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Genotoxicity of Two Arsenic Compounds in Germ Cells and Somatic Cells of *Drosophila melanogaster*

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Two arsenic compounds, sodium arsenite ($NaAsO_2$) and sodium arsenate (Na_2HAsO_4), were tested for their possible genotoxicity in germinal and somatic cells of *Drosophila melanogaster*. For germinal cells, the sex-linked recessive lethal test (SLRLT) and the sex chromosome loss test (SCLT) were used. In both tests, a brood scheme of 2-3-3 days was employed. Two routes of administration were used for the SLRLT: adult male injection (0.38, 0.77 mM for sodium arsenite; and 0.54, 1.08 mM for sodium arsenate) and larval feeding (0.008, 0.01, 0.02 mM for sodium arsenite; and 0.01, 0.02 mM for sodium arsenate). For the SCLT the compounds were injected into males. Controls were treated with a solution of 5% sucrose which was employed as solvent. The somatic mutation and recombination test (SMART) was run in the w^+/w eye assay as well as in the *mwh* +/+ flr^3 wing test, employing the standard and insecticide-resistant strains. In both tests, third instar larvae were treated for 6 hr with sodium arsenite (0.38, 0.77, 1.15 mM), and sodium arsenate (0.54, 1.34, 2.69 mM). In the SLRLT, both compounds were positive, but they were negative in the SCLT. The genotoxicity of both compounds was localized mainly in somatic cells, in agreement with reports on the carcinogenic potential of arsenical compounds. Sodium arsenite was an order of magnitude more toxic and mutagenic than sodium arsenate. This study confirms the reliability of the Drosophila in vivo system to test the genotoxicity of environmental compounds.

Key words: ***Drosophila melanogaster*, genotoxicity, sex-linked recessive lethal test (SLRLT), sex chromosome loss test (SCLT), somatic mutation and recombination test (SMART), sodium arsenite, sodium arsenate**

INTRODUCTION

Arsenic is a ubiquitous toxic, environmental pollutant; it is found in several chemical forms, with the most common being the trivalent and the pentavalent forms [Stokinger, 1981]. Important emission sources of arsenic into the environment include nonferrous smelting operations and certain coal-fired power plants [Alloway, 1990; Koren, 1991; Manahan, 1991]. Anthropogenic or natural arsenic pollution of water in different parts of the world has produced symptoms of poisoning in humans, as manifested by skin, gastrointestinal, and neurological disorders [Cebrian et al., 1983; Abdelghani et al., 1986; Frost et al., 1987; Roses et al., 1991].

Sodium arsenite induced mutations in WP2 and WP2 *uvrA* strains of *Escherichia coli*, but not in *CM571*, suggesting that the *rec* function is implicated in arsenic-induced mutagenesis, which may be dependent on error-prone (SOS) repair [Nishioka, 1975]. On the other hand, Rossman et al. [1980] reported that sodium arsenite did not increase the mutation frequency in five *E. coli* strains carrying different mutations for repair ability. In the Salmonella microsome assay, arsenite and arsenate were negative [Löfroth and Ames, 1978]; arsenite was also negative for gene conversion at the *trp* locus, and was weakly positive for reverse mutations in the *ivl* locus of *Saccharomyces cerevisiae* [Singh, 1983]. Arsenite also did not produce forward gene mutations in Chinese hamster cells at the *HPRT* or the sodium-potassium ATPase loci in vitro [Rossman et al., 1980]. Sodium arsenate was positive in the sex chromosome loss test of Drosophila [Ahmed and Walker, 1972; Walker and Bradley, 1969].

Arsenite is also clastogenic and induces sister chromatid exchanges (SCE) in a variety of cell types, including human cells in vitro [Crossen, 1983; Jha et al., 1992]; trivalent arsenic is approximately one order of magnitude more potent than the pentavalent form [Wan et al., 1982]. However, sodium arsenite does not appear to induce chromosomal aberrations in vivo in experimental animals [Poma et al., 1981], although several studies suggest that humans exposed to arsenic demonstrate higher frequencies of SCE and chromosomal aberrations in peripheral lymphocytes [Wan et al., 1982; Jha et al., 1992], and have an impairment of lymphocyte proliferation [Gonsebatt et al., 1992]. In addition, arsenic may inhibit the DNA repair process [Okui and Fujiwara, 1986; Nordenson and Beck-

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TABLE I. Frequencies of SLRLs in Adult *D. melanogaster* Males After Injection With $NaAsO_2$ and Na_2HAsO_4

Compound	Brood	Treated males	F_1 ♀♀	Fertile ♀♀	Lethals (%)	Activity*
Control	A	588	6,285	5,223	7 (0.13)	
	B	454	6.089	5,276	4 (0.08)	
	C	415	6,613	5,639	2 (0.03)	
	A + B + C	1,457	18,987	16,138	13 (0.08)	
$NaAsO_2$						
0.38 mM	A	259	2,450	2,183	3 (0.14)	
	B	180	2,622	2,228	3 (0.13)	
	C	165	2,589	2,122	0	
	A + B + C	604	7,661	6,533	6 (0.09)	
0.77 mM	A	367	3,171	2,516	12 (0.48)	$P < 0.01$
	B	240	2,860	2,401	10 (0.42)	$P < 0.01$
	C	199	2,275	1,885	6 (0.32)	$P < 0.01$
	A + B + C	806	8,306	6,802	28 (0.41)	$P < 0.01$
Na_2HAsO_4						
0.54 mM	A	300	4,190	3,611	10 (0.28)	
	B	186	2,014	1,829	1 (0.05)	
	C	180	2,486	2,214	1 (0.05)	
	A + B + C	666	8,690	7,654	12 (0.16)	
1.08 mM	A	300	3,574	3,065	7 (0.23)	
	B	158	2,139	1,943	0	
	C	147	1,836	1,688	5 (0.30)	$P < 0.05$
	A + B + C	605	7,549	6,696	12 (0.18)	$P < 0.05$

*Statistical diagnosis: Kastenbaum-Bowman test. All lethal mutations scored were independent.

