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Structure-activity relationships of several anisidine and dibenzanthracene isomers in the *w*/*w*+ somatic assay of *Drosophila melanogaster*

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Abstract

Several structurally related anisidine and dibenzanthracene isomers were evaluated for genotoxic effects in the somatic *w*/*w*+ assay of *Drosophila melanogaster* employing insecticide-susceptible (IS) and insecticide-resistant (IR) tester strains. In addition, and in order to find whether or not at the genetic level a regulatory effect is found, crosses between IS × IR strains and IR × IS strains were done. Chemicals tested were the aromatic amines (AAs) *ortho*-anisidine (*o*-AN), *meta*-anisidine (*m*-AN), and *para*-anisidine (*p*-AN) and the polycyclic aromatic hydrocarbons (PAHs) 1,2;3,4-dibenzanthracene (1,2;3,4-DBA) and 1,2;5,6-dibenzanthracene (1,2;5,6-DBA). As positive control *N*-nitrosodimethylamine (DMN) was used. Our results show that the genotoxic activity of DMN was higher in the IR than in the IS strain. There seems to be a tendency for slightly lower values as measured by clone induction in crosses between IS × IR and IR × IS. *o*-AN was positive in the IS strain and in crosses between IS × IR and IR × IS but negative in the IR strain. *m*-AN, *p*-AN and 1,2;3,4-DBA proved to be not recombinogenic in all strains and crosses while 1,2;5,6-DBA was positive at the highest concentration tested in all the crosses assayed. These findings show that the recombinogenic activity of the anisidine isomers depends on the position of the chemical group relative to one another and that the position of the benzene ring seems to be structurally relevant for genotoxicity of DBA isomers. With respect to IR and IS strains it remains to be determined to what extent the spectrum of metabolizing capacity really differs between the strains of the test assay. Thus more information is needed about the regulation and expression of the cytochrome-P450 genes and action at the molecular level taking place in the eye imaginal disc as well as between insecticide susceptible and resistant strains after exposure to genotoxic chemicals. © 2002 Published by Elsevier Science B.V.

Keywords: *Drosophila melanogaster*; Somatic *w*/*w*+ assay; Insecticide-susceptible strain (IS); Insecticide-resistant strain (IR); *N*-Nitrosodimethylamine (DMN); *ortho*-Anisidine (*o*-AN); *meta*-Anisidine (*m*-AN); *para*-Anisidine (*p*-AN); 1,2;3,4-Dibenzanthracene (1,2;3,4-DBA); 1,2;5,6-Dibenzanthracene (1,2;5,6-DBA)

1. Introduction

Aromatic amines (AAs) and polycyclic aromatic hydrocarbons (PAHs) represent classes of potential bacterial and rodent carcinogens which gain their biological activity upon metabolic conversion by phase I and phase II enzymes [1]. Subsequent to cytochrome-P450-dependent hydroxylation and epoxidation mainly catalyzed by enzymes of family 1 electrophilic products are produced that may react with nucleophilic sites of DNA [2,3]. *N*-Oxidation by cytochrome-P450 is the first step in the biotransformation of aromatic amines [4] AAs may also be activated by peroxidases [5]. Aniline and its derivatives are widely used as

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intermediates in dyestuffs and a variety of polyurethane products and anisidines are used in the manufacture of azo dyes [6]. *ortho*-Anisidine (*o*-AN) has been shown to be a potent human bladder carcinogen, genotoxic in vitro and positive in CHO clastogenicity and SCE assays [7]. *o*-AN was also shown to be active in the $w/w+$ assay of *Drosophila melanogaster* [8]. PAHs are ubiquitous compounds widely distributed in the environment mainly produced by incomplete combustion of petroleum derived products [9]. Activation of particular PAHs may lead to monofunctional epoxides or to vicinal diol-epoxides with high mutagenic activity [10].

