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Contribution to the study of the changes in the gills of zebrafish (*Danio rerio*) when exposed to dioxin

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*This study determined cell changes in the gills of zebrafish (*Danio rerio*, Hamilton, 1822) as a result of a dietary supply of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). 50 zebrafish were divided into five groups, one control group and four experimental ones. The experimental groups were supplied 10, 40, 100 and 270 ng/day/fish TCDD, respectively. Structural, ultrastructural and morphometric studies of the fish gill were carried out, indicating that, in low concentrations, fish gills presented hyperemia with progressive erosion of the endothelium of the capillaries resulting in edema and microhaemorrhages. In the non-respiratory gill, in those groups with low concentrations, it could be observed that the chloride cells presented hypertrophy as a result of dilatation of the intracellular canal and swelling of the microvilli, losing their activity in the degradation of chemical substances (according to some authors) in those groups with a higher concentration. Mucous cells presented hyperplasia and hypertrophy. The sensory buds were not directly affected, but, due to the presence of interstitial edema, swelling of the supporting cells occurred without the involvement of the neurosensory cells. We concluded that dioxins affect the gills to a greater or lesser extent depending on their concentration in the fish's diet, which alters their functionality.*

Key words: PCB, gill, histopathology, ultrastructure, cell morphology

INTRODUCTION

It has been observed that a large number of synthetic chemicals affect biological systems in both animals and humans (COLBORN *et al*., 1993). Following the publication of "Silent Spring" by CARSON (1962), numerous studies have been published, proving the evidence of the impact of these synthetic substances on wildlife (CAMPBELL & HUTCHINSON, 1988; COLBORN *et al.*, 1993; GUILLETTE, 2000; EDWARDS *et al*., 2006).

There are numerous chemicals with effects on those systems among which are the following: Dioxins, derived from the production of chloride and chlorinated compounds such as PVC, organochloride pesticides, bleaching of

paper pulp and waste incineration. One of the dioxins is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). It is made up of two rings linked by two benzyl ethers with four chlorides in positions 2, 3, 7 and 8. These substances are eliminated through river and seawater and tend to precipitate to the bottom. They are then ingested by fish and crustaceans and become deposited in their adipose tissue (bioaccumulation), with TCDD thus being incorporated into the trophic chain.

TANABE (1988) established that fish and other aquatic organisms could play an important role as primary carriers of dioxins and compounds related to human populations in several countries. Therefore, we suggest that there is an existing relationship between the variation of dioxin in the environment and cell changes in gills. However, from their observations on the gill, other authors suggest that its basic functional unit l is the primary filament or lamella, which supports rows of secondary lamellae in the form of a plate (WILSON & LAURENT, 2002; TORRES *et al.*, 2010). Several authors make both histological and functional description of the primary lamella or filament and secondary lamellae (FERNANDES & PERNA-MARTINS, 2001; WILSON & LAURENT, 2002; EVANS *et al.*, 2005). These descriptions indicate that the epithelium of the filament is non- respiratory, and that of the secondary lamella is structurally adapted to gas exchange (FERNANDES & PERNA-MARTINS, 2001).

In other studies, both mitochondria-rich cells (also known as chloride cells in teleost fish) and mucous cells are described both in their morphometry and in their distribution within the gill (FERNANDES & PERNA-MARTINS, 2001; EIRAS-STOFELLA & FRANK-DE-CARVALHO, 2002). Other studies compare chloride cells to those in the yolk sac and in the skin (KATOH *et al.*, 2000). Some studies on different fish species have investigated the variation in number and location of chloride cells within the gill, depending on environmental water conditions, i.e. both its salinity and oxygen concentration (FERNANDES & PERNA-MARTINS, 2001; ERKMEN & KOLANKAYA, 2009). Chloride cells are believed to play a secretory role of NaCl in teleost seawater. Chloride cells can also be found in freshwater teleosts, but these are characterized by different forms and functions from those found in teleost seawater (EVANS *et al.,* 2005).

In the literature, countless articles refer to the pollution levels of different substances in the aquatic environment. Similarly, some of them refer to the involvement of fish or other aquatic animals (LOONEN *et al.*, 1991), but only a few articles of this kind are focused from a histopathological point of view (HILL *et al.*, 2003). In fact, we have not found any works dealing with the histopathological study of TCDD in fish gills in both the respiratory lamellae and the non-respiratory filament. Based on experiments with organophosphorus pesticides (methyl parathion) in teleosts, it has been observed that the effect on the gill epithelium was drastic: with structural changes in the gill lamellar organization, epithelial detachment, necrosis, hyperplasia, loss of microridges and changes in their cell morphology being noted (MANCHADO & FANTA, 2003).

Our main goal was to prove whether, the same as with other chemical substances in teleost gill tissue of both filaments and lamellae, changes were produced by varying the concentration of TCDD mixed with food. Furthermore, we aimed to verify whether the non-respiratory gill tissue of teleost fish was affected in the same way as the respiratory tissue.

Our study was based on the hypothesis that an increase in the concentration of 2,3,7,8 -tetrachlorodibenzo-p-dioxin mixed with food affected the respiratory and non-respiratory tissues of the gills.

MATERIAL AND METHODS

In this study, 50 adult individuals of zebrafish (*Danio rerio*, Hamilton, 1822) were used. Size and weight of the 50 fish were within the general pattern of the individuals. The 50 fish used for the study were all in good health and free from malformations.

