

Evaluation on acute toxicity of tetrabutylammonium bromide ionic liquid at histological structure of some organs in zebrafish (*Danio rerio*)

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Abstract. In this work, we evaluated the acute toxicity caused by zebrafish (*Danio rerio*) exposure to various concentrations of tetrabutylammonium bromide. The ionic liquid's acute toxicity on zebrafish was assessed according to the lethal effects after a 96-hour exposure. To perform the test on tetrabutylammonium bromide's acute toxicity, we carried out three series of experiments; during each series, we used five different ionic liquid concentrations, respectively: 10; 25; 50; 75; 100 mg L⁻¹ for the experiment I, 200; 400; 600; 800; 1000 mg L⁻¹ for the experiment II and 1500; 2000; 3000, 4000 and 5000 mg L⁻¹ for the experiment III. All these batches were compared with the control variant. The histopathological examination was performed on individuals from the control group and on all the dead fish. The results of the acute toxicity test reveal that the LC50 value after 96 hours is between 2500 and 3000 mg L⁻¹. The fish exposed to 4000, respectively 5000 mg L⁻¹ tetrabutylammonium bromide died during the first 24 hours after exposure. The values obtained for tetrabutylammonium bromide are much smaller than the data obtained for LC50 at 96 hours for other solvents like acetone (30642 mg L⁻¹), respectively DMF (12220 mg L⁻¹), but much bigger than the toluene (60 – 313 mg L⁻¹), benzene (203mg L⁻¹), phenol (5 mg L⁻¹), etc. The histopathological study on gill, skin, liver, kidney and intestine reveal that the changes occurred within the structure of these organs are intensified with the increase of tetrabutylammonium bromide concentration.

Key Words: ionic liquids, tetrabutylammonium bromide, *Danio rerio*, acute toxicity, histopathology.

Résumé. Dans cette étude, nous avons évalué la toxicité aiguë causée par l'exposition à poisson zèbre (*Danio rerio*) à différentes concentrations tetrabutylammonium bromure. La toxicité aiguë du liquide ionique sur le poisson zèbre a été évaluée en mesurant les effets létaux de 96 heures après l'exposition. Pour atteindre aiguë bromure de tetrabutylammonium tests de toxicité ont fait trois séries d'expériences de chaque série en utilisant cinq concentrations différentes de chaque liquide ionique, respectivement: 10, 25, 50, 75, 100 mg L⁻¹ pour l'expérience I, 200; 400, 600, 800, 1000 mg L⁻¹ pour l'expérience II et 1500, 2000, 3000, 4000 et 5000 mg L⁻¹ pour l'expérience III. Tous ces groupes ont été comparés au contrôle. Histopatologie a été réalisée sur des individus dans le groupe témoin et tous les poissons morts. Les résultats d'essais de toxicité aiguë montrent que la valeur CL50 pendant 96 heures se situe entre 2500 et 3000 mg L⁻¹. Les poissons exposés à 4000, 5000 mg L⁻¹, respectivement tetrabutylammonium bromure sont morts dans les 24 heures après l'exposition. Les valeurs obtenues pour le tetrabutylammonium bromure sont beaucoup plus faibles par rapport aux données obtenues sur la CL50 à 96 heures pour d'autres solvants comme l'acétone (30642 mg L⁻¹) et du DMF (12220 mg L⁻¹), mais significativement plus élevé par rapport à le toluène (60 à 313 mg L⁻¹), le benzène (203 mg L⁻¹), le phénol (5 mg L⁻¹), etc. Étude histopathologique des branchies, la peau, le foie, les reins et l'intestin a révélé que les changements dans la structure de ces organes augmentent avec l'augmentation de la concentration tetrabutylammonium bromure.

Mots-clés: liquides ioniques, tetrabutylammonium bromure, *Danio rerio*, histopathologie.

Rezumat. În lucrarea de față, noi am evaluat toxicitatea acută cauzată de expunerea peștelui zebură (*Danio rerio*) la diferite concentrații de bromură de tetrabutylammonium. Toxicitatea acută a lichidului ionic asupra peștelui zebură a fost evaluată prin măsurarea efectelor letale ale acestuia după o expunere de 96 ore. Pentru realizarea testului de toxicitate acută a bromurii de tetrabutylammonium am realizat trei serii de experimente, în fiecare serie utilizând câte cinci concentrații diferite ale lichidului ionic, respectiv: 10; 25; 50; 75; 100 mg L⁻¹ pentru experimentul I, 200; 400; 600; 800; 1000 mg L⁻¹ pentru experimentul II

