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CELLULAR AND MOLECULAR BIOLOGY

Toxicity of commercial atrazine in rattus novergicus organs as a function of concentration: histopathological, ultrastructural and hematological evaluation

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Abstract: The effect of commercial Atrazine (ATR) on Rattus Novergicus organs was determined for a concentration of c_{ATR} of 1, 3, 13, 30, and 50 ppb. ATR was dissolved in drinking water. The rats were allowed to drink from it ad libitum for an uninterrupted period of 28 days as established by the Office of Environmental Protection protocol under the number EPA OPPTS 870.3050. In the 28 days of the test, data on the behavior of the medicated animals was recorded before the extraction of sample tissues (heart, liver, spleen, brain, and testicles) for histological analysis. A direct correlation of c_{ATR} and organ damage was found. The study showed that even for the smallest doses (1ppb), commercial ATR produced several lesions in the studied animals. The rats showed hepatic periacinar necrosis with acute coagulation, hepatocyte lipidosis, severe portal lymphocytic inflammation, coronary periarteritis, and lymphocytic meningoencephalitis for high concentrations. In the male reproductive system, testicular degeneration with mild acute tubular necrosis was observed.

Key words: Atrazine, microscope alterations, River la Villa, organ damage, herbicide, toxicity.

INTRODUCTION

Atrazine (ATR), 2-chloro-4- (ethylamine) -6- (isopropylamine) -1,3,5-triazine, is a herbicide widely used in agriculture. In plants, their main mode of action is the inhibition of photosynthesis. In animals, it behaves as an endocrine disruptor, inducing mammary tumors. Several other animals have shown that exposure to ATR alters the estrous cycle and plasma levels of sex hormones. For instance, in Iowa, the United States, a community accidentally exposed to ATR dissolved in drinking water was at increased risk of aging and other congenital disabilities (Danzo 1997). The same community was also exposed to 2.2 µg / L of ATR drinking water between 1984 and 1990. The exposure caused delayed uterine development, cardiac and urogenital defects, and in some cases, reduction of extremities and premature births (Munger et al. 1997, Ross & Filipov 2006). In a group of farmers in Ontario, Canada, exposed to ATR, there was no alteration in the sex ratio in the newborn children (Savitz et al. 1999, Stoker 2002). The data in animals suggest that the carcinogenicity of the ATR is specific according to the species, strain, and sex (Black et al. 2010, Song et al. 2014). However, there are still controversies regarding the adverse effects of this herbicide on human and animal health. (Danzo 1997, Denoyelles et al. 1982).

There is little information on the toxicity of inhaled ATR. Only two human studies (no animal studies) have been performed to the best of our knowledge. These ecological studies

examined reproductive and developmental toxicity endpoints in farmers using ATR (Curtis et al. 1999). In both studies, exposure to ATR was poorly characterized; no monitoring data was provided; and the direction probably involved inhalation, oral, and dermal. In couples of farmers exposed to ATR, Savitz reports an increase in delayed intrauterine growth (Savitz et al. 1999). Van Leeuwen et al. indicate that exposure to ATR has been associated with an increased incidence of stomach cancer.

Unfortunately, these studies are inadequate for determining the Maximum Residual Limits (MRL) for this type of exposure. An MRL of 0.01 mg/kg/day has been recorded for oral exposure to ATR with acute duration (14 days or less) in humans. The report only documents the intentional ingestion of a sole human (Pommery et al. 1993). As mentioned earlier, those can explain why ATR has not been classified as a carcinogenic compound for humans and is located in Group 3 of the International Agency for Research on Cancer (IARC).

Studies in animals for acute duration focus their evaluation on damage to the endocrine and reproductive system. Endocrine effects mainly included increased pituitary gland weight and alterations in reproductive hormone levels. An increase in pituitary weight was observed in rats receiving 120 mg/kg/day doses for seven consecutive days (Babić-Gojmerac et al. 1989, Kniewald et al. 2000). ATR also affects the nervous system. In female progenitors of fisher rats, neurobehavioral effects were observed. Their offspring also showed a deficiency in nervous development and behavior (Shafer et al. 1999).

The lowest level of observable adverse effect (LOAEL), identified in the acute toxicity database, is 5 mg/kg/day for maternal toxicity in rabbits that received doses of ATR on gestational days 7-19 (Infurna et al. 1988). They experienced a decrease in body weight gain and food consumption. This study also identified a No Observed Adverse Effect Levels (NOAEL) of 1 mg/kg/day for maternal toxicity.