The experimental phase of this study was conducted in the facilities of the Central Experimental Animal Service, which is located in the Rabanales Campus at the University of Córdoba. The Central Experimental Animal Service is registered as a breeding facility, supplier and

user of animals for experimentation and other scientific purposes, complying with the Spanish Royal-Decree Law 199/2005 of 20 September.

The whole experimental phase was conducted according to both, the Spanish Royal-Decree Law 1201/2005 of 10 October on the protection of animals used for experimental or other scientific purposes, and the Directive 2010/63/UE of the European Parliament and of the Council of September 22, 2010, on the same theme.

The fish underwent a period of two-week acclimation in aquariums under the following conditions: pH 7-8, water temperature 26 ± 1 º C, and water hardness 50-250 mg $CaCO_3$ / l. The fish were subjected to photoperiod treatments of 16 hours of light and 8 hours of darkness. They were fed twice a day, in the morning and in the evening. The diet consisted of Supervit Mini Granulat®, a multi-ingredient flake food containing fish and fish derivatives, mollusks and crustaceans, meat and animal derivatives, dried yeast, algae, vegetable oil and fats, mineral substances, vitamin A6 500 IU / kg, vitamin E 190 mg/kg, 40% crude protein, 8.5% crude fat, 3.5% crude fiber, 10.5% crude ash and 8% moisture. After the completion of this two-week phase, the fish underwent a pre-exposure three-week phase. During this time, they were fed *Artemia nauplii* and trout feed daily 2,3,7,8 - tetrachlorodibenzo-p-dioxin, previously diluted, was mixed with the food and fed to the fish by evaporating the acetone from the mixture, following the protocol designed by FERNANDEZ *et al.* (1998) and adding some modifications. The fish were fed this compound during a period of three weeks, in accordance with the OCDE Test No. 230.

The experiments were carried out in two different phases: pre-exposure and exposure. At the beginning of the experiments, we used 10 adult specimens of zebrafish in every exposure group (control, 1, 2, 3, and 4). During the exposure phase, the fish were fed (all together) with trout food. The trout food contained, following the order of the above-mentioned groups: 0 (only diluted in acetone), 10, 40, 100, and 270 ng of [^{3}H] - TCDD/g of food (ppb) and *artemia nauplii*. During a period of three weeks, the fish were fed until satiated. Depending on the mean amount of food consumed, the groups from 1 to 4 were given an estimated dosage of 0.08, 0.32, 0.80, and 2.16 ng TCDD/fish/day, respectively. In the course of the experiment, fish food intake was not controlled. However, the fish did not show any significant changes in weight (within 2 standard deviations from the average), suggesting that every individual received a similar dose in each treatment group.

The fish were randomly placed in 25-liter fish tanks, divided into 5 groups of 10 specimens. In order to prevent stress or other behavioral changes in them, the water conditions in the fish tank were the same as those of the water used during the acclimation phase.

Every fish tank used for this research had an internal filter (Eheim PickUp 2006-02®), which, by means of a machine, sucked up the water and carried it through a sponge filter. The clean water was then returned to the fish tank, by an adjustable diffuser. In addition, in order to control its temperature, every fish tank had a glass thermometer with a suction pad (Ica ka 21®). It also had an aerator (Sonic silent powerful 9905), which produced machine-made bubbles and distributed the oxygen uniformly, improving the fish's oxygen intake and gas exchange.

After 3 weeks of exposure, the fifty specimens of zebrafish were euthanized, following the recommendations of the Federation of European Laboratory Animal Science Associations (FELASA). This was done with a solution of the anesthetic MS-222® (Tricaine methanesulfonate, CAS: 886-86-2) at a concentration of 100-500 mg/l and sodium bicarbonate at a concentration of 300 mg/l. The latter was used to reduce the irritation of mucous membranes and possible tissue damage from the MS-222®. Right after the euthanasia, the fish were weighed and - measured. They were then immediately dissected and their gills were removed for histopathological and morphometric examinations.

Histopathological structural and ultra-structural examination

Some portions of the removed gills were analyzed under an optical microscope for a histopathological examination. Some other por-

tions underwent electron microscope examination, including transmission, scanning and morphometric analysis.

In order to carry out the optical microscope examination, the gill samples were fixed in a 10% buffered formaldehyde solution at room temperature. The gills were dehydrated in an ascendant series of ethanol and preserved in paraffin wax.

The first sections of each block (4 μm) were stained with hematoxylin/eosin for morphometric and histological study. The sections were observed and photographed by a Leitz Ortholux photomicroscope.

In the ultrastructural examination, both transmission electron microscope (TEM) and scanning electron microscope (SEM) techniques were used. In both cases, gill samples were fixed in 2% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.4) solution at 4 ºC. The gill samples remained in this solution until the following day, when they were post-fixed in osmium tetroxide, in a 0.1 M phosphate buffer (pH 7.4) solution for 3 minutes. After the dehydration of the samples in an ascendant alcohol series, the samples were included in Araldite. Thin and semi-thin cuts were obtained by the ultramicrotome LKB. The semi-thin cuts were stained with blue toluidine, whereas the ultra thin cuts were double stained with uranyl acetate and lead citrate. The cuts used for the ultrastructural examination were analyzed and photographed with a scanning electron microscope Jeol JSM 6300.

Quantitative assessment

The quantitative assessment was made by an image analysis system, which consisted of a trinocular Leitz Ortholux microscope connected to an image digitizing software by means of a SONY SSC-C370P® video camera. The Visilog 5® software was employed.

