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Comparative Toxicity of Rhodamine B and Rhodamine 6G to the House Fly (*Musca domestica* L.)

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Within the past decade, a great deal of research has been conducted on the photooxidative toxicity of xanthene dyes to insects. This toxicity has been attributed to the catalytic effect of visible light on dyes in insects (BARBIERI 1928). It has been proposed that the relative population of the dye in the first excited triplet state during illumination determines the effectiveness of the dye as a toxicant (CALLAHAM et al. 1977). Therefore, the more phosphorescent dyes should be more toxic than the more fluorescent dyes.

Coincidentally, several studies have shown that some of the same dyes cause insect mortality due to a slower, less efficient mechanism in the absence of light (BROOME et al. 1975, 1976). The rhodamine series of xanthene dyes are highly fluorescent and should not be, therefore, very toxic due to light catalyzed reactions. The toxicity observed in populations of insects that had been fed on either rhodamine B or rhodamine 6G and subsequently been held in the dark (YOHO et al. 1973; FONDREN & HEITZ 1978) warranted a more complete comparison of the toxicity caused by these two compounds.

This study was designed to determine the effectiveness of ingested rhodamine B and rhodamine 6G in the presence and absence of light and to evaluate toxicities when administered by feeding or by injection to house flies.

MATERIALS AND METHODS

One-day old adult house flies (Musca domestica L.) from a laboratory reared colony maintained in the Department of Biochemistry were used in these studies. Flies were reared on a

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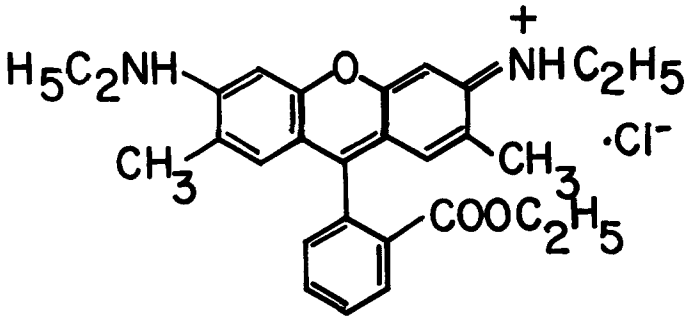
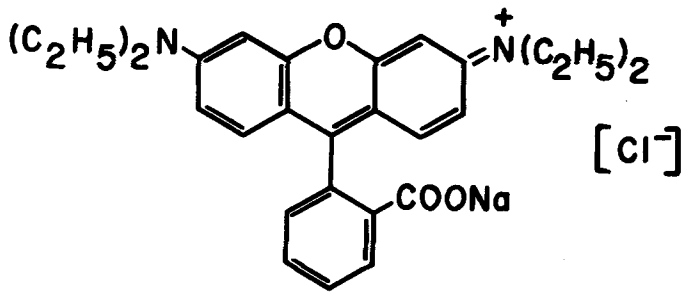
mixture of wheat middlings, yeast and dimalt and were held at temperatures ranging between 21 and 27°C. Reagent grade rhodamine B (Hilton-Davis Chemical Co.) and rhodamine 6G (Eastman Organic Chemicals) were evaluated. The chemical structures are shown in Figure 1.



RHODAMINE 6G



RHODAMINE B

Figure 1. Structures of rhodamine 6G and rhodamine B.

Feeding Experiments

Five molar concentrations of each dye were prepared in an aqueous 10% sucrose solution, placed in small plastic cups containing cotton, and were fed to the adult house flies for approximately 24 h in the dark. Each concentration was administered to two groups of 10 flies which were then confined in 300 mL plastic containers covered with wire screen. Flies fed only the 10% sucrose solution served as controls. After feeding, one group was exposed to light from two 40-W GE Cool White fluorescent lights at an intensity of about 1200 μeinsteins/m^2 sec (measured with an EG & G, Inc. Photometer). Mortalities were recorded at various times up to 24 h. Each treatment was replicated 5 times. The second group of flies was kept in the dark continuously. The feeding

dishes were moistened with the respective dye solution every other day. Mortalities were recorded daily for 7 to 8 days. Each treatment was replicated 9 times.