Other effects were observed in rats and pigs after oral exposure with intermediate duration. Pigs presented degeneration of the myocardial fibers and chronic interstitial hepatitis at a dose of 2 mg/kg/day (Ćurić et al. 1999), as well as lymphoid depletion of lymph nodes and spleen with a dose of 2 mg/kg/day (Ćurić et al. 1999). Lymphopenia was also observed in rats being dosed with 15.4 mg/kg/day (Vial et al. 1996), and a decrease in body weight gain at 2.7 mg/kg/day (Cantemir et al. 1997, Cooper et al. 1996, Goldman et al. 1999). When exposed to 6.9 mg/kg/day of ATR, 18-month-old rats increased estrous time (Wetzel et al. 1994). In addition to the endocrine/reproductive effects, a decrease in body weight was observed in rats exposed to 25 mg/kg/day and higher doses (Greim et al. 2015, Pintér et al. 1990). Acute ingestion of ATR (100 mg/kg/day) also produced subtle changes in the neurological functions of rats (Pommery et al. 1993). No behavioral changes have been observed in rats fed up to 75 mg/kg/day ATR.

Two human studies with chronic exposure to ATR (Munger et al. 1997) described liver damage when exposed to high levels of triazine in drinking water. These studies have limited utility for risk assessment since the concentrations of ATR were not quantified, and the subjects were part of a population exposed to other chemicals in the drinking water.

The association between drinking water contaminated with ATR (average concentration was 162.74 ng / L) and the increased risk of cancer was determined in Ontario, Canada (Van Leeuwen et al. 1999). There is a positive correlation with stomach cancer, with an increased risk of 0.6 cases for men and 1.0 cases for women per 100,000 people per year. Furthermore, a study in Kentucky reported an association between

breast cancer and water contaminated with ATR (Kettles et al. 1997). Only a few epidemiological and environmental exposure studies have been carried out on this herbicide in humans.

The present study is motivated by several reports from La Estrella de Panamá, a local newspaper in Panamá (Dixon 2014), evidencing the uncontrolled use of herbicides in the sugar cane fields in La Villa River (Province of Los Santos, Panamá). La Villa River is the primary source of drinking water for the community. Back then, the Ministry of Health determined that the water in the river had a c_{ATR} =13 μg L^{-1}, which is a factor of ten above the healthy reference levels established by the World Health Organization (2 μg L^{-1}) (*WHO guidelines for drinking- water quality.* 2006).

The objective of this study is **1.** Determine the histological modifications in animal organs subjected to the dose of c_{ATR} =1, 3, 5, 13, 30, and 50 μg L^{-1}; **2.** Assess the blood chemistry of the animals **3.** Determine if ATR affects the behavior of the animals. **4.** Determine whether the ATR concentration found in La Villa River is a potential health risk.

MATERIALS AND METHODS

Ethical statement

The study was carried out according to the procedure stated in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Universidad Central de Venezuela, Department of Veterinary Sciences, June 2018 via an acceptance letter. *(Guid. Care Use Lab. Anim., 2011)*

Chemicals

Commercial liquid Atrazine (2-chloro-4-ethylamino-6-isopropylamine-s-triazine) of a 50%w/w quality was purchased from a local store (Grupo MELO, RUC 650-529-126088-77, Panamá) and transported to Venezuela to be administered to the rats. Via spectroscopy, we found that the substance had other molecules (e.g., metabolites, surfactants), namely: 2-butyl (C_4H_6), 2-Pentenenitrile (C_5H_7N), 3,3'-Oxydianiline ($C_{12}H_{12}N_2O$), Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$),Tridecanoicacid($C_{14}H_{28}O_2$),Hexadecanoic acid ($C_{17}H_{34}O_2$), n-Decanoic acid($C_{10}H_{20}O_2$), alpha.-D-Glucopyranoside ($C_{12}H_{22}O_{11}$), beta.-D-Glucopyranoside ($C_{13}H_{24}O_6$), Methyl 4,6-ethylidene ($C_9H_{16}O_6$), n-Hexadecanoic acid ($C_{16}H_{32}O_2$, 7-Octadecenoic acid ($C_{19}H_{36}O_2$), 2-Ethoxypyridine N-oxide ($C_7H_9NO_2$), 2,5-Dimethoxy-p-cymene ($C_{12}H_{18}O_2$), phosphonochloridothioic acid, 1-piperidinyl-, s-methyl ester ($C_6H_{13}ClNOPS$), N,N'-Dimethyl-decane-1,10-diamine ($C_{12}H_{28}N_2$), 1,11-Undecanediamine ($C_{11}H_{26}N_2$), Diethyl benzamidomalonate ($C_{14}H_{17}NO_5$), Diethyl benzamidomalonate ($C_{14}H_{17}NO_5$), Trimethylsilyl 4-methoxy-2-(2-oxo-2-((trimethylsilyl) oxy)ethoxy)benzoate ($C_{16}H_{26}O_6Si_2$), Tetradecamethylheptasiloxane ($C_{14}H_{44}O_6Si_7$), 3,5'-Dimethyl-4'-nitro-1'H-1,3'-bipyrazole ($C_8H_9N_5O_2$), 1,2-Dimethyl cyclopropene (C_5H_8), Nortriptyline ($C_{19}H_{21}N$), 6-Methyltricosane ($C_{24}H_{50}$), among others (Riera & Molino 2016).

