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CONTENTS

Introduction

Review Of Literature

- Lethal dose studies
- Factors effecting mortality rates
- Pathological and other studies

Materials and Methods

- Experimental facilities
- Experimental procedures
- Experimental design

Results

- Lethal dose experiments
- Radiation vs sodium halide factorials
 - Radiation vs sodium bromide
 - Radiation vs sodium chloride
 - Radiation vs sodium fluoride
 - Radiation vs sodium iodide

Effect of radiation on oxygen uptake

Discussions

- Lethal dose experiments
- Radiation vs sodium halide factorials
 - Radiation vs sodium bromide
 - Radiation vs sodium chloride
 - Radiation vs sodium fluoride
 - Radiation vs sodium iodide

Effect of radiation on oxygen uptake

Summary and Conclusions

Literature Cited

Appendix

INTRODUCTION

Fish may be affected by radioactive wastes in their environment through one or more of the following routes:

1. Direct radiation from disposed radioactive materials:
2. Ingestion of food organisms containing concentrated radioisotopes:
3. Direct irradiation in water containing radioactive ions or particles:
4. Contamination by bottom materials rich in precipitated radioisotopes:

High doses of radiation may cause mortality. Normally lethal doses are not accumulated by fish, however, sublethal doses which are accumulated can cause cellular changes which in fish may be expressed physiologically.

The halides are an important constituent of the aquatic environment. Chloride is found in a wide range of concentrations in all natural waters. Fluorides are also quite common and occur in concentration from 0.1 ppm to 20 ppm. These fluorides are leached from sedimentary phosphate deposits, cryolite and apatite. They are also introduced into the aquatic habitat as a pollutant from phosphate fertilizer plants, distilleries, steel mills, aluminium refineries etc. Although Iodides and Bromides are less common, they occur as pollutants from various sources such as photo-, dye-, and petroleum industries and medical - and research laboratories. Since the halides usually occur in the aquatic environment in the simple ionic state they are readily available for uptake and can be toxic to fish. This project was designed to ascertain the lethal gamma ray, dose levels, describe the effects of lethal and sublethal doses, and study the effects of the interactions of lethal and sublethal gamma ray doses and halide concentrations on fish.

Review of Literature

Welander et. al., (n. d.), as a result of their experiments with X-rays on rainbow trout for a period of 64 weeks, concluded that mortalities were significantly higher in fish exposed to 2000 r or more. Fish receiving 2,500 r did not survive for more than 13 weeks.

In work done by Scechmeister, Watson, Cole, and Jackson (1962) on Carassius auratus the L.D. 50 for 30 days was found to be 2315 rads. In their work the survival time decreased with increase in X-ray dose; exposure to 100 r resulted in 100% mortality in 365 days and the fish exposed to 10,000 r succumbed in 11 to 14 days. Etoh and Egami (1962) and Egami et. al., (1962) have shown that small doses of X-rays (500 r or less) do not exert lethal effects on fish at least during a period of 30 days after irradiation while very large doses (64,000 r or more) killed the irradiated fish instantaneously. Between these extremes several dose ranges are distinguishable from each other by the difference in effects on the post irradiation survival time. The doses ranging from 4,000 r to 32,000 r come under the "dose independent range" since the survival time about 10 days at 23 degrees centigrade of fish following irradiation with different doses within this range are approximately the same irrespective of dose level.

Bonet-Maury (1963) studying the biological effects of ionizing radiations on the golden carp established that the mean survival time decreases very sharply with the dose. At 5,000 r it is no more than 22

days.. At doses above 100,000 r some deaths were observed during irradiation. At 15°C the LD₅₀ for 30 days was found to be 3,500 r for Co⁶⁰ gamma rays.

Etoh (1965) subjected Oryseas Latipes to a single dose of 100,000 r of Co⁶⁰ gamma rays. All the fish died immediately after the exposure but they survived for a period of about 10 days after irradiation with 50,000 r. Etoh and Egami (1965) have shown that at 23°C regardless of X-ray dose and of water temperature during irradiation, all fish exposed to 4,000 r or more of X-rays died within 20 days. No significant differences in survival time being found among different doses. Approximately 70% of the fish irradiated with 2,000 r survived until the 30th day after irradiation.

Factors effecting the mortality rate of irradiated fish.

Ch. M. Gros et al., (1958 and 1959) while studying the effects of ionizing radiations on goldfish have shown that lower temperature increases number of surviving fish. Data of survival at 3°C indicate that irradiation gives protection against cold. LD₅₀ at 18°C was found to be 1800 r. As a result of their studies these workers have established that low temperature has a protective effect against ionizing radiations and conversely that radiation gives protection against cold. Later similar type of work was done by Bonet-Maury (1963) on golden carp and he also concluded that increase in temperature causes a reduction in the mean survival time. In his studies the mean survival time for a dose of 10,000 r of X-rays was found to be 17 days at 15°C and 7.1 days at 25°C. Hyodo (1965) studied

the effects of post irradiation temperature on survival time and rate of development of intestinal damage in gold fish given 8,000 r of X-rays at room temperature; majority of these fish survived for more than 100 days if they were kept at 4°C whereas most of similarly irradiated fish died when kept at 22°C. He concluded that the cool thermal surroundings inhibited the development of histological damage of the intestinal epithelium that occurred in warm environment.

Franciscis (1961) has shown that in carps the post irradiation injection of carp spleen homogenate in fish treated with total body X-ray dose of 9,576 r and 38,600 r was able to delay the onset of death. Etoh and Egami (1963) studying the effects of X-rays on gold fish concluded that the hypophyseal-adrenal system plays an important role in prolonging the life of X-irradiated fish and that the removal of either the pituitary or the adrenals prior to irradiation obliterates the "dose independent range" in the dose survival time curve in gold fish as in mammals.

Srivastava and Tachi (1964) noticed that the doses of 8,000 r of X-radiation increased the excretion of Na^{22} through the gills of gold fish. Mean survival time after a dose of 8,000 r was approximately 9 days. They also conducted partial body irradiation experiments on gold fish and finally concluded that if the head was shielded and body was exposed to X-radiation the sodium loss was similar to the un-irradiated fish but if body was shielded and head was exposed the fish lost sodium excessively. According to these authors the loss of sodium may lead to a significant lowering of osmotic pressure of the body fluids and hence may be one of the major causes of death after irradiation in fish.

Working on the fractionation of the Co^{60} gamma ray doses on Orizeas Latipes. Etoh (1965) administered two doses of 50,000 r each separated by 72 hour interval, the survival curve similar to the case of a single dose of 50,000 r was obtained. If kept at 11°C during interval all the individuals died just after the second dose. He concluded that the rate of recovery process from injury after a massive dose of irradiation is likely to depend on temperature.

Pathological and other effects.

Bacterimia associated with leucocytosis was observed by Preston (1959) in plaice following exposure to 8,000 r of X-radiation. A feature of the acute radiation syndrome, according to him, in cold blooded vertebrates is the extended period between exposure and the onset of symptoms. Shechmeister et. al., (1962) observed an increase in the susceptibility of irradiated animals to an experimentally induced bacterial infection (Aeromonas salmonicida) and to a naturally acquired ectoparasitic trematode (Gyrodactylus spp.). They also observed external pigmentation development in irradiated fish. The swim bladder was found in the deflated condition in the fish receiving doses greater than 500 r resulting in the loss of boyancy.

Egami et. al., (1962) have shown that in gold fish the secretion of adrenocorticotrophic hormone from the pituitary is stimulated by exposing fish to X-radiation and that the melanophores in the skin are produced by this over excretion. Bonet-Maury (1963) observed an abnormal pigmentation in golden-carp at the end of a time period variable with dose.

2. In U.S.A.E.C. report number UWPL-4 (1964) it has been stated that 1000 r dose was not sufficient to produce any noticeable histological changes in tissues within 15 days. However, Welander et. al., (n. d.) report that injuries symptomatic of radiation were observed in fish treated with 500 r or more. The injuries (hemorrhage, necrosis, growth of fungus, and intestinal damage) were proportional to the X-ray dose.

