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Effects of Short-Term Field Applications of Acrolein and 2,4-D (DMA) on Flavor of the Flesh of Rainbow Trout

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Many of the irrigation and reservoir systems of the Western United States provide a suitable environment for the growth and proliferation of aquatic plants. This vegetation must be controlled so that water can be delivered for irrigation and to meet the municipal, industrial, and recreational demands for which these systems were designed and constructed. Without proper weed control, these systems would become useless. The most efficacious and economical method of controlling the unwanted vegetation has been with the use of herbicides.

Two herbicides that have been frequently employed to control unwanted algae and rooted macrophytes are acrolein (2-propenal) and 2,4-D (DMA) (the dimethylamine salt of 2,4-dichlorophenoxyacetic acid). Recent literature reviews on acrolein (FOLMAR 1977) and 2,4-D (SCHULTZ & HARMAN 1974) have presented abundant information on the acute toxicity of these chemicals to fish and aquatic insects. However, these reviews contained little information on sublethal effects that may be imparted by these chemicals.

The presence of certain organic and inorganic chemicals may impart objectionable taste, odor, or color to the flesh of fish or other edible aquatic organisms. The undesirable attributes can occur at concentrations of the chemical below those recognized as being stressful to the organism. Both petroleum derivatives (GATZLOFF et al. 1935, FETTEROLF 1962, KRISHHNAWAMI & KUPCHANKO 1969, WALSH et al. 1977, MALIGALIG et al. 1975) and phenols (BOETIUS 1954, BANDT 1955, SCHULTZE 1961, FETTEROLF 1964, SHUMWAY 1966, SHUMWAY & PALENSKY 1973) have been associated with off flavor problems of fish and shellfish in both freshwater and marine situations.

Since edible fish may be present in reservoirs or connecting canals where acrolein or 2,4-D (DMA) may be applied, it was of interest to determine if a short term field application of these chemicals would produce an off flavor in the flesh of a representative fish, the rainbow trout (Salmo gairdneri).

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METHODS AND MATERIALS

Experimental fish were obtained from the U.S. Fish and Wildlife Service's Fish Genetic Laboratory at Beulah, Wyoming. Upon arrival, the fish were distributed in the experimental canals in groups of nine and allowed to acclimate for a period of 30 days. At the time of distribution, the fish were 46.2 $\pm$ 0.6 cm and 1259 $\pm$ 51 g ($\bar{x}$ $\pm$ SEM) in total length and weight, respectively, and were in good health.

Herbicide exposures were conducted during February 1976 at the Agricultural Research Service, Bureau of Reclamation, and Fish and Wildlife Service Cooperative Aquatic Weed and Fish Experimental Station near Berthoud, Colorado. The 150-ft (46-m) long experimental canals have been described elsewhere (FOLMAR et al. 1979). The water quality at the time of exposure was as follows: temperature, 9.5°C; pH, 7.9; total dissolved solids, 120 mg/L; hardness, 79 mg/L as $CaCO_3$; and dissolved oxygen, 7.2 mg/L. All exposures were conducted for 4 h to simulate actual field application procedures. The test herbicides (technical grade) were administered by Meriotte bottle proximal to the water inlet pipe at the head of each canal. The drip rates were calculated to deliver 0.02, 0.05, and 0.1 mg/L for acrolein and 0.05, 0.1, and 0.5 mg/L for 2,4-D (DMA). The actual concentrations delivered into the canals, as determined by analytical methods (BOWMER et al. 1974, FRANK et al. 1970), were 0.02, 0.07, 0.09 mg/L for acrolein and 0.05, 0.09, 0.5 mg/L for 2,4-D (DMA). No residues were detected in water samples collected at 1, 4, and 7 days post-exposure.

Three fish from each exposure concentrations were sacrificed at 1, 4, and 7 days post-exposure, with the exception of 7-day samples treated with 0.05 mg/L acrolein, which were lost to predators. Both fillets were removed from the sacrificed fish; one fillet was utilized for organoleptic determination and the other was subjected to residue analysis. Tissue residue determinations were made at the USFWS, Columbia National Fisheries Research Laboratory, Route 1, Columbia, MO 65201 (HOGAN, J., unpublished). Organoleptic determinations were conducted at Colorado State University, Dept. of Food Science and Nutrition, Ft. Collins, Colorado, by a panel of 12 individuals trained in the sensory evaluation of food. The fillets (skin removed) were prepared by baking at 450°F for 10 min with no seasoning added. Samples were then randomly presented to the panel, and they were instructed to rank overall acceptability of the samples in question on a hedonic scale of one (very desirable) to six (very undesirable). The numerical ratings of each panel were then summed (rank totals) and compared by time and concentration using analysis of variance.