The histological cuts of every specimen were systematically sampled to select the microscopic images, which were digitized with 10x (N.A. 0.25) and 40x (N.A. 0.70) zooms. In every section, an average of 7 fields was taken. A total of 28 fields were taken, since the average number of sections per animal was 4. Both the number of chloride cells and their average diameter were quantified.

Statistical analysis

In order to establish the effects of TCDD on the exposed groups, data were analyzed by means of the statistical analysis software Statgraphic® (Centurion XVI®).

The Analysis of Variance Test F (ANOVA) was used to determine whether there were any significant value differences. Subsequently, the Scheffe multiple comparison test was used to compare the different groups. The results were expressed as mean ± standard deviation (SD) and $p < 0.05$ was considered to be significant.

RESULTS

Histopathological study
Lamellae

In the lamellae of groups 1, 2 and 3, all kinds of cell types corresponding to these components could be identified, such as epithelial, pillar, endothelial, chloride and mucous cells (Figs. 1C and 1E). However, some cell types in the group 3 were in the necrosis phase and many of them were becoming detached, especially the epithelial lining cells (Fig. 2D). In the group 4, the one with the highest concentration of TCDD, a destructuring of the lamellae, and even their partial destruction both on the basal and the apical sides, could be observed. In this group, every cell component showed degenerative or even necrotic phases (Fig. 1E). The highest degree of alteration in the lamellae took place at the capillary level, where a small dilatation with small basal hyperemia could be seen, generating a certain level of edema in the group 1 (Fig. 1B). The alteration degree was higher in the group 2, where a generalized hyperemia, both in big and small capillaries, presenting edema and microhemorrhages, could be observed. Due to these hyperemic phases, some bulges in the apical sides of the lamellae (Fig. 1C) were detected. Compared to the other groups, the morphology

of the lamellae in the group 2 changed drastically, since more serious vascular alterations were noted. The capillaries in this group were hyperemic, but there was no alteration in the general morphology on the basal side of the lamellae. On the apical side, the hyperemia appeared in such a way that the capillaries formed large hyperemic bags, which modified its morphology, with bulbous protuberances (Fig. 1D). Group 4 showed the greatest alteration, as the blood vessels were severely affected. In this group, not only the capillaries showed hyperemia, but also the artery and vein terminations. The most intense hyperemia could be found on the apical side of the lamellae (Fig. 1E), with hemorrhagic regions on the basal sides (Fig. 1E). Similarly, the whole connective tissue showed a severe edema. With the scanning electron microscope (SEM), it was observed that the surface of the lamellae was swollen in all the groups. The loss of the outline was more significant in groups 3 and 4, or even the loss of surface structures in the last group. Analyzing the groups in order, an increase in the cell components was noted. Although there were only some small flanges on the surface of the

