TRICAINÉ-S
(tricaine methanesulfonate)
KEEP TIGHTLY CLOSED USE ONLY FRESH SOLUTION
Before using this drug, read package insert for complete product information.
Store at room temperature (Approximately 25°C (77°F))
KEEP OUT OF THE REACH OF CHILDREN
TRICAINÉ-S is intended for the temporary immobilization of fish, amphibians, and other aquatic, cold-blooded animals (poikilotherms) as an aid in handling during manual spawning (fish and amphibians), weighing, measuring, marking, surgical operations, transport, photography, and research.

WARNINGS
Do not use within 21 days of harvesting fish for food. Use in fish intended for food should be restricted to Ictaluridae, Salmonidae, Esoxidae, and Percidae, and water temperature exceeding 10°C (50°F). In other fish and in cold-blooded animals, TRICAINÉ-S should be limited to hatchery or laboratory use. Avoid inhaling or getting into eyes.

CHEMISTRY
TRICAINÉ-S is the methanesulfonate of meta-amino benzoic acid ethyl ester, or simply ethyl-m-amino benzoate. It is thus an isomer of benzocaine having the formula C₈H₇NO + CH₃SO₃H and the following structure:

![Chemical Structure of Tricaine Methanesulfonate]

TRICAINÉ-S is a fine white crystalline powder. Its molecular weight is 261.3. Soluble to 11%, it forms clear, colorless, acid solutions in water.

TOXICOLOGY
Comparative toxicologic studies carried out on fish and frogs gave the following results:
FISH TOXICITY STUDIES - The toxicity of tricaine methanesulfonate was measured by standard methods in laboratory bioassays with rainbow trout, brown trout, brook trout, lake trout, northern pike, channel catfish, bluegill, largemouth bass, and walleye. The 24, 48 and 96 hour LC₅₀ (lethal concentration for 50 percent of the animals) values for trout ranged from 52 to 31 mg/liter; for northern pike, from 56 to 48 mg/liter; for catfish, from 65 to 50 mg/liter; for bluegill and largemouth bass, from 61 to 39 mg/liter; and for walleye, the values were 49 to 46 mg/liter.

Safety index: The safety indices for tricaine methanesulfonate refer to the margin between concentrations which cause anesthesia and mortality. They are expressed by the quotient of the lethal concentration for 50 percent of the fish (LC₅₀) and the effective concentration for 50 percent of the fish (EC₅₀).

Table 1 - Concentrations Required for Rapid Anesthesia continued

<table>
<thead>
<tr>
<th>Fish</th>
<th>Temperature</th>
<th>Concentration (mg/liter)</th>
<th>Max. tolerated exposure time* (min)</th>
<th>Recovery time in fresh water (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonidae</td>
<td>7 – 17°C</td>
<td>80 – 135</td>
<td>4 – 12</td>
<td>3 – 19</td>
</tr>
<tr>
<td>(Pacific and Atlantic salmon; trout; chars; etc.)</td>
<td>(45 – 63°F)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esocidae</td>
<td>8 – 12°C</td>
<td>150</td>
<td>8 – 28</td>
<td>8 – 31</td>
</tr>
<tr>
<td>(Northern Pike; muskellunge)</td>
<td>(46 – 54°F)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Maximum tolerated exposure time (in minutes) of fish to TRICAINÉ-S solution.

Table 2 - Concentrations Required for Moderately Rapid Anesthesia

<table>
<thead>
<tr>
<th>Fish</th>
<th>Temperature</th>
<th>Concentration (mg/liter)</th>
<th>Max. tolerated exposure time* (min)</th>
<th>Recovery time in fresh water (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonidae</td>
<td>7 – 17°C</td>
<td>50 – 60</td>
<td>3 – 12</td>
<td>3 – 19</td>
</tr>
<tr>
<td>(Pacific and Atlantic salmon; trout; chars; etc.)</td>
<td>(45 – 63°F)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ictaluridae</td>
<td>7 – 27°C</td>
<td>70 – 80</td>
<td>10 or &gt;</td>
<td>1 – 10</td>
</tr>
<tr>
<td>(Channel catfish)</td>
<td>(45 – 81°F)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3 - Concentrations Required for Sedation (Induction within 15 minutes used in fish transport)

<table>
<thead>
<tr>
<th>Fish</th>
<th>Temperature</th>
<th>Concentration (mg/liter)</th>
<th>Maintenance of Sedation (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonidae</td>
<td>7 – 17°C</td>
<td>15 – 30</td>
<td>6</td>
</tr>
<tr>
<td>(Pacific and Atlantic salmon; trout; chars; etc.)</td>
<td>(45 – 63°F)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esocidae</td>
<td>10 – 27°C</td>
<td>20 – 40</td>
<td>6</td>
</tr>
<tr>
<td>(Chain pickerel)</td>
<td>(45 – 81°F)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Additional exposure following medullary collapse may result in mortality. A rough estimate of the safe total exposure can be made by multiplying the time required for anesthesia by a factor of 2 or 3.

