A NOTE ON THE TOXICITY OF CHLOROPICRIN VAPORS TO *GLOEOPHYLLUM SAEPIARIUM* AND *PORIA* SP. IN WOOD

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ABSTRACT

Southern pine wafers infected with *Gloeophyllum saepiarium* and *Poria* sp. were exposed to a range of chloropicrin vapor concentrations for 4, 8, 12, 16, and 24 h. The minimum lethal dosages of chloropicrin for both fungi were determined at each exposure period. The range of lethal concentration/time values, calculated at each exposure period, was small for *Poria* sp. but much broader for *G. saepiarium* over the 24-h test limits. This difference indicates that increasing the length of exposure to chloropicrin has a greater affect on *G. saepiarium* (susceptibility increases) than on *Poria* sp. (susceptibility remains relatively constant).

Keywords: Chloropicrin, *Gloeophyllum saepiarium*, *Poria* sp., southern pine, fungitoxicity, fumigant, concentration/time value, gas chromatographic analysis.

INTRODUCTION

In recent years, chloropicrin, a volatile fungicide, has been used as an in-service treatment for the control of internal decay in wood poles, piles, laminated timbers, and other wood products (Graham and Corden 1977). Although the effectiveness of chloropicrin in controlling decay fungi has been proven in laboratory and field studies, little information is available concerning the lethal concentrations of the chemical vapors to wood decay fungi.

This note describes experiments performed to determine the minimum lethal dosages (MLD) of chloropicrin vapors required to kill two decay fungi, *Gloeophyllum saepiarium* (Wulf. Ex. Fr.) Karst., and *Poria* sp., in infected wood samples. Information on the MLD will be useful in developing better methods of chloropicrin treatment and in determining when retreatment is necessary.

METHODS AND MATERIALS

Preparation and treatment

Edge-grain southern pine wafers, 0.5 mm thick and 5 mm square, were cut with end-grain exposed along two of the 0.5 × 5 mm surfaces. Equal amounts of earlywood and latewood were exposed in each wafer. The wafers were inoculated as follows: Prior to each test 125 wafers were soaked in tap water, drained, and autoclaved for 20 min. After cooling, the wafers were divided into three groups and aseptically transferred to the mycelial surface of 1-month-old agar cultures of two decay fungi, *G. saepiarium* and *Poria* sp., isolated from decaying southern pine wood (Goodell 1979). Following a 7-day incubation, the wafers were removed from the culture, and excess agar and mycelium were scraped from their surfaces. Five wafers from each culture were speared with sharpened wire hooks

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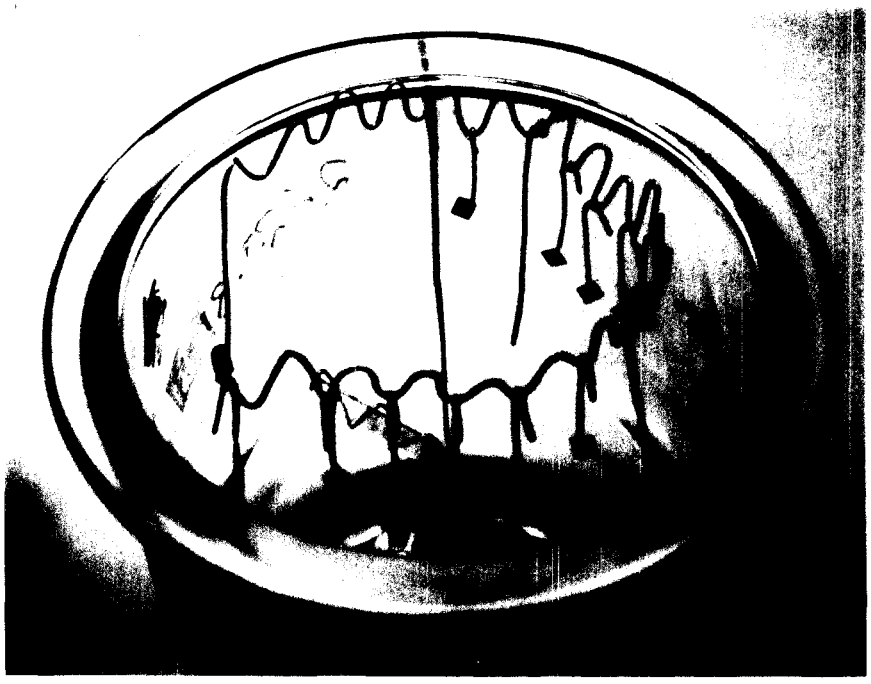

FIG. 1. *Positioning of infected wafers in a desiccator.*

and placed on a wire rack inside 2.5-liter desiccators (Fig. 1). An angled glass plate connected to a magnetic stirring bar, which would act as a fan when rotated, was placed in each desiccator to facilitate air circulation, and the desiccators were sealed with a ventable lid.