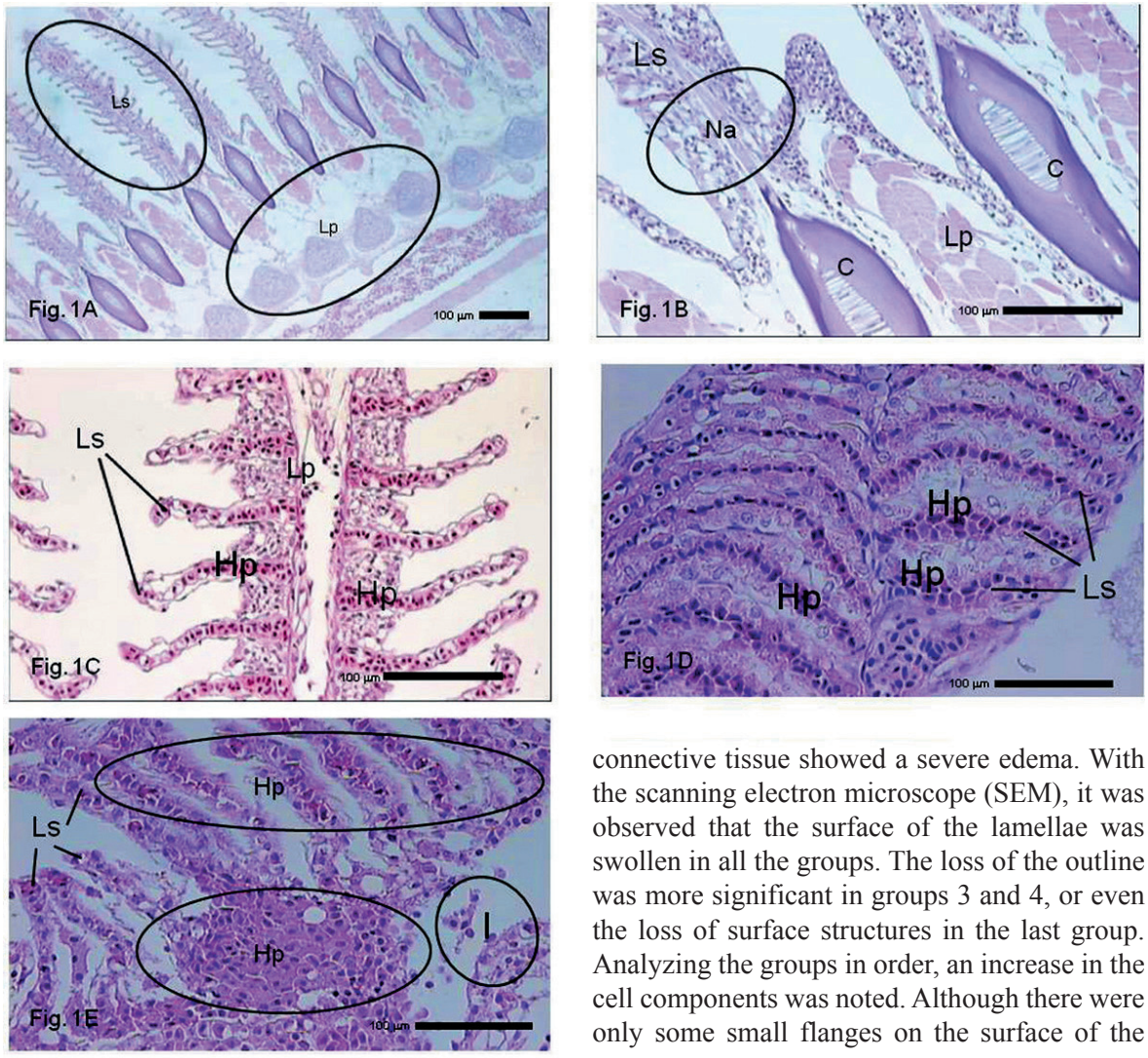
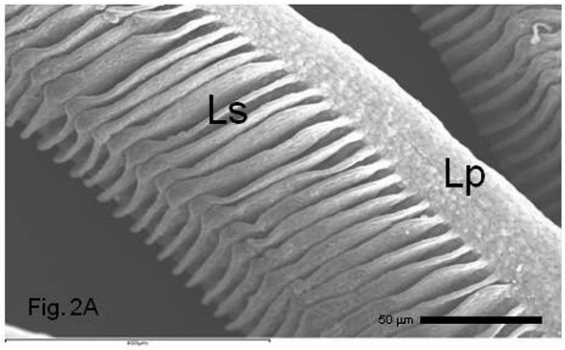
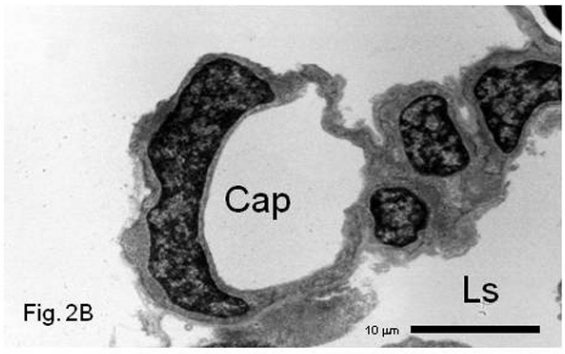
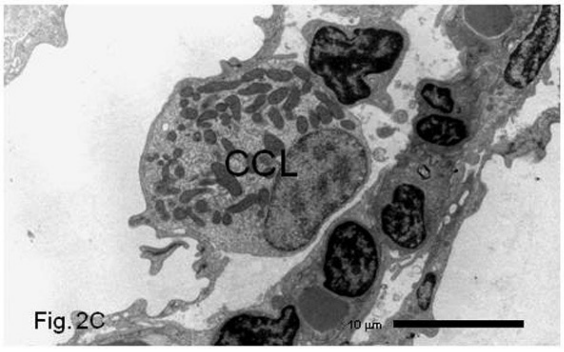


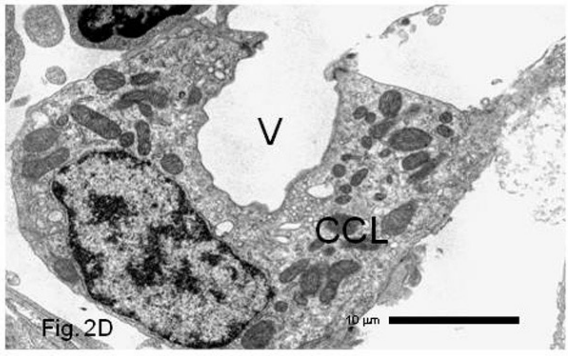
Fig.1. An image cuts of respiratory gills or lamellae optical microscope (OM). In the lamellae of groups 1, 2 and 3 all kinds of cell types corresponding to these components could be identified, such as: epithelial, pillar, endothelial, chloride and mucous cells. 1A) Lot control primary lamellae or filaments (Lp) and secondary lamellae or lamellae (Ls). 1B) Lot 1, lamella with the cartilage and the birth of the secondary lamella (Na). 1C) Lot 2, Image gills, showing details of the respiratory lamellae (secondary lamellae) with hyperemic processes. 1D) Lot 3, detail of gills, where arranged as a mosaic and very hyperemic secondary lamellae are appreciated. Some cell types in group 3 were in the necrosis phase. 1E) Lot 4, detail of secondary lamellae presenting high hyperemia and inflammatory processes. In this group, every cell component showed degenerative or even necrotic phases. Lp: primary lamella or filament; Ls: secondary lamella or lamella; c: cartilage; Na: basal part of middle lamella; Hp: hyperemic processes; I: inflammatory processes.

