ELSEVIER

Mutation Research 395 (1997) 229–242

Genetic Toxicology and Environmental Mutagenesis

Genotoxic activation of hydrazine, two dialkylhydrazines, thiourea and ethylene thiourea in the somatic *w / w +* assay of *Drosophila melanogaster*

Rosario Rodriguez-Arnaiz *

Laboratorio de Genética, Facultad de Ciencias, UNAM Coyoacán 04510, México, D.F., Mexico

Received 27 February 1997; revised 8 August 1997; accepted 18 August 1997

Abstract

Genotoxic activation of hydrazine (HZ), two symmetrical dialkylhydrazines, namely, 1,2dimethylhydrazine and 1,2-diethylhydrazine (SDMH and SDEH), thiourea (TU) and ethylene thiourea (ETU) has been evaluated by means of the *w / w +* somatic assay of Drosophila. Both low bioactivation insecticide-susceptible (IS) and high biotransformation insecticide-resistant (IR) strains were used. The combined application of insecticide-susceptible and insecticide-resistant strains should, in principle, detect somatic cell recombinagens in the *Drosophila melanogaster* in vivo *w / w +* assay. The IS strain was more susceptible to toxicity induced by the test chemicals than the IR stocks. Its performance in the biotransformation of the chemicals tested was rather poor. TU was inactive in all strains. With the active compounds, spot frequencies increased approximately linearly with dose for each spot type. SDEH gave a strong positive result in all three female genotypes exposed. HZ, ETU and SDMH were overall weakly positive in the IR strain Haag-79 (HG-R). Interestingly, ETU was clearly positive in the IR Hikone-R (HK-R) strain. A comparison of the recombinagenic potencies between the active and the weakly positive compounds, and among strains, showed pronounced genotype-dependent differences between the low and the high bioactivation strains. The ability of Drosophila to express several procarcinogens in relation to insecticide-resistance after activation catalyzed by CYP450 enzymes is discussed. © 1997 Elsevier Science B.V.

Keywords: Drosophila melanogaster; Somatic in vivo *w / w +* assay; Insecticide-susceptible strain; Insecticide-resistant strain; Biotransformation; CYP450 enzyme

1. Introduction

The eye mosaic *white / white +* (*w / w +*) assay of *Drosophila* is an in vivo short-term test measuring genetic damage in somatic cells of *Drosophila* after treatment of larvae [1]. The use of strains with naturally determined metabolic capacities has been shown to improve the assay [2,3]. Biotransformation of different classes of progenotoxins, is thought to involve oxidative metabolism by cytochrome P450 enzymes, a phenomenon also shown to be genotype-dependent in this species [3].

Hydrazine and its derivatives are frequently found in our environment. They are used as raw materials and/or intermediates in many industrial syntheses,

* Tel.: +52 (5) 622-4906; Fax: +52 (5) 622-4828; E-mail: rra@hp.fciencias.unam.mx

PII S1383-5718(97)00159-9

pesticides and medicines [4]. They occur also in nature, for example, in some fungal metabolites, such as gyromitrins isolated from *Gyromitra esculenta* [5] or agaritine from *Agaricus bisporus* [6]. After the first finding of colon carcinogenesis produced by 1,2-dimethylhydrazine [7,35], many hydrazines were tested for their carcinogenic activity [8,9].

Hydrazine (HZ) and 1,2-dimethylhydrazine (SDMH) need to be metabolized in vivo to become potent carcinogens. SDMH is a potent carcinogen used to induce selectively angiosarcomas and colon tumors in experimental animals [10–12]. Cytochromes P450 IIB1 and IIA1 seem to activate this colon-specific model mutagen in human colon microsomes [13]. Oxidation of several substituted hydrazines by neutrophils from rat peritoneal exudates, led to the formation of alkyl radicals [14]. Methylhydrazines are bacterial mutagens, the lesions induced are the target of DNA repair MTases, which probably include mutagenic and carcinogenic lesions such as O^6MeG and/or O^4MeT [15,16]. The carcinogen 1,2-diethylhydrazine (SDEH) has been reported to induce tumors in the olfactory bulb, brain and nasal cavity of rats [8,17], as well as other tumors and mammalian malignancies [18].

Thiourea (TU) is employed as a photographic fixing agent to remove stains from negatives, in the manufacture of resins, and among other uses, as vulcanization accelerator. TU is an hydroxyl scavenger [19], which has been shown to reduce the toxicity at various concentrations of hydrogen peroxide. It also produces a remarkable inhibition of the lethal response exerted by H_2O_2 at higher doses in *Escherichia coli* [20]. NADPH and oxygen-dependent flavin-containing monooxydases, catalyze the oxidation of TU to formamidine sulfenic acid [21]. Contradictory results were obtained on the mutagenicity of this compound in somatic assays of *Drosophila melanogaster*, it was genotoxic in the unstable zeste-white [22], while weakly-positive in the $w/w+$ test [1].

Ethylene thiourea (ETU) is used extensively in the rubber industry as an accelerator in the vulcanization of elastomers. It is also a trace contaminant and metabolic degradation product of a widely used class of ethylene bisdithiocarbamate nonsystemic fungicides which are used worldwide for crop protection [23]. ETU causes thyroid tumors in rodent and liver tumors in mice [24,25]. It inhibits thyroid peroxidase leading to decreased circulating levels of thyroid hormone and compensatory increased secretion by the pituitary of thyroid stimulating hormone (TSH). In *Drosophila* it has been shown to be inactive in the $w/w+$ assay [1]. Several independent and regulatory authorities have evaluated the

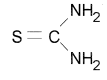
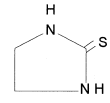
Compound abreviation	Molecular formula	Structural formula
Hydrazine, HZ	H_4N_2	$H_2N\text{-}NH_2$
1,2-dimethyl hydrazine SDMH	$C_2H_8N_2$	$CH_3\text{-}NH\text{-}NH\text{-}CH_3$
1,2-diethyl hydrazine SDEH	$C_4H_{12}N_2$	$CH_3\text{-}CH_2\text{-}NH\text{-}NH\text{-}CH_2\text{-}CH_3$
Thiourea, TU	CH_4N_2S	
Ethylene thiourea ETU	$C_3H_8N_2S$	

Fig. 1. Abbreviation, molecular and structural formula of the compounds tested.

ETU genetic toxicology database. The NTP did find some positive genotoxicity evidence for ETU [24]. IARC declared the evidence limited for mutagenicity [26]. And MAFF concluded that ETU showed no genotoxicity in vivo [27]. Recently, Dearfield [28] reviewed the extensive database of genetic toxicology tests conducted on ETU, and concluded that it is not a potent genotoxic agent. It was also suggested that additional in vivo studies should be performed before a conclusion on the activity of ETU is reached.

