Introduction

HEALTH HAZARDS BY AMMONIUM PERCHLORATE

Perchlorate is readily soluble in water and is quickly absorbed through the digestive tract. The mechanism by which perchlorate is transferred from the digestive system to the blood has not been investigated. The perchlorate ion is similar in size to the iodide ion and can therefore be taken up in place of it by the mammalian thyroid gland. In this way, perchlorate can disrupt the production of thyroid hormones and thus disrupt metabolism.

Since Durand (1938) detected perchlorate in the urine of subjects 10 minutes after oral administration, it seems likely that absorption of perchlorate may begin in the stomach and continue in the small intestine. Anbar et al. (1959) determined that perchlorate eliminated in the urine 3 hours after an oral dose had not been metabolized. Whether micro flora of the gut or intestinal enzymes, modify perchlorate that is finally eliminated in the feces has not been investigated.

Whatever the mechanism of absorption, perchlorate is distributed throughout the body via the circulation. It apparently is not metabolized (Anbar et al. 1959) and it binds only weakly to cations. Concentrations of perchlorate rise above serum levels only for those tissues that are equipped with the anion transporter mechanism that
normally takes up iodide. The effects of perchlorate on the thyroid
gland are known from studies on humans and animals (see Section
3.2); perchlorate levels in the thyroid reach a maximum several hours
after administration. Chow and co-workers (Chow and Woodbury
1970; Chow et al. 1969) determined that perchlorate is taken up from
interstitial fluid by active transport at the base of thyroid follicular
cells, which then actively transport it out into the follicular lumen. The
effects of perchlorate on the transfer of maternal iodide in milk have
been studied in rats and cattle (Clewell et al. 2003b; Howard et al.

The accumulation of perchlorate in ducts of the salivary gland
has been described in mice (Lazarus et al. 1974). Studies on rodents
have demonstrated that perchlorate can cross the placental barrier
and affect the thyroid gland of the fetus. The mechanism of transport
of perchlorate into the thyroid gland and other tissues is unknown.
Perchlorate is accumulated in thyroid follicle cells and lumen against
an electrochemical gradient, indicating an active transport mechanism,
and possibly different mechanisms at the basolateral and luminal
membranes (Chow et al. 1969; Chow and Woodbury 1970; Clewell et

Developmental Effects of Perchlorate:

Thyroid hormones are essential for normal development of the
nervous system, lung, skeletal muscle, and possibly other organ
systems (Forhead et al. 2002; Hume et al. 2001; Porterfield and
Hendrich 1993). The fetus is dependent on maternal thyroid hormones
at least until the fetal thyroid begins to produce T4 and T3 (Zoeller and
Crofton 2000). In humans, this occurs at approximately 16–20 weeks
of gestation. Brain development begins in humans prior to the onset of
fetal thyroid hormone production, with a major growth spurt occurring between 12 and 18 weeks of gestation with the beginning of neuron multiplication (Pintar 2000). This is followed by glial cell multiplication, myelination, and formation of dendritic extensions and synapses, which begin at approximately 18 weeks, reaching their peak near the end of gestation and continuing through postnatal years 1 and 2 (Boyages 2000; Fisher and Brown 2000; Oppenheimer and Schwartz 1997).

The perchlorate could potentially disrupt fetal thyroid hormone status by three mechanisms. Perchlorate inhibition of maternal thyroid iodide uptake, and the resulting suppression in production and levels of maternal thyroid hormones, could limit the availability of thyroid hormones needed for normal fetal development prior to the onset of fetal thyroid hormone production if thyroid function in the mother is compromised. Perchlorate can also cross the placenta and may directly inhibit fetal thyroid iodide uptake and, secondarily, fetal thyroid hormone production. By inhibiting NIS in breast tissue, perchlorate may also limit the availability of iodide to nursing infants, who depend entirely on breast milk for the iodide needed to produce thyroid hormone (Agency for Toxic Substances and Disease Registry 2004). No information is available on the doses in humans that might decrease iodide uptake into breast milk.

