. Smoking retards fetal growth (Nash and Persaud, 1988; Wilcox, 1993), producing low birth weight offspring (Chen, *et al.*, 1989; Lazzaroni, 1990) and increases the risks of spontaneous perinatal death and neonatal morbidity (Abel, 1980; U.S. Department of Health, Education, and Welfare, 1979). Whether or not pregnant women exposed to sidestream smoke expose the fetus to such risks is less clear. Recent research seems to indicate that fetal growth is reduced by exposure to sidestream smoke in pregnant women (Chen, *et al.*, 1989; Chen and Petitti, 1995; Fortier, *et al.*, 1994). Passive smoking during pregnancy is also a risk factor for persistent pulmonary hypertension in the newborn (Bearer, *et al.*, 1997). A variety of studies on pregnant animals exposed to sidestream smoke have demonstrated some of the same deleterious offspring effects experienced by the fetus of an active smoker (Abel, 1980; Bassi, *et al.*, 1984; Essenberg, *et al.*, 1940; Leichter, 1993; Mays, 1986; Mays, *et al.*, 1988, 1993; Mays, *et al.*, 1994; Reznik and Marquard, 1980; Vahakangas, *et al.*, 1982).

Although recent research has substantiated individual variability among both humans and other animals to the effects of tobacco smoke and nicotine (Overstreet, 1995; Parrot, 1994; Perkins, 1995; Shiffman, 1989; Swan, *et al.*, 1993), concern is growing that the potential effect of passive smoking among pregnant women is a serious issue (Chen and Petitti, 1995). This concern exists because of the prevalence of passive smoking among women of reproductive age and the fact that some substances in tobacco smoke can cross the placental barrier (Smith and Austen, 1982).

Any study of the effects of an environmental factor on phenotypic variation (V_P) has the inherent problem of separating the genetic variation (V_G) from that of the treatment (V_E). In this investigation, we used three inbred strains of mice and their hybrids to evaluate whether or not maternal exposure to sidestream smoke affects pup survival and to see if genetically based differences in survivorship exist.

MATERIALS AND METHODS

Three sets of crosses involving three inbred mouse strains, BALB/cAnHsd, C57BL/6NHsd, and C3H/HeNHsd, were performed following Hayes (1987).

Briefly, the three strains were crossed in all possible combinations, and the pregnant females were exposed to sidestream smoke. For any one set of crosses, the pups either contained 50% of their genes from each parental strain (i.e., hybrid pups) or 25% of their genes from one of the parental strains and 75% of their genes from another strain. We also collected data on purebred pups for comparison. Throughout the paper the crosses will simply be referred to as either BALB/c x C57BL, C57BL x C3H, or C3H x BALB/c with symbol designates as: B = BALB/c, C = C57BL, 3 = C3H, and H = hybrid. The first letter of a cross represents the dam. For example, BxH(BxC) is the cross of a BALB/c dam and a hybrid (H) sire whose mother was BALB/c and whose father was C57BL. Seventy-five percent of the genes in the pups from such a cross will be BALB/c in origin.

Virgin female mice were mated overnight with appropriate males. The day a copulation plug was visually observed was designated as gestation day 1. Pregnant mice were maintained in separate cages under controlled environmental conditions for room temperature (20-25° C), humidity (55-60%), and photoperiod (12 hr light, 12 hr dark). They were fed Lab Blox pellets (Wayne Feed Division, Continental Grain Co.) and water *ad libitum* between trials.