TABLE II. Frequencies of SLRLs After *D. melanogaster* Larval Feeding with $NaAsO_2$ and Na_2HAsO_4

Compound	Brood	Treated males	F_1 ♀♀	Fertile ♀♀	Lethals (%)	Activity*
Control	A	497	4,050	3,167	2 (0.06)	
	B	449	3,163	2,620	2 (0.08)	
	C	418	3,157	2,516	0	
	A + B + C	1,364	10,370	8,303	4 (0.05)	
$NaAsO_2$						
0.008 mM	A	159	1,249	965	0	
	B	133	1,404	1,022	5 (0.49)	$P < 0.05$
	C	125	756	556	0	
	A + B + C	417	3,409	2,543	5 (0.20)	$P < 0.05$
0.01 mM	A	166	1,173	863	1 (0.12)	
	B	125	988	584	0	
	C	103	592	382	0	
	A + B + C	394	2,753	1,829	1 (0.05)	
0.02 mM	A	100	776	489	0	
	B	89	643	455	4 (0.88)	$P < 0.01$
	C	75	175	101	0	
	A + B + C	264	1,594	1,045	4 (0.38)	$P < 0.01$
Na_2HAsO_4						
0.01 mM	A	200	1,945	1,599	12 (0.75)	$P < 0.01$
	B	181	2,299	1,980	1 (0.05)	
	C	168	1,398	1,115	0	
	A + B + C	549	5,642	4,694	13 (0.28)	$P < 0.01$
0.02 mM	A	200	2,151	1,727	3 (0.17)	
	B	188	2,080	1,801	0	
	C	180	1,535	1,218	1 (0.08)	
	A + B + C	568	5,766	4,746	4 (0.08)	

*Statistical diagnosis: Kastenbaum-Bowman test. All lethal mutations scored were independent.

TABLE III. Results Obtained in the SCLT After $y/B^{S}Yy^{+}$ Males Were Injected With $NaAsO_2$*

Compound	Brood	F_1 ♀♀	F_1 ♂♂	F_1 total	CL %	PL %	♀♀ XXY
Control	A	4,408	3,547	7,955	7 (0.09)		1 (0.01)
	B	3,733	4,123	7,856	2 (0.03)		2 (0.02)
	C	4,688	4,084	8,772	4 (0.05)	1 (0.01)	3 (0.03)
	A + B + C	12,829	11,754	24,583	13 (0.05)	1 (0.004)	5 (0.02)
$NaAsO_2$							
0.38 mM	A	2,636	2,084	4,720	6 (0.13)		1 (0.02)
	B	2,267	1,992	4,259	2 (0.05)		1 (0.02)
	C	2,604	2,292	4,896	1 (0.02)		3 (0.06)
	A + B + C	7,509	6,368	13,875	9 (0.06)		5 (0.04)
0.77 mM	A	2,409	2,087	4,496	3 (0.06)		
	B	2,462	2,132	4,594	3 (0.06)		
	C	2,450	2,138	4,588	4 (0.09)	2 (0.04)	
	A + B + C	7,321	6,357	13,678	10 (0.07)	2 (0.01)	
1.15 mM	A	2,826	2,356	5,182	3 (0.06)		2 (0.04)
	B	2,533	2,266	4,799	1 (0.02)		
	C	2,651	2,399	5,050	2 (0.04)		1 (0.02)
	A + B + C	8,010	7,021	15,031	6 (0.04)		3 (0.02)
Na_2HAsO_4							
0.54 mM	A	1,573	1,390	2,963		2 (0.07)	1 (0.03)
	B	1,238	925	2,163	3 (0.14)		
	C	1,421	1,167	2,588	1 (0.04)		2 (0.08)
	A + B + C	4,232	3,482	7,714	4 (0.05)	2 (0.03)	3 (0.04)
1.34 mM	A	1,688	1,359	3,047	2 (0.07)		
	B	1,280	1,093	2,373	1 (0.04)		1 (0.04)
	C	1,552	1,252	2,804			
	A + B + C	4,520	3,704	8,224	3 (0.04)		1 (0.01)
2.69 mM	A	1,444	1,262	2,706	1 (0.04)		1 (0.04)
	B	1,100	1,129	2,229		1 (0.04)	2 (0.09)
	C	1,248	1,132	2,380	1 (0.04)		1 (0.04)
	A + B + C	3,792	3,523	7,315	2 (0.03)	1 (0.01)	4 (0.05)

*Females: $y^2\ w^a/y^2\ w^a$

TABLE IV. Frequency and Number of Mosaic Spots Induced in Wings of Transheterozygous Larvae (*mwh* +/+ *flr*^{3}) After Treatment With Two Arsenic Salts

Compound conc. (mM)	No. of wings	Spots per wing (number of spots): Small single spots (1–2 cells), m = 2	Large single spots (>2 cells), m = 5	Twin spots, m = 5	Total spots, m = 2	Mean clone size class	Frequency of clone formation × 10^{-5}: Observed	Control corrected
5% sucrose	294	0.21 (62)	0.04 (13)	0.02 (5)	0.27 (80)	1.88	1.1	
$NaAsO_2$								
0.38	80	0.19 (15)	0.03 (2)		0.21 (17)	1.65	0.9	−0.2
0.77	80	0.26 (21)	0.03 (2)		0.29 (23)	1.45	1.1	0.0
1.15	77	0.35 (27)$^{+}$	0.03 (2)		0.38 (29)^{i}	1.29	1.5	0.4
Na_2HAsO_4								
0.54	77	0.27 (21)	0.01 (1)		0.27 (21)	1.50	1.1	0.0
1.34	80	0.33 (26)^{i}			0.33 (26)	1.12	1.3	0.2
2.69	79	0.30 (24)^{i}		0.01 (1)	0.32 (25)	1.48	1.3	0.2

*Exposure: 72 × 6 hr. Statistical analysis according to Frei and Würgler [1988]. +, positive; i, inconclusive; m, multiplication factor; m = 2 for small single and total spots; m = 5 for large single and twin spots. Probability levels: alpha = beta = 0.05. One-side statistical tests.

man, 1991; Lee-Chen et al., 1993]. Epidemiological studies have correlated skin and lung cancer with occupational and local exposures; however, no clear association with other types of cancer has been observed [IARC, 1980; Weisburger, 1990; Tinwell et al., 1991].

