

EFFECTS OF ANAESTHETICS ON THE BEHAVIOUR OF MULLET FINGERLINGS AND THE SCOPE OF USING THESE IN DIFFERENT FISHERY PROCEDURES

II. Effects of Tertiary Amyl Alcohol, Ether, Quinaldine, Paraldehyde, Pentobarbital Sodium, Phenobarbital Sodium and MS-222 Sandox.

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INTRODUCTION

THE present paper is the continuation of the work by the author published earlier (Durve and Dharma Raja, 1966). Here, seven more anaesthetics in different concentrations have been tried in order to study their effects on the behaviour of fingerlings of the mullet *Liza tade*. This has enabled the author to select suitable anaesthetics—out of thirteen so far experimented with—for fishery work such as transport of live fish, tagging, fin-clipping, weighing, stripping and operating. Earlier work on the subject has been discussed by Durve and Dharma Raja (*op.cit.*). At the end of the present paper, a general review of all the thirteen anaesthetics is given. Material and methods are the same as mentioned in the earlier paper (Durve and Dharma Raja *op. cit.*). All experiments were carried out in freshwater and at temperatures ranging from 27 to 28° C.

PROPERTIES OF THE ANAESTHETICS AND THEIR MODE OF ACTION

Tertiary Amyl Alcohol

It is a hydrocarbon in the form of a volatile liquid with a characteristic odour and burning taste. It is soluble in water and miscible in Ethanol, Benzene, Ether, Chloroform and Glycerol. It has failed to establish itself as a hypnotic for humans but is reported to sedate herbivores and excite dogs and cats. Its contact irritates whereas inhalation causes headache and circulatory collapse at higher dosage. It is a depressant of central nervous system and reduces the volume of air-breathed.

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Ether

A highly inflammable, volatile, organic solvent, soluble in water to some extent and requiring thorough mixing to ensure solution. It is predominantly a narcotic for central nervous system, affecting first the cerebral centres, then the spinal cord and finally the vital centres in the medulla. The site of action appears to be the posterior hypothalamus.

Quinaldine

A colourless oily liquid of quinoline odour that turns reddish-brown on exposure to air. It is very slightly soluble in water but readily dissolves in chloroform, Ether and Acetone. Its mode of action is unknown but is supposed to act like barbiturates as depressant of the central nervous system especially the respiratory centres.

Paraldehyde

A colourless liquid with a characteristic aromatic odour and warm but disagreeable taste. It is soluble in water to some extent but is completely miscible in organic solvents like Ether, Benzene and Acetone.

Pentobarbital Sodium

A barbiturate in the form of a crystalline granular or white powder with a slightly bitter taste, freely soluble in water. It is a hypnotic of moderate duration of action and is largely used in veterinary science.

Phenobarbital sodium

A barbiturate in the form of bitter, slightly hygroscopic crystals or white powder, soluble in water. It is widely used in medical science as a sedative, tranquillizer of prolonged action.

MS-222 Sandoz

A methanesulphonate of *meta*-aminobenzoic acid ethylester in the form of a fine crystalline powder, very soluble in water. It is variously known as Tricaine-Sandoz, Tricaine methane-sulphonate, Metacaine and Metacaine methanesulphonate. It is recommended as a local anaesthetic for humans where it acts on nerve terminals. It is known to reduce activity in cold-blooded animals.

The information given above has been compiled from Merck Index (1960), Bell (1964) and Bove (1965). The behavioural changes in mullet

fingerlings during anaesthesia and the description of the anaesthetic stages are the same as given in the earlier paper by Durve and Dharma Raja (*op. cit.*). These are more or less on a similar pattern as observed by McFarland (1959, 1960).

RELATIONSHIP BETWEEN DOSAGE AND ANAESTHESIA

Tertiary Amyl Alcohol (Appendix I A)

It will be seen from the table that at the concentration of 0.05 ml./100 ml., the majority of fishes remained in light sedation (stage 1). While there was a small percentage of fish remaining in deep sedation (stage 2), those remaining normal (stage 0) also formed a sizable percentage. The next higher concentration of 0.08 ml./100 ml. appears to be ideal as 81.66% fish remained in stage 1 and 18.33% remained in stage 2. There were no fish reaching any higher stage. As for the stage 4 (total loss of equilibrium), the maximum number of fish were in this stage at the concentration of 0.2 ml./100 ml., while there was a small percentage remaining in stage 3 (partial loss of equilibrium). The mortality at this concentration was somewhat high being 18.33%. The number of fish remaining in stage 3 was the highest in the concentration of 0.15 ml./100 ml. The lethal dose was 0.50 ml./100 ml., at which all fish died within three minutes of exposure.

Ether (Appendix I B)

Only four concentrations could be tried in Ether because of its extreme volatility. The lowest was 0.20 ml./100 ml., wherein though the fish reaching stage 2 were more, those remaining in stage 0 were also considerable in number. It could be seen from the table that in three out of four concentrations, there was some percentage of fish remaining in stage 0. This appears to be due to the volatility of the anaesthetic resulting in its disappearance from the water after some time. The lethal dose was 0.60 ml./100 ml. for the majority of fish. A small percentage of fish remained in stage 3.