Other systemic effects:

Absolute and relative heart weights were significantly decreased in rats treated with 2% potassium perchlorate (approximately 2,327 mg perchlorate/kg/day) in the drinking water for 6 weeks (MacDermott 1992). No gross or microscopical alterations were observed in the heart of rats administered ammonium perchlorate in
the drinking water at doses of up to 8.5 mg perchlorate/kg/day for up to 90 days (Siglin et al. 2000); the weight of the heart was also not affected by exposure to perchlorate. Two recent controlled acute exposure studies in euthyroid volunteers provide information of hematological effects of perchlorate in humans. No alterations in hematological parameters (complete blood count and routine chemistries) were observed in a group of nine male subjects who consumed once a day for 14 consecutive days a solution of potassium perchlorate that provided 10 mg of perchlorate/day (Lawrence et al. 2000).

MacDermott (1992) observed a decrease in membrane potential and in intracellular potassium ion activity in skeletal muscle from rats treated with 2% potassium perchlorate (approximately 2,327 mg perchlorate/kg/day) in the drinking water for 6 weeks. The observed changes are consistent with a decrease in the number of sodium-potassium pump units in the muscle. No evidence of liver toxicity, as judged by blood chemistry tests, was observed in a group of nine volunteers who ingested approximately 0.14 mg of perchlorate/kg/day as potassium perchlorate for 14 consecutive days (Lawrence et al. 2000). Other endocrine effects, other than thyroid, reported in perchlorate-treated animals included pituitary hypertrophy and hyperplasia (Pajer and Kalisnik 1991), reduced serum growth hormone levels (Ortiz-Caro et al. 1983), and reduced serum insulin (Tarin-Remohi and Jolin 1972).

**MECHANISMS OF TOXICITY**

Perchlorate is an inhibitor of NIS, the primary mechanism by which iodide enters thyroid follicle cells from the blood, and the first step in the uptake of iodide into the thyroid and formation of thyroid
hormones (Carrasco 1993; Taurog 2000; Wolff 1998). All toxic effects of perchlorate on the thyroid hormone system derive directly or secondarily from this mechanism. The thyroid hormone, T3, is essential for normal development of the nervous system and for the regulation of metabolism of cells in nearly all tissues of the body. Adverse effects on a wide variety of organ systems can result from disruption in the availability of T3 to target tissues. Organ systems affected by disturbances in T3 levels include the skin, cardiovascular system, pulmonary system, kidneys, gastrointestinal tract, liver, blood, neuromuscular system, central nervous system, skeleton, male and female reproductive systems, and numerous endocrine organs, including the pituitary and adrenal glands.

T3 exerts its wide range of actions by binding to thyroid hormone receptors (TRs) in the cell nucleus, which, when bound with hormone, modulate the transcription of a variety of genes (Anderson et al. 2000). TRs consist of a family of structurally similar proteins within the so-called steroid receptor superfamily that includes receptors for steroid hormones, vitamin D, retinoic acid, and peroxisomal proliferator activators (Lazar 1993). Each receptor has DNA binding domains capable of forming two zinc fingers; the sequence of the latter determine hormone receptor specificity to response elements on DNA that modulate gene transcription of hormone-sensitive genes. A ligand binding domain is responsible for conferring specificity for hormone binding.

Modulation of gene expression occurs when the T3–TR complex binds to a region of DNA associated with a thyroid hormone response element (TRE). Studies in humans and experimental animals have identified TREs associated with a variety of genes, including growth hormone, myelin basic protein, α-myosin heavy chain, malic enzyme
and protein S14 (important in lipogenesis), sarcoplasmic reticulum Ca$_2^+$ ATPase, Pcp-2 (in Purkinje cells), Na+/K+-ATPase, and TSH (Anderson et al. 2000; Klein and Levey 2000; Schwartz et al. 1994). Adverse effects on cell metabolism and growth can result from either understimulation or overstimulation of target tissues by T3. The amount of T3 available to target tissues is highly controlled by feedback regulation of the production, secretion, and elimination of both T3 and its metabolic precursor, T4.

Major components of this mechanism include negative feedback control mediated by T4 and T3 of the synthesis and release of thyrotropin-releasing hormone (TRH) in the hypothalamus and of TSH in the pituitary. TRH stimulates the synthesis and secretion of TSH in the pituitary and modulates hypothalamus and the synthesis and secretion of thyroid stimulating hormone (TSH) in the thyrotrophs of the anterior pituitary. Most of the T3 in these tissues derives from local deiodination of T4; as a result, TRH and TSH synthesis and secretion are sensitive to circulating levels of both T3 and T4.

