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BIOCHEMICAL ALTERATIONS INDUCED BYSHORT AND LONG TERM EXPOSURE OF CHROMIUM SULPHATEON THE FRESHWATER FISH *CIRRHINUS MRIGALA* (HAM.)

Dr.B.Dhanalakshmi

Assistant professor, Post Graduate and Research Department Zoology, Nirmala College for Women, Coimbatore-18.

ABSTRACT: The present study was aimed to observe the impact of chromium (Cr) on the cholesterol level of liver, kidney, muscles, gills and brain of *Cirrhinusmrigala* fingerlings and experiments were designed having a control without Chromium sulphate 0.25ppm, respectively in triplicates. Among the selected tissues decreasing trends were observed in the cholesterol levels of experimental fish during short term and long term exposures in all tissues.During short term exposure within the tissues the decrease in cholesterol trend was noted to be Gills> Liver> Muscles> Brain > Kidney while long term exposure it was observed to be Gills> Kidney> Liver> Brain > Muscles.

Key words: Heavy metal**,** *Cirrhinusmrigala,* fish tissues, exposure period

*Corresponding author: Dr.B.Dhanalakshmi, Post Graduate and Research Department Zoology, Nirmala College for Women, Coimbatore-18, India

INTRODUCTION

Aquatic pollution due to heavy metals contamination is a unique problem that it is now threatening to become wide spread castrophe(Mukherjee, 1922) by bringing devastating effects on the ecological balance of therecipient environment, on diversity of aquatic organisms (Farombi *et al*. 2007) and have been recognized as a serious pollution problem along the food chain especially with fish.Biomolecules (Proteins, carbohydrate, and cholesterol) derived from fish which constitute the major component of the body, plays an important role in body construction and energy metabolism.All the biomolecules are involved in major physiological events and therefore assessment of fish biomolecules especially cholesterol can be considered as a diagnostic tool todetermine the physiological phases of organism (Kapilamanoj and Ragothaman, 1999) as fishes form the major component of the food chain.Fish, major macro-vertebrate of aquatic food chain when exposed to elevated levels of metals in a polluted aquatic ecosystem, they tend to take these metals up from their direct environment (Seymore 1994). The metals are brought into contact with the organs and tissues of the fish and consequently accumulated to a different extent in different organs and tissues of the fish and brings change in biochemical compositions. Therefore an attempt has been made to study the possible impact of heavy metal Chromiumsulphate on some biochemical aspect of a fresh water fish *Cirrhinusmrigala* (Hamilton).The present work involved the determination of Cholesterol in the tissues like liver, muscles, gills, kidney and brain of the fresh water fish *Cirrhinusmrigala* (Hamilton) exposed to acute and chronic period.

MATERIALS AND METHODS

In the present study (8±1.04 g of weight, 12±3.55 cm in total lengthas mean ± S.E), ranging *Cirrhinusmrigala* were procured from the local lake in and around Coimbatore city and also from Aliyar reservoir and were transferred to the laboratory in suitable polythene bags containing oxygenated water. The laboratory was illuminated for 12h with fluorescent lamps.The fishes were acclimatized to the laboratory condition for 2 weeks in large cement tank (6'x 4' x 3') at 24±3°C with dechlorinated water. The tank was washed using 1% $KMno_4$ to prevent fungal infection prior to stocking.

The fishes were fed with ad-libitum and minced boiled egg. Fishes of about the same size irrespective of sexes were selected for the experiment. Experimental tanks contained 150 liter of dechlorinated aerated water with sustainable temperature of 23.2±0.1°C, pH 7.8±0.5, dissolved oxygen 7.7±0.6 mg/l and alkalinity 115±3.7 mg/l. Appropriate narrow range of concentrations 10-50 mg was used to find the median lethal concentration, using a minimum of 6 fishes, for each concentration and the mortality was recorded for every 24 hrs upto 96 hrs. It was found as 25 mg for 96 hrs using probit analysis method (Finney, 1971).

