The Comparative Effects of Chloramines on a Range of Nematodes[1]

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Abstract: Chloramine-T (sodium p-toluene sulfonchloramide) was a good surface sterilant for *Ditylenchus dipsaci,* however it was somewhat nematicidal. These properties were presumably associated with its properties as an oxidizing chlorine. Other chloramines tested were also toxic. Its possible use as a nematicide is suggested in relation to dosage and phytotoxicity. The comparative effects of chloramines on a wide range of freeliving soil nematodes and freeliving infective larvae of animal parasitic forms are included.

In attempting to sterilize phytoparasitic nematodes for inoculation onto aseptic callus tissue culture, the use of chloramines, especially chloramine-T, was investigated. The chloramines are widely used as bacteriocides, fungicides and as general antiseptics. The structural formulae of those tested is given in Table 1: potassium dichloroisocyanurate, trichloromelamine, N-chlorosuccinimide, chloramine-T, and 1,3-dichloro-5,5-dimethyl hydantoin.

Our studies followed three main lines of inquiry: (i) to determine the efficacy of chloramines as surface sterilants, (ii) to evaluate their nematocidal activity to *Ditylenchus dipsaci,* and other nematodes, and (iii) to test the phytotoxicity of these compounds on garlic cloves and scales.

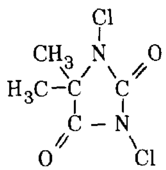
THE INFLUENCE OF CHLORAMINES ON D. DIPSACI

MATERIALS AND METHODS: *The Axenization of D. dipsaci.—D. dipsaci* were extracted from garlic scales in a mist chamber. The nematodes were treated with chloramines at several concentrations and times of exposure (Table 2). Following treatment, they were rinsed in sterile, distilled water and divided into two batches. One batch (NS) was subdivided and transferred to 8 tubes each of growth media NIH and APT (S). A second batch was screened (S) aseptically and similarly placed in the growth media.

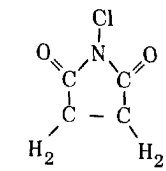
Motility Tests on D. dipsaci.—An experiment was designed to establish the tolerance of *D. dipsaci* to Chloramine-T treatment.

TABLE 1. Chloramine compounds used for the axenization and nematocidal tests.

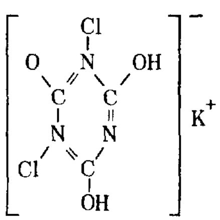
1,3 dichloro-5,5-dimethyl hydantoin



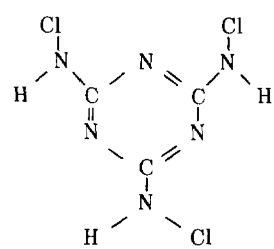
N-chlorosuccinimide



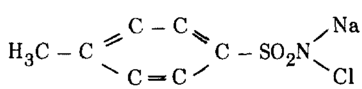
Potassium dichloroisocyanurate



Trichloromelamine



Chloramine-T
Sodium p-Toluenesulfonchloramide



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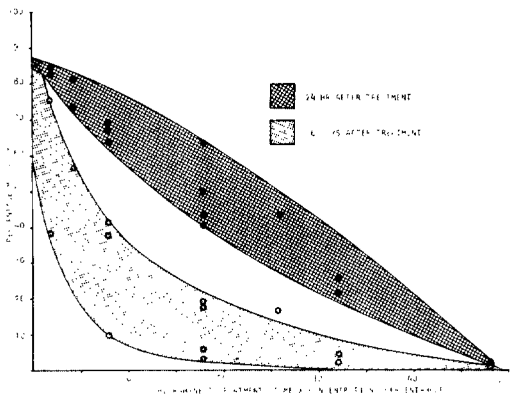

FIG. 1. The "primary" and "secondary mortality" of *Ditylenchus dipsaci*, after one day and six days following treatment in Chloramine-T. Motility is taken as an assessment of those nematodes alive. Control motility 75% (± 2%) throughout.