Experimental mice (pregnant dams) were placed in a Plexiglas smoking chamber (25.5 cm x 30.5 cm x 21.0 cm) containing 6 air holes, each 6.5 mm in diameter, on two opposite sides and exposed each day of gestation (21 days) to sidestream smoke from one commercial brand filter-tip cigarette. The tar and nicotine content of the cigarettes used was 15.1 mg and 0.13 mg, respectively. These values are approximately in the mid-range of the tar and nicotine content of 25 popular commercial cigarette brands (ranging from a low of 1.0 mg tar/0.13 mg nicotine to a high of 29.8 mg tar/2.03 mg nicotine (Federal Trade Commission, 1997)). The duration of each smoking session was 40 minutes. At the end of each treatment period, the dams were returned to their respective cages. Treatment was terminated at parturition, and the dams were allowed to nurture their pups until they were weaned at day 21. On postnatal day 1 (within 16 hr of birth), each experimental pup was sexed and weighed. Percent survivorship values were calculated for each litter and were based on the number of pups alive at day 21 relative to the number of pups born. Control mice were treated in the same manner as the experimentals except that the smoking chamber contained an unlit cigarette.

Statistical analyses were made using the Minitab program. Percent survivorship values were arcsine transformed and analyzed separately for each set of crosses using a Model I ANOVA. The outcome of the ANOVA served as the basis for Tukey's multiple comparison test (Zar, 1984) to test for differences in the percent survivorship among control and smoke-exposed pups with different percent genetic composition values. Differences in percent survivorship between the control and experimental pups at different percent genetic composition values were also determined by this method. Experiment-wise error rates were set at $\alpha = 0.05$.

Table 1. Mean pup survivorship based on % BALB/c genetic composition produced by BALB/c (B) x C57BL (C) crosses and backcrosses.

Treatment	% BALB/c Genetic Composition	N	Cross	Mean % Survivorship[1]	Standard Deviation[2]
CON[3]	0% (100% C57BL)	$\sum$ = **70**	CxC	$\bar{x}$ = **56.445**	2.679
	25% (75% C57BL)	20	CxH(BxC)	74.740	
		23	CxH(CxB)	80.594	
		36	H(BxC)xC	84.130	
		17	H(CxB)xC	84.618	
		$\sum$ = **96**		$\bar{x}$ = **81.021**	2.288
	50% (50% C57BL)	32	BxC	72.725	
		38	CxB	79.364	
		43	H(BxC)	80.238	
		17	H(CxB)	79.508	
		$\sum$ = **130**		$\bar{x}$ = **77.959**	1.966
	75% (25% C57BL)	34	BxH(BxC)	77.849	
		29	BxH(CxB)	78.670	
		14	H(BxC)xB	84.956	
		16	H(CxB)xB	80.542	
		$\sum$ = **93**		$\bar{x}$ = **80.504**	2.325
	100% (0% C57BL)	$\sum$ = **40**	BxB	$\bar{x}$ = **76.400**	3.545
EXP[3]	0% (100% C57BL)	$\sum$ = **34**	CxC	$\bar{x}$ = **40.120**	4.314
	25% (75% C57BL)	16	CxH(BxC)	66.607	
		33	CxH(CxB)	50.367	
		16	H(BxC)xC	81.461	
		14	H(CxB)xC	75.483	
		$\sum$ = **79**		$\bar{x}$ = **68.480**	2.522
	50% (50% C57BL)	67	BxC	68.547	
		30	CxB	73.662	
		85	H(BxC)	71.006	
		13	H(CxB)	76.148	
		$\sum$ = **195**		$\bar{x}$ = **72.341**	1.605
	75% (25% C57BL)	12	BxH(BxC)	80.239	
		16	BxH(CxB)	71.659	
		29	H(BxC)xB	81.707	
		15	H(CxB)xB	79.529	
		$\sum$ = **72**		$\bar{x}$ = **78.284**	2.642
	100%	$\sum$ = **26**	BxB	$\bar{x}$ = **67.781**	4.396

[1] Arcsine transformed values.

[2] Standard deviations determined by a Model I ANOVA.

[3] CON = Control; EXP = Experimental.