Congo red stain, Trichrome of Gomoril stain, and Nissl stain were used as markers for the tissues. Ether was employed to suffocate the rats (sacrifice by anesthesia suffocation).

Standard LATI pelleted food and water were given ad libitum to the rats.

Animals

Wistar Albina (Rattus Norvegicus) of both sexes weighing 203.4 ± 5.146g, obtained from the Animal Unit of the Universidad Central de Venezuela Bioterium in Maracay, Venezuela, were employed for the study. Following randomization, the 60 animals were placed in their cages.

Devices

To analyze the chemical composition of commercial ATR, an *HP 5973 Gas Chromatography/ Mass Spectrometer System* and an *HP - Agilent 6890 FID TCD* gas chromatographer, was employed. A Sout Voyage Mass balance was used to record the weight of the animals and a Beurer infrared thermometer to measure the temperature of the rats. A Nikon Labophot 2 fluorescence and phase contrast trinocular microscope was employed.

Methods

All 60 rats were randomly divided into separate cages and went through 5 days of acclimatization before starting the trial. Food was provided ad libitum; the condition was artificial lighting and ventilation; the bed was made of rice husk to avoid damaging the legs (it was changed when wet). The temperature of the trial was set at 30° C, and the relative humidity was 65%. The rats were frequently monitored. The test was performed at the Universidad Central de Venezuela Bioterium located in Maracay, Venezuela.

Five trials were performed, each comprised of 10 rats (5 males and five females). Each rat was placed in independent cages and fed with 50g of food concentrate for rodents, and all pens had bottles of 300mL of water. Each group was administered with different concentrations of Atrazine in water, c_{ATR} =1, 3, 5, 13, 30, and 50 μg L^{-1} for 28 days as established by the Office of Environmental Protection (EPA OPPTS 870.3050). The sixth group was a control group fed with 50g of food concentrate for rodents and a bottle of 300mL of water without ATR. Body weights were recorded throughout the trial.

During the administration period, the animals were frequently observed to find signs of toxicity or changes in behavior. After 28 days, they were sacrificed via suffocation (ether anesthesia) as established in the American Veterinary Medical Association. Then a necropsy was performed on the animals. Those animals that died before 28 days were also analyzed.

Samples of testis, liver, spleen, heart, pancreas, and brain were taken, freed of adhering fat, washed free from blood traces, and preserved in 10% formalin. Subsequently, a histopathological examination was performed. We analyzed the lesions to determine the tissue's inflammatory infiltrate, macrophages, edema, angiogenesis, collagen deposition, fibrosis, hemorrhage, and hemosiderin.

Each rat had a different name; the five names employed was Head, Foreleg, Back, Hind Leg, and Tail (those were the part of the bodies where the animal was tagged).

Furthermore, the animals did not show signs of fatigue or sneeze during the manipulation. If they had an injury and were sacrificed, such damage would be evident in the installation of bacterial or viral diseases since the lung is already sensitized.

The data was analyzed using a Statistical Analysis Package, SSPS by IBM. A Kolmogorov-Smirnov test was used to determine whether the collected data had normal blood chemistry distribution.

RESULTS

Metabolism

Table I summarizes the weight evolution for a group of female and male animals administered with different concentrations of c_{ATR}. For a concentration of **1** μg $L^{-1,}$ the necropsy showed no evidence of organic lesions correlated to intoxication with ATR. Notice that, in some cases, the rat's weight is not shown because the rat died during the experiment. Immediately a necropsy was performed, and there was no evidence of death by ATR intoxication nor organic lesions. No correlation between the weight and c_{ATR} was found.

During the experiments, the rats never showed any symptoms of intoxication due to

Table I. Animals weight for both sexes.