Doses of perchlorate those decrease circulating levels of T3 or T4 can trigger the HPT feedback mechanism to stimulate thyroid growth, including hypertrophy and hyperplasia of follicle cells. Chronic stimulation of thyroid growth is thought to be contributor to the development of thyroid tumors in rats exposed to perchlorate.

**Materials and method**

According to the OECD guide lines for the testing of chemicals

1. Fish, toxicity test: 14-day study
2. Algae growth inhibition test: 15-day study
**FISH TOXICITY TEST**

The test is done according to the OECD guidelines (203 Section 2, July 1992) for measurement of lethal and other observed effects in fish exposed to test substances. Objective of the test was to check the lethal potentiality of the treated effluent of ammonium perchlorate after bio-remediation by each of the 3 bacterial strains. Care was taken to keep the mortality in the controls not to exceed 10 per cent at the end of the test. The dissolved oxygen concentration was maintained with aeration throughout the experiment.

**Test animal:**
Species: Fresh water Fish  
Strains: *Poecillia reticulata*, (Guppy)  
Source: Local fish farm  
Size: 10 to 20mm length  
Sex: Male and females  
Number: 90 fish  
Housing: 10 per aquarium  
Diet: Standard fish food  
Water: Chlorine free water  
Room temperature: 21-25 degrees Celsius  

The species of fish used were selected on the basis their ready availability throughout the year, ease of maintenance, convenience for testing and any relevant economic, biological or ecological factors. The fish were in good health, active and free from any apparent malformation. The fish selected in this study was *Poecillia reticulata* commonly known as guppy fish (Plate 5.1). Test tanks were made of glass and of a capacity to hold 9-10 fish at a time. Calibrated
thermometer and pH meter were used for determination of temperature and pH respectively. Temperature was maintained by heating bulb. Care was taken to restrict maximum loading of 1.0g fish/liter.

The fish were acclimatized for 15 days before use. All fish were exposed to water of the quality to be used in the test for seven days before they were used. Any disturbance that may change the behavior of the fish was avoided.

Ninety fish were exposed to 12 to 16 hours photoperiod daily. The fish were fed once daily. Following a 48-hours settling-in period, mortalities were recorded for the selection of the whole batch. (As per the OECD rules the following criteria was applied: greater than 10 per cent of population in seven days: rejection of entire batch; between 5 and 10 per cent of population: acclimatization must be continued for seven additional days; less than 5 per cent of population acceptance of batch). There was less than 5 percent death and so the entire batch was selected for the test. A fish was presumed to be dead if no respiratory movement and no reaction to a slight mechanical stimulus can be detected.

Effects other than lethal effects: these include all effects observed on the appearance, size and behaviour of the fish that make them clearly distinguishable from the control animals, e.g. different swimming behaviour, different reaction to external stimuli, changes in appearance of the fish, reduction or cessation of food intake, changes in length or body weight. The fish were inspected at least once a day for mortality. Dead fish were removed when observed and mortalities were recorded. Daily records were kept of all observed effects. Measurements of pH and temperature were carried out at least twice a week. Representative samples of the test population should be
weighed and measured before the test starts. All survivors were weighed at the termination of the test.

Duration of the study: Total duration of 14 days.
Number of fish per group: Nine per group.
The fish were grouped in the following manner:
- Control negative: Fresh water
- Control positive: Untreated effluent (150ppm)
- Test group 1A (Treated with *Pseudomonas stutzuri*): 24hrs of treated effluent.
- Test group 2A (Treated with *Pseudomonas stutzuri*): 60hrs of treated effluent.
- Test group 1B (Treated with *Arthrobacter atrocyaneus*): 24hrs of treated effluent.
- Test group 2B (Treated with *Arthrobacter atrocyaneus*): 60hrs of treated effluent.
- Test group 1C (Treated with *Arthrobacter sp. ArthoaeA3*): 24hrs of treated effluent.
- Test group 2C (Treated with *Arthrobacter sp. ArthoaeA3*): 60hrs of treated effluent.