The aim of this study has been the characterization of hydrazine, two symmetrical dialkylhydrazines, as well as, thiourea and ethylene thiourea (Fig. 1 shows their structural and molecular formulas) with regard to their ability to induce interchromosomal recombination in somatic cells. The *white / white +* (*w / w +*) in vivo mosaic assay [29,30] was used, employing an insecticide-susceptible (IS) and two insecticide-resistant (IR) strains.

2. Materials and methods

2.1. Drosophila strains

Three *Drosophila* stocks were used: one wild-type low bioactivation insecticide-susceptible (IS) Leiden Standard (ST) strain, and two high biotransformation insecticide-resistant (IR) strains, namely Hikone-R (HK-R) and Haag 79-R (HG-R). Crosses were done between white females and yellow (LS, HG-R) or wild-type (HK-R) males. Stocks were maintained at 25°C and 60% humidity.

2.2. Chemicals

Hydrazine (HZ, CAS No. 10217-52-4), 1,2-dimethylhydrazine (SDMH, CAS No. 306-37-6), 1,2-diethylhydrazine (SDEH, CAS No. 7699-31-2), thiourea (TU, CAS No. 62-56-6), and ethylene thiourea (ETU, CAS No. 96-45-7) were obtained from Aldrich (Milwaukee, WI, USA).

2.3. The w / w + somatic assay

Chemicals were administrated by chronic exposure. Fifteen pair of flies were permitted to lay eggs for three days on standard food supplemented with the test substance dissolved in a mixture of 3 parts ethanol 1 part Tween 80. Growing cultures were exposed to each compound during all three instar stages of larval development. Two 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 transferred to fresh medium and scored 1 to 5 days later. The scoring of etherized flies was carried out in a liquid containing 90 parts ethanol, 1 part Tween 80 and 9 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 spots was done on the basis of small spots (2–4 ommatidia), large spots (> 4 ommatidia affected) and total spots. Spots separated by at least four nonmutated ommatidia were considered as independent events [1]. For an indirect estimation of the genotoxic effectiveness of the chemicals, the frequency of clones per 10^4 cells was calculated. The chi square for proportions, was used for statistical evaluation of the data [31]. Test responses were classified into four categories: [1] positive, + a recombinagenic response and a dose-response relation was found, [2] weakly positive, w + the clone frequency was enhanced compared with concomitant controls, [3] inconclusive, i no acceptance at the same time of two mutually exclusive hypotheses, and [4] negative −, no effects under the conditions of the test.

When a dose–response relationship was obtained a comparison of the genotoxic activities of agents was calculated separately for each dose point, and a mean value listed apart after the highest dose. In cases of levelling-off effect, the high dosage groups were not included in this evaluation [1]. Thus recombinagenic potency is expressed as the number of spots induced per millimole chemical.

3. Results and discussion

The *white / white +* (*w / w +*) mosaic assay [29,30] used in this study, monitors mosaic light

Table 1
Frequencies of mosaic clones in different female genotypes from unexposed and treated larvae with several hydrazines and thioureas in the *w / w +* assay of *Drosophila melanogaster*

Compound (dose mM) / genotype tested	No. of eyes scored	No. of spots						Spots (%)			Average clones size	Clones per 10 000 cells[b]	Activity[c]	Spots induced per mM[d]
		Total	Size (ommatidia)					Size classes[a]						
			1–2	3–4	5–8	9–64	> 64	T	S	L				
Hydrazine, HZ														
ST														
C	612	58	35	10	7	6		9.47	7.35	2.12	5.27	12.49		
0.5	442	47	32	14	1			10.64	10.41	0.23	2.45	6.51	−	
1.0	518	58	36	11	5	5	1	11.19	9.07	2.12	5.22	14.61	−	
5.0[e]	–	–									–	–		
HK														
C	500	53	45	6	2			10.60	10.20	0.40	2.30	6.09		
0.5	500	55	45	7	3			11.00	10.40	0.60	2.44	6.70	−	
1.0	500	52	44	6	1	1		10.40	10.00	0.40	2.40	6.24	−	
5.0	500	55	42	9		4		11.00	10.20	0.80	3.24	8.90	−	
HG														
C	578	57	33	16	6	1	1	9.86	8.48	1.38	4.24	10.44		
0.5	470	50	30	10	5	5		10.78	8.51	2.13	4.58	12.18	−	
1.0	468	66	36	20	9	1		14.24	12.39	1.71	3.08	10.86	w +	
5.0	500	76	49	17	5	4	1	15.20	13.20	2.00	4.38	16.64	+	2.43
1,2-Dimethylhydrazine, SDMH														
ST														
C	594	56	39	11	5	1		9.43	8.42	1.01	3.21	5.93		
0.5	576	58	39	8	6	5		10.07	8.16	1.91	3.98	10.02	−	
1.0	450	62	37	11	5	9		13.77	10.66	3.11	7.69	26.49	+	2.81
5.0	408[f]	38	25	5	5	3		9.31	7.35	1.96	4.50	10.48	−	
HK														
C	696	70	31	15	16	8		10.06	6.61	3.45	5.20	13.07		
0.5	532	58	27	10	12	9		10.90	6.95	3.95	6.95	18.94	−	
1.0	508	63	31	18	9	5		12.40	9.64	2.75	5.09	15.78	−	
5.0	652	85	54	12	10	7	2	13.03	10.12	2.91	5.77	18.90	−	
HG														
C	500	115	92	13	7	3		23.00	21.00	2.00	2.80	16.10		
0.5	500	146	119	19	5	3		29.20	27.60	1.60	2.61	19.04	i	
1.0	500	121	106	11	3	1		24.20	23.20	1.00	2.28	13.78	−	
5.0	500	193	167	15	7	4		38.60	36.40	2.20	2.51	24.22	+	
1,2-Diethylhydrazine, SDEH														
ST														
C	560	49	33	11	5			8.75	7.86	0.89	2.63	5.75		
0.5	524	77	51	14	8	4		14.69	12.40	2.29	3.05	11.21	+	
1.0	506	169	106	40	14	9		33.40	28.85	4.55	3.76	31.40	+	
5.0	500	362	199	92	48	23		72.40	58.20	14.20	4.18	75.66	+	19.26
HK														
C	500	40	33	7				7.64	7.60	0.04	2.55	5.10		
0.5	500	304	213	46	31	14		60.80	51.80	9.00	3.42	51.98	+	
1.0	500	380	226	79	51	23	1	76.00	61.00	15.00	4.28	81.32	+	
5.0	500	1181	564	297	258	53	9	236.20	168.20	68.00	4.02	237.38	+	73.46
HG														
C	560	96	40	31	17	8		17.14	12.68	4.46	5.22	22.37		
0.5	260	205	101	57	37	8	2	78.85	60.77	10.08	4.53	89.29	+	
1.0	160	220	100	68	36	15	1	143.75	105.00	38.75	4.83	166.00	+	
5.0	64	212	49	40	60	62	1	331.25	139.06	192.19	9.17	759.39	+	104.28

