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Histological Responses of Golden Apple Snail (*Pomacea canaliculata*) to Copper

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Histopathological changes in kidney, digestive gland, foot, and gills of *Pomacea canaliculata* due to copper exposure were studied to assess copper's effects on tissues of its different organs and also for the possibility of being used as a biomarker. Three-month-old snails were exposed to copper (67.5µg L^{-1}) for seven days and were excised and fixed in 10% formalin. Routine histological preparation and examination exhibited varied forms and degrees of aberrations. These include hydropic degeneration, disintegration and loss of cells, elongation of kidney tubules, hyperplasia of K corpuscles in the digestive gland, flattened epithelium, and muscular bundles in foot in complete disarray, dilations and folding of the gill filaments. Gross and subtle tissue alterations in these organs may lend support to the role of *P. canaliculata* as a biomarker for copper contamination.

Key words: copper, digestive gland, foot, gills, kidney, *Pomacea canaliculata*

INTRODUCTION

Golden apple snail (*Pomacea canaliculata*) is a common freshwater snail and a notorious agricultural pest in the Philippines and other countries in Asia (Mochida 1988, 1991; Naylor 1996). Concerted efforts have been undertaken to annihilate them but they still persist and even spread naturally and intensively. Since this snail is ecologically important, persistent and possesses attributes of a biomonitor – (a) they are big enough to provide sufficient material (soft tissue) for analyses, (b) easy to handle, collect, culture, (c) live long, (d) abundant, (e) sedentary, (f) can survive for a long time without food, and (g) can found in almost any freshwater ecosystem in many countries – this needs an extensive study for ecological management and its potential as a metal biomonitor.

Copper (Cu) is an essential trace mental but exposure to levels greater than the required amount may prove to be fatal to most living organisms. Sediment in some rivers and other freshwater ecosystems in the Philippines have varied Cu concentrations from as low as 28 µg/g in Marikina River in year 1992 (Prudente et al. 1994) to as high as 44,800 µg/g in Boac-Makulapnit River in 2002 (David 2003) (Table 1). Some of the sediment Cu concentrations in the Philippine rivers exceeded the sediment quality guidelines (65-270 mg/kg) of ANZECC/ARMCANZ (2000) Guidelines for Fresh and Marine Water Quality. These high values and variability call for a monitoring program to check the level of Cu contamination in the rivers and other freshwater ecosystems.

Early detection of Cu pollution would save a lot of aquatic organisms and eventually human beings. Use of biomarkers would help attain this purpose. Biomarkers at the molecular or biochemical levels are often regarded as early warning indicators

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Table 1. Sediment Cu concentration in some rivers/ freshwater ecosystems in the Philippines.

Location	Cu Concentration (μg g^{-1} dry out)	Date Taken	Author
Boac-Makulapnit River	843-14,500	2001	David (2003)
Boac-Makulapnit River	1,390- 44,800	2002	David (2003)
Manila Bay	32-118	May 1992	Prudente et. al. (1994)
Marikina River	28-79	May 1992	Prudente et. al. (1994)
Pasig River	110-189	May 1992	Prudente et. al. (1994)
Rivers in Bulacan	36-98	May 1992	Prudente et. al. (1994)
Laguna Lake	98	-	Vicente-Beckett et al. (1991)
Laguna Lake	100	-	Pfeiffer et al (1988)
Laguna Lake	115	-	LLDA (1978)
Lake Tributary Rivers	81	-	Vicente-Beckett et al. (1991)
Lake Tributary Rivers	98	-	Vicente-Beckett et al. (1991)
Pasig River	108	-	LLDA (1978)
Marikina River	83	-	Pfeiffer et al (1988)
Lu Silangan Streams	65	-	Pfeiffer et al (1988)
Agno River	561	-	Santos et al (1985)
Bued River	504	-	Santos et al (1986a)
Patalan River	34		Dela Rosa et al (1985)

for long-term ecological effects (Lopez-Barea & Pueyo 1998) due to their rapid response to stress and their potentially high toxicological relevance (Lau & Wong 2003). Histopathological analysis provides a vital link between the biochemical effects and the effects measured in the whole organism (Lowe 1988). Histopathology has been recommended as a physiological approach to pollution investigation and enables the researcher to rapidly examine multiple potential sites of injury (Sindermann 1985; Hinton 1993 as cited by Teh et al. 1999).