Hyodo (1965) studied the development of intestinal damage in gold fish after X-irradiation with 8,000 r at different temperatures. In the cool thermal surroundings, the development of characteristic histological damage of the intestine seldom took place during a period of 100 days after irradiation, but in fish examined at 181 days or 201 days marked histological damage had occurred in the intestine. The epithelial cells were increased in size but reduced in number.

Retardation of growth in length and weight has been reported as a sensitive measurement of radiation damage by Welander et. al. (n. d.) and is directly proportional to the amount of radiation dose.

MATERIALS AND METHODS

Facilities

Irradiation facility.

The irradiator used as a radiation source for exposing fish to gamma radiation is shown in Figure 1. This irradiator is loaded with 1306.8 curies Cs^{137} arranged in such a manner as to give an even flux of gamma ray dose in the 30.5 x 29 x 10 cms. irradiation chamber. The gamma rays from this source have energy of 0.66 Mev. The irradiation chamber is placed in the irradiating position with a hand operated rack and pinion gear. The irradiation chamber is also fitted with a water inflow and outflow system which can be maintained at a desired rate.

The dose standardization of the irradiator was accomplished by the use of Bausch and Lomb and Toshiba glass rod radiation dosimeters and a Turner fluorometer. The rods were exposed for various time intervals in the irradiation chamber. The change in fluorescence was read on the fluorometer. A graph was obtained by plotting fluorescence against exposure time. This curve was compared to a similar curve obtained from a standard source. The estimated dose rate obtained by the method described above is 322 roentgens per minute (in January 1967).

An assessment was also made of the exposure dose at various locations of the irradiation chamber. Twenty-seven glass rod dosimeters were arranged in the irradiation chamber in three layers, each layer consisting of three rows and three columns. These were exposed for 16 minutes

Figure 1. Fish Irradiator

and the change in fluorescence was recorded. It was concluded that the gamma flux varied little at the 27 locations with a slightly higher rate at the center of each layer and maximum at the center of the bottom layer. However, the differences are so small that for practical purposes the gamma flux was assumed to be evenly distributed throughout the chamber.

The dose rate inside the fish was also determined. The glass rod dosimeter was placed inside the body cavity of the fish with a #13 cannulating needle. The fish were exposed in the irradiation chamber, the rods were recovered from the fish and the change in the fluorescence was recorded. This was done for 7 cms and 28 cms size fish. Very little difference was noted in the dose rate outside and inside the fish. The energy of Cs^{137} gamma ray (0.66 Mev) is in such a range that a rad of the absorbed dose is almost equivalent to a roentgen of the exposure dose. It is therefore assumed that the dose read on the glass rod dosimeter exposed inside the fish is proportional to the absorbed dose.

Fish Holding Facilities.

Fish over 150 gms were held in a circular tank made of fiberglass having diameter of 121 cms and depth of 74 cms. The depth of water column in this tank was maintained at 66 cms.

Fiberglass channels, 208 x 34 x 18 cm, were used to acclimate fish smaller than 150 gms. Depth of the water was maintained at 13 cms. The rate of flow of water varied from 300 to 450 liters per hour. The incoming water was splashed at the upper end of the channel through a nozzle connected to the water distribution system of the laboratory. The concentration of the dissolved oxygen was constant (at saturation) throughout

the course acclimation and experiments. The water temperature varied from 45° F to 55° F during the course of a year. Each channel was covered with wire gauze screens. The light source was from fluorescent lamps. A diurnal effect was maintained with light on between 8 AM to 5 PM.

Lethal Dose Studies.

Wall aquaria were used for fish weighing over 150 gms each. The size of each aquarium was 102 x 51 x 51 cms. The depth of the water column was maintained at 42 cms. Each aquarium had its own inlet and outlet system and the compressed air facility. The incoming water was splashed at the top of the water column. The outlet was designed to keep the water in circulation. The water temperature of these aquaria also varied from 45° F to 55° F during the course of a year. The flow rate in these aquaria was approximately 120 liters per hour for each aquarium. Water was aerated during the course of an experiment by passing the compressed air through a carborundum stone. The front of all these aquaria was covered by a white gauze cloth to prevent disturbance to the fish by personnel working in this laboratory. The troughs were used for fish less than 150 gms.

Facilities for Radiation vs Sodium Halide Factorial Experiments.

A cold room (287 x 285 x 218 cms) was used for conducting these experiments. Plastic containers were used as aquaria for these experiments. Seventy-eight such aquaria were arranged on the shelves with compressed air for each aquarium. The aquaria were filled to 20 liters.

For the twenty liter mark the depth of the water column in these aquaria was 28 cms. All the experimental units were covered with wire gauze screens during the experimental periods. The temperature of the cold room fluctuated between 51° and 53° F. Light was held at a low intensity throughout the course of the experiments.

EXPERIMENTAL PROCEDURES

Procurement and Acclimatization of the Test Animal.

Salmo gairdneri (rainbow trout) were used as experimental test animals for these studies. The eggs of this species were obtained in Soap Lake, Washington and were hatched at the Logan, Utah Experimental Fish Hatchery. Fry were reared in fiberglass troughs until they weighed 1 gm each and then were released in the concrete hatchery tanks. These fish were fed daily and were checked periodically for signs of bacterial infections or other diseases. The fish were transported to the laboratory as and when required. In the laboratory smaller fish were held in troughs whereas the circular holding tank was used for the bigger fish.

The fish were acclimatized to the laboratory conditions for 3 to 5 days. While in the laboratory the fish were maintained on the same feed as at the hatchery. The fish used for lethal dose studies were given a maintenance diet every day prior to and during the course of an experiment. The fish used for the factorial experiments were kept off feed for 72 hours before they were used. No feed was given to fish during an interaction experiment.

Irradiation Procedure.

The inlet of the chamber was connected to the water supply of the holding troughs and the circular tank. The flow rate was adjusted to insure an adequate rate of change of water in the irradiation chamber. The fish were held in the chamber in the irradiating position for the time required to expose the fish to a particular dose.

The number of fish irradiated in a single batch varied according to the size of the fish. It was possible to irradiate 200 fish in one batch when they weighed 0.7 gms each whereas only two fish weighing 450 gms each could be irradiated in a single batch. For the ease of operation the smaller fish were first kept in a small wire gauge cage and the whole cage was then lowered in to the irradiation chamber. After the required exposure the cage was pulled out. The cage was slightly smaller in size than the irradiation chamber.

Procedure for Lethal Dose Studies.

After exposing the fish to various doses, the fish were transferred to randomly assigned channels of the fiberglass troughs. One channel was assigned to each dose. As mentioned earlier the fish were given a maintenance diet every day during the experiment (32 or 64 days). Mortality was recorded at 24-hour intervals. The dead fish were removed every day. The channels were cleaned by siphoning with minimum disturbance to the fish. Observations were also made regarding signs of radiation sickness and change in behavioral patterns.

Procedure for Radiation vs Sodium Halide Factorial Experiments.

The sodium halide concentrations were made in 20 liters. Hard water was used for dissolving sodium salts of chloride, iodide, and bromide

whereas sodium fluoride was dissolved in soft water. The fish after receiving the radiation dose were transferred to various concentrations of halides in the aquaria in the cold room. The fish were not fed during the experiment. Mortality was recorded and dead fish were removed after every 8 hours (12 hours for some of the sodium chloride experiments, refer to table no.). Each experiment lasted for 128 hours (192 hours in cases where mortality was recorded at 12 hour intervals).

Zepherine chloride was used as a disinfectant in this laboratory. Care was taken to use hygienic procedures with the fish in all laboratory situations.

EXPERIMENTAL DESIGN

Lethal Dose Studies.

A completely randomized design in a factorial arrangement of size, time, and dose was utilized for the size effect experiment. Because of space limitations only one replicate of each dose was conducted for each size of fish. It was therefore necessary to use the three factor interaction as the experimental error term (Table 1).