		1 µg L^{-1}				3 µg L^{-1}				10 µg L^{-1}				30 µg L^{-1}				50 µg L^{-1}			
		♀			♂	♀			♂	♀			♂	♀			♂	♀			♂
	DAYS	0	7	14	0	0	7	14	0	0	7	14	0	0	7	14	0	0	7	14	0
Head		193	204	218	185	199	200	214	200	200	205	210	207	200	204	215	204	200	205		207
Foreleg		190	185	215	195	204	197	221	195	197	220	209	199	204	185	212	220	207	215	224	204
Back		197	202	224	200	200	207	210	212	203	227	213	200	209	202	219	217	211	219	228	211
Hind leg		200	197	217	205	218	216	230	204	211	215	220	209	211	197	221	212	213	220	232	205
Tail					193	197	210	214	199	207	220	215	197	207	220	215	200	217	223	228	202
					201	216	206	217	199	211	207	211	207	212	210	207	212	212	207	210	212
					216	216	208	217	200	215	205	224	217	218	220	229	233	221	209	222	221

Atrazine, regardless of the concentration. The rats' behavior was normal, they were not aggressive towards the lab technicians nor against other rats. Self-mutilation, nor photophobia, or intolerance to noise was observed. Furthermore, the statistical analysis revealed that the animals did not lose weight, which is a clear indication of the effect of c_{ATR} on the rat's metabolism.

Hematological analysis

The blood chemistry of the animals was analyzed against the standard blood chemistry data for Rattus Novergicus by comparison of the means. As c_{ATR} increases, the concentration of the enzymes in the blood increases as well, which indicates liver damage even for c_{ATR} as low as 1 µgL^{-1}. This implies that for the present study, there is no safe c_{ATR}. Table II shows the reference values compared to those rats in which water had c_{ATR} =1, 3, 5, 13, 30, and 50 µg L^{-1}.

A Kolmogorov-Smirnov test analysis was performed. The results from the test can be found in Table III.

From this analysis, it was concluded the distribution is normal. Figure 1 shows the amount of hemoglobin, triglyceride, Cholesterol, glycemia, uric acid, and TGP in rats as a function of c_{ATR}.

Further study comparing hemoglobin levels as a function of ATR dosages in water shows that increasing c_{ATR} reduces the level of hemoglobin, which indicates a spinal cord depletion.

A similar tendency can be observed for cholesterol and triglyceride levels. Notice that a c_{ATR} < 13 µg L^{-1} in water will not modify the resistance and metabolism of total lipids.

When studying means of blood glucose levels and urea in animals (with 8 hours of fasting before sacrificing them), it was found that even with a c_{ATR} = *1* µg L$^{-1,}$ the behavior of insulin metabolism at the liver level is altered. Likewise, it also modifies the value of protein metabolism (the amount of urea changed).

Table II. Hematology and blood chemistry.

Parameter	Control group	1 µg L^{-1}	3 µg L^{-1}	13 µg L^{-1}	30 µg L^{-1}	50 µg L^{-1}
Red Blood Cells	8020 mm^3	8020 mm^3	8020 mm^3	7500 mm^3	7210 mm^3	6550 mm^3
Hemoglobin	12.54 gr	12.50 gr	12.50 gr	12.00 gr	11.45 gr	11,20 gr
Hematocrit	39.2 %	39.10 %	39.10 %	38.40 %	37.50%	37,10 %
MCHC	31.98 gr dl^{-1}	31.98 gr dl^{-1}	31.80 gr dl^{-1}	30.10 gr dl^{-1}	28.75 gr dl^{-1}	28,40 gr dl^{-1}
Segmented Neutrophils	40 %	40 %	37 %	35 %	34 %	33 %
Lymphocytes	60 %	60 %	63 %	65 %	66 %	67 %
Total bilirubin	0.995 mg dl^{-1}	0.995 mg dl^{-1}	0.995 mg dl^{-1}	0.995 mg dl^{-1}	0.995 mg dl^{-1}	0,995 mg dl^{-1}
Direct bilirubin	0.78 mg dl^{-1}	0.78 mg dl^{-1}	0.78 mg dl^{-1}	0.78 mg dl^{-1}	0.78 mg dl^{-1}	0,78 mg dl^{-1}
Indirect bilirubin	0.33 mg dl^{-1}	0.33 mg dl^{-1}	0.33 mg dl^{-1}	0.33 mg dl^{-1}	0.33 mg dl^{-1}	0,33 mg dl^{-1}
Total protein	8.05 gr dl^{-1}	8.05 gr dl^{-1}	8.05 gr dl^{-1}	8.05 gr dl^{-1}	9.10 gr dl^{-1}	9,25 gr dl^{-1}
Albumin	3.7 gr dl^{-1}	3.7 gr dl^{-1}	3.7 gr dl^{-1}	3.90 gr dl^{-1}	3.90 gr dl^{-1}	4,10 gr dl^{-1}
Globulin	4.3 gr dl^{-1}	4.3 gr dl^{-1}	4.3 gr dl^{-1}	4.80 gr dl^{-1}	4.80 gr dl^{-1}	5,00 gr dl^{-1}
A/G ratio	0.86	0.86	0.86	0.81	0.81	0,86
TGO	24 Ul	24 Ul	24 Ul	27 Ul	29 Ul	35 Ul
Tryglicerides	124 mg dl^{-1}	124 mg dl^{-1}	124 mg dl^{-1}	155 mg dl^{-1}	170 mg dl^{-1}	180 mg dl^{-1}
Cholesterol	129 mg dl^{-1}	129 mg dl^{-1}	129 mg dl^{-1}	145 mg dl^{-1}	155 mg dl^{-1}	165 mg dl^{-1}
Glycemia	142 mg dl^{-1}	142 mg dl^{-1}	142 mg dl^{-1}	160 mg dl^{-1}	165 mg dl^{-1}	160 mg dl^{-1}
Urea	100 mg dl^{-1}	100 mg dl^{-1}	110 mg dl^{-1}	115 mg dl^{-1}	120 mg dl^{-1}	125 mg dl^{-1}
Creatinine	0.94 mg dl^{-1}	0.94 mg dl^{-1}	0.94 mg dl^{-1}	1.10 mg dl^{-1}	1.15 mg dl^{-1}	1,20 mg dl^{-1}
Uric acid	4.0 mg dl^{-1}	4.0 mg dl^{-1}	4.0 mg dl^{-1}	4.15 mg dl^{-1}	4.75 mg dl^{-1}	5,10 mg dl^{-1}
TGP	22 µL	27 µL	28 µL	28 µL	30 µL	35 µL

