Safety assessment of propyl-propane-thiosulfonate (PTSO): 90-days oral subchronic toxicity study in rats

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- A 90 days oral toxicity study was conducted with propyl-propane-thiosulfonate
- PTSO did not induce toxic effects at the highest dose assayed (55 mg/Kg/day)
- NOAEL for PTSO was estimated to be ≥ 55 mg/Kg/day

Abstract

Propyl-propane-thiosulfonate (PTSO) is one of the main organosulfur compounds present in *Allium* essentials oil. Different applications in the food sector have been proposed for PTSO, such as food and feed additive and as active packaging. However, the authorization of its use depends on its toxicity profile. Thus, as a part of its safety assessment, in this work a repeated dose 90-day oral toxicity study has been conducted for the first time in rats following the OECD guideline 408. PTSO was administered to groups of 10 male and 10 female rats at dose levels of 0, 14, 28, and 55 mg/Kg/day. No clinical signs or mortality and no changes in body weight, food consumption and feed conversion efficiency were detected through the study. Moreover, no treatment-related changes in hematological and biochemical parameters were observed, for either sex or dose groups. The histopathology study performed revealed no differences in organ weights, and no morphological and histopathological changes were observed. Based on these results, the no-observed-adverse-effect level (NOAEL) of PTSO was judged to be ≥ 55 mg/Kg/day for both sexes.

Keywords: Subchronic toxicity; 90-day; Organosulfur compounds; *Allium*; Propyl-propanethiosulfonate

1. Introduction

Essentials Oils (EO) extracts from plants have been the focus of numerous studies due to their potential in the pharmaceutical and food industries. Several works and patents have been developed specifically for the application of EO and their components into the food sector (Ribeiro-Santos et al., 2017; Llana-Ruiz- Cabello et al., 2015; Maisanaba et al., 2017). Globally, their relatively safe status, properties and acceptance by consumers which demand natural compounds to replace synthetic ones have piqued the interest of industries and consumers (Sacchetti et al., 2005; Benkeblia and Lanzotti, 2007; Debiagi et al., 2014). Among those beneficial plants, *Allium* sp. is a genus well-known for its antimicrobial, antiviral, antiprotozoal, antifungal or antioxidant properties (among others). These properties are mainly due to their content of organosulfur compounds (OSC), which are secondary phytochemical metabolites, biosynthesized for defensive purposes against biotic and abiotic stressors. They are mainly formed for the action of the enzyme alliin (stored in vacuoles) on cytoplasmic compounds like alk(en)yl cysteine sulfoxides (ACOs) once the vegetable tissue is hurt (Putnik et al., 2019) (Fig. 1). Otherwise, these compounds are not available in intact cells as they are toxic for the plant (Ramirez et al., 2017).

One of the main components of *Allium* essential oil is propyl-propane-thiosulfonate (PTSO), that corresponds to the molecular formula $C_6H_{14}O_2S_2$ (Fig. 1). This compound has been stabilized and characterized by DMC Research Center SLU (Granada, Spain) to be used for different applications, taking advantage of its beneficial properties. Thus, this product has been reported to show mainly antioxidant and antimicrobial activities, being able to inhibit the growth of Gram (–) and Gram (+) bacteria as well as molds and yeast (Peinado et al., 2012, 2013; Llana-Ruiz-Cabello et al., 2015). Its antibacterial activity *in vitro* in humans has also been demonstrated (Sorlozano-Puerto et al., 2018).

Proallium AP®, a commercial *Allium* sp. extract with a 14.5% PTSO content, has been proposed as a biopreservative in active food packaging for human food commodities mainly due to its antioxidant and antimicrobial activity (Llana-Ruiz-Cabello et al., 2018). The packaging material used in these systems can incorporate components intended to be released into the food from the package, allowing foods to arrive at the consumers with their original or enhanced organoleptic properties, with longer shelf-life and safety (Ribeiro-Santos et al., 2017). Previously, another study carried out by Seydim & Sarikus, (2006) tested the antimicrobial activity of garlic EO in combination with oregano EO in films made with whey protein isolate, and showed antimicrobial activity in a concentration of 4% (w/v). But the incorporation of garlic EO and their components in active food packaging can result in a higher human exposure and consequently, more research is needed to stablish the safety concentration. An additional proposed application for PTSO is as sensory additive in animal nutrition, improving the palatability of feed, and also as a zootechnical additive, being an alternative to the use of antibiotics (Peinado et al., 2012), contributing to reduce resistance generated by their excessive use in livestock. Since the ban in the European Union (EU) of the use of antibiotics as growth promoters, the search for new alternative products that ensure similar production levels and food security without generating unwanted effects, including human resistances, has been fostered, being additives of natural origin a good alternative. The regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 sets the parameters of use of the additives used in animal nutrition. Recent studies have shown that some feed additives can help animals to maintain good physiological conditions and improve animal welfare. This has led to an amendment in the abovementioned regulation on 12th June 2019, establishing new functional groups of feed additives to improve

physiological condition of animals. In this regard, other properties demonstrating the potential use of PTSO in animal nutrition sector are the anti-methanogenic effect described during the fermentation process in rumen (Martínez-Fernández et al. 2013) and the immunomodulatory and anti-inflammatory properties (Vezza et al., 2019).

However, the successful development of any application of this compound must be accompanied by an extensive toxicological evaluation, that guarantees its safety for the final consumers, both humans and livestock.

In this regard, the toxicological profile of PTSO has been investigated by cytotoxicity assays in human cell lines (Llana-Ruiz-Cabello et al., 2015) as well as its genotoxicity and mutagenicity *in vitro* (Mellado-García et al., 2015) and genotoxicity *in vivo* (Mellado-Garcia et al., 2016b). Furthermore, its acute toxicity *in vivo* has been also evaluated (Llana-Ruiz-Cabello et al., 2015). Notwithstanding, safe doses of use have been not yet established for PTSO. Thus, a repeated dose 90-days oral toxicity study in rodents of PTSO would be necessary to clearly characterize its toxicity, being also a requirement in the authorization application processes of the European Food Safety Authority (EFSA, 2012; 2016a; 2016b; 2017).

The aim of the present study is, therefore, to further explore the toxicity potential of PTSO and, for the first time, to conduct a subchronic dietary toxicity assay of PTSO in rats following internationally recognized test guidelines (Organisation for Economic Co-operation and Development, OECD 408, 2018). Considering these facts, in the present study several parameters have been evaluated, including body weight changes, food and water consumption, feed conversion efficiency, organ weight ratios and biochemistry and hematology parameters. In addition the histopathology of various tissues has been also studied. The results from the complete assessment of this

subchronic study would allow to get an estimation of a non-observed-adverse-effect-level (NOAEL) of exposure to establish safety conditions for human exposure to PTSO.

2. Materials and Methods

2.1. Test item and doses preparation

PTSO was supplied by DMC RC SLU (Granada, Spain) with a 96% of purity. Commercial powder neutral gelatin from pork protein (Jesus Navarro S.A., Alicante, Spain) was employed as the vehicle for the test substance in all groups including the controls.

For the 90-days study, the doses were prepared daily for each animal during the 13 weeks, and on Fridays they were also prepared for the weekend. The dose was mixed in 3 mL of liquid gelatin. The volumes of PTSO (µL) to add to the gelatin depended on the dose selected for each group, and the gelatin could solidify at 4 ºC overnight. Homogeneity of the dietary dose formulations and their stability were confirmed to be at least 5 days.

2.2. Animals conditions and husbandry services

The rats were supplied by Charles River laboratories S.L. (Kings, NY, USA), 40 males and 40 females of Sprague-Dawley strain. They were approximately 7 weeks old and were stabilized for an acclimatization period of 7 days during which they were examined by a veterinary surgeon. When the first week of dosage began the rats body weight mean was 320 g ± 11.3 for males and 227 ± 11.9 for females.

Animals were individually housed in cages type 3H with Souralit 29/12 plus (souralit S.L., Gerona, Spain) aspen wood bedding and food completely available without restriction using standard dry pellet diet for rodents Scientific (Panlab, S.L.U., Cornella de Llobregat, Barcelona, Spain). They were kept in a room with controlled conditions of hygiene behind a barrier system, a range of temperature of 21±2ºC, with a 10-15 air changes per hour, and a relative humidity between 30-70% under 12 h light/dark cycle. Each cage contained an information card which contained study code (19-CAM-11-animal number), sex, dose, group, and individual animal identification. Community tap water (EMACSA, Cordoba Water company, Córdoba, Spain), filtered and autoclaved was available *ad libitum.*

2.3. Study design

The maximum tolerable dose (MTD) for PTSO in rats orally exposed to PTSO (gavage) was previously set at 55 mg/kg by Llana-Ruiz-Cabello et al., (2015) following the OECD 425 (2008) test guideline (oral toxicity study: Up and Down procedure), and it was used as a reference to establish the test doses. Accordingly, this dose was selected as the highest one to be tested, and also descending doses using a 2-fold interval factor according to the guideline OECD 408 (2018) recommendations: 14, 28, 55 mg/kg/day. Rats (10/sex/group) were orally administered the selected doses and the control group received only the vehicle (pork gelatine).