Injections of liquid chloropicrin were made through a rubber serum stopper that sealed the vent in the desiccator lid. Immediately following injection, the stirring bar/glass-plate "fan" was started rotating by placing the desiccator on a magnetic stirrer. Air circulation was continued until all liquid chloropicrin had evaporated and the vapor concentration, determined by gas chromatographic analysis of vapor samples withdrawn through the serum stopper, had come to equilibrium (usually 15 to 20 min). A Hewlett-Packard[1] 5750 research gas chromatograph with flame ionization detector was used for the analysis. Operating conditions were: 91.5 cm × 3 mm o.d. stainless steel column packed with 3% OV-17 on a Gaschrome Q solid support; helium flow-rate, 25 cm^3/min; column temperature, 75 C; injection port and detector temperatures, 129 and 163 C, respectively. Because some of the chemical was adsorbed on the glass surfaces within the desiccators, vapor concentrations could not be calculated directly from the injection volumes. Therefore, a calibration curve was prepared to provide an estimate of the injection quantities needed to effect a certain vapor concentration in the desiccators. Temperature was maintained at 21 ± 1 C throughout testing.

[1] Mention of commercial products does not imply endorsement by Oregon State University.

TABLE 1. *Minimum lethal dosages and lethal CT factors for Chloropicrin vapors at five exposure periods for two decay fungi growing in wooden wafers.*

Hours exposure	Fungus	Minimum lethal dosage of chloropicrin (μg/ml)	Lethal CT factor
24	*Gloeophyllum saepiarium*	0.064 (0.047, 0.080)*	1.54
	Poria sp.	0.61 (0.59, 0.64)	14.64
16	*G. saepiarium*	0.106 (0.097, 0.115) 0.125 (0.123, 0.127) 0.131** (0.127, 0.135)	1.93
	Poria sp.	0.55 (0.54, 0.55) 0.77 (0.75, 0.78)	10.56
12	*G. saepiarium*	0.83 (0.79, 0.87) 0.90 (0.89, 0.91)	10.56
	Poria sp.	1.39 (1.35, 1.43)	16.68
8	*G. saepiarium*	1.70 (1.50, 1.89)	13.60
	Poria sp.	1.77 (1.59, 1.94) 1.95 (1.94, 1.95) 2.26 (2.11, 2.41)	15.95
4	*G. saepiarium*	2.95 (2.76, 3.13) 3.62 (3.57, 3.67)	13.14
	Poria sp.	2.94 (2.76, 3.13)	11.76

* Numbers in parentheses represent the actual dosages that were averaged to obtain the MLD.
** More than one value is given where successive tests did not agree on one lethal concentration for that exposure period.

Fungal assay

Following vapor-exposure periods of 4, 8, 12, 18, and 24 h, the desiccators were vented and allowed to air for 3 to 4 h. Preliminary tests showed that after an initial equilibration period, the concentration of chloropicrin could be maintained in the sealed desiccators for at least 1 week, ensuring that a constant vapor concentration would be maintained over the maximum exposure period. After removal from the desiccators, the wafers were flamed lightly to destroy surface contaminants and aseptically transferred to malt-benomyl nutrient agar[2] to discourage nonhymenomycete growth and increase isolation of decay fungi. Control wafers from all culture plates were placed in a desiccator with no chloropicrin for the maximum 24-h exposure as a check on infection and sterilization procedures. The cultures were observed frequently for 3 to 4 weeks.

Because the number of viable fungal cells in each treated wafer could not be determined, the MLD (concentration at which no decay fungi could be isolated from the wafers) was used to express the fungitoxic concentration of vapors for a particular exposure period rather than the ED_{90} or ED_{50} concentrations more commonly used to express fungitoxicity. Because vapor concentrations in the desiccators could not be reproduced with accuracy in successive tests, a range

[2] Malt extract agar with 10 ppm benomyl solution.