RESULTS

The organoleptic evaluations of the individual samples are presented as rank totals in Table 1. Columns 1, 2, and 3 represent triplicate determinations of each sample. The numerical values in each column are the rank totals of 12 individual estimations by the evaluation panel. Those samples with rank totals in the range of 37 to 52 were considered acceptable and did not taste significantly different from one another ($P \leq 0.05$, analysis of variance). Those samples with rank totals greater than 52 were considered inferior in taste; whereas, those samples with rank totals less than 37 were considered to taste significantly better than those in the aceptable range. Samples from the high concentrations of both 2,4-D (0.5 mg/L) and acrolein (0.1 mg/L) were considered inferior in the 1- and 4-day samples, and at 7 days in the high 2,4-D treatment. Controls were considered to taste significantly better than the acceptable fish at all three sample times. Those fish in the lowest acrolein concentration were also considered to taste significantly better than the acceptable fish at day 7.

At the lowest 2,4-D treatment level (0.05 mg/L) residues of 0.01 mg/g were detected in all the samples from day 1 and one sample each from the 4- and 7-day collections. Only the day-1 samples in the 0.1 and 0.5 mg/L treatments contained quantifiable residues and those were inconsistent, ranging from 0.03 to 0.22 mg/g. Residues were absent in all control samples. The lack of a reliable method for the analysis of tissue acrolein residues prevented a similar comparison with the acrolein treated fish.

Figure 1 is a linear regression analysis of 2,4-D (DMA) off-flavor rank totals and time. The regression equations were as follows:

$$\hat{Y} = 69.8 - 0.072x \text{ (0.5 ppm)}, \quad \hat{Y} = 46.1 - 0.023x \text{ (0.1 ppm)}$$

$$\hat{Y} = 41.8 - 0.028x \text{ (0.05 ppm) and } \hat{Y} = 29.3 - 0.014x \text{ (control)}$$

Fish from the 0.05 and 0.1 mg/L treatments were considered acceptable throughout the sampling period; however, these fish were considered less desirable than the control fish. The 0.5 mg/L treatment imparted a significantly objectional taste to the test fish throughout the sampling period. It was estimated that at least a 10-day depuration period would be required for the test fish to return to an acceptable level of palatability.

Figure 2 is a linear regression analysis of acrolein off-flavor rank totals and time. The regression equations were as follows:

$$\hat{Y} = 72.4 - 0.17x \text{ (0.1 ppm)}, \quad \hat{Y} = 456.1 - 0.086x \text{ (0.02 ppm)}$$

and

TABLE 1

RANK TOTAL COMPARISONS
CONTROL VS. ACROLEIN AND 2,4-D AT ALL LEVELS

Compound	Treatment level (mg/L)	Days post exposure	Rank totals[a] Replicate 1	2	3
Control	--	1	27[b]	30[b]	25[b]
2,4-D	0.05	1	39	41	42
Acrolein	0.02	1	42	42	42
2,4-D	0.1	1	45	47	43
Acrolein	0.05	1	50	48	45
Acrolein	0.1	1	66[c]	64[c]	65[c]
2,4-D	0.5	1	68[c]	65[c]	70[c]
Control	--	4	31[b]	30[b]	33[b]
2,4-D	0.05	4	38	40	42
Acrolein	0.02	4	40	40	45
Acrolein	0.05	4	47	45	52
2,4-D	0.1	4	53	47	55
Acrolein	0.1	4	60[c]	62[c]	64[c]
2,4-D	0.5	4	64[c]	62[c]	65[c]
Control	--	7	25[b]	27[b]	24[b]
Acrolein	0.02	7	30[b]	29[b]	31[b]
2,4-D	0.05	7	37	35	38
Acrolein	0.1	7	39	40	41
2,4-D	0.1	7	49	50	46
2,4-D	0.5	7	56[c]	55[c]	61[c]

[a]7 samples, except in the 7-day series where samples from fish subjected to 0.05 mg/L were not supplied, ranked from best to worst for overall acceptability by 12 judges. Non-significant range for p=0.05 is 37-59. For 7-day series non-significant range for 6 samples at p=0.05 is 32-52.

[b]Significantly better than other samples within each day/replicate block.

[c]Significantly inferior than other samples within each day/replicate block.