Table 1 (continued)

Compound (dose mM)/ genotype tested	No. of eyes scored	No. of spots						Spots (%)			Average clones size	Clones per 10 000 cells[b]	Activity[c]	Spots induced per mM[d]
		Total	Size (ommatidia)					Size classes[a]						
			1–2	3–4	5–8	9–64	> 64	T	S	L				
Thiourea, TU														
ST														
C	500	39	24	10	4	1		7.80	6.80	1.00	2.92	5.69		
0.25	500	48	36	7	3	2		9.60	8.60	1.00	2.79	6.70	−	
0.50	500	50	39	3	2	6		10.00	8.40	1.60	3.72	9.30	−	
1.0	500	48	34	8	4	2		9.60	8.40	1.20	2.83	6.79	−	
HK														
C	500	37	27	6	3	1		7.40	6.60	0.80	2.97	5.49		
0.25	500	49	30	10	5	4		9.80	8.00	1.80	3.68	9.46	−	
0.50	500	59	45	11	2		1	11.80	11.20	0.60	3.64	10.74	−	
1.0	200[f]	25	15	7	2	1		11.50	11.00	0.50	3.32	10.37	−	
HG														
C	484	72	43	19	9	1		14.87	12.81	2.06	3.01	11.31		
0.25	424[f]	59	38	11	8	2		13.91	11.55	2.36	3.49	12.14	−	
0.50	364[f]	71	38	26	5	2		19.50	17.58	1.92	3.03	14.77	−	
1.0	162[f]	30	19	6	5			18.52	15.43	3.09	2.83	13.10	−	
Ethylenethiourea, ETU														
ST														
C	500	46	27	10	6	3		9.20	6.80	2.40	3.98	9.15		
0.25	490	46	31	9	3	3		9.39	8.16	1.22	3.63	8.52	−	
0.50	466[f]	48	34	8	3	3		10.30	9.01	1.29	3.90	9.88	−	
1.0	334[f]	35	21	12	2			10.48	9.28	1.20	2.63	6.89	−	
HK														
C	500	39	35	2	2			7.44	7.40	0.04	2.38	4.64		
0.25	486	53	38	9	4	2		10.90	9.67	1.23	2.17	5.92	i	
0.50	490	56	43	8	4	1		11.43	10.41	1.02	2.61	7.46	+	
1.0	120[f]	21	18	3				17.50	15.00	2.50	2.52	11.07	+	10.63
HG														
C	636	122	76	26	15	5		19.18	17.72	3.14	3.40	16.30		
0.25	700	144	95	24	16	9		20.57	17.00	3.57	3.47	17.84	−	
0.50	704	149	93	33	14	9		21.16	17.89	3.27	3.30	17.46	−	
1.0	148[f]	42	19	17	5	1		28.38	24.32	4.05	3.19	22.63	+	6.24

[a] Size classes: T, total spots; S, small spots 1–4 ommatidia affected; L, large > 4 ommatidia.
[b] Calculated according to the formula $f = 2$ nm/NC.
[c] Activity: + positive, − inactive, i inconclusive, $w+$ weakly positive.
[d] Expressed as spots induced per millimole per 100 eyes.
[e] Dose that induced sterility.
[f] Reduced survival in relation to control series.

spots in the eyes of adult females, resulting from the loss of heterozigosity and the expression of white in female genotypes heterozygous for this marker. The various recombinagenic events which may lead to loss of heterozygosity, and thus to expression of white are: mitotic crossing over, the reciprocal exchange of genetic information between homologous chromosomes at the four-strand stage; unequal sister-strand recombination between the chromatids carrying the wild-type allele; and gene conversion, the nonreciprocal transfer of information from one DNA duplex to another [32].

Each of the five compounds tested was assayed in at least two independent experiments using three

different concentrations. No differences between repetitions were noticed, and the data were pooled. The results obtained with the compounds tested in the three genotypes employed, and the statistical evaluation are presented in Table 1. Large variation in spontaneous occurrence of mosaic light spots in the three stocks under test was observed. As in previous studies [2,3] drastic variation in spot frequencies in