Drosophila melanogaster has shown to have a versatile metabolic capacity to activate most classes of promutagens and genotoxic procarcinogens. However, two classes of procarcinogens, the polycyclic aromatic hydrocarbons (PAHs) and the aromatic amines (AAs), were difficult to detect in *Drosophila* assays measuring genotoxic damage in germ cells [11]. The low sensitivity of the *Drosophila* assays for aromatic compounds characterizes the problem of the low detection capacity for most AAs and PAHs [12]. This situation has changed with the development of novel and more economic *Drosophila* assays measuring genetic alterations in somatic cells [13]. The somatic genotoxicity assay systems of *Drosophila melanogaster* developed over the past two decades are well validated, [14,15]. It is estimated at present that over 400 chemical compounds of different chemical classes have been analyzed in these assays. The advantages of the somatic systems are, among others, that only one generation is needed to perform the tests. Two well suited somatic tissues (wing and eye) permit a large number of cells to be analyzed in an individual fly (approximately 25,000 cells in one wing and approximately 800 ommatidia in one eye). In particular the $w/w+$ somatic assay makes use of larave that carry one recessive cell marker in the eye imaginal disk cells. This marker is then, in turn, manifested as mutant clones or spots in the eyes of the resulting adult flies when mutation at the $w+$ locus or interchange between the X chromosomes give rise to homozygosis of the w marker. It has been shown by comparison of induced spot frequencies in different genotypes ($w/w+$ and $w+$/In-w) that homologous interchromosomal mitotic recombination constitutes the major mechanism involved in mosaic light spot formation in the $w/w+$ assay [16]. Gene mutations and deletions affecting the *white* locus seem to represent only a minor fraction of events leading to clone formation [17,18].

In addition *Drosophila* is used in a short-term in vivo test that has its own endogenous metabolic activation system. There are at present several strains with different metabolic capacities which can be employed to perform the $w/w+$ assay. It has been shown that these strains have a high constitutive cytochrome-P450-dependent bioactivation capacity which is responsible for insecticide resistance as well as for a general increase in xenobiotic metabolism. The use of these strains has demonstrated that when compounds whose genotoxic action depends on metabolic activation the genetic background of the strain used play a key role [13,19,20].

Insecticide resistance might be correlated to overexpression of cytochrome-P450 genes. Overexpression of P450 in insecticide resistant strains is well documented at the biochemical level and also has been documented for several genes such as *Cyp6a2* and *Cyp4e2* in *Drosophila* [21]. Cyp6A2 has a broad substrate specificity typical of an enzyme involved in detoxification. Although, *Cyp6a2* message and Cyp6A2 proteins are present at low levels in wild type adult flies, the expression of the gene is regulated by a variety of factors [22]. At the genetic level crosses between IS × IR and IR × IS could show an effect on the regulation of these genes.

The aim of this study has been the characterization of several structurally related anisidines and dibenzanthracenes with regard to their ability to induce recombination between X chromosomes in somatic cells of *Drosophila melanogaster*. The white/white+ ($w/w+$) in vivo mosaic assay was used, employing larvae from the standard insecticide-susceptible strain (IS) and from an insecticide-resistant strain (IR) as well as from crosses between IS × IR and IR × IS.

2. Materials and methods

2.1. Chemical compounds

N-Nitrosodimethylamine (DMN, CAS No. 62-75-9), *ortho*-anisidine (*o*-AN, CAS no. 90-04-0), *meta*-anisidine (*m*-AN, CAS no. 536-90-3), *para*-anisidine (*p*-AN,CAS no. 104-94-9), 1,2;3,4-dibenzanthracene

(1,2;3,4-DBA, CAS no. 215-58-7) and 1,2;5,6-dibenzanthracene (1,2;5,6-DBA, CAS no. 53-70-3) were purchased from Sigma (St. Louis, MO, USA). Compounds were dissolved in a mixture of 1% Tween-80 and 3% ethanol immediately prior to use.