Quinaldine (Appendix C I)

The concentrations required of this chemical to induce any stage of sedation were the lowest. The concentrations of 0.0009 and 0.001 ml./100 ml. were the best for inducing stage 1 or 2 while that of 0.003 was found suitable for inducing the stage 4, for 92.49% fish reached this stage. The induction and recovery times were also low. The higher concentrations showed increasing mortality and the lethal dose was found to be 0.005 ml./

100 ml. at which mortality was 100% within 5 minutes of exposure. Fairly large proportion of fish reached stage 3 at the concentration of 0.002 ml. 100 ml.

Paraldehyde (Appendix I D)

The observed lowest concentrations were 0.01 and 0.05 at which the majority of fish remained either in stage 1 or 2. Significant increase in the percentage of fish remaining in stage 0 at the concentration of 0.05 could perhaps be an observational error. The highest concentrations suitable for the induction of stage 4 were 0.25 and 0.30 ml./100 ml. The induction and recovery times for these concentrations were low. The sizable number of fish reached stage 3 at the concentration of 0.15 ml./100 ml. Lethal concentration was 0.35 ml./100 ml. wherein all the fish died within 4 to 13 minutes of exposure.

Pentobarbital Sodium (Appendix I E)

The maximum number of concentrations were tried in this anaesthetic. The lowest concentration observed was 0.0005 g./100 ml. and here negligible percentage of fish remained either in stage 1 or 2. It could be seen from the table that even at higher dosage, the fish remaining in stage 0 were significant in number. Concentrations suitable for inducing stage 4 were 0.005 and 0.01 g./100 ml. The periods of induction and recovery for stage 4 were appreciable. The lethal concentration was 0.10 g./100 ml. wherein 90.01% fish died within 30 minutes of exposure. The fish reaching stage 3 were observed only in three concentrations and their percentage was negligible.

Phenobarbital Sodium (Appendix I F)

The lowest concentration of 0.025 g./100 ml. induced stage 1 in a majority of fish but could not sedate sizable number of fish. A slightly higher concentration of 0.05 was better and had some fish reaching stage 3 or even 4. The maximum number of fish reached stage 4 in the concentrations of 0.125 and 0.15 g./100 ml. However, in both these concentrations there was some percentage of fish reaching stages 3 and 2. The induction and recovery times were fairly long. The lethal limit was found to be at 0.20 g./100 ml. at which 85% fish died after 30 minutes of exposure, i.e., the fish did not recover from the stage 4.

MS-222 Sandoz (Appendix I G)

This well known anaesthetic of proved use for cold-blooded animals was found to induce stages 1 and 2 in the majority of fish at concentrations

0.003 and 0.004 g./100 ml. The concentration useful for inducing the stage 4 was 0.01 g./100 ml., wherein 91.00% fish reached the required stage; while there was a small percentage of fish reaching stage 3. The time required for the induction of stage 4 and recovery was appreciably less in the concentration of 0.01 g./100 ml. The lethal limit was at 0.015 g./100 ml. at which 100% fish died within 8 to 10 minutes of exposure. Owing to the non-availability of a sufficient quantity of this chemical, experiments within the concentration range of 0.01 to 0.015 could not be carried out.

Table I gives the selected concentrations of each anaesthetic useful to induce stage 4 (total loss of equilibrium). It will be noticed from the table that the lowest concentrations are required in the case of Quinaldine and Pentobarbital Sodium, followed by MS-222, Phenobarbital, Tertiary Amyl Alcohol and Paraldehyde. Ether requires the highest concentration of 0.40 ml./100 ml. The average time required to reach stage 4 is the highest in Phenobarbital Sodium closely followed by the other barbiturate, Pentobarbital Sodium. The induction time is the lowest in Quinaldine, followed by MS-222. Paraldehyde has a moderately low induction time while Tertiary Amyl Alcohol and Ether have more or less the same induction periods. Since, in all experiments, the time of exposure was fixed at 30 minutes, the recovery time could be compared and it was found that the average recovery time is the shortest in Quinaldine, being only 6.37 minutes at the lowest concentration (Table I). Tertiary Amyl Alcohol stands second, the third place being occupied by Ether. MS-222 also has a short recovery period. In the case of Paraldehyde, the recovery appears to be quick but the values obtained are somewhat misleading. Very long recovery periods were noticed in the case of both the barbiturates. It was more in Phenobarbital than in Pentobarbital. From this, it could be inferred that out of seven anaesthetics studied, Quinaldine appears to be the best from the point of view of the time required to induce anaesthesia and for recovery. It is also economical as the quantity required is considerably low.

With reference to Table I and those in *Appendix*, it could be said that increased dosage of anaesthetics decreases the induction time of stage 4. This observation is in agreement with that of McFarland (1959) and supports the earlier observation of Durve and Dharma Raja (*op. cit.*). It can be inferred from the experimental data that the time required for recovery is more or less proportional to the concentration of the anaesthetic with the exceptions of Tertiary Amyl Alcohol, Ether and Paraldehyde (*see also Durve and Dharma Raja, op. cit.*). As in the earlier series of experiments (Durve and Dharma Raja, *op. cit.*), the fact whether the duration of exposure affects

TABLE I

Concentrations found useful to induce the total loss of equilibrium (stage 4) in the fingerlings of the mullet *Liza tade* with different anaesthetics and the time required for the recovery of the fishes