In another experiment, the flies were anesthesized with CO_2 and separated according to sex. Males and females were held separately in groups of 10 in the same type of container used in the previous experiment and fed in the dark with various concentrations of rhodamine 6G in aqueous 10% sucrose solution. Each treatment was replicated at least 6 times. The same number of flies fed only a 10% sucrose solution served as controls. The flies were fed continuously and kept in the dark during the entire length of the experiment. Mortalities were recorded daily.

Feeding vs. Injection Experiment

In this study only female flies were used. Flies were fed separately with four concentrations of rhodamine 6G for 24 h in the dark, after which they were fed the 10% sucrose solution. In the injection experiment, flies were anesthesized with CO_2. The same concentrations of rhodamine 6G used in the feeding experiment were prepared in distilled water. Two µL of each concentration were injected into the females just beneath the integument of the pronotum. A 50-µL Hamilton syringe equipped with a 32-guage stainless steel needle and fitted to a Hamilton repeating dispenser was used for injection. Each fly was held on the needle for 5 to 10 sec after delivery of the solution to reduce bleeding. Fifty female flies were injected with each concentration. The same number of flies, fed or injected only with the 10% sucrose solution or with distilled water, respectively, served as controls. The flies were confined in groups of 10 in plastic containers, provided with half-inch dental roll moistened with the 10% sucrose solution, and kept in the dark for 7 days. Mortalities were recorded daily.

All observed mortalities were corrected for control mortality (ABBOTT 1925). The median lethal concentrations (LC_{50}) and their fiducial limits at the $P = 0.05$ level were calculated by probit analysis described by FINNEY (1952).

Tissue Level of Dye

The tissue levels of rhodamine 6G or rhodamine B in house flies fed continuously in the dark were determined. Flies were fed 4 and 9 x 10^{-4} M concentrations of rhodamine 6G and rhodamine B, respectively. These concentrations were the predetermined LC_{10} levels based on data recorded on the 8th day of continuous feeding. After each feeding interval, 20 flies were homogenized in methanol (adjusted to basic pH with 1N KOH) using a Tekmar SDT Tissumizer. After filtration with a sintered-glass filter, the dye concentration was determined optically on a Beckman Acta V spectrophotometer. Standard curves for each dye were determined by a least square regression analysis using a TRS-80 micro computer system.

RESULTS AND DISCUSSION

Table 1 shows the oral toxicity of rhodamine 6G and rhodamine B to adult house flies after light exposure and also in the absence of light. In both light and dark reactions, higher mortalities were recorded in house flies fed with rhodamine 6G than those fed with rhodamine B. At 1.0 x 10^{-3}M concentration, the percent mortalities for rhodamine 6G were about 11 and 7 times as great as those observed for rhodamine B after 24 h of light exposure and in the absence of light, respectively. A similar observation was reported by YOHO et al. (1973), where rhodamine 6G was more toxic to house flies than rhodamine in the absence of light. However, the same investigators reported that rhodamine was more toxic than rhodamine 6G in the presence of light, which is contrary to the present observations. The rhodamine referred to by YOHO et al. (1973) was indeed rhodamine B (BUTLER 1981).

Rhodamine 6G was more toxic to the flies in the absence of light than when exposed to light for the same period. Twenty-four hours after the initial feeding period there was a 2-fold difference in mortality at all concentrations except at 0.3 x 10^{-3}M, where the mortality was equal. This result is also contrary to the findings of YOHO et al. (1973), where they concluded that rhodamine and rhodamine 6G were more toxic to house flies in the presence of light than in darkness. Differences in these observations might be attributed to variations in dye formulations, dye purities, and also in the genetic strain of house flies used.

TABLE 1. Mortality of dye-fed house flies after light exposure and in the absence of light.