2.2. Drosophila strains

Two *Drosophila* stocks were used in the assay: one insecticide-susceptible (IS) Leiden Standard (ST) and one insecticide-resistant (IR) Haag 79-R (HG) strain. Crosses were made between *white* homozygous females and red ($w+$) hemizygous males. Stocks were maintained at 25 °C and 60% humidity.

2.3. Somatic assay

Chemicals were administrated by chronic exposure. Fifteen pairs of flies were permitted to lay eggs for 3 days on standard food supplemented with the test substance dissolved in a mixture of three parts ethanol, one part Tween-80. Growing cultures were exposed to each compound during all three instar stages of larval development. Two or three separate experiments were conducted with each single chemical at the same exposure dose. For each experiment a concurrent control was run, where larvae were treated with the solvent alone. Newly hatched females were transfered to fresh medium and scored 1–5 days later. The scoring of etherized flies was carried out in a liquid containing 90 parts ethanol, one part Tween-80 and nine parts water. The eyes of adult females were inspected for mosaic light spots under a dissecting microscope at a magnification of 120×, with optical fiber illumination.

2.4. Data analysis and statistics

Adult females are heterozygous for white and were inspected for the occurrence of white in their compound eyes. Classification of mosaic light spots was done on the basis of small spots (2–4 ommatidia), large spots (>4 ommatidia) and total spots. Spots separated by at least four non-mutated ommatidia were counted as independent clones [13].

Proliferation of pre-ommatidia cells in the imaginal discs of developing larvae increases the number of target cells from about 20 at the end of the first instar, to 100–150 cells in the second instar, and reaches a final number of 780–800 pre-ommatidia cells at the end of the third instar [23]. Thus, primordial cells of the adult compound eye divide continuously throughout the larval period. Mosaic *white* clones induced in early larvae will be large in size but less frequent, whereas those produced later will successively be smaller and more frequent, as the number of potential target cells increases with larvae age. The size of *white* clones and their distribution among size classes provides therefore information on the time point of induction of the loss of heterozygosity event. This analysis was carried out through a comparison of the total frequency, the average clone size, and the distribution of spots in several size classes. For an indirect estimation of the genotoxic effectiveness of the chemicals, the frequency of clones per 10^4 cells were calculated [15].The chi square for proportions was used for statistical analysis of the data collected [24].

3. Results and discussion

It has been shown in earlier studies that the use of strains with increased cytochrome-P450-dependent metabolism is an approach that improved the detectability of promutagens in the somatic $w/w+$ eye assay. Thus the insecticide resistant tester strains allowed the detection as genotoxic compounds, although with small increase of sensitivity, of several AAs such as 2-naphthylamine, *o*-anisidine, *o*-toluidine and 4,4′-oxydianiline, and PAHs such as benzo(a)pyrene, 7,12-dimethylben(a)anthracene and 9,10-dimethylanthracene [8,13,20].

The results of experiments performed in the present study are compiled in Table 1. The distribution of *white* spots as well as average clone size, clones per 10^4 cells and statistical diagnosis are also shown in Table 1. DMN was positive in all the assayed concentrations being the genotoxic activity of DMN in the IR strain higher than in the IS strain. Bioactivation of DMN as measured by clone induction shows a tendency, although non-significant, for slightly lower values in crosses between IS × IR as compared with the values obtained from crosses between IS × IS. Clone induction from the IR × IS cross was around four times lower at 0.1 mM and comes two times down at the highest concentration tested as compared with results from the IR × IR cross.

