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Research article

Genotoxicity and cytotoxicity evaluation of two thallium compounds using the *Drosophila* wing somatic mutation and recombination test

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ABSTRACT

Thallium (Tl) is a heavy and toxic metal and a byproduct of several human activities, such as cement production, mining, and coal combustion. Thallium is found in fruits, vegetables, and animal fodder with high Tl contamination; therefore, it is an environmental pollution issue and a toxicological contamination problem for human beings and other organisms when exposed to it. The mutagenic potential of Tl and its compounds is controversial, and there are few *in vivo* studies on its effects. We conducted the animal bioassay *Drosophila* wing somatic mutation and recombination test (SMART) to test for genotoxicity and assessed the genotoxic effects of Tl acetate ($TlCH_3COO$) and Tl sulfate (Tl_2SO_4) on *Drosophila melanogaster*. Third instar larvae from the SMART standard cross (ST) were fed Tl acetate [0.2, 2, 20, 200, 600 and 1200 μM] and Tl sulfate [0.2, 2, 20, 200, and 600 μM]. Hexavalent chromium [CrO_3, 500 μM] served as the positive control, and Milli-Q water served as the negative control. Only the high Tl_2SO_4 [600 μM] concentration resulted in genotoxicity with 87.6% somatic recombination, and both salts disrupted cell division of wing imaginal disc cells, showing the expected cytotoxic effects. Genotoxic risks due to high metal levels by bioaccumulation of Tl^{+1} or its compounds require further evaluation with other *in vivo* and *in vitro* assays.

1. Introduction

Thallium (Tl) is a rare, very reactive, highly toxic heavy metal that is not found free in the environment because it forms mono- and trivalent compounds. Tl is mainly found in Asia, Europe, and North America [1, 2] in the form of sulfides in minerals and rocks. It is a polluting metal and a byproduct of coal combustion [3] and a high variety of industries, such as cement, fireworks, electronic devices, mercury lamps, low-temperature thermometers, special glasses, mixed crystals, radioactive isotopes, mining, steel and sulfuric acid production [2]. Some Tl salts have been used as epilators and to treat venereal diseases, such as typhus, tuberculosis, malaria and ringworm. Tl sulfate (Tl_2SO_4) has been included as an active compound in insecticides and rodenticides (2.5%) since 1920, and its use was prohibited in the USA in 1972 [4]; nevertheless, it is still used as a rodenticide in some countries given its low price [2]. Recently, it has been reported that the inks used in tattoos contain this metal [5]. The concentration of Tl in wastewater can reach 3,000 μg/L [1], and frequently, Tl contaminants generated by human activities end up in the trophic chain due to their absorption and accumulation in plants [6, 7]. The Tl concentration in foods, such as fruits and vegetables [6], increase by bioaccumulation, which depends on the ground concentrations, ground pH and cultivated species and varieties. In humans, an 8 mg/kg concentration may be indicative of poisoning, and a 10–15 mg/kg concentration is lethal [2]. Tl tends to form the +3 and +1 oxidation states, and it has been suggested that the majority of Tl^{+3} is converted to Tl^{+1} [8]. Its salts, such as sulfate, carbonate and acetate, are very soluble in water, but there are other salts, such as sulfide and iodide, that are slightly soluble in water. Tl^{+1} and cation compounds, such as Tl acetate ($TlCH_3COO$) and Tl sulfate (Tl_2SO_4) (Figure 1), are more stable in water solution at neutral pH compared with Tl^{+3} compounds.

In general, greater than 90% Tl is absorbed through the skin and oral mucosa, but it is also acquired by inhalation. Tl accumulates mainly in

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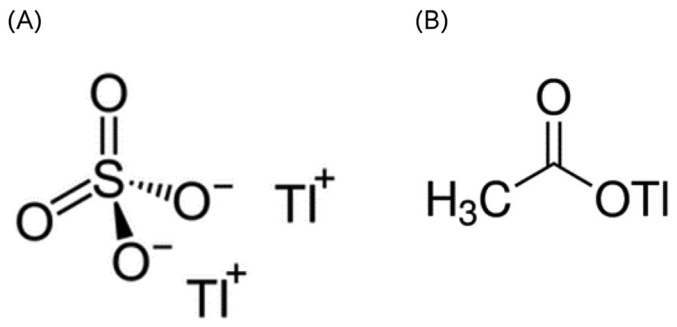