Golden apple snail is a benthic, macrophytophagous organism; so it is an appropriate tool for monitoring Cu in the river sediment, from the water, and vegetation present in the river. It would give a composite bioavailable Cu of the river. Lastly, it is a good accumulator of copper from various matrices: sediment, food, and water (Ruangareerat 2004; Sumritdee 2007; Neeratanaphan & Phalaraksh 2008; Peña & Pocsidio 2008; Kruatrachue et al. 2011; Dummee et al. 2012).

Accumulation study of Cu in different organs (kidney, foot, gill, and digestive gland) of snails (Peña & Pocsidio 2008) from different matrices shows that the accumulated Cu do not vary significantly in the different organs, though the gill accumulates the highest amount from the aqueous solution. To see its effects on the tissues of these organs using the same Cu concentration (67.5 μg L^{-1}), histological study had been done. The result of this study could be used for ecological management as the snail being an agricultural pest and as a potential metal biomonitor analogous to the mussel watch in marine ecosystems.

METHODS

Five egg masses of *P. canaliculata* were obtained from the field in Naga City, Philippines (123^{o}11'18"E, 13^{o}37'15"N, 293^{o}NE). The hatchlings were cultured in a laboratory until they became three months old. They were fed with young sweet potato (*Ipomoea batatas*) leaves ad libitum (Lacanilao 1988). The water in the basins was changed daily. Three replicates containing ten snails each were maintained. The experimental groups were given a nominal Cu concentration of 67.5 μg L^{-1} (Peña & Pocsidio 2008) for seven days. They were not fed during the experimental period. The temperature ranged 28-30 ^{o}C at 6.0–6.7 pH while the dissolved oxygen (DO) was 6-7 ppm. The posterior kidney, digestive gland, foot, and gills of the control and treated snails were dissected and fixed in 10% formalin. Routine histological preparation was conducted.

RESULTS AND DISCUSSION

The kidney of *P. canaliculata* is composed of numerous tubules lined with non-ciliated cuboidal cells (Toribio 1979). The kidney tubules of the control were intact and of almost the same size and shape (Figure 1A). However, the one exposed to Cu underwent several histopathological changes (Figure 1B): cells appeared swollen and assumed spherical profile, loss of their normal integrity and structure, disappearance and clustering of cells within the lumen and

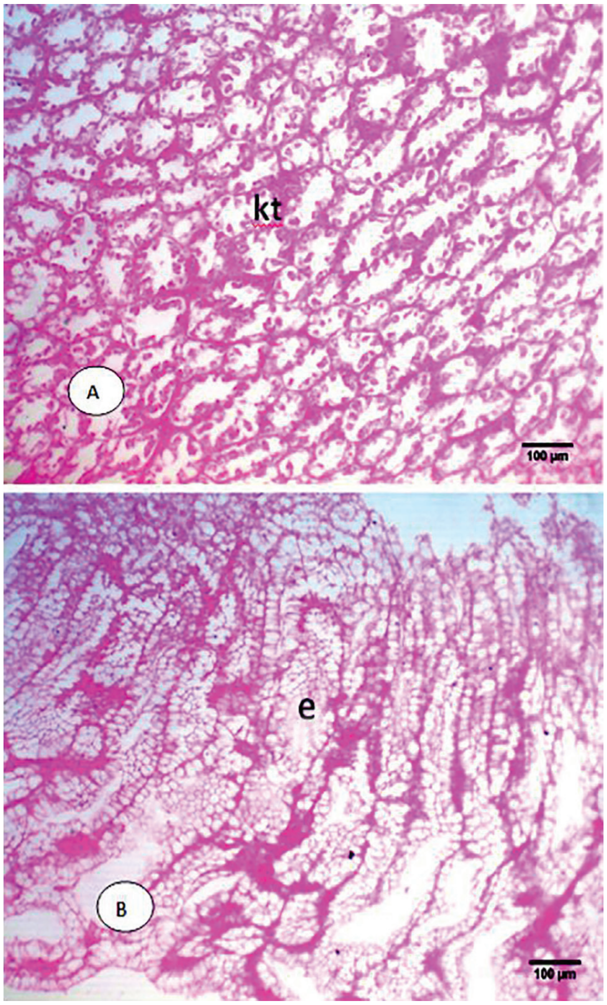

Figure 1. Light micrograph of a portion of the kidney of the control (A) *P. canaliculata* and the one exposed (B) to 67.5 µg L^{-1} Cu in seven days. Fig. 1A shows intact kidney tubules (kt) while Fig. 1B shows disappearance of kidney envelope (upper portion), highly elongated (e) kidney tubules, swollen cells, disappearance and clustering of cells within the lumen, and the disintegration of tubules. (A) & (B) x100