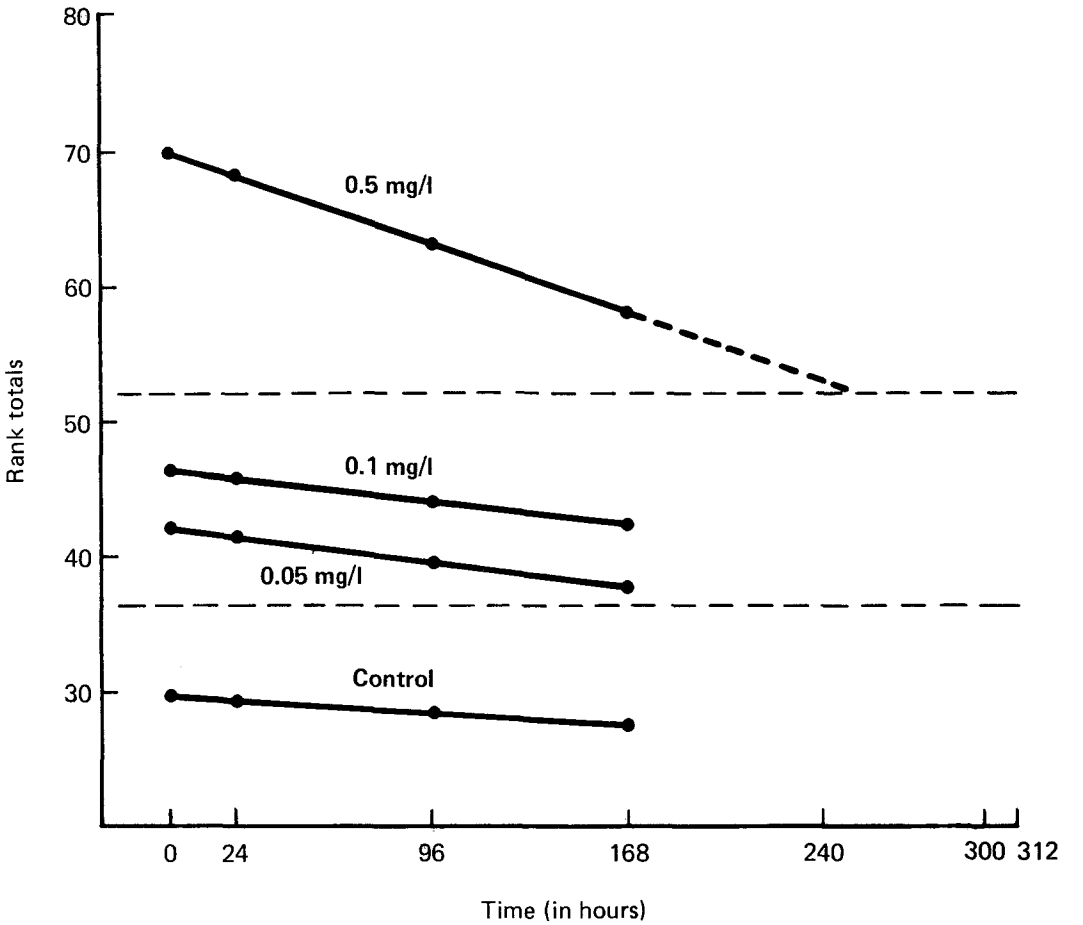

FIGURE 1.--A linear regression analysis of flavor impairment rank totals of 3 concentrations of 2.4-D (DMA) and time. Sample rank total values between 37 and 52 were considered acceptable. Those samples with rank totals greater than 52 were considered inferior in taste; whereas, those samples with rank totals less than 37 were considered to taste significantly better than those in the acceptable range.