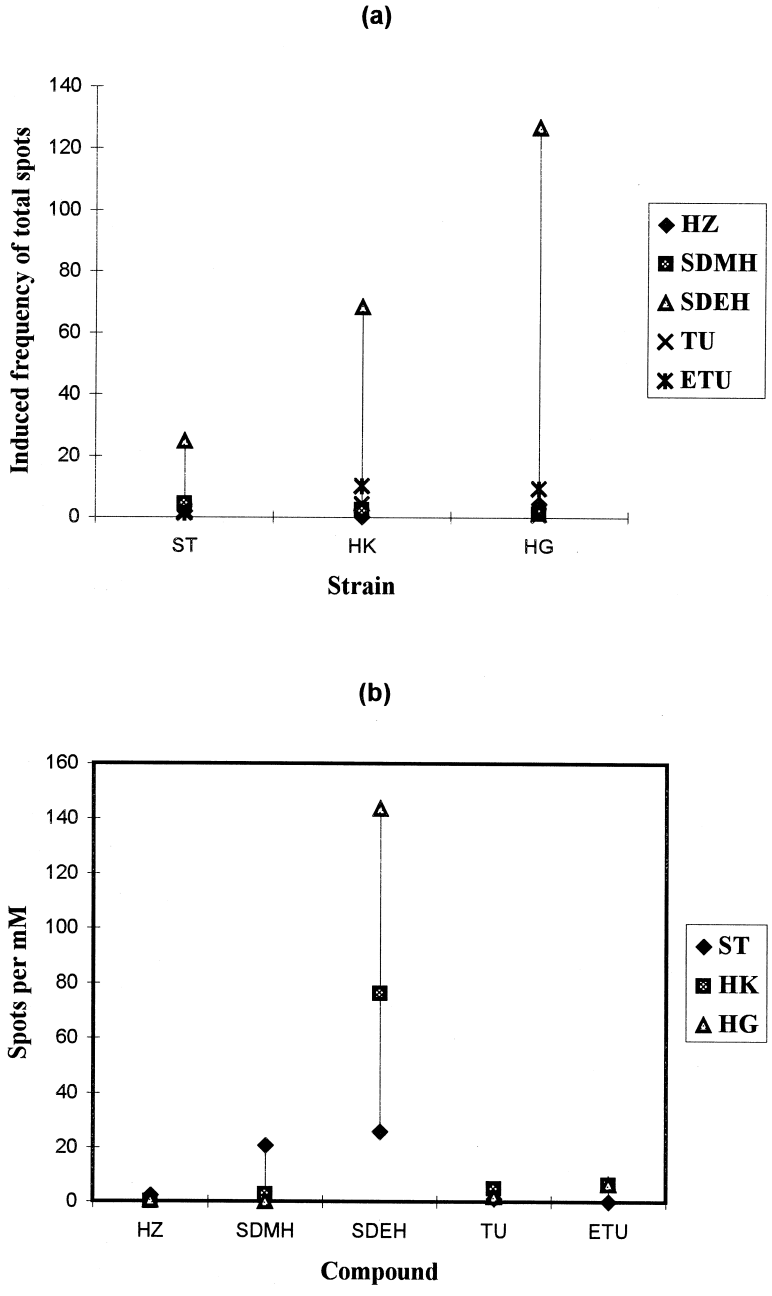


Fig. 2. Induced frequency of total spots (a) and induced clones per 10 000 cells (b) of the five compounds tested in different female genotypes.

non-exposed larvae was noticed, thus it is necessary to run concurrent controls. In the susceptible-insecticide strain ST spontaneous clone frequencies varied from 7.80 to 9.5, while in the resistant-insecticide HK-R variations between 7.40 to 10.60 were obtained. More pronounced differences were observed in the high bioactivation IR strain HG-R. Spontaneous white mosaic spots varied from 9.9 to 23.0. The majority are small spots, large spots are about five times less frequent in ST, near six times less frequent in HG, and about seven times in HK-R.

The principal ability of the $w/w+$ system is to detect genotoxic agents depending on metabolic conversion to DNA-reactive species. The compounds tested in the present study are of this type. The inert agents were applied by chronic exposure in order to assure metabolic activation. Hydrazines and dialkylhydrazines were tested at the same concentrations

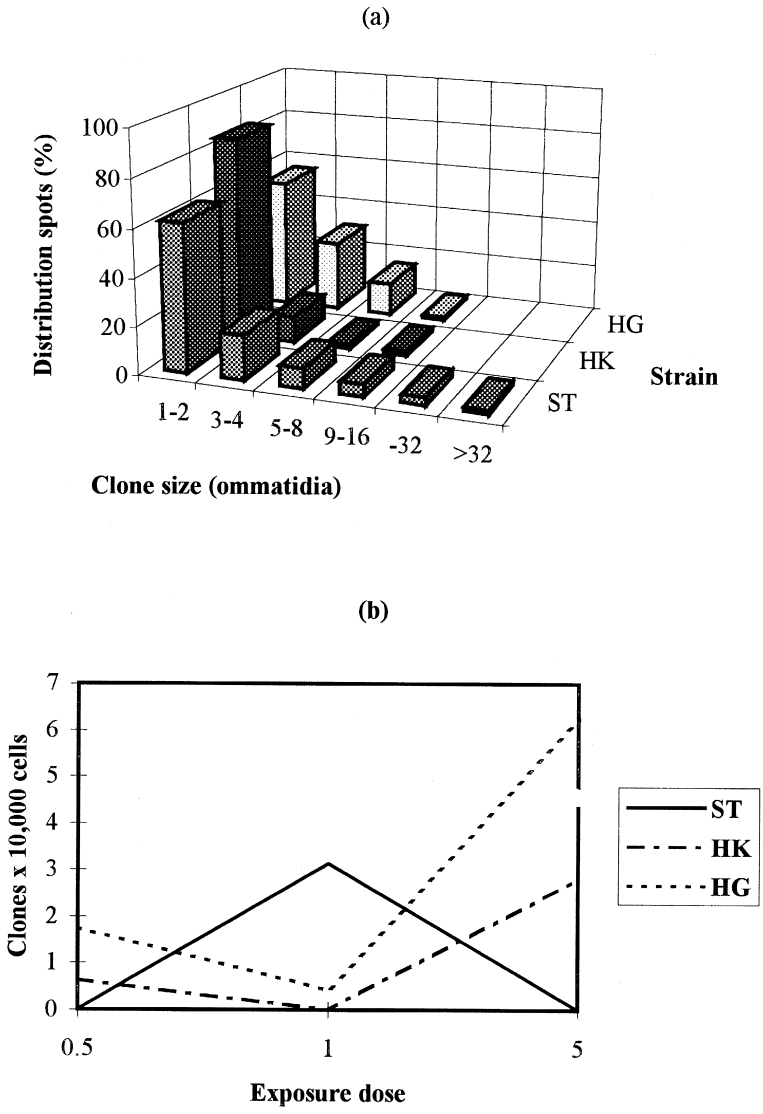


Fig. 3. Relative distribution in size classes of induced mosaic light spots (a), and genotoxic effectiveness (b) in female genotypes exposed to three different doses of hydrazine.

ranging between 0.5 and 5 mM, while the more toxic thiourea and ethylenethiourea, were tested at concentrations between 0.25 and 1 mM. For a comparison between strains, the induced frequency of total spots for the 1 mM exposure dose is shown in Fig. 2a. The corresponding clones induced per 10 000 cells are plotted in Fig. 2b. A strong positive response was obtained with 1,2-diethylhydrazine (SDEH) in the three female genotypes exposed. Marginal positive results (only at the highest concentration tested) were obtained with 1,2-dimethylhydrazine (SDMH) in both ST and HG-R. Hydrazine (HZ) was inactive in ST and in HK-R, while in HG-R the frequencies of total spots per 100 eyes were significantly increased over the control from 1.0 mM and higher. Thiourea (TU) proved to be non-genotoxic, the chemical was probably biotransformed and detoxified. It also induced a drop in spot frequencies at the highest concentration