Table 4 - Methods of Application

<table>
<thead>
<tr>
<th>Method of Application</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery</td>
<td>Ocular</td>
</tr>
<tr>
<td>Methods of Application</td>
<td>Description</td>
</tr>
</tbody>
</table>
| General anesthesia    | For most situations where rapid or moderately rapid anesthesia is required, TRICAINÉ-S may be applied in bath, i.e., the fish are immersed in a saline solution of tricaine methanesulfonate, or when the solutions become fouled with mucus or excrement. Additionally, excess exposure as avoided by observing the following sensory and motor responses of the fish which characterizes progressively deeper levels of anesthesia:

- Sedation - Decreased reactivity to visual and vibrational stimuli; opercular activity reduced.
- Total loss of equilibrium - Fish turns over; locomotion ceases; fish swims or extends fins in response to pressure on caudal fin or peduncle.
- Total loss of reflex - No response to pressure on caudal fin or peduncle; opercular rate slow and erratic.
- Medullary collapse - Opercular activity ceases. Laboratory and field investigators have shown that the action of TRICAINÉ-S is readily reversed when the fish are transferred to fresh water before they are overanesthetized. Excessive exposures are avoided by observing the following sensory and motor responses of the fish which characterize progressively deeper levels of anesthesia:

- Sedation - Decreased reactivity to visual and vibrational stimuli; opercular activity reduced.
- Total loss of equilibrium - Fish turns over; locomotion ceases; fish swims or extends fins in response to pressure on caudal fin or peduncle.
- Total loss of reflex - No response to pressure on caudal fin or peduncle; opercular rate slow and erratic.
- Medullary collapse - Opercular activity ceases. Laboratory and field investigators have shown that the action of TRICAINÉ-S is readily reversed when the fish are transferred to fresh water before they are overanesthetized. Excessive exposures are avoided by observing the following sensory and motor responses of the fish which characterize progressively deeper levels of anesthesia:

- Sedation - Decreased reactivity to visual and vibrational stimuli; opercular activity reduced.
- Total loss of equilibrium - Fish turns over; locomotion ceases; fish swims or extends fins in response to pressure on caudal fin or peduncle.
- Total loss of reflex - No response to pressure on caudal fin or peduncle; opercular rate slow and erratic.
- Medullary collapse - Opercular activity ceases. Laboratory and field investigators have shown that the action of TRICAINÉ-S is readily reversed when the fish are transferred to fresh water before they are overanesthetized. Excessive exposures are avoided by observing the following sensory and motor responses of the fish which characterize progressively deeper levels of anesthesia:

- Sedation - Decreased reactivity to visual and vibrational stimuli; opercular activity reduced.
metabolic wastes. Also, some workers suggest pre-tranport sedation for several hours to lower metabolism. With distribution units, the fish may be fasted and sedated prior to loading. The anesthetic solution is prepared in the distribution unit and oxygenated. Then, the fish are added and temperature acclimated.

In air shipments, the anesthetic solution is placed in a suitable plastic bag, the sedated fish are added, the bag inflated with oxygen, tied securely, and placed in a second bag. This bag is also tied, and then placed on ice in insulated containers. A modification of this method involves complete anesthesia of the fish, and placing them in water bags which contain no anesthetic. In any case, upon arrival, the fish should be acclimated slowly to new environmental temperatures.

**PREPARATION OF TRICAINE-S SOLUTIONS**

Prior to use, TRICAINE-S may be weighed out into amounts which are convenient for the volume of water to be used. A handy unit is 2 g since this quantity in 5 gallons of water yields a concentration of about 100 mg/liter. For rough approximations, one level teaspoonful contains 2.0 to 2.5 g. Thus, a level teaspoonful of anesthetic in 5 gallons gives a concentration of about 120 mg/mtlter.

To convert mg/liter into a ratio of TRICAINE-S to water: divide 1,000,000 by the number of mg.

To convert mg/liter into a ratio of TRICAINE-S to water: divide 1,000,000 by the number of mg.

To get a concentration of about 120 mg/liter.

With distribution units, the fish may be fasted and sedated prior to distribution.

**LIMITATIONS IN USE**

Since TRICAINE-S is taken up into the blood of fish, residues of the drug may occur in edible tissues. However, the residues dissipate rapidly after the fish are placed in fresh water. Use in fish intended for food should be restricted to Ictaluridae, Salmo, Equus, and Percidae, and water temperature exceeding 10°C (50°F).

