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Acute and Chronic Toxicities of Colchicine in *Brachydanio rerio*

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Colchicine is an alkaloid produced by a plant of the Liliacae family (*Colchicum automnale*). Its antimitotic activity has been known for a long time and is caused by a dysfunction of the chromatin system and an action on tubulin (Farrell and Wilson 1980; Avila *et al.* 1987). At the functional level, colchicine acts on the autonomous nervous system (Mundy and Tilson 1990), resulting in a series of pathological effects on the digestive tract, including capillary vasodilatation, exaggerated peristalsis, etc. It may also act on central nervous system, resulting in a variety of motor disorders.

Colchicine is highly toxic to mammals, and more generally to warm blooded species (Wallace and Singer 1988). The oxidized form of the substance, oxycolchicine is the most toxic. In fish as in other poikilotherms, published data are rare. Acute toxicity tests have shown that colchicine has a low toxicity, since these species cannot oxidize this molecule (Derivaux and Liegeois 1962).

This work was intended to show that chronic exposure resulted in high increased toxicity. For this purpose the lethal concentration (LC-50) of colchicine to fish and its evolution with time were determined, using a standardized method: NF T90-303 (AFNOR, French Standards Bureau : Association Française de Normalisation, Tour Europe, Cedex 7, F92049 Paris La Défense) on *Brachydanio rerio*. These results were used to calculate the concentration considered as having no lethal effect.

MATERIALS AND METHODS

Fresh water zebrafish (*Brachydanio rerio*, adults, 3 cm ± 1), were obtained from local enterprise specialized in fish aquaria equipment. They were acclimated to test conditions during 10 d. French standard NF T90-303 (AFNOR 1985a) was rigorously applied for the determination of the 24- to 96-hr LC50. Tests were done in "dilution water", also called "synthetic river water" ($CaCl_2\ 2H_2O$: 11.76 g/L, $MgSO_4\ 7H_2O$: 4.93 g/L, $NaHCO_3$: 2.59 g/L, KCl : 0.23 g/L). The tests were run in 5-L tanks with a range of five concentrations determined in preliminary tests. Temperature was held constant at 23°C.

Five fishes were placed in 2 L of "dilution water" previously aerated with air, 10 animals being used for each concentration of colchicine and for control. At the end of the exposure period, dead fish were counted, and pH and dissolved oxygen

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were determined. No changes in pH (7.8) or in oxygen levels (saturation) were observed. No mortality was observed in control fish.

In parallel and according to the test requirements, the toxicity of potassium dichromate (reference substance) was determined in order to verify the compliance of the group of animals and determine its reactivity. The 24-hr LC50 of potassium dichromate for the fish population was between 130 and 257 mg/L (Table 1). These values were within the range of AFNOR standards and argued that the sample population complied with the required qualities.

For longer exposure, fish were held in 2 L of newly prepared medium for 6-d periods during which the medium was continuously aerated by bubbling. Ten animals were used for each concentration of colchicine and for control. The threshold concentration was sought during a maximum period of 18 d. None of the fish, including controls, received food. This condition was chosen to avoid possible interactions of toxicant with food pellets or feces. At the end of the exposure, no mortality was observed in control starved fish.

Test concentrations of colchicine were 0, 10 mg/L, 13.3 mg/L, 25 mg/L, 50 mg/L, 100 mg/L, 600 mg/L, 800 mg/L, 1000 mg/L, 1200 mg/L and 1500 mg/L. Determinations of pH and dissolved oxygen were made at the end of each period and revealed no changes over time.

A gaussian-logarithmic relationship between percentages of mortality and drug concentration was established and was used with the computerized probits method to calculate the LC10, LC50 and LC90 values, as well as their 95% confidence limits (Finney 1981).

Colchicine was graciously obtained by Roussel UCLAF Society (Paris, France). Dichromate potassium and salts necessary for "dilution water" were purchased from Farmitalia Carlo Erba Firm (Milan, Italy).

RESULTS AND DISCUSSION

Acute toxicity tests on *Brachydanio rerio* showed that LC50 of colchicine was relatively high after 24-hr exposure : between 838 and 1382 mg/L. Increased duration of exposure resulted in severely enhanced toxicity as shown by the steep decrease of LC50 values between 24 and 96 hr. The curve of lethal concentrations as a function of exposure time (Fig.1) cannot be described by a single logarithmic or exponential curve. It shows two phases.

Between 1-d and 6-d the curve shows an abrupt drop. Its equation is :
$Y = 3488 * 10^{(-0.328*X)}$, where Y = LC50 (mg/L) and X = days. Its regression coefficient is 0.97.
Between 7-d and 18-d, LC50 decreases more slowly. The curve can be described by an exponential function whose equation is : $Y = 41{,}1 * 10^{(-0.037*X)}$, (where Y = LC50 (mg/L) and X = days) with a regression coefficient R=0.96.