Anaesthetic	Concentration per 100 ml. of water	Time required to reach the stage 4 (minutes)	Period of exposure to the stage (minutes)	Time required for recovery to stage 0 after the change of water (minutes)	
Tertiary Amyl Alcohol	0.15 ml.	45.10	30	7.62	
	0.20	7.99	30	7.47	
	0.50	0.35	Fishes died within three minutes
Ether	0.40 ml.	46.81	30	10.81	
	0.60	2.83	30	8.4	
Quinaldine	0.002 ml.	7.53	30	6.37	
	0.003	7.09	30	12.13	
	0.004	1.54	30	12.80	
	0.005	1.00	Fishes died within one to seven minutes of exposure
Paraldehyde	0.20 ml.	19.56	30	31.87	
	0.25	6.46	30	19.31	
	0.30	3.89	30	10.00	
	0.35	4.50	Fishes died within four to thirteen minutes of exposure
Pentobarbital Sodium	0.002 g.	108.80	30	55.00	
	0.0025	91.61	30	75.14	
	0.005	46.44	30	60.16	
	0.01	32.79	30	122.50	
	0.10	10.00	30	205.00	Majority of fishes died by 30 minutes of exposure
Phenobarbital Sodium	0.125 g.	151.1	30	204.2	
	0.15	150.93	30	179.28	
	0.20	93.7	30	338.6	Majority of fishes died by 30 minutes of exposure
MS-222 Sandoz	0.01 g.	10.09	30	13.24	
	0.015	3.10	Fishes died within eight to ten minutes of exposure

the time of recovery, could not be tested here as the exposure time for the fish reaching stage 4 was fixed at 30 minutes throughout the work.

Some fish of varying sizes were allowed to reach the stage 5 (loss of reflex reactivity) in MS-222 and then quickly transferred to the freshwater. All the fish recovered fully within about 8 minutes. This supports the earlier observation of Durve and Dharma Raja (*op. cit.*).

Another important observation, though made in the earlier study but not recorded there, was confirmed during the present study. A reversal of anaesthesia or sedation was noticed in the case of some fish. That means, the fish which reached the stage 3 or 4 were at times found to return to the earlier stages of 1 or 2 and remain in these latter stages for rest of the duration of experiment. Such a reversal of sedation was frequent in MS-222.

Table II gives the concentrations suitable for inducing stage 1 or 2 (light and deep sedations). No induction and recovery time was measured at these concentrations except in the case of a few chemicals discussed later. Here, it will be seen that the dosage of Quinaldine and Pentobarbital were the lowest followed by MS-222. The highest dose was of Ether. In all cases, the fish were exposed for the same duration, *i.e.*, 5 hours, and they were observed to tolerate the concentrations very well. The mortality was at a considerably low level except in MS-222 and Phenobarbital where it was a little more. However, on studying the general trend of mortality in these two anaesthetics (also refer *Appendix*, Tables VI and VII), it is felt that the higher mortality at the lowest concentrations of these chemicals is of little significance. Similar mortality was also noticed in the earlier study (Durve and Dharma Raja, *op. cit.*). It will be seen from tables in the *Appendix* that some fish reached stages 3 and 4 even at low concentrations but their percentage was low. The probable reason for this could be the same as discussed in the earlier paper (Durve and Dharma Raja, *op. cit.*).

The presumption in the earlier paper that the anaesthetic in which the induction time for stage 4 is the shortest will also have the shortest induction time for stages 1 and 2, was tested in the present investigation. These tests were made for MS-222. Quinaldine and Phenobarbital Sodium, the first two being quick actors and the third one a slow actor anaesthetic. The presumption was found to be correct. This was also found true for the time of recovery.

TABLE II

Concentrations found useful to induce light or deep sedation (stages 1 and 2) in the fingerlings of the mullet *Liza tade* with different anaesthetics, the period of exposure to these stages and the mortality during and after the experiments

Anaesthetic	Concentration per 100 ml. of water	Period of exposure	Mortality during the experiment and within next 24 hours % fishes
Tertiary Amyl Alcohol	0.05 ml.	5 hr.	Nil
	0.08	5	Nil
	0.10	5	2.50
Ether	0.20 ml.	5	1.67
	0.30	5	1.67
Quinaldine	0.0009 ml.	5	2.50
	0.001	5	2.50
Paraldehyde	0.01 ml.	5	1.67
	0.05	5	Nil
Pentobarbital Sodium	0.0005 g.	5	1.67
	0.001	5	1.67
	0.0015	5	1.69
	0.0016	5	Nil
	0.0017	5	Nil
Phenobarbital Sodium	0.025 g.	5	1.92
	0.05	5	4.989
MS-222 Sandoz	0.003 g.	5	1.27
	0.004	5	10.00
	0.005	5	7.499

TOLERANCE OF FISH TO ANAESTHETICS AND THE NARCOTIC POTENCY

It is needless to mention here the importance of the tolerance of fish to anaesthetics. To study this, experiments as designed by Durve and Dharma Raja (*op. cit.*), were performed and the results are tabulated in Table III. No experiments were performed using Ether as it evaporates and fish which should have been in sedation, look normal after some time,

Further, the experiments with Ether has hazards of heavy inhalation. While in all anaesthetics the tolerance experiments were run for full 24 hours for sixty fish, in Phenobarbital Sodium the majority of fish reached total loss of equilibrium (stage 4) during sometime in experimentation and hence were removed. The reversal of sedation seen in Pentobarbital Sodium (Table III) was observed during the initial hours of the experiment. The mortality