A completely randomized split plot design was used for a similar experiment but with different size ranges of fish (Table 4). The split plot Analysis of Variance was computed with time as the whole plot and size and dose as the sub plots.

Radiation vs Sodium Halide Factorial Experiments.

For these experiments various combinations of radiation doses and sodium halide concentrations were used in a factorial arrangement in a

Randomized Split Plot Design. In computing the Analysis of Variance, Time was taken as the whole plot and radiation dose and the sodium halide concentrations being the sub plots.

TABLE 1. Analysis of variance of rainbow trout mortality as a result of combination of gamma dose, time and size of fish.

Source	D. F.	M. S.	Significance
Total	179	54.75	.
Size	5	226.14	.05
Time	5	149.22	.05
Dose	4	389.85	.01
S X T	25	29.62	
S X D	20	66.93	.05
T X D	20	59.98	.05
Error	100	30.86	

TABLE 2. Mean number 5 to 154 of gamma rainbow trout dying per treatment combination of time and dose. Dose (rads)

		0	21	207	2070	20700
Time (days)	1	0	0	1.66	0	7.16
	2	0.83	0.83	0	0	5.33
	4	2.50	2.50	4.50	0	8.33
	8	5.0	0.83	3.33	0.83	5.83
	16	5.83	0.83	8.66	1.66	16.66
	32	2.50	4.16	2.33	1.16	18.33

Table 3. Mean number of rainbow trout dying per treatment combination of weight and dose for a 32 day experiment. Dose (rads).

	0	21	207	2070	20700
Size (gms)					
0.5	0	0	1.33	0.33	0
2.0	0	0	0	0	0
20.5	10.83	5.00	6.66	0	10.83
38.0	0	0.83	9.16	1.66	16.66
94.5	0.83	0	0.83	0	12.50
154.0	5.00	3.33	2.50	1.66	12.50

Table 4. Mean number of rainbow trout dying per treatment combination of time and size for a 32 day experiment. Time (days).

	1	2	4	8	16	32
Size (gms)						
0.5	1.6	0.4	0.4	0	0.4	1.2
2.0	0	0	0	0	0	0
20.5	1.0	3.0	3.0	9.0	15.0	9.0
38.0	5.0	1.0	6.0	5.0	9.0	8.0
94.5	1.0	1.0	0	0	6.0	9.0
154.0	2.0	3.0	3.0	5.0	10.0	7.0

Table 5. Analysis of variance of rainbow trout mortality as a result of combination of gamma dose and time.

Source	D.F.	M.S.	Significance
Total	139	117.96	
Replication	1	25.71	
Time	6	409.52	.05
Error (a)	6	85.71	
Size	1	71.43	
Dose	4	667.14	.05
SXD	4	107.14	
SXT	6	51.43	
DXT	24	207.14	.01
SXTXD	24	70.48	
Error (b)	63	51.75	

Table 6. Mean number of 248-454 gram rainbow trout dying per treatment combination of time and dose. Dose (rads).

		0	21	207	2070	20700
Time (days)	1	0	0	0	0	0
	2	0	0	0	0	0
	4	0	0	0	5.0	0
	8	5.0	0	5.0	0	5.0
	16	0	0	0	15.0	25.0
	32	0	0	0	10.0	15.0
	64	0	0	15.0	5.0	40.0

Table 7. Analysis of variance of 1 gram rainbow trout dying in treatment combinations of time, gamma dose and sodium bromide concentrations.

Source	D.F.	M.S.	Significance
Total	249	204.31	
Replications	1	10.00	
Time	4	1723.15	.01
Error (a)	4	28.25	
Dose	4	2.90	
Concentration	4	3535.65	.01
T x D	16	16.84	
T x C	16	1681.77	.01
D x C	16	4.96	
T x D x C	64	14.37	
Error (b)	120	12.72	

Table 8. Mean number of 1 gram rainbow trout dying in treatment combinations of time and sodium concentrations.

		0	20	200	2000	20000
Time (Hours)	8	0	0	0	0	0
	16	0.5	0	0	0.5	1.0
	32	0	0	0	0	11.5
	64	0.5	0	1.0	0	70.0
	128	0.5	1.0	1.5	2.5	13.5

Table 9. Analysis of variance of 2 gram rainbow trout dying in treatment combinations of gamma dose, time and sodium bromide concentration.

Source	D.F.	M.S.	Significance
Total	199	172.11	
Replication	1	24.50	
Time	4	797.81	.01
Error (a)	4	58.56	
Dose	3	49.33	
Concentration	4	2641.56	.01
T x D	12	79.65	.05
T x C	16	697.19	.01
D x C	12	10.48	
T x D x C	48	84.14	.01
Error (b)	95	39.91	

Table 10. Mean number of 2 gram rainbow trout dying in treatment combinations of time and sodium bromide concentration.

		Concentration (ppm)				
		0	20	200	2000	20000
Time (hours)	8	0	0	0	0	0
	16	1.8	1.2	0	0	0.6
	32	1.8	0	0.6	0.6	33.1
	64	2.5	1.8	0.6	0	48.1
	128	1.8	3.1	0	1.2	13.1

Table 11. Mean number of 2 gram rainbow trout dying in treatment combinations of time and dose in sodium bromide experiment.

		Dose (in Rads)			
		0	8000	16,000	32,000
Time (hours)	8	0	0	0	0
	16	0	0	1.5	1.5
	32	0.5	6.5	11.0	11.0
	64	12.5	8.5	8.0	13.5
	128	6.5	3.5	2.0	3.5

Table 12. Analysis of variance of 1 gram rainbow trout dying in treatment combinations of time, gamma dose and sodium chloride concentration.

Source	D.F.	M.S.	Significance
Total	249	185.57	
Replications	1	0.40	
Time	4	624.60	.05
Error (a)	4	57.90	
Dose	4	13.10	
Concentration	4	3554.35	.01
T x D	16	110.35	
T x C	16	708.47	.01
D x C	16	4.79	
T x D x C	64	105.63	
Error (b)	120	77.23	

Table 13. Mean number of 1 gram rainbow trout dying in treatment combinations of time and sodium chloride concentrations.

		Concentration (ppm)				
		0	20	200	2000	20000
Time (hours)	8	0.5	0.5	0.5	0	11.0
	16	1.5	0	0	0	23.0
	32	0	0	0	0	49.5
	64	0.5	0	0	0.5	11.0
	128	0.5	1.0	1.0	0.5	1.5

Table 14. Analysis of variance of 1.5 gram rainbow trout dying in treatment combinations of time, gamma dose and sodium chloride concentrations.

Source	D.F.	M.S.	Significance
Total	249	174.13	
Replications	1	0.40	
Time	4	967.10	.01
Error (a)	4	14.40	
Dose	4	5.35	
Concentration	4	3506.60	.01
T x D	16	78.04	.01
T x C	16	950.85	.01
D x C	16	1.60	
T x D x C	64	75.85	.01
Error (b)	120	33.68	

Table 15. Mean number of 1.5 gram rainbow trout dying in treatment combinations of time and sodium chloride concentrations.

		Concentration (ppm)				
		0	20	200	2000	20000
Time (hours)	8	0	0	0	0	0
	16	0.5	0	0	0.5	47.5
	32	0	0	0.5	0	37.0
	64	0.5	0.5	0.5	0	8.0
	128	0	0	0.5	0	2.0

Table 16. Mean number of 1.5 gram rainbow trout dying in treatment combinations of time and gamma dose in sodium chloride experiment.

		Dose (rads)				
		0	21	207	2070	20700
Time (hours)	8	0	0	0	0	0
	16	14.5	10.5	5.0	5.0	13.5
	32	4.0	6.0	12.5	8.5	6.5
	64	1.0	1.5	2.0	3.0	2.0
	128	0	1.0	0.5	1.0	0

Table 17. Analysis of variance of 2.6 gram rainbow trout dying in treatment combinations of time, gamma dose and sodium chloride concentrations.