From this stock solution various sublethal concentration were prepared for bioassay study.Four groups of fishes in each experimental tank containing 10 were exposed to 0.25 ppm ($1/10^{th}$ of 96 hrs, Lc_{50} values) concentration of the metal Chromium sulphate for 24 hrs 48 hrs, 72 hrs and 10 days,20 days and 30 days respectively. Another group was maintained as control. At the end of each exposure period, fishes were sacrificed and tissues such as liver, gill, muscles, kidney and brain were dissected and removed. The tissues (10 mg) were homogenized in 80% methanol, centrifuged at 35 rpm for 15 minutes and the clear supernatant was used for the analysis of different parameters.Cholesterol was estimated based on enzymatic method using cholesterol esterase Cholesterol oxidase and peroxidase (Richmond, 1973).

RESULTS AND DISCUSSION

In the present investigation the effect of a heavy metal Chromium sulphate on the biochemical nature of cholesterol in the different tissues(liver, kidney, muscles, gills and brain) of freshwater carp, *Cirrhinusmrigala* upon exposure to short term periods have been studied and the values were tabulated and presented as figures (Fig:1-10) and the results were statistically analyzed.

In liver tissue 80.75 mg/dl, 80.72 mg/dl and 80.61mg/dl of cholesterol was recorded in control and 50.10mg/dl, 30.60 mg/dl and 24.60 mg/dl in experiment after 24hrs, 48 hrs and 72 hrs respectively. In long term, 80.32 mg/dl, 80.33 mg/dl and 80.27 mg/dl of cholesterol in control and 40.52 mg/dl, 20.30 mg/dl and 11.43 mg/dl in experimental fish after 10 days, 20 days and 30 days. The mean control values in short term and long term was 80.69 mg/dl and 80.31 mg/dl. Muscle tissue was found to contain 28.25 mg/dl, 28.21 mg/dl and 28.35 mg/dl and 28.33 mg/dl, 28.38 mg/dl and 28.25mg/dl of cholesterol in the different concentration in control for 24hrs, 48 hrs and 72 hrs and 10 days, 20 days and 30 days respectively. The mean control values short in term and long term was 28.27 mg/dl and 28.32 mg/dl.Gills tissues when exposed to 0.25ppm of chromium sulphate recorded 45.23 mg/dl, 45.20 mg/dl 45.30 mg/dl in control and 26.18 mg/dl 22.31 mg/dl and 13.50 mg/dl in 24hrs, 48 hrs and 72 hrs respectively. The amount of cholesterol in gills in long term exposure were 45.50 mg/dl, 45.36 mg/dl and 45.20 mg/dl in control and 20.33 mg/dl 11.55 mg/dl and 4.43 mg/dl in experimental fish after 10 days, 20day and 30days exposure respectively.

In kidney tissues the values were decreased and recorded as 30.63 mg/dl, 23.60 mg/dl and 20.25 mg/dl from control 35.05 mg/dl, 35.10 mg/dl and 35.20 mg/dl. The mean control value in short term in treated fishes was 35.12 mg/dl and long term was 35.37 mg/dl. In long term exposure, kidney recorded as 35.47 mg/dl, 35.33 mg/dl and 35.30 mg/dl of cholesterol in control fishes and it was found to decrease as 15.38 mg/dl, 8.33 mg/dl and 3.49 mg/dl after 10 days, 20day and 30days exposure respectively.The amount of cholesterol in the brain of the control fishes recorded were 25.05 mg/dl, 25.15 mg/dl and 25.25 mg/dl in short term while 25.29 mg/dl, 25.42 mg/dl and 25.42 mg/dl in long terms exposures. In experimental fish the values recorded were 15.61 mg/dl, 20.25 mg/dl and 13.92 mg/dl in 24hrs, 48 hrs and 72 hrs and 13.32 mg/dl, 9.27 mg/dl and 5.45 mg/dl for 10 days, 20day and 30days exposure respectively.

The values were also found to be decreased in short term exposures in all tissues. Within the tissues the decrease in cholesterol trend was Gills> Liver> Muscles> Brain > Kidney. The values were found to decrease in long term exposures also in all tissues. Within the tissues the decrease in cholesterol trend was Gills> Kidney> Liver> Brain > Muscles.The percentage values ranges within 69.48>37.93 mg/dl in liver, 54.85>12.89 mg/dl in muscle, 76.99>42.12 mg/dl in gills, 42.47>12.61 mg/dl in kidney 44.87>37.69 mg/dl in brain during short term exposure.