This study was performed at the Central Service of Experimental Animals from the University of Cordoba (SAE, Cordoba, Spain) in which all animals received human care in accordance with the guidelines for the protection of animals used for the science

purposes (Directive, 2010/63 EU, Decision, 2012/707/UE, and RD 53/2013). All procedures have been approved by the Ethical Animal Experimentation Committee of the University of Córdoba and by the Junta de Andalucía (project nº 20/10/2015-348).

2.4. Clinical observations

Each animal was observed twice daily for morbidity and mortality and once daily for clinical signs, such as changes in skin, fur, eyes or mucous membranes; secretions; changes in gait, posture, or handling response; abnormal, clonic, or tonic movements, and stereotypes or bizarre behavior. Ophthalmic examinations were performed on all animals before initiating the study and in the control and in the highest dose group at the end of treatment.

2.5. Body weight, food and water consumption

These three parameters were checked weekly in order to avoid stress. The mean body weight per group and sex were calculated weekly and prior to necropsy from individual animals' data, as well as the food and water consumption. The total food consumed per cage was recorded and the weekly mean intake per rat was calculated. The feed conversion efficiency (FCE) ratio was determined according to Escobar et al., (2015) by the ratio of food intake (g)/ weight gained (g).

2.6. Hematology and Biochemistry

Blood samples were extracted from the heart by an intracardiac injection under light isofluorane anesthesia at week 13. Then, the hematological parameters were

estimated on an automatic hematology analyzer Cell-Dyn 3700 (Abbot, GMI, MI, USA): red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hematoglobin (MCH), mean corpuscular hematoglobin concentration (MCHC), blood platelet count (PLT), red cell volume distribution (RDW), prothrombin time (PT), cefaline time (CT), white blood cell count (WBC), Neutrophils (NE), Lymphocites (LY), Monocytes (MO), Eosinophils (EO) and Basophils (BA).

An automatic chemistry analyzer Cobas 6000 (Roche Diagnostics, IN, USA) was used to determinate the following standard biochemistry parameters: glucose (GLUC), blood urea nitrogen (UREA), creatinine (CREAT), bile acids (BILI-T), total cholesterol (CHOL), triglycerides (TRIGL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKP), albumin (ALB), total protein (TOT PROT), sodium (Na^+), potassium (K^+) and Calcium (Ca^{2+}) ions.

2.7. Necropsy and Organ weight

The animals were fasted for 18 h before the sacrifice and profoundly anaesthetized with isofluorane, then exsanguinated by intracardiac injection. All rats were given a complete pathology examination through the necropsy. The following organs were collected from animals at necropsy and weighed wet immediately after dissection: brain, liver, heart, spleen, kidneys, thymus, adrenal glands, uterus with cervix and ovaries (females) and testes and epididymis (males). In addition, tissue samples of the following organs were taken from the control and the highest dose (55 mg/kg/day) group for histopathological examination under light microscopy: liver, kidney, spleen, heart, brain, pituitary, stomach, intestine, lung, testicle/ovary and

skeletal muscle. Samples were fixed in 10% formalin for 24h at 4ºC, and then immediately dehydrated in graded series of ethanol, deep in xylol and embedded in paraffin wax using an automatic processor. Sections of 3-5 µm were mounted. After they had been deparaffinized, the sections were rehydrated, stained with hematoxylin and eosin, and mounted with Cristal/Mount (Paraplat, Oxford Labware, St. Louis, MO.).

2.8. Statistical analysis

All statistical analysis were carried out using Graph-Pad Instant software (GraphPadSoftware Inc., La Jolla, USA). Continuous variables, such as body weight, body weight gain, food and water consumption, hematology, clinical chemistry, and organ weight, were summarized using standard measures of central tendency and dispersion, mean and standard deviation (ST. DEV.), and were reported by sex and dosage. One-way analysis of variance (ANOVA) was carried out to test differences in continuous variables. Normality assumption was tested using Kolmogorov-Smirnov's test. If non-normality, comparison was performed with Kruskal Wallis test. In case of significant differences, multiple comparisons were performed using Tukey-Kramer/Dunn's Multiple Comparisons Tests. Differences were considered significant from $P < 0.05$.

3. Results

3.1. Survival and clinical observations

No unscheduled deaths occurred during the study. Light clinical signs were observed in some animals. Thus, sporadic alopecia was observed in rats 47, 59 and 62; small wounds in the ear of rat 52 and hematoma in the ear of rat 14 satisfactorily

recovered. These signs were not considered to be related to the test item, and were not biologically significant. Ophthalmologic examination revealed no compound-related lesions in the highest dose group of animals. No other clinical observations were noted.

3.2. Body weight, body weight gain, food and water consumption, feed consumption efficiency

Body weight increased along the study period following a usual pattern for this species. The test item did not induce any statistically significant alteration in this parameter (Fig. 2). Similarly, the body weight gain increased along the 13 weeks, with no differences between the control and the exposed groups in both sexes (Fig. 3).

Food and water consumption per week did not show significant differences between control and treated groups, male and female, throughout the duration of the study, following the usual pattern (data not shown). The feed consumption efficiency also did not reveal any remarkable change for animals (Table 1). Thus, the test item did not have a negative impact on these parameters.

3.3. Hematology and blood chemistry

Hematology parameters evaluated in rats exposed to PTSO are provided in Table 2. All variables considered remained unaltered in males. However, in females HCT (%) showed a significant increase ($p<0.5$) in groups 2 and 3 (14 and 28 mg/Kg/d, respectively) in comparison to group 4 (50 mg/Kg/d), and also the MCH (pg) experienced a significant decrease ($p<0.5$) in group 2 (14 mg/Kg/d) in comparison to group 3 (28 mg/Kg/d).

The differential White blood cells count did not show any change in any of the exposed groups and neither in males nor females (Table 3).

Table 4 includes the clinical biochemistry values obtained for the parameters analyzed after the oral subchronic exposure of rats to PTSO. Most of them were not modified by the treatment. Only in males, a significant ($p<0.01$) decrease of Chol (mg/dL) values was observed in the highest dose group (55 mg/Kg/day), and also a significant increase ($p<0.5$) of TRIGL (mg/dL) in the lowest dose group (14 mg/Kg/day), in comparison to the control group.

In general, as the significant changes observed were minimal, sporadic, not present in all dose groups, neither in both sexes, they were considered incidental and not indicative of toxicity.

3.4. Necropsy, organ weights and histopathology

No gross pathologies were observed during the necropsy in any of the experimental animals. Also, organ weights were not altered by the treatment and only the mean heart weight of males of group 3 (28 mg/Kg/day) showed a slight but significant ($p<0.5$) decrease in comparison to the control group (Table 5). This was considered to be not related to the test item. Moreover, no significant changes were recorded in the organ weight/body weight ratio (Table 6) neither in the organ weight/brain weight ratio (Table 7).

Regarding to the histopathological study performed, tissues of the rats, both male and female, exposed to the highest dose, did not revealed alterations in comparison to the control group in any of the organs examined by light microscopy (Fig. 4).

4. Discussion

The repeated dose 90-days oral toxicity study in rodents provides information on the possible health hazards likely to arise from repeated exposure over a prolonged period of time covering post-weaning maturation and growth into adulthood of the test animals. The study provides, among others, information on the major toxic effects, identification of target organs and also can provide an estimate of a non-observed-adverse-effect level (NOAEL) of exposure which can be used in selecting dose levels for chronic studies and for establishing safety criteria for human exposure (OECD 408, 2018). Moreover, an oral 90-days subchronic toxicity assay is usually included among the basic set of toxicity tests required in the evaluation of chemical substances with potential applications in the agri-food sector before their authorization. This is the case for example of food (EFSA, 2012a) and feed additives (EFSA, 2017), migrating food contact materials (EFSA, 2016a), or novel food (2016b). All this highlights the relevance of the study performed with PTSO, as this compound has shown beneficial properties with different potential applications previously described.

The general absence of toxic effects observed after the treatment allows to estimate that the NOAEL for PTSO is ≥ 55 mg/Kg/day. Considering a safety factor of 100 usually applied in food additives to derive human safety values from animal data (EFSA, 2012b), a dose of 0.55 mg/Kg/day could be suggested as a safe human exposure (~ 38,5 mg/day for a 70 kg b.w. person). However, this value is underestimated taking into account that a NOAEL could not be established from the assay performed and could be higher than 55 mg/Kg/day.