Source	D.F.	M.S.	Significance
Total	159	397.50	
Replications	1	46.98	
Time	4	3710.21	.01
Error (a)	4	48.41	
Dose	3	1333.47	.01
Concentration	3	2277.94	.01
T x D	12	233.99	.01
T x C	12	2142.97	.01
D x C	9	162.42	.01
T x D x C	36	158.15	.01
Error (b)	75	21.45	

Table 18. Mean number of 2.6 gram rainbow trout dying in treatment combinations of time and sodium chloride concentration.

		Concentration (ppm)			
		0	200	2000	20000
Time (hours)	12	0	0	0	0
	24	7.5	5.0	9.1	79.9
	48	5.8	13.3	14.1	17.5
	96	1.6	3.3	0.8	0.8
	192	4.1	3.3	0.8	0

Table 19. Mean number of 2.6 gram rainbow trout dying in treatment combination of time and gamma dose in sodium chloride experiment.

		Dose (rads)			
		0	16000	32000	64000
Time (hours)	12	0	0	0	0
	24	14.1	20.8	24.1	42.4
	48	10.0	6.6	5.8	28.3
	96	0	0	0.8	5.8
	192	0	0	0	8.3

Table 20. Mean number of 2.6 gram rainbow trout dying in treatment combinations of gamma dose and sodium chloride concentrations.

		Concentration (ppm)			
		0	200	2000	20000
Dose (rads)	0	0	0	0	19.3
	16,000	0.7	1.3	0.7	19.3
	32,000	2.7	1.3	0.7	20.0
	64,000	12.0	17.3	18.7	20.0

Table 21. Analysis of variance of 12.0 gram rainbow trout dying in treatment combinations of time, gamma dose and sodium chloride concentrations.

Source	D.F.	M.S.	Significance
Total	159	53.5	
Replication	1	6.9	
Time	4	250.4	.01
Error (a)	4	6.9	
Dose	3	367.0	.01
Concentration	3	38.1	.01
T x D	12	229.9	.01
T x C	12	52.0	.01
D x C	9	35.8	.01
T x D x C	36	51.6	.01
Error (b)	75	9.3	

Table 22. Mean number of 12.0 gram rainbow trout dying in treatment combinations of time and sodium chloride concentrations.

		Concentration (ppm)			
		0	100	1000	10000
Time (hours)	12	0	0	0	0
	24	0	0	0	0
	48	0	0	0	0
	96	5.8	0.8	0	0
	192	4.2	10.0	10.8	0.8

Table 23. Mean number of 12.0 gram rainbow trout dying in treatment combinations of time and gamma dose in sodium chloride experiment.

Time (hours)	Dose (rads)			
	0	10000	20000	40000
12	0	0	0	0
24	0	0	0	0
48	0	0	0	0
96	0	0.8	0	5.8
192	0	0	0.8	25.0

Table 24. Mean number of 12.0 gram rainbow trout dying in treatment combinations of gamma dose and sodium chloride concentration.

Dose (rads)				
	0	100	1000	10000
0	0	0	0	0
10,000	0.7	0	0	0
20,000	0.7	0	0	0
40,000	6.7	8.7	8.7	0.7

Table 25. Analysis of variance of 16.0 gram rainbow trout dying in treatment combinations of time, gamma dose and sodium chloride concentrations.

Source	D.F.	M.S.	Significance
Total	159	39.7	
Replication	1	5.6	
Time	4	156.3	.01
Error (a)	4	5.6	
Dose	3	350.6	.01
Concentration	3	45.6	.05
T x D	12	137.1	.01
T x C	12	27.9	.05
D x C	9	45.6	.01
T x D x C	36	32.1	.01
Error (b)	75	12.3	

Table 26. Mean number of 16.0 gram rainbow trout dying in treatment combinations of time and sodium chloride concentrations.

		Concentration (ppm)			
		0	100	1000	10000
Time (hours)	12	0	0	0	0
	24	0	0	0	0
	48	0	0	0	0
	96	3.8	6.3	8.8	0
	192	5.0	5.0	2.5	0

TABLE 27. Mean number of 16.0 gram rainbow trout dying in treatment combinations of time and gamma dose in sodium chloride experiment.

		Dose (rads)			
		0	10,000	20,000	40,000
Time (hours)	12	0	0	0	0
	24	0	0	0	0
	48	0	0	0	0
	96	1.2	0	0	17.5
	192	0	0	0	12.5

TABLE 28. Mean number of 16.0 gram rainbow trout dying in treatment combinations of gamma dose and sodium chloride concentration.

		Concentration (ppm)			
		0	100	1,000	10,000
Dose (rads)	0	1.0	0	0	0
	10,000	0	0	0	0
	20,000	0	0	0	0
	40,000	6.0	9.0	9.0	0

TABLE 29. Analysis of variance of 35 gram rainbow trout dying in treatment combinations of gamma dose, time and sodium chloride concentration.

Source	D. F.	M. S.	Significance
Total	79	307.9	
Replication	1	1.9	
Time	4	869.1	
Error (a)	4	246.1	
Dose	1	1876.9	.01
Concentration	3	90.5	
T x D	4	753.9	.01
T x C	12	311.8	.05
D x C	3	564.5	.05
T x D x C	12	365.9	.05
Error (b)	35	139.2	

TABLE 30. Mean number of 35 gram rainbow trout dying in treatment combination of time and sodium chloride concentration.

Concentration (ppm)

		0	100	1,000	10,000
Time (hours)	12	0	3.1	0	0
	24	0	0	0	0
	48	6.3	0	0	13.0
	96	34.4	9.4	0	13.0
	192	9.4	21.9	25.00	6.3

TABLE 31. Mean number of 35 gram rainbow trout dying in treatment combinations of time and dose in sodium chloride experiment.

		Dose (rads)	
		0	40,000
Time (hours)	12	0	1.6
	24	0	0
	48	4.7	4.7
	96	6.3	21.9
	192	0	31.3

TABLE 32. Mean number of 35 gram rainbow trout dying in treatment combinations of gamma dose and sodium chloride concentration.

		0	100	1,000	10,000
Dose (rads)	0	0	0	0	8.8
	40,000	20.0	13.8	10.0	3.8

TABLE 33. Analysis of variance of 9 gram rainbow trout dying in treatment combinations of gamma dose, time and sodium chloride concentration.

Source	D. F.	M. S.	Significance
Total	79	14.6	
Replication	1	5.0	
Time	4	20.0	
Error (a)	4	5.0	
Dose	1	5.0	
Concentration	3	18.3	.05
T x D	4	30.0	.01
T x C	12	26.7	.01
D x C	3	31.7	.01
T x D x C	12	23.3	.01
Error (b)	35	5.0	.01

TABLE 34. Mean number of 9 gram rainbow trout dying in dying in treatment combinations of time and sodium chloride concentration.

		Concentration			
		0	100	1,000	10,000
Time (hours)	12	0	0	0	0
	24	0	0	0	0
	48	0	5.0	0	0
	96	0	0	0	0
	192	10.0	0	0	0

TABLE 35. Mean number of 9 gram rainbow trout dying in treatment combinations of time and gamma dose in sodium chloride experiment.

		Dose (rads)	
		0	40,000
Time (hours)	12	0	0
	24	0	0
	48	0	2.5
	96	0	0
	192	5.0	0

TABLE 36. Mean number of 9 gram rainbow trout dying in treatment combinations of gamma dose and sodium chloride concentration.

		Concentration (ppm)			
		0	100	1,000	10,000
Dose (rads)	0	4.0	0	0	0
	40,000	0	2.0	0	0

TABLE 37. Analysis of variance of 0.7 gram rainbow trout dying in treatment combinations of gamma dose, time and sodium fluoride concentrations.

