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# Effects of Acute Toxicity of Chemical Treatments on the Cultured Oliver Flounder, *Paralichthys olivaceus*

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# 화학제 처리가 성장기 넙치(*Paralichthys olivaceus*)에 미치는 급성독성 효과

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**This study was carried out for the purpose of developing environmental friendly and effective chemical treatment method for the disease control in the land-based flounder culture which is industrially popular in the coastal area in Korea. The chemicals such as formalin, chlorine dioxide and hydrogen peroxide were treated to the 1 year old cultured flounder, *Paralichthys olivaceus* and their effects on the fish based on the 24hr-$LC_{50}$, $LT_{50}$, 24-hour survival rate at each experimental concentration, recovery rate of the survived individual from chemical treatment, and the histological change of the gill after chemical treatment were investigated and analyzed. The 24hr-$LC_{50}$ was 321. 65 ppm for formalin, 419.62 ppm for chlorine dioxide, and 395.97 ppm for hydrogen peroxide, respectively. The $LT_{50}$ was 15-hour for formalin, 17-hour for chlorine dioxide and 24-hour for hydrogen peroxide, respectively. Fishes exposed to the experimental concentration of three chemicals were quickly susceptible in the order of formalin, chlorine dioxide and hydrogen peroxide with a trend of shorter half lethal time at higher concentration. Initial survival rate of the flounder soon after chemical treatment was the highest in the hydrogen peroxide treatment compared with the other two chemicals. The histological damage by the hydrogen peroxide treatment was negligible compared with the other two chemicals. Accordingly, hydrogen peroxide treatment showed the lowest toxicity compared with the other two chemicals to the experimental fishes.**

**Key words : Acute toxicity, Hydrogen peroxide, Flounder, 24hr-$LC_{50}$, Histopathology**

## Introduction

Formalin treatment is routinely employed in the fish farm in the season of disease attack and dipping method mixed with Malachite green is widely used to exterminate the parasites of Trichordina and Protozo a etc. in the eel and tilapia farm (Leteux and Meyer, 1972 ; Fox et al., 1985). And in the land-based culture systems suitable for the highly-

intensive stocking of flounder, *Paralichthys olivaceus*, formalin dipping is ordinarily perfo-rmed for the prevention and extermination of parasitic and pathogenic disease (Park et al., 1995). But the formalin treatment in the fish farm often renders the consumers on the cultured fish fearful of being toxic and contaminative to the human and environment and decreasing consumption of cultured fish produced in the formalin treated farm. Accordingly, new method by alternative chemicals, not harmful to the fish as well as to the safe food stuff as raw fish preferred by the many Korean and Japanese people, have been long required to be developed and studied among the aquaculturists and fisheries scientists. Prior to the development of new chemical method, appropriate target species for the treatment should be chosen first, and then fish size at the remedy time should be taken into consideration. In addition to the exact treatment concentration and exposure time to the desired chemicals, and the influences on the cultivated water and surrounding water quality around in the effluent seawater are to be considered (Park et al., 1995).

Generally speaking, in the toxicity experiments by chemical treatment the effects of the used chemicals on the targeted live organisms are recommended to be evaluated in a short time and to be tested for the various organisms over than 100 species depending on the standard method (ASTM, 1980 ; OECD, 19 81). In case of fish, targeted fish including flounder for this chemical experiment were mainly confined to the adult fish, but many toxicity experiments were given to the fries or fingerlings sensitive to the metabolic activity before and after hatching (Mckim and Benoit, 1977 ; Park and Park, 1987).

Conclusively, this study was aimed at the develoment of new chemical treatment method and providing the possible adopted criteria in the use of these new tested chemicals especially in the summer season, frequently encountering various disease occurrence, in the culture ground for flounder. Thus, we investigated the effects of three kinds of chemical treatments such as formalin, chlorine dioxide and hydrogen peroxide on the 1 year old cultured flounder based on the $LC_{50}$, 24hr-$LT_{50}$, survival rates, recoverly rates of the survived fish after experiencing chemical treatment and histological change of the gill tissue before and after chemicals treatment.

