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Mutagenicity and recombinogenicity evaluation of bupropion hydrochloride and trazodone hydrochloride in somatic cells of *Drosophila melanogaster*

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ABSTRACT

The aim of the present study was to appraise the mutagenic and recombinogenic potential of bupropion hydrochloride (BHc) and trazodone hydrochloride (THc). We used standard (ST) and the high bioactivation (HB) crossings from *Drosophila melanogaster* in the Somatic Mutation and Recombination Test. We treated third-instar larvae from both crossings with different concentrations of BHc and THc (0.9375 to 7.5 mg/mL). BHc significantly increased the frequency of mutant spots in both crossings, except for the lowest concentration in the ST crossing. ST had also the mostly recombinogenic result, and in the HB, BHc was highly mutagenic. On the other hand, THc significantly increased the frequency of mutant spots in both the ST and HB crossings at all concentrations. The three initial concentrations were recombinogenic and the highest concentration was mutagenic for the THc. BHc and THc at high concentrations were toxic, even though their mutagenicity was not dose-related. THc significantly increased the frequency of mutant spots when metabolized, probably as a result of the production of 1-(3′-chlorophenyl) piperazine. BHc was essentially recombinogenic and when metabolized, it became mutagenic. THc was recombinogenic in both crossings. Further studies are needed to clarify the action mechanisms from BHc and THc.

1. Introduction

Depression is a disease associated to a chronic and intermittent mood disorder with meaningful impact on the life quality of depressed individuals and their surroundings. The worldwide increasing number of depression cases comprises a significant public health issue as a result of the sociological implications (Aguiar et al., 2011). According to the World Health Organization, in 2017 4.4% of the world's population was depressed, being most predominant in women and elder people.

The pathophysiological mechanisms that led to depression were greatly studied in the late 1950s, making it possible for the antidepressants discovery. Clinical treatment for depression through pharmacological intake was finally a reality (Moreno et al., 1999; Ribeiro et al., 2014).

There are several classes of antidepressants, namely: tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs), selective norepinephrine reuptake inhibitors (SSNIs), selective dopamine reuptake inhibitors (SDRIs), selective serotonin-norepinephrine reuptake inhibitors (SSNRIs) and tetracyclic antidepressants (TeCA) (Then et al., 2017; Wu et al., 2012).

Bupropion Hydrochloride (BHc) belongs to the SSNRIs class of antidepressants (Fig. 1A) and besides being used for depression treatment, it is also applied for quitting smoking therapies (Bhattacharya et al., 2013). Even though the BHc action mechanisms have not yet been fully elucidated, it is known to be a nicotinic antagonist, which decreases smoking-related abstinence symptoms. BHc is metabolized in the liver and owing to its lack of interplay with histamine and cholinergic receptors, it is highly acceptable by the organism (Jefferson et al., 2005).

Although being an important antidepressant, BHc may trigger a handful of detrimental effects such as toxicity, hepatotoxicity, neurotoxicity, cardiovascular toxicity, tachydysrhythmia, severe cardiac arrhythmias, agitation, seizures, drowsiness, hallucinations, convulsions and apoptotic cell death (Anandabaskaran and Ho, 2018; Jang et al., 2011; Tracey et al., 2002). Likewise, the *in vitro* genotoxicity and cytotoxicity of BHc in human peripheral lymphocytes and human cortical neuron have also been pointed out (Bhattacharya et al., 2013).

Additionally, a further research showed that BHc conferred

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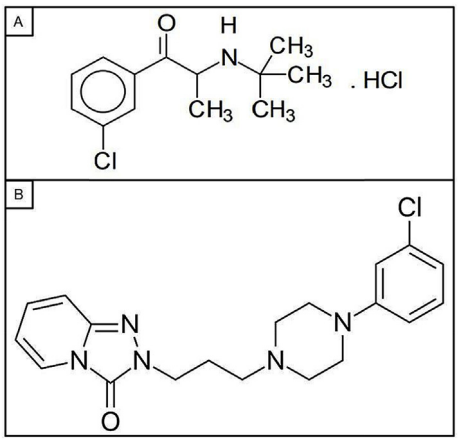

Fig. 1. Structural formula of (A) bupropion hydrochloride, and (B) trazodone hydrochloride.

cardiotoxic effects during fetal development, advocating for BHc avoidance during child-bearing periods (Qureshi et al., 2014). As a consequence, in 2009 the Food and Drug Administration (FDA) from the United States issued a Black Box Warning (BBW) on BHc due to the reasonable evidence of its hazardous effects. On the contrary of the expectations, the BHc consumption did not reduce until 2014, despite the FDA's warning (Shah et al., 2017).