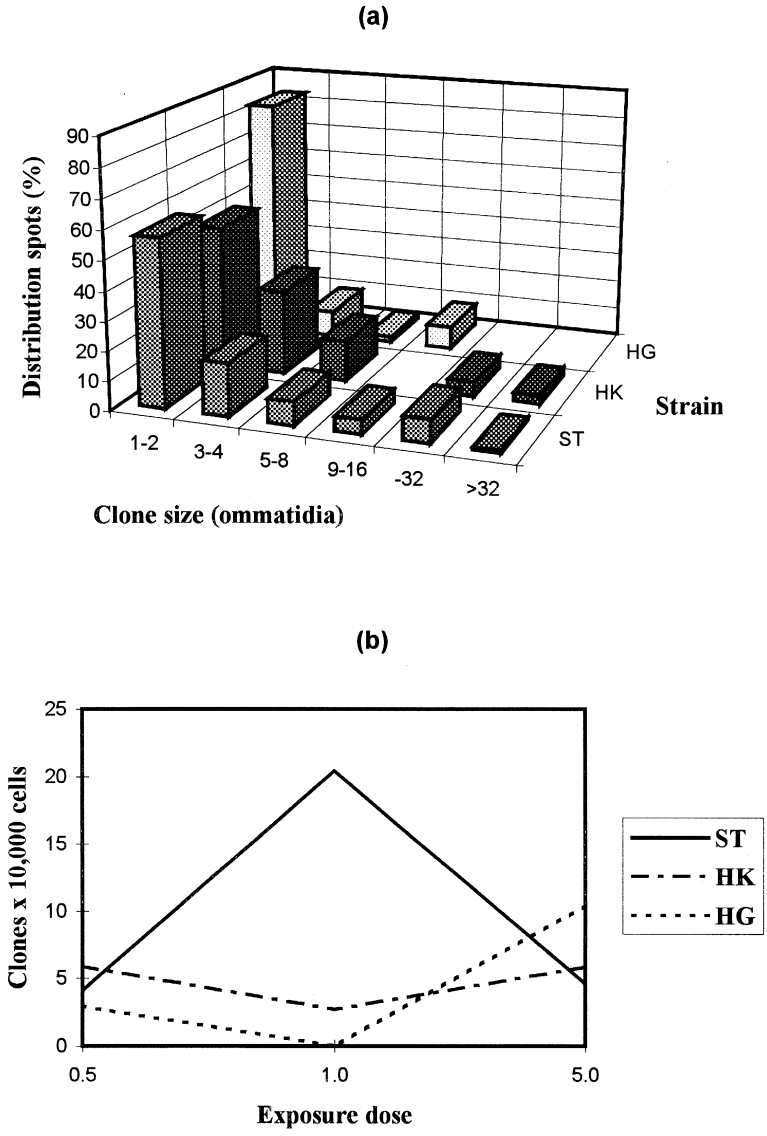


Fig. 4. Relative distribution in size classes of induced mosaic light spots (a), and genotoxic effectiveness (b) in female genotypes exposed to three different doses of 1,2-dimethylhydrazine.

tested, an effect probably due to its pronounced toxicity. Interestingly, ethylene thiourea (ETU) reduced viability at higher doses, was inactive in strain ST, was clearly genotoxic in HK-R, and active only at toxicity levels in the IR strain HG-R.

In Figs. 3–7a the spot size distribution for spots are given for the 1 mM treatment with each of the 5 compounds in the three stocks employed. The genotoxic effectiveness of the compounds in relation to exposure dose is also shown (Figs. 3–7b). In the treated series the predominance of small spots was maintained, large spots were only more abundant at the high effective exposure dose for SDEH in HG-R.

As expected, the IS strain was much more susceptible to induced sterility at high exposure doses than the IR strains. All the compounds were tested at

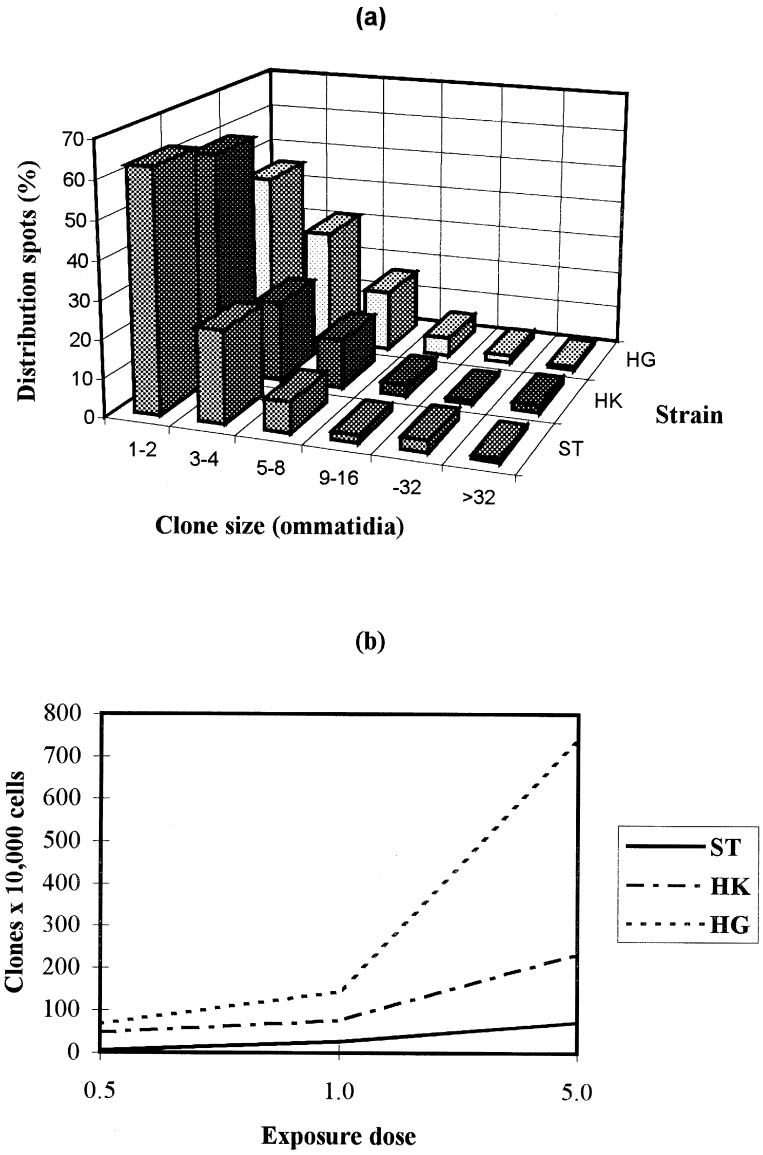


Fig. 5. Relative distribution in size classes of induced mosaic light spots (a), and genotoxic effectiveness (b) in female genotypes exposed to three different doses of 1,2-diethylhydrazine.

several exposure levels; doses producing cytotoxicity can be identified by the low number of individuals obtained for eye inspection. With the exception of TU spot frequency increased approximately linearly with dose for each spot type. Low exposure doses sometimes produced weak effects, were inconclusive or clearly negative. No particular attempts were made to determine the highest dose tested (HDT) for those chemicals inactive in the assay [1].