Table 1
Summary of results obtained in the *Drosophila* $w/w+$ eye test[a]

Genotype	Compound concentration (mM)	Number of eyes	Distribution of spots size (ommatidia)								Spots per 100 eyes size classes[b]			Average clone size	Clones per 10^4 cells[c]	Statistical diagnosis[d]
			1–2	3–4	5–8	9–16	17–32	33–64	>64	Total	S	L	T			
DMN																
IS	Control	772	36	13	4	2	0	0	0	55	6.35	0.77	7.12	3.11	5.54	
	0.01	220	61	21	9	4	0	1	0	96	37.27	6.36	43.93	3.56	3.88	+
	0.1	674	211	88	48	15	2	1	1	368	44.36	10.24	54.6	3.89	54	+
	1	194	117	51	34	13	2	0	0	213	87	24.85	111.85	3.67	102.63	+
IS × IR	Control	320	19	9	0	0	1	0	0	29	8.75	0.31	9.06	2.93	6.64	
	0.01	94	29	19	11	1	2	0	0	62	51.06	14.89	65.95	3.82	62.99	+
	0.1	190	97	41	21	3	0	0	0	160	72.63	20	84.21	3.09	65.05	+
	1	68	27	17	11	3	0	0	0	58	64.7	20.59	85.29	3.83	81.67	+
IR	Control	738	86	30	7	4	0	0	0	121	15.04	1.49	16.53	3.01	12.44	
	0.01	696	194	121	69	22	10	8	0	424	45.26	15.66	60.92	4.84	7.37	+
	0.1	236	201	76	36	16	3	2	0	334	117.37	24.15	141.52	3.65	129.14	+
	1	80	109	49	33	13	5	3	0	212	197.5	67.5	265	4.86	321.97	+
IR × IS	Control	758	64	8	4	1	2	1	0	80	9.5	1.05	10.55	3.71	9.79	
	0.01	674	206	81	34	18	3	1	0	343	42.58	8.31	50.89	3.55	45.16	+
	0.1	680	252	79	28	11	5	1	0	376	48.68	6.61	55.29	3.29	45.48	+
	1	136	158	60	35	2	3	2	0	260	160.29	30.89	191.18	3.48	166.32	+
ortho-Anisidine (*o*-AN)																
IS	Control	744	32	11	10	1	0	2	1	57	5.78	1.88	7.66	6.02	11.53	
	1	548	51	16	11	7	3	0	0	88	12.77	3.29	16.06	4.32	17.34	+
	2	564	63	20	9	5	6	0	0	103	14.72	3.54	18.26	4.3	19.63	+
	4	506	75	27	16	5	6	1	2	132	20.16	5.93	26.09	7.3	47.61	+
IS × IR	Control	774	32	8	3	2	1	0	0	46	5.17	0.77	5.94	3.13	4.65	
	1	886	54	13	7	2	2	0	1	79	7.56	1.36	8.92	4.15	9.26	−
	2	636	61	27	13	1	5	0	0	107	13.84	2.48	16.82	3.85	16.19	+
	4	584	67	14	8	4	2	0	0	95	13.87	2.4	16.27	3.4	13.83	+
IR	Control	674	44	13	8	3	1	0	1	70	8.46	1.92	10.38	4.43	11.5	
	1	740	57	6	3	6	3	0	0	75	8.51	1.62	10.13	3.72	9.43	−
	2	734	51	19	7	1	1	0	0	79	9.24	1.52	10.76	3.04	8.18	−
	4	613	54	20	11	0	1	1	1	88	12.07	2.28	14.35	6.62	23.76	−
IR × IS	Control	648	54	9	3	3	1	0	0	70	9.72	1.08	10.8	3.23	8.72	
	1	630	58	15	10	7	2	0	0	92	11.59	3.01	14.6	4.03	14.55	−
	2	674	114	19	13	7	2	1	1	157	19.73	3.56	23.29	4.18	24.5	+
	4	708	102	21	13	2	2	1	1	142	17.37	2.69	20.06	3.72	18.65	+