Figure 1. Thallium sulfate (A); thallium acetate (B).

the kidney and reaches the brain after systemic administration [9]. Galván-Arzate *et al.* [10] induced significant lipoperoxidation (LPO) in the brains of rats treated with daily sublethal doses of Tl [0.8 mg/kg]; at [1.6 mg/kg], all brain regions studied presented a significant increase in LPO compared to the control, suggesting that oxidative stress is involved in Tl toxicity. Thalliotoxicosis diagnosis and treatment are important, although alopecia is the distinctive symptom of Tl poisoning [4, 11]. The addition of 10–15 nmol of Tl^{+1} per mg/mitochondrial protein inhibits K^{+1} translocation without affecting oxidative phosphorylation [12]; when Tl^{+1} enters the cell, it induces oxidative stress and affects glutathione (GSH) metabolism, K^{+1}-regulated homeostasis and cellular and mitochondrial membrane integrity [13]. In PC12 cells incubated for 1–72 h at a single dose of Tl^{+1} or Tl^{+3} [10–250 μM], H_2O_2 increased in the mitochondria, which resulted in higher levels of reactive oxygen species (ROS) in the cytoplasm [14]. Tl^{+1} [25–200 μM] in hepatocyte mitochondria induced ROS and decreased ATP, which triggered cytochrome c release and the activation of apoptosis [15]. Korotkov *et al.* [16] assumed that Tl^{+1} might deform complex I in "Ca^{+2}-loaded" mitochondria and that Tl^{+1} to Tl^{+3} oxidation is involved in this event. Pourahmad *et al.* [17] studied rat hepatocytes and associated Tl^{+1}, Tl^{+3}, GSH and CYP2E1 with the interruption of the electron transport chain in mitochondria and the mutual damage between mitochondria and lysosomes that resulted from an increase in ROS. The metabolism and toxicity of Tl^{+1} are positively related to the induction of oxidative stress, apoptosis and LPO. Tl and its compounds are not classifiable as carcinogens to humans based on "inadequate information to assess its carcinogenic potential" [4]. Despite all the toxic effects described above, which mainly involve ROS increases related to DNA damage [18], there is a lack of data on the mutagenic effects of thallium compounds in humans [13]. Furthermore, very few studies evaluate Tl compound genotoxicity *in vitro* and *in vivo* [19]. We assessed the *in vitro* genotoxic effect of $TlCH_3COO$ and Tl_2SO_4 in human leucocytes cultured at [0.5, 1, 5, 10, 50 and 100 μg/mL] based on structural and numerical chromosome aberrations, sister chromatid exchange and comet assays (pH > 13 and 12.1) and showed that both salts have effects on the mitotic index, exert cytotoxic, cytostatic, and clastogenic activity and cause DNA damage [20, 21]. Given these previous results, the aim of this study is to gain insight into the possible *in vivo* genotoxicity of $TlCH_3COO$ and Tl_2SO_4 through an alternative animal test that uses the insect *Drosophila melanogaster* to realize the wing somatic mutation and recombination test (SMART) standard cross (ST) with Cyp450 basal levels [22] by pairwise comparison with negative dissolvent control results. This bioassay detects mutations, deletions, aneuploidies, and recombinations in wing imaginal disc cells and allows cytotoxicity to be determined based on alterations in cellular division through cumulative frequency analysis of *mwh* clones [23, 24].

2. Materials and methods

2.1. Chemicals

$TlCH_3COO$ (CAS No: 563-68-8, reagent-grade 98%) and Tl_2SO_4 (CAS No: 7446-18-6, reagent-grade 99.99%) were purchased from Sigma-Aldrich® (St. Louis, MO, USA). Drosophila Instant Medium (DIM) was purchased from Carolina Biological Supply Co. (Burlington, North Carolina, USA). Chromium trioxide (CrO_3), (CAS No: 1333-82-0, reagent-grade ≥ 98%) was purchased from Sigma-Aldrich® (St. Louis, MO, USA).