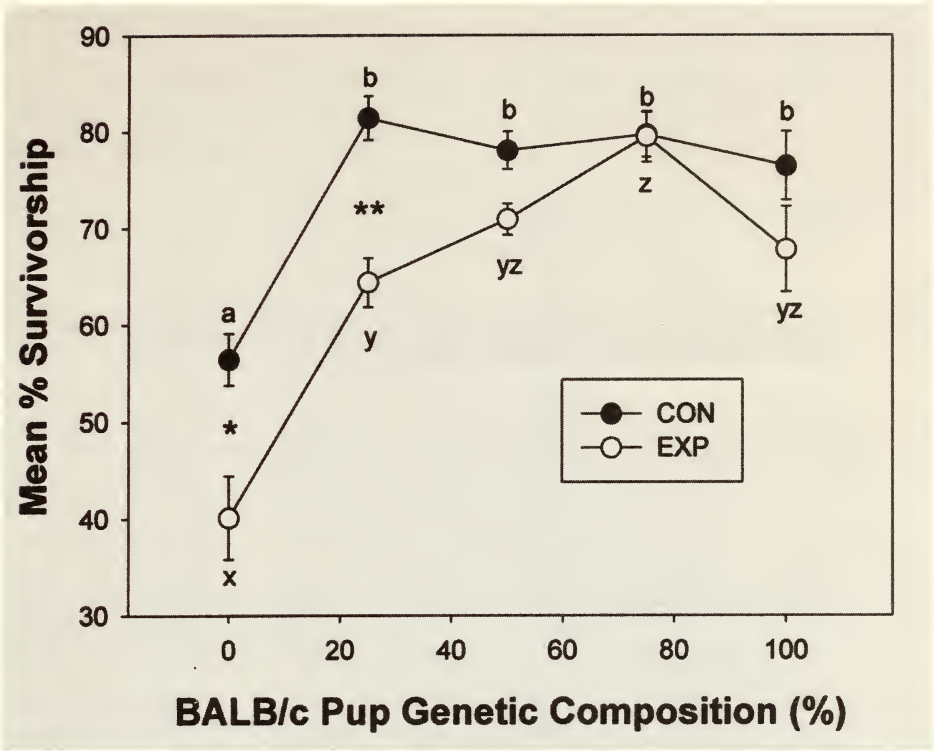

Figure 1. A comparison of the arcsine tranformed control and experimental pup mean percent survivorship values based on the percent BALB/c genetic composition produce by BALB/c x C57BL crosses and backcrosses. The *P* values were determined using Tukey's multiple comparison test (* = significant at $P < 0.05$; ** = significant at $P < 0.001$). Means with no letters in common are significantly different at $P < 0.001$.

RESULTS

Arcsine transformed percent survivorship values for the BALB/c x C57BL, C57BL x C3H, and C3H x BALB/c crosses are presented in Tables 1-3, respectively. Analysis of variance of the BALB/c x C57BL crosses indicated that both the percent genetic composition of the pups and sidestream smoke exposure significantly affected pup percent survivorship ($P < 0.001$; Table 1). Analysis of variance also indicated significant interaction between the two factors (Table 4a). Tukey's multiple comparison tests were performed to distinguish differences in the percent survivorship values both among and between control and smoke-exposed pups with different percent genetic composition values. The percent survivorship of control pups with 0% BALB/c (100% C57BL) genes differed significantly ($P < 0.001$) from that of pups with 25%, 50%, 75%, and 100% BALB/c genes (Table 5). Experimental pups displayed similar percent survivorship differences, but in addition, the percent survivorship of pups with 25% BALB/c genetic composition differed significantly ($P < 0.001$) from that of pups

Table 2. Mean pup survivorship based on % C57BL genetic composition produced by C57BL x C3H crosses and backcrosses.