și 1500, 2000; 3000, 4000 și 5000 mg L⁻¹ pentru experimentul III. Toate aceste loturi au fost comparate cu lotul martor. Examenul histopatologic a fost efectuat pe indivizi din lotul control și pe toți peștii morți. Rezultatele testului de toxicitate acută relevă faptul că valoarea LC50 la 96 de ore se situează între 2500 și 3000 mg L⁻¹. Peștii expuși la 4000, respectiv 5000 mg L⁻¹ bromură de tetrabutilamoniu au murit în primele 24 ore după expunere. Valorile obținute pentru bromura de tetrabutilamoniu sunt mult mai mici comparativ cu datele obținute privind LC50 la 96 ore pentru alți solvenți cum ar fi acetona (30642 mg L⁻¹), respectiv DMF (12220 mg L⁻¹), dar mult mai mari comparativ cu toluenul (60 – 313 mg L⁻¹), benzenul (203 mg L⁻¹), fenolul (5 mg L⁻¹) etc. Studiul histopatologic al branhiilor, pielii, ficatului, rinichiului și intestinului relevă faptul că modificările care apar în structura acestor organe se intensifică o dată cu creșterea concentrației bromurii de tetrabutilamoniu.

Cuvinte cheie: lichide ionice, bromură de tetrabutilamoniu, *Danio rerio*, histopatologie.

Introduction. The ionic liquids are organic salts consisted of an organic cation with big molecular mass, like the cations alkyl and aryl – ammonium, imidazolium, pyridinium, piperidinium, pyrrolidone, pyrazolium, phosphonium, sulphonium etc., and an organic or anorganic anion. Different combinations between the multitude of anions and cations lead to a big number of ionic liquids, whose properties are strongly influenced by cation's and anion's nature, by the nature and size of the alkyl chain attached to the cation and also by the size of anion's molecular mass (Brennecke et al 2001; Carda-Broch et al 2003; Gathergood et al 2004). Since their apparition, the ionic liquids have had a constantly increasing influence on organic chemistry, biochemistry and "green chemistry" (environmental chemistry), due to their unique physical-chemical properties expressed through their typical structure (Pernak & Chawala 2003).

During the last years, the ionic liquids (IL), due to their characteristic properties, which cannot be met in other substances, have been more used in chemical processes as new-generation solvents, by replacing the conventional organic solvents with significant emissions of toxic vapours (VOCs). Of all the important properties of ionic liquids, we may mention: insignificant vapour pressure, big thermal stability, big ionic and thermal conductivity, non-inflammability, melting point below 100°C for most ionic liquids and very good solvent properties, for the organic compounds and for the anorganic ones as well (Bernor et al 2005).

They may be widely applied in various chemical industry branches (the industry of paints and plastics, synthesis of solvents and catalysers, metal processing, for nanomaterials and electrolytes for electronic devices etc.); they are more preferred than the traditional solvents like benzene, acetone, toluene etc.

Due to the neglectable vapour pressure (approx. 10⁻¹¹ Pa), the ionic liquids are considered to belong to the category of "green" solvents and they do not participate to air pollution. However, they are water-soluble and may enter the environment through the phreatic water, representing a toxic potential for the aquatic ecosystems (Alfassi et al 2003; Sheldon 2005). Moreover, due to their great stability in water, the ionic liquids may become persistent pollutants in residual waters. Ionic liquids' toxic character requires supplementary eco-toxicological studies in various species, in order to improve the "projection rules" in the synthesis of ionic liquids with minimal toxicity on the environment-integrated organisms. This is the reason why different specialists, at international level, have tried during the last years to test the ionic liquids' toxic potential on different organism and microorganism types. A relatively reduced number of reports on ionic liquids' toxicological properties have been presented so far. For example, Pretti et al (2005) test the aquatic vertebrates' response to the administration of 15 types of ionic liquids with different anions and cations. Bernot (2005) studies the toxic potential of ionic liquids with imidazolic and pyridinic nuclei by using various aquatic organisms as biological samples (*Physa acuta*; *Daphnia magna*; *Vibrio fischeri*; *Lemna minor*; *Pimephales promelas*). Couling et al (2006) determine the relationship between ionic liquids' degree of toxicity and the length of the alkyl chain attached to the imidazolic and pyridinic nuclei and to the quaternary ammonium cation of these acids; they also prove that the toxicity of ionic liquids, in the bacterium *Vibrio fischeri* and in the crustacean *Daphnia magna*, increases gradually with the number of nitrogen atoms present in the aromatic cationic ring. So, the toxicity increases starting from the ionic liquids with ammonium ion > pyridinic nucleus > imidazolic nucleus >

