

## Comparative Study of Triazine Biocides

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Three substituted triazines, hexahydro 1,3,5 tris (2-hydroxyethyl)-s-triazine [G], hexahydro 1,3,5 tris ethyl-s-triazine [V], and hexahydro 1,3,5 tris (2-hydroxypropyl)-s-triazine [P] were examined for their activity against three species of fungi (*Rhizopus nigricans*, as well as a *Fusarium* sp. and *Cephalosporium* sp., which were isolated from industrial cutting fluid). All triazines were totally ineffective against the three species up to levels of 0.1%. However, 1-hydroxypyridine-2-thione [O] was effective against all three species, but there was minimal difference in inhibition zones in concentrations from 0.005% to 0.1%. When [V], [G], and [P] were mixed with 0.005% [O], there was no reduction in antifungal activity of [O] against *R. nigricans* but there was minimal decrease against the *Cephalosporium* and *Fusarium* species. Compounds [V], [G], and [P] were also compared in their formaldehyde-releasing abilities, a presumed mode of action. A specific formaldehyde releasing reagent, 5,5 dimethyl 1,3 cyclohexanedione, was added to all three triazines in acid and alkaline solutions. Triazines [G] and [P] gave precipitates, indicating formaldehyde release, only in acid pH; [V] gave none at any pH. Results indicated that antibacterial activities of [G], [P], and [V] were equal in cutting fluid. Unexplained is the mode of action above pH 7, the ambient pH of [V], [G], and [P] in cutting fluid.

### INTRODUCTION

In an earlier study (Rossmoore and Williams, 1971), hexahydro 1,3,5 tris (2-hydroxyethyl)-s-triazine [G] (Grotan<sup>®</sup>, Lehn & Fink, Industrial Products Div.) proved extremely effective in controlling bacterial populations responsible for cutting fluid deterioration. This evaluation was done in relatively small (300-gal) systems in which fluid turnover was at least 100% in 2 weeks. A field evaluation in a 100,000-gal central system had mixed success; reduction in bacterial counts resulted in the appearance of nuisance fungal growth. Although the role of fungi in the breakdown of oil/water emulsions has not been established, the esthetic problems caused by their odor and appearance were sufficient, in this instance, to force the discontinuance of both the germicide and the coolant. Recently, Druskeit and Eggenesperger (1971) investigated [G] in the laboratory where the replacement rate approximated turnover in the small system reported above. They found no fungi. Thus, it appears from these few reports that the sudden onset of fungal populations may be related to two factors: the lack of competition (in the coolant milieu) from more aggressive bacterial species; and the lower rate of fluid replacement

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typical of large central systems. Perhaps, the major contributory factor, resulting from slow rate of fluid replacement, is presumptive drop in [G] concentration with time. This work will be reported in detail in a later publication. However, a paradox remains; [G] was presumed to be a broad spectrum biocide, and in fact a compound closely related to [G], hexahydro 1,3,5 tris ethyl-s-triazine [V] (Vancide TH<sup>®</sup>, R. T. Vanderbilt Co.) was also purported to be fungicidal as well as bactericidal. An earlier report by Wolf et al. (1955) described a preliminary study on the fungicidal activity of substituted s-triazines, which suggested that under certain conditions and with selected species, some derivatives were potentially useful.

The availability of a third s-triazine, an experimental compound, with a presumptive structure of hexahydro 1,3,5 tris (2-hydroxypropyl)-s-triazine, [P] now made it possible to examine three compounds which differed either by a methyl or a hydroxy group. Our interest was directed toward three areas: does this type of compound release formaldehyde in acting, as suggested by Smith (1969); are differences in structures reflected in different minimal antibacterial inhibitory concentrations; and does their reactivity against pure cultures of selected fungal species differ?

#### MATERIALS AND METHODS

Each of the three compounds, [G], [V], and [P] was checked for aldehyde release at pH values from 5-9 by the method of Pasto and Johnson (1969). To a solution of 100 mg of the presumptive aldehyde in 4 ml of 50% ethanol, add 0.3 mg of methone (5,5-dimethyl-cyclo-hexane-1,3-dione) and one drop of piperidine. Boil the mixture gently for 5 min. If the solution is clear at this point, add several drops of water until cloudy. Cool the mixture in an ice bath until crystallization (if it occurs) is complete. Crystallization is often slow and one should allow, if necessary, 3 to 4 hr, before reporting negative results wait 24 hr. To the preceding, one modification is added. The appropriate pH values are reached by dropwise addition of 0.1 N NaOH or 0.1 N HCl to the 50% ethanol prior to adding the compounds being tested.