Treatment	% C57BL Genetic Composition	N	Cross	Mean % Survivorship[1]	Standard Deviation[2]
CON + EXP[3]	0% (100% C3H)	32 (Con)	3x3	64.394	
		34 (Exp)	3x3	61.865	
		Σ = 66		**$\bar{x}$ = 63.130**	3.038
	25% (75% C3H)	20 (Con)	3xH(Cx3)	61.272	
		19 (Con)	3xH(3xC)	67.740	
		21 (Con)	H(Cx3)x3	87.367	
		20 (Con)	H(3xC)x3	88.105	
		80 (Con)		**76.121**	
		20 (Exp)	3xH(Cx3)	48.407	
		21 (Exp)	3xH(3xC)	53.411	
		21 (Exp)	H(Cx3)x3	78.423	
		21 (Exp)	H(3xC)x3	86.311	
		83 (Exp)		**66.638**	
		Σ = 163		**$\bar{x}$ = 71.380**	1.933
	50% (50% C3H)	16 (Con)	Cx3	62.455	
		18 (Con)	3xC	76.512	
		12 (Con)	H(Cx3)	77.943	
		18 (Con)	H(3xC)	85.000	
		64 (Con)		**75.478**	
		29 (Exp)	Cx3	55.970	
		21 (Exp)	3xC	76.513	
		21 (Exp)	H(Cx3)	75.605	
		22 (ExpP	H(3xC)	78.302	
		93 (Exp)		**71.598**	
		Σ = 157		**$\bar{x}$ = 73.538**	2.003
	75% (25% C3H)	16 (Con)	CxH(Cx3)	78.821	
		21 (Con)	CxH(3xC)	76.641	
		21 (Con)	H(Cx3)xC	85.458	
		21 (Con)	H(3xC)xC	83.425	
		79 (Con)		**81.092**	
		20 (Exp)	CxH(Cx3)	60.633	
		14 (Exp)	CxH(3xC)	69.255	
		17 (Exp)	H(Cx3)xC	79.330	
		14 (Exp)	H(3xC)xC	87.068	
		65 (Exp)		**74.073**	
		Σ = 144		**$\bar{x}$ = 48.283**	2.066
	100% (0% C3H)	70 (Con)	CxC	54.445	
		34 (Exp)	CxC	40.121	
		Σ = 104		**$\bar{x}$ = 48.283**	2.794

[1] Arcsine transformed values.

[2] Standard deviations determined by a Model I ANOVA.

[3] CON + EXP = Control + Experimental.