During long term exposure the percentage values ranges between 85.76>49.55 mg/dl in liver, 73.74>35.65 mg/dl in muscle, 90.20>55.32 mg/dl in gills, 90.11>56.64 mg/dl in kidney 78.56>47.33 mg/dl in brain respectively. The value of cholesterol was found to be significant in all tissues and maximum percentage decrease was recorded as 76.99% in gills at 72hrs exposures during short term period. During long term exposure the maximum percentage decrease was recorded as 90.20% in gills in 30days exposures during long term period.
The natural physiological functioning of organism gets disturbed on exposure to toxicant stress. A toxicant induces its effect first at cellular or even at molecular level, but ultimately causes physiological, pathological and biochemical alterations. It is therefore necessary to focus attention on changes in biochemical composition of fresh water and marine water organisms, which are constantly under pollutant threat (Yeragi *et al*., 2003).The aquatic medium is a very efficient solvent for many chemical compounds. Consequently aquatic organisms are extremely vulnerable to toxic effects resulting from absorption or oral intake of these contaminants from the immediate environment. Various chemical compounds had been investigated to determine their potential toxicity to certain organisms, especially fish (David *et al*., 1969; Rand and Petrocell, 1985).The release of heavy metals as byproducts of small scale industries near by the water bodies and consequent contamination of water bodies into which the effluents are released have created great metabolic hazards in aquatic organisms (Chandravathy and Reddy,1994; Das *et al*., 1994;Sultana and Umadevi,1995; Virk and Kaur,1999; Manoj and Ragothanan,1999).

Cholesterol is an important normal body constituent, used in the structure of cell membranes, synthesis of bile acids and synthesis of steroid hormones. It is an important and prominent lipid present in all living systems and plays an important role in body metabolism. It is known to control cell permeability, hormone regulation and protection against injuries and diseases (West *et al*., 1974). An alteration in biochemical values in fish gives an indication and help to understand the mode of action and type of pollutants (Shareef *et al.*, 1986). To get an insight into the nature of changes that are taking place in the biochemical and physiological nature of various tissues in organisms as a result of pollution by Chromium sulphate, biochemical studies were carried out in the tissues of the fish, *Cirrhinusmrigala* (Hamilton). The results were statistically analyzed and presented in the form of figures.In the above investigation, heavy metal treated *Cirrhinusmrigala* a sharp decline in lipid content was noted in all the tissues on compared to control Fig (1-10). The percentage decrease was found minimum in kidney (12.61%) in 24 hrs exposure and maximum in gills (76.99%) in 72 hrs in short term exposure while in long term exposure the minimum percentage decrease was found in muscle (35.65%) during 24 hrs exposure and maximum in gills (90.19%) during 30 days exposure. Similar reduction in lipids in various tissues was studied by various authors. According to Rao *et al*. (1985) and Rao and Rao (1981) decrease in lipid content of muscles indicates that lipid hydrolysis might be accelerated to derive the energy to overcome pesticidal stress.

Decline in liver cholesterol level has been associated with depressed denovo synthesis, along with greater mobilization of liver cholesterol for the synthesis of corticosteroids (or) their conversion to bile acids, which forms the main route for the excretion of cholesterol (Kondal *et al*., 1988). Decrease in the lipid level of various tissues of *Channapunctatus* and *Tilapia mossambicus* when exposed to atrison and atrizin was observed by Ram and Sathyanasen (1987) and Srinivasan *et al*. (1991).Surinder Virk (1999) have reported the rapid and periodical decline in lipid content of liver when exposed to mixture of metals (Ni and Cr) on *Cyprinuscarpio*. Depletion in the total cholesterol in the brain and liver of *Channapunctatus* on exposure to zinc has been reported by Gargisen *et al*. (1994). Saraswathi (2004) reported the decreased level of cholesterol in various tissues of the fish *Labeorohita* on exposed to the detergent Commando. The titanium dioxide, industrial effluent was found to influence the lipid metabolism in the fish *Oreochromismossambicus* and *Etroplusmaculatus* (VijayaMohanan and Achuthan Nair, 2000). Ansari (1984) in *Mystusvittatus* was observed the dysfunction of several physiological biochemical and behavioural processes on exposed to copper. A similar decline in lipid content in tissues of *Anabas testudineus* upon exposure to Malathion was analysed by Mirsh *et al.* (2004). Arockia Rita and John Milton (2006) have showed decline trend of lipid content in the brain, gill, kidney and muscles upon exposure to Carbomate in fish *Oreochromismossambicus* .Saradhamani *et al*. (2007) observed the decline trend of cholesterol in the tissue of the fish *Labeorohita*.