disintegration of tubules. The swelling of the tubules could be attributed to increased proliferation of the cells in response to the presence of toxicant. The hydropic degeneration may imply impaired osmotic and ionic regulation or as Popper (1982) suggests that there can be an inhibition of ion pump in the cell organelles. The mitochondria and rough endoplasmic reticulum (rER) of such cells are said to be dilated and are associated with a decrease in ATP production. Vesicularization of rER is accompanied by accumulation of lipid droplets. This may account for the observed vacuolation of the cells. The disintegration, disappearance, and the clustering of cells within the lumen in this study could be presumed that these cells were severely damaged internally which led to their death.

A very large coiled organ, the digestive gland (hepatopancreas) of *P. canaliculata* is composed of digestive tubules joined by loose connective tissue. The digestive tubules of the control appeared round or oval with slit-like or star-shaped lumen with more C corpuscles and fewer K corpuscles (Figure 2A). Cu-exposed digestive tubules contained a lot more K corpuscles (Figure 2C, 2D) than that in the control. It appeared to have a greater number of K corpuscles clumped together at the base of the lumen. Exposure to Cu led to a marked degree of degeneration and disintegration of the tubule with widening lumen. Figures 2B and 2D were pictures focusing on a single digestive tubule. Figure 2B was of the control containing more C corpuscles than K corpuscles while Figure 2D was from the treated showing more K corpuscles than C corpuscles.

The midgut gland (hepatopancreas or liver) of *P. canaliculata* contains two cell types: the greenish spherules and the brown concretions. The former cell type produces digestive enzymes and absorbing soluble products of digestion while the later type is excretory in function (Andrews 1965). The greenish spherules are referred to as C corpuscles and the brown concretions as K corpuscles (Castro-Vasquez et al. 2002). C corpuscles are also referred to as digestive cells while K corpuscles are the basophilic or calcium cells (Soto et al. 2005). The digestive cell is also referred to as acidophilic cell (Owen 1972). The C corpuscles in the midgut gland alveoli are contained within columnar cells while K corpuscles are found within pyramidal cells. The digestive cells are primarily involved in intracellular food digestion whereas basophilic cells (or calcium cells) are thought to be secretory cells that contribute to extracellular digestion and metabolic regulation (Soto et al. 2005). The calcium spherule serves to accumulate toxicants like excess copper to detoxify it to minimize its effect (Viarengo et al. 1981). Thus the increase in K corpuscles after exposure to copper solution (67.5 µg L^{-1}) could be a way of detoxifying the Cu to minimize its effects. Increase of K corpuscles, however, was not exclusive to copper contamination. Increase of K corpuscles (dark granules) is also seen in *P. canaliculata* exposed to sediments contaminated with heavy metals (Kruatrachue et al. 2011; Dummee et al. 2012). Dark granules are lysosomal residual bodies, suggesting the accumulation of toxicants such as heavy metals or molluscicides (Hamed et al. 2007).

Chi-square showed that the number of C corpuscles and K corpuscles in a single digestive tubule was dependent on the presence or absence of Cu (p>0.05).

The foot of *P. canaliculata* was a large muscular organ covered by a thick epidermal layer of ciliated columnar epithelium with mucous cell. The epidermis of the control foot was smoothly wavy in appearance. The core of the