The results obtained in the present study complete the information available in relation to the toxicity profile of PTSO. Thus, Llana-Ruiz-Cabello et al. (2015)

performed an acute oral toxicity test (Up-and-Down Procedure) following the OECD 425 guideline (2008) and 55 mg/Kg b.w. was established as the maximum tolerated dose (MTD) in rats. However, in that case the exposure was by gavage whereas in the present study it was with the diet, a more realistic human exposure scenario.

Mellado-García et al. (2015) performed a thorough *in vitro* genotoxicity assessment of PTSO including 4 different tests, among them the Ames test and the Micronucleous (MN) assay. This is the basic battery indicated by EFSA (2012) as they cover the three genetic endpoints required: gene mutations and both structural and numerical chromosome aberrations. They also performed the Mouse Lymphoma assay (MLA) and the Comet assay. They concluded that PTSO was not mutagenic in the Ames test, but it was mutagenic in the MLA assay after 24 h of treatment. The parent compound did not induce MN on mammalian cells; however, its metabolites induced positive results. Due to inconclusive results, a follow-up of positive *in vitro* results by *in vivo* testing was performed. The genotoxicity of PTSO in rats following an oral administration of 5.5, 17.4 and 55 mg/kg was evaluated by a combined *in vivo* comet assay and MN test (Mellado-Garcia et al., 2016a) and the results revealed no genotoxicity.

All these results suggest a safety profile of PTSO for food applications at the doses assayed, but in a risk assessment frame it is well known that risk depends not only on the hazard but also in the human exposure level. In this regard, the level of PTSO to use, and therefore the potential exposure, will depend on the specific application considered. For instance, in the active packaging of lettuce, Llana-Ruiz-Cabello et al. (2015) estimated that in the worst-case scenario a human could ingest 6.87 mg PTSO, this means only a 18% of the dose calculated as safe in the present study.

The results obtained agree with those of Mellado et al. (2016b) who evaluated the safety of Proallium AP®, an *Allium*-based commercial product in a 90-days feeding study with rats. PTSO is actually the major organosulfur compound present in Proallium AP® (14.5%). Similarly, neither clinical signs nor any other changes on general, biochemical, hematological or histopathological parameters were detected, and the authors derived a NOAEL higher than 400 mg/Kg/day at the conditions assayed. Both studies show a good correlation as in the present study the test item had a 7-fold higher content of PTSO than Proallium® AP (100 versus 14.5%) and the NOAEL derived for Proallium was 7-fold higher. This suggests that PTSO has an important role on the toxicity of *Allium* extracts. Actually, a MTD of 55 mg/kg in rats for PTSO has been established as previously indicated. And higher doses tested according to the OECD 425 guideline (2000 mg/kg and 175 mg/kg) resulted in the death of the animal and evident hepatotoxicity. On the contrary, a single dose of 55 mg/kg did not induce remarkable damage (Llana-Ruiz-Cabello et al, 2015). Mellado-García et al. (2016a) observed that in rats treated with 55 mg/kg (3 doses at 0, 24 and 45h and euthanized at 48h) an increase in the glycogen storage was noticeable in the liver and also a slight degenerative process in the chief cells of the stomach.

In this regard, at the dose levels assayed, histopathological lesions were absent. This could be explained by the exposure way employed in this study, using gelatin as vehicle and with the feed. In the previous trials, oral gavage with a stomach tube after a fasting period was used following the recommendations of the corresponding OCDE guidelines. The bolus could have a more deleterious effect on the gastrointestinal system as there is a direct contact.

Differences in toxicity between PTSO and Proallium AP® could be explained as the toxicity shown by components of an essential oil can be modulated by the other

constituents by synergistic/antagonistic phenomena (Escobar et al., 2015; Pavlidou et al., 2004). The interest of PTSO in comparison to Proallium® is based on its different chemical properties (higher hydrophilia) and in its higher efficiency on its antioxidant and antimicrobial properties.

Other authors have shown the potential toxicity of aqueous extracts of different medicinal plants, including *Allium sativum*, in Wistar rats. Thus, Sulaiman et al. (2014) administered orally to the animals 10 mg/kg of *A. sativum* extract for 30 days and observed alteration in the activities of marker enzymes: AST increased in liver, kidney and heart, ALT in serum and liver, and ALP activity was reduced in serum, heart, kidney and liver. They concluded that caution was required in using unrefined extracts of these herbs in traditional settings.

There are scarce *in vivo* toxicity data regarding other OSC. Thus, Guyonnet et al. (2000) demonstrated the effects of some of them (DAS, DADS, dipropylsulfiide (DPS) and dipropyl disulfide (DPDS)) on the activation of several mutagens in male Wistar rats exposed to 1 mmol/kg by gavage for 4 days. They explained the results based on the induction of cytochrome (CYP) and phase II enzymes activities. This effect, the alteration of CYP activity, was pointed out by other authors as well (Davenport and Wargovich, 2005). Moreover, they observed hepatotoxicity induced by DAS (bile duct obstruction, hyperproliferation and focal points of necrosis) in rats gavaged daily with 200 mg/kg for 1, 4, or 8 weeks. On the contrary, 8 weeks of exposure to lower doses (50 and 100 mg/kg) did not induced liver histophatological damage. This suggest that liver could be the target organ of OSC as both, DAS (Davenport and Wargovich, 2005) and PTSO (Llana-Ruiz-Cabello et al, 2015), have shown liver toxicity when a threshold dose is exceeded. Also, Wu et al. (2001) exposed rats orally to garlic oil (GO, 200 mg/Kg) and 3 allyl compounds, DAS (20 and 80 mg/kg), DADS (80 mg/kg), and diallyl

trisulfide (DATS, 70 mg/kg) 3 times a week for 6 weeks and examined the antioxidation system in rat livers and red blood cells. They found that GO, DADS and DATS significantly induced the glutathione content (GSH) in blood cells but neither GO nor any of its OSC affected the GSH-related antioxidant enzymes. Hepatic GSH was not influenced by garlic components. But DADS and DATS significantly increased the activity of GSH-reductase and GSH-transferase and decreased GSH peroxidase. In the present study the hematological parameters were not influenced by the PTSO exposure and scarce scientific data dealing with PSTO are available to compare.

5. Conclusions

In conclusion, the results obtained confirm the already reported safety profile of PTSO for some food applications at the conditions considered. Thus, PTSO did not promote toxic effects as seen from body weight changes, food and water consumption, feed conversion efficiency, biochemical and blood parameters as well as organ toxicity and histological examinations of main organs that could eventually be affected by its subchronic administration (90 days). NOAEL was estimated to be ≥ 55 mg/Kg/day.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Figure captions

Fig. 1. Biochemical pathways and chemical structures of organosulfur compounds. (Alk(en)yl cysteine sulfoxides (ACOs); Propyl-propane-thiosulfonate (PTSO); Propyl-propane-thiosulfinate (PTS)).

Fig. 2. Mean body weights (g) of A) male and B) female rats orally exposed to 0, 14, 28 and 55 mg/kg b.w./day PTSO and control rats for 90 days.

Fig. 3. Mean body weight gain (%) of A) male and B) female rats orally exposed to 0, 14, 28 and 55 mg/kg b.w./day PTSO and control rats for 90 days.

Fig. 4. Histopathological study of liver, kidney, spleen, heart, brain, pituitary, stomach, intestines, lung, testes, and and skeletal muscle of untreated Sprague Dawley rats, control (A), and treated (B) with PTSO (55 mg/Kg/day) for 90 days (bars= 50 μm). In the liver, normal hepatic cords and normal polyhedral hepatocytes with central nucleus and clear cytoplasm are seen in control and exposed animals. In the kidney, the renal parenchyma with normal glomeruli and renal tubules is shown in control rats (A), as well as in rats treated with the highest dose of PTSO (B). Detail of the apparently normal spleen parenchyma in control and treated rats (A, B). In the heart, normal cardiac fibers were observed in all groups (A, B). In the brain, the motions of the cerebral cortex are normal (A, B). Details of the apparently normal pituitary are observed in control and treated rats (A, B). Detail of the stomach with apparently normal mucous and glandular cells in control and treated rats (A, B). Intestinal villi with abundant apparently normal enterocytes are shown in all groups. Detail of the control

bronchial epithelium, without alterations in the bronchi and alveoli in the untreated rats (A) as well as in the rats treated with the highest dose (B). Male rat testes showed normal seminiferous tubules and interstitial space (A) that is maintained in treated male rats (B). The ovaries of the treated and control female rats (A, B) presented normal follicles in all groups. Detail of the normal striated skeletal muscle of the treated and control rats (A, B).

Table captions

Table 1. Effect of 90 days oral exposure to PTSO on body weight and food consumption in rats. Values represent the mean ±SD of 10 rats/sex/group. Differences between control and treated groups for male and female rats were evaluated by Kruskal-Wallis test (K.W.) or by ANOVA test (F values).