triazolic nucleus > tetrazolic nucleus. Cho et al (2007) assess the toxicity of some ionic liquids with imidazolic nucleus (1-propyl-3-methylimidazol [PMIM] bromide, 1-butyl-3-methylimidazol [BMIM] bromide, 1-hexyl-3-methylimidazol [HMIM] bromide and 1-octyl-3-methylimidazol [OMIM] bromide) on the freshwater alga *Selenastrum capricornutum*. Li Xiao-Lu et al (2007) study the toxic effect of 1-methyl-3-octylimidazol [C₈mim] [Br] bromide on the precocious embryo development in the black-spotted frog *Rana nigromaculata*.

Material and Method. In this work, we evaluated the acute toxicity caused by zebrafish (*Danio rerio*) exposure to different concentrations of tetrabutylammonium bromide. The ionic liquid's acute toxicity on zebrafish was assessed according to the lethal effects occurred after a 96-hour exposure. All tests were performed in concordance with the procedure 7346 of the International Standard Organization. The fish involved were maintained in aquariums with water recomposed according to the NF EN ISO 7346 norms. This was obtained by diluting the following components in demineralised water: CaCl₂ x 2H₂O (294.0 mg L⁻¹); MgSO₄ x 7H₂O (123.3 mg L⁻¹); NaHCO₃ (63.0 mg L⁻¹) and KCl (5.5 mg L⁻¹). The aquariums were endowed with air-conditioning systems, to maintain a constant temperature of 26^oC, with illuminating system, with vibrators to maintain an O₂ concentration of over 60% of air saturation volume and with filtering pumps. Water's physical-chemical parameters (pH, O₂, hardness, conductometry) were daily measured. Fish feeding was performed with the dry feed Tetramin Rpro. The first step of this experiment was represented by the **determination of batch validity criteria** (Meyer et al 1993; Dir. 2001/59/EC). For this, we determined fish mortality for 12 days before the experiment. The mortality was 0%. After these 12 days, we performed the **sensibility test** in all fish batches, with the utilization of potassium dichromate, for 24 hours. We used concentrations of 120, 150, 200, 260, 350, 400 mg L⁻¹, and the potassium dichromate CL50 (24h) was between 150 and 200 mg L⁻¹. To carry out the tetrabutylammonium bromide **acute toxicity test**, we performed a series of three experiments, with five different ionic liquid concentrations in each series: 10; 25; 50; 75; 100 mg L⁻¹ for the experiment I, 200; 400; 600; 800; 1000 mg L⁻¹ for the experiment II and 1500, 2000; 3000, 4000 and 5000 mg L⁻¹ for the experiment III. All these batches were compared with the control batch. For this, we used 9-liter aquariums, and the fish were observed at 1, 12, 24, 48, 72 and 96 hours. The fish were not fed during the test. The acute toxicity was expressed as mean lethal concentration (LC50). The histopathological examination was performed in individuals from the control batch and in all the dead fish. The fish were fixed in neutral formalin 10% and included in paraffin blocks, after a previous dehydration in increasing ethylic alcohol solutions (70^o, 80^o, 90^o, 100^o) and clearance in two benzene washes. Then they were longitudinally laid down in the paraffin blocks, which were successively sectioned with the manual rotating microtome Leica, at the width of 5 μ. After adhesion, the histological sections were stained with the trichromic Mallory method and examined with the research microscope Olympus CX41, endowed with digital photo camera and software for image analysis.

Results and Discussion. After 96 hours, we observed that any of the concentrations used exerted a lethal effect, in the case of the experiments I and II. The results of the acute toxicity test reveal, in the experiment III, that LC50 value at 96 hours was between 2500 and 3000 mg L⁻¹. The fish exposed to 4000, respectively 5000 mg L⁻¹ tetrabutylammonium bromide died during the first 24 hours after exposure. The values obtained for tetrabutylammonium bromide are much smaller than the LC50 values at 96 hours for other solvents like acetone (30642 mg L⁻¹), respectively DMF (12220 mg L⁻¹), and much bigger than for toluene (60 – 313 mg L⁻¹), benzene (203mg L⁻¹), phenol (5 mg L⁻¹) etc. Moreover, in the experiment III, we observed behavioural disorders like balance loss, inordinate swim, long-lasting inertia.