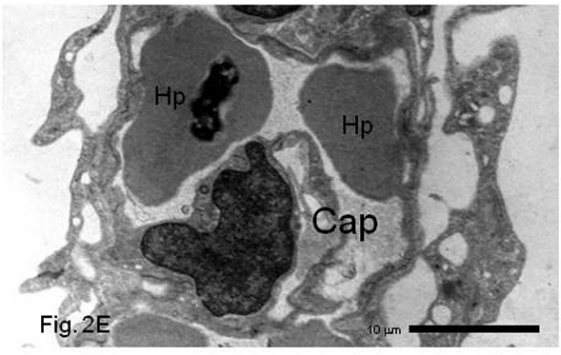
first group, on examining the other groups, some microfilaments of a vascular origin, adhering to the surface of the lining cells, were noticed. Simultaneously, it was on the basal sides of the lamellae of groups 3 and 4 where most of the cell detachment could be found, especially vascular infiltration, with a predominance of red blood cells. In addition, there were wider regions of cellular exudates and red blood cell extravasation in the group 4. Regarding the vascular alteration, it was noted that this consisted of both vascular disorganization on the basal side of the lamellae, including a detachment of apical cells, microhemorrhages, and large edema and hemorrhage regions gradually appeared as we advanced in our examination of the different groups. The scanning transmission microscope confirmed the morphology previously described







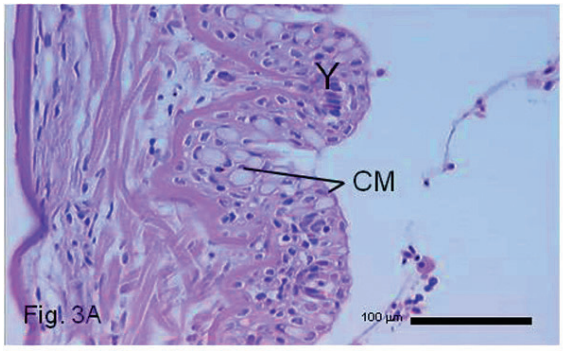


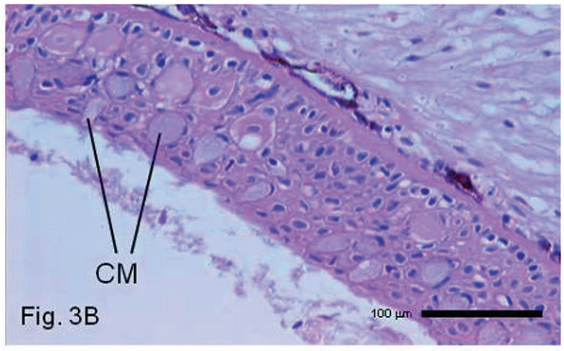


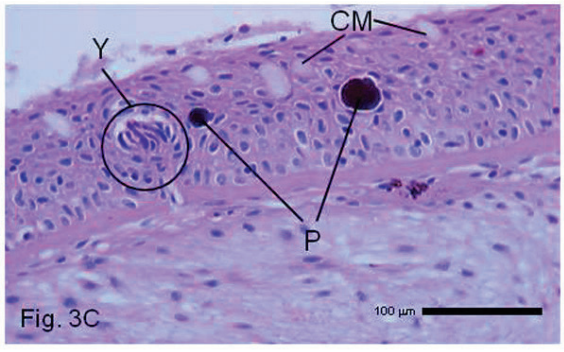
under the light microscope and the electron scanning microscope (Figs. 2B, 2C, 2D and 2E). In the capillaries, from both a normal appearance with flat lining epithelium and some red blood cells inside (Fig. 2B), to capillary hyperemia and interstitial edema with a general alteration in the endothelial cells (Fig. 2E), could be observed. As the different groups were examined, the hyperemia gradually increased, as well as the

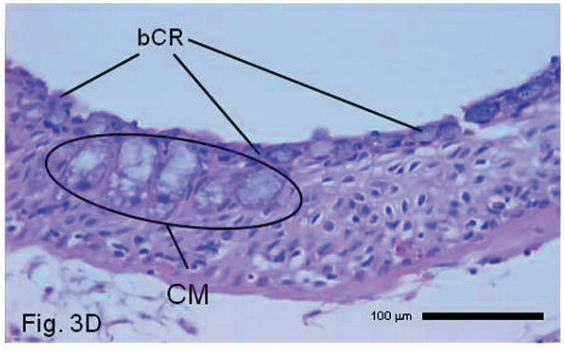
Fig .2. Images lamellae or respiratory cuts electron microscopy (TEM and SEM) gills. In control groups 1 and 2, a cell organization including all its components, chloride cells and mucous cells could be perceived, whereas in groups 3 and 4, there were alterations in all the cell components.2A) Lot control primary lamellae or filaments (Lp) and secondary lamellae or lamellae (Ls). 2B) Lot 1, capillaries of a secondary lamella are observed. 2C) Lot 2, chloride cells apparently normal are observed. 2D) Lot 3, a chloride cell is observed with loss of intracellular channels and showing strong vacuolization. 2E) Lot 4, disruption of capillaries is observed hyperemia. Lp: primary lamella or filament; Ls: secondary lamella or lamellae, Cap: capillaries; CCL: chlorine cells, V: vacuolization, Hp: hyperemic processes.

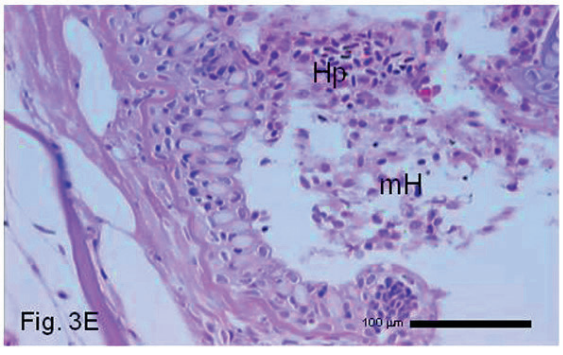
interstitial edema and the progressive swelling of the endothelial cells. In control groups 1 and 2, a cell organization including all its components, chloride cells and mucous cells (Figs. 2B and 2C) could be perceived, whereas, in groups 3 and 4, there were alterations in all the cell components. In these latter groups, the epithelial lining tissue was swollen and had a vacuolated cytoplasm. Due to the swelling shown in these cells, the light-projected images were irregular. The pillar cells were also swollen and both the core and the cytoplasm increased their electron density. Due to the cell swelling, the cell gaps almost disappeared (Fig. 2D). They were found in the group 4, in the regions where previously there had been chloride cells, with expansions of intracellular channels (Fig. 2E).