The cytotoxicity, mutagenicity, clastogenicity, and car-

TABLE V. Frequency and Number of Mosaic Spots Induced in Wings of Transheterozygous Larvae (ORR, *flr*^{3}/*mwh*) After Treatment With Two Arsenic Salts*

Compound conc. (mM)	No. of wings	Spots per wing (number of spots) Small single spots (1–2 cells), m = 2	Large single spots (>2 cells), m = 5	Twin spots, m = 5	Total spots, m = 2	Mean clone size class	Frequency of clone formation × 10^{-5} Observed	Control corrected
5% sucrose	80	0.43 (34)	0.11 (9)	0.01 (1)	0.55 (44)	1.73	2.3	
$NaAsO_2$								
0.38	72	0.29 (21)	0.06 (4)		0.35 (25)	1.75	1.4	−0.9
0.77	70	0.55[i] (40)	0.07 (5)	0.03 (1)	0.64 (45)	1.73	2.7	0.4
1.15	80	0.45 (36)	0.06 (5)		0.51 (41)	1.61	2.1	−0.2
Na_2HAsO_4								
0.54	80	0.12 (10)	0.04 (3)		0.16 (13)	1.69	0.7	−1.6
1.34	76	0.39 (30)	0.12 (9)	0.2 (1)	0.51 (39)	2.12	2.1	−0.2
2.69	80	0.32 (26)	0.06 (5)	0.01 (1)	0.40 (32)	1.72	1.6	−0.7

*Exposure: 72 × 6 hr. Statistical analysis according to Frei and Würgler [1988]. i, inconclusive; m, multiplication factor; m = 2 for small single and total spots, m = 5 for large single and twin spots. Probability level: alpha = beta = 0.05. One-side statistical tests.

TABLE VI. Mosaic Light Spots Induced in *w*/*w*$^+$ Females and *w*$^+$/*Y* Males After Treatment With Sodium Arsenite*

Compound (mM)	No. of eyes	Spots per 100 eyes[a] S 1–4	L >4	T[b]	Average clone size	Clones per 10^4 cells
Females						
5% sucrose	1,116	7.8 (87)	1.8 (20)	9.6 (107)	4.0	9.7
0.38	384	12.8 (49)	3.9 (15)	16.7$^+$ (64)	3.9	16.3
0.77	593	19.1 (113)	5.4 (32)	24.5$^+$ (145)	3.9	23.8
1.15	436	17.7 (77)	3.4 (15)	21.1$^+$ (92)	4.2	22.2
Males						
5% sucrose	839	6.3 (53)	1.7 (14)	8.0 (67)	3.7	7.4
0.38	428	16.4 (70)	6.5 (28)	22.9$^+$ (98)	4.5	25.8
0.77	511	10.8 (55)	2.7 (14)	13.5$^+$ (69)	4.1	13.8
1.15	411	17.0 (17)	2.4 (10)	19.5$^+$ (80)	3.01	14.6

*Exposure: 72 × 6 hr.

[a]Sizes classes: S, small spots (1–4 ommatidia); L, large spots (>4 ommatidia); T, total spots.

[b]Statistical analysis according to Frei and Würgler [1988]. +, positive. Probability levels: alpha = beta = 0.05. One-side statistical tests.

cinogenicity induced by various compounds are enhanced by arsenic salts, which suggests that As is a comutagen [Léonard and Lauwerys, 1980; Lee et al., 1985, 1986; Okui and Fujiwara, 1986; Jan et al., 1990; Li and Rossman, 1991; Lin and Tseng, 1992; Yang et al., 1992; Lee-Chen et al., 1993]. Sodium arsenite is a potent toxin, a carcinogen, and a strong inducer of heat shock proteins and enzymatic activity [Deaton et al., 1990; Laszlo, 1992].

Drosophila melanogaster provides an in vivo test system in which it is possible to analyze heritable mutations and chromosomal aberrations in the same population of treated germ cells [Clark, 1982; Hällström et al., 1984; Zijlstra and Vogel, 1988b]. The sex-linked recessive lethal test (SLRLT) is considered the most reliable and sensitive assay in the fruit fly [Woodruff et al., 1984]. In addition, changes in number (nondisjunction and total loss) and integrity (partial loss) of sex chromosomes can be detected with the sex chromosome loss test (SCLT) [Zimmering, 1987]. It is also possible to distinguish between mutagens (direct action) and promutagens (requiring metabolic activation) employing the brood system [Abrahamson et al., 1983; IPCS, 1985]. In addition, chemical compounds can be administrated by feeding or injecting adult flies or larvae, which addresses different metabolic barriers that chemicals cross to interact with biological macromolecules [Zijlstra and Vogel, 1988a,b]. On the other hand, the somatic mutation and recombination test (SMART) is reliable for determining the somatic cell damage that chemicals induce. Wing and eye cells can be used to evaluate the induction of somatic mutations, deletions, nondisjunction, and mitotic recombinational events [Graf et al., 1984; Vogel and Zijlstra, 1987a,b; Graf and Van Shaik, 1992; Vogel and Nivard, 1993]. In

TABLE VII. Mosaic Light Spots Induced in w/w^+ Females and w^+/Y Males After Treatment With Sodium Arsenate*

Compound (mM)	No. of eyes	Spots per 100 eyes[a] S 1–4	L >4	T[b]	Average clone size	Clones per 10^4 cells
Females						
5% sucrose	1,116	7.8 (87)	1.8 (20)	9.6 (107)	4.0	9.7
0.54	236	9.7 (23)	4.7 (11)	14.4+ (34)	5.2	18.8
1.34	382	13.6 (52)	4.2 (16)	17.8+ (68)	3.8	17.0
2.69	481	15.0 (72)	5.8 (28)	20.8+ (100)	5.5	28.6
Males						
5% sucrose	839	6.3 (53)	1.7 (14)	8.0 (67)	3.7	7.4
0.54	291	13.7 (40)	3.8 (11)	17.5+ (51)	3.7	16.2
1.34	492	8.1 (40)	2.6 (13)	10.8 (53)	4.1	11.0
2.69	776	7.3 (57)	1.5 (12)	8.9 (69)	3.5	7.8

*Exposure: 72 × 6 hr.
[a]Size classes: S, small spots (1–4 ommatidia); L, large spots (>4 ommatidia); T, total spots.
[b]Statistical analysis according to Frei and Würgler [1988]. +, positive. Probability levels: alpha = beta = 0.05. One-side statistical tests.