Pretti et al (2006, 2009) test the aquatic vertebrate response to the administration of 15 types of ionic liquids with different anions and cations ([bmim][OTf], [bmim][Tf₂N], [bmim][BF₄], [bmim][N(CN)₂], ECOENG212, AMMOENG 100, [bpyrr][Tf₂N], [emim][OTs], AMMOENG 130, AMMOENG 110, AMMOENG 112, [bmim][OTf],

[BPy][Tf₂N], [bm₂ im][PF₆], [bmim][NO₃]), and compare their toxic effect with the one of the classic organic solvents. Of the 15 ionic liquids tested by Pretti et al (2005), 13 presented LC50 values (96 h) bigger than 100 mg L⁻¹. The highest mortality was observed in the batches treated with ionic liquids with ammonium cation, AMMOENG 100TM and AMMOENG 130TM. The lethal concentrations (LC) were similar for both batches, namely 1.9 – 13.9 mg L⁻¹ for AMMOENG 130TM and 2.2 – 15.4 mg L⁻¹ for AMMOENG 100TM. LC50 was 5.2 and 5.9 mg L⁻¹ for AMMOENG 130TM and respectively AMMOENG 100TM. These values were much smaller than the LC50 values at 96 h of other organic solvents like methanol (12700 – 29400 mg L⁻¹), dichloromethane (>100 mg L⁻¹), acetonitrile (>100 mg L⁻¹), aniline (up to 100 mg L⁻¹) and triethylamine (44 mg L⁻¹), indicating a high lethal potential of the ionic liquids tested on zebrafish populations.

The ionic liquids generate completely different effects on zebrafish, according to their chemical structure. The ones with imidazolic, pyridinic and pyrrolidonic nucleus presented LC>100 mg L⁻¹ so that these ones cannot be considered as presenting very high lethality on zebrafish. On the contrary, AMMOENG 100TM and AMMOENG 130TM presented LC50 values that were remarkably smaller than the ones reported for organic solvents and for tertiary amines; consequently they present high lethal potential for fish.

The microscopic study reveals, in the case of the control batch, the **morphology of gills** consisted of primary and secondary lamellae with a uniform aspect. The secondary lamellae are covered up with a bilayer epithelium.

In the case of the dead fish, taken from the batches exposed to concentrations of over 3000 mg L⁻¹ tetrabutylammonium bromide, we observed the disordered aspect of the secondary lamellae (Fig. 1). The epithelium which covers these lamellae is frequently detached from the basal membrane (Fig. 2) and, on large areas, it presents dystrophies, an aspect suggested by numerous hypertrophies and cell vacuolisations (Fig. 3). Nearby the base of the secondary lamellae, the disepithelisation process is complete (Fig. 4). Within the primary lamellae conjunctiva, we could observe vascular hypertrophies with numerical erythrocyte increase; the secondary lamellae chorion presents frequent lymphocyte infiltrates.

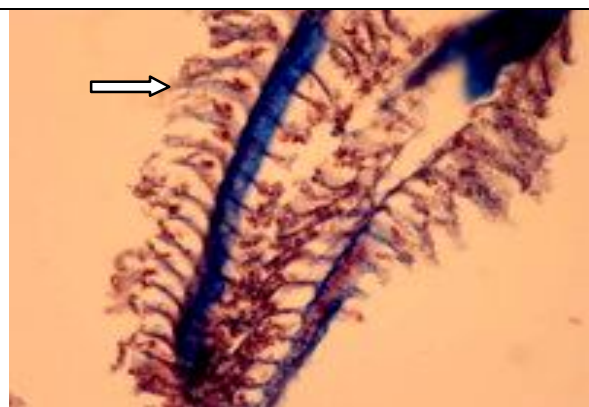


Figure 1. Histological sections of gills at the treated fish. The desorganization aspect of secondary lamellae (trichromic Mallory; 400x)



Figure 2. Histological sections of gills at the treated fish. The epithelium which covers secondary lamellae is frequently detached from the basal membrane (trichromic Mallory; 1000x)

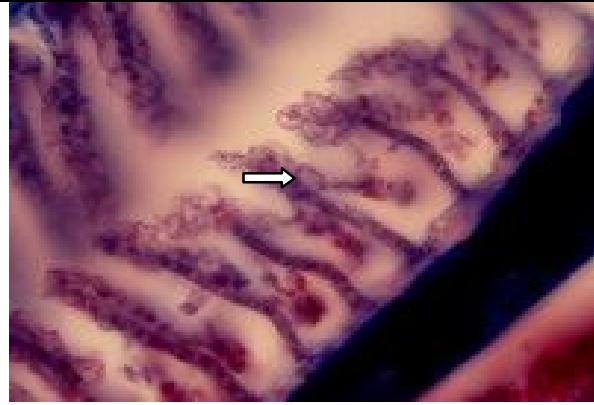


Figure 3. Histological sections of gills at the treated fish. Numerous hypertrophies and cell vacuolisations in the epithelium (trichromic Mallory; 1000x)