Table 2. Hematology parameters of male and female rats fed with 0, 14, 28 and 55 mg/kg b.w./day PTSO for 90-days. Values are mean ± SD for 10 rats/sex/group. The differences between control and treated groups for male and female rats were evaluated by Kruskal-Wallis test (K.W.) or by ANOVA test (F values). The significance levels observed are & in comparison to group 4 (55 mg/Kg/d) when $p<0.05$, and # in comparison to group 3 (28 mg/Kg/d) when $p<0.05$.

Table 3. Differential White blood cells count data of male and female rats fed with 0, 14, 28 and 55 mg/kg b.w./day PTSO for 90-days. Values are mean ± SD for 10 rats/sex/group. The differences between control and treated groups for male and female rats were evaluated by Kruskal-Wallis test (K.W.) or by ANOVA test (F values).

Table 4. Clinical biochemistry of male and female rats fed with 0, 14, 28 and 55 mg/kg b.w./d PTSO for 90-days. Values are mean ± SD for 10 rats/sex/group. The differences between control and treated groups for male and female rats were evaluated by Kruskal-Wallis test (K.W.) or by ANOVA test (F values). The significance levels observed are *$p < 0.05$ and **$p < 0.01$ in comparison to control group values.

Table 5. Absolute organ weight of male and female rats fed with 0, 14, 28 and 55 mg/kg b.w./day PTSO for 90-days. Values are mean ± SD for 10 rats/sex/group. The differences between control and treated groups for male and female rats were evaluated by Kruskal-Wallis test (K.W.) or by ANOVA test (F values). The significance levels observed are *$p < 0.05$ in comparison to control group values.

Table 6. Relative organ weight/body weight of male and female rats fed with 0, 14, 28 and 55 mg/kg b.w./day PTSO for 90-days. Values are mean ± SD for 10 rats/sex/group. The differences between control and treated groups for male and female rats were evaluated by Kruskal-Wallis test (K.W.) or by ANOVA test (F values).

Table 7. Relative organ weight/brain weight of male and female rats fed with 0, 14, 28 and 55 mg/kg b.w./day PTSO for 90-days. Values are mean ± SD for 10 rats/sex/group. The differences between control and treated groups for male and female rats were evaluated by Kruskal-Wallis test (K.W.) or by ANOVA test (F values).

Table 1

Table 1

PARAMETERS	MALE				FEMALE			
	Group 1 (0 mg/Kg/day) N=10	**Group 2 (14 mg/Kg/day) N=10**	**Group 3 (28 mg/Kg/day) N=10**	**Group 4 (55 mg/Kg/day) N=10**	**Group 1 (0 mg/Kg/day) N=10**	**Group 2 (14 mg/Kg/day) N=10**	**Group 3 (28 mg/Kg/day) N=10**	**Group 4 (55 mg/Kg/day) N=10**
Inicial body weight (g)	320.1±10.8	317.4±8.7	321.4±14.0	320.3±14.7	226.5±9.2	228.6±14.6	227.3±10.1	225.3±12.3
	F(36.3)=0.19 p=0.90; N.S.				**F(36.3)= 0.14 p=0.94; N.S.**			
Final body weight (g)	633.8±38.3	623.1±28.7	611.5±26.5	625.9±48.1	326.8±11.5	338.0±29.1	321.1±23.8	321.3±18.3
	F(36.3)=0.64 p=0.59; N.S.				**F(36.3)=1.33 p=0.28; N.S.**			
Body weight gain	313.7 ±35.4	305.7±29.5	290.1±29.2	305.6±38.2	100.3± 13.5	109.4±19.9	93.8±23.3	96.0±15.5
	F(36.3)=0.88 p=0.46; N.S				**F(36.3)=1.40 p=0.26; N.S.**			
Total feed intake (g)	2977.2±197.2	2998.0±207.0	2822.1±249.2	2810.5±155.5	1948.4±174.2	1994.9±258.8	1928.3±210.7	1888.3±142.6
	KW=7.17 p<0.066; N.S				**KW=1.94 p=0.58; N.S.**			
Feed conversion ratio	9.6±0.8	9.9±1.4	9.8±1.0	9.3±0.9	19.8±3.2	18.5±2.5	21.3±3.7	20.3±4.6
	F(36.3)=0.65 p=0.59; N.S.				**KW=2.51 p=0.47; N.S.**			

Values are mean ± SD for 10 rats/sex/group. F: Statistics ANOVA test; K.W: Kruskal-Wallis Statistic; N.S.: Not Significant.

Table 2

HAEMATOLOGY DATA SUMMARY									
		MALE				FEMALE			
		Group 1 (0 mg/Kg/day) N=10	Group 2 (14 mg/Kg/day) N=10	Group 3 (28 mg/Kg/day) N=10	Group 4 (55 mg/Kg/day) N=10	Group 1 (0 mg/Kg/day) N=10	Group 2 (14 mg/Kg/day) N=10	Group 3 (28 mg/Kg/day) N=10	Group 4 (55 mg/Kg/day) N=10
RBC	MEAN	9.08	9.08	9.13	8.78	8.40	8.81	8.48	7.75
$10^6/\mu l$	ST. DEV.	0.53	0.35	0.59	0.64	0.29	0.53	0.20	1.43
			F(36.3)=0.82 p=0.49; N.S.				**KW=6.57 p=0.09; N.S.**		
HGB	MEAN	14.79	15.02	15.08	14.72	14.30	14.76	14.88	13.26
g/dL	ST. DEV.	1.11	0.64	0.91	0.76	0.62	0.59	0.49	2.41
			F(36.3)=0.35 p=0.79; N.S.				**KW= 7.03 p=0.07; N.S.**		
HCT	MEAN	70.10	70.20	70.63	68.89	70.00	72.89[&]	72.70[&]	64.10
%	ST. DEV.	3.48	2.78	3.74	3.10	3.35	3.76	2.91	11.88
			F(36.3)=0.46 p=0.72; N.S.				**KW= 8.77 [&] p<0.03.**		
MCV	MEAN	77.29	77.42	77.46	78.57	83.23	82.80	85.95	82.50
fL	ST. DEV.	2.54	2.12	3.50	3.34	2.52	2.66	3.14	2.27
			F(36.3)=0.39 p=0.76; N.S.				**KW=7.67 p=0.05; N.S.**		
MCH	MEAN	16.29	16.55	16.55	16.81	17.02	16.78[#]	17.53	17.12
pg	ST. DEV.	1.26	0.40	0.74	0.66	0.52	0.76	0.55	0.26
			F(36.3)=0.62 p=0.61; N.S.				**F(34.3)=3.12 [#]p<0.04.**		
MCHC	MEAN	21.07	21.39	21.35	21.36	20.47	20.26	20.40	20.68
g/dL	ST. DEV.	1.22	0.38	0.38	0.35	0.30	0.44	0.40	0.33
			F(36.3)=0.43 p =0.73; N.S.				**F(34.3)=2.12 p=0.12 ; N.S.**		
PLT	MEAN	891.50	912.50	1001.00	899.00	666.67	780.67	810.50	726.00
$10^3/\mu l$	ST. DEV.	247.31	219.09	221.36	241.41	345.75	283.76	252.44	407.29
			F(36.3)=0.40 p=0.76; N.S.				**F(34.3)=0.29 p=0.83; N.S.**		
RDW	MEAN	16.67	17.26	16.90	15.98	14.02	14.62	14.46	15.02
%	ST. DEV.	1.11	1.03	1.53	1.52	0.34	1.11	0.89	1.50
			F(36.3)=1.61 p=0.21; N.S.				**KW= 2.49 p=0.48; N.S.**		
T PRO	MEAN	22.33	22.49	21.27	24.79	21.68	22.55	21.78	22.10
seg	ST. DEV.	4.92	3.77	0.62	4.22	1.41	1.03	1.19	0.49
			KW=4.19 p=0.24; N.S.				**F(34.3)=1.01 p=0.40; N.S.**		
T CEF	MEAN	34.30	35.38	34.12	27.65	36.03	33.45	33.80	33.63
seg	ST. DEV.	6.52	8.00	7.61	6.94	3.86	2.85	4.01	3.55
			KW=4.97 p=0.17; N.S.				**F(34.3)=0.96 p=0.46; N.S.**		

RBC: Erythrocyte count; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: platelet (thrombocyte) count; RDW: red blood cell distribution width; T PRO: prothrombin time; T CEF: cephalin time. F: Statistics ANOVA test; K.W: Kruskal-WallisStatistic; N.S.: Not Significant; [&]Significantly different in comparison to group 4 when $p<0.05$; [#] significantly different in comparison to group 3 when $p<0.05$.