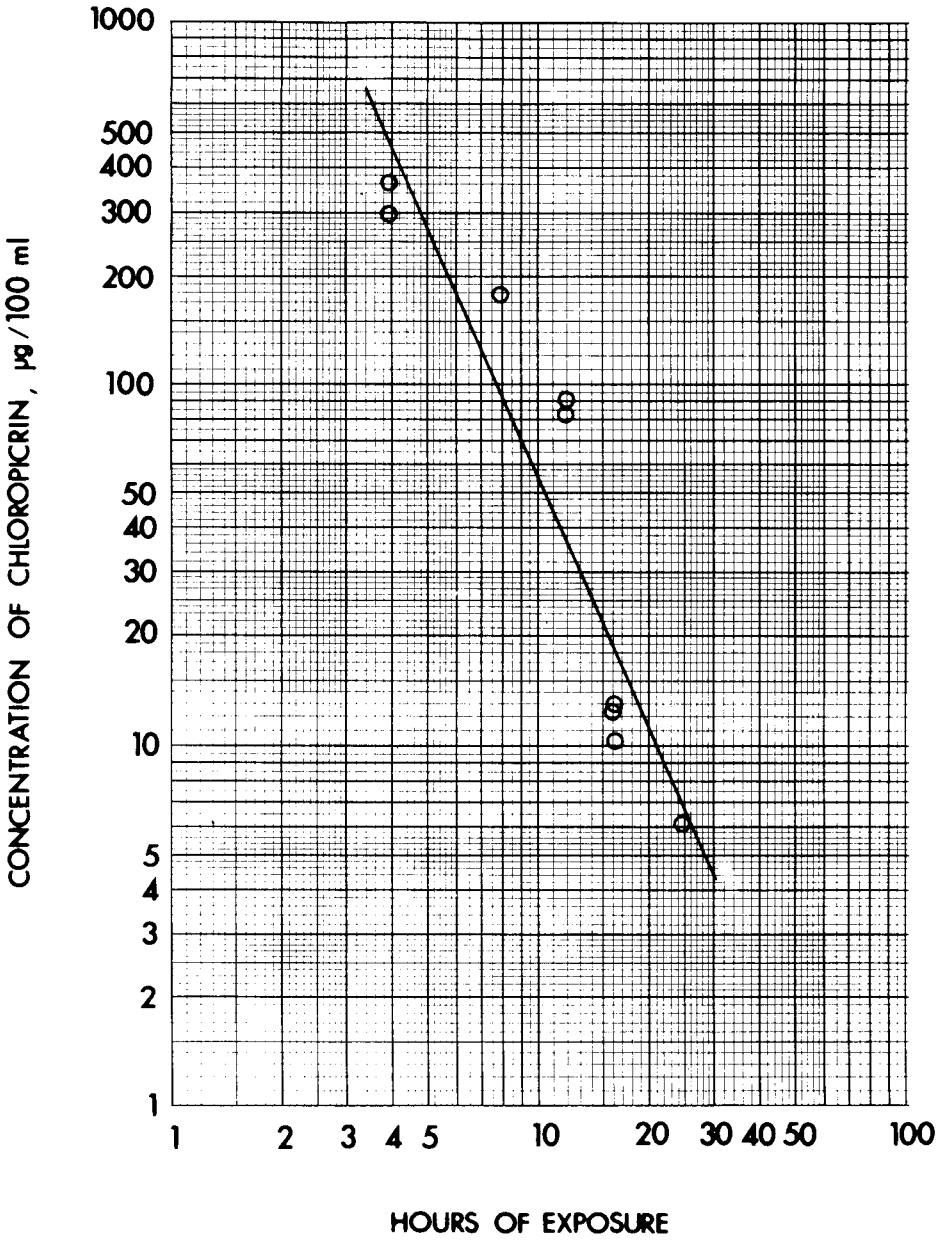

FIG. 2. Minimum lethal dosages of chloropicrin to *Gloeophyllum saepiarium* over time.

of vapor concentrations that bracketed the MLD dosage was developed instead. The MLD values were then calculated using data from successive tests by averaging the lowest chloropicrin dosages that prevented fungal growth from the wafers and the next lowest dosages. These values are presented in Table 1. More than one MLD value was used in the data analysis when successive tests did not agree on a single value for a particular exposure period. The average MLD value