TABLE VIII. Mosaic Light Spots Induced in w/w^+ Hikone-R Females After Treatment With Sodium Arsenite and Sodium Arsenate*

Compound (mM)	No. of eyes	Spots per 100 eyes[a] S 1–4	L >4	T[b]	Average clone size	Clones per 10^4 cells
5% sucrose	1,025	16.0 (164)	2.3 (24)	18.3 (188)	3.1	14.2
$NaAsO_2$						
0.38	500	23.0 (115)	4.6 (23)	27.6+ (138)	3.0	20.7
0.77	630	22.4 (141)	4.3 (27)	26.7+ (168)	3.3	22.0
1.15	638	26.6 (170)	6.6 (42)	33.2+ (212)	3.1	25.8
Na_2HAsO_4						
0.54	612	19.0 (116)	4.1 (25)	23.0+ (141)	3.8	21.9
1.34	754	19.1 (144)	4.5 (34)	23.6+ (178)	3.8	22.4
2.69	570	16.8 (96)	2.5 (14)	19.3 (110)	2.8	13.5

*Exposure: 72 × 6 hr.
[a]Size classes: S, small spots (1–4 ommatidia); L, large spots (>4 ommatidia); T, total spots.
[b]Statistical analysis according to Frei and Würgler [1988]. +, positive. Probability levels: alpha = beta = 0.05. One-side statistical tests.

addition, considerable genetic variation existing between various Drosophila strains may provide a useful tool for manipulating the xenobiotic-metabolizing enzymes in this mutagenicity test system. A relationship between insecticide resistance and sensitivity towards promutagens has been suggested by Vogel [1980].

The aim of this study is the characterization of the genetic toxicity of a trivalent (sodium arsenite, $NaAsO_2$) and a pentavalent (sodium arsenate, Na_2HAsO_4) arsenic compound. Their ability to induce sex-linked recessive lethal mutations and sex chromosome loss, after mutagen exposure of postmeiotic (adult treatment) and premeiotic (larval treatment) male germ cells of *Drosophila melanogaster,* and to induce somatic mutations and mitotic recombination in the *mwh* +/+ *flr*^{3} wing test and in the w^+/w eye assay employing standard and insecticide-resistant flies, was determined.

MATERIALS AND METHODS

Chemicals

Sodium arsenite ($NaAsO_2$, CAS 7784-46-5) and sodium arsenate (Na_2HAsO_4, CAS 10048-95-0) were obtained from Baker, Mexico.

Strains

The following strains and matings of *Drosophila melanogaster* were used: (i) for SLRLT: ♀♀ *Basc* [*In(1) sc*^{51L} *sc*^{8R} *+S, sc*^{51} *sc*^{8} *w*^{a} *B*] × ♂ Canton-S; (ii) for SCLT: ♀♀ *y*^{2} *w*^{a}/*y*^{2} *w*^{a} × ♂♂ *y*/*B*^{S}*Yy*$^+$; for SMART, four matings were used: (iii) ♀♀ *mwh*/*mwh* × ♂♂ *flr*^{3}/*TM3, Ser,* standard wing cross (SC); (iv) ♀♀ *ORR*/*ORR; flr*^{3}/*TM3, Ser* × ♂♂ *mwh*/*mwh,* improvement wing high bioactivation cross (HB); (v) ♀♀ *y*/*y* × ♂♂ *w*/*Y,* standard eye cross (ST); (vi) ♀♀ *Hikone-R w*/*w* × ♂♂ *Hikone-R* +/*Y,* insecticide resistance cross (IR).

For detailed information on genetic markers, see Lindsley and Zimm [1992]. The stocks were maintained at a constant temperature of 25 ± 1°C and 60% humidity. Food medium was prepared with sugar (30.3%), agar (6.5%), corn meal (45.5%), yeast (14.3%), Nipagin in 10% alcohol solution (1.7%), and propionic acid (1.7%).

Toxicity

The compounds were dissolved in a 5% sucrose solution which was also used as the negative control. Solutions were always freshly prepared immediately before use. Groups of 10 48-hr-old Canton-S (CS) males were starved for 4 hr and were then treated for 48 hr in vials containing filter papers soaked with the solution being tested; every 8 hr the flies were transferred to a newly-prepared solution. The surviving males were counted in order to obtain the LC_{50}. Three repetitions were performed