## Material and Methods

Experimented fish were 360 normal and active flounders and acclimated for 2weeks in the experimental tanks after transportation from hatchery before use. The average total length and weight of these fishes reached 17. 59±0.99 ㎝ and 51.93±10.94 g (n=30) respectively. The experimental vessels used were 200ℓ rectangular FRP tanks with the temperature maintenance at 21±1℃ and pH of 7.8. Tested chemicals were formalin containing 30.0% aldehyde, 3% chlorine dioxide and 33~35% hydrogen peroxide. All three chemicals were treated in the 5 concentration divisions of 300, 400, 500, 600, and 700 ppm with an additional division of 350 ppm in formalin, 450 ppm in chlorine dioxide and hydrogen peroxide, and 20 individuals of fish were stocked in each experimental concentration of three chemicals.

The 24hr-$LC_{50}$, $LT_{50}$ and survival rates for

24hr, recovery rate from the chemical treatments depending on the tested chemicals were investigated at an hour interval after chemical treatment. And the number of survived individuals were counted by eliminating the dead ones by every 1 hour. The data collected were analyzed using SPSS statistic program with Probit method. For the observation of histological change of the gill, the gills of normal fish and the fish exposed to chemical at each concentration were fixed in the bouin's solution and dehydrated with ethanol series after washing. Preparation of gills were done with paraffin method. This paraffin blocks were cut to be 5~6 ㎛ thick and the sections were stained in the Mayer's hematoxylin-eosin.

## Results and Discussion

**24hr-$LC_{50}$ dependent on the chemical treatment**

The results of 24hr-$LC_{50}$ affected from the tested chemicals (formalin, routinely employed in the fish culture, chlorine dioxide and hydrogen peroxide) when treated to the flounders were shown in Fig. 1. No dead fish was found in the control (no treatment) and the 24hr-$LC_{50}$ were 321.65 ppm for formalin, 419 ppm for chlorine dioxide and 395.97 ppm for hydrogen peroxide. Chlorine dioxide was found to be less toxic with no significant differences from the hydrogen peroxide exposed groups ($P>0.05$). Comparatively, formalin treated groups showed lower 24hr-$LC_{50}$ than the other two chemicals. Park et al. (1995) reported the 24hr-$LC_{50}$ with 141 ppm when treated with formalin in the flounder fingerlings sized 6.1±0.5 ㎝ with the big difference from that of this study. These discrepancies can be explained by the fact that the size of the tested fish in this study was much larger than that of the former research. The degree of fish susceptibility to the chemical was quite different depending on the fish size (Heath, 1987). From these facts, the concentration was recommended to be regulated with the size of the target fish for chemical treatment.

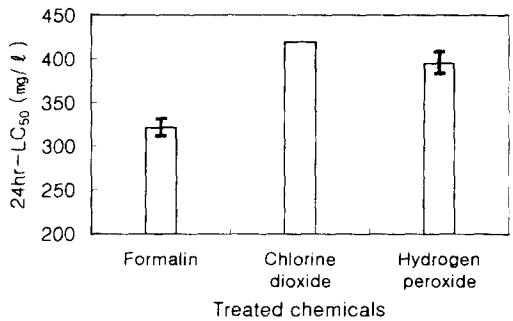


**Fig. 1. 24h-$LC_{50}$ of olive flounder exposed to the three chemical treatments with several concentrations.**

**$LT_{50}$ dependent on the chemical treatment**

The results of $LT_{50}$ affected from the tested chemicals when treated to the flounders were shown in Fig. 2. The $LT_{50}$ of the flounder treated with 300, 400, 500 ppm of formalin appeared 27, 13, 10hrs after formalin treatment respectively. The $LT_{50}$ of the flounder treated with 400, 500, 600 ppm of chlorine dioxide showed 21, 7, 5hrs after chlorine dioxide treatment respectively. And the $LT_{50}$ of the flounder treated with 400, 500, 600 ppm of hydrogen peroxide showed 30, 20, 13hrs after hydrogen peroxide treatment respectively. The $LT_{50}$ of the flounder treated with 3 kinds of chemicals appeared earlier in the order of formalin, chlorine dioxide, and hydrogen peroxide except for the division of 300 ppm. The required time for the $LT_{50}$ was re-

cognized to be shorter with higher concentration of chemicals tested. The $LT_{50}$ inferred from the average 24hr-$LC_{50}$ of each chemical was 15hrs for formalin, 17hrs for chlorine dioxide, 24hrs for hydrogen peroxide. 24hr-$LC_{50}$ of the chlorine dioxide was a little higher than that of hydrogen peroxide. On the contrary, the $LT_{50}$ of chlorine dioxide appeared very rapidly compared with that of hydrogen peroxide. Hydrogen peroxide showed stable trend to the high concentration over 500 ppm and chlorine dioxide showed half lethal motality within 7hours with the toxicity at this high concentration. This fact seemed to result from the unstable chemical components of chlorine dioxide and its existence in the ClO state. In our study, In that higher hydrogen peroxide concentration showed consistently shorter half lethal time with its concentration, hydrogen peroxide is likely to be chemically stable state.