TABLE III
Results of the tolerance test

No. Anaesthetic	Concentration per 100 ml. of water	Total fishes	Average exposure hours	Mortality %		Stage reached	Remarks
				During experiment	Within next 24 hours		
1. Tertiary Amyl Alcohol	0.08	60	24	Nil	5.00	Light sedation	
2. Ether				No experiments were performed			
3. Quinaldine	0.0009	60	24	3.34	Nil	Light sedation	
4. Paraldehyde	0.05	60	24	1.67	1.66	Light sedation	
5. Pentobarbital Sodium	0.0015	60	24	Nil	3.34	Deep and light sedation	Some fishes reached stage 4 during experiment but returned to deep sedation within a short time
6. Phenobarbital Sodium	0.025	60	24	Nil	Nil	Deep sedation	Only 35.0% fishes tolerated the concentration for 24 hours. Rest lost their equilibrium during the course of the experiment and hence were removed.
7. MS-222 San-do	0.003	60	24	Nil	Nil	Light sedation	

was either nil or insignificant in the tolerance tests. The stage of sedation reached was either 1 or 2.

The experiments tend to indicate the suitability of all the anaesthetics except perhaps Phenobarbital Sodium, so far as the tolerance of fish to these chemicals is concerned. Similar observations were made in respect of six other anaesthetics by Durve and Dharma Raja (*op. cit.*) and no further discussion appears necessary regarding tolerance.

The narcotic potency has been calculated for all the anaesthetics used in the present investigation, based on the potency value of 1 given to Tertiary Butyl Alcohol in the earlier investigation (Durve and Dharma Raja, *op. cit.*). Table IV gives the potency values of all the anaesthetics. It will be seen that

TABLE IV

Minimal and lethal doses, narcotic potency and molecular weights of different chemicals used in the study

Sl. No.	Chemicals (Anaesthetics)	Molecular weight	Narcotic potency	Minimal dose ml. or g. % (M.D.)	Lethal dose ml. or g. % (L.D.)
1.	Tertiary Amyl Alcohol ..	88.15	5.99	0.05	0.50
2.	Ether ..	74.12	1.50	0.20	..
3.	Quinaldine ..	143.18	333.40	0.0009	0.005
4.	Paraaldehyde ..	132.16	30.00	0.01	0.35
5.	Pentobarbital ..	248.26	599.90	0.0005	0.10
6.	Phenobarbital Sodium ..	254.22	12.00	0.025	0.20
7.	MS-222 Sandoz	261.30	100.50	0.003	0.015

the highest value (599.90) is for Pentobarbital Sodium next comes Quinaldine (333.40), followed by MS-222 (100.50). The lowest value of 1.50 is for Ether. The earlier observation (McFarland, 1959; Durve and Dharma Raja, 1966) that potency of an anaesthetic is somewhat related to the molecular weight is not well supported by the present observations though some trend in that direction is evident. Ether having the lowest potency has also the lowest molecular weight. Similarly, Tertiary Amyl Alcohol having slightly higher molecular weight has a higher narcotic potency. This trend

TABLE V
A comparative chart of merits and suitabilities of all the anaesthetics studied

No.	Anaesthetic	Solubility	Concentrations useful in inducing stage 4 (For tagging, fin-clipping and operative procedures) g. or ml./100 ml.	Time of induction min.	Time of recovery min.	Permissible exposure min.	Concentrations useful for transport g. or ml./100 ml.	Tolerance Tested hrs.	Narcotic potency	Lethal dose L.D.	Remarks about suitability and reasons
1.	MS-222 Sandoz	Water, very soluble	0.01 g. 0.015	10.09 3.10	13.24 ..	30 Lethal for 8 to 10	0.003 0.004 0.005	24 5 5	100.50	0.015	Suitable for all fishery work but is costly
2.	Chloral Hydrate	Water, very soluble	0.20 g. 0.25	116.87 81.42	20.67 30.92	30 30	0.02 0.025	24 5	30.00	..	Suitable for transport
3.	Tert. Amyl Alcohol	Water, very soluble	0.15 ml. 0.20 0.50	45.10 7.99 0.35	7.62 7.47 ..	30 30 3	0.05 0.08 0.10	5 24 5	5.99	0.50	Suitable for all fishery work
4.	Chlorobutanol	Hot water or Organic solvent	0.010 g. 0.015 0.019 0.020	28.43 5.06 4.25 3.61	11.54 8.50 3.94 ..	30 30 30 Lethal for 30	0.002 .. 0.003	24 .. 5	150.00	0.020	Suitable for all fishery work
5.	Quinaldine	Organic solvent	0.002 ml. 0.003 0.004 0.005	7.53 7.09 1.54 1.00	6.37 12.13 12.80 ..	30 30 30 Lethal for 1 to 7	0.0009 .. 0.001	24 .. 5	333.40	0.005	Suitable for all fishery work
6.	Paraldehyde	Organic solvent	0.20 ml. 0.25 0.30 0.35	19.56 6.46 3.89 4.50	31.87 19.31 10.00 ..	30 30 30 Lethal for 4-13	0.01 .. 0.05	5 .. 24	30.00	0.35	Suitable for all fishery work