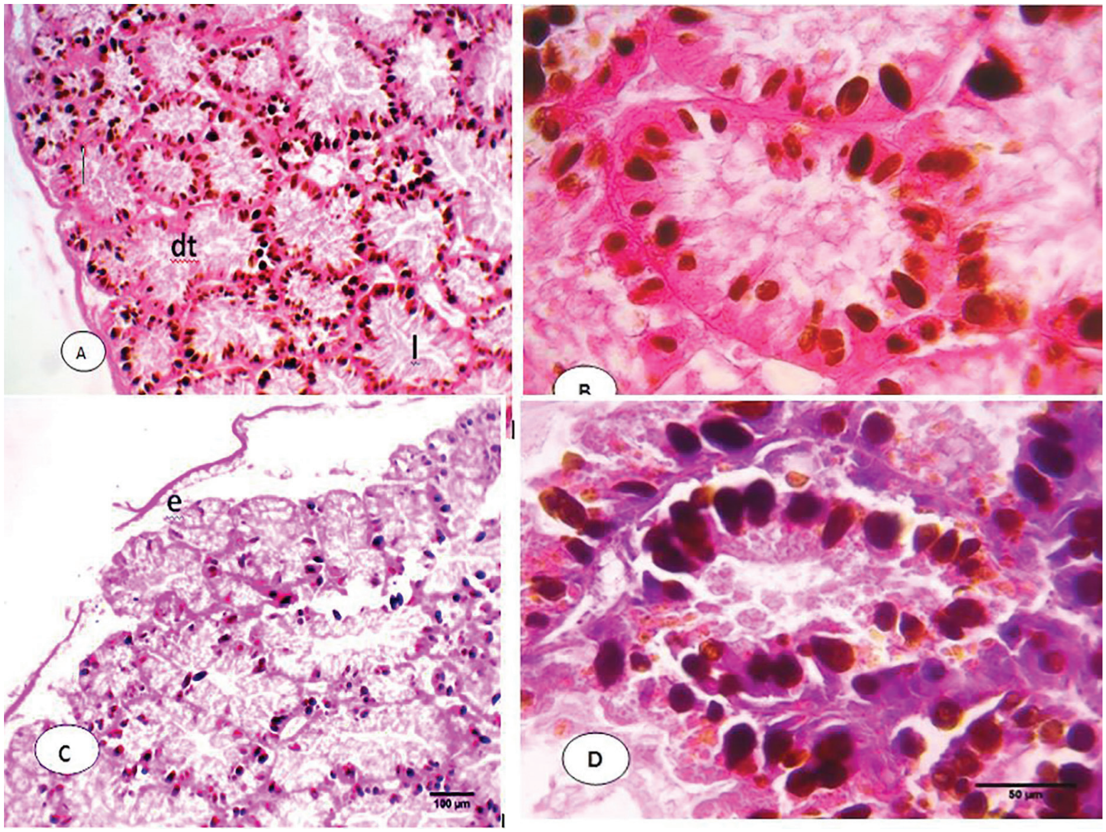

Figure 2. Light micrograph of the digestive gland of control *P. canaliculata* showing undisruptive digestive tubules (dt). Digestive tubule of the control showed intact columnar cells with C corpuscles (light colored) and the dark colored K corpuscles (A). B is a single digestive tubule of the control showing more C corpuscles than K corpuscles. C is Cu-exposed digestive tubules with disrupted digestive envelope (e), widening of digestive tubules, disrupted columnar cells apparently emptying both the C and K corpuscles to the lumen and hyperplasia of K corpuscles. D is a single digestve tubule of treated *P. canaliculata* showing more K corpuscles than C corpuscles and a disrupted tubule. (A) & (C) X 100, (B) & (D) x 50

foot was occupied by several bundles of muscle cells and adipose tissue enmeshed in fibrous dense connective tissue. The adipose tissue appeared as a larger mass of clear cells among the muscles (Figure 3A). In the sub-epidermal tissue are three kinds of gland cells: mucocytes, calciferous (lime cells), and pigment cells (Barrington 1979). Cu-exposed foot was found to have disintegrated epidermis and disorganized muscle bundles exhibiting longitudinal splitting or separation (Figure 3B). The smooth wavy epidermis in the control had irregular deep folds with muscle cells seen to be in disarray. A marked separation of the muscular cells and disrupted adipose tissues were observed.

The folding of the wavy epidermis into irregular folds could be attributed to cell proliferation or the snail's mechanism to alleviate pain or other negative effects. Among the organs, the foot seemed to be the prime site of entry of Cu because of its large surface area and its direct contact with the substrate or the food. However, the *P. canaliculata* exposed to Cu-contaminated food, sediment contaminated with heavy metals and dissolved Cu suggested that the gill was the prime site of entry of Cu. The gill's uptake of Cu is higher than that of the foot (Peña and Pocsidio 2008). This study suggested that the gill, not the foot, is the primary site of entry of Cu in *P. canaliculata*.