A fall in lipid level is due to a consequence of high demand of energy during stress condition and when there is minimum of food intake the stored energy like lipid is readily used up to meet the energy demand (Peter Tytler and Peter Calow,1985).

In the present investigation the reduction of lipid content can be taken as a meaning for biochemical indices of Chromium sulphate toxicity to assess heavy metal pollution of that aquatic environment. The present study conclusively deduced the changes in Cholesterol levels in the tissues of heavy metals treated fish reveals that the fish species have the tendency to accumulate heavy metals which will naturally affect the nutritive value of their proximate compositions and all the metabolites studied are found to be sensitive biochemical indicators which reflect change in the normal activities of various functional systems.

Fig. 1. Level of Cholesterol content (mg/dl) in liver tissue of *Chrrinus mrigala* exposed to 0.25ppm of chromium sulphate

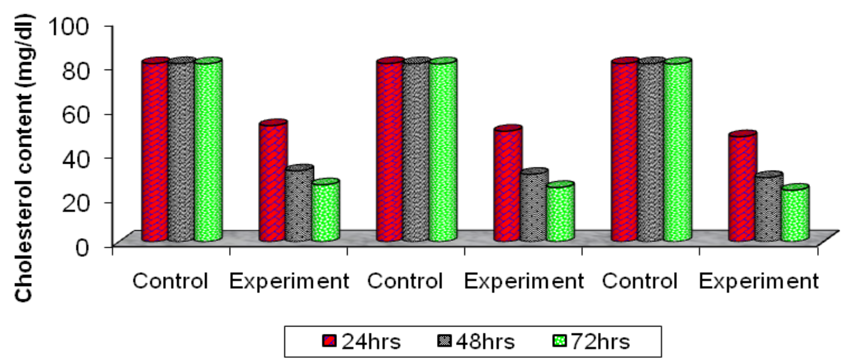


Fig. 2. Level of Cholesterol content (mg/dl) in liver of *Chrinnus mrigala* exposed to 0.25ppm of chromium sulphate

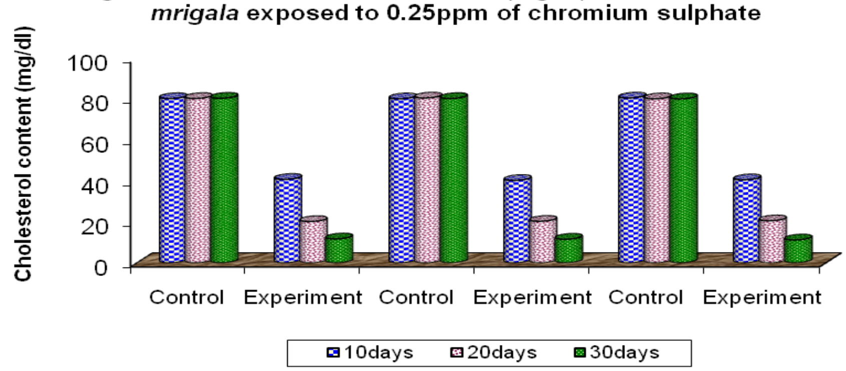


Fig. 3. Level of Cholesterol content (mg/dl) in muscle tissue of *Chrinnus mrigala* exposed to 0.25ppm of chromium sulphate

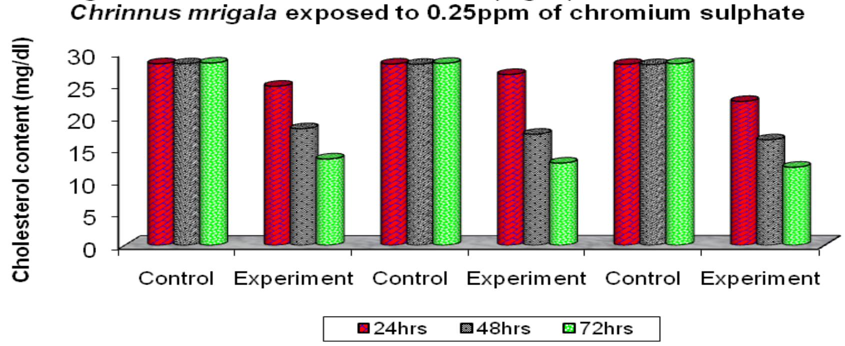


Fig. 4. Level of Cholestrol content (mg/dl) in muscle tissue of *Chrinnus mrigala* exposed to 0.25ppm of chromium sulphate