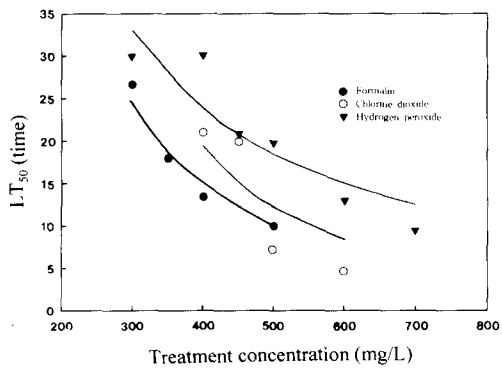


**Fig. 2. The $LT_{50}$ of olive flounder exposed to the three chemical treatments with several concentrations.**

**Initial survival rate dependent on the chemical treatment**

The required time for the chemical treatment in the fish farm is routinely completed within 4~5 hours. Thus, the effects of the treated chemicals on the initial survival rate is sure to be important. When three chemicals were treated, the survival rates within 24 hours were shown in Fig. 3.

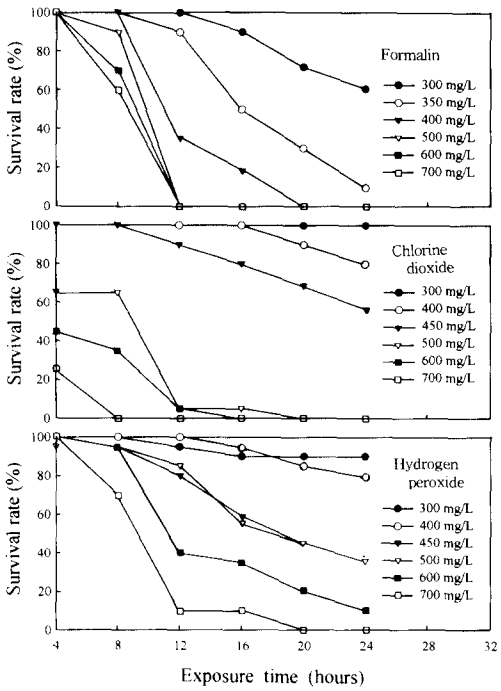


**Fig. 3. Survival rates of olive flounder exposed to the three chemical treatments with several concentrations.**

The fish exposed to formalin did not die within 4hours at the all tested concentrations. At the concentration of 500 ppm of formalin, the survival rate showed 90% during 8hours of treatment and it showed all the mortality during 12hours. The fish exposed to the formalin of 350 and 400 ppm showed sharp decrease in the survival rate of 10~15% with the lapse of time. The fish in the concentrations over 350 ppm showed low survival rates with

time and resulted in the lethal toxicity.

In the fish treated with chlorine dioxide, the survival rate at each concentration was 100% within 4 hours of exposure at the concentration less than 450 ppm. The fish exposed to the concentration over 500 ppm was rapidly affected by the lethal toxicity and showed all the mortality within 12 hours of treatment. The chlorine dioxide exhibited strong toxicity as well as formalin. The survival rates of the fish treated with chlorine dioxide were not consistent with its concentration. This seemed to be due to the chemical unstablity of chlorine dioxide itself.

In the treatment with hydrogen peroxide, the fish exposed to the concentration less than 600 ppm showed higher survival rate even 8hours after treatment. The fish exposed to the concentration at 600 and 700 ppm for 12 hours showed the unproportional survival rates of 40 and 10% with a trend of stable initial motality rate. The survival rates with time at the concentrations of 300 and 400 ppm showed 90 and 80% respectively with a similar trend when treated with chlorine dioxide. Even at relatively high concentration of 500 ppm the fish could sustain the survival rate over than 50%. From these results, hydrogen peroxide was characterized by less toxicity to the fish in a wide concentration range compared with the formalin which is very acutely lethal to the fish in its narrow range of low concentration. Even when fish aquaculturists happen to treat hydrogen peroxide at over than 500 ppm, this chemical which showed less acutic toxicity and consistent survival rates dependent on the concentration can be evaluated to be safe to the fish at the higher concentration than the formalin and chlorine dioxide.