Table III. Kolmogorov -Smirnoff Test.

		Hb	TG	HDL	Glycemia	UA	TGP
N		**6**	**6**	**6**	**6**	**6**	**6**
	Mean	12.0317	146.17	142.00	151.83	4.3333	28.33
	Stdev	.58789	25.553	15.582	10.926	0.47504	4.227
Máximas diferencias extremas	Absolute	.287	0.307	0.298	0.316	0.317	0.210
	Positive	.194	0.307	0.298	0.316	0.317	0.198
	Negative	-.287	-0.193	-0.202	-0.273	-0.241	-0.210
Test Statistic		.287	-0.307	0.298	0.316	0.317	0.210
Asymptotic significance (bilateral)		.133[a]	-0.080[a]	0.104 [a]	0.062[a]	0.060[a]	0.200[a,b]
Significance		0.080[b]	0.080	0.104[b]	0.062[b]	0.060[b]	0.200

Notes: The test distribution is normal. a. Lilliefors significance correction. b. Asymptotic Significance is observed and above 0.05 (positive correlation between the physical parameter and c_{ATR}).

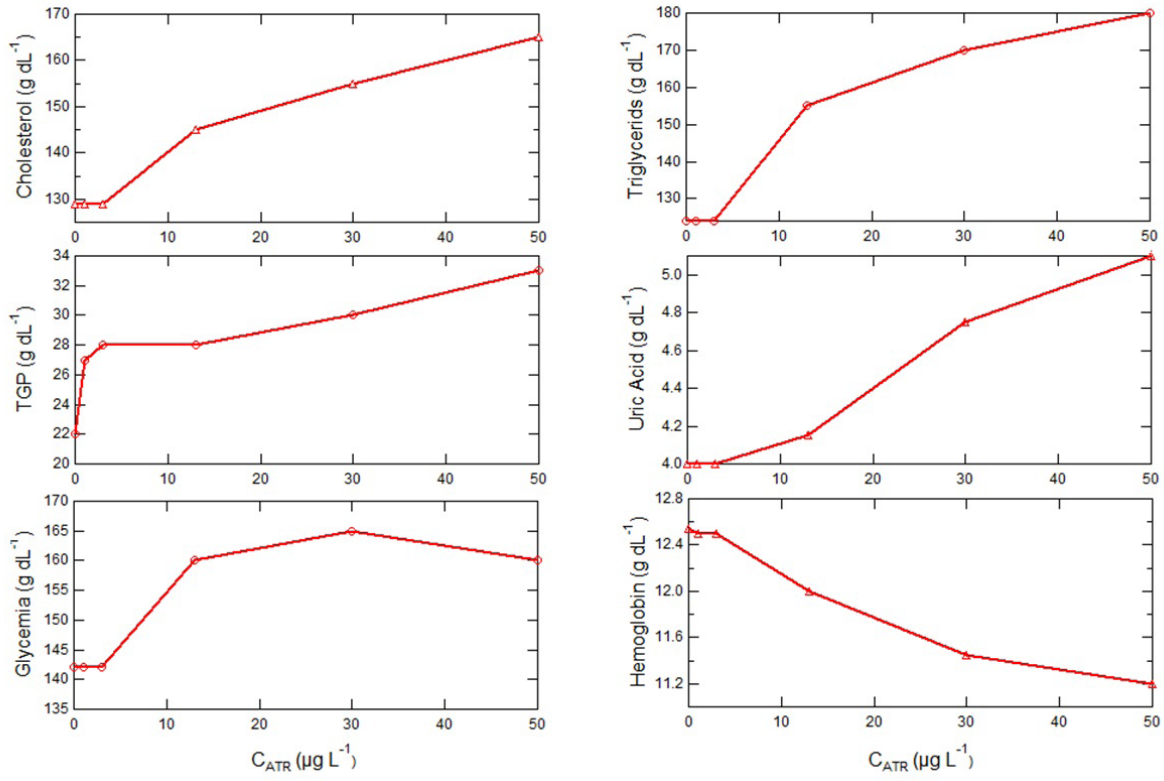

Figure 1. The effect of cATR on several blood chemistry indicators.