Another highly used pharmaceutical drug is the trazodone hydrochloride (THc), which is also metabolized within the liver and it belongs to the SSRI class (Fig. 1B). THc is clinically prescribed to the insomnia treatment, as a result of its strong association to sleep apnea improvement (Kaynak et al., 2004; Smales et al., 2015). THc desensitizes and decreases the number of beta-adrenergic and 5-HT2A receptors. Besides that, THc also inhibits the following receptors, namely: catecholamine, serotonin, alpha-1-adrenergic and antihistaminic, (Karhu et al., 2011). THc has also been linked to cardiac HERG channels blockade, which is the structure responsible for normalizing electrical activities in human hearts, making THc not suitable for people with cardiovascular diseases (Zitron et al., 2004).

Likewise, THc may also bring about detrimental effects within the organism, such as dizziness, headache, sedation, sweating, weight changes, nausea, vomiting, orthostatic hypotension and syncope (Carvalho et al., 2016; Mittur, 2011). In hepatic cells, THc has been associated to hepatotoxicity and mitochondrial disorders, mainly motivated by increased production of reactive oxygen species (ROS) (Dykens et al., 2008). Just as any other pharmaceutical drug, THc is dose-dependent, and high doses of THc have been correlated to cardiac arrhythmias, ventricular tachycardia (Zitron et al., 2004) and significantly increased risk of upper gastrointestinal bleed (Coupland et al., 2018). Priapism, even though being rare, has also been associated to THc intake (Jayaram and Rao, 2005), as well as *in vitro* clastogenic, mutagenic and cytotoxic effects on human lymphocytes (Yilmaz et al., 2017).

Mammalian use for toxicological and genetic research has decreased over the last decades, which makes it necessary for alternative *in vivo* practices (Siddique et al., 2005). In parallel, *Drosophila melanogaster* has been the study system in genetics and developmental biology for almost a century (Roberts, 2006). Needless to say, *D. melanogaster* has become highly recommended by virtue of its increased possibility to evaluate the toxicity and mutagenicity of various xenobiotics (Demir et al., 2013; Morais et al., 2016; Naves et al., 2018; Oliveira et al., 2017; Reis et al., 2015; Vasconcelos et al., 2017).

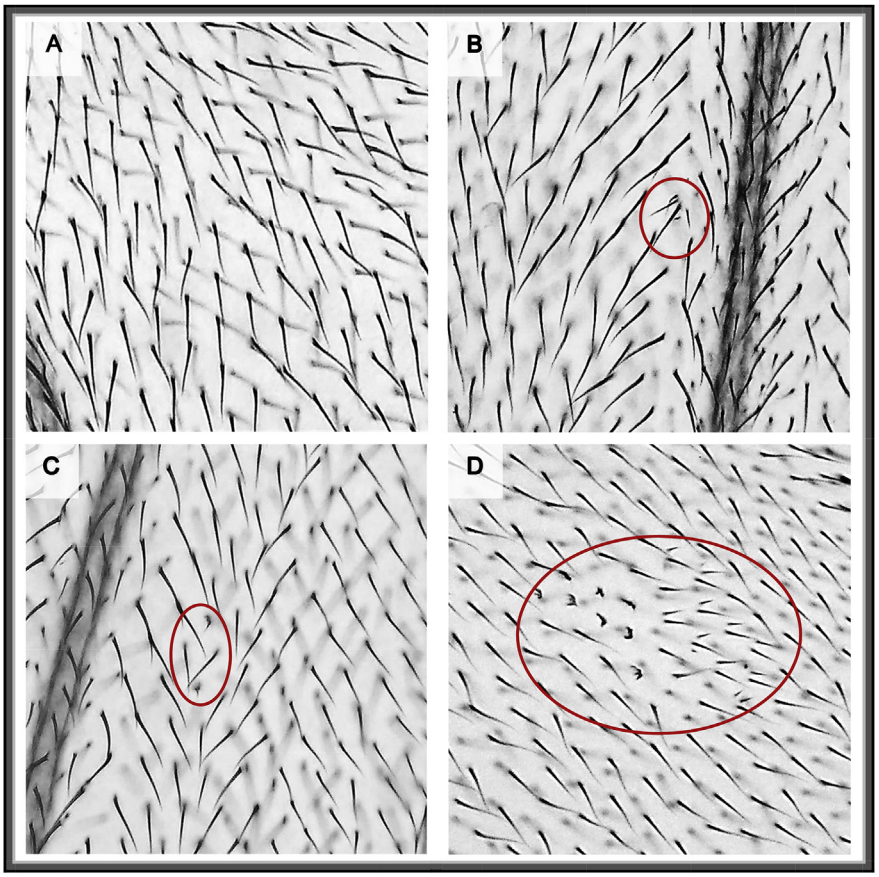

Fig. 2. Photomicrographs (400X magnification) of *D. melanogaster* wings obtained by light microscopy. (A) Normal hair; (B) Small single spot, “mwh” type; (C) Small single spot, “flare” type; (D) Twin spot with “mwh” and “flare” hairs.