7. Tert. Butyl Alcohol	Water	0.50 ml.	97.50	8.08	30			1.00	..	Suitable for transport
		0.60	39.36	10.82	30	0.30	24			
		0.70	32.84	11.32	30	0.35	5			
8. Sodium Amytal	Water, scarcely soluble	0.015 g.	28.60	41.75	30	0.0035	24	85.70	0.035	Suitable for work only in freshwater
		0.020	34.47	82.10	30					
		0.030	24.18	101.73	30	0.0040	5			
		0.035	24.50	145.50	Lethal for 30 min.					
9. Pentobarbital Sodium	Water, very soluble	0.0025 g.	91.61	75.14	30	0.001	5	599.90	0.10	Unsuitable
		0.005	46.44	60.16	30	0.0015	24			
		0.01	32.79	122.50	30	0.0016	5			
		0.10	10.00	205.00	Lethal for 30					
10. Phenobarbital Sodium	Water, very soluble	0.125 g.	151.1	204.2	30	0.025	24 (?)	12.00	0.20	Unsuitable
		0.15	150.93	179.28	30	0.05	5			
		0.20	93.7	338.6	Lethal for 30					
11. Sodium Barbital	Water	0.30 g.	216.00	195.00	30	0.10	16.67	3.00	..	Unsuitable
						0.125	5			
						0.150	5			
12. Urethane	Water, very soluble	0.40 g.	18.70	14.80	30	0.15	24	2.00	0.85	Unsuitable because of carcinogenic properties
		0.70	3.00	20.55	30	0.175	5			
		0.80	3.18	30.75	30	0.200	5			
		0.85	4.00	30.34	Lethal for 30 min.					
13. Ether	Water, scarcely soluble	0.40 ml.	46.81	10.81	30	0.20	5	1.50	..	Unsuitable because of hazards to the worker and rapid volatilization
		0.60	2.83	8.4	30	0.30	5			

holds good up to Paraldehyde, beyond which there appears to be little relation. MS-222 having the highest molecular weight has relatively low potency when compared to Pentobarbital or Quinaldine. Phenobarbital Sodium which has a very high molecular weight has a low potency. It appears that barbiturates are exceptions to this relation of molecular weights to potencies, as this was also evident in the case of Sodium Barbitol in the earlier investigation (Durve and Dharma Raja, *op. cit.*). Further, it could be said that a smaller quantity of anaesthetic is required to induce equinarcotic levels as the molecular weight increases. This supports the earlier observation of Durve and Dharma Raja (*op. cit.*) and several others.

Observations of McFarland (1959) that the lower the molecular weight the lesser is the induction time and *vice versa*, could not be confirmed during the present investigation. Quinaldine and MS-222 having very high molecular weights were found to be quick-acting anaesthetics while Ether and Tertiary Amyl Alcohol with their low molecular weights were found to be slow acting. This is in conformity with the earlier observations of Durve and Dharma Raja (*op. cit.*).

It was found that the size of the fish has little relation to the rate of induction. The smaller fish may attain the stage 4 of the anaesthesia sooner than the larger fish or *vice versa* supporting thereby the earlier observation of Durve and Dharma Raja (*op. cit.*).

Experiments on the metabolism of the anaesthetized fish were not expected to give any different picture than already obtained by Durve and Dharma Raja (*op. cit.*). Hence, these experiments were not repeated in the present investigation.

DISCUSSION

Tertiary Amyl Alcohol has been used in fishery procedures by McFarland (1960). He suggests the dose of 4 to 5 ml./gal. for the total loss of equilibrium (stage 4). Saunders as reported by Bell (1964) found this anaesthetic cheap and effective for tagging Atlantic Salmon smolts. The dosage of 48 ml./gal. has been reported to immobilise sockeye salmon within 2 minutes, that of 5.5 to 6.0 ml./gal was found effective for immobilisation within 8 to 12 minutes and of 5 to 6 ml./litre within 20 to 25 minutes (Bell, 1964). The recovery time ranged from 10 to 30 minutes depending upon the concentration. Bell (*op. cit.*) writes that this anaesthetic has a relatively long induction period and fish show some hyperactivity while recovering,

In the present investigation, dose varying from 0.15 to 0.20 ml./100 ml. is recommended for the fishery work requiring the stage 4 for the fish, provided the fish is to be kept in this stage for not more than 30 minutes. Both induction and recovery time are low. Concentrations ranging from 0.05 to 0.10 ml./100 ml. are recommended for transport. The concentration of 0.08 appears to be the best suited as the fish could tolerate this concentration for 24 hours without any adverse effects or significant mortality (Table III). McFarland (1959) finds the narcotic potency of the chemical as moderate being 3.2 while in the present investigation, it was found to be 5.99. He (McFarland, 1960) recommends this anaesthetic as best suitable for live fish transport at a concentration of 2 ml./gal.

Ether has been employed for anaesthetizing fishes by Griffiths *et al.* (1940), Eschmeyer (1953) and Cherkin and Catchpool (1964). Eschmeyer (*op. cit.*) advantageously used 1.00% aqueous solution of Ether to anaesthetize lake trout *Salvelinus namaycush* for fin clipping. Bell (1964) reports the dosage range of 1.5 to 2.00% v/v which gives immobilization within 1 to 2 minutes and righting within 3 to 20 minutes. In the present investigation, Ether was found to induce stage 4 at the concentrations 0.40 and 0.60 ml./100 ml. However, this anaesthetic was not found suitable for several reasons. It is difficult to get a thorough solution as some Ether evaporates in spite of all precautions. Inhalation of Ether is hazardous but unavoidable when handling it. Fish which appear to sedate return to normalcy after some time when the Ether evaporates. Aeration, if employed, flushes off the Ether. This chemical thus does not find its place in the list of suitable anaesthetics.