**Recovery rate of flounder after exposure to the three tested chemicals**

As recovery of the fish from acute toxicity experiment at each chemical concentration was somewhat related to the possibile application for disease remedy (Park et al., 1995). The survived fish from the chemical treatment experiment at each chemical concentration were restocked in the fresh natural seawater for 24 hrs after complement of chemical treatment and the physiological

**Table 1. Survival rates of oliver flounder restocked in chemical-free seawater after 24 hours treatment with formalin, chlorine dioxide, and hydrogen peroxide**

| Chemicals | Concentration (mg/ℓ) | Survival No. after exposure | Survival No. after 24h | Survival rates |
|---|---|---|---|---|
| Formalin | 300 | 14 | 5 | 35.71 |
| | 350 | 2 | 0 | 0 |
| Chlorine dioxide | 300 | 20 | 19 | 95.0 |
| | 400 | 16 | 15 | 93.75 |
| | 450 | 11 | 9 | 81.81 |
| Hydrogen peroxide | 300 | 18 | 17 | 94.4 |
| | 400 | 16 | 12 | 75.0 |
| | 450 | 8 | 5 | 62.5 |
| | 500 | 7 | 2 | 28.57 |
| | 600 | 2 | 0 | 0 |

recovery of the survived fish were investigated (Table 1).

In the formalin treatment, the survived individuals of 14 at 300 ppm concentration, only 5 individuals could be recovered with the survival rate of 35.7%. But the fish treated at over than 350 ppm showed no survival in the recovery experiment. The chlorine dioxide treatment showed relatively higher survived individuals of 20, 16 and 11 at 300, 400 and 450 ppm respectively, the fish showed high recovery rate of 95~81.8%. The fish treated with hydrogen peroxide showed a little lower recovery rate than that at the same concentration of chlorine dioxide with the similar trend between these two chemicals. Kim (1997) reported that when the juvenile abalones once exposed to the phenol treatment were moved to the phenol-free seawater after chemical treatment of 96 hours, abalones experienced the concentration of 20 ㎎/ℓ and 5 ㎎/ℓ acquired full recovery without death after 72 hours and 58 hours, respectively and then showed rapid increase in the respiration.

The experimented species in this study was different from that of the former. But the survived individuals from the three chemical treatments (Formalin : 300~350 ppm, Chlorine dioxide : 300~450 ppm, Hydroge peroxide : 300~500 ppm) showed a little dead individuals within 24 hours after recovery experiment and then became a stable condition. This required time for the recovery was showed to be within 24 hours.

**Histological change of gill with chemical treatment**

The one of main reasons of the fish adaptation to the variable water environment is that the gill is playing a vital role in location of their habitat, gas transportaion, acid-base balance and ion regulation (Perry and Laurent, 1993). Accordingly, the morphological change of the gill in the fish was characterized by its functional regulation, and this morpho-histological change of the gill suggests that acute material like chemicals can directly and importantly affect the respiration of the chemical-loaded fish (Heath, 1987). Primary filament and epithelial cells of secondary lamella of the fish in the controls (not chemical exposed) showed normal arrangement (Fig. 4a). Contrary to these fish, the gill of fish exposed to the formalin 8 hours at 300 ppm showed hyperplasia of primary filaments, and desquamation in epithelial layers of secondary lamella (Fig. 4b). Desquamation and necrosis of epthelial layers were recognized also in fish exposed 8hours at the chlorine dioxide with 450 ppm. And clubbing phenomenon on the tip of the secondary lamella were also appeared (Fig. 4c). Hypertropy on primary filament and a little clubbing on secondary lamella was appeared in fish exposed 8hours at the hydrogen peroxide with 400 ppm (Fig. 4d). Gills from 8 hours after exposing to each chemical approach to 24hr-$LC_{50}$, the histological state of the gill exposed to hydrogen peroxide was shown to be negligibly morbid. Histological impact by hydrogen peroxide was relatively little compared with the other two chmicals. Another characteristics in the hydrogen poroxide treatment are the increase in the dissolved oxygen in the cultivated water due to the active oxidation reaction. In the application of hydrogen pero-