There is a greatly used test in *D. melanogaster*, namely the wing Somatic Mutation and Recombination Test (SMART), also known as the wing spot test, which is deeply recognized to assess mutagenicity (Parvathi and Rajagopal, 2014). SMART was developed by Graf et al. (1984) and improved by Graf and van Schaik (1992). The basic premise of the test is to detect heterozygous marker genes impairment. Therefore, whenever the cell is totally differentiated in *D. melanogaster*, heterozygous marker gene loss may drive wing hair (or trichomes) phenotype alteration (Graf et al., 1996; Spanó and Graf, 1998).

Such changes in the phenotype are classified as: "multiple wing hairs" or "flare" simple spots (Fig. 2B and C). Which expresses only one of the mutant genes, such as the *mwh* or *flr*^{3}, being either a small (with one or two mutants trichomes) (Fig. 1S A and B; Fig. 2S A and B) or large (with more than two mutants trichomes) (Fig. 1S C and D; Fig. 2S C and D). On the other hand, twin spots (Fig. 2D) are visualized as multiple trichomes, adjacent to flare, occurring exclusively by recombination (Graf et al., 1984).

Even though being widely used, we still lack knowledge about the genotoxic potential of antidepressants. Furthermore, genotoxicity tests for antidepressants is still understudied though (Yilmaz et al., 2017). Therefore, the main objective of the present study was to evaluate the mutagenic and recombinogenic potential of two pharmaceutical drugs, specifically: BHc and THc in wing somatic cells of *D. melanogaster*.

2. Material and methods

2.1. Chemical compounds and culture media

The antidepressants used in the analysis were: bupropion hydrochloride (BHc) ($C_{13}H_{18}NClO$ - Bupium®), CAS Number: 31677-93-7; EMS Sigma Pharma Ltda., Hortolândia/SP, Brazil, Allotment: 801094, and trazodone hydrochloride (THc) ($C_{19}H_{22}ClN_5O$ - Donaren®), CAS Number: 25332-39-2, Apsen Pharmaceuticals, São Paulo/SP, Brazil, Allotment: 16090258.

Urethane (Ethyl carbamate – CAS Number: 51-79-6, Buchs, Switzerland) was used as a positive control and ultrapure water (18.2 MΩ), obtained from the MilliQ System (Millipore, Vimodrone, Milan, Italy), as the negative control. The flake-mashed potatoes, used as an alternative culture medium, were obtained from Yoki Alimentos S.A. - São Bernardo do Campo, SP, Brazil.

2.2. Somatic Mutation and Recombination Test (SMART) in Drosophila melanogaster

2.2.1. Strains of Drosophila melanogaster

Three different strains were used: (i) *mwh* (*mwh/mwh*); (ii) *flr*^{3} (*flr*^{3}/*In* (3LR) TM3, *r1p sep I (3) 89Aa bx*^{34e} and *Bd*^{S}); and (iii) *ORR*; *flr*^{3}; (*ORR; flr*^{3}*/In* (3LR) TM3, *ri pp sep (3) 89Aa bx*^{34e} and *Bd*^{S}). The mutant strains were kept in a B.O.D. (Biologic Oxygen Demand) greenhouse (Tecnal - Equipamentos para Laboratórios Ltda., Piracicaba, SP, Brazil), with 12 h of light and 12 h of dark and temperature of 25 ± 1 °C.

2.2.2. Crossings and treatments

Two crossings were performed: the Standard (ST) cross, which uses *mwh* males and *flr*^{3} females (Graf et al., 1989); and the High Bioactivation (HB) cross, which uses *mwh* males and *ORR*; *flr*^{3} females (Graf and van Schaik, 1992). HB crossings are characterized by high constitutive levels of cytochrome P450 enzymes. Correspondingly, the ST and the HB produced two types of progeny, trans-heterozygous marked individuals (MH - *mwh* + / + *flr*^{3}) and balanced-heterozygous flies (BH - *mwh* + / + TM3, *Bd*^{S}).

We performed a pilot study to test the toxicity in the SMART, due to the lack of investigations using BHc and THc. The antidepressant concentrations were based on survival assays in *D. melanogaster* (Fig. 3).

To calculate the survival rates upon exposure, larvae were counted before the distribution in treatment tubes containing different concentrations of BHc or THc (0.9375; 1.875; 3.75; 7.5 or 15 mg/mL). The hatched flies were counted and stored in 70% ethanol. The following concentrations 0.9375, 1.875 and 3.75 mg/mL did not alter survival rates, when compared to the negative control. Therefore, we chose these concentrations to be used within the SMART, having BHc and THc tested in two independent experiments. Although the concentration of 7.5 mg/mL of THc have significantly altered the survival rate in the HB cross, this concentration was also included in the SMART.