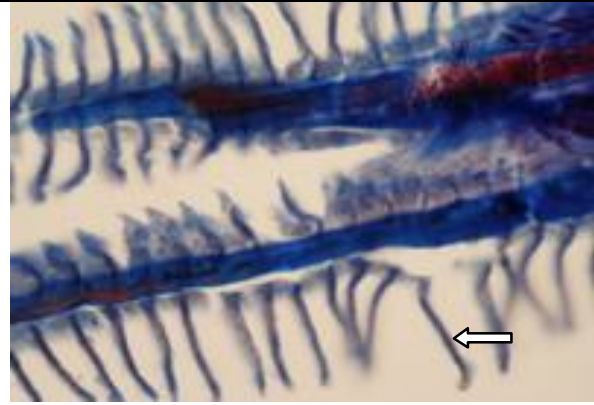


Figure 4. Histological sections of gills at the treated fish. The disepithelisation process is complete in a large number of lamellae (trichromic Mallory; 400x)

The microscopic analysis of **skin** microscopic sections reveals, in the case of the control batch, the presence of epidermis consisted of several cell layers with calciform cells.

The tetrabutylammonium bromide concentrations of 3000 mg L⁻¹ and over determine a series of changes at skin level. On some areas, the epithelial cells within the epidermis structure get overloaded with keratin, they lose their nucleus, become very flat and lose the contacts between them; this last aspect determines cell desquamation. On large areas, we may observe intense erosion processes in the cells from the superficial epidermis layers (Figs 5, 6), turning the epidermis into a very thin one; the predominant cells are the mucus-releasing ones. Intra-epithelial oedema appears on reduced areas.

The histological modifications induced at gills and skin level by big tetrabutylammonium bromide concentrations (3000 – 5000 mg L⁻¹) are similar with the ones mentioned by Pretti et al (2006). The cell degradation induced by ionic liquids at skin level, especially by ammonium salts, alters skin functions by reducing the breathing area, respectively by interrupting external barrier integrity. Also, the changes induced at gill lamellae epithelium level affect fish ability of involvement in gas inter-exchange. The toxic effect exerted by the cationic surfactants suggests that the changes occurred in ionic liquids' cationic part is responsible for the toxic behaviour of these new solvents. This observation is concordant with recent publications dealing with ionic liquid's biodegradability and with their effect on other aquatic trophic levels. The ionic liquid-induced histological changes, especially by the ones with ammonium cation, may be correlated with their well-known surfactant action on membranes, enhancing membrane permeability for the external ions, affecting lipid bilayer's physical properties (Pretti et al 2006).

The microscopic sections through **liver** do not reveal histological changes in the individuals from the control batch and in the individuals exposed to reduced tetrabutylammonium bromide concentrations. On the contrary, in the individuals exposed to tetrabutylammonium bromide concentrations of 3000, respectively 4000 mg L⁻¹, the hepatocytes present intensely granular cytoplasm, disposed in cordons delimited by large areas where the sinusoid capillaries are hypertrophic (Fig. 7); in their lumen, we may observe erythrocyte hyperplasia. At intra-lobular and especially inter-lobular levels, we may observe perivascular fibroses, regions including numerous fibroblasts and collagen fibers (Fig. 8). The hepatic parenchyma contains lymphocyte infiltrative cells and scattering hepatic degenerescences.

In the individuals exposed to the tetrabutylammonium bromide concentration of 5000 mg L⁻¹, the fibrosis processes are much extended and more intense (Figs 9-10), so that the collagen fibers are disposed in a thick layer that surrounds and isolates vessels and cell groups. Hepatocytes are small, with vacuolar cytoplasm disposed in uneven

cordons, separated by large areas. The hepatic necrosis processes extending over large areas (Figs 9-10) and the sinusoid capillaries are hypertrophic, with content.