Histopathological analysis

A complete histopathological examination of the organs and tissues, preserved in 10% formalin, was performed. All macroscopic and microscopic lesions were examined.

a. 1 µg L^{-1}

The rats at the epicardium level showed mild congestion (Figure 2-b). In the case of the spleen, adequate follicular lymphoid reactivity was observed (Figure 2-c). Figure 2 (a-f) summarizes the results for the given concentration. At this concentration, the animals did not show nervous or erratic symptomology.

b. 3 µg L^{-1}

Lesions were observed in the renal glomeruli, turgidity was found as well. Figure 3(a-c) In the distal renal tubules, there was hydropic degeneration. (Figure 3c).

c. 13 µg L^{-1}

At the central nervous system level, lesions are not yet evident for the cells; in the case of the lungs, the alveoli are preserved. However, the spleen presented follicular lymphoid depletion. The liver showed fatty degeneration. There was damage at the level of the centrilobular vein. At the kidneys, a dramatic decrease in the

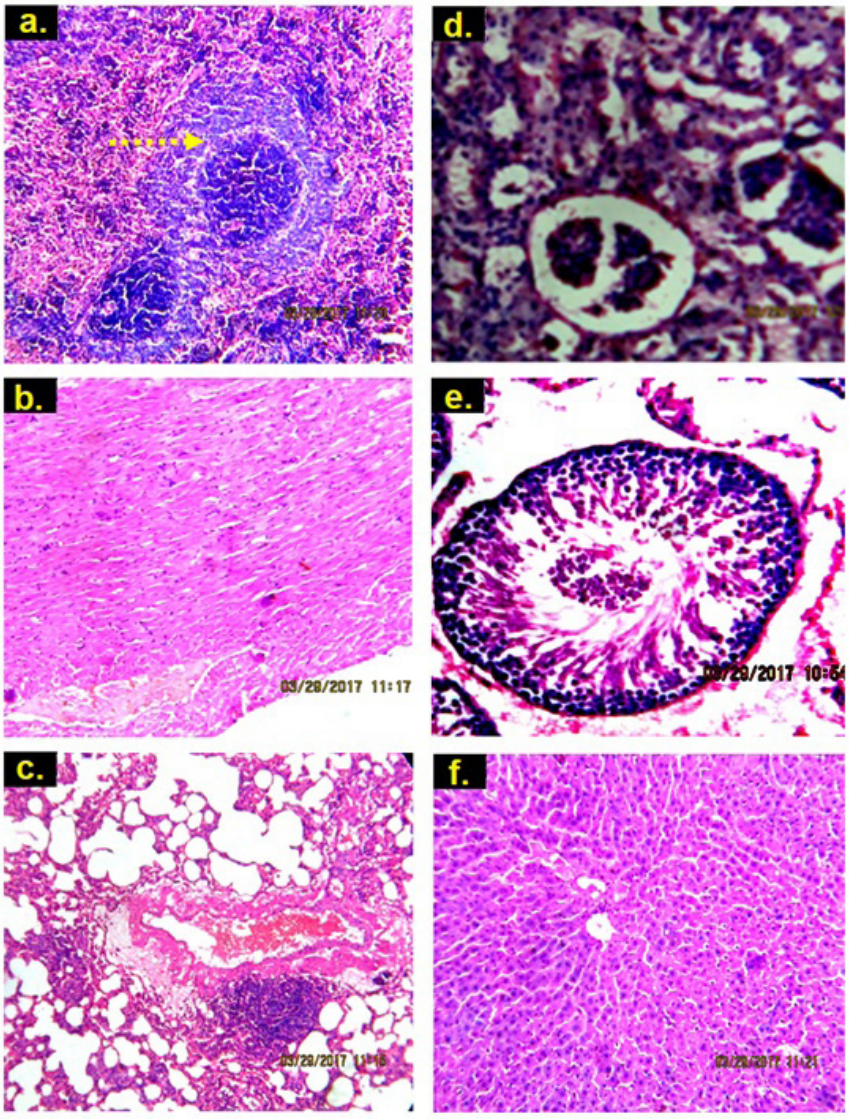

Figure 2. a. Liver with hepatocytes without lesions, b. mild congestion in epicardium c. Perivascular mononuclear focuses. d-e. Kidneys without alterations. f. Conserved architecture.