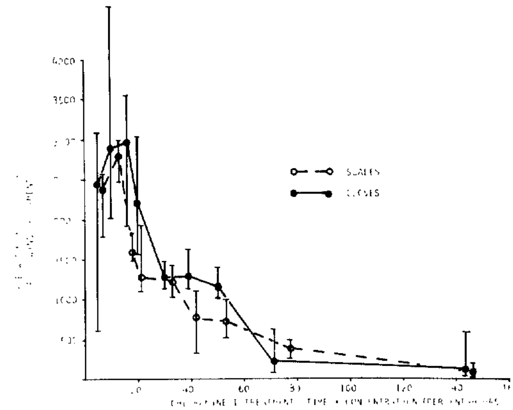

FIG. 2. Mean line *Ditylenchus dipsaci* recovered after 6 days from 1.5 g garlic scales and 5 garlic cloves following treatment in varying times and concentrations of Chloramine-T.

Nematodes extracted from garlic scales, after different treatments with Chloramine-T, were stored in distilled water at 15 C. At daily intervals the percentage motility (1) was determined. Untreated controls were stored at 15 C in distilled water.

Nematocidal and Phytotoxicity Tests on D. dipsaci in Infected Garlic Scales and Cloves. —Heavily infested garlic scales and cloves from bulbs, were treated in series of 6 cm meshed stainless steel baskets. One series contained 1.5 gm of scales each, the other

TABLE 2. The percentage of *Ditylenchus dipsaci* showing microorganisms contamination 7 days after treatment in various chloramines.

Chloramine concentration		x				2x				4x			
Microbial growth media		NIH		APT		NIH		APT		NIH		APT	
Screened —S Not screened—NS		S	NS	S	NS	S	NS	S	NS	S	NS	S	NS
Chloramine compound	Length of treatment (hr)												
Potassium dichloro-isocyanurate	4	0	0	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0	0	0	0
x = .1%	16	0	0	0	0	0	0	0	0	0	0	0	0
Trichlormelamine	4	100	0	75	0	88	0	63	0	50	0	25	0
	8	13	0	50	0	25	0	0	0	25	0	25	0
x = .025%	16	0	0	13	13	0	13	0	0	25	0	0	0
1,3-dichloro-5,5-di-methylhydantoin	4	100	100	100	100	100	25	100	13	100	88	100	75
	8	100	100	100	100	100	100	88	100	75	25	88	25
x = .0525%	16	100	100	100	100	88	100	100	100	88	75	75	25
N-chlorosuccinimide	4	88	0	88	0	0	0	13	0	0	0	0	0
	8	0	0	13	0	0	0	13	0	0	0	0	0
x = .0875%	16	50	0	13	0	25	0	13	0	13	0	13	0
Chloramine-T	4	63	0	38	0	100	0	100	13	13	0	38	0
	8	38	50	0	0	63	0	75	0	0	13	13	0
x = 1%	16	0	0	0	0	25	0	38	0	0	0	38	0

five garlic cloves. Four replicates for each of nine treatments were included. The baskets were soaked at 25 C in .1% Vatsol® (wetting agent) for 2 hr. After draining off the Vatsol, the baskets were shaken at 27.5 C in various time concentration treatments of chloramines. Following treatment, the baskets were rinsed in distilled water, air dried for 1 hr and stored in polyethylene bags at 15 C for six days.

Twenty cloves from each treatment were planted in pots to investigate the phytotoxicity of the treatments. After six days *D. dipsaci* were extracted from the treated scales and cloves on a Baermann funnel, and the number passing through was counted after 48 hr.

RESULTS: The percentage microbial contamination on the growth media seven days after inoculation with treated *D. dipsaci* is given in Table 2.

All treatments with Chloramine-T were toxic to *D. dipsaci,* the toxicity increasing with both time and concentration (Table 3). After one day the reduction in motility was approximately linear with respect to the treatment time concentration product (Fig. 1). We call this first day effect the "primary mortality" to distinguish it from the "secondary mortality" observed in the six days following treatment.