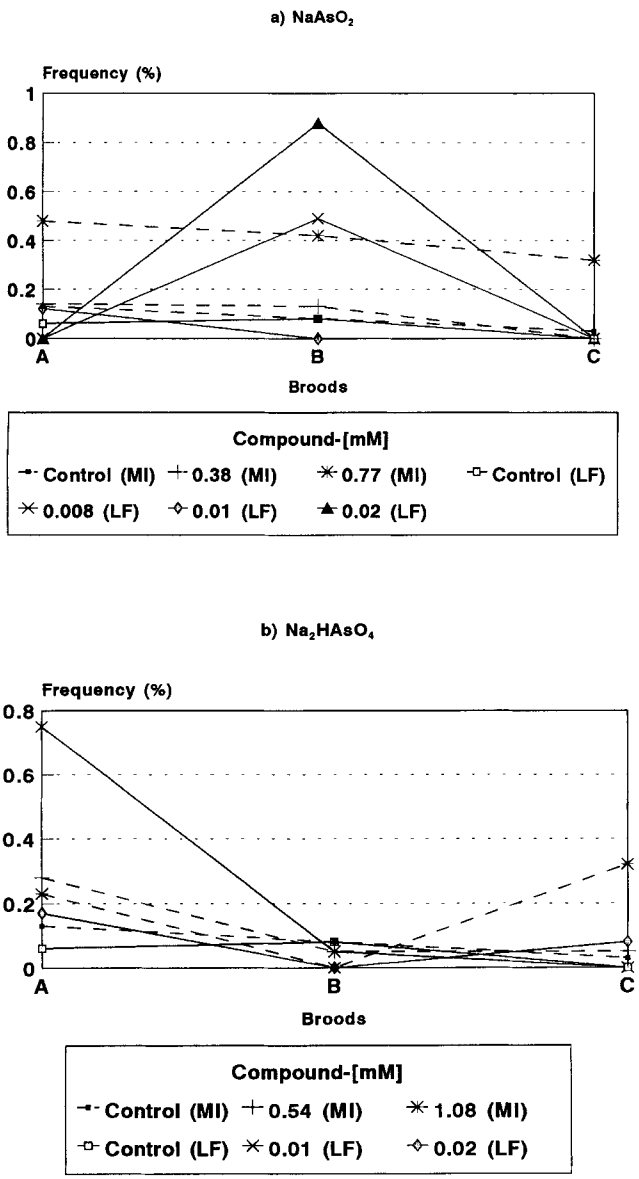

Fig. 1. Frequency of SLRL mutations in Canton-S males after treatment with (**a**) sodium arsenite and (**b**) sodium arsenate. MI, male injection; LF, larval feeding.

with 100 males per concentration to confirm the response. The LC_{50} value for sodium arsenite was 1.15 mM, and for sodium arsenate it was 2.69 mM. Two additional concentrations were tested: 0.38 and 0.77 mM for sodium arsenite, and 0.54 and 1.34 mM for sodium arsenate.

Germinal Assays

Treatments

Male injection. Forty-eight-hr-old Canton-S males were injected with approximately 4 µl of the test solutions, were allowed 4 hr to recover, and were then mated individually with 72 ± 4-hr-old *Basc* virgin females.

Larval feeding. Eggs were collected from a Canton-S culture for 8 hr, and 48 hr later 1 ml of the test solution was poured into the bottle to cover the food surface. Adult CS males from these cultures were mated with *Basc* virgin females. With this procedure, where the arsenic concentration was not completely uniform throughout the media, the final concentrations were estimated to be 0.008, 0.01, and 0.02 mM for sodium arsenite and 0.01 and 0.02 mM for sodium arsenate.

Experimental Procedure

Treated and control males were mated with virgin females in a ratio of 1 ♂:3 ♀♀ for 2 days (brood A). Males were transferred to bottles with virgin females for two additional periods of 3 days each (broods B and C), and then discarded. Females were retained in bottles for egg laying for 5 days.

For the sex-linked recessive lethal test (SLRLT), Canton-S males treated as adult or as larvae (and control males) were mated individually with virgin *Basc* females. The F_1 progeny were intercrossed, and sex-linked recessive lethals were scored in the F_2 according to standard procedure [Woodruff et al., 1984]; when necessary, the occurrence of a lethal mutation was checked using the F_3. To detect the occurrence of clustered lethals, the progeny of each individual P male was identified and analyzed as described by Gocke et al. [1982].

For the sex chromosome loss test (SCLT), $y/B^S Yy^+$ males injected with the test solution (and control males) were mated to virgin $y^2\ w^a/y^2\ w^a$ females in mass cultures. F_1 progeny were individually scored for total and partial sex chromosome loss.

Somatic Assays

Treatments

SMART. Seventy-two ± 4-hr-old larvae from each one of the iii–vi matings were collected from culture bottles according to Nöthiger [1970] and introduced into a glass tube with a fine nylon gauze at one end and a rubber stopper at the other. Each tube containing approximately 50–150 larvae was put in a 10-ml glass beaker with 60 mg of powdered cellulose (Merck, Darmstadt) and 0.3 ml of the test solution. Larvae fed for 6 hr through the fine gauze on the wet cellulose. Larvae were then removed from vials, washed with tap water, and transferred to fresh culture bottles until adult emergence.

Preparation and Microscopic Analysis of Wings

Transheterozygous adults (wild phenotype) were fixed in 70% ethanol. Both wings from each fly were dissected and mounted in Faure's solution [Graf et al., 1984]. Both surfaces of the wings were scored under a microscope at 400× magnification for the presence of single spots, with *mwh* or *flr* phenotype and twin spots, with adjacent *mwh* and *flr* areas. Two spots were considered independent if separated by at least three wild-type trichome rows.

Microscopic Analysis of Eyes

Progeny of both sexes from the standard cross (v), but only females descending from the insecticide-resistant cross (vi), were used to score light spots in the eyes. Scoring of eyes was carried out in a solution consisting of Tween-80, ethanol, and water, in a 1:9:90 ratio, under a dissecting microscope at 120× magnification, using an optical fiber light (Fiberlite M. 170D, Dolan-Jenner, USA) to detect the presence of light spots. Two spots were considered independent if separated by at least four wild-type ommatidial rows.

Statistical Analysis

For germ cells, statistical analysis of the results was performed using the Kastenbaum-Bowman test [Kastenbaum and Bowman, 1970]. For somatic cells, data evaluation and statistical analyses were performed for the wings with the SMART computer program [Würgler, unpublished], and for wings and eyes, with the multiple-decision procedure described by Frei and Würgler [1988]. For the wing spot test, the spots were classified as small (1–2 cells) and large (>3 cells) single spots,

