Castañeda, P.L., G.L.E. Muñoz, D.A. Durán, P.M.E. Heres, and G.I.E. Dueñas. 2001. LD_{50} in *Drosophila melanogaster* fed on lead nitrate and lead acetate. Dros. Inf. Serv. 84: 44-48.

LD_{50} in *Drosophila melanogaster* fed on lead nitrate and lead acetate.

Castañeda, P.L.[1], G.L.E. Muñoz[1], D.A. Durán[1], P.M.E. Heres[1], and G.I.E. Dueñas[1]. [1]Lab. Genética Toxicológica, UNAM-Iztacala. Av. de los Barrios S/N. Los Reyes Iztacala,Tlalnepantla. Edo. de México, México; Castañeda Partida Laura. Tel: 56231197; Lab. Genética Toxicológica, Dept. Biología. UNAM-Iztacala. Av. de los Barrios S/N. Los Reyes Iztacala, Tlalnepantla. Edo. de México, México

Introduction

The use of eukaryote organisms for the screening of chemical compounds for toxicity screening is restricted for such reasons as economy, generation times, housing and handling of organisms, number of individuals needed to obtain representative statistical data, and others.

The fruit fly *Drosophila melanogaster* is an eukaryote widely used in genetics. It requires simple facilities, inexpensive culture media, it has a short generation time (±10 days at 25ºC), it breeds a large number of individuals per generation, and *in vivo* assays can be done easily. Among the mutant strains developed for genotoxicity research there are two of extreme importance: Oregon (ORR(1)/ORR(2); flr^3/Bd^S) and *flare* (flr^3/Bd^S). Oregon is a DDT-resistant mutant strain that shows high constitutive levels of cytochrome P450. This is a group of enzymes that are part of the detoxication system (Parke *et al.*, 1990) carried in chromosomes 1 and 2 (Frölich and Würgler, 1989). The strain *flare* carries a recessive mutation lethal in homozygotic condition affecting the phenotype of wing hairs located in the left arm of chromosome 3 (3-38.8) that also shows regulated levels of cytochrome P450 (Graf and van Shaik, 1992). The *flare* strain remains heterozygotic, because it carries the balancer chromosome TM3 with multiple inversions. Cytochrome P450 is present in every eukaryote organism studied (Gonzalez and Gelboin, 1992). During Phase I of metabolism, it inserts one or more polar functional groups into lipophilic molecules. Through these reactions the parental compound becomes an ideal substrate for the conjugation enzymes of Phase II. The conjugated products have an increased polarity that enhances their excretion out of the cell and the body.

Lead is a pollutant heavy metal, which can be absorbed by the digestive system in a 10% (Corey and Galvao, 1989). When incorporated by cells, it produces free radicals, H_2O_2 and ·OH (Roy, 1992), which can be eliminated through different pathways, but free radicals can also produce simple breaks in the DNA chains and adducts (Friedberg *et al.*, 1995). If administrated in excess it can compete with calcium (Pounds, 1984), turn into a poison (Foulkes, 1993), inhibit the heme group synthesis (Alvares *et al.*, 1972; Goldberg *et al*., 1977), and produce cell death.

Based on the methods proposed by Graf and Singer (1992) for the wing Somatic Mutation and Recombination Test (SMART), the two mutant strains of the eukaryote *Drosophila melanogaster* formerly described were used to perform an easy, short term, alternative test for the toxicity screening of two lead salts.

Materials and Methods

Flies were grown and aged in culture bottles containing standard medium at 25ºC. Eggs were collected from these flies by shaking them without anesthesia into bottles containing an approximately 2 cm layer of fermenting fresh baker's yeast supplemented with sucrose (Graf *et al*., 1991). The egg collection bottles were then kept undisturbed in the dark for 8 h at 25ºC. After removing the parental flies, the egg collection bottles were taken back to 25ºC where they remained at a relative humidity of 65% for the rest of their development. Three days later, the 72 h larvae were collected by washing them out the bottles with tap water at room temperature through a fine-meshed stainless steel strainer. They were thoroughly washed free of yeast with tap water while still in the strainer. Immediatly after that, the larvae were transfered to vials (10 larvae/vial) containing 0.5 g of *Drosophila* Instant Medium (Carolina Biological Supply Co, NC, USA) prepared with the solutions of the test compounds, 2 ml of lead acetate (Fluka Nº Cat. 15334) or lead nitrate (Fluka Nº Cat. 21194) at 0, 0.625, 1.25, 2.5, 5.0, 10 mM. Five replications were made for each concentration in five independent experiments for each lead salt. The treatment vials were kept at 25ºC and at a relative humidity of 65%. The surviving flies were collected from the vials on days 10 to 12 after egg laying and shaken into a flask containing 70% ethanol to quantify mortality. The LD_{50}'s for each strain and lead salt were calculated using logistic regression with all five replications of every concentration. LD_{50}'s obtained from the five experiments were analyzed with a two-way ANOVA (one factor being the strain, the other the salt).