Table 3

DIFFERENTIAL WHITE BLOOD CELLS COUNT DATA SUMMARY									
		MALE				FEMALE			
		Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 1**	**Group 2**	**Group 3**	**Group 4**
		(0 mg/Kg/day)	**(14 mg/Kg/day)**	**(28 mg/Kg/day)**	**(55 mg/Kg/day)**	**(0 mg/Kg/day)**	**(14 mg/Kg/day)**	**(28 mg/Kg/day)**	**(55 mg/Kg/day)**
		N=10	**N=10**	**N=10**	**N=10**	**N=10**	**N=10**	**N=10**	**N=10**
WBC	**MEAN**	11.43	11.84	12.03	11.70	7.19	6.02	6.83	4.54
$10^3/\mu L$	**ST. DEV.**	3.38	1.70	1.88	3.80	3.67	1.93	2.16	2.70
			KW= 0.60 p=0.90; N.S.				**F(34.3)=1.86 p=0.16; N.S.**		
NE	**MEAN**	16.43	14.59	16.07	16.95	30.24	31.03	24.66	24.99
%	**ST. DEV.**	5.07	2.80	3.53	5.88	27.30	15.46	15.28	11.31
			KW=1.47 p=0.69; N.S.				**F(34.3)=0.35 p=0.79; N.S.**		
LY	**MEAN**	71.87	80.02	78.09	74.52	64.73	62.00	68.32	67.00
%	**ST. DEV.**	14.48	4.25	5.78	8.01	26.04	17.24	14.42	12.00
			KW= 5.07 p=0.17; N.S.				**F(34.3)=0.25 p=0.86; N.S.**		
MO	**MEAN**	3.01	1.52	2.87	2.94	1.44	1.07	0.75	1.57
%	**ST. DEV.**	2.24	1.81	2.33	2.16	1.66	0.74	0.77	1.78
			KW=2.71 p=0.44; N.S.				**KW=2.22 p=0.53; N.S.**		
EO	**MEAN**	3.38	3.22	2.03	4.58	3.17	6.33	4.52	6.94
%	**ST. DEV.**	2.33	1.69	0.58	2.88	2.52	4.53	2.90	5.23
			KW=6.70 p=0.08; N.S.				**KW=3.14 p=0.37; N.S.**		
BA	**MEAN**	0.57	0.70	0.95	1.07	0.41	0.54	0.80	0.51
%	**ST. DEV.**	0.50	0.44	1.39	1.09	0.41	0.41	0.65	0.39
			KW=1.42p=0.70; N.S.				**F(34.3)= 1.01 p=0.40; N.S.**		

WBC: total leukocyte count; NE: neutrophil; LY: lymphocyte; MO: monocyte; EO: eosinophil; BA: basophil; F: Statistics ANOVA test; K.W: Kruskal-Wallis Statistic; N.S.: Not Significant.

Table 4

Table 4

CLINICAL BIOCHEMISTRY DATA SUMMARY									
		MALE				FEMALE			
		Group 1 (0 mg/Kg/day) N=10	Group 2 (14 mg/Kg/day) N=10	Group 3 (28 mg/Kg/day) N=10	Group 4 (55 mg/Kg/day) N=10	Group 1 (0 mg/Kg/day) N=10	Group 2 (14 mg/Kg/day) N=10	Group 3 (28 mg/Kg/day) N=10	Group 4 (55 mg/Kg/day) N=10
GLUC mg/dL	MEAN	143.50	146.50	128.40	123.60	125.60	121.60	105.20	104.00
	ST. DEV.	22.73	27.44	26.31	20.30	16.64	17.20	27.59	44.93
			F(36.3)=2.12 p=0.12; N.S.				**KW=4.90 p=0.18; N.S.**		
UREA mg/dl	MEAN	35.22	31.67	33.99	35.37	30.26	31.87	34.90	34.22
	ST. DEV.	4.83	2.95	4.37	3.16	4.63	3.36	7.02	4.14
			F(36.3)=1.92 p=0.14; N.S.				**F(36.3)=1.85 p=0.16; N.S.**		
CREAT mg/dL	MEAN	0.30	0.28	0.29	0.29	0.34	0.35	0.35	0.34
	ST. DEV.	0.04	0.02	0.03	0.03	0.06	0.03	0.07	0.02
			F(36.3)=0.48 p=0.70; N.S.				**F(36.3)=0.26 p=0.86; N.S.**		
BILI-T mg/dL	MEAN	0.21	0.31	0.21	0.22	0.26	0.28	0.25	0.26
	ST. DEV.	0.02	0.32	0.02	0.03	0.03	0.02	0.03	0.01
			KW=1.41 p=0.70; N.S.				**F(36.3)=2.15 p=0.11; N.S.**		
CHOL mg/dL	MEAN	107.50	95.40	93.50	88.50**	104.20	101.80	112.40	99.20
	ST. DEV.	13.83	15.36	10.60	8.50	11.12	20.75	20.58	15.36
			F(36.3)=4.25 p<0.01**				**F(36.3)=1.08 p=0.36; N.S.**		
TRIGL mg/dL	MEAN	156.40	163.40*	131.80	123.00	78.60	104.80	84.40	77.80
	ST. DEV.	33.81	36.16	27.59	20.96	18.87	28.06	21.53	16.94
			KW=10.48 p<0.05*				**F(36.3)=1.85 p=0.16; N.S.**		
AST U/L	MEAN	147.52	145.76	135.39	183.73	264.52	246.91	251.94	264.62
	ST. DEV.	17.74	26.66	17.66	101.03	94.90	92.11	73.01	122.10
			KW=3.44 p=0.33; N.S.				**KW= 0.39 p=0.95; N.S.**		
ALT U/L	MEAN	30.46	38.36	33.42	31.44	38.31	35.40	37.03	35.22
	ST. DEV.	6.26	16.86	6.09	2.42	10.34	17.81	5.26	10.55
			KW=3.12 p=0.38; N.S.				**KW=2.41 p=0.49; N.S.**		
ALKP U/L	MEAN	91.20	98.20	88.30	89.60	62.00	62.90	66.40	60.60
	ST. DEV.	9.62	18.50	26.97	15.21	12.14	9.99	17.22	9.13

		KW=0.56 p=0.65; N.S.				**F(36.3)=0.39 p=0.76; N.S.**			
ALB	MEAN	3.93	3.88	3.98	4.00	4.80	4.59	4.66	4.72
g/dl	ST. DEV.	0.47	0.25	0.32	0.29	0.35	0.34	0.59	0.34
		KW=1.54 p=0.67; N.S.				**F(36.3)=0.47 p=0.70; N.S.**			
TOT PROT	MEAN	6.24	6.26	6.26	6.20	6.46	6.78	6.78	6.54
g/dl	ST. DEV.	0.34	0.48	0.37	0.29	0.44	0.35	0.68	0.51
		F(36.3)=0.06 p=0.98; N.S.				**F(36.3)=1.05 p=0.38; N.S.**			
Na^+	MEAN	134.80	133.70	133.60	132.80	142.60	139.60	140.20	139.40
mmol/L	ST. DEV.	10.40	3.59	3.24	4.08	4.62	7.12	11.64	7.78
		KW=2.00 p=0.57; N.S.				**KW=1.65 p=0.65; N.S.**			
K^+	MEAN	7.87	8.89	7.90	8.38	9.68	12.81	14.93	10.34
mmol/L	ST. DEV.	1.58	1.62	0.77	1.29	6.97	6.18	7.61	5.67
		F(36.3)=1.26 p=0.30; N.S.				**KW=6.35 p=0.10; N.S.**			
Ca^{++}	MEAN	10.87	10.28	10.82	10.51	11.30	11.42	10.76	11.25
mg/dL	ST. DEV.	1.04	0.66	0.64	0.47	1.06	1.26	0.87	1.36
		KW=4.43 p=0.22; N.S.				**KW=2.34 p=0.51; N.S.**			

GLUC: glucose; CREAT: creatinine; Bili-T: Bilirubin, total; CHOL:cholesterol, total; TRIGL: triglycerides; AST:aspartate aminotransferase; ALT: alanine aminotransferase; ALKP: alkaline phosphatase; ALB:albumin; TOT PROT: protein, total; Na^+:sodium; K^+:potassium; Ca++: calcium.