The respiratory system of snails consists of a set of gills or ctenidia of about 32-40 folds diagonally located on the left anterior part of the body immediately under the mantle (Toribio 1979). The ctenidia had a double-walled layer of ciliated columnar cells interspersed with mucous cells. They were arranged parallel to each other and separated by a narrow space (Figure 4A). Gills of *P. canaliculata* exposed to Cu were marked with an expansion of the connective tissue between the gill filaments and gill arch (Figure 4B). Gill filaments were separated/diluted in varied degrees from slight to severe. The ctenidia also exhibited zigzag-like folds, cutting/splitting, and loss of cilia (Figure 4C). The varying separations/dilations seen in the gill filaments might imply varying susceptibility

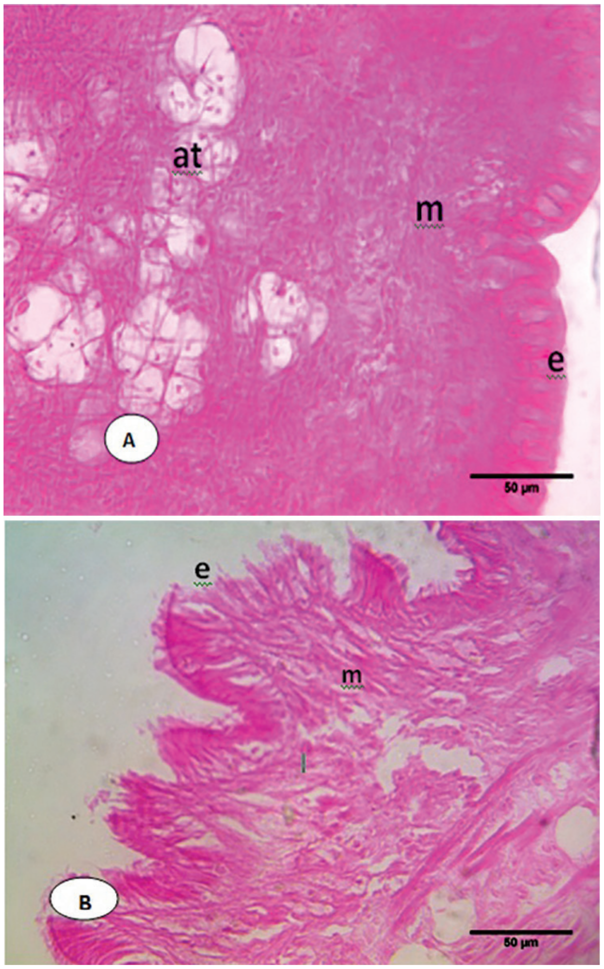

Figure 3. Light micrograph of the foot of control (A) *P. canaliculata* showing the epidermis (e) core of muscles (m) and adipose tissue (at), (B) disintegrated epidermis (e), muscle cells in disarray, longitudinal splitting of muscle bundles, irregular folding of epidermis and disrupted adipose tissue. (A) & (B) x 50.

to the toxicant even within the same gill. This dilation or occurrence of wider hemolymph space is also seen in *P. canaliculata* exposed to heavy metal contaminated sediment (Kruatrachue et al. 2011). Production of more mucous cells in the Cu-exposed gills provided a direct evidence of active mucous secretion that served to trap Cu from the environment rendering it less toxic. This increase in the number of mucous cells and the degeneration of columnar cells of gill filament are also seen in *P. canaliculata* exposed to sediment contaminated with heavy metals (Dummee et al. 2012). The ctenidial folds may be interpreted as a form of clustering of cells. The expansion of the connective tissue between the gill filaments and gill arch may be attributed to cell proliferation leading to fusion. Similar findings are reported in the Black Sea mussels (Ciocan 1997). The toxicity of Cu lies in the fact that it acts as a respiratory