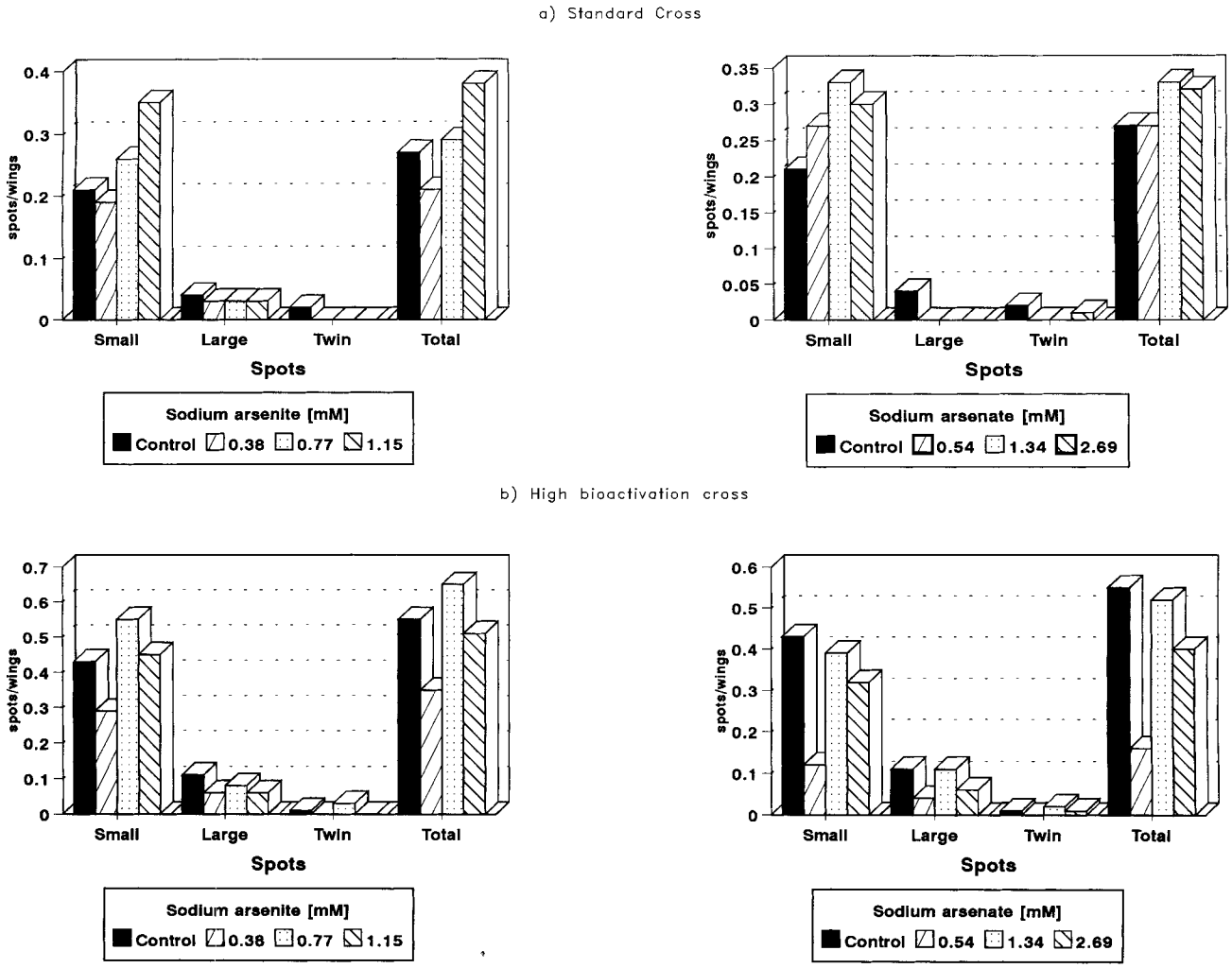

Fig. 2. Spots per wing after exposure to sodium arsenite and sodium arsenate. **a:** Standard cross. **b:** High metabolic cross.

and as twin spots. Because the frequencies of small single and total spots were higher than those of large single and twin spots, data had to be analyzed separately. m implies the magnitude of the difference that should be obtained to consider a significant response; m = 2, for small single and total spots; and m = 5, for large single and twin spots.

RESULTS

The high toxicity of both arsenic salts made it difficult to test all the concentrations. Table I shows the data obtained in the SLRLT after adult male injection with sodium arsenite and sodium arsenate. Sodium arsenite induced SLRL in the three broods analyzed at 0.77 mM ($P < 0.01$). Sodium arsenate produced an increase in the frequency of SLRL only at 1.08 mM, in brood C, and for pooled A–C broods ($P < 0.05$).

For larval feeding (Table II), sodium arsenite was positive only in brood B at 0.008 mM, and for pooled A–C broods ($P < 0.05$); the same was found at the concentration of 0.02 mM ($P < 0.01$). Sodium arsenate increased the frequency of lethal mutations at 0.01 mM in brood A, and in A–C broods ($P < 0.01$). In the SCLT, neither sodium arsenite nor sodium arsenate induced total or partial sex chromosome loss (Table III).

In the wing spot test employing the standard cross, sodium arsenite increased the frequency of small single spots only at 1.15 mM, but the statistical diagnosis of total spots was inconclusive. Sodium arsenate was inconclusive for small single spots at 1.34 mM and 2.69 mM, and negative for large single, twin, and total spots (Table IV). Both sodium arsenite and sodium arsenate were negative when the HB cross was used (Table V).

In the eye somatic assay employing the standard cross (ST), the frequencies of mosaic light spots were obtained separately for females and males (Table VI). All concentrations tested for sodium arsenite induced significantly increased ($P < 0.05$) numbers of light spots in both sexes. In females, a decrease in the induction of small, large, and total spots was observed at the highest concentration (1.15 mM). In males, the lowest concentration gave the highest induction of light spots. The genotoxic potency