Figure 5. Histological sections of the skin at the treated fish with 3000 mg L⁻¹ tetrabutylammonium bromide. Erosion of the superficial layers (trichromic HEA; 1000x)

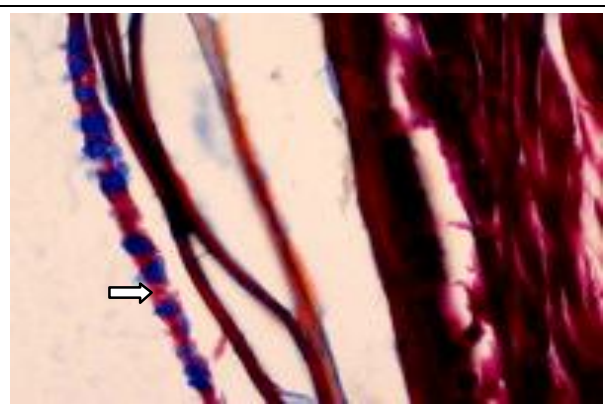


Figure 6. Histological sections of the skin at the treated fish with 4000 mg L⁻¹ tetrabutylammonium bromide. Intense erosion process (trichromic Mallory; 1000x)

Similar results regarding the ionic liquids' toxic effect on liver histological structure are mentioned by Pretti et al (2006), successive to studies performed on zebrafish (*Danio rerio*), respectively by Yu et al (2008) successive to the studies on the toxic effect exerted by 1 octyl - 3 methylimidazolium on hepatic parenchyma in mouse. Recent studies reveal the implication of ionic liquids in enzymatic system inhibition at cell level, especially the activity performed by acetylcholinesterase (Arning et al 2008; Jastorff et al 2005; Matzke et al 2007; Ranke et al 2007; Stasiewicz et al 2008; Stock et al 2004; Torrecilla et al 2009; Zhang & Malhotra 2005), AMP deaminase and by the liver enzymatic antioxidant system (superoxide-dismutase, catalase, glutathione-peroxidase and glutathione S - transferase) (Yu et al 2009).

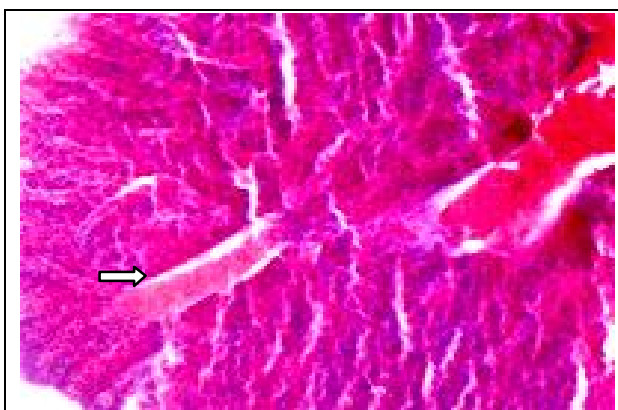


Figure 7. Histological sections of the liver at the treated fish with 4000 mg L⁻¹ tetrabutylammonium bromide. The hepatocytes present intensely granular cytoplasm and the sinusoid capillaries are hypertrophic; in their lumen observed erythrocyte hiperplasia (trichromic Mallory; 400x).

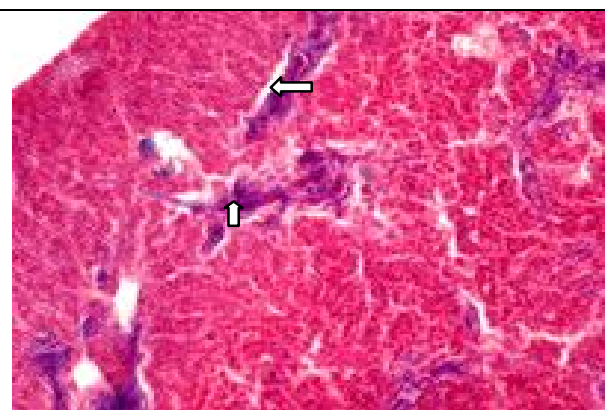


Figure 8. Histological sections of the liver at the treated fish with 4000 mg L⁻¹ tetrabutylammonium bromide - perivascular fibroses (trichromic Mallory; 400x)



Figure 9. Histological sections of the liver at the treated fish with 5000 mg L⁻¹ tetrabutylammonium bromide. Perivascular fibroses and necrotic parenchima (trichromic Mallory; 400x)