Quinaldine has been acclaimed as one of the most suitable fish anaesthetics. Its first use is reported by Muench (1959) who employed the concentrations of 5 to 12 ppm. to induce light or deep sedation. He found no ill effects on fish even after a prolonged treatment of 2 to 3 days. Natarajan and Renganathan (1960) employed Quinaldine at the concentration of 5 ppm. for the transport of breeders. They observed that the concentration required varies with the species of fish, size of fish, the nature of water, etc. Investigation carried out here also indicates Quinaldine as highly suitable for inducing any stage of sedation. Concentrations ranging from 0.002 to 0.004 ml./100 ml. are useful for inducing total loss of equilibrium while those of 0.0009 and 0.001 ml./100 ml. were found the best for transport. The suitability of the former concentration is supported by the tolerance tests. In Quinaldine, both induction and recovery times are the lowest amongst

chemicals studied. The narcotic potency is also high. The drawback is that chemical needs a vehicle like acetone for going into aqueous solution.

Paraldehyde has not yet appeared to have been used in the fishery work though its use in mammals and amphibians is known (Koppanyi and Karczmar, 1948). The author is perhaps the first to try this in fishery work. The chemical requires moderately low concentrations to induce sedation and the induction and recovery periods are low. There are no side effects and the fish tolerate the chemical well. This chemical also requires a vehicle for proper solubility in water.

Pentobarbital Sodium is next to Quinaldine in its ability to induce sedation at low concentrations. Earlier work with this chemical is by Bové (1965) who used this for studying its toxicity for frogs. As discussed earlier the anaesthetic has long induction and recovery times and hence is not suitable for fishery work, though its narcotic potency is the highest (599-90).

Phenobarbital Sodium has been used by McFarland (1959) who reported calcium antagonism in respect of all barbiturates. In the present investigation, the Phenobarbital is rejected as unsuitable for fishery work not only because it belongs to barbital group but also because of its excessive induction and recovery periods. Its narcotic potency is low being only 12.00. The tolerance of fish to this anaesthetic is also not appreciable as indicated by the tolerance tests (Tables III).

MS-222 Sandoz, also known as Methane tricainsulfonate, has been widely used as an anaesthetic in cold-blooded animals. Baudin (1932) was the first to study the action of MS-222 on the oxygen consumption of *Carassius auratus*. After this, it has been employed for variety of purposes in cold-blooded animals. A comprehensive bibliography of MS-222 has been given by Bell (1964) and Bové (1965). The latter author gives a comparative chart of the concentrations of the chemical useful for anaesthetizing a variety of cold-blooded animals as observed by different workers. The paper by Bové (*op. cit.*) is perhaps the best that could be recommended for knowing everything about MS-222. The concentrations ranging from 0.25 to 1.0 g./U.S. gal. or 1:15,000 to 1:3,800 have been recommended for anaesthetizing salmon, trout and bass. U.S. government agency recommends the concentration of 1:17,000 for fin marking in salmon fingerlings and of 1:3,785 for subcutaneous tagging in rainbow trout. The concentrations recommended for the transport of tropical ornamental fish ranged from 1:12,000 to 1:24,000 depending upon the time of transport, species and crowding (Bové, *op. cit.*).

Quite spectacular results on the use of MS-222 Sandoz are of Pickford and Atz (1957) and Gilbert and Wood (1957) who used this anaesthetic at the concentration of 4 mM/L. for quieting elasmobranchs as large as 400 pounds. In the present investigation, the concentration of 0.01 g./100 ml. was found suitable to induce loss of equilibrium in mullet fingerlings and keeping them in this stage for 30 minutes. Higher concentration reduces the exposure time (Table I). Concentrations suitable for transport range from 0.003 to 0.005 g./100 ml. The former being ideal for longer duration as evidenced by tolerance tests.

Having discussed the merits and demerits of the seven anaesthetics investigated in the present work, it will be worthwhile to have an overall view of all the thirteen chemicals so far tried in this project. This could be best obtained by a glance over Table V. The basic attributes for any anaesthetic to be declared as most suitable for fishery work could be listed below.

1. The anaesthetic should be water-soluble.
2. Dosage required should be low to ensure economy in the use of the chemical.
3. The time of induction and recovery should be low, *i.e.*, the chemical should be quick-acting and its effect should vanish in the shortest possible time so as to enable the chemical to qualify itself for use in tagging, fin-clipping and operative procedures.
4. The fish should tolerate the chemical well—even for several hours at lower concentrations, if the chemical is to be used for live-fish transport.
5. The chemical should not show any side effects on fish.
6. The lethal concentration should be fairly high so that fish are not accidentally killed even if slightly higher concentration is added by mistake, especially in the case of long transport.