**Algae Growth Inhibition Test**

The purpose of this test is to determine the effects of a substance on the growth of freshwater microalgae. Exponentially growing test organisms are exposed to the test substance in batch cultures over a period of normally 60 hours. In spite of the relatively brief test duration, effects over several generations can be assessed.

The system response was the reduction of growth in a series of algal cultures (test units) exposed to various concentrations of a test substance. The response was evaluated as a function of the exposure
concentration in comparison with the average growth of replicate, unexposed control cultures. For full expression of the system response to toxic effects (optimal sensitivity), the cultures were allowed unrestricted exponential growth under nutrient sufficient conditions and continuous light for a sufficient period of time to measure reduction of the specific growth rate. Growth and growth inhibition were quantified from measurements of the algal biomass as a function of time. Algal biomass is defined as the dry weight per volume, e.g. mg algae/liter test solution. The test endpoint was inhibition of growth decided on the parameter of total biomass weight at the end of the study.

The algal growth control study was performed according to the OECD test 201 (ref OECD1984 section 2). Commonly available algae *Chlorella vulgaris* (Plate5.2) was used for this study. The duration of the test was 14 days.

The test vessels were glass flasks of volume of 1000ml were used. The items were thoroughly washed to ensure that no organic or inorganic contaminants may interfere with the algal growth or composition of the test solutions.

Preparation of growth medium:

For the preparation of growth medium four solutions were essential as defined in Appendix 1. Stock solutions (Appendix 1) were sterilized by membrane filtration (mean pore diameter 0.2 μm) and stored in the dark at 4°C. Stock solutions 2 and 4 were prepared and sterilized by membrane filtration.

Growth medium was prepared by adding an appropriate volume of the stock solutions 1-4 to water: 500 ml of sterilized water was supplemented with 10 ml of stock solution1; 1 ml of stock solution 2;
1 ml of stock solution 3; 1 ml of stock solution 4 and the volume was made up to 1000mL with sterilized water. Sufficient time for equilibrating the medium with the atmospheric CO₂ was given, by bubbling with sterile, filtered air for some hours.

Preparation of inoculum culture:

In order to adapt the test alga to the test conditions and ensure that the algae were in the exponential growth phase when used to inoculate the test solutions, an inoculum culture in the test medium was prepared 2-4 days before start of the test. The inoculum culture was incubated under the same conditions as the test cultures. The increase in biomass was measured to ensure that growth is within the normal range for the test strain under the culturing conditions.

Preparation of test solutions:

All test solutions contained the same concentrations of growth medium and initial biomass of test alga. Test solutions of the chosen concentrations were prepared by mixing a stock solution of the test substance with growth medium and inoculum culture. Stock solutions were prepared by dissolving the substance in test medium.

Untreated effluent containing 150ppm perchlorate was prepared by mixing it with stock solution. Similarly 24 hrs and 60 hrs treated test groups were prepared for all the three test groups in stock solutions. Fifteen grams of algae was inoculated into each of the flask.

Duration of the study: Total duration of 14 days.
Weight per group: 14gms.
The study was divided into the following groups:
Control negative: Fresh water
Control positive: Untreated effluent (150ppm)
Test group 1A (Treated with *Pseudomonas stutzuri*): 24hrs of treated effluent
Test group 2A (Treated with *Pseudomonas stutzuri*): 60 hrs of treated effluent
Test group 1B (Treated with *Arthrobacter atrocyaneus*): 24hrs of treated effluent
Test group 2B (Treated with *Arthrobacter atrocyaneus*): 60 hrs of treated effluent
Test group 1C (Treated with *Arthrobacter sp. ArthroaeroA3*): 24 hrs of treated effluent
Test group 2C (Treated with *Arthrobacter sp. ArthroaeroA3*): 60 hrs of treated effluent

The study is done in triplicate.

Incubation:

The test vessels were capped with air-permeable stoppers. The vessels were shaken and placed in the culturing apparatus. During the test it was necessary to keep the algae in suspension and to facilitate transfer of CO2. To this end constant shaking or stirring was done. The cultures were maintained at a temperature in the range of 21 to 24°C, controlled at ± 2°C in an incubator shaker for 15 days. The pH of the control medium was maintained by ensuring an adequate CO2 mass transfer rate from the surrounding air to the test solution, e.g. by increasing the shaking rate. The surface where the cultures were incubated received continuous, uniform fluorescent illumination.