meta-Anisidine (*m*-AN)																
IS	Control	502	19	16	4	3	1	0	0	43	7.77	0.79	8.56	4.05	8.67	
	1	524	29	11	5	2	3	3	0	53	8.59	1.52	10.11	6.24	15.78	–
	2	518	28	11	9	1	0	0	0	49	9.27	0.19	9.46	3.11	7.35	–
	4	494	50	12	3	2	3	0	0	70	12.55	1.62	14.17	3.51	12.43	–
IS × IR	Control	628	37	13	6	0	3	0	0	59	7.96	1.43	9.39	3.95	9.28	
	1	750	60	10	5	1	0	0	0	76	9.33	0.8	10.13	2.6	10.97	–
	2	654	57	9	4	4	2	0	0	76	10.09	1.53	11.62	3.47	10.08	–
	4	542	45	7	2	0	1	2	0	57	9.59	0.92	10.51	4.44	11.67	–
IR	Control	530	61	15	10	1	0	1	1	89	14.34	2.45	16.79	5.45	22.88	
	1	522	71	15	0	6	2	2	0	96	16.47	1.92	18.39	4	18.39	–
	2	530	52	45	8	5	4	1	1	116	18.3	3.59	21.89	5.3	29	–
	4	554	89	21	6	3	0	1	0	120	19.85	1.81	21.66	3.06	16.57	–
IR × IS	Control	650	52	10	5	1	3	0	0	71	9.54	1.38	10.92	3.66	9.99	
	1	508	74	15	4	5	1	0	0	99	17.52	1.97	19.49	3.09	15.05	–
	2	500	59	26	7	5	1	0	0	98	17	2.6	19.6	3.5	16.9	–
	4	504	55	16	10	3	3	2	0	89	14.09	3.57	17.66	5.03	22.2	–
para-Anisidine (*p*-AN)																
IS	Control	400	19	5	2	3	2	0	0	31	6	1.75	7.75	5.03	9.74	
	1	510	24	11	4	3	2	3	0	47	6.86	2.35	9.21	6.89	15.87	–
	2	400	26	7	1	3	2	1	0	40	8.25	1.75	10	4.92	12.3	–
	4	204[e]	17	7	3	2	2	1	0	32	11.76	3.91	15.67	5.78	22.66	–
IS × IR	Control	510	30	5	5	2	2	4	0	48	6.86	2.55	9.41	7.39	17.39	
	1	510	37	6	8	3	5	0	0	59	8.43	3.04	11.57	5.13	14.84	–
	2	554	35	12	5	2	2	2	0	58	8.48	1.99	10.47	5.1	13.35	–
	4	358[e]	27	7	2	2	0	0	0	38	9.5	1.11	10.61	2.97	7.88	–
IR	Control	500	38	5	3	2	0	0	0	48	8.6	1	9.6	2.71	6.5	
	1	534	52	17	10	5	1	1	1	87	12.92	3.37	16.29	5.26	21.42	–
	2	460	46	9	7	3	0	0	0	65	12.61	1.52	14.13	3.03	10.7	–
	4	500	40	15	3	2	2	0	0	62	11	1.4	12.4	3.61	11.19	–
IR × IS	Control	384	26	3	4	1	0	1	0	35	7.55	1.56	9.11	3.74	8.52	
	1	400	33	10	5	2	1	0	0	51	10.75	2	12.75	3.71	11.82	–
	2	276	28	12	8	2	0	2	0	52	14.49	4.35	18.84	4.54	21.38	+
	4	456	36	8	12	2	1	2	0	61	9.65	3.72	13.37	3.79	12.67	–
1,2;3,4-Dibenzanthracene (1,2;3,4-DBA)																
IS	Control	534	32	1	2	1	0	0	0	36	6.18	0.56	6.74	2.5	4.21	
	1	544	28	4	7	1	0	0	0	40	5.88	1.47	7.35	3.15	5.79	–
	2	546	32	6	6	5	4	0	0	53	16.96	2.75	19.71	5.23	12.69	+
	4	612	46	14	8	2	3	1	0	74	9.8	2.29	12.09	4.58	13.84	–