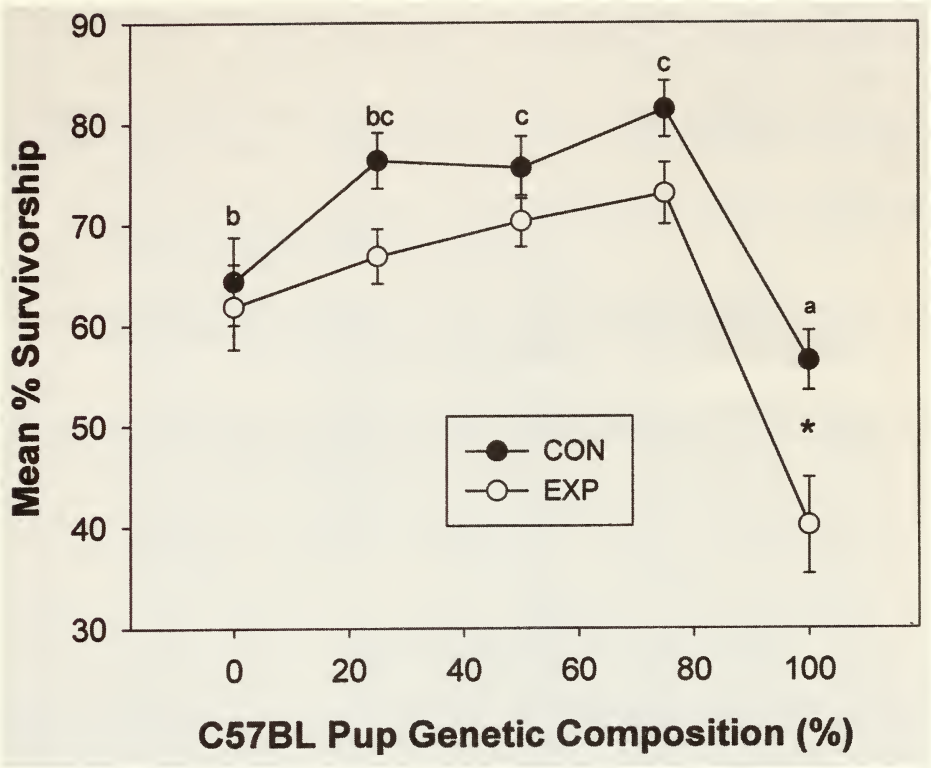

Figure 2. A comparison of the arcsine transformed control and experimental pup mean percent survivorship values based on the percent C57BL genetic composition produced by C57BL x C3H crosses and backcrosses. The values of P were determined using Tukey's multiple comparison test (* = significant at $P < 0.05$). Means with no letters in common are significantly different at $P < 0.05$.

with 75% BALB/c genetic composition. Control pups with 0% and 25% BALB/c genetic composition had significantly higher percent survivorship values than did the corresponding experimental pups. At 0% genetic composition, control pup percent survivorship was 56.5%, and experimental pup percent survivorship was 40.1% ($P < 0.05$); at 25% genetic composition, control pup percent survivorship was 81.0%, and experimental pup percent survivorship was 68.5% ($P < 0.001$; Figure 1).

Analysis of variance of the C57BL x C3H crosses showed that both the percent genetic composition of the pups and the effects of sidestream smoke exposure significantly affected pup percent survivorship ($P < 0.001$; Table 2). No interaction between the percent C57BL genetic composition of the pups and smoke exposure was indicated (Table 4b). Therefore, Tukey's multiple comparison test was performed on the pooled percent survivorship values of the control and experimental pups to determine differences in percent survivorship between groups with different genetic compositions. The percent survivorship

Table 3. Mean pup survivorship based on % C3H genetic composition produced by C3H x BALB/c crosses and backcrosses.

Treatment	% C3H Genetic Composition	N	Cross	Mean % Survivorship[1]	Standard Deviation[2]
CON + EXP[3]	0% (100% BALB/c)	40 (Con)	BxB	76.400	
		26 (Exp)	BxB	67.781	
		Σ = 66		**$\bar{x}$ = 72.091**	3.000
	25 % (75% BALB/c)	24 (Con)	BxH(Bx3)	77.172	
		31 (Con)	BxH(3xB)	70.772	
		32 (Con)	H(Bx3)xB	83.969	
		32 (Con)	H(3xB)xB	82.806	
		119 (Con)		**78.681**	
		30 (Exp)	BxH(Bx3)	61.570	
		22 (Exp)	BxH(3xB)	61.227	
		26 (Exp)	H(Bx3)xB	74.389	
		20 (Exp)	H(3xB)xB	78.000	
		98 (Exp)		**68.797**	
		Σ = 217		**$\bar{x}$ = 73.739**	1.624
	50% (50% BALB/c)	20(Con)	Bx3	75.761	
		13 (Con)	3xB	74.113	
		30 (Con)	H(Bx3)	75.711	
		32 (Con)	H(3xB)	71.256	
		95 (Con)		**74.210**	
		25 (Exp)	Bx3	74.191	
		36 (Exp)	3xB	62.475	
		24 (Exp)	H(Bx3)	74.014	
		28 (ExpP	H(3xB)	65.395	
		113 (Exp)		**69.041**	
		Σ = 208		**$\bar{x}$ = 71.626**	1.648
	75% (25% BALB/c)	31 (Con)	3xH(Bx3)	59.308	
		32 (Con)	3xH(3xB)	44.143	
		26 (Con)	H(Bx3)x3	79.594	
		30 (Con)	H(3xB)x3	72.328	
		119 (Con)		**63.843**	
		21 (Exp)	3xH(Bx3)	19.945	
		24 (Exp)	3xH(3xB)	23.065	
		23 (Exp)	H(Bx3)x3	72.773	
		25 (Exp)	H(3xB)x3	68.247	
		93 (Exp)		**46.007**	
		Σ = 212		**$\bar{x}$ = 54.925**	1.648
	100% (0% BALB/c)	32 (Con)	3x3	64.394	
		34 (Exp)	3x3	61.865	
		Σ = 104		**$\bar{x}$ = 63.130**	2.933