Withdrawal in fresh water is unnecessary for non-food fishes such as goldfish, bilt, and ornaments. Also, withdrawal is unnecessary for sublegal sizes of the following species of fish because they are not used as food immediately following anesthesia (Table 4).

**PRECAUTIONS**

1. Avoid inhaling TRICAINE-S or getting it into the eyes.
2. Always conduct preliminary tests with TRICAINE-S to determine desired rates of anesthesia and optimal length of exposure.
3. Do not overexpose fish to lethal levels of TRICAINE-S.
4. Do not overexpose fish to lethal levels of TRICAINE-S. Too long a period of anesthesia for the time noted.
5. Do not discard TRICAINE-S solutions into water supplies or natural waters.

REFERENCES

   b. Butler, E.S., Princeton University, Dept. of Biology, Princeton, N.J.
   d. Del, W., City College, Dept. of Biology, New York, N.Y.
   e. Cosil, R.D., Brown University, Providence, R.I.
   f. Kollros, J.J., State University Iowa, Iowa City.
   h. Ross, S.M., University of Illinois, Urbana, Ill.
   i. Schatzmann, J.H., Harvard Medical School, Boston, Mass.
   j. Taylor, A.C., Rockefeller Institute of Medical Research, New York, N.Y.
   k. Thornton, C.S., Kenyon College, Dept. of Biology, Gambier, Ohio.
   o. Knight, A.E.: Intracellular hemoglobin crystallization in two centrarchids, the large-mouth bass and the bluegill. Progressive Fish Culturist 26:115 (no. 3) 1964.
   r. Webb, R.T., Distribution of bluegill treated with tricaine methanesulfonate (MS-222), Progressive Fish-Culturist 20:69 (no. 2) 1958.

**II. GUIDELINES FOR USE ON AMPHIBIANS**

**Table 5 - Effects of Varying Concentrations of TRICAINE-S on Amphibians**

<table>
<thead>
<tr>
<th>Species</th>
<th>Concentration</th>
<th>Anesthesia Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ambystoma opacum</strong></td>
<td>1:3,000</td>
<td>2 days</td>
</tr>
<tr>
<td><strong>Ambystoma tigrinum</strong></td>
<td>1:3,000</td>
<td>10 - 15 min.</td>
</tr>
<tr>
<td><strong>Ambystoma maculatum</strong></td>
<td>1:2,000</td>
<td>30 min.</td>
</tr>
<tr>
<td><strong>Ambystoma maculosus</strong></td>
<td>1:2,000</td>
<td>30 min.</td>
</tr>
<tr>
<td><strong>Mudpuppy</strong></td>
<td>1:2,000</td>
<td>30 min.</td>
</tr>
</tbody>
</table>

*When an individual of any of the species listed is exposed at the designated concentration, the data available suggests that the animal may be safely maintained under anesthesia for the time noted. Prolonging exposure to the anesthesia beyond the time indicated may cause deaths. See PRECAUTIONS.*

**AVAILABILITY OF TRICAINE-S**

Bottles of 1 kilogram, 100 grams, 50 grams, and 5 grams.

**Table 6 - Effects of Varying Concentrations of TRICAINE-S on Frogs**

<table>
<thead>
<tr>
<th>Species</th>
<th>Concentration*</th>
<th>Anesthesia Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EBRYO</strong></td>
<td>1:1,000</td>
<td>2 days</td>
</tr>
<tr>
<td><strong>TADPOLES</strong></td>
<td>1:1,000</td>
<td>3 days</td>
</tr>
<tr>
<td><strong>Rana sp.</strong></td>
<td>1:3,000</td>
<td>5 days</td>
</tr>
<tr>
<td><strong>Rana pipiens</strong></td>
<td>1:3,333*</td>
<td>10 - 15 min.</td>
</tr>
<tr>
<td><strong>ADULTS</strong></td>
<td>1:1,000</td>
<td>30 min.</td>
</tr>
<tr>
<td><strong>Leopard frog</strong></td>
<td>1:3,000</td>
<td>30 min.</td>
</tr>
<tr>
<td><strong>Eastern wood frog</strong></td>
<td>1:1,000</td>
<td>5 - 10 min.</td>
</tr>
</tbody>
</table>

*When an individual of any of the species listed is exposed at the designated concentration, the data available suggests that the animal may be safely maintained under anesthesia for the time noted.

**ANADA 200-226, Approved by FDA**

Manufactured by:
Syndel USA
1441 W. Smith Road Ferndale, WA 98248
(800) 283-5292 (360) 384-5898
FAX (360) 384-0270
www.syndel.com
ver. 101118