HZ and SDMH were positive according to statistical criteria at the higher exposure dose in HG-R. Methylhydrazines can be oxidized to active DNA-methylating derivatives which generate methylphosphotriesters in DNA [15]. DNA alkylating agents represent one of the largest classes of environmental chemical carcinogens. The treatment of cells with simple alkylating agents can produce several DNA lesions [33] among wich O^6-alkylguanine and O^4-al-

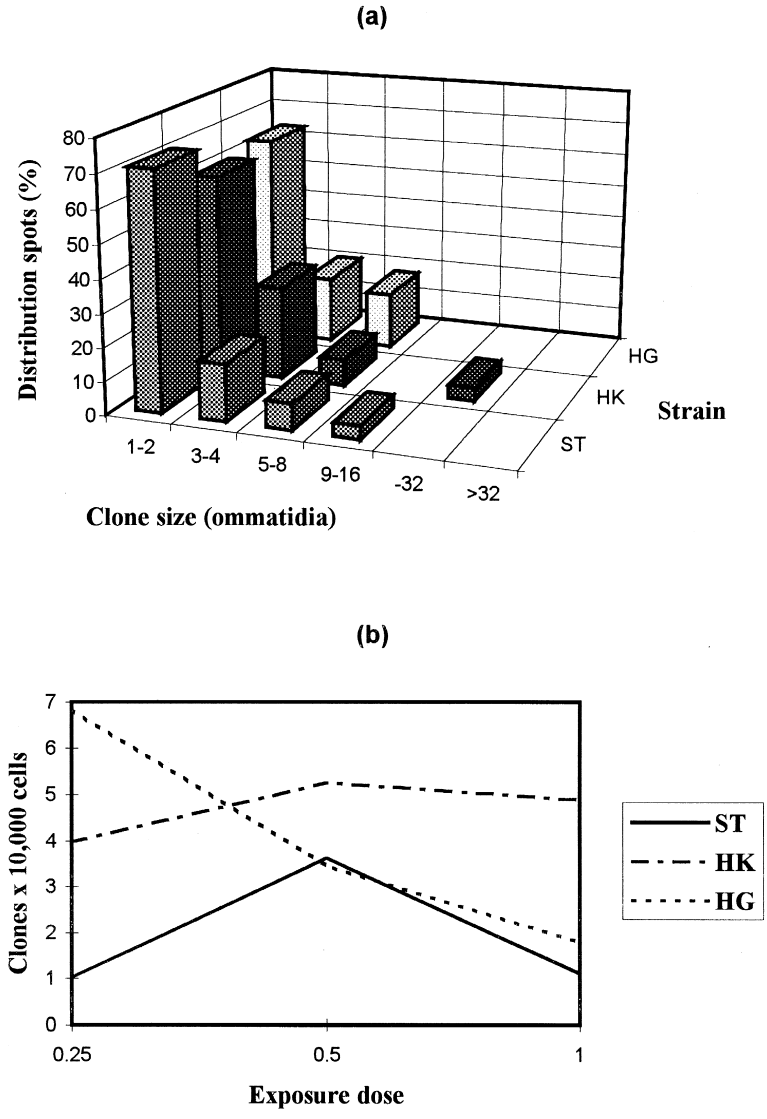


Fig. 6. Relative distribution in size classes of induced mosaic light spots (a), and genotoxic effectiveness (b) in female genotypes exposed to three different doses of thiourea.

kylthymine are considered to be the most mutagenic since they tend to mis pair with deoxythymine and deoxyguanine respectively during DNA replication and cause transition mutations [16]. A strong mutagenicity of the ethyl derivative was obtained in all three female genotypes tested. Thus, with both symmetrical hydrazines the genotoxic mechanism probably is through DNA alkylations. The diethyl substituted hydrazine is shown in the present study to be much more reactive than the disubstituted methylhydrazine (Fig. 1).

ETU is known to produce thyroid follicular cell neoplasms in rats and liver neoplasms in mice, also produces adenomas of the pars distalis of the pituitary gland following long-term administration [24]. Mutagenicity associated with toxic levels was observed for ETU in the present study. In the IR strain HG-R it produced an elevated frequency of spots at

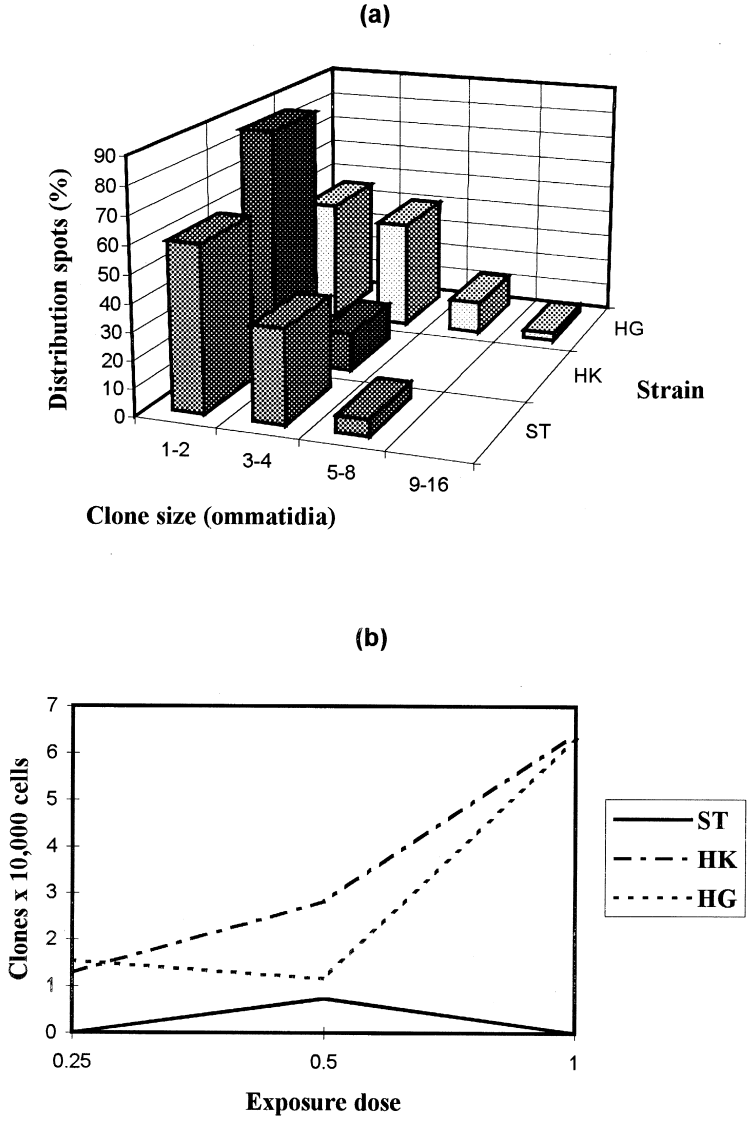


Fig. 7. Relative distribution in size classes of induced mosaic light spots (a), and genotoxic effectiveness (b) in female genotypes exposed to three different doses of ethylene thiourea.