[1] Arcsine transformed values.

[2] Standard deviations determined by a Model I ANOVA.

[3] CON + EXP = Control + Experimental

Table 4. Summary of results generated by a Model I ANOVA of arcsine transformed % survivorship values attributed to the percent genetic composition of three inbred mouse strains: **A**) %BALB/c genetic composition (BALB/c x C57BL crosses and backcrosses); **B**) % C57BL genetic composition (C57BL x C3H crosses and backcrosses); and **C**) % C3H genetic composition (C3H x BALB/c crosses and backcrosses). Significance at $P < 0.05$.

	ANOVA: Linear Model I	*F*	*P*
A	BALB/c Genetic Comp (%)	27.36	0.000
	BALB/c Smoke Effects	27.56	0.000
	Interaction (Genetic Comp and Smoke Effects)	3.60	0.006
B	C57BL Genetic Comp (%)	20.09	0.000
	C57BL Smoke Effects	15.22	0.000
	Interaction (Genetic Comp and Smoke Effects)	0.900	.462
C	C3H Genetic Comp (%)	20.14	0.000
	C3H Smoke Effects	18.63	0.000
	Interaction (Genetic Comp and Smoke Effects)	1.74	0.139

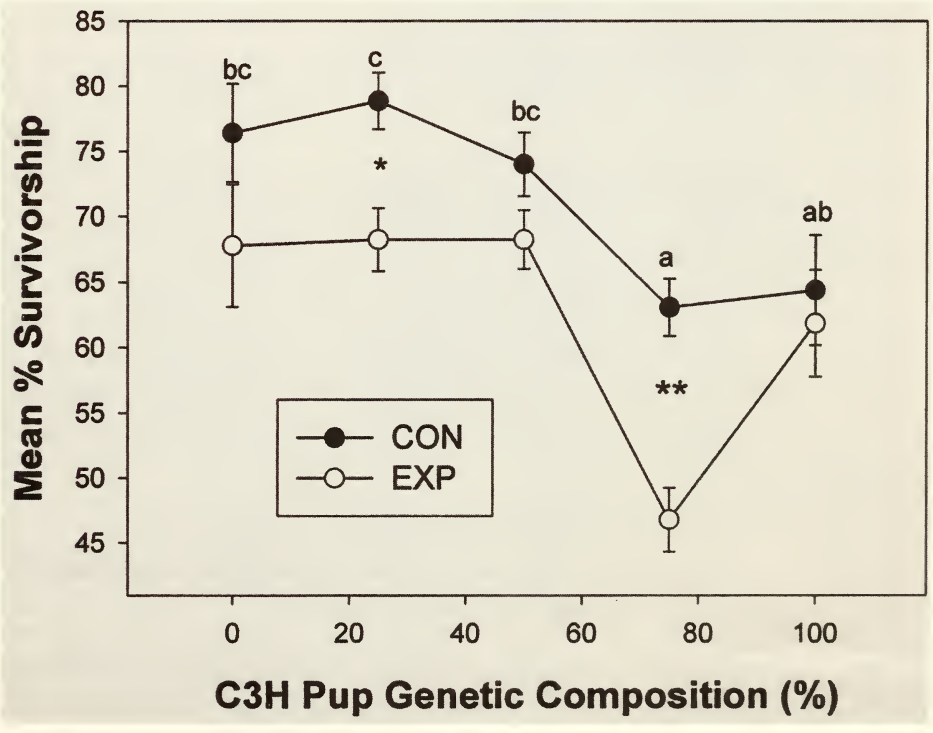

Figure 3. A comparison of the arcsine transformed control and experimental pup mean percent survivorship values based on the percent C3H genetic compostion produced by C3H x BALB/c crosses and backcrosses.The *P* values were determined using Tukey's multiple comparison test (* = significant at $P < 0.05$; ** = significant at $P < 0.001$). Means with no letters in common are significantly different at $P < 0.05$.

Table 5. Tukey's multiple comparison tests of the differences between pup survivorship at different percent BALB/c genetic composition values produced by BALB/c x C57BL crosses.

Treatment	Groups Compared (% BALB/c Genetic Composition)	q
Controls	0 vs 25	10.017**
	0 vs 50	9.186**
	0 vs 75	9.244**
	0 vs 100	6.300**
	25 vs 50	1.598