Due to the complexity of the evolution of LC50 with time, it is not possible to calculate the threshold LC50, i.e., the LC50 for infinite time. Nevertheless, it can be empirically estimated using the LC50 obtained for the last periods (15 to 18-d). These values are close to 9 mg/l and are approximately 10% of the 96-hr LC50. We have used the 15-d LC10 and its confidence interval to determine the

concentration without effect, that can be estimated at 0.9 mg/L. The high values found for 24-hr and 48-hr LC50 of colchicine (1000 mg/L), tend to show that this substance is relatively harmless for acute exposure. Similar results were obtained in frogs (Fühner 1920). The LC50 decreased abruptly between 24-hr and 96-hr, suggesting the existence of a latency time between the administration of the product and the manifestation of its acute toxicity.

The threshold LC50 was below $1/100^{th}$ of the 24h-LC50. In other terms, prolonged exposure to very low concentrations and without special precautions concerning preservation of the product in solution, caused low chronic mortality.

Table 1. LC10, LC50, LC90 rates (mg/L) of potassium dichromate and colchicine in *Brachydanio rerio*, obtained by probit method. Nf= number of fishes, Nc= number of concentrations used, [ ] = 95% confidence limits when Nc>3.

Chemical	Nf	Nc	LC10	LC50	LC90
Potassium dichromate: 24-hr LC	10	5	102.8 [42.3-249.9]	182.9 [130.1-257.3]	325.5 [176.9-599.0]
Colchicine : 24-hr LC	10	4	673.4 [378.2-1199.0]	1075.8 [837.6-1381.7]	1718.6 [929.4-3177.9]
48-hr LC	10	4	709.9 [125.7-4009.7]	964.8 [449.8-2070.1]	1311.3 [281.1-6117.8]
72-hr LC	6	3	373.5	686.2	3613.0
96-hr LC	6	3	51.2	112.1	626.4
5-d LC	6	3	20.1	90.0	243.6
6-d LC	6	4	13.2 [0.82-212.7]	32.4 [10.6-99.0]	79.4 [8.8-714.7]
7-d LC	6	4	17.5 [1.2-255.0]	25.2 [4.8-133.2]	36.3 [2.5-530.1]
8-d LC	6	3	11.0	18.6	31.4
12-d LC	6	4	12.9 [4.5-36.4]	15.0 [5.8-39.4]	17.6 [3.6-86.6]
13-d LC	6	3	11.8	14.2	17.2
14-d LC	6	3	9.2	13.3	19.3
15-d LC	6	4	8.4 [0.9-78.8]	10.9 [4.7-25.9]	14.3 [2.0-103.6]
16-d LC	6	2	8.5	10.0	11.7
17-d LC	6	2	8.5	10.0	11.7
18-d LC	6	2	7.8	9.4	11.4

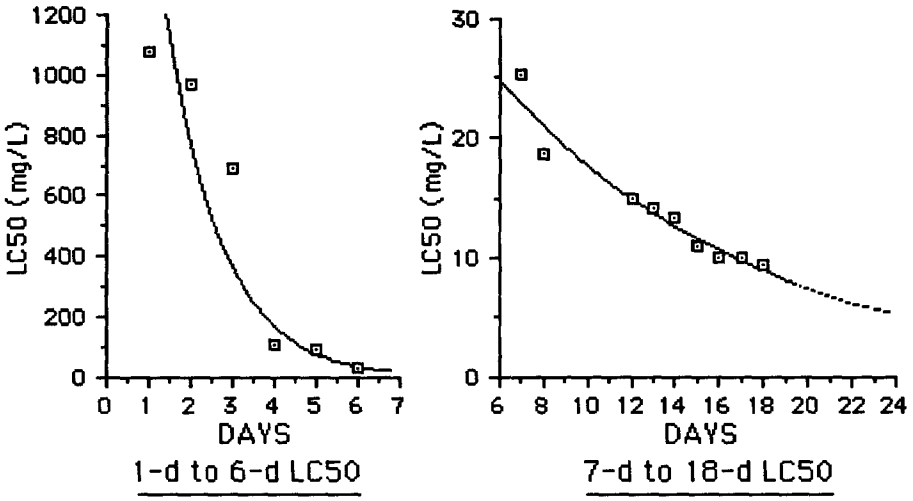

Figure 1. Time dependence of colchicine LC50 in *Brachydanio rerio*. (Results of table 1 were used to plot the evolution of LC50 between 1 and 6 days and between 7 and 18 days).

Questions are thus in order concerning this delay which could result from a low absorption of colchicine from the water, which is undoubtedly related to the temperature of the surrounding environment, or from a low degradation of this molecule as generally accepted. The octanol/water partition coefficient for colchicine : 1.03, suggests that this compound is probably poorly absorbed during the first days of exposure.

Concerning the mechanism of action of this substance, data are lacking in fish. The low toxicity in the initial hours of treatment, in contrast to its very low threshold LC50, suggests a severe physiological dysfunction occurring after a delayed absorption in fish. The analysis of significant blood parameters of a salt water species (AFNOR 1985b), *Dicentrarchus labrax*, whose colchicine resistance is equivalent to that of *Brachydanio rerio* (48h-LC50 = 1020 mg/l), is now in progress by Roche and Bogé.

In conclusion, these results have shown that colchicine has low toxicity in *Brachydanio rerio* after short exposure, but leads to considerable lethality after prolonged exposure.

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