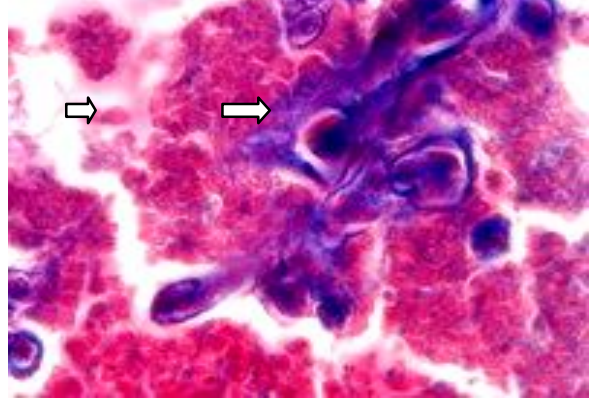


Figure 10. Histological sections of the liver at the treated fish with 5000 mg L⁻¹ tetrabutylammonium bromide. Perivascular fibroses and necrotic parenchima (trichromic Mallory; 1000x)

Within the renal parenchyma, we observed dramatic histological changes in the individuals exposed to tetrabutylammonium bromide concentrations of 3000, 4000, respectively 5000 mg L⁻¹. The renal corpuscles present reduced capsular areas, and the vascular glomerulus seem disordered (Figs 11-12), presenting abundant lymphocyte infiltrates. On large areas, we may observe peritubular oedema (Fig. 11). At uriniferous tubule level, the nephrocytary epithelium is detached from the bazal membrane; at this level, we may observe hyperplasic hypertrophies, so that the tubular lumen is very narrow, being occupied with cells and infiltrative elements. The peritubular capillary network is hypertrophic and the vessel lumen is occupied by numerous erythrocytes (Fig. 9). We may notice frequent extended hemorrhagic regions and abundant leukocyte infiltrates within the interstitial space.

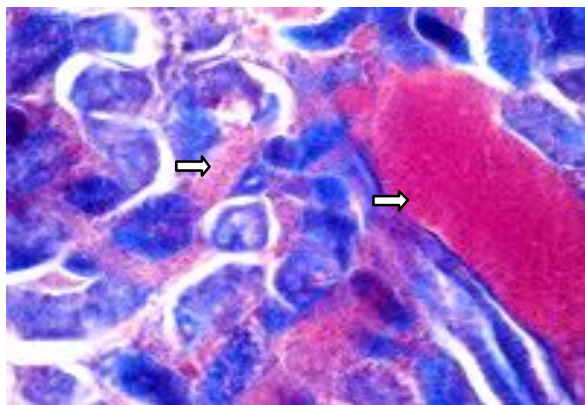


Figure 11. Histological sections of the kidney at the treated fish with 4000 mg L⁻¹ tetrabutylammonium bromide. The peritubular capillary network is hypertrophic and the vessel lumen is occupied by numerous erythrocytes (trichromic Mallory; 400x)

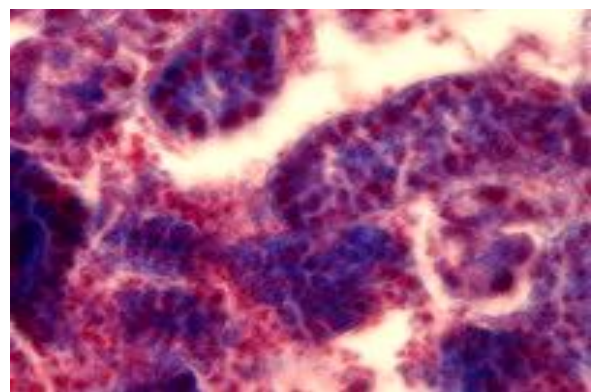


Figure 12. Histological sections of the kidney at the treated fish with 5000 mg L⁻¹ tetrabutylammonium bromide. The infiltrative cells and necrotic parenchima (trichromic Mallory; 1000x)

Zebrafish exposure to tetrabutylammonium bromide concentrations of 3000 and 4000 mg L⁻¹ induces changes at **intestinal** level, as well. In this viewpoint, the histological sections reveal the presence of tall intestinal villusities, coated with a prismatic mono-layered epithelium (Fig. 13), where we may notice a big number of caliciform cells (Fig. 14). At the apical pole, the cells present an evidently ribbed plateau. The villositary chorion is reduced to thin lamellae of lax conjunctival tissue (Fig. 12), where we may observe collagen fibers, capillaries with small lumen and numerous infiltrative cells, predominant in the subepithelial conjunctival tissue (Fig. 14). The basal chorion, actually very reduced, contains periglandular oedema.

In the case of the individuals exposed to a tetrabutylammonium bromide concentration of 5000 mg L⁻¹, the alterations produced at intestinal mucous level are much obvious. These appear as epithelial degenerescence processes (Fig. 15), with profound digestion and absorption alteration.