periglomerular space was observed. The renal tubules showed degeneration of the hydropic type; the renal glomeruli were swollen, the bowman's capsule presented atypical adhesion, the glomerular ball was plethoric. The distal contoured tubule showed mild hydropic degeneration. In the case of the testicles, there was a marked decrease in the germinal epithelium. There was no presence of sperm.

d. 30 µg L^{-1}

At the level of the distal tubules, there is a marked hydropic degeneration, the behavior of naked cells, which have lost the brush border, are now aggravated by the presence of hemorrhage at the level of the renal pelvis, regarding the integrity of the bowman's capsule is presented with abnormal adhesions. At the same time, the glomerular ball appears plethoric. At the level of the distal contoured tubule, the same degeneration of a hydropic type occurs. Finally, at the level of the testicular tissue, there was a dramatic decrease in the germinal epithelium, and the absence of sperm, despite being young animals.

e. 50 µg L^{-1}

The rats present fatty degeneration in the liver, subcapsular hemorrhage, considerable damage at the renal level (hemorrhagic renal

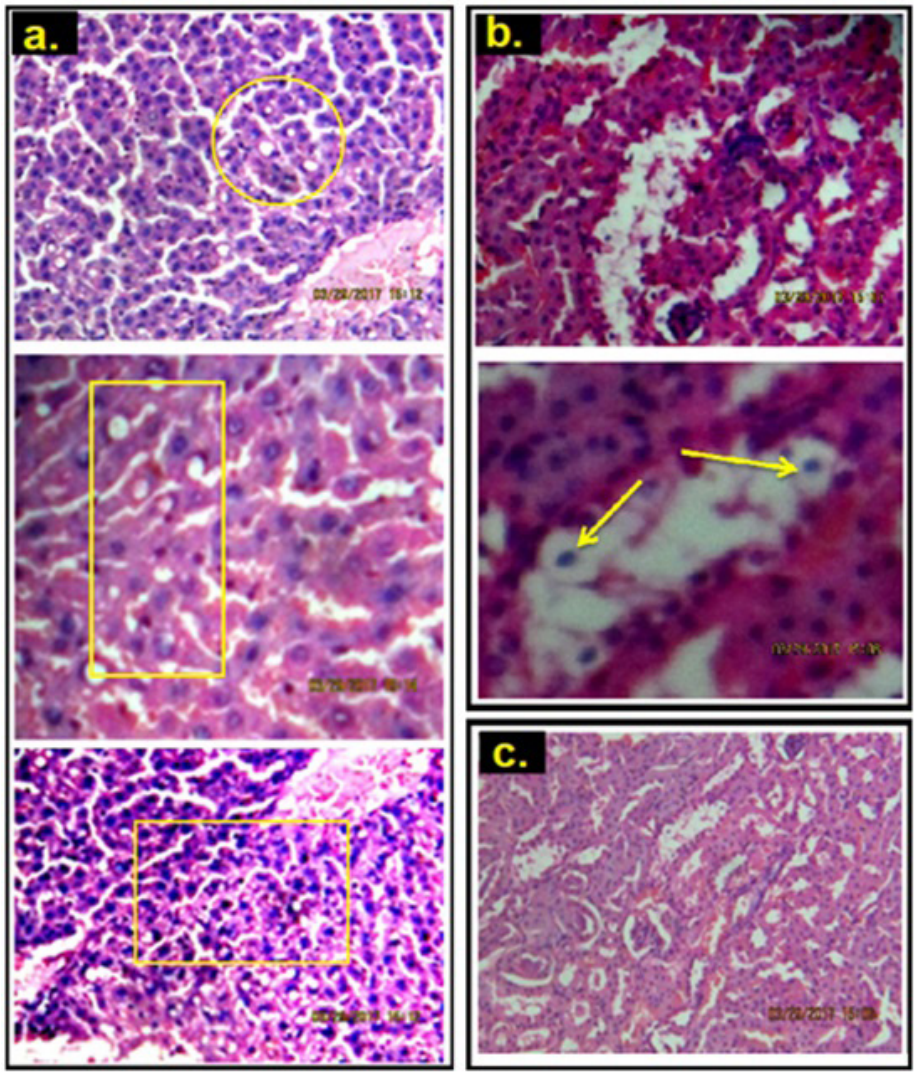

Figure 3. a. Mild fat degeneration of hepatocytes. b. Distal renal tubules with hydropic degeneration c. Mild fat degeneration of hepatocytes.

glomeruli), decrease in periglomerular space, significant damage in the distal contoured tubules, hydropic degeneration, and hemorrhage at the level of the renal pelvis. At the testicles, germinal epithelial necrosis is also evident. Figure 4(a-e) shows the results. It is worth mentioning that the rats drank a little less water at this concentration than the rest. It is possible that at this concentration, the water changed its taste.