F: Statistics ANOVA test; K.W:Kruskal-WallisStatistic; N.S.: Not Significant

*Significantly different from control. *when p<0.01

**Significantly different from control. **when p<0.01

Table 5

ORGAN WEIGHT DATA SUMMARY											
MALE						FEMALE					
		Group 1 (0 mg/Kg/day) N=10	Group 2 (14 mg/Kg/day) N=10	Group 3 (28 mg/Kg/day) N=10	Group 4 (55 mg/Kg/day) N=10			Group 1 (0 mg/Kg/day) N=10	Group 2 (14 mg/Kg/day) N=10	Group 3 (28 mg/Kg/day) N=10	Group 4 (55 mg/Kg/day) N=10
BODY W.	**MEAN**	633.80	623.10	611.5	625.90	**BODY W.**	**MEAN**	326.80	338.00	321.10	321.30
(g)	**ST. DEV.**	38.25	28.71	26.50	48.14	**(g)**	**ST. DEV.**	11.50	29.08	23.81	18.31
		F(36.3)=0.19 p=0.90; N.S.						**F(36.3)=1.34 p=0.29; N.S.**			
BRAIN	**MEAN**	2.09	2.13	2.12	2.07	**BRAIN**	**MEAN**	1.96	2.03	2.01	2.06
(g)	**ST. DEV.**	0.21	0.14	0.17	0.23	**(g)**	**ST. DEV.**	0.21	0.10	0.12	0.15
		KW=0.09 p=0.99; N.S.						**KW=2.35 p=0.50; N.S.**			
LIVER	**MEAN**	21.06	18.49	18.40	18.41	**LIVER**	**MEAN**	8.41	9.54	8.90	8.48
(g)	**ST. DEV.**	4.86	1.45	1.77	2.40	**(g)**	**ST. DEV.**	1.36	1.09	1.08	0.83
		F(36.3)=1.99 p=0.13; N.S.						**F(36.3)=2.21 p=0.10; N.S.**			
HEART	**MEAN**	2.14	1.98	1.82*	1.99	**HEART**	**MEAN**	1.21	1.28	1.20	1.22
(g)	**ST. DEV.**	0.18	0.19	0.19	0.32	**(g)**	**ST. DEV.**	0.13	0.13	0.14	0.16
		F(36.3)=3.11 *p<0.05						**F(36.3)=0.67 p=0.59; N.S.**			
SPLEEN	**MEAN**	1.17	1.16	1.16	1.45	**SPLEEN**	**MEAN**	0.80	0.86	0.74	0.75
(g)	**ST. DEV.**	0.10	0.15	0.14	0.93	**(g)**	**ST. DEV.**	0.11	0.19	0.07	0.09
		KW=0.2426 p=0.9704; N.S.						**F(36.3)=1.71 p=0.18; N.S.**			
KIDNEYS	**MEAN**	4.05	4.10	3.99	4.06	**KIDNEYS**	**MEAN**	2.13	2.30	2.15	2.06
(g)	**ST. DEV.**	0.30	0.41	0.37	0.28	**(g)**	**ST. DEV.**	0.23	0.23	0.20	0.14
		F(36.3)=0.17 p=0.92; N.S.						**F(36.3)=2.48 p=0.08; N.S.**			
THYMUS	**MEAN**	0.88	0.80	0.83	0.71	**THYMUS**	**MEAN**	0.67	0.70	0.66	0.60
(g)	**ST. DEV.**	0.25	0.12	0.15	0.27	**(g)**	**ST. DEV.**	0.08	0.13	0.20	0.10
		F(36.3)=1.20 p=0.32; N.S.						**F(36.3)=1.02 p=0.40; N.S.**			
TESTES	**MEAN**	3.92	3.95	3.77	3.83	**UTE./CERV.**	**MEAN**	0.87	0.76	1.03	0.94
(g)	**ST. DEV.**	0.44	0.29	0.32	0.28	**(g)**	**ST. DEV.**	0.24	0.17	0.81	0.32
		F(36.3)=0.62 p=0.61; N.S.						**KW=1.32 p=0.73; N.S.**			
EPIDIDIMS	**MEAN**	1.88	2.05	2.36	2.07	**OVARIES**	**MEAN**	0.26	0.31	0.23	0.25
(g)	**ST. DEV.**	0.24	0.52	0.98	0.28	**(g)**	**ST. DEV.**	0.0.8	0.10	0.06	0.07
		KW=4.00 p=0.26; N.S.						**F(36.3)= 1.90 p=0.15; N.S.**			
ADRENALS	**MEAN**	0.11	0.13	0.12	0.12	**ADRENALS**	**MEAN**	0.15	0.12	0.10	0.12
(g)	**ST. DEV.**	0.04	0.05	0.07	0.05	**(g)**	**ST. DEV.**	0.08	0.04	0.02	0.08
		KW=0.72 p=0.87; N.S.						**KW=4.95 p=0.18; N.S.**			

F: Statistics ANOVA test; K.W: Kruskal-Wallis Statistic; N.S.: Not Significant. * Significantly different from group 3 in comparison to group 1 when p<0.05.

Table 6

ORGAN WEIGHT/BODY WEIGHT RATIO DATA SUMMARY											
MALE						FEMALE					
		Group 1 (0 mg/Kg/day) N=10	Group 2 (14 mg/Kg/day) N=10	Group 3 (28 mg/Kg/day) N=10	Group 4 (55 mg/Kg/day) N=10			Group 1 (0 mg/Kg/day) N=10	Group 2 (14 mg/Kg/day) N=10	Group 3 (28 mg/Kg/day) N=10	Group 4 (55 mg/Kg/day) N=10
BRAIN	**MEAN**	0.33	0.34	0.36	0.33	**BRAIN**	**MEAN**	0.60	0.60	0.63	0.64
(%)	**ST. DEV.**	0.03	0.03	0.06	0.04	**(%)**	**ST. DEV.**	0.07	0.05	0.6	0.05
		KW=4.99 p=0.17; N.S.						**F(36.3)=1.17 p=0.34; N.S.**			
LIVER	**MEAN**	3.31	2.97	3.22	2.93	**LIVER**	**MEAN**	2.58	2.82	0.77	2.64
(%)	**ST. DEV.**	0.71	0.15	0.71	0.21	**(%)**	**ST. DEV.**	0.42	0.23	0.27	0.24
		KW=2.03 p=0.57; N.S.						**F(36.3)=1.47 p=0.24; N.S.**			
HEART	**MEAN**	0.33	0.31	0.32	0.32	**HEART**	**MEAN**	0.37	0.38	0.38	0.38
(%)	**ST. DEV.**	0.04	0.02	0.05	0.05	**(%)**	**ST. DEV.**	0.04	0.05	0.06	0.05
		F(36.3)=0.64 p=0.59; N.S.						**KW=1.12 p=0.77; N.S.**			
SPLEEN	**MEAN**	0.18	0.19	0.20	0.23	**SPLEEN**	**MEAN**	0.25	0.25	0.23	0.23
(%)	**ST. DEV.**	0.02	0.02	0.03	0.14	**(%)**	**ST. DEV.**	0.03	0.05	0.02	0.03
		KW=2.15 p=0.54; N.S.						**F(36.3)=0.76 p=0.52; N.S.**			
KIDNEYS	**MEAN**	0.63	0.65	0.70	0.65	**KIDNEYS**	**MEAN**	0.65	0.68	0.67	0.64
(%)	**ST. DEV.**	0.05	0.05	0.15	0.06	**(%)**	**ST. DEV.**	0.07	0.06	0.06	0.06
		KW=1.42 p=0.70; N.S.						**KW=2.53 p=0.47; N.S.**			
THYMUS	**MEAN**	0.14	0.13	0.15	0.11	**THYMUS**	**MEAN**	0.21	0.21	0.21	0.19
(%)	**ST. DEV.**	0.02	0.02	0.05	0.04	**(%)**	**ST. DEV.**	0.03	0.04	0.06	0.03
		KW=4.80 p=0.19; N.S.						**F(36.3)=0.68 p=0.57; N.S.**			
TESTES	**MEAN**	0.62	0.63	0.66	0.61	**UTE./CERV.**	**MEAN**	0.27	0.23	0.32	0.29
(%)	**ST. DEV.**	0.07	0.05	0.14	0.07	**(%)**	**ST. DEV.**	0.07	0.05	0.25	0.10
		KW=1.16 p=0.76; N.S.						**KW=2.47 p=0.48; N.S.**			
EPIDIDIMS	**MEAN**	0.30	0.32	0.41	0.33	**OVARIES**	**MEAN**	0.08	0.09	0.07	0.08
(%)	**ST. DEV.**	0.04	0.08	0.18	0.05	**(%)**	**ST. DEV.**	0.02	0.03	0.02	0.02
		KW=5.544 p=0.1361; N.S.						**KW=3.53 p=0.32; N.S.**			
ADRENALS	**MEAN**	0.02	0.02	0.02	0.02	**ADRENALS**	**MEAN**	0.05	0.04	0.03	0.04
(%)	**ST. DEV.**	0.01	0.01	0.01	0.01	**(%)**	**ST. DEV.**	0.02	0.01	0.01	0.02
		KW=0.45 p=0.93; N.S.						**KW=4.49 p=0.21; N.S.**			

F: Statistics ANOVA test; K.W: Kruskal-Wallis Statistic; N.S.: Not Significant.