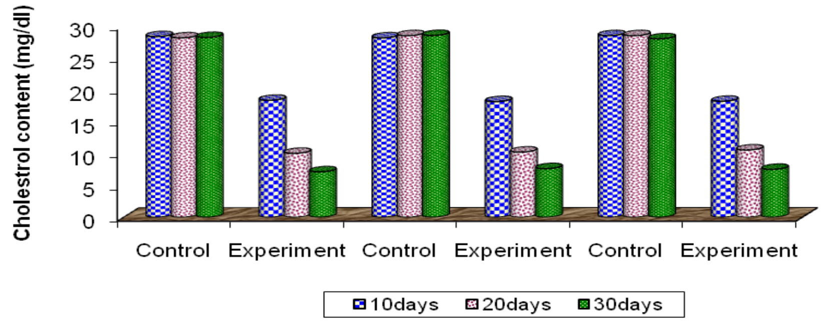


Fig. 5. Level of Cholesterol content (mg/dl) in gill tissue of *Chrinnus mrigala* exposed to 0.25ppm of chromium sulphate

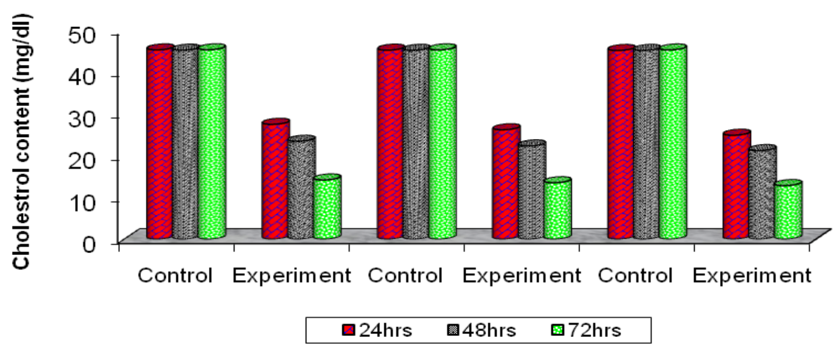


Fig. 6. Level of Cholestrol content (mg/dl) in the gills of *Chrinnus mrigala* exposed to 0.25ppm of chromium sulphate

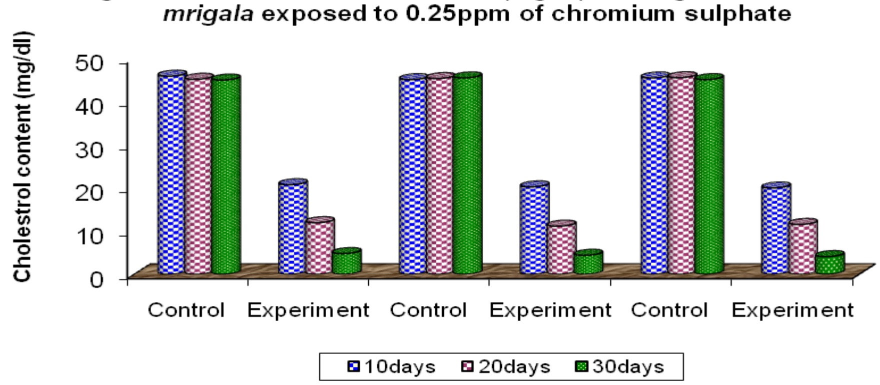
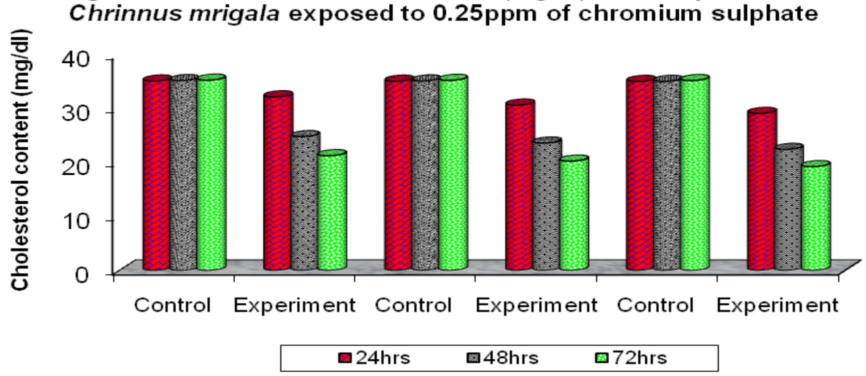