Filaments

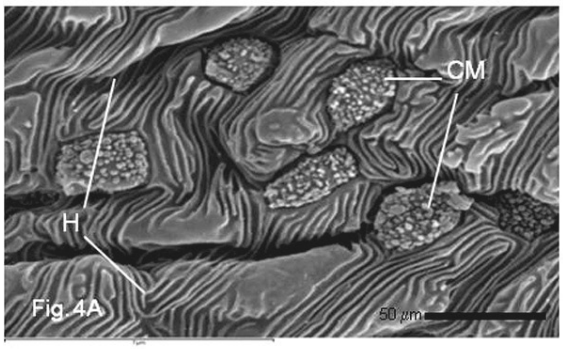
The histopathological examination in control groups 1 and 2 of the gill spine filaments and the primary lamellae did not reveal any significant alterations in their cell components, epithelial lining cells, taste buds, goblet cells, mucous cells or chloride cells. Only a slight edema over the surface was detected (Fig. 3C).

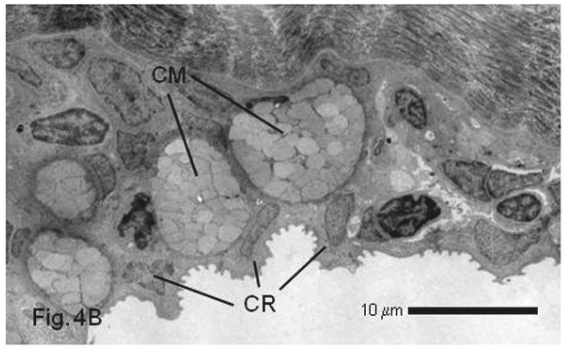
Fig. 3. Sectional images sensitive or non-respiratory gill optical microscopy (OM). In control groups 1 and 2 of the gill spine filaments and the primary lamellae did not reveal any significant alterations in their cell components, only a slight oedema over the surface was detected. In groups 3 and 4, there were vascular-type alterative processes. 3A) Lot control mucosa with abundant mucous cells and nerve buds. 3B) Lot 1, where mucous cells were observed. 3C) Lot 2 mucous cells are observed with strong nerve and yolk pigmentation groups. 3D) Lot 3, an increase of mucous cells, and increased cell basophilia observed coating. 3E) Lot 4, hyperemia and microhaemorrhage area is observed. Y: taste buds, CM: mucous cells, P: pigmentation groups; bCR: basophilia of lining cells, Hp: hyperemia, mH: microhaemorrhage.

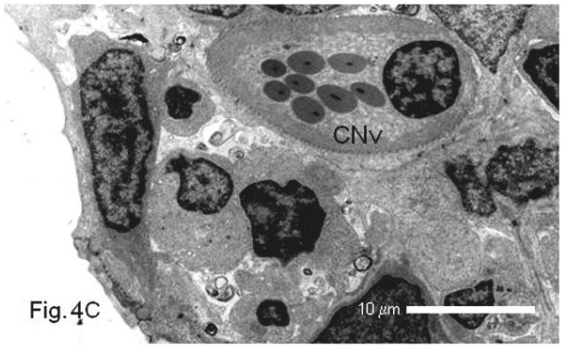
In the groups 3 and 4, there were vascular-type alterative processes, with hyperemia and edema in the *lamina propria*. The edema separated the connective elements, which made up the *lamina propria*. Regarding the epithelium, top necrosis with cell detachment was observed. In this epithelium, the increase in the goblet cells was noteworthy (Fig. 3D). The mucous cells were placed in superficial layers, in contact with the epithelium light. Chloride cells were found on the basal side and were swollen with some vacuolations. Taste buds were partially disorganized in the group 4 (Fig. 3E).

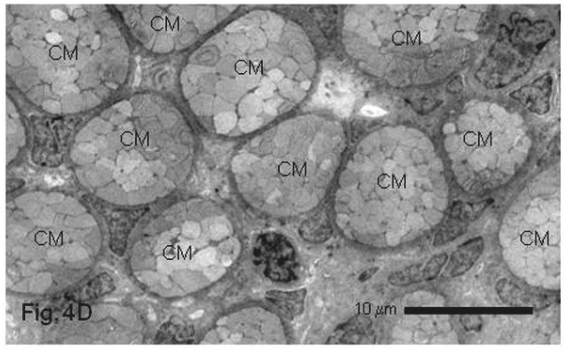
The scanning electron microscope showed that, in groups 1 and 2, the epithelium surface was scored with very deep creases like fingerprints. Attention was drawn to the long pedicels of the taste bud neuroepithelial cells that emerged from the stomata, although these prolongations were short. The large protuberances with an irregular surface corresponding to the mucous cell secretion were noticed.