End point analysis- weight of biomass:

The algal biomass was calculated by removing the excess moisture from the biomass. This was performed by keeping the
biomass in Petri dish and incubating in 35°C for 1 day taking care of not dehydrating the algae completely. After this the algae was weighed on an mono-pan balance (Mettler) before and after the test was over. The difference of weight before and after the test was used as an end point in this study as discussed above. The study was done in triplicate.

**Results and Discussion**

Bioremediated product were to be discarded through aqueous medium and the selected sites were in the sea as discussed earlier thus the toxicity study performed was related to the significance of bioremediated product of ammonium perchlorate on aquatic animals and plant. The whole toxicity study was focused on two organisms that is the fish and algae.

In the Piscean study it was found that the group which was control negative and was untreated could survive through out the test period of 14 days with no change in its behavioral aspects and also increase in body weight. The control positive group of *Poecilia reticulate* could not survive and 6 fishes died within 24hrs. The 3 left died on the second day. Their movement became totally sluggish and food intake was stopped after the first feed. The test 1 A group was exposed to the effluent which was treated for 24 hrs for bioremediation. Six out of nine fish died within 6-7 days. Test group I B and C five out of nine fish were found dead within 5-6 day. Food intake decreased slowly from day to day starting from fourth day and on the sixth day all the fishes except a few stopped taking food. The few which were till taking food were feeding very infrequently. It seemed to be that they had lost the appetite may be due to lack of
nutrition or due to systemic effect of remnant perchlorate. Their alertness to external stimuli decreased (Table 5.1).

The fish which died were subjected to body weight estimation immediately after death both control positive group and test 1 group and the fish which survived in test 1 group was subjected to body weight estimation after 14th day. Their were no change in body weight of six fish which died in the test 1 group and a marginal increase in the body weight was seen in the 3 guppy fish. The forth group, the test 2 group as discussed earlier was exposed to effluent treated for 60 hrs (Table 5.1). There were no mortality seen in test 2 group. The swimming behavior was normal not slow or tilted and misbalanced at one side, food intake was normal and so at the end of the study the Pisceans were weighed their was increase in the body weight, this was similar for all the three groups A, B and C (Table 5.2). They also reacted promptly to external stimuli and all the reflexes were present and so no overall behavioral changes were observed. The whole group was active through out the study.

At the end of the study all the fish were subjected to autopsy of thyroid gland by histological method. Section through Control group and Test 2A, 2B and 2C showed areas of muscule, salivary gland and thyroid tissue. The thyroidal tissue showed acini lined by single to double layer of the cuboidal epithelium. Colloid was seen inside the acini. The impression was of normal looking thyroid (Plate 5.3a and 5.3d).

Section through Test 1A, 1B and 1C showed areas of muscule, salivary gland and thyroidal tissue. It shows acini lined by cells which showed karyolitic and piknotic nuclei. Focal necrotic areas were seen. The impression was of partial necrosis of thyroid (Plate 5.3c).
Sections through Control-positive showed areas of muscule, salivary gland tissue with necrosis and thyroidal tissue with complete necrosis. The lining of the acini did not show any lining nuclei. The impression was of complete necrosis of thyroid (Plate 5.3b).

The inference we could draw from the Fish toxicity test was that the effluent treated by all the three organisms for 60 hrs showed no toxicity at all and the 24 hrs of treatment still proved to be toxic with the presence of the Perchlorate which correlates with the study undertaken in the previous chapter, where the presence of perchlorate was analyzed with the help of ion chromatography.

The rate of algal growth and its increase in biomass is considered to a healthy parameter for the proliferation of *Chlorella vulgaris* in non toxic environment. *Chlorella vulgaris* is highly sensitive to the pollution and its growth is hampered immediately. To study the toxic potentiality of the bioremediated end products *Chlorella vulgaris* was selected as recommended by OECD. The algal toxicity study reveals the similar picture of toxicity as seen above. The weight of the algae in the test group 2A, 2B, 2C had increased by 2.5, 2.2 and 2.8mg respectively. In test group 1A, 1B and 1C the increase in biomass by 1.8, 1.9 and 1.9mg, whereas control group had shown increased biomass of 2.5gms in 14 days. The control positive did not show any growth but at the same time signs of browning of the green algae were observed at the end of the 2nd week at the end of the study. These evidences were clear enough to prove the non toxic potential of bioremediated effluent treated for 60 hrs to algae, one of the most sensitive and primitive stage of a plant kingdom.
Conclusion