Three fungal strains were used for the studies reported here. *Rhizopus nigricans*, from the Wayne State culture collection, a *Cephalosporium* sp. and a *Fusarium* sp., each isolated from a spoiled cutting fluid emulsion. The cultures were maintained on Sabouraud Dextrose Agar slants (SDA) (Difco), incubated at 25 C, and transferred every 5 days until ready for use. The spores were washed from the slants with 1-ml sterile water, added to 100 ml of Sabouraud Broth (Difco) in a 500-ml Erlenmyer flask, and incubated 2 days at 25 C without shaking. The resultant growth was subsequently used for evaluation of the triazines.

The three triazines, [V], [G], and [P], were dispensed in final concentrations of 50, 250, 500, and 1000 mg/l, as was the sodium salt of 1-hydroxypyridine 2-thione [O] (Omadine<sup>®</sup>, Olin Corp.). This latter compound has been used in cutting fluid preservation and, in addition, is reported to control a rather lengthy list of fungal species. It has been included in this study (50 mg/l), alone and in combination with the triazines, to check its compatibility for potential combined use in cutting fluids. We evaluated the compounds in SDA plates inoculated with 1 ml of the previously described broth cultures; the various inhibitor concentrations were added to ¼-inch diameter wells, four or five wells to each plate. Incubation was at 25 C and zones of inhibition were read at 2 days and again at 5 days before discarding.

TABLE 1. *Detection of putative formaldehyde release from s-triazines*

Triazine	pH value				
	5	6	7	8	9
Hexahydro 1, 3, 5 tris (2-hydroxyethyl)-s triazine [G]	+ <sup>a</sup>	+	—	—	—
Hexahydro 1, 3, 5 tris ethyl-s-triazine [V]	— <sup>b</sup>	—	—	—	—
Hexahydro 1, 3, 5 tris (2-hydroxypropyl)-s-triazine [P]	+	+	—	—	—

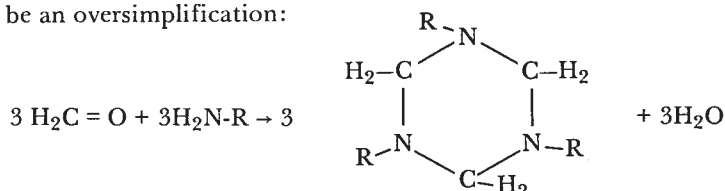
<sup>a</sup>+ aldehyde complex with 5,5 dimethyl cyclohexane 1,3-dione produces visible crystals within 5 min of cooling.

<sup>b</sup>— No reaction with above reagent, even after 24 hr.

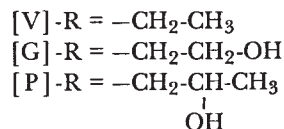
Each of the three triazines was added to 5% v/v oil in water emulsion (Sunseco, Sun Oil Co.) so that the final concentration was 1000 mg/l. To 90 ml of the three emulsions, and a fourth without inhibitor, was added 10 ml of a spoiled, mixed cutting fluid. This latter served as an inoculum. After periods of 1, 2, and 3 hr, at 25 C, 1 ml was removed from each emulsion for total plate counts. These were done in Trypticase Soy Agar (TSA) Baltimore Biological Laboratory, and incubated 2 days at 30 C.