In groups 3 and 4, (Fig.4E), the fingerprint grooves on this surface were partially maintained, but what stood out the most was the









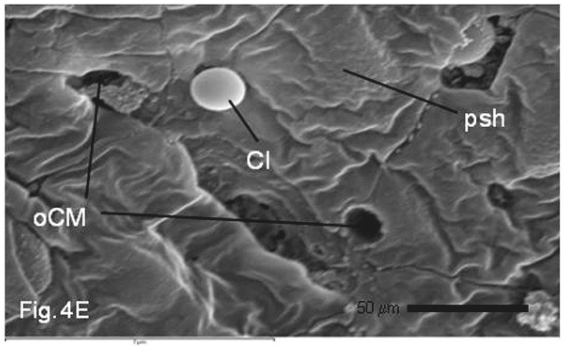


Fig.4-*. Sectional images sensitive or non-respiratory gill electron microscopy (SEM and TEM). In groups 1 and 2, the chloride cells changed with respect to the control group morphology, expanding the channel and partially losing intracellular mitochondria and other cytoplasmic organelles. In groups 3 and 4 changed the morphology of most of the epithelial cells.* ***4A****: Lot control lining cells folds was presented by way of fingerprints around mucous cells.* ***4B****: Lot 1, where mucosal and lining cells observed.* ***4C****: Lot 2 sensory bud detail with nerve cell is observed.* ***4D****: Lot 3, detail of increase of mucous cells was observed.* ***4E****: Lot 4, swelling was observed with losses grooves "footprints" and recesses of mucosal cells, also observed inflammatory exudate cells. H: folds lining cells, CM: mucous cells, RR: lining cells; CNV: nerve cell; psh: loss of fingerprint ridges; oCM: recesses of mucous cells, CI: inflammatory exudate cells.*

numerous amount of mucous cell areas next to the taste bud surfaces, which were exuding their mucous secretion. Using the scanning transmission microscope, the cell morphology in groups 1 and 2 seemed to be normal. With this scanning method, it could also be seen that the fingerprint spicules of the epithelial lining cells and the taste buds and the mucous cells appeared to be normal. However, the chloride cells, changed with respect to the control group morphology, expanding the channel and partially losing intracellular mitochondria and other cytoplasmic organelles (Fig. 4B and 4C)

In the groups 3 and 4 (Fig. 4D), it is worth noting that the epithelium showed capillary hyperemia and edema, which changed the morphology of most of the epithelial cells. The biggest changes could be found in chloride cells, with the loss of large numbers of mitochondria, in particular their smooth endoplasmic reticulum, with all its lights dilated. Sensory buds also had a marked edema, with a wrinkling of cells in the apical portion of the yolk, and separation of cells and nerve teloglia, although the latter showed no changes at this time.

Morphometric study

There was an increase in numbers of chloride cells in groups 2 and 3, and a decreased number in the group 4 (Fig. 5). A Scheffer test for this parameter showed that there were no significant differences between the control group and group 1. Thus, the variation in the number of chloride cells at this concentration of TCDD was not affected. On the other hand, significant differences ($p < 0.05$) were observed, if compared to the control groups, in the number of chloride cells in groups 2, 3 and 4.

Furthermore, the mean diameters of chloride cells, were significantly higher in groups 3 and 4, these being equal to each other (Fig. 6).

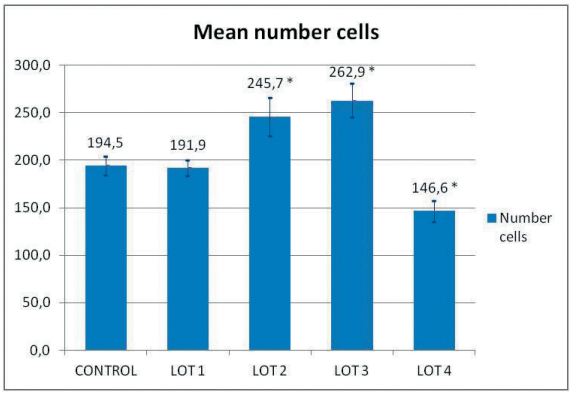


*Fig 5. Graphical representation of the variation in chloride cell number in each group. There was an increase in numbers of chloride cells in groups 2 and 3, and a decreased number in group 4. A Scheffer test for this parameter showed that there were no significant differences between the control group and group 1. Thus, the variation in the number of chloride cells at this concentration of TCDD was not affected. Significant differences ($p < 0.05$) were observed, if compared to the control groups, in the number of chloride cells in groups 2, 3 and 4. *Significantly different from the control with $p<0.05$.*

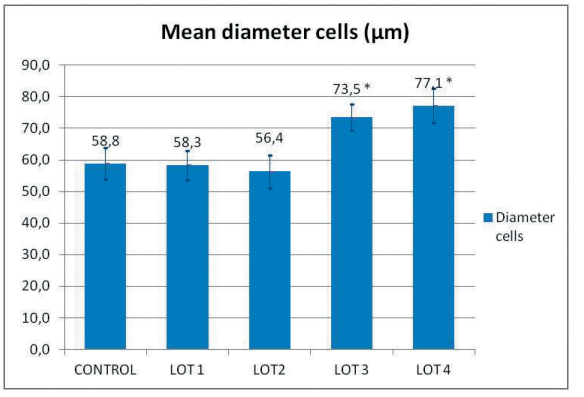


*Fig 6. Graphical representation of the variation of the average chloride cell diameter in each of the lots. The mean diameters of chloride cells were significantly higher in groups 3 and 4, these being equal to each other. *Significantly different from the control with $p<0.05$.*