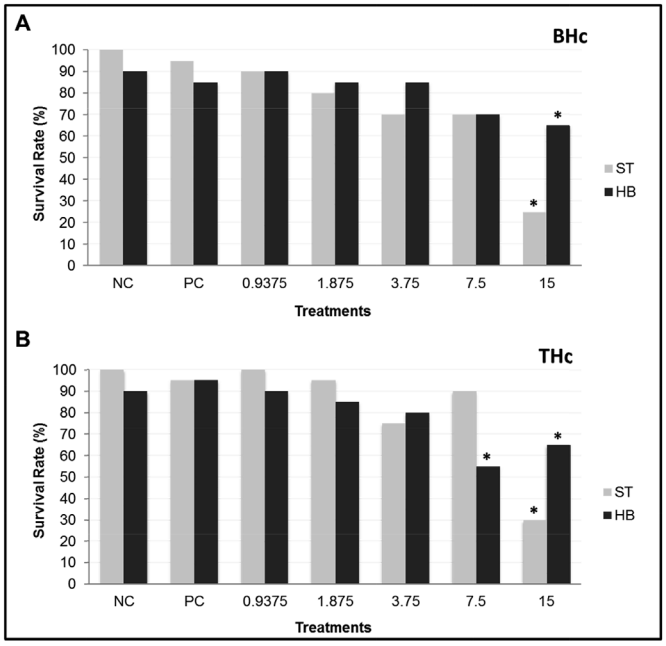

Fig. 3. Survival rate of individuals from the ST and HB crossings treated with different concentrations of (A) bupropion hydrochloride and (B) trazodone hydrochloride. NC: Negative control; PC: Positive control; *$p < 0.05$.

We used 1.5 g flasks with the mashed potato alternative culture media (Yoki Hikari®), along with 5 mL of each tested concentration. Ultrapure water was used as a negative control and 10 mM of urethane was used as a positive control.

2.2.3. Slides preparation and analysis

We gathered every emerged adult from each concentration, storing in flasks containing 70% ethanol. The wings were removed, aligned on microscope slides with Faure's solution (30 g of arabic gum, 20 mL of glycerol, 50 g of chloral hydrate and 50 mL of water), and inspected under 400x magnification for the presence of spots.

2.2.4. Statistical analysis

The mutant spot frequency (single small, single large or twin spots) was compared with the negative control using the conditional binomial test, following the protocol described by (Kastenbaum and Bowman, 1970), with $p = 0.05$ (Frei and Würgler, 1988, 1995), which is used to decide whether a result is positive, inconclusive or negative. Chis-quared-test was performed for statistical comparisons of the survival rate ratios for independent samples (De Rezende et al., 2011). Each statistical test was evaluated at the 5% of significance level. Based on the clone induction frequency per 10^5 cells, the recombinogenic activity was calculated as follows: Frequency of mutation (FM) = frequency of clones in BH flies/frequency of clones in MH flies. Frequency of recombination (FR) = 1 – frequency of mutation (FM) (Sinigaglia et al., 2006).

3. Results and discussion

Our results for the survival test indicated that at the concentration

of 15 mg/mL, the highest used one, for BHc and THc, there was a high toxicity in ST and HB crossing. (Fig. 3), even though being more noticeable in the individuals from the ST crossing, suggesting that metabolites generated were less toxic than BHc or THc.

Previous *in vitro* studies have already demonstrated that BHc is cytotoxic at high concentrations for hepatocytes. The toxicity caused by BHc is mainly associated to changes in the cytoskeleton and cell membrane, increased oxidative stress, lipid peroxidation, glutathione depletion, as well as lesions in the mitochondrial membrane (Ahmadian et al., 2017).

On the other hand, THc was also toxic at 7.5 mg/mL in the HB cross (Fig. 3B). Nevertheless, since this concentration was not toxic in the ST cross, it was included in the SMART. Thus, we tested BHc and THc, at 0.9375; 1.875; 3.75 and 7.5 mg/mL, in two independent experiment, and we found no significant differences between replications.

With regards to the results from the urethane treatment, individuals from the HB crossing have a high Cyp450-dependent bioactivation capacity, which in turns increases the frequency of mutant spots. Our results are also in accordance with previous studies (Frölich and Würgler, 1990;Graf and van Schaik, 1992; Machado et al., 2016; Morais et al., 2016; Naves et al., 2018; Reis et al., 2016).

We did not find an association between antidepressant doses (BHc and THc) and the induction of mutant spots, regardless of the utilized crossing experiment. Although BHc and THc significantly increased the frequencies of mutant spots in both crosses, the frequencies of total spots show a plateau. This may be a reflection of the limited bioactivation capacity of the larvae for these types of compounds. The same phenomenon has been observed previously with different compounds (Frölich and Würgler, 1990; Graf and Singer, 1992; Machado et al., 2016; Morais et al., 2016; Naves et al., 2018).