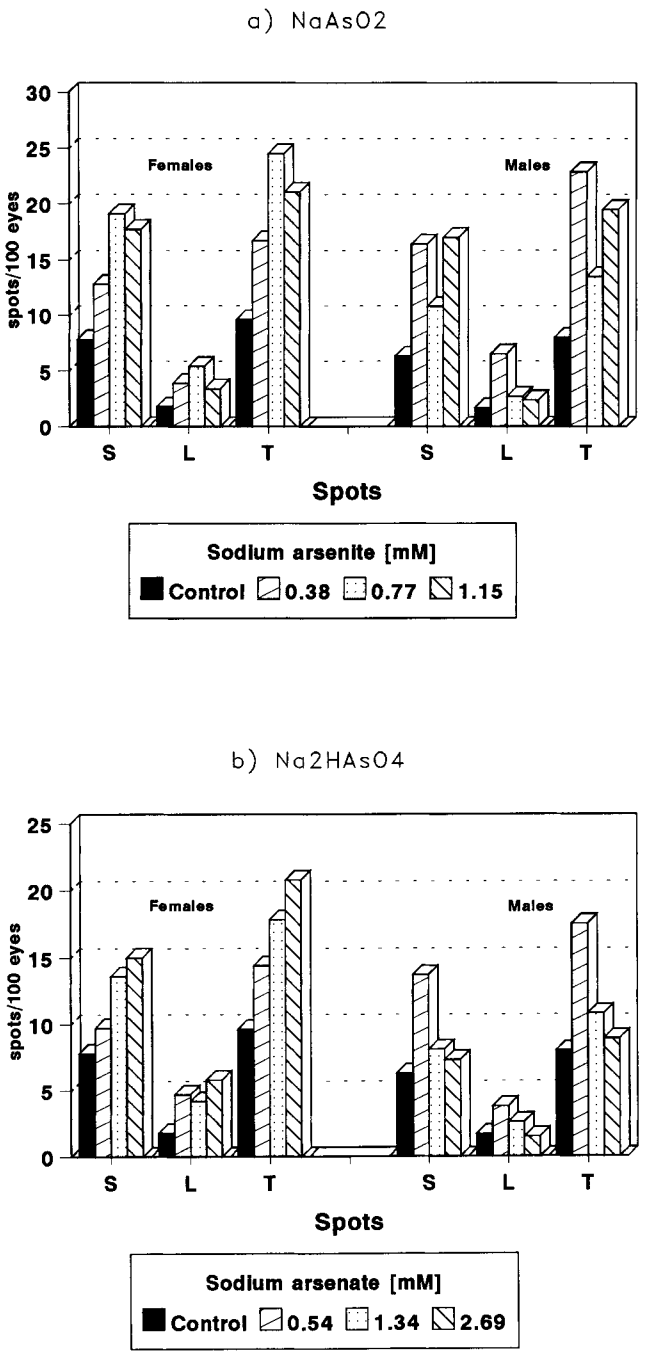

Fig. 3. Mosaic light spots in standard females and males after treatment with (**a**) sodium arsenite (**b**) sodium arsenate.

measured by the number of clones per 10^4 cells in females was different from that obtained in males. For both sexes, the average clone size was similar to that obtained in controls. Sodium arsenate induced a significant dose-response relationship for small and total light spots in females; this relationship was not observed in males. A similar tendency was found for the number of clones per 10^4 cells (Table VII).

When insecticide-resistant females (IR) were used, sodium arsenite was positive for the induction of mosaic light spots at all concentrations tested, while sodium arsenate was positive only for the two lower concentrations (Table VIII). Again sodium arsenite was more potent than sodium arsenate, as seen by the number of clones produced per 10^4 cells.

DISCUSSION

Sodium arsenite increased the frequency of SLRL when injected into males (acute exposure); the absence of a differential response among broods suggests that the salt does not need metabolic activation, and seems to be very stable (Fig. 1a). The slight negative slopes obtained through broods in males treated by injection (MI) could partially be due to excretion, or repair of damage. In contrast, larval feeding (LF), which represents a chronic exposure, preferentially induced lethals in brood B, an effect that could be associated with the accumulation of arsenite in exposed larvae; however, the arsenite could probably be detoxified afterwards. In fact, different treatments could be responsible for different response levels, with LF giving the highest response in this study.

Sodium arsenate was a weak inducer of lethals when injected into males; again, the magnitude of induction of lethals was higher when fed to larvae, which may suggest a biotransformation from sodium arsenate to sodium arsenite (Fig. 1b). As demonstrated by Léonard and Lauwerys [1980], arsenate (pentavalent form) is reduced in vivo to the mutagenic trivalent form (arsenite). Oberly et al. [1982] found, for the thymidine kinase locus in mouse L5178Y cells, that treatments with sodium arsenite gave a positive response in absence of S9 mix, while treatments with sodium arsenate were positive only with the S9 mix. Several reports [EPA, 1984; IARC, 1987] on in vitro systems have failed to show the mutagenicity of As; however, data obtained in this study indicate that sodium arsenite and sodium arsenate were mutagenic in the Drosophila in vivo system.

Both sodium arsenite and sodium arsenate were negative in the SCLT. In comparison with the SLRLT, the former requires higher concentrations to produce clastogenic effects [Vogel and Natarajan, 1979]. It has also been proposed that part of the male germinal cell damage induced by chemicals is repaired during the first replication before fertilization [Kammermeyer and Zimmering, 1985]. In addition, Phillips and Pitt [1985] suggest that species-specific maternal RNA polymerases can influence the embryonic development of Drosophila progeny despite their genetic dowry.

Data concerning chromosomal aberrations induced by arsenic are contradictory. Positive responses with sodium arsenite and sodium arsenate have been obtained primarily in in vitro assays [Wan et al., 1982; Lee et al., 1985; IARC, 1987; Eastmond and Pinkel, 1990]; by contrast, only a slight increase in the frequencies of chromosomal aberrations has been recovered after in vivo exposure in