2.2. LC_{50}

The LC_{50} of each Tl salt was determined by feeding until pupation *D. melanogaster* third instar larvae (72 ± 4 h) of the flare strain with 0.5 g of DIM plus 2 mL of fresh solution at eight concentrations of Tl_2SO_4 or $TlCH_3COO$ [1.9, 7.8, 31.2, 125, 500, 1000, 2000 and 4000 μM] running three independent experiments with five replicates for each compound at 25 ± 2 °C and 65% relative humidity (RH) until emergence. One-factor ANOVA ($P < 0.5$) was performed to compare results among experiments, and the LC_{50} was determined by regression.

2.3. SMART

To develop the *Drosophila* wing SMART, the ST cross was performed by mating virgin females of the flare strain (*flr*^{3}/TM3, *Bd*^{S}) to males of the mwh strain (*mwh/mwh*) originally donated by Dr. Ulrich Graf (ETH, Zurich, Switzerland). Eggs collected from fresh yeast media from the cross were incubated at 25 ± 2 °C and 65% relative humidity (RH); equal batches of third instar larvae (72 ± 4 h) were chronically exposed by feeding until pupation with 0.5 g of DIM plus 2 mL fresh solution of $TlCH_3COO$ [0.2, 2, 20, 200, 600 and 1200 μM] or Tl_2SO_4 [0.2, 2, 20, 200 and 600 μM] dissolved in Milli-Q water, which was also used as a negative control. Hexavalent chromium [CrO_3, 500 μM] was used as a positive control [22]. Transheterozygous flies (MH, marker-heterozygous flies: *mwh*+/+*flr*^{3}: wild-type wings) were randomly selected, and wings were dissected and mounted in Entellan® and scored at 40x under a microscope (double-blind). Relative spot per fly frequencies were compared through statistical analysis using SMART v2.1. software (Frei and Würgler, unpublished), which is based on the Kastenbaum-Bowman test that contrasts the expected frequencies [25]. A Mann-Whitney-Wilcoxon U test was performed to corroborate the results [26]. When the twin spot frequency was statistically significant, we mounted and scored the BH of balancer-heterozygous flies (*mwh*+/TM3, *BdS*) with serrated wings to determine the recombinogenic activity of the compound [22]. Similarly, the size distribution of *mwh* clones was analyzed using the Kolmogorov-Smirnov test [23]. $P < 0.05$ was considered statistically significant in all tests.

3. Results

3.1. LC_{50}

To determine the test concentrations, first, we determined the LC_{50} for $TlCH_3COO$ [919 μM] by quadratic regression and for Tl_2SO_4 [460 μM] by linear regression (Figure 2).

3.2. SMART

Based on these data, we exposed third instar larvae from the ST cross of the SMART bioassay to six concentrations of $TlCH_3COO$ [0.2, 2, 20, 200, 600 and 1200 μM] or five concentrations of Tl_2SO_4 [0.2, 2, 20, 200, and 600 μM] using Milli-Q water and CrO_3 as negative and positive controls, respectively. Tables 1 and 2 show the effect of treatments with the metal compounds and controls.

As expected, the relative frequencies of spots in the CrO_3 treatment were significantly increased for each type of spot compared to the remainder of the treatments, which confirms the mutagenic and recombinogenic effects of this positive control [22, 29]. Regarding Tl, only the highest concentration of Tl_2SO_4 [600 μM] was clearly genotoxic because all the spot per fly relative frequencies were significantly higher. Considering that the twin spots are produced exclusively by somatic