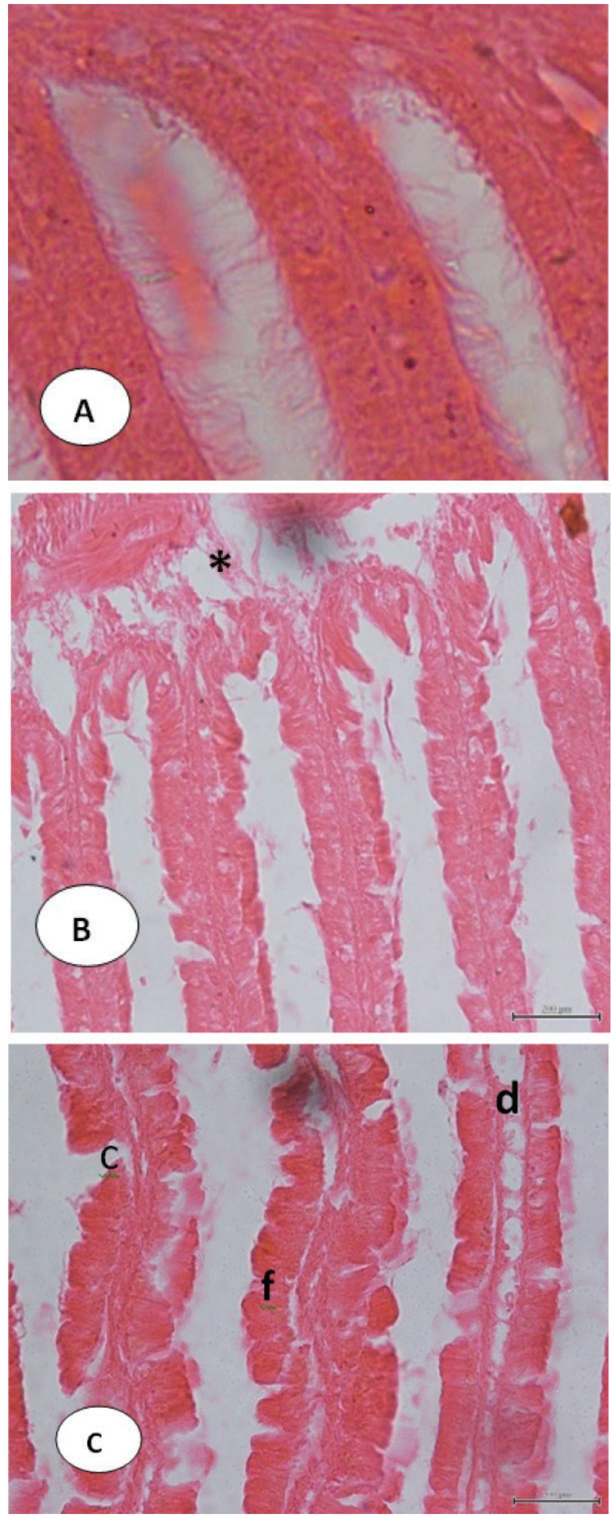

Figure 4. Portion of the gills of the control (**A**) *P. canaliculata* showing double –walled layer of ciliated columnar cells parallel to each other and separated by a narrow space interspersed with mucous cells. (**B**) expansion (*) of connective tissues between the gill filament and gill arch of treated snail and (**C**) with different degrees of dilation (d) of gill filaments, zigzag-like ctenidial folds (f) and cutting/splitting (c) of the ctenidia. (**A**), (**B**) & (**C**) x 50.

poison on the snails. The disruption of gill epithelium will certainly hinder gas exchange; hypoxia will follow, and eventually, death of the snail.

All four organs: kidney, digestive gland, foot, and gill though exposed to the same Cu concentration showed slight to moderate, and to severe/extreme tissue aberrations caused by Cu exposure.

CONCLUSION

After a seven-day exposure to 67.5 µg L^{-1} Cu, the kidney, digestive gland, foot and gills of *P. canaliculata* exhibited various degrees of histopathological aberrations. These include loss of structural integrity of the cells, hyperplasia of K corpuscles, flattened epithelium and muscular bundles in foot in complete disarray, loss of cilia, and dilations and folding of the gill filaments. Gross, moderate and subtle tissue alterations in these organs might lend support to the role of *P. canaliculata* as a biomonitor for Cu in the environment.

As there was no significant difference in the accumulated Cu in the different organs so were the effects on the tissues of the different organs. All organs exhibited slight to moderate and to severe alterations.

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