	25 vs 75	0.774
	25 vs 100	1.689
	50 vs 75	0.759
	50 vs 100	0.572
	75 vs 100	1.093
Experimentals	0 vs 25	7.668**
	0 vs 50	9.466**
	0 vs 75	10.994**
	0 vs 100	6.350**
	25 vs 50	3.078
	25 vs 75	6.516**
	25 vs 100	0.905
	50 vs 75	3.904
	50 vs 100	0.946
	75 vs 100	3.209
Controls vs Experimentals	0 vs 0	4.549*
	25 vs 25	7.060**
	50 vs 50	3.976
	75 vs 75	0.080
	100 vs 100	2.158

* Significant at $P = 0.05$
** Significant at $P = 0.001$

of the pups with 0% C57BL genetic composition differed significantly from that of pups with 50% ($P < 0.05$), 75% ($P < 0.005$), and 100% ($P < 0.005$) C57BL genetic composition. The percent survivorship of pups with 25%, 50%, and 75% C57BL genetic composition differed significantly from that of pups with 100% C57BL genetic composition ($P < 0.001$; Table 6). Control pups with 100% C57BL genetic composition had a significantly higher percent survivorship than corresponding smoke-exposed pups (54.5% in contrast to 40.1%; $P < 0.05$; Figure 2).

Analysis of variance of the C3H x BALB/c crosses showed that both the percent C3H genetic composition of the pups and sidestream smoke exposure sig-

Table 6. Tukey's multiple comparison tests of the differences between pup survivorship at different percent C57BL genetic composition values produced by C57BL x C3H crosses.

Treatment	Groups Compared (% C57BL/Genetic Composition)	q
Controls + Experimentals	0 vs 25	3.355
	0 vs 50	3.901*
	0 vs 75	5.445**
	0 vs 100	5.384**
	25 vs 50	0.710
	25 vs 75	2.847
	25 vs 100	10.573***
	50 vs 75	2.118
	50 vs 100	11.061***
	75 vs 100	12.775***
Controls vs Experimentals	0 vs 0	0.597
	25 vs 25	3.483
	50 vs 50	1.949
	75 vs 75	3.209
	100 vs 100	4.493*

* Significant at $P = 0.05$
** Significant at $P = 0.005$
*** Significant at $P = 0.001$