#### RESULTS AND DISCUSSION

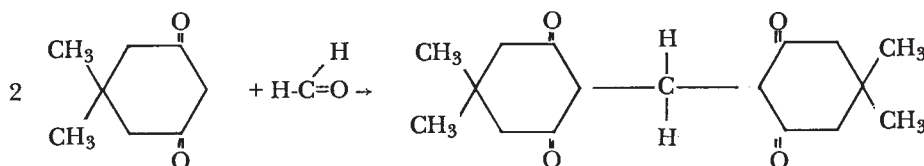
The detection of aldehyde release by methone complexing was positive only in acid pH with [G] and [P], but not with [V] (Table 1). Although it is presumed that all three triazines are condensation products of formaldehyde and monoalkylamine, we are not privy to the synthetic processes of the three manufacturers. Thus the following reaction may be an oversimplification:



From the above we would have no reason to expect a difference among [V], [G], and [P]. However, only a very tentative explanation can be offered at this time, based solely on structural differences among the three compounds where:



With respect to stability and/or subsequent reaction with methone, it appears that the 2-hydroxy group is necessary. However, only the putative aldehyde is involved in the complexing reaction:



This reaction can be used for quantitatively determining formaldehyde. Thus, it would appear that [V] is not releasing formaldehyde. This is not in agreement with the statement in the literature accompanying [V], that it can be converted to ethylamine and formaldehyde by adjusting the pH of the substrates to 6.3 or lower. No method is stated for this conversion. It is conceivable that the [V] formulation is somehow not compatible with the methone method. This latter point needs further investigation. Formaldehyde release, only at acid pH, is reminiscent of the utility of hexamethylenetetramine, (the progenitor of formaldehyde-releasing biocides) which is only active below pH 6 where it releases formaldehyde. Thus, its use as an urinary antiseptic requires concomitant treatment with a compound such as mandelic acid, to bring urine to a reactive pH. Nevertheless, the results for all three triazines in alkaline pH are convincing and should point to an alternate mode of action other than "formaldehyde release" at those pH values (Goodman and Gilman, 1958).

The results of the fungal inhibition studies are shown in Figures 1-7, with a key to follow well concentrations shown in Figure 8. All three triazines proved ineffective in inhibiting the test species (Figs. 1-3), while the opposite is true (Fig. 4) with [O]. Rossmore et al. (1971) reported on the antibacterial activity of [G] in cutting fluid, the potential for resident fungal flora to achieve ascendancy and that [O] and [G] were compatible in cutting fluid. In Figures 5, 6, and 7, 50 mg/l [O] has been added to each of

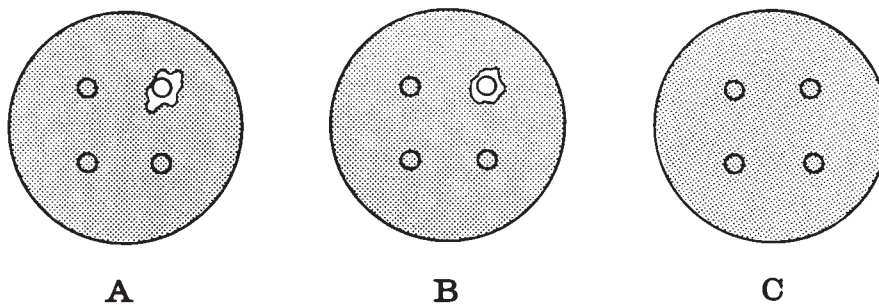


FIG. 1. Fungal growth in the presence of triazine [G]. (A-*Cephalosporium* sp., B-*Fusarium* sp., and C-*Rhizopus* sp.)

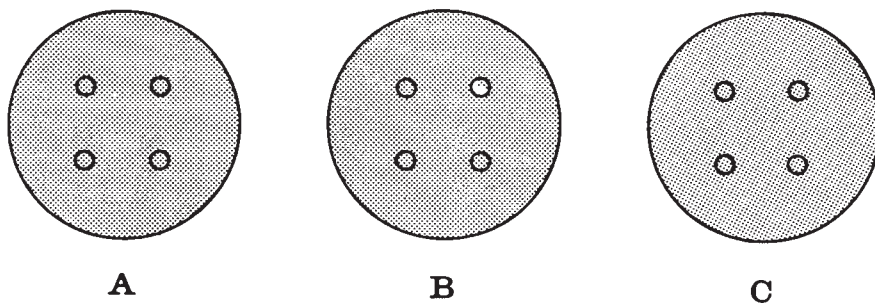


FIG. 2. Fungal growth in the presence of triazine [V]. (A-*Cephalosporium* sp., B-*Fusarium* sp., and C-*Rhizopus* sp.)