Dye	Concentration (10^{-3}M)	% Mortality[a]				
		Light Exposure (h)				Dark (h)
		1	3	5	24	24
Rhodamine 6G	3.0	4	10	16	26	--
	1.0	8	12	12	22	40
	0.7	0	8	10	20	36
	0.5	2	2	2	12	26
	0.3	0	2	2	2	2
Rhodamine B	5.0	0	0	0	6	2
	3.0	2	2	2	2	1
	1.0	0	0	0	2	6
	0.9	0	0	0	2	0
	0.7	0	0	0	2	0

[a]Corrected for control mortality by Abbott's formula (1925). Control mortalities were 0 and 1 percent in light and dark, respectively.

More detailed studies on the oral toxicity of rhodamine 6G and rhodamine B to house flies in the absence of light indicated that rhodamine 6G is statistically more toxic than rhodamine B (Table 2). Based on the LC_{50} values, rhodamine 6G was approximately 28 times as toxic as rhodamine B on day 2 and from 9 to 15 times in the succeeding days of observation.

TABLE 2. Oral toxicity of rhodamine 6G and rhodamine B to house fly adults in the absence of light.[a]

Day interval[b]	Rhodamine 6G LC_{50} (10^{-3}M)	Rhodamine 6G Fiducial limits (P = 0.05)	Rhodamine B LC_{50} (10^{-3}M)	Rhodamine B Fiducial limits (P = 0.05)
1	0.87	0.77-0.99	c	c
2	0.60	0.56-0.65	17.08	15.75-18.54
3	0.44	0.40-0.49	4.47	3.74- 5.34
4	0.39	0.36-0.43	3.51	3.01- 4.09
5	0.29	0.24-0.34	2.62	2.34- 2.95
6	0.21	0.18-0.24	2.34	2.09- 2.62
7	0.16	0.13-0.19	2.16	1.93- 2.43
8	0.13	0.11-0.15	1.96	1.77- 2.17

[a]Control mortalities ranged from 1.2 to 5.0 percent at different day intervals.
[b]After the initial 24 h feeding period.
[c]Value not determined due to low mortalities.

The differential toxicity of rhodamine 6G and rhodamine B to the house flies may be related to the several differences in structure between the dyes, such as the presence of substituted amino and carboxyl groups. Esterification as in the case of rhodamine 6G may possibly enhance the movement of the dye to the site of action and thereby increase the toxicity.

Table 3 shows the tissue concentrations of rhodamine 6G and rhodamine B in house flies fed continuously in the dark for different time intervals. The concentration in terms of moles/g wet weight of the fly indicates that the flies ingested comparatively higher amounts of rhodamine B than rhodamine 6G, particularly at days 1 to 5, where consumption was at least four times as high. However, the amount of dye present in the tissue could also be a function of metabolism, degradation, excretion, and bioaccumulation rates which were not determined in this study. The observation that rhodamine 6G is more toxic than rhodamine B is made despite a lowered consumption of rhodamine 6G relative to the consumption of rhodamine B. This would indicate that rhodamine 6G is even more toxic than it appears relative to rhodamine B, since less rhodamine 6G is consumed.

TABLE 3. Tissue concentrations of rhodamine 6G and rhodamine B in house flies fed continuously in the dark at different time intervals.

Day Interval	Rhodamine 6G (10^{-4} Moles/ g wet weight)	Rhodamine B
0	0.93	3.69
1	1.43	7.94
2	1.11	6.66
3	1.27	6.76
4	1.32	5.96
5	1.39	6.05
6	1.61	4.23
7	1.84	4.76

Table 4 presents LC_{50} values and fiducial limits for ingested rhodamine 6G on male and female flies in the absence of light. On each day of observation, males and females were very nearly equally susceptible to rhodamine 6G.