Results

Table 1 shows the LD_{50}'s for each strain and salt. Figure 1 shows the mortality rates and LD_{50}'s for lead acetate that were 2.72 mM for the Oregon strain and 2.91 mM for the *flare* strain, respectively. Figure 2 shows the mortality rates and LD_{50}'s for lead nitrate that were 2.93 mM for the Oregon strain and 2.70 mM for the *flare* strain, respectively. On the other hand, the ANOVA results show significant differences between salts ($F = 48.77$, $p = 0.000003$) and a strain-salt interaction ($F = 10.38$, $p = 0.0046$), but no significant differences between strains ($F = 0.011$, $p = 0.9183$). The Tukey test shows significant differences among all four LD_{50}'s.

Table 1. LD_{50}´s of *Drosophila melanogaster* Oregon and *flare* strains fed on lead nitrate and lead acetate.

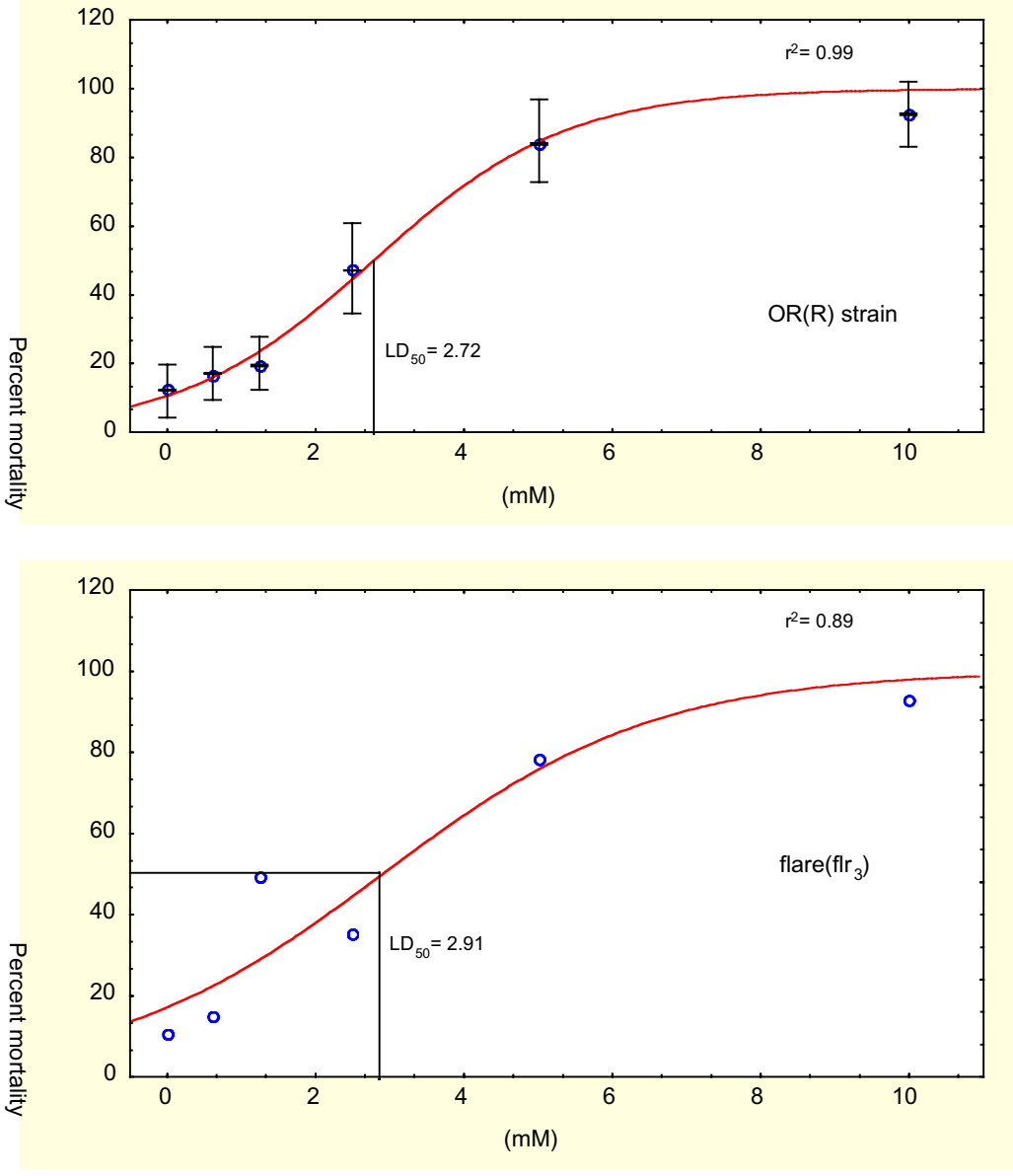
Strain	$Pb(NO_3)_2$ LD_{50} (mM)	$Pb(CH_3COO)_2$ LD_{50} (mM)
Oregon	2.93	2.72
flare	2.70	2.91