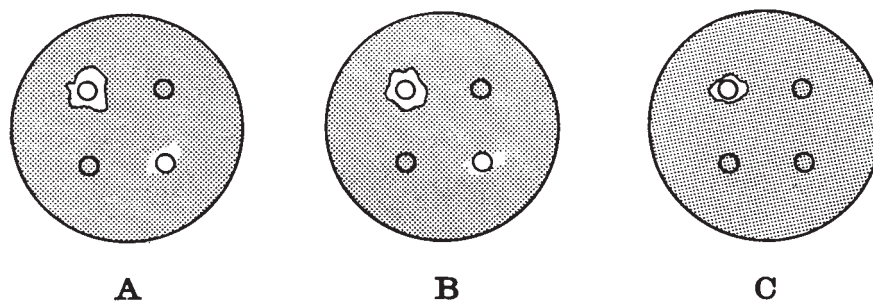


FIG. 3. Fungal growth in the presence of triazine [P]. (A-*Cephalosporium* sp., B-*Fusarium* sp., and C-*Rhizopus* sp.)

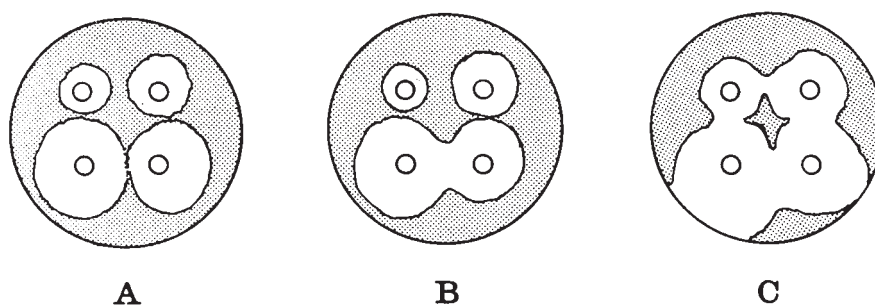


FIG. 4. Inhibition of fungal growth by 1-hydroxypyridine-2-thione [O]. (A-*Cephalosporium* sp., B-*Fusarium* sp., and C-*Rhizopus* sp.)

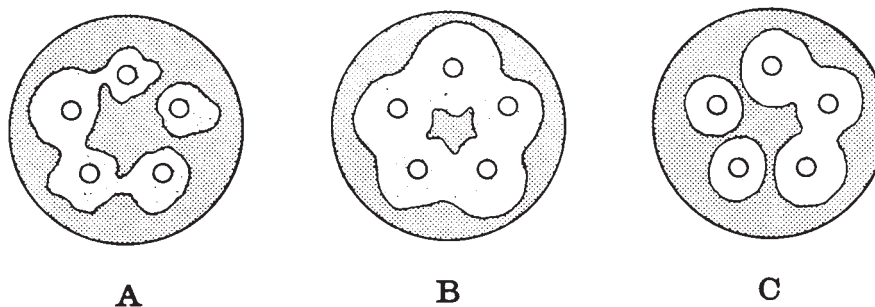


FIG. 5. Inhibition of fungal growth by 1-hydroxypyridine-2-thione [O] in the presence of triazine [G]. (A-*Cephalosporium* sp., B-*Fusarium* sp., and C-*Rhizopus* sp.)

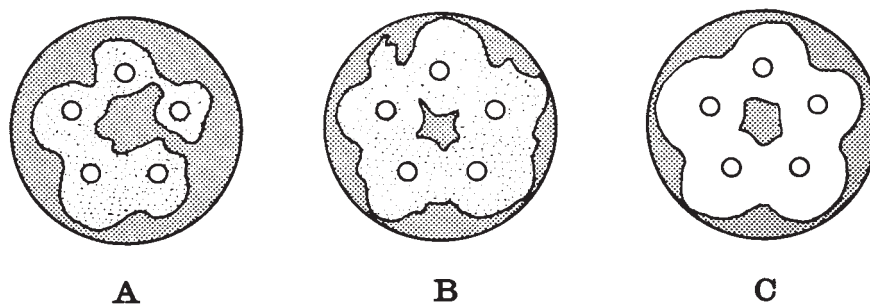


FIG. 6. Inhibition of fungal growth by 1-hydroxypyridine-2-thione [O] in the presence of triazine [V]. (A-*Cephalosporium* sp., B-*Fusarium* sp., and C-*Rhizopus* sp.)