After analyzing the MH progeny of both crosses, only 0.9375 mg/mL of the BHc in the ST cross group did not increase the number of mutant spots (Table 1). On the other hand, in the HB cross, all concentrations altered the frequency of mutant spots when compared to the negative control ($p < 0.05$; Table 2). THc has a slightly different result from the BHc, being mutagenic for both ST and HB crossing at all analyzed concentration (Tables 3 and 4).

In MH individuals, mutant spots ("*mwh*" or "*flr*^{3}") can be produced by mutation, chromosomal deletion, recombination or non-disjunction. The presence of twin spots indicated the occurrence of recombination (between the centromere and the "*flare*" locus), while recombination between the "*flare*" locus and "*mwh*", produces *mwh* single spots only (Graf et al., 1984). In order to quantify the contribution of recombination in the total mutant spots, an analysis of balancer-heterozygous (BH) descendants was done. Due to the multiple inversions in the TM3 chromosome, all the recombinational events are suppressed. Thus, the frequency of mutant spots observed in the BH progeny when compared to the frequency of mutant spots of the MH group, allowed us to quantify the contribution of recombinant events in the total number of observed spots (Graf et al., 1984).

Although the frequencies of twin spots observed in the series treated with BHc were not statistically significant, BH progeny analysis suggests that, when considering the ST cross, BHc is preferentially recombinogenic (Fig. 4A) and, when metabolized by the Cyp450 complex enzymes, it becomes essentially mutagenic (Fig. 4B). According to Graf et al. (1984), we concluded that, for ST cross, BHc preferentially induces distal recombination, leading to the formation of single spots mwh.

In the HB cross, the frequency of mutations remained constant at all concentrations, including at 7.5 mg/mL. However the recombination rate decreased on 1.875 mg/mL.

The BHc in the ST group showed to be essentially recombinogenic and, in the HB cross, it becomes mutagenic (Fig. 4). The resulted metabolites generated by the BHc are: hydroxybupropion (OHBUP), threohydrobupropion and erythrohydrobupropion (Fokina et al., 2016; Sager et al., 2016). Ahmadian et al. (2017) showed an increase in the production of ROS in hepatocytes, which could explain the genotoxic potential of BHc when metabolized by enzymes of the Cyp450 complex.

The BH individuals, treated with THc, showed that for the ST cross, in lowest concentrations (0.9375, 1.875 and 3.75 mg/mL) the drug was essentially recombinogenic, being mutagenic only at the highest concentration (7.5 mg/mL) (Fig. 5A). The same was observed for the HB cross (Fig. 5B), but the recombination contribution was lower when compared to the ST cross.

The toxicity might have been linked to by-products from THc metabolization, as a result of the influence of the enzymes from the Cyp450 complex in HB crossing. As a consequence, the survival rate, recombination rate and total mutant spots decreased with increasing concentration of THc, showing that the cytochrome P450 enzyme complex directly influences the phenotype and the survival rate.

Taziki et al. (2013) showed that THc is more toxic when metabolized in liver cells. ROS production and lipid peroxidation are significantly increased, and the mitochondrial membrane potential decays dramatically. 1- (3′-chlorophenyl) piperazine (*m*-CPP) is the most abundant circulating active metabolite in the body, arising from the metabolism of THc by the liver, mediated by Cyp3A4 and Cyp2D6 (Wen et al., 2008).

In both crosses, the THc showed to be essentially recombinogenic for lower concentrations (Fig. 5). 7.5 mg/mL concentration at the HB cross was toxic, suggesting the toxicity of possibles metabolites produced in *D. melanogaster*. Data obtained in the present study confirmed the genotoxic (clastogenic and mutagenic) potential suggested by *in vitro* assays performed in lymphocyte culture (Yilmaz et al., 2017).

4. Conclusion

Based on our results, both of the analyzed antidepressants (BHc and THc) were toxic under high concentrations. However, the mutagenic effect was not associated to the administered concentration. When taking only BHc into account, we encountered no interference from the cytochrome P450 in the induction of mutant spots. On the other hand, THc has exhibited a significant increase in the mutant spot frequency when metabolized, which was probably associated to the production of *m*-CPP. When considering the balanced-heterozygous progeny (BH), we showed that within the ST crossing, BHc was essentially recombinogenic, becoming mutagenic in the HB cross. Differently, THc suggested a recombinogenic property in both ST and HB crossings at the lowest analyzed concentrations. Therefore, given the lack of data in the literature and the increased use of antidepressants by the world's population, further studies are needed to elucidate the mechanisms of action associated to the damage onto genetic material caused by BHc and THc.

Conflicts of interest

There is no conflict of interest.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.06.004.