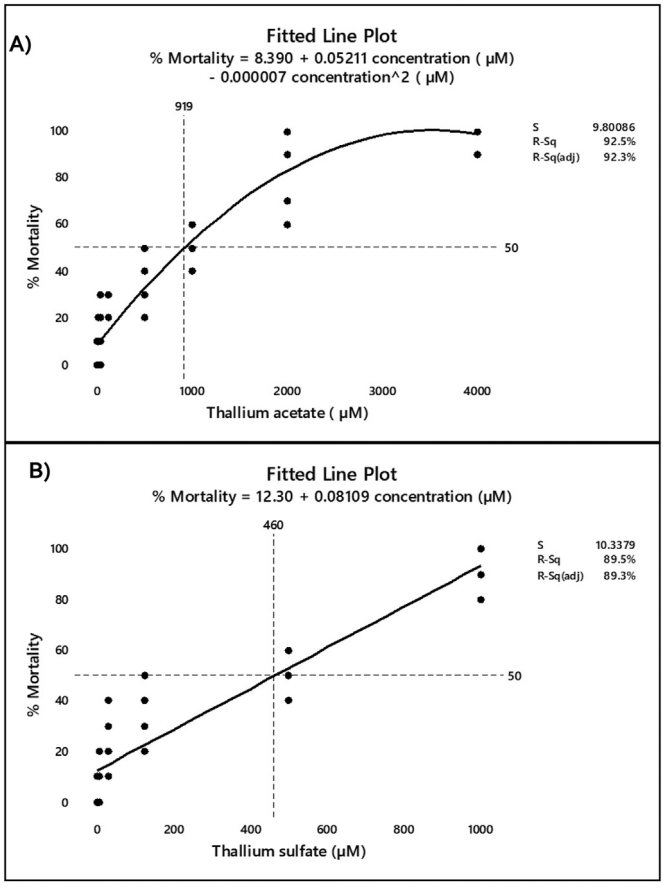

Figure 2. Survival rates after feeding *D. melanogaster* third instar larvae of the flare strain with (A) $TlCH_3COO$ and (B) Tl_2SO_4 [1.9, 7.8, 31.2, 125, 500, 1000, 2000 and 4000 µM] until pupation. The LC_{50} was determined by quadratic regression for $TlCH_3COO$ [919 µM] and by linear regression for Tl_2SO_4 [460 µM]. All data (•) from the three experiments and replicates were plotted, and regressions were calculated for each compound.

recombination between the marker *flr* and the centromere [22, 24], only yielded a high frequency in Tl_2SO_4 [600 µM], we scored the BH balancer-heterozygous flies (*mwh*+/TM3, Bd^S) with serrated wings to calculate the percentage of spots induced by somatic recombination [22, 30] dividing the frequency of total *mwh* spots on the BH wings by that on the MH wings (0.47*100/3.8) [22] obtaining a percentage of somatic mutation of 12.4% and 87.6% of recombination. The size distribution of the *mwh* clones and the statistical comparison of the experimental results against the negative control allowed us to detect whether the treatment altered the cellular division from the imaginal discs from the wing. The data in Table 2 show the results of the *mwh* clone cumulative frequency analysis that compared the results of the maximum difference between the cumulative distributions of the negative control with respect to treatments through a Kolmogorov-Smirnov test [23, 24]. With both salts, all the treatments were statistically significant, with higher means at [600 µM]; in contrast, the [2.0 µM] treatment had no effect on any of the salts. Of note, only $TlCH_3COO$ [0.2 µM] showed an effect, so we considered this result a false positive.