Fig. 7. Level of Cholesterol content (mg/dl) in kidney tissue of *Chrinnus mrigala* exposed to 0.25ppm of chromium sulphate



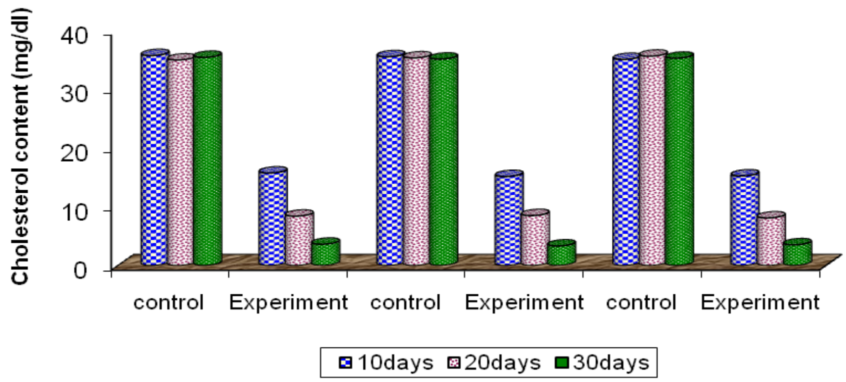


Fig. 8. Level of Cholesterol content (mg/dl) in kidney of *Chrrihnus mrigala* exposed to 0.25ppm of chromium sulphate

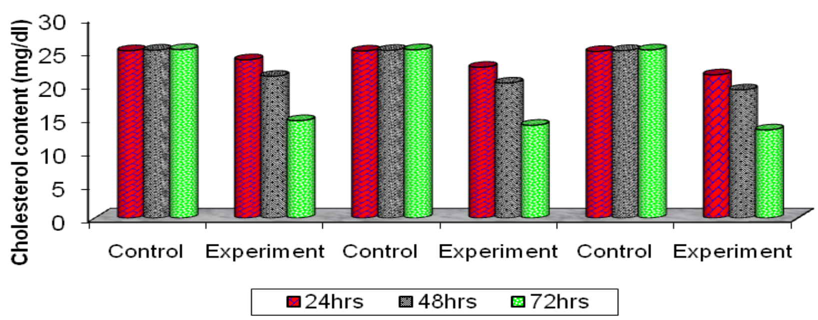


Fig. 9. Level of Cholesterol content (mg/dl) in brain tissue of *Chrinnus mrigala* exposed to 0.25ppm of chromium sulphate

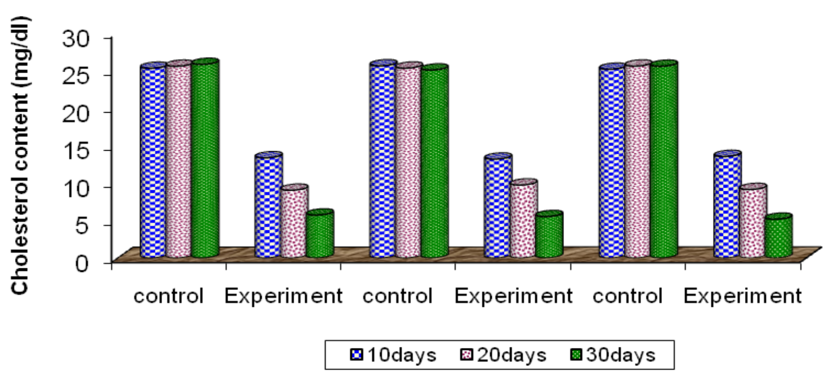


Fig. 10. Level of Cholesterol content (mg/dl) in brain of *Chrinnus mrigala* exposed to 0.25ppm of chromium sulphate

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