Source	D. F.	M. S.	Significance
Total	249	681.0	
Replication	1	22.50	
Time	4	29754.7	.01
Error (a)	4	94.3	
Dose	4	64.7	
Concentration	4	617.0	.01
T x D	16	680.0	.01
T x C	16	975.6	.01
D x C	16	89.3	
T x D x C	64	138.4	.05
Error (b)	120	88.7	

TABLE 38. Mean number of 0.7 gram rainbow trout dying in treatment combinations of time and sodium fluoride concentration.

		Concentration (ppm)				
		0	2	4	8	16
Time (hours)	8	1.0	0	0.5	0.5	0.5
	16	1.0	1.5	2.5	2.5	6.5
	32	2.0	8.0	10.0	23.0	41.5
	64	41.5	65.5	68.5	71.5	51.5
	128	12.5	11.0	13.0	2.5	0

TABLE 39. Mean number of 0.7 gram rainbow trout dying in treatment combinations of time and gamma dose in sodium fluoride experiment.

		Dose (rads)				
		0	21	207	2,070	20,700
Time (hours)	8	0	0	0	0.5	2.0
	16	0.5	0	1.5	0.5	11.5
	32	16.5	12.5	10.0	16.0	29.5
	64	55.5	69.5	71.0	64.5	38.0
	128	8.0	9.0	10.0	10.5	1.5

TABLE 40. Analysis of variance of 1 gram rainbow trout dying in treatment combinations of gamma dose, time and sodium fluoride concentration.

Source	D. F.	M. S.	Significance
Total	119	562.9	
Replication	1	0.2	
Time	4	24057.1	.01
Error (a)	4	103.6	
Dose	4	3.0	
Concentration	3	6.5	
T x D	16	264.9	.01
T x C	12	361.1	.01
D x D	12	3.3	
T x D x C	48	36.1	
Error (b)	95	52.6	

Table 41. Mean number of 1 gram rainbow trout dying in treatment combinations of time and sodium fluoride concentration.

		Concentration (ppm)			
		0	4	8	16
Time (hours)	8	0	0	0	0
	16	20.0	25.5	30.5	41.5
	32	56.0	60.5	63.0	53.0
	64	19.5	13.5	6.5	6.0
	128	1.0	0	0	0

Table 42. Mean number of 1 gram rainbow trout dying in treatment combinations of time and gamma dose in sodium fluoride experiment.

		Dose (rads)				
		0	21	207	2070	20700
Time (hours)	8	0	0	0	0	0
	16	28.2	30.0	20.6	26.9	41.3
	32	56.9	56.3	70.0	61.3	46.3
	64	12.5	10.6	9.4	11.9	12.5
	128	1.3	0	0	0	0

Table 43. Analysis of variance of 1 gram rainbow trout dying in treatment combinations of gamma dose, time and sodium fluoride concentration.

Source	D.F.	M.S.	Significance
Total	199	396.9	
Replication	1	2.0	
Time	4	14857.8	.01
Error (a)	4	163.6	
Dose	4	14.4	
Concentration	3	120.8	
T x D	16	322.6	.01
T x C	12	127.4	
D x C	12	16.0	
T x D x C	48	79.2	
Error (b)	95	82.0	

Table 44. Mean number of 1 gram rainbow trout dying in treatment combinations of time and gamma dose in sodium fluoride experiment.

		Dose (rads)				
		0	21	207	2070	20700
Time (hours)	8	0	0	0	0	0
	16	15.6	13.1	10.0	24.4	26.9
	32	52.5	41.2	43.7	44.4	56.9
	64	24.4	33.1	31.2	23.7	13.1
	128	1.9	3.1	6.2	1.9	1.2

Table 45. Analysis of variance of 1 gram rainbow trout dying in treatment combinations of gamma dose, time and sodium fluoride concentration.

Source	D.F.	M.S.	Significance
Total	199	675.8	
Replication	1	0.5	
Time	4	28073.9	.01
Error (a)	4	42.1	
Dose	4	10.8	
Concentration	3	4.3	
T x D	16	185.3	
T x C	12	346.5	.05
D x C	12	4.6	
T x D x C	48	82.5	
Error (b)	95	114.0	

Table 46. Mean number of 1 gram rainbow trout dying in treatment combinations of time and sodium fluoride concentration.

		Concentration (ppm)			
		0	4	8	16
Time (hours)	8	0	0	0	0
	16	7.6	6.5	12.0	11.5
	32	53.0	63.5	64.5	72.5
	64	35.5	28.0	23.5	16.0
	128	1.5	0	0.5	0

Table 47. Analysis of variance of 2.5 gram rainbow trout dying in treatment combinations of gamma dose, time and radium fluoride concentration.

Source	D.F.	M.S.	Significance
Total	249	712.91	
Replication	1	1.02	
Time	4	33903.91	.01
Error (a)	4	33.22	
Dose	4	68.95	
Concentration	4	64.65	
T x D	16	854.50	.01
T x C	16	553.20	.01
D x C	16	59.94	
T x D x C	64	129.99	.01
Error (b)	120	78.57	

Table 48. Mean number of 2.5 gram rainbow trout dying in treatment combinations of time and sodium fluoride concentrations.

		Concentration (ppm)				
		0	4	8	16	32
Time (hours)	8	0	0	0	0	0
	16	0.5	4.6	4.0	3.5	3.0
	32	48.0	46.0	51.0	67.5	71.5
	64	38.0	47.5	43.0	29.0	25.5
	128	0	0	0	0	0

Table 49. Mean number of 2.5 gram rainbow trout dying in treatment combinations of time and gamma dose in sodium fluoride experiment.

		Dose (rads)				
		0	21	207	2070	20700
Time (hours)	8	0	0	0	0	0
	16	6.1	3.0	1.5	1.5	3.5
	32	56.5	47.5	51.0	51.5	77.5
	64	23.5	49.0	46.5	45.0	19.0
	128	0	0	0	0	0

Table 50. Analysis of variance of 15.6 gram rainbow trout dying in treatment combinations of gamma dose, time and sodium fluoride concentrations.

Source	D. F.	M. S.	Significance
Total	159	763.65	
Replication	1	3.90	
Time	4	15011.23	.05
Error (a)	4	1246.57	
Dose	3	15.62	
Concentration	3	15.62	
T x D	12	1227.37	.01
T x C	12	813.96	
D x C	9	15.62	
T x D x C	39	436.36	.01
Error (b)	75	212.63	

Table 51. Mean number of 15.6 gram rainbow trout dying in treatment combination of time and sodium fluoride concentration.

		Concentration (ppm)			
		0	8	16	32
Time (hours)	8	6.2	1.6	12.5	21.9
	16	15.6	28.1	37.3	35.9
	32	64.1	65.6	48.4	34.4
	64	7.8	3.1	0	7.8
	128	0	1.6	1.6	0

Table 52. Mean number of 15.6 gram rainbow trout dying in treatment combinations of time and gamma dose in sodium fluoride experiment.

		Dose (rads)			
		0	10000	20000	40000
Time (hours)	8	0	4.9	20.3	17.2
	16	10.9	39.1	43.7	23.4
	32	68.7	53.1	31.2	59.4
	64	14.1	1.6	3.1	0
	128	0	1.6	1.6	0

Table 53. Analysis of variance of 1.4 gram rainbow trout dying in treatment combinations of gamma dose, time and sodium iodide concentration.

Source	D.F.	M.S.	Significance
Total	249	35.50	
Replications	1	0.00	
Time	4	264.25	.01
Error (a)	4	0.25	
Dose	4	37.75	.01
Concentration	4	250.75	.01
T x D	16	20.43	.01
T x C	16	212.50	.01
D x C	16	26.62	.01
T x D x C	64	23.45	.01
Error (b)	120	8.11	

Table 54. Mean number of 1.4 gram rainbow trout dying in treatment combination of time and sodium iodide concentration.