4. Discussion

Thallium exerts potent toxic effects on a wide variety of *in vitro* and *in vivo* biological systems through different routes of exposure [4, 19]. Considered an industrial and mining polluting byproduct [31], thallium is present in the environment at subtoxic levels, but it can be found in food [32], plants [6, 7], and animals [33] because of its bioaccumulation, posing threats to human health and the environment. Therefore, it was important to assess whether it poses a genotoxic risk to human health and to other organisms. Given the few *in vivo* reports on Tl genotoxicity, an alternative animal bioassay, the *D. melanogaster* wing spot test, was performed with the aim of collecting robust data using this alternative eukaryotic model organism, which shares 77% of the genes that cause disease in humans [34] and 80–90% or more of conserved functional domains in nucleotides and proteins [35]. The relative spontaneous spot frequencies obtained in the negative Milli-Q water control were compared against those of the positive control, CrO_3, a potent carcinogen that produces chromosomal alterations through oxidative stress and whose toxic, teratogenic and mutagenic effects have been confirmed in humans, birds [36] and *D. melanogaster* [22]. The frequencies obtained with both salts show that only Tl_2SO_4 [600 µM] was genotoxic. To explain the fact that $TlCH_3COO$ did not result in genotoxicity, we considered that each sulfate molecule has two Tl atoms compared to one atom in each acetate molecule. Thus, [300 µM] Tl_2SO_4 would correspond to the same amount of Tl atoms in [600 µM] $TlCH_3COO$.Accordingly, we also evaluated Tl_2SO_4 [300 µM] and compared its results with $TlCH_3COO$ [600 µM] and obtained negative and statistically similar results (data not shown). In addition, we compared the positive data of Tl_2SO_4 [600 µM] of progeny: marker-transheterozygous flies (MH, *mwh* +/+ flr^3) wild-type wings and balancer-heterozygous flies (BH, *mwh*+/TM3, Bd^S) serrate-type wings to determine the percentage of somatic recombination induced by the compound at that concentration. This analysis was performed by dividing the total frequency of *mwh* spots on the BH wings by that on the MH wings [22], and the results showed 12.4% (0.47*100/3.8) mutation and 87.6% recombination rates for Tl_2SO_4 [600 µM]. These outcomes do not support the hypothesis that differences between SMART results of Tl_2SO_4 [600 µM] and $TlCH_3COO$ [600 µM] are due to the double number of Tl atoms in Tl_2SO_4 molecule but demonstrate increased somatic recombination events with Tl_2SO_4 [600 µM]. We do not have clear explanations for these differences, but we propose that these findings could be related to the corresponding molecule dissociation and/or the contribution of sulfur to a putative generation of genotoxic sulfur compounds [37], such as hydrogen sulfide (H_2S) [38] or sulfur dioxide (SO_2) [39]. It has been demonstrated that Tl exhibit a certain affinity for guanines in the major groove of B-DNA and for thymine, which could affect the molecule. Thus, the genotoxic effect observed with Tl_2SO_4 [600 µM] could be related to the reported adduct formation between Tl and DNA nitrogenous bases [40] but also to ROS increases that generate DNA damage [18]. In addition, Yildirim *et al.* [41] found significant differences in chromosome aberrations and sister chromatid exchanges three days after administration of Tl to patients exposed to this metal for a myocardial perfusion study. Decreases in the mitotic index and replicative index *in vitro* were demonstrated by Rodríguez-Mercado *et al.* [20, 21], who suggested that Tl^{+1} induces genetic damage and is cytotoxic. The genotoxic effects of Tl_2SO_4 [600 µM] in this *in vivo* study are evidence of the potential effects of Tl_2SO_4 that have been reported in *in vitro* studies at [1.8–369.8 µM] [21, 42]. The differences found in this study might be attributed to the compound's absorption distribution, metabolism, and excretion (ADME) of this metal salt when administered *in vivo*.

The *mwh* clone cumulative frequency analysis showed a significant effect of both Tl salts (Table 2); this parameter is related to cytotoxicity that induces cell division disruption, aneuploidy [24, 27, 28] or apoptosis in imaginal wing cells. These cytotoxic effects are consistent with published reports about Tl^{+1} toxicity caused by its cellular accumulation [43]. To explain these results, we also considered putative damage derived from Tl^{+1} toxicity as glutathione (GSH) depletion in the mitochondrial redox balance [44, 45]. On the other hand, the production of ROS [14, 15] and its known effects on mitochondria could have induced apoptosis, which would account for the cell division disruption observed (Table 2). Hantson *et al.* [27] detected an increase in micronuclei in lymphocyte binucleated cells of a patient who had ingested Ti_2SO_4 and associated it with the ability of metals to induce aneuploidy.

Table 1. Summary of results obtained from the standard (ST) cross[b] of the *Drosophila* wing SMART after scoring marker-heterozygous (MH) flies (*mwh* +/+ *flr*^{3}, wild-type wings) treated with Tl acetate ($TlCH_3COO$) [0, 0.2, 2, 20, 200, 600 and 1200 μM] and Tl sulfate (Tl_2SO_4) [0, 0.2, 2, 20, 200 and 600 μM]; balancer heterozygous (BH) flies (*mwh*+/TM3, *BdS*)[d]. Milli-Q water and chromium trioxide (CrO_3) [500 μM] served as negative and positive controls, respectively.