DISCUSSION

The indiscriminant use of different chemical substances in various industries favors the storage of these substances in the body. It is a well-known fact, as reported by COLBORN *et al.*, 1993; CAMPBELL & HUTCHINSON, 1998; GUILLETTE, 2000; EDWARDS *et al.*, 2006, etc., that these substances, among which are dioxins, modify various organic systems, especially the sexual organs, both male and female. As poisoning usually takes place gradually (ANTUNEZ *et al.*, 2001; JACOBS *et al.*, 2002; VIVES *et al.*, 2005), few lesions

are known in the organs through which the toxic substances enter, particularly the digestive and respiratory tracts. In mammals and fish, lesions in the gastrointestinal tract (HENRY *et al.*, 1997; ARELLANO *et al.*, 2001; ZODROW *et al.*, 2004) have been cited. However, no modifications have been observed in airways.

In our approach, we made different groups depending on the doses administered to the fish. The work of CAMEÁN *et al.* (2004), detected by immunocytochemistry, suggest that toxic substances entered through the gill, and even that part of these substances are absorbed by the chloride cells., The reason may be that, as they had a large number of mitochondria and, especially, of cisternae of the smooth endoplasmic reticulum, they degraded the toxic substances. In the quantification of chloride cells made in our experiment, especially in the respiratory tract, there was a progressive increase in this cell population (VAN DEN HEUVEL *et al.*, 2000), which inferred a reaction against the dioxins for their elimination. The reduction in the group 4 corroborated what was described by histological observation, in which cell degeneration and necrosis occurred in the last group. However, as the doses increased so the cells became hypertrophic (AL-GHAMBOUSI *et al.*, 2012). This was also shown in the histopathological study, in which swelling of the epithelial tissue and swelling and vacuolization of the chloride cells appeared. In electron - microscopy studies, the membranous reticulum system (smooth endoplasmic reticulum and microvilli of the intracellular channel), and initially remained normal but in the last two groups hypertrophy was due to dilatation of the intracellular channel with swelling of the microvilli. Thus the degradation activity of dioxin was lost (as established by CAMEAN *et al.* (2004)), which meant that the first retention barrier against the toxic substance was also lost.

Similarly, too many fish poisoning cases, and non-specifically, both the primary and secondary lamellae, showed marked hyperemia (AL-GHANBOUSI *et al.*, 2012), which in our case ended in an erosion of the lining and capillary epithelium producing edema and microhemorrhages (MALLATT, 1985). Especially prominent was the swelling in the lamellae (ZODROW *et al.*, 2004; AL-GHANBOUSI *et al.*, 2012).

Another defense system of the gills that started to operate was that related to the mucous cells. Again, as we saw, as poisoning became accentuated, it produced a mucosal inflammation with hyperplasia and hypertrophy of the surface and deep mucosal cells (LICHTENFELS *et al.*, 1996).

Everything seemed to indicate that the passage of the toxic substance through the gills did not directly affect the sensory buds, although in the last groups, due to the presence of interstitial edema, they produced swelling of the supporting cells, without any changes occurring in the neurosensory cells. This was accentuated in the respiratory lamellae terminations.

CONCLUSIONS

It was concluded that dioxins affect zebrafish gills by significantly increasing the number of chloride cells, with the supply of 40 ng of TCDD in the fish's diet. Similarly, there was a significant variation in the mean diameter of the chloride cells when administering 100 ng of TCDD in the diet. We hypothesized that both circumstances altered the functionality of the gills.

Furthermore, we established that the neurosensory cells of the taste buds were not directly affected but that adjacent supporting cells with interstitial edema did indirectly affect the functionality of the taste buds.

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Prilog istraživanju promjena na škrgama zebrice (*Danio rerio*) prilikom izloženosti dioksinu

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SAŽETAK

Ovim istraživanjem utvrđene su promjene na stanicama škrga zebrice (*Danio rerio,* Hamilton, 1822) kao rezultat hranjena 2,3,7,8 tetraklorodibenzo p-dioksinom (TCDD). Pedeset jedinki zebrice podijeljeno je u pet grupa, jednu kontrolnu grupu i četiri eksperimentalne. Eksperimentalnim grupama davano je 10, 40, 100 i 270 ng/riba/dan TCDD. Provedena su strukturalna, ultrastrukturalna i morfološka istraživanja škrga ribe, ukazujući da su škrge riba pokazivale hiperemiju s progresivnom erozijom endotel kapilara koja je rezultirala edemima i mikrokrvarenjima. Kod grupa kojima je dana manja koncentracija, u škrgama koje ne služe za disanje može se primijetiti da kloridne stanice pokazuju hipertrofiju kao posljedicu dilatacije međustaničnog kanala i oticanje mikroresica kojima se smanjuje aktivnost tijekom razgradnje kemijskih tvari (prema mišljenjima nekih autora) u onim grupama koje su bile izložene većim koncentracijama. Mukozne stanice pokazuju hiperplaziju i hipertrofiju. Osjetni pupoljci nisu bili izravno pogođeni, ali, zbog prisutnosti intersticijskog edema, oticanja potpornih stanica bez uključivanja neurosenzornih stanica. Zaključak je da dioksini utječu na škrge u većoj ili manjoj mjeri ovisno o njihovoj koncentraciji u ishrani ribe, što mijenja njihovu funkcionalnost.

Ključne riječi: PCB, škrge, patološka histologija, ultrastruktura, stanična morfologija