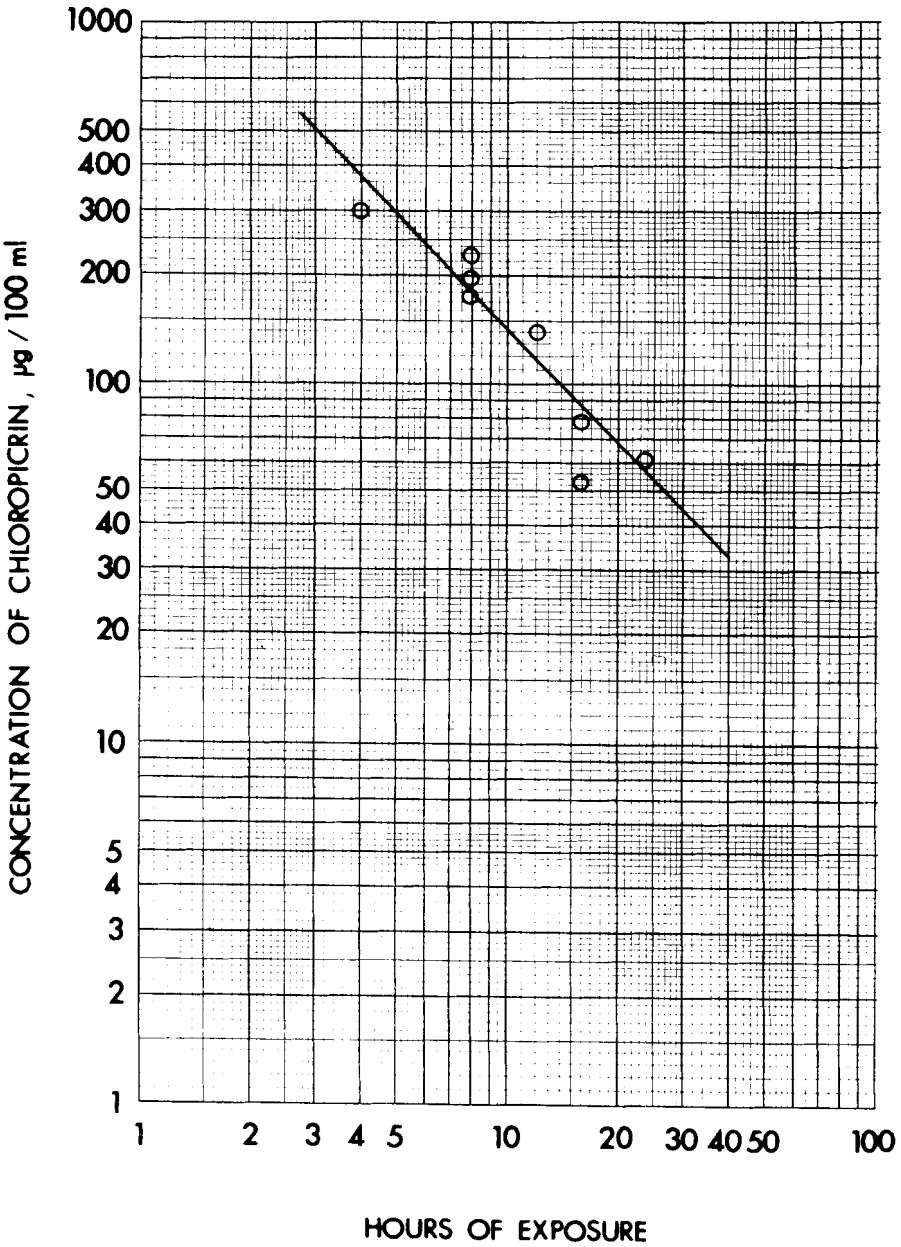

FIG. 3. Minimum lethal dosages of chloropicrin to *Poria* sp. over time.

is perhaps more useful than an ED value from a practical standpoint because it corresponds to the chloropicrin concentration required to stop decay in a timber.

Fumigant vapors must be contained in the treated substrate for a minimum time period to be effective. For this reason a concentration/time (CT) value was used to express fumigant effectiveness. The lethal CT is obtained by multiplying the MLD by the length of time necessary to effect death of the fungi. Ideally, the

lethal CT value would be constant over time for any particular organism and fumigant; however, susceptibility for a particular fungus varies with the exposure period and dosage so that the lethal CT value may increase or decrease as these parameters vary.

RESULTS AND DISCUSSION

MLD values with respective exposure periods for *G. saepiarium* and *Poria* sp. are plotted in Figs. 2 and 3. Both chloropicrin concentration and time axes are scaled logarithmically to obtain a linear plot for both fungi. The expected lethal CT values for *G. saepiarium* and *Poria* sp. can be calculated by using the dosage and time coordinates from the two regressions. Representative values are listed in Table 1.

Hypothetically, the lethal CT values for a particular fungus should remain constant as the exposure time to the fumigant varies, except at the extremes of the time exposure range (Goring 1967). The CT values for *Poria* sp. support this hypothesis in that the lethal CT range is small between the 4- and 24-h test limits. However, the data indicate that *G. saepiarium* is more susceptible to chloropicrin after longer exposure periods; thus, these data do not support the hypothesis.

Since chloropicrin vapors appear to remain in wood in service for many years (Graham and Corden 1977), it may be assumed that the MLD values for the 24-h exposure periods listed in Table 1 would be adequate to control decay by *G. saepiarium* and *Poria* sp. in in-service timbers. By analyzing the concentration of chloropicrin in these timbers, it should be possible to determine if adequate amounts of the chemical are being applied and when retreatment may be necessary.

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