Compound	Conc. (μM)	Number of flies	Spots per Fly (Number of Spots) Statistical Diagnosis[a]					Mean *mwh* clone size class	Clone formation per 10^5 cells per cell division[c]	
Cross & Progeny Type			Small single spots (1–2 cells) m = 2	Large single spots (>2 cells) m = 5	Twin spots m = 5	Total spots m = 2	*mwh* clones		Observed	Control corrected
Negative and positive controls										
ST, MH										
Milli-Q Water	0	52	0.42 (022)	0.13 (007)	0.00 (000)	0.56 (029)	28	1.79	1.10	
CrO_3	500	58	3.28 (190) +	4.57 (265) +	4.28 (248) +	12.12 (703) +	659	3.28	23.30	22.20
Tl acetate treatments										
ST, MH										
Milli-Q Water	0	45	0.49 (022)	0.02 (001)	0.00 (000)	0.51 (023)	23	1.39	1.05	
$TlCH_3COO$	0.2	41	0.29 (012) -	0.24 (010) +	0.02 (001) -	0.56 (023) -	23	2.52	1.15	0.10
$TlCH_3COO$	2	68	0.32 (022) -	0.01 (001) -	0.00 (000) -	0.34 (023) -	23	1.30	0.70	-0.35
$TlCH_3COO$	20	47	0.47 (022) -	0.06 (003) -	0.02 (001) -	0.55 (026) -	26	1.85	1.15	0.10
$TlCH_3COO$	200	34	0.65 (022) -	0.09 (003) -	0.09 (003) -	0.82 (028) -	28	1.93	1.70	0.65
$TlCH_3COO$	600	58	0.57 (033) -	0.03 (002) -	0.03 (002) -	0.64 (037) -	36	1.67	1.25	1.15
$TlCH_3COO$	1200	62	0.45 (028) -	0.05 (003) -	0.00 (000) -	0.50 (031) -	31	1.61	1.05	0.00
Tl sulfate treatments										
ST, MH										
Milli-Q Water	0	60	0.32 (019)	0.05 (003)	0.03 (002)	0.40 (024)	24	2.04	0.8	
Tl_2SO_4	0.2	59	0.34 (020) -	0.03 (002) -	0.00 (000) -	0.37 (022) -	21	1.43	0.75	-0.1
Tl_2SO_4	2	56	0.30 (017) -	0.12 (007) -	0.02 (001) -	0.45 (025) -	22	2.18	0.80	0.0
Tl_2SO_4	20	55	0.20 (011) -	0.05 (003) -	0.00 (000) -	0.25 (014) -	14	1.64	0.50	-0.3
Tl_2SO_4	200	50	0.22 (011) -	0.10 (005) -	0.04 (002) -	0.36 (018) -	17	2.18	0.70	-0.1
Tl_2SO_4	600	54	2.13 (115) +	1.13 (061) +	0.87 (047) +	4.13 (223) +	206	2.49	7.80	7.0
Tl sulfate treatment										
ST, BH										
Milli-Q Water	0	60	0.12 (007)	0.00 (000)	0.00 (000)	0.12 (007)	7	0.71	0.25	
Tl_2SO_4	600	60	0.45 (027) +	0.02 (001) -	0.00 (000)-	0.47 (028) +	28	0.57	0.90	0.65

[a] Statistical diagnoses according to [25] m: minimal risk multiplication factor for the assessment of negative results. For the final statistical diagnosis of all positive (+) and negative (-) results the nonparametric Mann-Whitney and Wilcoxon *U*-test with significance levels α and β = 0.05 was used to exclude false positive or negative diagnoses [26]. One side binomial test, significance levels α and β: significant results: + ($\alpha \leq 0.05$); no significant results: - ($\beta \leq 0.05$).

[b] ST: standard cross.

[c] Clone frequencies per fly divided by the number of cells examined per fly (48,800) provides an estimate of formation frequencies per cell and per cell division in chronic exposure experiments [26].

[d] BH flies were scored to calculate the percentage of somatic mutation and recombination in this treatment [22].