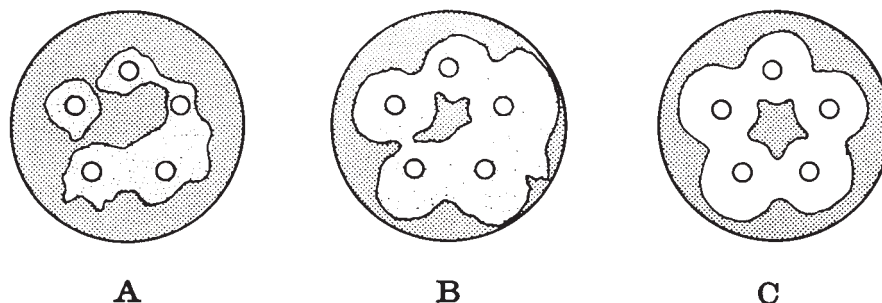


FIG. 7. Inhibition of fungal growth by 1-hydroxypyridine-2-thione [O] in the presence of triazine [P]. (A-*Cephalosporium* sp., B-*Fusarium* sp., and C-*Rhizopus* sp.)

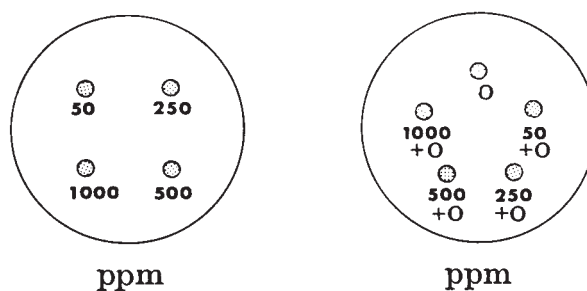


FIG. 8. Key to Figs. 1-7. Plates with four wells, additive levels as indicated. Plates with five wells have 1-hydroxypyridine-2-thione, at 50 mg/l, in wells labeled O and the appropriate triazine compound in wells at 50, 250, 500, and 1000 ppm.

the triazines to ascertain if that compatibility extended to the fungicidal action of [O]. From the zones with [O] alone and [O] plus varying concentrations of triazines against all three fungal species, it may be seen that there is either minimal or no interference with [O] antifungal action. With *Rhizopus nigricans*, the combinations completely inhibited growth, while *Fusarium* sp. and *Cephalosporium* sp. grew in light-scattered patches within the zones of inhibition.

TABLE 2. Bactericidal activity of *s*-triazines in 5% oil/water cutting fluid

Triazine	Survival after		
	1 hr	2 hr	3 hr
Hexahydro 1, 3, 5 tris (2-hydroxyethyl)- <i>s</i> -triazine [G]	0.89%	0.27%	0.15%
Hexahydro 1, 3, 5 tris ethyl- <i>s</i> -triazine [V]	0.35	0.27	0.23
Hexahydro 1, 3, 5 tris (2-hydroxypropyl)- <i>s</i> -triazine [P]	1.54	0.43	0.20
None	70.0	50.0	46.0

More extensive results using [G] plus [O] with species of *Mucor*, *Penicillium*, *Geotrichum*, *Aspergillus*, as well as others in progress, reinforce the results reported here. Thus, combined use of [O] and these triazines seems a promising solution to the fungal problem associated with triazine use in cutting fluids.

The last comparative study is shown in Table 2. There is no apparent difference among the three triazines in extent or rate of kill of the mixed aerobic bacteria from the cutting fluid inoculum. Since in all four samples the initial pH was 9.0, the results confirm further than formaldehyde release is not a *sine qua non* for antibacterial activity.

#### CONCLUSION

No biological difference among the three triazine compounds was shown. They all were totally ineffective against the fungal species at the level of 0.1%; however, they were equally effective against bacteria in the cutting fluid emulsion. Based on these results, we do not anticipate any biological differences. However, until more work on their specific modes of action is completed, that possibility cannot be ruled out. The ultimate selection of one of the triazines for use in cutting fluids may well depend on other factors such as cost, toxicity, and stability.

#### ACKNOWLEDGMENT

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