When these tests are applied to the chemicals investigated in the project, only six can be considered as successful anaesthetics. These are, MS-222, Chloral Hydrate, Tertiary Amyl Alcohol, Tertiary Butyl Alcohol, Sodium Amytal and Urethane. However, some of these have other drawbacks. Sodium Amytal being a barbiturate has calcium antagonism and hence cannot be used in sea-water. Urethane has been proved to be a carcinogen and its use has, therefore, to be discouraged (Wood, 1956). Only four chemicals are thus left, namely, MS-222, Chloral Hydrate, Tertiary Butyl Alcohol and Tertiary Amyl Alcohol. Chloral Hydrate and Tertiary Butyl Alcohol have a prolonged induction time (Table V) though the recovery is comparatively

quicker and hence their use in tagging, fin-clipping and operative procedures is doubtful. However, the use of Chloral Hydrate in transport is advisable (McFarland, 1960 and Durve and Dharma Raja, *op. cit.*). Thus only MS-222 and Tertiary Amyl Alcohol can be classified as ideal for fishery work both at higher and lower dosages. MS-222 is very costly and great economy has to be ensured in its use. This leaves Tertiary Amyl Alcohol as the only anaesthetic useful for all fishery work. This supports the observation of McFarland (1960).

McFarland (1960) observed MS-222 as the best suitable for operative procedures but rejected it for live-fish transport on the ground that if proper attention was not paid to the concentration, a slightly more concentration might induce the whole lot of fish to lose its equilibrium, the stage most undesirable for transport. While agreeing with this view, it is felt that proper attention and preliminary testing are pre-requisites for fishery work involving the use of anaesthetics. Moreover, during transport, pretreatment is necessary (Durve and Dharma Raja, *op. cit.*) and this itself will forewarn if the concentration is not maintained at the appropriate level.

The aforesaid discussion does not, however, mean that other excellent chemicals such as Quinaldine, Chlorobutanol and Paraldehyde should be totally eliminated. The only drawback of these chemicals is that they require a vehicle to go into aqueous solution. Except for this drawback, the author finds these anaesthetics excellent for any fishery work. All the above chemicals do go into aqueous solutions with a little difficulty and the solutions thus prepared are equally good as those prepared by using an organic vehicle.

The author excludes all barbiturates, with the exception of Sodium Amytal, as useless for any fishery work. Ether, likewise, is useless because of its various drawbacks discussed earlier. The comparative chart of all the chemicals given in Table V is expected to give reasonable guidance about the anaesthetic to be used for any fishery work. However, it may be noted that the recommendations made here are based on work with mullet fingerlings at the specified experimental conditions and therefore, may not apply fully to other fishes.

SUMMARY

Seven anaesthetics, namely, Tertiary Amyl Alcohol, Ether, Quinaldine, Paraldehyde, Pentobarbital Sodium, Phenobarbital Sodium and MS-222

Sandoz, are experimented at different concentration levels on the fingerlings of the mullet *Liza tade*.

The results obtained are compared with those of the earlier work of the author to arrive at the most suitable anaesthetic in fishery work. Tertiary Amyl Alcohol is found to be the ideal anaesthetic in all respects. Merits of other anaesthetics have been discussed.

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* Not referred to in original.

APPENDIX I

Experimental data for finding out the minimal and maximal doses of anaesthetics and also the relationship of dosage with anaesthesia

A.—Tertiary amyl alcohol

No.	Concentration ml./100 ml.	No. of fishes	Weight range and Average weight	Mortality % fishes	Loss of equilibrium % fishes	Average time required (minutes)	Average time for recovery (minutes)	Partial loss % fishes	Deep sedation % fishes	Light sedation % fishes	Normal % fishes
1.	0·05	59	0·8–8·9 (3·20)	Nil	Nil	Nil	6·78	64·40	28·80
2.	0·08	60	0·5–8·0 (3·69)	Nil	Nil	Nil	18·33	81·66	Nil
3.	0·10	80	1·00–9·80 (3·88)	2·50	Nil	1·25	48·75	47·50	Nil
4.	0·15	60	0·50–11·0 (2·27)	6·67	68·32	45·10	7·62	20·00	5·00	Nil	Nil
5.	0·20	60	0·90–11·20 (3·35)	18·33	79·98	7·99	7·47	1·67	Nil	Nil	Nil
6.	0·50	20	1·00–4·40 (1·81)	100·00	Nil	0·35	..	Nil	Nil	Nil	Nil Died within three minutes.

B.—Ether

No.	Concentration ml./100 ml.	No. of fishes	Weight range and Average weight	Mortality % fishes	Loss of equilibrium % fishes	Average time required (minutes)	Average time for recovery (minutes)	Partial loss % fishes	Deep sedation % fishes	Light sedation % fishes	Normal % fishes
1.	0·20	60	0·4-9·00 (2·40)	1·67	Nil	Nil	23·33	41·57	33·33
2.	0·30	60	0·6-6·0 (1·85)	1·67	5·00	200·0	10·3	1·67	41·66	38·32	11·66
3.	0·40	59	0·75-5·65 (2·04)	10·17	37·28	46·81	10·81	5·08	25·42	16·94	5·08
4.	0·60	40	0·7-3·2 (1·69)	57·49	39·99	2·83	8·4	..	2·50	Nil	Nil

C.—Quinaldine

No.	Concentration ml./100 ml.	No. of fishes	Weight range and Average weight	Mortality % fishes	Loss of equilibrium % fishes	Average time required (minutes)	Average time for recovery (minutes)	Partial loss % fishes	Deep sedation % fishes	Light sedation % fishes	Normal % fishes
1.	0·0009	79	0·50–5·40 (2·205)	2·5	Nil	Nil	27·85	67·10	2·531
2.	0·001	40	1·0–4·40 (2·228)	2·5	Nil	Nil	52·49	45·0	Nil
3.	0·002	40	1·35–4·40 (2·79)	Nil	57·49	7·53	6·37	42·49	Nil	Nil	Nil
4.	0·003	40	0·8–3·65 (1·94)	2·5	92·49	7·09	12·13	4·999	Nil	Nil	Nil
5.	0·004	40	1·45–4·50 (2·78)	52·49	47·50	1·54	12·80	Nil	Nil	Nil	Nil
6.	0·005	10	2·70–17·95 (8·185)	100	Nil	1·00	..	Nil	Nil	Nil	Nil Died within 5 minutes of exposure.