Table 7

ORGAN WEIGHT/BRAIN WEIGHT RATIO DATA SUMMARY											
MALE						FEMALE					
		Group 1 (0 mg/Kg/day) N=10	Group 2 (14 mg/Kg/day) N=10	Group 3 (28 mg/Kg/day) N=10	Group 4 (55 mg/Kg/day) N=10			Group 1 (0 mg/Kg/day) N=10	Group 2 (14 mg/Kg/day) N=10	Group 3 (28 mg/Kg/day) N=10	Group 4 (55 mg/Kg/day) N=10
LIVER	**MEAN**	1011.66	874.10	876.04	892.82	**LIVER**	**MEAN**	437.12	470.10	444.59	411.74
(%)	**ST. DEV.**	229.58	103.29	131.13	92.16	**(%)**	**ST. DEV.**	104.65	48.18	56.83	41.47
		KW=4.08 p=0.25; N.S.						**F(36.3)=1.27 p=0.30; N.S.**			
HEART	**MEAN**	103.79	93.72	86.79	97.94	**HEART**	**MEAN**	62.93	63.41	60.00	59.58
(%)	**ST. DEV.**	16.99	12.97	10.89	23.79	**(%)**	**ST. DEV.**	13.12	8.56	8.83	9.81
		KW=6.19 p=0.10; N.S.						**F(36.3)=0.37 p=0.78; N.S.**			
SPLEEN	**MEAN**	56.36	54.78	55.10	55.42	**SPLEEN**	**MEAN**	41.83	42.21	37.10	36.61
(%)	**ST. DEV.**	7.85	6.83	7.14	7.75	**(%)**	**ST. DEV.**	9.96	9.33	3.40	6.17
		F(36.3)=0.084 p=0.97; N.S.						**F(36.3)=1.52 p=0.23; N.S.**			
KIDNEYS	**MEAN**	195.30	193.90	190.21	198.22	**KIDNEYS**	**MEAN**	110.18	113.37	107.39	100.25
(%)	**ST. DEV.**	22.29	27.64	30.06	26.91	**(%)**	**ST. DEV.**	18.71	12.13	13.40	12.68
		F(36.3)=0.15 p=0.93; N.S.						**KW=5.94 p=0.11; N.S.**			
THYMUS	**MEAN**	42.63	37.87	39.25	34.33	**THYMUS**	**MEAN**	34.57	34.51	33.29	28.27
(%)	**ST. DEV.**	13.08	6.61	7.85	12.04	**(%)**	**ST. DEV.**	5.25	5.96	12.14	4.26
		F(36.3)=1.13 p=0.35; N.S.						**KW=5.75 p=0.12; N.S.**			
TESTES	**MEAN**	189.99	187.00	179.45	187.33	**UTE./CERV.**	**MEAN**	44.81	37.40	51.51	46.40
(%)	**ST. DEV.**	33.59	23.50	24.34	28.69	**(%)**	**ST. DEV.**	12.42	8.40	40.13	18.00
		F(36.3)=0.26 p=0.85; N.S.						**KW=2.62 p=0.45; N.S.**			
EPIDIDIMS	**MEAN**	89.92	96.89	112.09	100.46	**OVARIES**	**MEAN**	13.04	15.32	11.31	12.10
(%)	**ST. DEV.**	7.49	24.61	46.97	12.46	**(%)**	**ST. DEV.**	3.734	5.41	3.20	3.43
		KW=3.96 p=0.27; N.S.						**F(36.3)=1.85 p=0.16; N.S.**			
ADRENALS	**MEAN**	5.42	5.40	5.91	5.75	**ADRENALS**	**MEAN**	8.00	6.10	4.82	5.98
(%)	**ST. DEV.**	2.59	2.83	3.99	2.77	**(%)**	**ST. DEV.**	4.87	2.02	1.33	3.97
		KW=0.50 p=0.92; N.S.						**KW=4.27 p=0.23; N.S.**			

F: Statistics ANOVA test; K.W: Kruskal-Wallis Statistic; N.S.: Not Significant.