		Concentration (ppm)				
		0	2	20	200	2000
Time (hours)	8	0	0	0	0	0
	16	0	0.5	0	0.5	0.5
	32	0	0	0	0	0
	64	0.5	0	0	0	1.5
	128	0.5	0	0.5	1.5	24.0

Table 55. Mean number of 1.4 gram rainbow trout dying in treatment combinations of time and gamma dose in sodium iodide experiment.

		Dose (rads)				
		0	21	207	2070	20700
Time (hours)	8	0	0	0	0	0
	16	0	0	0	0	1.5
	32	0	0	0	0	0
	64	0.5	0	0	0.5	1.0
	128	2.5	3.5	4.0	5.5	11.0

Table 56. Mean number of 1.4 gram rainbow trout dying in treatment combinations of gamma dose and sodium iodide concentration.

		Concentration (ppm)				
		0	2	20	200	2000
Dose (rads)	0	0	0	0	0	3.0
	21	0.5	0	0.5	0.5	2.0
	207	0	0	0	0	4.0
	2070	0	0	0	0.5	5.5
	20700	0.5	0.5	0	1.0	11.5

Table 57. Analysis of variance of 15.6 gram rainbow trout dying in treatment combinations of gamma dose, time and sodium iodide concentration.

Source	D.F.	M.S.	Significance
Total	119	320.85	
Replications	1	46.87	
Time	4	512.36	
Error (a)	4	89.19	
Dose	2	692.70	.01
Concentration	3	2161.45	.01
T x D	8	490.88	.01
T x C	12	968.96	.01
D x C	6	192.70	.05
T x D x C	24	286.02	.01
Error (b)	55	77.88	

Table 58. Mean number of 15.6 gram rainbow trout dying in treatment combinations of time and sodium iodide concentration.

		0	100	1000	10000
Time (hours)	8	0	0	0	0
	16	0	0	0	31.2
	32	0	0	2.1	45.8
	64	2.1	12.5	27.1	0
	128	2.1	6.2	2.1	22.9

TABLE 59. Mean number of 15.6 gram rainbow trout dying in treatment combinations of time and gamma dose in sodium iodide experiment.

		Dose (rads)		
		0	20,000	40,000
Time (hours)	8	0	0	0
	16	1.5	10.9	10.9
	32	10.9	12.5	12.5
	64	0	1.5	29.7
	128	12.5	3.1	9.4

TABLE 60. Mean number of 15.6 gram rainbow trout dying in treatment combinations of gamma dose and sodium iodide concentration.

		Concentration (ppm)			
		0	100	1,000	10,000
Dose (rads)	0	0	0	0	20.0
	20,000	0	1.2	1.2	20.0
	40,000	2.5	10.0	17.5	20.0

TABLE 61. Analysis of variance of 1.7 gram rainbow trout dying in treatment combinations of gamma dose, time and sodium iodide concentration.

Source	D. F.	M. S.	Significance
Total	249	195.36	
Replications	1	0.17	
Time	4	1178.83	.01
Error (a)	4	44.08	
Dose	4	5.61	
Concentration	4	3842.94	.01
T x D	16	88.08	.01
T x C	16	1263.47	.01
D x C	16	2.07	
T x D x C	64	84.92	.01
Error (b)	120	10.55	

TABLE 62. Mean number of 1.7 gram rainbow trout dying in treatment combinations of time and sodium iodide concentration.

Concentration (ppm)

		0	10	100	1,000	10,000
Time (hours.)	8	0.72	0	0	0	0
	16	0	0	0	1.3	3.3
	32	0	0.7	0	0	40.0
	64	0	0	0	0	53.3
	128	0.7	1.3	0	0.7	2.7

TABLE 63. Mean Number of 1.7 gram rainbow trout dying in treatment combinations of time and gamma dose in sodium iodide experiment.

		Dose (rads)				
		0	21	207	2070	20700
Time (hours)	8	0	0	0	0	0.7
	16	0	0	0	0	4.7
	32	6.0	8.7	4.7	8.0	13.3
	64	13.3	10.7	14.7	11.3	3.3
	128	2.7	0.7	0	0.7	1.3

TABLE 64. Analysis of variance of routine oxygen uptake by rainbow trout in treatment combination of gamma dose and fish density.

Source	D. F.	M. S.	Significance
Total	53	0.95	
Replications	1	.07	
Dose	2	5.43	.05
Density	2	1.69	
D x D	4	3.71	.05
Sampling	36	.40	
Error	8	.84	

TABLE 65. Mean routine oxygen uptake for rainbow trout in treatment concentration of gamma dose and fish density.

		Density (#)		
		4	8	16
Dose (rads)	0	4.23	2.15	2.41
	8280	1.48	1.87	2.34
	20700	2.26	2.20	1.82

TABLE 66-A. List of gamma ray lethal dose experiments.

EXPT.	DATE	WEIGHT (gm)	RADIATION DOSE (r).	FISH/ UNIT	TOTAL DURATION (days)	EXPT. LOCATION
1	2-2-66	0.5	0,21,207,2070, 20700	50	32	troughs
2	3-25-66	2.0	0,21,207,2070, 20700,41400	25	64	"
3	8-3-65	20.5	"	20	32	"
4	9-7-65	38.0	1,21,207,2070 20700	20	32	"
5	10-21-65	94.5	"	"	"	"
6	12-1-65	134.0	"	"	"	"
7	1-17-68	248.5	"	5 x 2	64	Wall Aquarium
8	3-29-66	428.5	0,21,207,2070, 20700,41400	5 x 2	64	"

TABLE 66-B. List of sodium halide vs. radiation dose interaction experiments.

Expt. #	Halide used	Date	Weight (Gm)	Radiation Dose (r)	Halide Conc. (ppm)	Time (hrs.)
9	NaBr	2-22-66	1.0	0,21,207,2070,20700	0,20,200,2000,20000	8
10	NaBr	3-5-67	2.0	0,8000,16000,32000	0,20,200,2000,20000	8
11	NaBr	4-1-67	4.0	0,8000,16000,32000	0,1000,2000,4000,8000	8
12	NaCl	2-9-66	1.0	0,21,207,2070,20700	0,20,200,2000,20000	8
13	NaCl	3-12-66	1.5	0,21,207,2070,20700	0,20,200,2000,20000	8
14	NaCl	6-20-66	2.6	0,16000,32000,64000	0,200,2000,20000	12
15	NaCl	7-6-66	12.0	0,10000,20000,40000	0,100,1000,10000	12
16	NaCl	7-29-66	16.0	0,10000,20000,40000	0,100,1000,10000	12
17	NaCl	9-6-66	35.0	0,40000	0,100,1000,10000	12
18	NaCl	8-19-66	9.0	0,40000	0,100,1000,10000	12
19	NaF	2-15-66	0.7	0,21,207,2070,20700	0,2,4,8,16	8
20	NaF	9-29-66	1.0	0,21,207,2070,20700	0,4,8,16	8
21	NaF	10-7-66	1.0	0,21,207,2070,20700	0,4,8,16	8
22	NaF	10-7-66	1.0	0,21,207,2070,20700	0,4,8,16	8
23	NaF	5-2-66	2.5	0,21,207,2070,20700	0,4,8,16,32	8
24	NaF	9-12-66	15.6	0,10000,20000,40000	0,8,16,32	8
25	NaI	3-1-66	1.4	0,21,207,2070,20700	0,2,20,200,2000	8
26	NaI	9-13-66	15.6	0,20000,40000	0,100,1000,10000	8
27	NaI	2-21-67	1.7	0,21,207,2070,20700	0,10,100,1000,10000	8

Figure 1. Dose-response relationship of 0.5 to 154.0 gram rainbow trout for 32 day period.

Figure 2. Dose-response relationship of 248.5 to 428.5 gram rainbow trout for 64 day period.

Figure 3. Dose-response relationship of 0.5 to 154.0 gram rainbow trout exposed to gamma dose.

Figure 4. Time-response relationship for 248.5 to 428.5 gram rainbow trout exposed to gamma dose. (for experiment no. 7-8 of table 66 A).