Table 1 (*Continued*)

Genotype	Compound concentration (mM)	Number of eyes	Distribution of spots size (ommatidia)								Spots per 100 eyes size classes[b]			Average clone size	Clones per 10^4 cells[c]	Statistical diagnosis[d]
			1–2	3–4	5–8	9–16	17–32	33–64	>64	Total	S	L	T			
IS × IR	Control	558	32	8	3	0	0	0	0	43	7.17	0.54	7.71	2.46	4.74	
	1	614	38	5	5	1	0	0	0	49	7	0.98	7.98	2.71	5.41	−
	2	472	34	5	3	0	0	0	0	42	8.26	0.74	9	2.4	5.34	−
	4	520	34	15	5	1	0	0	0	55	9.42	1.16	10.58	2.87	7.59	−
IR	Control	636	62	6	7	1	0	0	0	76	10.69	1.26	11.95	2.54	7.59	–
	1	584	48	30	12	4	2	0	0	96	13.36	3.08	16.44	3.89	15.99	−
	2	632	26	50	13	2	0	1	0	92	12.02	2.53	14.55	3.77	13.74	−
	4	636	62	22	9	8	0	0	0	101	13.21	2.67	15.88	3.83	15.2	
IR × IS	Control	560	54	20	4	7	1	0	0	86	13.21	2.15	15.36	3.55	13.63	–
	1	630	88	14	5	5	3	0	0	114	16.19	1.9	18.09	3.38	15.29	−
	2	586	73	24	11	0	3	1	0	110	16.55	2.22	18.77	3.82	17.93	−
	4	542	89	18	10	2	1	1	0	122	19.74	2.58	22.32	4.7	19.23	−
1,2;5,6-Dibenzanthracene (1,2;5,6-DBA)																
IS	Control	644	32	16	0	4	2	0	0	54	7.45	0.93	8.38	3.79	7.94	
	1	712	64	18	4	2	0	0	0	78	11.52	0.84	12.36	2.79	8.62	−
	2	608	41	28	7	2	1	0	0	69	11.35	1.64	12.99	3.57	11.59	−
	4	680	82	16	11	4	4	0	0	108	14.41	2.94	17.35	3.46	15.01	+
IS × IR	Control	500	21	10	3	1	0	0	0	35	6.2	0.8	7	3.09	5.41	
	1	832	35	24	13	3	1	1	0	79	7.09	2.4	9.49	5.08	12.06	−
	2	734	48	18	15	3	0	1	0	87	8.99	2.86	11.85	3.94	11.67	−
	4	604	44	12	10	3	3	0	1	88	9.27	5.3	14.57	4.33	17.39	+
IR	Control	552	40	8	4	6	1	0	0	59	8.69	1.99	10.69	4.02	10.74	
	1	568	67	25	5	2	2	1	2	100	16.19	1.41	17.6	5.37	23.63	−
	2	544	63	18	10	4	3	0	0	119	14.89	7	21.87	2.94	16.08	+
	4	538	83	18	3	2	1	0	0	107	18.77	1.3	19.89	2.79	13.87	+
IR × IS	Control	724	94	12	15	1	3	3	0	108	14.64	0.28	14.92	5.07	18.91	–
	1	576	79	23	9	1	2	0	0	114	17.71	2.09	19.79	3.23	15.98	−
	2	624	105	11	4	3	3	1	0	127	18.59	1.76	20.35	3.19	16.23	−
	4	230[e]	57	8	5	1	1	0	0	72	28.26	3.04	31.3	2.86	22.38	+

[a] Chronic feeding with DMN, different anisidine and dibenzanthracene isomers. Genotypes tested standard (IS) and Haag-R (IR).
[b] Size classes: S, small 1–4 ommatidia affected; L, large >4 ommatidia; T, total spots.
[c] Calculated according to the formula $f = 2\,\text{nm/NC}$.
[d] (+) positive; (−) negative.
[e] Reduced survival in relation to control series.