CONCLUSIONS

In all chronic toxicity tests carried out for 28 consecutive days in white rats Wistar strains with an average weight of 203.4 ± 5.146 gr, females and males, it is concluded that none of the applied doses is safe. Even in the smallest administered dose, which was 1 μg L^{-1}, it generated biochemical changes of the animals under study compared to the typical values from the control group.

There are significant changes in blood chemistry in triglyceride levels, cholesterol, and glycemia, which indicates that the product directly impacts the liver and its functions.

No clinical symptoms were observed during the trial. The organs had no atypical lesions, friable liver, cystic lesions in the kidneys, or erosive lesions of the intestinal mucosa. Nonetheless, even at c_{ATR} of 1 μg L^{-1}, the micrographs showed mild to moderate coronary periarteritis. As for the spleen, a mild to moderate lymphodepletion was observed. At the level of the testicular tissue, moderate acute testicular degeneration was evidenced, renal tubule degeneration at a mild level is also evident, and the rest of the organs do not present significant lesions. When doing comparative studies with the control group animals, they do not show these lesions in the organs, which indicates that these were not pre-existing conditions typical of a genetic line (degenerating or is in the process of degeneration).

From those above, it can be understood that a c_{ATR} =13 μg L^{-1}, the concentration found in the Rio La Villa (La Villa River) on 28 January 2015, represents a potential health risk for the population. Rats that drank from c_{ATR}=13 μg L^{-1} water showed acinar and periacinar hepatic necrosis. Acute coagulation accompanied by hepatocyte lipidosis was observed as well. Severe lymphocytic portal inflammation was

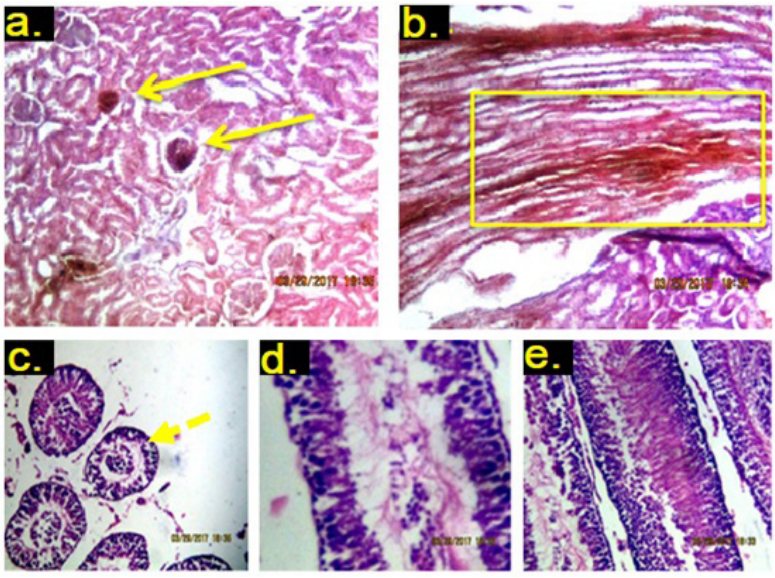

Figure 4. The decrease in periglomerular space. a. Renal tissue hemorrhagic glomeruli, decreased periglomerular space, distal tubules with hydropic degeneration, naked, b. hemorrhage in the renal pelvis. c-e. Testicular tissue necrosis of the germinal epithelium (magnification of 10x, 50x, 100x immersion respectively).

also observed, as well as moderate coronary periarteritis. Mild to moderate lymphocytic encephalitis was also observed. In the case of the male reproductive system, testicular degeneration with moderate acute tubular necrosis was found at this dose. As for the other organs, there were no significant microscopic lesions. In general, as c_{ATR} increases, physical health decreases.

It is essential to mention that none of the animals presented unexpected changes in behavior during daily activities (bed change, tail manipulation, weekly weighing, attacks against operators), which indicates that the used concentrations of Atrazine did not change the behavior. Similarly, no cases of cannibalism nor self-mutilation, nor alteration or erratic behavior in animal groups, were observed. The histopathological studies of nerve tissue showed no damage to the central nervous system's tissues or associated structures.

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JR and JM wrote the first draft, including the conceptualization, data curation, formal analysis, investigation, methodology, project administration, investigation, resources, supervising, validation, visualization. EM, and LM evaluated and analyzed the data. All authors read and approved the final manuscript.