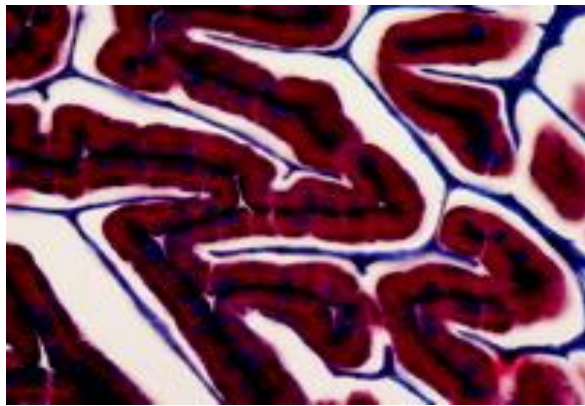


Figure 13. Histological sections of the intestine at the treated fish with 4000 mg L⁻¹ tetrabutylammonium bromide. Intestinal villosities (trichromic Mallory; 400x)

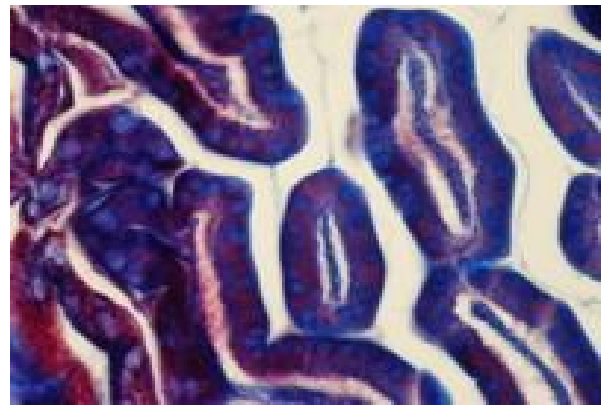


Figure 14. Histological sections of the intestine at the treated fish with 4000 mg L⁻¹ tetrabutylammonium bromide. Infiltrative cells, predominant in the subepithelial conjunctival tissue. (trichromic Mallory; 400x)



Figure 15. Histological sections of the intestine at the treated fish with 5000 mg L⁻¹ tetrabutylammonium bromide. Degenerescence processes (trichromic Mallory; 400x)

Conclusions

1. The LC50 value at 96 hours for tetrabutylammonium bromide is between 2500 and 3000 mg L⁻¹. This is much smaller than the data regarding LC50 at 96 hours for other solvents, like acetone (30642 mg L⁻¹), respectively DMF (12220 mg L⁻¹), but much bigger than toluene (60 – 313 mg L⁻¹), benzene (203 mg L⁻¹) and phenol (5 mg L⁻¹).
2. The high tetrabutylammonium bromide concentrations (3000 – 5000 mg L⁻¹) determine disorganisation at gills secondary lamellae level, desquamations, hypertrophies and cell vacuolisations, with numerical erythrocyte increase.
3. At skin level, the high ionic liquid concentrations determine intense erosion processes in the superficial keratinocytes and, on reduced areas, intraepithelial oedema.
4. The microscopic sections through liver reveal, in the individuals exposed to tetrabutylammonium bromide concentrations of 3000, respectively 4000 mg L⁻¹, hepatocytes with intensely granular cytoplasm, disposed in cordons delimited by large spaces where the sinusoid capillaries are hypertrophic and whose lumen includes erythrocyte hyperplasia, perivascular fibroses, lymphocyte infiltrative cells and hepatocytary degenerescences.
5. In the individuals exposed to the concentration of 5000 mg L⁻¹, the fibroses processes within the liver are much extended and more intense, hepatocytes are smaller, with vacuolar cytoplasm, disposed in uneven cordons separated by large areas.
6. The renal corpuscles present reduced capsular areas, and the vascular glomerulus seem disordered, presenting abundant lymphocyte infiltrates. On large areas, we may observe peritubular oedema, nephrocitary hyperplastic hypertrophies and infiltrative cells. The peritubular capillary network is hypertrophic and the vessel lumen is occupied by numerous erythrocytes. We may notice frequent extended hemorrhagic regions and abundant leukocyte infiltrates within the interstitial space.
7. Zebrafish exposure to tetrabutylammonium bromide concentrations of 3000 and 4000 mg L⁻¹ reveals intestinal periglandular oedema, leukocyte infiltrates and epithelium detachment from the basal membrane, and a concentration of 5000 mg L⁻¹ determines epithelial degenerescence, with profound digestion and absorption alterations.

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