Transparency document

Transparency document related to this article can be found online at

Table 1
Results obtained through the Somatic Mutation and Recombination Test (SMART) in *Drosophila melanogaster* wing cells, with trans-heterozygous (MH) and balanced heterozygous progeny (BH) from the standard cross (ST), treated with ultrapure water (negative control), urethane 10 mM (positive control) and different concentrations of bupropion hydrochloride (BHc).

Genotypes and Treatments (mg/mL)	Number of flies	Spots per fly (number of spots); statistical diagnoses[a]				Spots with *mwh* clone[c]	Frequency of clone formation/10^5 cells per division[d]		Recombination (%)
		Small single spots (1–2 cells)[b]	Large single spots (> 2 cells)[b]	Twin spots	Total spots		Observed	Correct Control	
mwh/flr³									
Negative Control	40	0.45 (18)	0.05 (02)	0.00 (00)	0.50 (20)	18	0.92		
Positive Control	40	2.13 (85) +	0.23 (09) +	0.10 (04) i	2.45 (98) +	92	4.71	3.79	9.77
BHc									
0.937	40	0.58 (23) i	0.10 (04) i	0.03 (01) i	0.70 (28) i	25	1.28	0.36	
1.875	40	0.70 (28) i	0.10 (04) i	0.15 (06) +	0.95 (38) +	38	1.95	1.03	60.51
3.75	40	0.80 (32) +	0.08 (03) i	0.05 (02) i	0.90 (36) +	33	1.69	0.77	57.40
7.5	40	0.83 (33) +	0.05 (02) i	0.10 (04) i	0.98 (39) +	36	1.84	0.92	66.85
***mwh*/TM3**									
Negative Control	40	0.30 (12)	0.05 (02)		0.35 (14)	14	0.72		
Positive Control	40	2.03 (81) +	0.05 (02) i		2.08 (83) +	83	4.25	3.53	
BHc									
1.875	40	0.38 (15) i	0.00 (00) i		0.38 (15) i	15	0.77	0.05	
3.75	40	0.35 (14) i	0.00 (00) i		0.35 (14) i	14	0.72	0.00	
7.5	40	0.30 (12) i	0.00 (00) i		0.30 (12) -	12	0.61	−0.11	

Marker-trans-heterozygous flies (*mwh/flr³*) and balancer-heterozygous flies (*mwh*/TM3) were evaluated.

[a] Statistical diagnoses according to Frei and Würgler (1988): + positive. i inconclusive. – negative. M: multiplication factor. Significance levels $\alpha = \beta = 0.05$.

[b] Including rare *flr³* single spots.

[c] Considering *mwh* clones from *mwh* single and twin spots.

[d] Frequency of clone formation: clones/flies/48,800 cells (without size correction).

Table 2
Results obtained through the Somatic Mutation and Recombination Test (SMART) in *Drosophila melanogaster* wing cells, with trans-heterozygous (MH) and balanced heterozygous progeny (BH) from the hight bioactivation cross (HB), treated with ultrapure water (negative control), urethane 10 mM (positive control) and different concentrations of bupropion hydrochloride (BHc).

Genotypes and Treatments (mg/mL)	Number of flies	Spots per fly (number of spots); statistical diagnoses[a]				Spots with *mwh* clone[c]	Frequency of clone formation/10^5 cells per division[d]		Recombination (%)
		Small single spots (1–2 cells)[b]	Large single spots (> 2 cells)[b]	Twin spots	Total spots		Observed	Correct Control	
mwh/flr³									
Negative Control	40	0.58 (23)	0.03 (01)	0.03 (01)	0.63 (25)	24	1.23		
Positive Control	40	9.33 (373) +	0.75 (30) +	1.20 (48) +	11.28 (451) +	437	22.39	21.16	12.15
BHc									
0.937	40	0.80 (32) i	0.20 (08) +	0.08 (03) i	1.08 (43) +	42	2.15	0.92	16.77
1.875	40	0.85 (34) i	0.08 (03) i	0.10 (04) i	1.03 (41) +	41	2.10	0.87	34.28
3.75	40	0.93 (37) +	0.08 (03) i	0.00 (00) i	1.00 (40) +	39	2.00	0.77	20.50
7.5	40	0.78 (31) i	0.25 (10) +	0.00 (00) i	1.03 (41) +	41	2.10	0.87	17.14
***mwh*/TM3**									
Negative Control	40	0.75 (30)	0.00 (00)		0.75 (30)	30	1.54		
Positive Control	40	9.43 (377) +	0.18 (07) +		9.60 (384) +	384	19.67	18.13	
BHc									
0.937	40	0.85 (34) -	0.03 (01) i		0.88 (35) -	35	1.79	0.25	
1.875	40	0.65 (26) -	0.03 (01) i		0.68 (27) -	27	1.38	−0.16	
3.75	40	0.75 (30) -	0.03 (01) i		0.78 (31) -	31	1.59	0.05	
7.5	40	0.80 (32) -	0.05 (02) i		0.85 (34) -	34	1.74	0.20	

Marker-trans-heterozygous flies (*mwh/flr³*) and balancer-heterozygous flies (*mwh*/TM3) were evaluated.