Figure 5. Size-response relationship for rainbow trout exposed to gamma dose for 32 day period. (for experiment no 1-6 of table 66 A).

Figure 6. Time-response relationship of rainbow trout exposed to combinations of gamma dose and sodium bromide concentration. (for experiment no. 9 and for experiment no. 10 of table 66 B).

Figure 7. Concentration-response relationship of rainbow trout exposed to combinations of gamma dose and sodium bromide concentration for 128 hour period. (for experiment no. 9 and for experiment no. 10 of table 66 B).

Figure 8. Time-response relationship of rainbow trout exposed to combinations of gamma dose and sodium chloride concentration. (for experiment no. 12 and for experiment no. 13 of table 66 B)

Figure 9. Time-response relationship of rainbow trout exposed to combinations of gamma dose and sodium chloride concentrations. (for experiment no. 14 and for experiment 15, and for experiment no. 16 of table 66 B).

Figure 10. Concentration-response relationship of rainbow trout exposed to combinations of gamma dose and sodium chloride concentration for 128 hour period. (for experiment no. 12 and for experiment no. 13 of table 66 B).

Figure 11. Concentration-response relationship of rainbow trout exposed to combinations of gamma dose and sodium chloride concentrations for 192 hour period (for experiment no. 14 of table 66 B.)

Figure 12. Concentration-response relationship of rainbow trout exposed to combinations of gamma dose and sodium chloride concentration for 192 hour period. (for experiment no. 15, for experiment no. 16 and for experiment no. 18 of table 66 B).

Figure 13. Dose-response relationship of rainbow trout exposed to combinations of gamma dose and sodium chloride concentration for 192 hour period (for experiment no. 14 of table 66 B).

Figure 14. Dose-response relationship of rainbow trout exposed to combinations of gamma dose and sodium chloride concentration for 192 hour period (for experiment no. 15 and - - - - for experiment no. 16 of table 66B).

Figure 16. Time-response relationship of rainbow trout exposed to combinations of gamma dose and sodium iodide concentration. (— for experiment no. 25 and - - - for experiment no. 27 of table 66B).

Figure 17. Concentration-response relationship of rainbow trout exposed to combinations of gamma dose and sodium iodide concentration for 128 hour period (for experiment no 25 of table 66 B).

Figure 18. Concentration-response relationship of rainbow trout exposed to combinations of gamma dose and sodium iodide concentrations for 128 hour period (for experiment no. 27 of table 66 B).

Figure 19. Concentration-response relationship of rainbow trout exposed to combinations of gamma dose and sodium iodide concentrations for 128 hour period (for experiment no 26 of table 66 B).

Figure 20. Dose-response relationship of rainbow trout exposed to combinations of gamma dose and sodium iodide concentrations for 128 hour period (for experiment no. 25 of table 66 B).

Figure 21. Dose-response relationship of rainbow trout exposed to combinations of gamma dose and sodium iodide concentration for 128 hour period (for experiment no. 26 of table 66 B).

Figure 22. Effect of gamma dose on amount of oxygen consumed by rainbow trout (mgms of oxygen used per kilogram of fish per minute.)

RESULTS

Lethal Dose Experiments. Figure 2 is an illustration of the dose-response relationship. Mortality in the controls was followed by a reduced number of deaths in the 21 r dose. Mean response was higher in the next higher dose level of 207 r. In the group receiving 207 r the mean response was consistently low for all sizes of fish up to 150 gms. each. However, this consistently low mean response was followed by a sharp increase in response in the next higher dose of 20700 r. A steady increase in mean response was evident up to the 16th day followed by a decline till the 32nd day when the experiment was terminated (Figure 4).

The smaller sized fish (0.5 and 2.0 gms. each) exhibited a very low mean response for the 32-day period as compared to the bigger fish (20 gms. each and over) as is evident from the figure 2.

Time x Dose, Size x Dose, and Size x Time interactions were found to be highly significant.

Maximum mortality in the controls occurred between the 8th and the 16th day. The group receiving 21 r dose had low response up to the 15th day and the maximum response for the group occurred between 16th and the 32nd day. The group receiving a dose of 207 r showed irregular response pattern with a maximum between 8th and the 16th day. The next higher dose of 2070 r showed no response for first 4 days followed by a very low response for the rest of the period. Fish receiving 20700 r responded irregularly up to 8 days and then consistently higher response occurred between 8th and the 32nd day.

Controls showed significant mortality in 3 out of 6 sizes. However, fish up to 2 gms. each had no mortality in controls for the 32-day period. The overall picture (table 3) of the dose x size interaction is indicative of the trend exhibited by the main effect of dose. As the size of the fish increased, the mean response also increased, although not in a very linear fashion.

Maximum response for all sizes occurred between 8th and the 16th day followed by a decrease in response for the remaining period. 0.5 gms. fish showed very low response while 2 gms. fish failed to respond to any of the doses for the 32-day period.

Mortality in the controls was much less in these experiments as compared to experiment No. 501-506 followed by no response in the group of fish receiving 21 r dose. An increasing linear relationship between dose and response is evident for the groups of fish receiving 207, 2070, and 20700 r doses (figure 3).

The time-response relationship was very much similar to the set of experiments No. 504-506. However, after a decline in the response up to the 32nd day, a sharp increase was noticed from the 32nd to the 64th day (these experiments were run for a duration of 64 days).

Response was not significantly different in the two sizes of 248.5 and 428.5 gms. each.

The only significant interaction occurred between time and dose. Controls suffered mortality between 4th and the 8th day. The fish receiving 21 r dose suffered no mortality. An increasingly higher response was observed for other dose groups. A substantially significant response occurred between 8th and the 16th day followed by a decrease up to the 32nd day and again an increase up to the 64th day for 20700 r dose and a decrease for the same period in case of fish receiving 2070 r dose.

Radiation vs. sodium bromide. The maximum response in these experiments occurred at approximately 64 hours (Figure 7). A sharp increase in response between 32 and 64 hours followed by a decline till the termination of the experiments was noticeable.

Very low mean response was observed for the sodium bromide concentration up to 2,000 ppm for the 128 hour period. However, a very high response was evident for a maximum concentration of 20,000 ppm.

Radiation doses as high as 32,000 r failed to show any significant response in these experiments.

Time x concentration interaction was highly significant (Table 8). Mortality increased with increasing concentrations and similarly higher response occurred with increasing time up to 64 hours and then showed a decline for the rest of the period in most of the cases.

A highly significant time x dose interaction was evident in the experiment where the radiation dose range covered doses between 0 r and 32,000 r, even though the main effect of dose was not significant. Increase in the mortality was observed with increasing dose and increasing time up to 64 hours. After this period a decline in mortality occurred in all doses.

Dose x concentration interaction was not significant.

Radiation vs. sodium chloride. Time-response relationship was highly significant in 4, significant in 1, and insignificant in 2 experiments. The maximum response seems to occur between 16 and 32 hours.

Very low mean response was observed up to sodium chloride concentrations of 2,000 ppm; however, a sharp increase occurs at 20,000 ppm. In another set of experiments where the sodium chloride concentrations ranged from 0 ppm to 10,000 ppm an initially significant response at lower concentrations is followed by insignificant response at 10,000 ppm (Figure 13).

Noticeable response for the radiation dose was observed only for considerably higher doses of 40,000 r or 64,000 r for the 192 hour period. Effect of dose was not significant in experiments where lower doses of radiation were used.

Mortality increased with increasing concentrations and increasing time in the experiments where concentrations of sodium chloride ranged 0 ppm to 20,000 ppm. Relatively low response was observed for 2,000 ppm concentration. The mortality tends to decline after 48 or 64 hours in 2,000 and 20,000 ppm concentrations. In the experiments where concentrations of sodium chloride ranged between 0 and 10,000 ppm comparatively low mean response was observed for 10,000 ppm concentration. Most of the response seems to occur after 48 hours.