the highest dose, which is also the exposure that induced sterility due to toxicity, thus the response was overall negative. The performance of HK-R for this chemical was different: clear positive results were obtained at several doses. The agent showed to be inactive in the susceptible-insecticide strain ST.

A comparison of the recombinagenic effectiveness between the compounds active and weakly positive, and among strains employed in the assay, was made in terms of the number of spots induced per millimole chemical. Both types of activities showed to be genotype-dependent (Fig. 8). For the weakly mutagenic agents, the IS strain induced $\cong 2$ mosaic clones per millimolar exposure dose, while both IR strains produced between 6 to 10 mosaic clones $\times$ mM dose. More pronounced differences were found after mutant treatment with the active genotoxin, whereas in the low bioactivation strain mosaic clones were increased only 5 fold, with HK-R mosaic clones were increased 12 fold, and with HG-R about 17 fold. In conclusion the performance of the IR HG-R strain was clearly the best.

This range of genotype-determined variation in response to genotoxins could, in principle, be related to pesticide-resistance. Several P450 cDNA fragments have been cloned recently, revealing a variety of P450 genes from two families, CYP4 and CYP9, and seven subfamilies some of which are clustered in the *Drosophila melanogaster* genome. Putative allelic variants of several of the genes were found in different insecticide-resistant (HK-R and HG-R) and susceptible strains (Oregon R); sequences of the CYP4 family from mammals are always grouped outside the insect CYP4 clade, CYP6 and CYP9 families have only been obtained from insects to date [34]. Their role in the metabolic activation of progenotoxins and in insecticide-resistance have to be determined. Orthologous relationships between P450 genes across insect species shows that the CYP4D subfamily diverged early in Diptera and have representatives in *Drosophila melanogaster*, *Musca domestica* and *Anopheles albimatus*. The CYP4C subfamily has also representatives in cockroaches, mosquitos and *Drosophila*. In contrast the CYP4E subfamily has only been found in *Drosophila*, the genes are scattered in the genome of this species. Speculations on the functional roles of different P450 subfamilies in insects and vertebrates

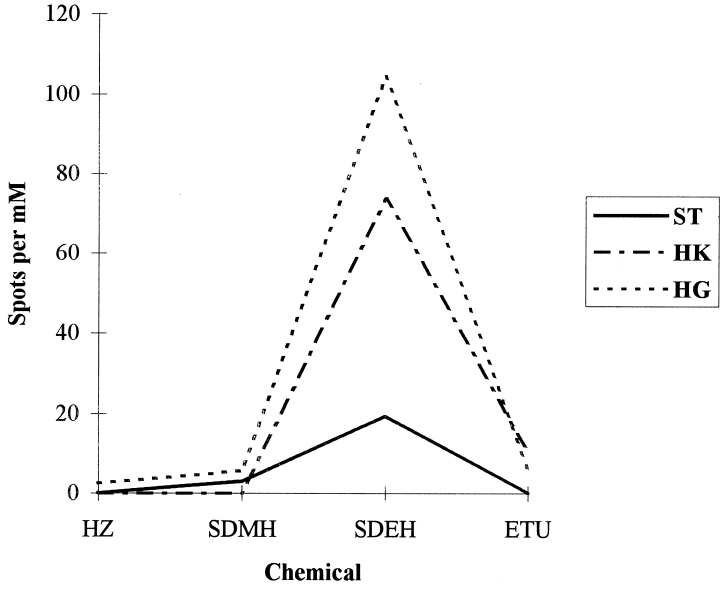


Fig. 8. Performance of the strains employed expressed as recombinagenic effectiveness.

is interesting. It seems that the CYP4 family in Diptera has the same role as that of the CYP2 family in mammals that of a rapidly evolving group of mostly detoxifying P450s [34].

Acknowledgements

The author wish to thank Judith Hernandez Aranda and Juan Carlos Gaytan Oyarzun for their technical assistance.