[a] Statistical diagnoses according to Frei and Würgler (1988): + positive. i inconclusive. – negative. M: multiplication factor. Significance levels $\alpha = \beta = 0.05$.

[b] Including rare *flr³* single spots.

[c] Considering *mwh* clones from *mwh* single and twin spots.

[d] Frequency of clone formation: clones/flies/48,800 cells (without size correction).

Table 3
Results obtained through the Somatic Mutation and Recombination Test (SMART) in *Drosophila melanogaster* wing cells, with trans-heterozygous (MH) and balancer heterozygous progeny (BH) from the standard cross (ST), treated with ultrapure water (negative control), urethane 10 mM (positive control) and different concentrations of trazodone hydrochloride (THc).

Genotypes and Treatments (mg/mL)	Number of flies	Spots per fly (number of spots); statistical diagnoses[a]				Spots with *mwh* clone[c]	Frequency of clone formation/10^5 cells per division[d]		Recombination (%)
		Small single spots (1–2 cells)[b]	Large single spots (> 2 cells)[b]	Twin spots	Total spots		Observed	Correct Control	
mwh/flr³									
Negative Control	40	0.43 (17)	0.03 (01)	0.00 (00)	0.45 (18)	17	0.87		
Positive Control	40	2.58 (103) +	0.35 (14) +	0.25 (10) +	3.18 (127) +	125	6.35	5.48	43.46
THc									
0.937	40	0.73 (29) i	0.33 (13) +	0.10 (04) i	1.15 (46) +	46	2.36	1.49	74.15
1.875	40	0.65 (26) i	0.28 (11) +	0.10 (04) i	1.03 (41) +	40	2.05	1.18	60.00
3.75	40	0.90 (36) +	0.30 (12) +	0.08 (03) i	1.28 (51) +	51	2.61	1.74	70.50
7.5	40	0.53 (21) i	0.35 (14) +	0.03 (01) i	0.90 (36) +	36	1.84	0.97	41.85
***mwh*/TM3**									
Negative Control	40	0.35 (14)	0.03 (01)		0.38 (15)	15	0.77		
Positive Control	40	1.63 (65) +	0.13 (05) i		1.75 (70) +	70	3.59	2.82	
THc									
0.937	40	0.23 (09) -	0.08 (03) i		0.30 (12) -	12	0.61	−0.16	
1.875	40	0.33 (13) -	0.08 (03) i		0.40 (16) i	16	0.82	0.05	
3.75	40	0.33 (13) -	0.05 (02) i		0.38 (15) -	15	0.77	0.00	
7.5	40	0.50 (20) i	0.03 (01) i		0.53 (21) i	21	1.07	0.30	

Marker-trans-heterozygous flies (*mwh/flr³*) and balancer-heterozygous flies (*mwh*/TM3) were evaluated.

[a] Statistical diagnoses according to Frei and Würgler (1988): + positive. i inconclusive. – negative. M: multiplication factor. Significance levels $\alpha = \beta = 0.05$.

[b] Including rare *flr³* single spots.

[c] Considering *mwh* clones from *mwh* single and twin spots.

[d] Frequency of clone formation: clones/flies/48,800 cells (without size correction).

Table 4
Results obtained through the Somatic Mutation and Recombination Test (SMART) in *Drosophila melanogaster* wing cells, with trans-heterozygous (MH) and balanced heterozygous progeny (BH) from the hight bioactivation cross (HB), treated with ultrapure water (negative control), urethane 10 mM (positive control) and different concentrations of trazodone hydrochloride (THc).

Genotypes and Treatments (mg/mL)	Number of flies	Spots per fly (number of spots); statistical diagnoses[a]				Spots with *mwh* clone[c]	Frequency of clone formation/10^5 cells per division[d]		Recombination (%)
		Small single spots (1–2 cells)[b]	Large single spots (> 2 cells)[b]	Twin spots	Total spots		Observed	Correct Control	
mwh/flr³									
Negative Control	40	0.43 (17)	0.05 (02)	0.03 (01)	0.50 (20)	20	1.02		
Positive Control	40	13.93 (557) +	1.98 (79) +	1.08 (43) +	16.98 (679) +	656	34.8	33.78	34.65
THc									
0.937	40	1.20 (48) +	0.23 (09) +	0.33 (13) +	1.75 (70) +	68	3.48	2.46	51.44
1.875	40	1.88 (75) +	0.25 (10) +	0.18 (07) +	2.30 (92) +	85	4.35	3.33	52.87
3.75	40	1.35 (54) +	0.30 (12) +	0.28 (11) +	1.93 (77) +	76	3.89	2.87	59.13
7.5	40	0.75 (30) +	0.28 (11) +	0.08 (03) i	1.10 (44) +	42	2.15	1.13	26.05
***mwh*/TM3**									
Negative Control	40	0.40 (16)	0.03 (01)		0.43 (17)	17	0.87		
Positive Control	40	10.63 (425) +	0.48 (19) +		11.10 (444) +	444	22.74	21.74	
THc									
0.937	40	0.80 (32) +	0.03 (01) i		0.83 (33) +	33	1.69	0.82	
1.875	40	0.98 (39) +	0.03 (01) i		1.00 (40) +	40	2.05	1.18	
3.75	40	0.75 (30) +	0.03 (01) i		0.78 (31) +	31	1.59	0.72	
7.5	40	0.65 (26) i	0.13 (05) i		0.78 (31) +	31	1.59	0.72	