D.—Paraldehyde

No.	Concentration ml./100 ml.	Nc. of fishes	Weight range and Average weight	Mortality % fishes	Loss of equilibrium % fishes	Average time required (minutes)	Average time for recovery (minutes)	Partial loss % fishes	Deep sedation % fishes	Light sedation % fishes	Normal % fishes
1.	0·01	60	0·5–11·70 (3·816)	1·666	1·666	34	6	1·666	81·66	11·66	1·666
2.	0·05	60	0·85–9·95 (3·208)	Nil	Nil	Nil	18·33	68·32	13·33
3.	0·15	20	1·40–8·40 (2·95)	Nil	45·00	30·5	14·1	25·00	25·00	5·00	Nil
4.	0·20	20	1·10–7·50 (2·985)	10·00	80·00	19·56	31·87	10·00	Nil	Nil	Nil
5.	0·25	60	1·10–18·50 (5·775)	15·00	83·33	6·46	19·31	1·666	Nil	Nil	Nil
6.	0·30	20	1·00–12·80 (5·99)	5·00	95·00	3·89	10·00	Nil	Nil	Nil	Nil
7.	0·35	10	1·40–9·00 (3·45)	100	Nil	4·50	..	Nil	Nil	Nil	Nil Died within 4–13 minutes of exposure.

E.—Pentobarbital sodium

No.	Concentration g./100 ml.	No. of fishes	Weight range and Average weight	Mortality % fishes	Loss of equilibrium % fishes	Average time required (minutes)	Average time for recovery (minutes)	Partial loss % fishes	Deep sedation % fishes	Light sedation % fishes	Normal % fishes
1.	0·0005	60	0·5-4·45 (1·84)	1·67	Nil	Nil	3·34	8·34	86·66
2.	0·001	60	0·5-8·6 (2·46)	1·67	Nil	Nil	Nil	38·32	59·99
3.	0·0015	59	0·3-6·3 (1·59)	1·69	1·69	280·0	25·00	Nil	8·47	62·70	25·42
4.	0·0016	19	1·0-5·0 (2·5)	Nil	Nil	Nil	5·26	63·16	31·58
5.	0·0017	39	0·5-5·20 (1·81)	Nil	2·56	135·00	40·00	5·13	5·13	61·53	25·64
6.	0·0020	20	0·9-5·8 (1·89)	Nil	65·00	108·80	55·00	5·00	10·00	20·00	Nil
7.	0·0025	58	0·5-4·25 (2·04)	1·72	56·90	91·61	75·14	Nil	12·07	22·42	6·898
8.	0·005	60	0·5-5·8 (1·75)	1·67	94·99	46·44	60·16	1·67	1·67	Nil	Nil
9.	0·01	59	0·55-3·40 (1·65)	18·64	81·34	32·79	122·5	Nil	Nil	Nil	Nil
10.	0·10	20	1·55-3·30 (2·26)	90·01	10·00	10·00	205·00	Nil	Nil	Nil	Nil

Died within 30 minutes.

F.—Phenobarbital sodium

No.	Concentration g./100 ml.	No. of fishes	Weight range and Average weight	Mortality % fishes	Loss of equilibrium % fishes	Average time required (minutes)	Average time for recovery (minutes)	Partial loss % fishes	Deep sedation % fishes	Light sedation % fishes	Normal % fishes
1.	0·025	52	0·90–5·10 (1·90)	1·92	Nil	Nil	1·92	63·46	32·69
2.	0·05	60	1·0–7·5 (3·16)	4·989	1·671	105	75	6·667	43·33	43·33	Nil
3.	0·1	40	0·8–6·0 (2·597)	Nil	34·99	182	119	52·49	12·50	Nil	Nil
4.	0·125	20	1·3–6·3 (2·56)	10·00	65·00	151·1	204·2	20·00	5·00	Nil	Nil
5.	0·15	40	1·0–6·3 (2·68)	2·5	87·50	150·93	179·28	Nil	10·00	Nil	Nil
6.	0·20	20	1·95–16·65 (7·715)	85·00	15·00	93·7	338·6	Nil	Nil	Nil	Nil Died after 30 minutes of exposure and did not recover.

G.—MS-222 Sandoz

1.	0·003	79	0·75–5·10 (2·083)	1·27	Nil	Nil	2·53	88·61	7·60
2.	0·004	40	0·7–3·45 (1·86)	10·00	Nil	Nil	57·49	32·49	Nil
3.	0·005	40	0·90–4·95 (2·295)	7·499	4·999	227·5	9·0	Nil	77·50	10·00	Nil
4.	0·01	100	0·50–4·50 (1·85)	5·00	91·00	10·09	13·24	4·00	Nil	Nil	Nil
5.	0·015	20	0·90–4·15 (1·87)	100·00	Nil	3·10	..	Nil	Nil	Nil	Nil Died within 8–10 minutes of exposure.