Figure 1

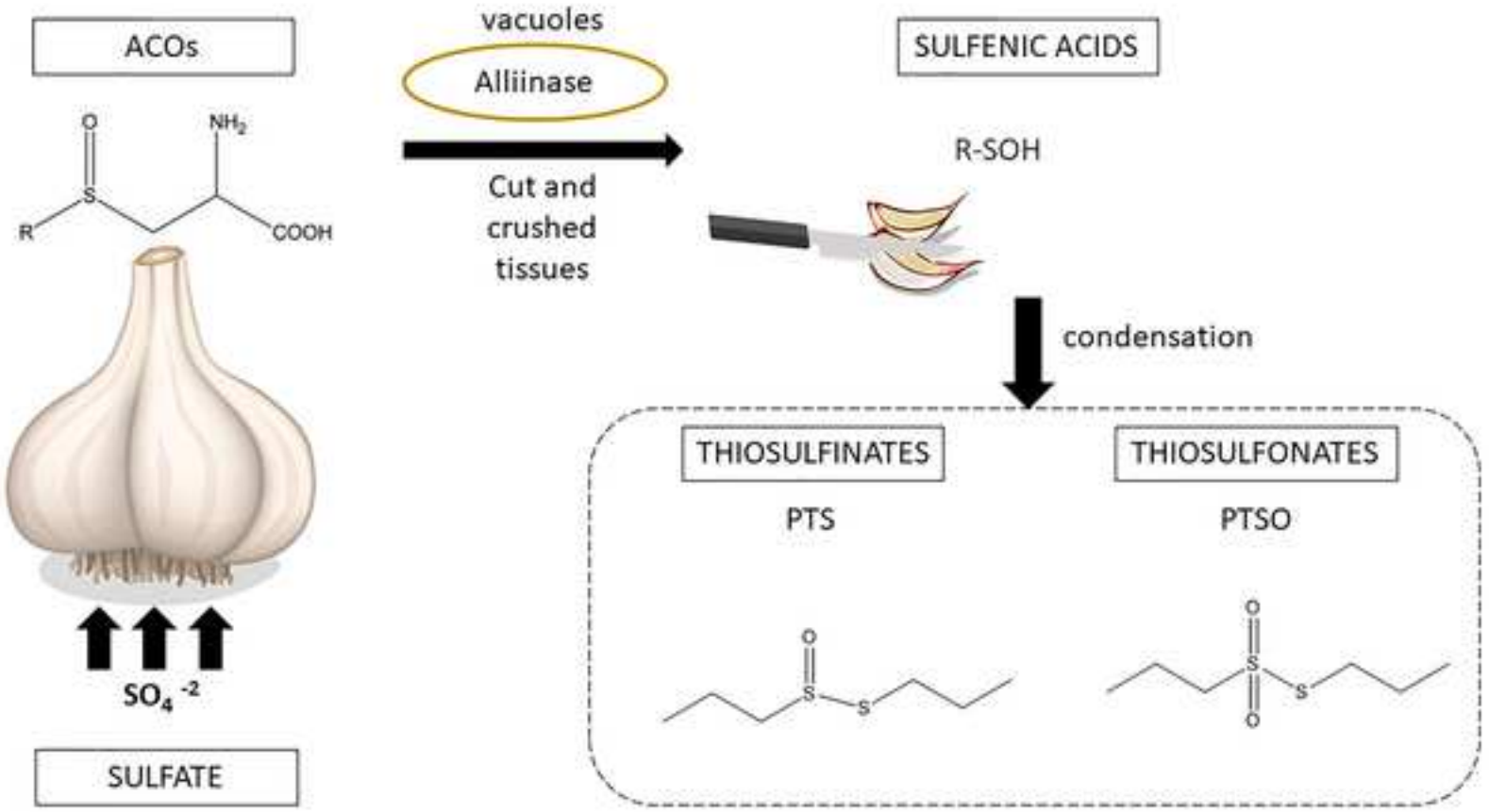


Figure 2

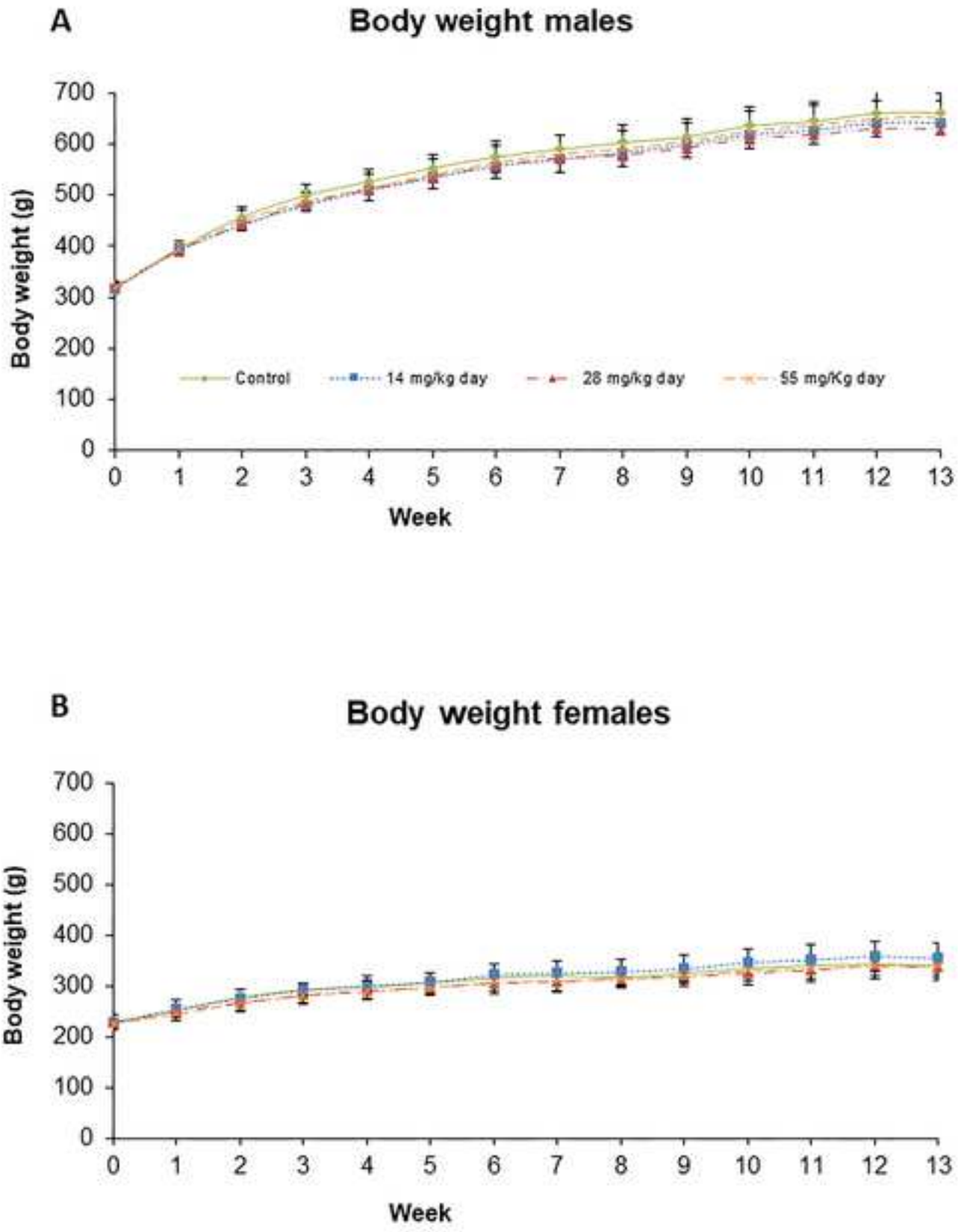
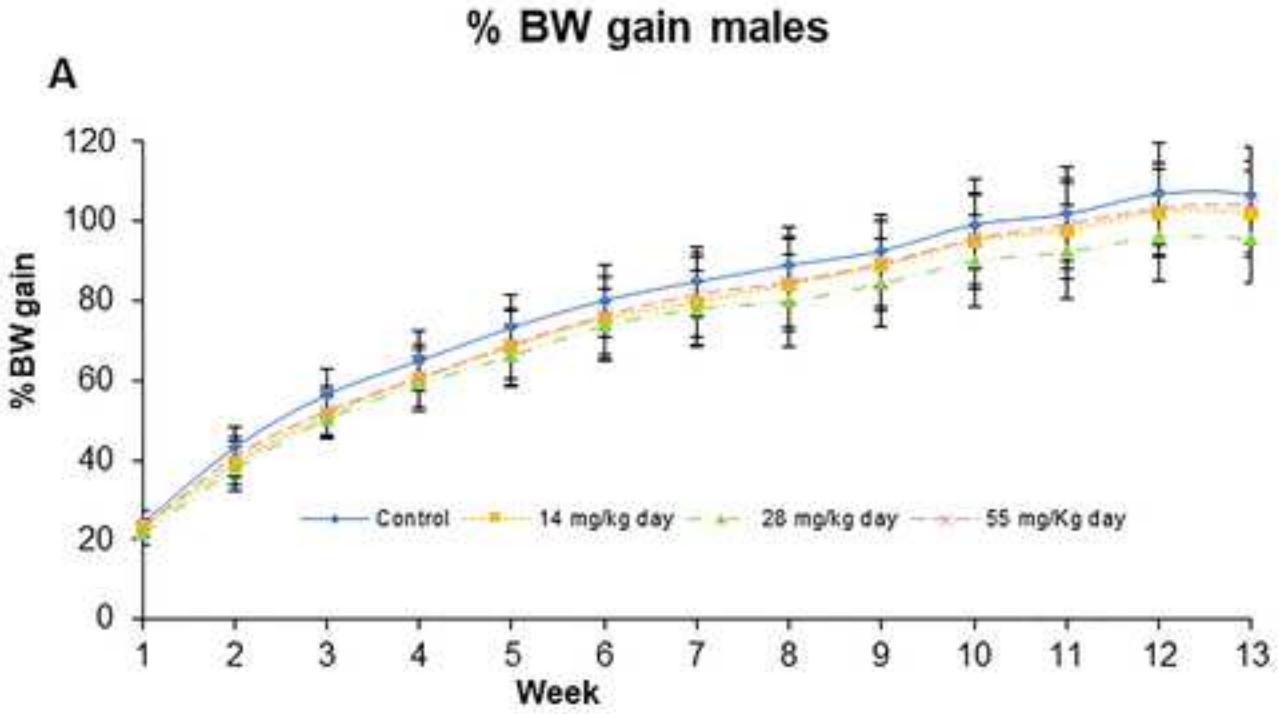


Figure 3

Click here to access/download;Figure;Figure 3. Mean body weight gain.tif



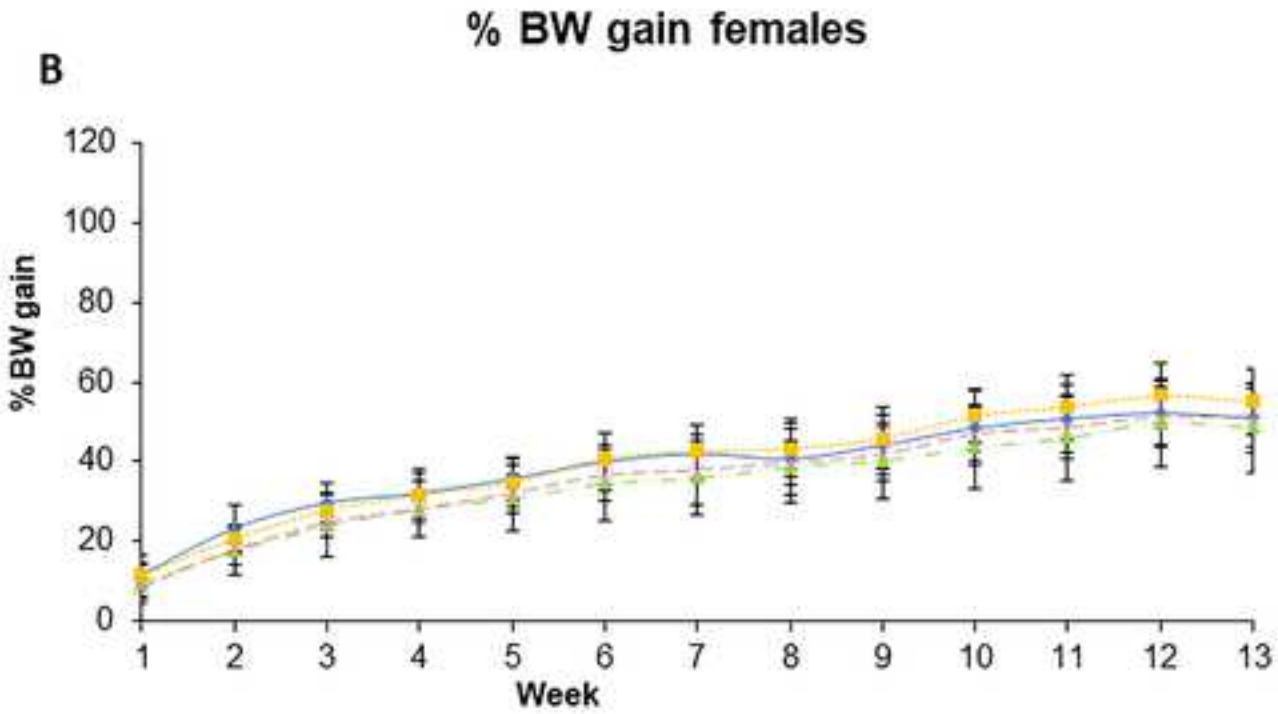


Figure 4

Click here to access/download;Figure;Fig 4 Histopathology.tif