In the highly significant time x dose interaction a usual trend is towards higher mortality with increasing time and dose. In 64,000 r dose, however, the maximum response occurs between 12 and 24 hours.

Dose x concentration interaction was highly significant in experiments where the radiation dose ranged between 0 r and 40,000 or 64,000 r. High mortality occurred with increasing dose and concentration. However, for 32,000 r dose an initially high response at 0 ppm concentration was followed by a decline up to and including 2,000 ppm concentration. In the experiments where 0, 100, 1000, and 10000 ppm of sodium chloride concentration and 0, 10000, 20000, and 40000 r of radiation dose was used, high mean response was observed for 0, 100, and 1000 ppm concentration and very low or no response occurred in 10,000 ppm concentration for the 40,000 r dose. In one experiment, using 35 gm fish, an initially high response in 40,000 r dose in low concentrations of sodium chloride followed a linear decreasing trend with increasing concentrations.

Radiation vs. sodium fluoride. Maximum response in these experiments occurs between 16 and 32 hours after exposure for all dose levels followed by a sharp decrease between 32 and 64 hours.

Main effects of radiation dose and sodium fluoride concentrations were not significant.

An increase in mortality occurred with increase in time and concentration up to 32 hours after exposure but after 32 hours decrease in mortality is significant with increase in time and concentration.

Although dose is not significant in any of these experiments, time x dose interaction was highly significant in 5 out of 6 experiments. An increase in mortality occurred with increase in dose and time up to 32 hours after exposure. However, after 32 hours a linear decreasing response was evident for all doses until the termination of the experiment.

Radiation vs. sodium iodide. A general increase in mortality with time, dose, and concentration is evident. The time x dose interaction and the time x concentration interaction were similar to those occurring in the sodium chloride experiment, but not as pronounced. The dose x concentration interaction was also slight, but it equated with an increasing mortality with increasing concentration for higher dose levels and a lesser rate of increase at lower dose levels.

DISCUSSIONS

Lethal Dose Studies

Significant deviations from a linear pattern were observed for the dose-response relationship (Figures 2 & 3). A decrease in response for lower doses of radiation as compared to the controls (where controls suffer mortality) has not been reported very definitely for fishes, although the results obtained by many workers manifest it very clearly.

In US AEC Report no. UWFL 4 it has been stated for male silver salmon that the controls died earlier than most of the irradiated fish (dose range 25 to 1000 r). However, it has also been reported that none of the deaths could justifiably be attributed to the X-ray treatment and that it is the normal occurrence for these salmon to die at about the stage at which these experiments were conducted. The results obtained by Welander et al., (n.d.) show a higher mortality in the controls than the fish receiving 50 and 100 r from the very beginning of their experiments and for the 64 week period controls suffered higher mortality as compared to fish receiving 750 r or less. These authors report that mortalities in fish irradiated with 50, 100, 500, and 750 r were less than mortalities occurring in the controls, but not significantly so.

Similar data were obtained by Welander (1945) where high response at 25 and 50 r was followed by a lower response at 100 r for the larvae of chinook salmon. Conte (1965) has reported 94.3% survival for the controls and 100 % survival for fish receiving 400 and 800 r in the experiments conducted with coho salmon in 3% salinity.

Grosch (1965) cites many examples for animals other than fish exhibiting this anomalous response. Davey (1919) discovered that irradiated flour beetles may survive their controls. Cork (1957) has found a favorable chronic dosage for beetle investigations and other investigators have demonstrated the phenomenon for additional types of insects. Carlson et al., (1957) have shown that rats receiving 0.1 r/hr of Co^{60} gamma rays for eight hours daily throughout adult life outlived their controls at 5 and 25°C.

Andrews (1961) has quoted Lornez's work (n.d.) where a daily dose of approximately 0.1 r increased the survival time of mice as compared to the non-irradiated animals.

The low doses mentioned above at which the response seems to be negative or non-linear, in fish, are varied. These range from 21 r (in present work) to 750 r (in work conducted by Welander et al.) Such diversity in results can very well be effected because of entirely different experimental conditions. It is our contention that for radiation studies the medium should be defined very rigorously because it is now quite obvious that radiation dose can have highly significant interactions with experimental techniques and physical and chemical properties of the environment.

Various explanations have been advanced and a few more can be added to the list to understand the nature of such response. Before any attempt is made to explain this anomalous response at low dosage of radiation, it must be emphasized that all radiations cause damage to the living tissue and it is yet to be proved that the low doses or the chronic doses of radiation have any direct beneficial effect on animals.

There seem to be no signs of rejuvenation or better condition of animals receiving these low doses. For the embryos and larvae of chinook salmon Welander (1945) noted measurable effects in exponential or direct proportional relationship to the amounts of röntgens administered in almost all the criteria used. Gain in weight and length in irradiated rainbow trout did not show any abnormal response at the low doses in question. Insects receiving favorable chronic dosage in Davey's work (1919) although survived longer than the controls but were not alert specimens at the peak of their physical activity. Experiments on the oxygen consumption by irradiated fish, conducted in this laboratory, indicate that the fish receiving 21 r had a lower rate of metabolism as compared to the controls and the group receiving the next higher dose of 207 r.

Possibility of adaptive organ system response has been suggested along with the hyper stimulation of repair mechanism which might more than offset the destructive effects of radiation (Andrews 1961 and Grosch 1965).

Ord and Stocken (1961) believe that immediate radiation damage is not a single, specific phenomenon and that radiation affects a number of different biochemical systems simultaneously and to an extent which varies independently for each system according to the dose. Possibility, therefore, can not be ruled out that at low dosage some enzymatic and / or hormonal activity could be triggered towards more beneficial direction, beneficial strictly in the sense of better life expectancy.

In the light of the work done on metabolism of the irradiated fish, in this laboratory, the low rate of oxygen consumption by the group of fish receiving 21 r indicates a lower rate of metabolism. (Neuhold and Sharma, 1967). Since rate of metabolism is directly proportional to the intensification of the radiation damage, the fish, although potentially carrying radiation damage, do not manifest radiation sickness because of comparatively low rate of metabolism. It is also quite likely that since they have a lower rate of metabolism they suffer less laboratory diuresis as compared to the controls during the course of the experiment.

In the set of experiments conducted in the fiberglass troughs a high response at 207 r dose was followed by a very low response at 2070 r dose. However, in the wall aquaria the response was more or less linear between 21 and 20700 r. It might be mentioned that the

wall aquaria were covered in front so as to minimize the disturbance to the fish by personnel working in the laboratory. The amount of light was also very much reduced by these covers. No such care was taken to prevent disturbance to fish in the troughs and therefore these fish were continually exposed to disturbance and sudden light intensity fluctuations during the working hours.

There can be two explanations for the reduced response at 2070 r dose in the troughs: (1) an interaction of gamma dose with increased activity of the fish, or (2) an interaction of gamma dose with quality, or fluctuations of light intensity.

It was noted that no such response was observed in the fish that were kept in a deep circular tank or the covered wall aquaria.

The experiments concerning the metabolism of irradiated fish in the troughs indicated very low metabolic activity for the fish receiving 2070 r, even lower than the 21 r group. The 2070 r dose does not seem to effect any vital organs and fish survive by virtue of their low rate of metabolism.

Although still lower rate of metabolism is apparent for fish receiving 20700 r dose, the extent of radiation damage is large enough to overcome any protection afforded by the low rate of metabolism.

In the results obtained (figures 4 and 5) first peak in mortality occurs between 8 and 16 days after exposure. This coincides with the region of infection death (Andrews 1961) and it is supposed to be due to the bacterial infection because bacterimia is a prominent finding at autopsy in most of the animals dying during this time period.

Mortality declines in the following period up to the 32nd day after exposure but heavy mortality is again encountered during 32nd to the 64th day. The period of low response between 16 and 32 days may be described as a "latent period" during which the intestinal damage develops causing a high mortality between 32nd and the 64th day.

Further discussion of the results is awaiting a more complete analysis of the data currently under way in our computer laboratory.

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