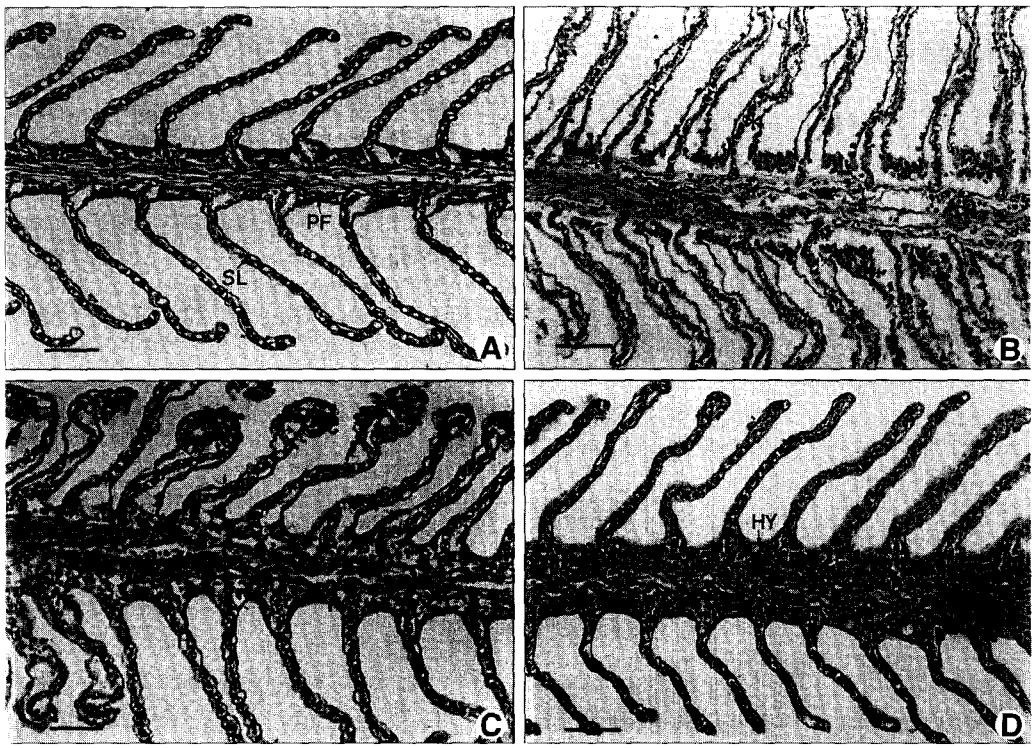


**Fig. 4. Sections of gill from olive flounder sampled after 8hrs exposure. A ; Control of gill, showing normal primary filaments (PF) and secondary lamella (SL). B ; Gill exposed at formalin of 350 ppm, showing hyperplasia of cells and desquamatiom(*) of epithelial layers in secondary lamella. C ; Gill exposed to chlorine dioxide of 450 ppm, showing desquamatiom of epithelial layers and clubbing(*) on the secondary lamella. D ; Gill exposed to hydrogen peroxide of 400 ppm, showing hypertrophy (Hy) on primary filament and a little clubbing on secondary lamella.**

xide in the fish farm for the control of fish disease, if the wasted water affer treatment would be diluted and discharged into the natural waters, it could be naturally resolved and become nontoxic. So, it can be a promising candidate chemical with a merit of being friendly to the water enviornment and providing security to the cultured fish. In addition, it was reported to be very effective and possibly available for the extermination of red tide dinoflagellates and their spores (Ichikawa et al, 1993 ; Ryu et al, 1998). When hydrogen peroxide was combined with water, it brings out free radical separated from hydrogen peroxide which could be adopted in the fish farm in the low oxygen concentration with safety and stability compared with the other two chemicals such as formalin and chlorine dioxide which caused mass mortality and toxicity to the fish even in the lower concentration.

Conclusively, considering the results of our further study on the chemical stability and parasitic control effectiveness (unpublished), we are sure to expect that the use of hydrogen peroxide could be a alternative for

the control of fish disease and red tide organism in the fish culture.

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