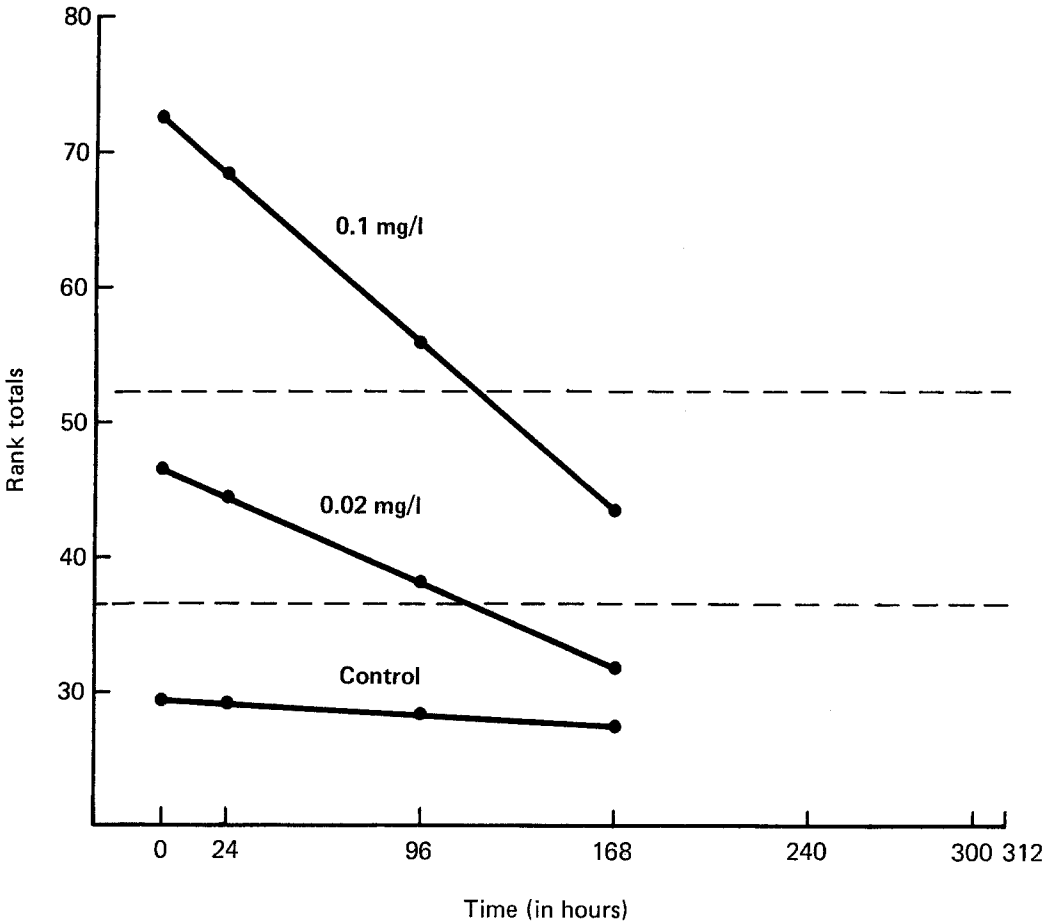

FIGURE 2.--A linear regression analysis of flavor impairment rank totals of 2 concentrations of acrolein and time. Sample rank totals values between 37 and 52 were considered acceptable. Those samples with rank totals greater than 52 were considered inferior in taste; whereas, those samples with rank totals less than 37 were considered to taste significantly better than those in the acceptable range.

$\hat{Y} = 29.3 - 0.014x$ (control).

Fish from the 0.2 mg/L treatment were considered acceptable in taste through the first 4 days of sampling and were considered comparable with the controls 7 days after treatment. Fish from the 0.1 mg/L treatment were considered significantly inferior in palatability through the first 4 days after toxicant administration, but returned to an acceptable level by day 7.

DISCUSSION

This study has demonstrated that the experimental concentrations of both acrolein (0.1 mg/L) and 2,4-D (0.5 mg/L) can significantly taint the flesh of experimental fish to make them unpalatable.

The fish exposed to the high concentration of 2,4-D required a longer period of time to return to an acceptable level of palatability than did those fish which were exposed to the high concentration of acrolein. However, the clearing rates in Figure 1 are comparable with clearing rates for other chemicals (SCHUMWAY & PALENSKY 1973).

The shorter time period required for acrolein treated fish to return to an acceptable level of palatability was probably related to its volatility. Unfortunately, off-flavor indices and tissue residues could not be compared to determine whether the unfavorable organoleptic ratings were due to the volatile or non-volatile fractions of acrolein (BOWMER et al. 1974).

Tissue residue analysis of the 2,4-D samples showed no significant accumulation of the parent compound at any of the test concentrations. Therefore, the high off-flavor ratings observed in the fish exposed to the highest 2,4-D concentration were apparently not related to residues of the parent compound. A recent investigation (STALLING & HUCKINS 1978) suggested that metabolic or reincorporation products of the parent chemical may be involved. They exposed fish to 2.0 mg/L of ^{14}C-2,4-D (DMA) for 12 weeks to determine degradation rates of the herbicide, and potential incorporation of the ^{14}C-fragments into natural biochemical products. Reincorporation of the ^{14}C-fragments into fatty acids, glycogen, and protein materials accounted for 85% of the ^{14}C-activity in the samples. These results suggested that carbon fragments of the parent compound may be incorporated into the lipid-protein structure of the muscle, or that the parent compound may have an indirect effect on metabolism, causing the accumulation of flavor impairing substances through an alternative pathway.

Observations have been made that indicate that rainbow trout can quickly concentrate organoleptically active compounds in their body tissues. The depuration of these chemicals varied with the concentration to which the fish were exposed. These results should be considered prior to the application of acrolein or 2,4-D

(DMA) in areas such as irrigation canals, reservoirs, governmental and commercial fish production facilities, or other waters where fish may be taken for human consumption.

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