Marker-trans-heterozygous flies (*mwh/flr³*) and balancer-heterozygous flies (*mwh*/TM3) were evaluated.

[a] Statistical diagnoses according to Frei and Würgler (1988): + positive. i inconclusive. – negative. M: multiplication factor. Significance levels $\alpha = \beta = 0.05$.

[b] Including rare *flr³* single spots.

[c] Considering *mwh* clones from *mwh* single and twin spots.

[d] Frequency of clone formation: clones/flies/48,800 cells (without size correction).

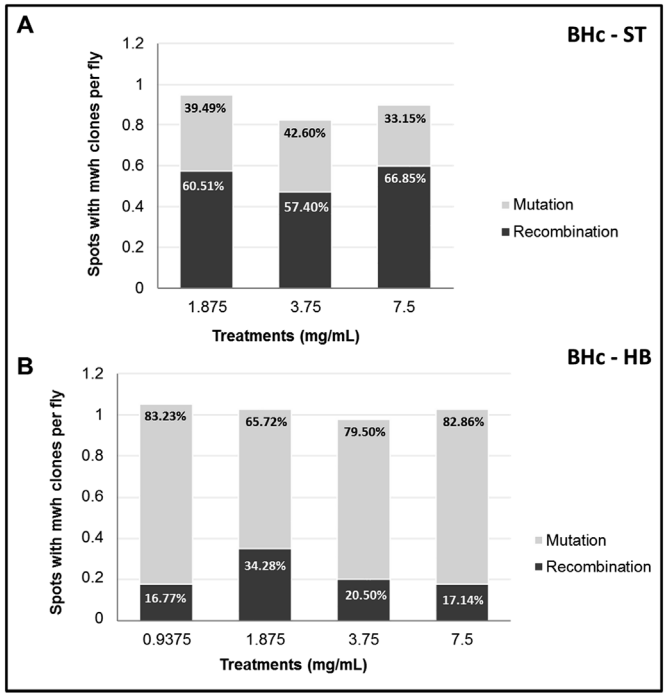

Fig. 4. Mutation and recombination contribution (%) for the total frequency of spots observed in the MH subjects from the: (A) ST and (B) HB crossings, treated with bupropion hydrochloride.

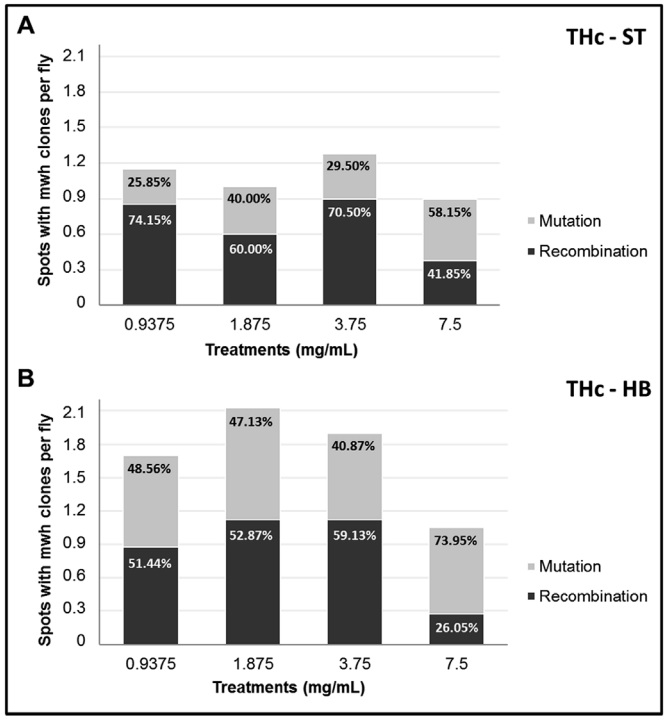

Fig. 5. Mutation and recombination contribution (%) for the total frequency of spots observed in the MH subjects from the: (A) ST and (B) HB crossings, treated with trazodone hydrochloride.

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