To conclude, the above toxicity tests against both the aquatic living organisms from animal and plant kingdom. As proved nontoxic remnants produced by bioremediation of ammonium perchlorate by the strains *Pseudomonas putida, Arthrobacter atrocyaneus* and *Arthrobacter sp. ArthroaeroA3* had proved to be eco-friendly and a most promising, economical, safe and fast bioremediation process proved till date for ammonium perchlorate.
<table>
<thead>
<tr>
<th>Groups</th>
<th>Control -ve</th>
<th>Control +ve</th>
<th>Test group 24hr treatment</th>
<th>Test group 60 hr treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality:</td>
<td>Nil</td>
<td>All within 24hrs</td>
<td>6 within 7 days</td>
<td>Nil</td>
</tr>
<tr>
<td>Swimming behavior:</td>
<td>Normal</td>
<td>Slow</td>
<td>Slow</td>
<td>Normal</td>
</tr>
<tr>
<td>Food intake:</td>
<td>Normal</td>
<td>No intake</td>
<td>Stopped from 4th day</td>
<td>Normal</td>
</tr>
<tr>
<td>Stimulus to external stimuli:</td>
<td>Immediate reflex</td>
<td>Sluggish</td>
<td>Sluggish</td>
<td>Normal reflex</td>
</tr>
<tr>
<td>Body weight (after 14 days):</td>
<td>Increased</td>
<td>No change</td>
<td>No change</td>
<td>Increased</td>
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Table 5.2: Fish mortality data

<table>
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<tr>
<th>Groups</th>
<th>Nos. treated</th>
<th>Days</th>
<th>Total dead</th>
<th>% dead</th>
<th>Dead/treated</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>Nil Nil Nil Nil Nil Nil Nil Nil Nil Nil Nil Nil 0 0 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control positive</td>
<td>9 2 7</td>
<td>9</td>
<td>100 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test 1 A</td>
<td>9 1 3 3 7</td>
<td>5 77.8 0.78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test 1 B</td>
<td>9 1 2 2</td>
<td>5 55.6 0.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test 1 C</td>
<td>9 2 2 1</td>
<td>5 55.6 0.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test 2 A</td>
<td>9 0 0 0</td>
<td>0 0 0</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Test 2 B</td>
<td>9 0 1 1</td>
<td>1 11.1 0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test 2 C</td>
<td>9 0 0 0</td>
<td>0 0 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Groups</td>
<td>Weight of the algae before treatment in mg</td>
<td>Weight of the algae after treatment in mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>--------------------------------------------</td>
<td>-------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>18 +/-0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control positive</td>
<td>14</td>
<td>14 +/-0.3</td>
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<tr>
<td>Test 1 A</td>
<td>14</td>
<td>15.8 +/-0.4</td>
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<td></td>
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<tr>
<td>Test 1 B</td>
<td>14</td>
<td>15.9 +/-0.5</td>
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<tr>
<td>Test 1 C</td>
<td>14</td>
<td>15.9 +/-0.7</td>
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<td></td>
</tr>
<tr>
<td>Test 2 A</td>
<td>14</td>
<td>16.5 +/-0.8</td>
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</tr>
<tr>
<td>Test 2 B</td>
<td>14</td>
<td>16.3 +/-0.7</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Test 2 C</td>
<td>14</td>
<td>16.8</td>
<td></td>
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</tbody>
</table>
Plate 5.1: Guppy Fish

Plate 5.2: Chlorella vulgaris

Courtesy: www.nies.go.jp/images/nies-0642.jpg
Plate 5.3a: Section through Thyroid of Control Negative

- Normal nuclei
- Normal follicles

Plate 5.3b: Section through Thyroid of Control Positive

- Complete necrosis of follicles
Plate 5.3c: Section through Thyroid of Test group 1
(24hrs of treated effluent)

Plate 5.3d: Section through Thyroid of Test group 2
(60 hrs of treated effluent)