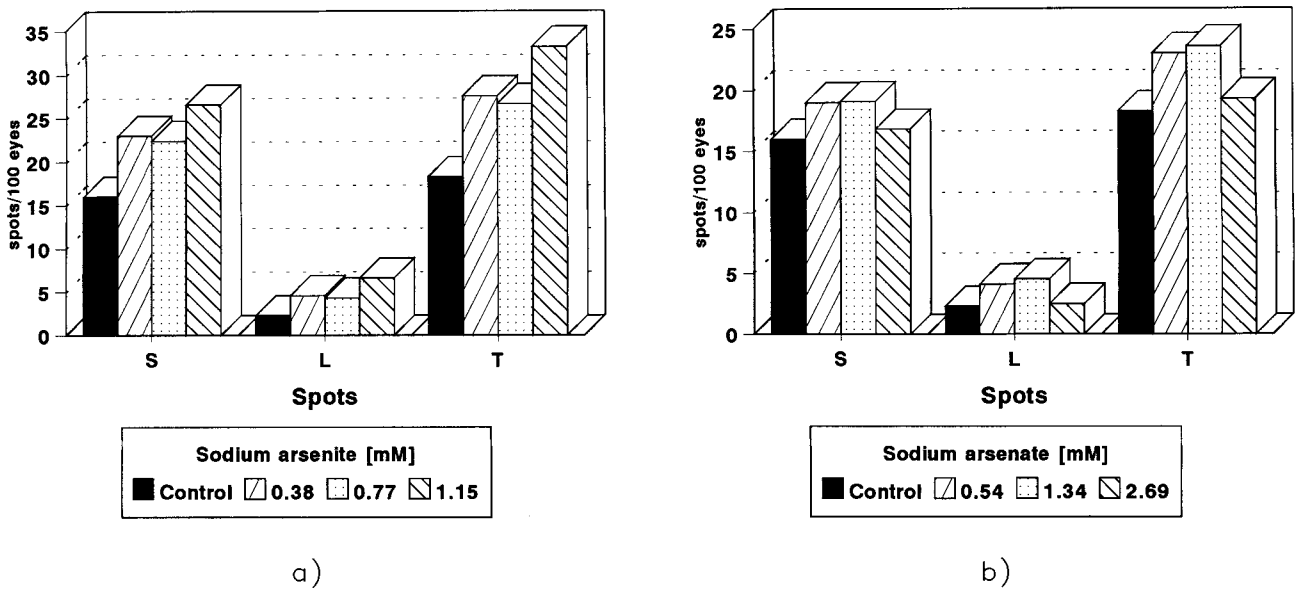

Fig. 4. Mosaic light spots induced in insecticide-resistant females after treatment with (**a**) sodium arsenite and (**b**) sodium arsenate.

TABLE IX. Summary of Results Obtained for Sodium Arsenite and Sodium Arsenate in Germinal and Somatic Tests in *Drosophila melanogaster

	Germinal tests									Somatic tests				
										SMART				
Compound	SLRLT						SCLT					Eyes		
(mM)	MI			LF			MI			Wings		S		IR
Brood	A	B	C	A	B	C	A	B	C	S	HB	♀♀	♂♂	♀♀
$NaAsO_2$														
0.008				−	+	−								
0.01				−	−	−								
0.02				−	++	−								
0.38	−	−	−				−	−	−	−	−	+	+	+
0.77	++	++	++				−	−	−	−	i	+	+	+
1.15							−	−	−	+	−	+	+	+
Na_2HAsO_4														
0.01				++	−	−								
0.02				−	−	−								
0.54	−	−	−				−	−	−	−	−	+	+	+
1.08	−	−	+											
1.34							−	−	−	i	−	+	−	+
2.69							−	−	−	i	−	+	−	−

*MI, male injection; LF, larval feeding; HB, high bioactivation; IR, insecticide-resistant; +, positive, $P < 0.05$; ++, positive, $P < 0.01$; −, negative; i, inconclusive.

mice [IARC, 1987]. Epidemiological studies have shown structural chromosomal aberrations in humans occupationally exposed to arsenicals [Nordenson and Beckman, 1982; Kazantzis and Lilly, 1986], although humans exposed to drinking water contaminated with arsenic did not show chromosomal aberrations [Vig et al., 1984].

In the wing test, sodium arsenite induced a significant increase of small single spots in the standard cross, but failed to produce large single or twin spots. These results are in contrast to those with sodium arsenate, which were negative (Fig. 2a). These results are different from those obtained by Tripathy et al. [1990], and can be explained, at least in part, on the basis of the type of exposure (acute vs. chronic) and, hence, the intensity of the treatment. When high bioactivation (HB) larvae were treated, both compounds were negative (Fig. 2b). The detoxification ability of the HB strain has been reported [Graf and Van Shaik, 1992].

In the w^+/w eye assay employing the standard strain, sodium arsenite induced light spots at all concentrations tested in females as well as in males; however, there is a difference in response between the sexes (Fig. 3a). Although the highest concentration tested was toxic, no correlation with dose-response was observed for arsenite in males; a positive correlation was found in females. Sodium arsenate shows a direct concentration-effect relationship in females, while in males an inverse relationship was obtained (Fig. 3b).

Gender differences for internal cancers in humans exposed to drinking water contaminated with arsenic were reported by Bates et al. [1992], with females being the most affected, with higher mortality rates in bladder, kidney, and lung cancers; and by Gonsebatt et al. [1994] for mitogen-induced lymphocyte proliferation, in which chronic As ingestion increases these differences. In our report, gender differences may be due to the flies' genetic constitution, i.e., heterozygous females and hemizygous males.

In the insecticide-resistant strain, both compounds were positive (Fig. 4a,b), maybe due to the higher susceptibility of these flies [Zijlstra et al., 1984a,b; Vogel et al., 1991; Rodríguez-Arnaiz et al., 1993].

The toxicity of sodium arsenite in the present study was higher than that of sodium arsenate. The genotoxicity of both compounds was localized mainly in somatic cells (Table IX). The recombinational events obtained could be related to the carcinogenic potential of arsenic compounds [IARC, 1987; Weisburger, 1990; Ostrosky-Wegman et al., 1991; Tinwell et al., 1991; Dong and Luo, 1993]. The eye somatic assay was more sensitive than the wing spot test for detecting the genotoxicity of arsenic salts. Genes on the Drosophila X chromosome showed higher spontaneous mutation frequencies than when the same genes were translocated to the third chromosome [García-Bellido and Merriam, 1971a,b]. On the other hand, it has been proposed that mitotic recombination constitutes the major event measured by the w^+/w assay [Vogel and Szakmàry, 1990]; nevertheless, the differential responses between females and males cannot be explained only by intragenic recombination.

Our study shows that both sodium arsenite and sodium arsenate are mutagenic in germinal as well as in somatic cells of *D. melanogaster,* in both cellular lineages. In addition, sodium arsenite was an order of magnitude more toxic and potent than sodium arsenate. It also confirms the reliability of the Drosophila in vivo system for testing the genotoxicity of environmental compounds.

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