nificantly affected percent survivorship ($P < 0.001$; Table 3). No interaction between the percent C3H genetic composition and smoke exposure was indicated (Table 4c). Due to the lack of interaction, Tukey's multiple comparison test was run on the pooled percent survivorship values of the control and experimental pups to determine differences in percent survivorship between groups with different genetic compositions. The percent survivorship of pups with 0% C3H genetic composition differed significantly from that of pups with 75% C3H genetic composition ($P < 0.001$). The percent survivorship of pups with 25% C3H genetic composition differed significantly from that of pups with C3H genetic composition values of 75% ($P < 0.001$) and 100% ($P < 0.025$), and the percent survivorship of pups with 50% C3H genetic composition differed significantly from that of pups with 75% C3H genetic composition ($P < 0.001$). Control pups with 25% and 75% C3H genetic composition had significantly higher percent survivorship values than did the corresponding experimental pups. At 25% C3H genetic composition, control pup percent survivorship was 78.7%, and experimental pup percent survivorship was 68.8% ($P < 0.05$). At 75% C3H genetic composition, control pup percent survivorship was 63.8%, and experimental pup percent survivorship was 46.0% ($P < 0.001$; Figure 3).

Table 7. Tukey's multiple comparison tests of the differences between pup survivorship at different percent C3H genetic composition values produced by C3H x BALB/c crosses.

Treatment	Groups Compared (% C3H Genetic Composition)	*q*
Controls + Experimentals	0 vs 25	0.619
	0 vs 50	0.40
	0 vs 75	7.241 ***
	0 vs 100	3.072
	25 vs 50	1.482
	25 vs 75	11.466 ***
	25 vs 100	4.420 **
	50 vs 75	9.870 ***
	50 vs 100	3.376
	75 vs 100	3.469
Controls vs Experimentals	0 vs 0	2.032
	25 vs 25	4.629 *
	50 vs 50	2.490
	75 vs 75	7.056 ***
	100 vs 100	0.613

* Significant at $P = 0.05$
** Significant at $P = 0.025$
*** Significant at $P = 0.001$

In order to determine if the sex of the pups had an effect on percent survivorship, we calculated the pup sex ratios for all control and experimental crosses and backcrosses for day 1 and day 21. In each case, the sex ratio was approximately 1.0. Therefore, the sex of the pups did not appear to have a significant effect on percent survivorship.

DISCUSSION

In this investigation, we examined the effects of sidestream smoke exposure *in utero* and pup genetic composition on mean percent survivorship of mouse pups produced by BALB/c (B) x C57BL (C), C57BL (C) x C3H (3), and C3H (3) x BALB/c (B) crosses (Tables 1-3). The results showed that pup percent survivorship values for all three crosses were significantly affected both by the genetic composition of the pups and by sidestream smoke exposure. In addition, our results indicated that significant interaction between the two factors (percent genetic composition and smoke exposure) occurred only with the BALB/c x C57BL crosses.

Variability in the percent survivorship of pups with different genetic composition was also apparent. For example, the percent survivorship of both the control (56.5%) and experimental pups (40.1%) with 0% BALB/c (100% C57BL) genetic composition differed significantly from the percent survivorship of the control (81.0%) and experimental pups (68.5%) with 25% BALB/c (75% C57BL) genetic composition (Table 5). Neither the C57BL x C3H nor the C3H x BALB/c crosses produced significant percent survivorship differences between 0% and 25% genetic composition. The only difference in the genetic composition of BALB/c, C57BL, and C3H pups that resulted in consistently significant differences in percent survivorship was between 0% and 75% genetic composition.

Several significant differences in percent survivorship between the control and smoke-exposed pups were also noted. In the BALB/c x C57BL crosses, both 0% BALB/c genetic composition (56.5% controls/40.1% experimentals) and 25% BALB/c genetic composition (81.0% controls/68.5% experimentals) produced significant differences in pup percent survivorship. The percent survivorship of the control pups produced by C57BL x C3H crosses that had 100% C57BL genetic composition was significantly different from the percent survivorship of smoke-exposed pups (54.5% controls/40.1% experimentals). Control pups with 75% C3H genetic composition produced by C3H x BALB/c crosses had a percent survivorship that differed significantly from that of smoke-exposed pups (63.8% controls/ 46.0% experimentals).

Overall, our results suggest that, while sidestream smoke clearly affects pup survival, genetic factors contribute to the variation in susceptibility of pups to the effects of maternal exposure to sidestream smoke.

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