Among anisidines *o*-AN induced a significantly increased frequency of light spots in a dose-dependant manner in the IS strain and also in crosses between IS × IR and IR × IS. Surprisingly the IR strain gave a clearly negative result. Again a regulatory effect was found in crosses between IS × IR where clone induction was almost four times lower than the results obtained from the cross between insecticide susceptible flies. A positive recombinogenic effect was induced by *o*-AN in larvae resulting from crosses between Haag-79 females and standard males, also a slightly reduction in clone induction was observed as compared with larvae from IR × IR cross where *o*-AN was clearly non-active. Haag-79 R strain is an insecticide resistant strain that is characterized by high P450 activity and at the molecular level expressed more than 10 cytochrome-P450s of families CYP4, CYP6 and CYP9, the three major xenobiotic metabolizing P450 families from insects [25]. The lack of response of this strain to *ortho*-anisidine could be due to intrinsic differences between strains in constitutive and inducible levels of the enzymes involved in the metabolism of this carcinogen. Interestingly in previous reports a recombinogenic effect was found in another IR strain, Hikone-R, treated with different AAs: 2-naphthylamine [13], *o*-anisidine, *o*-toluidine and 4,4,′-oxydianiline [8]. Thus, as stated earlier performance and variation in response to procarcinogens is genotype-dependent in this species [13].

Both *m*-AN and *p*-AN showed not to induce mitotic recombination in all the strains and combinations of crosses tested. Anisidine isomers reveal markedly different responses associated with the position of the methoxy group in the chemical structure, only *o*-methoxyaniline (*o*-AN) is shown to be recombinogenic while the *meta* and *para* isomers were not. The recombinogenic activity of these monocyclic aromatic amines depend on the positions of the chemical groups relative to one another. Isomers can differ appreciably from one another with respect to recombinogenicity, thus, relatively minor differences in the position of the substituent group can similarly cause major differences in biological activity.

DBAs are well known mutagenic and carcinogenic PAHs, in our study 1,2;3,4-DBA was negative in IS and IR strains and in crosses between them while 1,2;5,6-DBA proved to be positive at the highest concentration tested in the two strains and crosses assayed. These results show that the structural position of the benzene ring in the molecule seems to be relevant for genotoxicity.

The eye $w/w+$ somatic assay detects phenotypically visible light spots in the red eyes of adult females resulting from loss of heterozygosity (LOH) by mitotic recombination. The use of a multiply inverted X chromosome, which in XX cells supresses recombination between the two Xs identified inter-chromosomal mitotic recombination as the predominant cause generating loss of the $w+$ reporter gene [17]. In our study, classification of spots in size classes showed that homologous mitotic recombination was produced late during development as can be seen by the highest frequency of small 1-2 and 3-4 ommatidia spots produced.

With respect to structure-activity relationships which can be derived from the results obtained in the present study several conclusion can be drawn, for anisidines: (1) the position of the methoxy group is required for genotoxic activity, (2) the methoxy group in position *ortho* is recombinogenic while in positions *meta* and *para* are not. For DBAs: (1) the position of the benzene ring plays a role in the recombinogenic activity consequently, (2) 1,2;3,4-DBA was substantially inactive while 1,2;5,6-DBA was marginally positive.

Another conclusion is that although the somatic mutation and recombination $w/w+$ assay has been used for the evaluation of a broad number of chemicals with different action mechanisms the two classes of procarcinogens tested in this study, namely AAs and PAHs, are difficult and poorly detected by the assay. With respect to IR and IS strains it remains to be determined to what extent the spectrum of metabolizing capacity really differs between the strains of the test assay. More information is needed about the regulation and expression of the cytochrome-P450 genes and on the molecular nature taking place in the eye imaginal disc as well as between insecticide susceptible and resistant strains after exposure to genotoxic chemicals.

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