Table 2. Results obtained from analyzing the accumulation frequency in the *mwh* clones using the Kolmogorov-Smirnov test [24]. Treatments with Tl acetate ($TlCH_3COO$) and Tl sulfate (Tl_2SO_4). Milli-Q water and chromium trioxide (CrO_3) serve as negative and positive controls, respectively.

Treatments	Mean[1]	Std. Dev.[2]	*P*-level <0.05
Negative and positive controls			
Milli-Q water	22.10	2.51	
CrO_3 500 μM	508.90	216.48	<0.05
$TlCH_3COO$ (μM)			
Milli-Q water	22.10	2.51	
$TlCH_3COO$ 0.2	18.70	5.16	<0.05
Tl CH_3COO 2	22.30	1.88	ns
$TlCH_3COO$ 20	23.10	4.70	<0.05
$TlCH_3COO$ 200	23.40	3.47	<0.05
$TlCH_3COO$ 600	33.60	4.97	<0.05
$TlCH_3COO$ 1200	28.75	2.86	<0.005
Negative and positive controls			
Milli-Q water	20.00	2.26	
CrO_3 500 μM	875.50	317.43	<0.05
Tl_2SO_4 (μM)			
Milli-Q water	20.00	2.26	
Tl_2SO_4 0.2	20.80	2.82	ns
Tl_2SO_4 2	20.90	4.48	ns
Tl_2SO_4 20	13.10	1.52	<0.05
Tl_2SO_4 200	14.30	2.71	<0.05
Tl_2SO_4 600	152.20	41.18	<0.05

[1] Mean: statistical average.
[2] Std.Dev.: standard deviation; ns: nonsignificant.

The present findings in *D. melanogaster* are important because it has genes that are related to human diseases and conserved functional domains in nucleotides and proteins [34, 35, 46, 47]. Additionally, using this model can demonstrate the genotoxicity of compounds [48, 49] as well as alterations in metabolic pathways [50, 51, 52] given that enzymes related to energy metabolism are common between flies and humans [53]. Therefore, based on the aforementioned Tl effects and the data generated in this study, we consider that the genotoxic and cytotoxic risks [38] due to the high exposure by bioaccumulation of this heavy metal salt in nature, food and humans, mainly in certain regions of the world that have no control over the polluting sources, such as mining [7, 54], coal power stations and cement production [31], should be further assessed using more genotoxicity and cytotoxicity studies with other assays to increase our knowledge about the types of risk related to this metal.

Finally, this study evaluated a wide range of concentrations, including the Tl concentrations found in the blood or urine of intoxicated people, which reached values of 8.3–880 μg/L and 68–42,000 μg/L or greater, respectively [19, 55, 56]. Additionally, we believe that high concentrations must be tested because Tl accumulates in different tissues and may be found at even higher concentrations than those detected in biological fluids.

5. Conclusions

Survival rates obtained with *D. melanogaster* precisely demonstrated that different compound effects were related to the amount of Tl^{+1} in each compound. Our *D. melanogaster* wing SMART results showed that Tl_2SO_4 [600 μM] was genotoxic in all types of spots and induced a high recombinogenic effect, but $TlCH_3COO$ had no significant genotoxic activity at any concentration tested. Cell division disruption by both salts indicates cytotoxic effects of Tl^{+1}, and these findings are consistent with previous reports of the toxicity of this metal in other organisms and assays. Tl and its compounds have been proposed to be more cytotoxic than genotoxic, and the results obtained in this study support this view. However, more genotoxicity studies with other *in vivo* assays should be performed.

Declarations

Author contribution statement

María de los Ángeles Reyes-Rodríguez: Performed the experiments; Analyzed and interpreted the data.

Luis Felipe Santos-Cruz: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Carlos García-Castro1, Ángel: Performed the experiments; Wrote the paper.

Durán-Díaz: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Laura Castañeda-Partida:Contributed reagents, materials, analysis tools or data; Wrote the paper.

Irma Elena Dueñas-García: Conceived and designed the experiments; Performed the experiments.

María Eugenia Heres-Pulido:Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Juan José Rodríguez-Mercado:Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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