References

[1] E.W. Vogel, M.J.M. Nivard, Performance of 181 chemicals in a *Drosophila* assay predominantly monitoring interchromosomal mitotic recombination, Mutagenesis 8 (1993) 57–81.
[2] E.W. Vogel, M.J.M. Nivard, J.A. Zijilstra, Variation of spontaneous and induced mitotic recombination in different *Drosophila* populations: a pilot study on the effects of polyaromatic hydrocarbons in six newly constructed tester strains, Mutation Res. 250 (1991) 291–298.
[3] R. Rodriguez-Arnaiz, E.W. Vogel, A. Szakmary, Strong intra-species variability in the metabolic conversion of six procarcinogens to somatic cell recombinagens in *Drosophila*, Mutagenesis 8 (1993) 543–551.
[4] A. Poso, A. von Wright, J. Gynther, An empirical and theoretical study on mechanisms of mutagenic activity of hydrazine compounds, Mutation Res. 332 (1995) 63–71.
[5] H. Pyysalo, A. Niskanen, On the occurrence of *N*-methyl-*N*-formyl hydrazones in fresh and processed false morel *Gyromitra esculenta*, J. Agric. Food Chem. 25 (1977) 644–647.
[6] D. Ross, B. Nagel, A. Toth, Evidence for the ocurrence and formation of diazonium ions in *Agaricus bisporus* mushroom and its extracts, Agric. Food Chem. 30 (1982) 521–525.
[7] H. Druckrey, R. Preussmann, F. Matzkies, S. Ivankovic, Selektive Erzeugung von Darmkrebs bei Rattendurch 1,2-Dimethylhydrazin, Naturwissenschaften 54 (1967) 285–286.
[8] J.C. Arcos, Y.T. Woo, M.F. Argus (Eds.), Chemical Induction of Cancer, vol. III A: Aliphatic Carcinogens, Academic Press, New York, 1982, pp. 326–414 and 710–714.
[9] H. Matsushita, O. Endo, H. Matsushita, M. Yamamoto, M. Mochizuki, Mutagenicity of alkylhydrazine oxalates in Salmonella typhimurium TA100 and TA102 demonstrated by modifying the growth conditions of the bacteria, Mutation Res. 301 (1993) 213–222.
[10] W. Linsky, R.M. Kovatch, C.W. Riggs, Carcinogenesis by nitrosodialkylamines and azoxyalkanes given by gavage to rats and hamsters, Cancer Res. 47 (1987) 3968–3972.
[11] S.N. Newaz, W. Fang, H.W. Strobel, Metabolism of the carcinogen 1,2-dimethylhydrazine by isolated human microsomes and human colon tumor cells in culture, Cancer 52 (1983) 794–798.
[12] N.O. Davidson, C.A. Ifkovits, S.F. Skarosi, A.M. Hausman, X. Lor, M.D. Sitrin, A. Montag, T.A. Brasitus, Tissue and cell-specific patterns of expression of rat liver and intestinal fatty acid binding protein during development and in experimental colonic and small intestinal adenocarcinomas, Lab. Invest. 68 (1993) 663–675.
[13] D. Stralka, H.W. Strobel, Characterization of cytochrome P450 dependent dimethylhydrazine metabolism in human colon microsomes, Cancer 68 (1991) 2363–2369.
[14] M. Gamberini, L.C. Leite, Carbon-centered free radical formation during the metabolism of hydrazine derivatives by neutrophils, Biochem. Pharmacol. 45 (1993) 1913–1919.
[15] B. Sedgwick, Oxidation of methylhydrazines to mutagenic methylating derivatives and inducers of the adaptative response of *Escherichia coli* to alkylation damage, Cancer Res. 52 (1992) 3693–3697.
[16] W. Xiao, M. Nowak, S. Laferte, T. Fontanie, Mutagenicity and toxicity of the DNA alkylation carcinogens 1,2-dimethylhydrazine and azoxymethane in *Escherichia coli* and *Salmonella typhimurium*, Mutagenesis 11 (1996) 241–245.
[17] M.S. Zedeck, Hydrazine derivatives, azo and azoxy compounds, and methylazoxymethanol and cycasin, in: S. Searle (Ed.), Chemical Carcinogens, American Chemical Society, Washington, DC, 1984, pp. 915–944.
[18] V.N. Anasimov, Carcinogenesis induced by a single administration of 1,2-diethylhydrazine in female rats of various ages, Cancer Lett. 67 (1992) 21–25.
[19] A. Farhataziz, B. Ross, Selected specific rates of reactions of transitiens from water in aqueous solutions III Hydroxil radical and perhydrpxyl radical and their radical ions, Natl. Stand. Ref. Ser. 58 (1977) 1–113.
[20] G. Brandi, L. Salvaggio, F. Cattabeni, O. Cantoni, Cytocidal and filamentous response of *Escherichia coli* cells exposed to low concentrations of hydrogen peroxide and hydroxyl radical scavengers, Environ. Mol. Mutagen. 18 (1991) 22–27.
[21] Z. Guo, T.J. Smith, H. Ishizaki, C.S. Yang, Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) by cytochrome P450IIB1 in a reconstituted system, Carcinogenesis 12 (1991) 2277–2282.
[22] M. Batiste-Alentorn, N. Xamena, A. Creus, R. Marcos, Genotoxicity studies with the unstable zeste-white (UZ) system of *Drosophila melanogaster*: results with ten carcinogen compounds, Environ. Mol. Mutagen. 18 (1991) 120–125.
[23] C.M. Elia, G. Arce, S.S. Hurt, P.J. O'Neill, H.E. Scribner, The genetic toxicology of ethylenethiourea: a case study concerning the evaluation of a chemical's genotoxic potential, Mutation Res. 341 (1995) 141–149.
[24] NTP, National Toxicology Program, Toxicology and carcinogenesis studies of ethylene thiourea in F344/N rats and B6C3F1 mice (feed studies), US Department of Health and Human Services, Public Health Service, National Institutes of Health, NIH publication no. 92-2843, National Toxicology Program Techical Report Series No. 388, March 1992.
[25] R.S. Chhabra, S. Eustis, J.K. Haseman, P.J. Kurtz, B.D.

Carlton, Comparative carcinogenicity of ethylene thiourea with or without perinatal exposure in rats and mice, Fund. Appl. Toxicol. 18 (1992) 405–417.
[26] IARC, IARC Monographs on the Evaluation of Carcinogenic Risk to Humans: Genetic and Related Effects: Supplement 6, International Agency for Research on Cancer, Lyon, 1987, pp. 304–307.
[27] MAFF, Ministry of Agriculture, Fisheries, and Food, Position Document on consumer risk arising from the use of ethylenebisdithiocarbamates, MAFF, London, January 31, 1990.
[28] K.L. Dearfield, Ethylene thiourea (ETU). A review of the genetic toxicology studies, Mutation Res. 317 (1994) 111–132.
[29] E.W. Vogel, J.A. Zjilstra, Mechanistic and methodological aspects of chemically-induced somatic mutation and recombination in *Drosophila melanogaster*, Mutation Res. 182 (1987) 243–264.
[30] E.W. Vogel, J.A. Zjilstra, Somatic cell mutagenesis in *Drosophila melanogaster* in comparison with genetic damage in early germ-cell stages, Mutation Res. 180 (1987) 189–200.
[31] H. Frei, F.E. Würgler, Statistical methods to decide whether mutagenicity test data from *Drosophila* assays indicate a positive, negative or inconclusive result, Mutation Res. 203 (1988) 297–308.
[32] E.W. Vogel, A. Szakmary, Basic principles and evaluation results of assays measuring genotoxic damage in somatic cells of *Drosophila*, in: M.L. Mendelsohn, R.J. Albertini, (Eds.), Mutation and the Environment, part B, Wiley, New York, 1990, pp. 149–158.
[33] D.T. Beranek, Distribution of methyl and ethyl adducts following alkylation with monofunctional alkylating agents, Mutation Res. 231 (1990) 11–30.
[34] B.C. Dunkov, R. Rodriguez-Arnaiz, B. Pittendrigh, R.H. ffrench-Constant, R. Feyereisen, Cytochrome P450 gene clusters in *Dnrosophila melanogaster*, Mol. Gen. Genet. 251 (1996) 290–297.
[35] H. Druckrey, R. Preussmann, F. Matzkies, S. Ivankovic, Chem. Abstr. 67 (1967) 41747a.