Discussion

Results revealed an increase in mortality rates directly proportional to all different concentrations with a sigmoid-type curve (Figures 1 and 2). Interestingly, the mortality rate curves obtained showed that the *flare* strain exposed to these lead salts had a clear increase in the interval of the 0.0625 mM and 1.25 mM concentrations and then an important decrease in the interval of the 1.25 mM and 2.5 mM concentrations for both salts. This was not the case for the Oregon strain wich kept a constant increase in a sigmoid-type curve. Parke *et al*. (1990) have shown it takes a minimal concentration of the chemical compound to induce the cytochrome P450 synthesis in *in vivo* experiments, so that it can go on with detoxication or biotransformation of the chemical compound into a reactant agent. Considering the Oregon strain with constitutive levels of cytochrome P450, where enzymes are always present and the *flare* strain with regulated levels, that might account for the curves found in their mortality rates at different concentrations of both salts, but this remains to be proved in further biochemical experiments

and exposures to other compounds. At higher concentrations of both lead salts, the mortality rates in the *flare* strain are similar to those of the Oregon strain.

Fig.1 Mortality rates and LD50's of lead acetate (Pb(CH3COO)2) for Drosophila melanogaster Oregon OR(R) and flare (flr3) mutant strains.



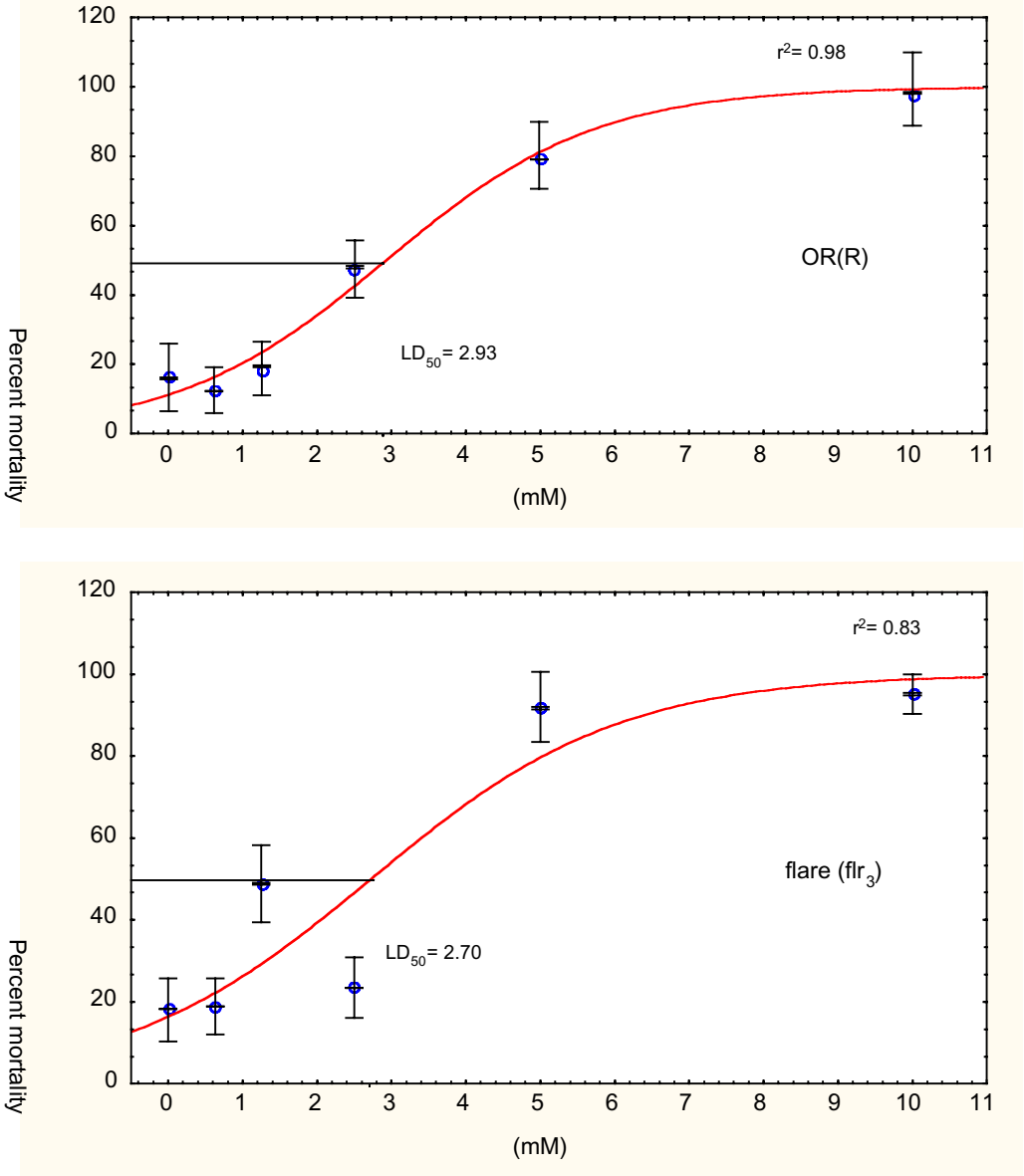
Conclusions

We found significant differences between salts, a strain-salt interaction, and differences among all four LD_{50}'s. These results mean this system could be used to screen many other well known chemical compounds to search how these mutant strain's mortality rates behave when exposed to