TABLE 4. Oral toxicity of rhodamine 6G to male and female house flies in the absence of light.[a]

	Male		Female	
Day interval	LC_{50} (10^{-3}M)	Fiducial limits (P = 0.05)	LC_{50} (10^{-3}M)	Fiducial limits (P = 0.05)
1	0.71	0.63-0.81	0.67	0.60-0.74
2	0.53	0.48-0.58	0.53	0.48-0.58
3	0.41	0.36-0.46	0.46	0.41-0.50
4	0.29	0.24-0.34	0.36	0.32-0.40

[a]Control mortalities were 1.7 percent on day 3 for males, 3.3 and 5.0 percent on days 2 and 5 for females.

The toxicity of rhodamine 6G administered by injection to house flies in the absence of light was also evaluated. The LC_{50} value of 1.34×10^{-3}M for day 1 was significantly higher than those calculated for the succeeding 6 days while the value for day 2 was likewise significantly higher than those calculated for days 4 to 7, thus showing a more gradual decrease in LC_{50} values from day 1 to 7 (Figure 2). These data suggest that injected dosages of rhodamine 6G exert a delayed toxic effect on the flies which is probably attributed to slower translocation of dye molecules to the site of action.

Among orally-treated flies, the LC_{50} values for days 1 to 7 were somewhat constant. No significant differences were recorded in LC_{50} values ranging from 0.80 to 0.62×10^{-3} M for days 1 to 5. However, the LC_{50} value of 0.80×10^{-3} M evaluated for day 1 was

slightly different from 0.59 x 10^{-3} M evaluated for days 6 and 7 (Figure 2). This observation indicates that oral dosages of rhodamine 6G have a more immediate effect on the flies suggesting that the alimentary canal may be the site of action and may have an important role in the mode of action of the dye. Previously, microscopic examination of the alimentary canal from mosquito larvae treated with acridine red revealed considerable destruction of the midgut wall without apparent cell differentiation (SCHILDMACHER 1950). WEAVER et al. (1976) observed a more rapid onset of moribundity in Periplaneta americana (L.) and Blatta orientalis L. injected with either rose bengal or erythrosin B than in those fed with the dyes. In the present study, the house flies were more rapidly affected when fed with rhodamine 6G.

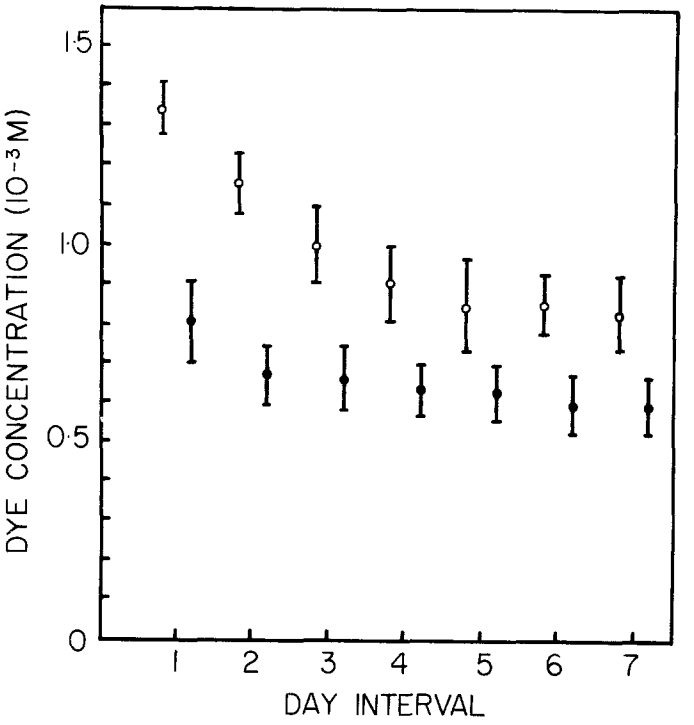


Figure 2. Injection and oral LC_{50} values of rhodamine 6G with corresponding fiducial limits for house fly adults in the absence of light.
—○— Injection; —●— Oral.

Although the toxicity of many phosphorescent xanthene dyes is dependent upon the synergistic effect of visible light, highly fluorescent dyes such as rhodamine B and rhodamine 6G are not dependent on light. The toxic action of the latter group may present an entirely different and as yet unknown mechanism.

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