different kinds of agents. It will also be interesting to validate these results with further experiments regarding increased levels of cytochrome P450 in the *flare* strain in relation with its mortality rates.

References: Alvares, A., S. Leigh, J. Cohon, and A. Kappas 1972, Jour. Experim. Med. 135: 1406-1409; Corey, O.G., and C.L. Galvao 1989, Plomo. Serie Vigilancia 8. Metepec, Edo. de México. Centro Panamericano de Ecología Humana y Salud. Org. Panam. Salud. O. M. S. 103 pp.;

Fig. 2 Mortality rates and LD50's of lead nitrate (PbNO3) for Drosophila melanogaster Oregon OR(R) and flare (flr3) mutant strains respectively.



Foulkes, E.C., 1993, *Biological Effects of Heavy Metals II. Metal Carcinogenesis*. CRC Press, Boca Ratón; Friedberg, E.C., G. Walker, and W. Siede 1995, *DNA Repair and Mutagenesis*. ASM Press, Washington, pp: 16-17; Frölich, A., and F.E. Würgler 1989, Mutat. Res. 216: 179-187; Goldberg, A.,

P.A. Meredith, S. Miller, M.R. Moore, and G.G. Thompson 1977, Proc. Biochem. Pharmac. Sci. 13th-15th July; González, F., Ch. Crespi, and H. Gelboin 1991, Mutat. Res. 247: 113-127; Graf, U., N. van Shaik, and R. Pacella 1991, Dros. Inf. Serv. 70: 247-248; Graf, U., and N. van Shaik 1992, Mutat. Res. 271: 59-67; Graf, U., and D. Singer 1992, Rev. Int. Contam. Ambient. 8(1): 15-27; Parke, V.D., C. Ionnides, and D.F.V. Lewis 1991, Pharmac. Manufact. Assoc. Can. Key. Lect. 69(19): 537-548; Pounds, J.G., 1984, Neurotoxicology 5: 295-332; Roy, N.K., 1992, Mutat. Res. 298: 97-103.