Chloramine-T, at 8% for 18 hr killed 94% of *D. dipsaci* in infected garlic scales (Table 4). The time between treatment and the extraction of the nematodes allowed for both the primary and second mortality (Fig. 2). Control was greater in scales than in cloves, probably through facilitated penetration of Chloramine-T into scales. The control effects of N-chlorosuccinimide and potassium dichloroisocyanurate are compared in Table 5.

TABLE 3. Percentage motility of *Ditylenchus dipsaci* 5 days after treatment in Chloramine-T at varying time-concentration products.

	Time (hr)					
Concn. (%)	1	2	4	8	12	16
1		41%	31%	15%	19%	6%
2	78%	62%	24%	15%	5%	5%
4	47%	35%	13%	1%	0%	
8	37%	18%	3%			

TABLE 4. *Ditylenchus dipsaci* surviving in garlic after treatment in Chloramine-T.

Chloramine-T treatment	Time concentration product	Mean worms/ml		Percent kill	
		Cloves	Scales	Cloves	Scales
Nontreated		2547	3513		
2 hr 2%	4	2325	2013	9%	43%
2 hr 4%	8	2940	2768	0%	21%
6 hr 2%	12	3063	1610	0%	54%
2 hr 8%	16	2123	1378	17%	61%
6 hr 4%	24	1255	1255	51%	64%
18 hr 2%	36	1490	775	42%	78%
6 hr 8%	48	1382	1010	46%	71%
18 hr 4%	72	333	625	87%	82%
18 hr 8%	144	310	220	88%	94%

Prolonged treatments in chloramines gave some phytotoxicity (Chloramine-T, 18 hr, 8%; N-chlorosuccinimide, 16 hr, 0.1%; potassium dichloroisocyanurate, 16 hr, 0.6%). At the lower concentrations, Chloramine-T showed some nematode control and the level of phytotoxicity did not differ significantly from the untreated controls.

THE INFLUENCE OF CHLORAMINES ON SOIL INHABITING NEMATODES AND ANIMAL PARASITIC NEMATODES

MATERIALS AND METHODS: Nematodes from widely separated taxonomic groups were isolated from soil samples in Davis, including these seven genera: *Rhabditis, Acrobeles, Tylenchus, Criconemoides, Tripyla, Dorylaimus,* and *Mononchus.* These nematodes were treated in potassium dichloroisocyanurate, N-chlorosuccinimide, trichloromelamine, Chloramine-T, and dimethyl hydantoin for 4, 8, and 16 hr at each

TABLE 5. Percent of *Ditylenchus dipsaci* surviving in garlic 6 days following treatment in N-chlorosuccinimide and potassium dichloroisocyanurate.

Treat-time	2 hr		6 hr		16 hr	
Concentration	Cloves	Scales	Cloves	Scales	Cloves	Scales
.1% N-chlorosuccinimide	28%	34%	55%	28%	70%	21%
.3% potassium dichloroisocyanurate	100%	32%	100%	7%	30%	2%
.6% potassium dichloroisocyanurate	89%	3%	100%	8%	51%	7%

of three concentrations (Fig. 3). Six days after treatment, the relative numbers of survivors was estimated.

To further examine the toxicity of Chloramine-T on nematodes, a selection of animal parasitic infective third-stage larvae from cattle were treated: *Cooperia, Ostertagia, Trichostrongylus,* and *Bunostomum.* They were treated together as "animal parasitic larvae" and following treatment their percentage motility was calculated. A period of five min on the microscope was included before the counts were made to standardize effects of photo-stimulation by the microscope illumination (1).

RESULTS: The chloramines were toxic to the soil and animal parasitic larvae used; potassium dichloroisocyanurate was totally toxic to them all. *Tylenchus* and *Rhabditis* were most tolerant, while *Mononchus, Tripyla,* and *Criconemoides* were most readily killed. Chloramine-T had no measurable toxicity at 2% for 2 hr on the animal parasitic larvae; above this its nematocidal properties increased with increasing time concentration product.

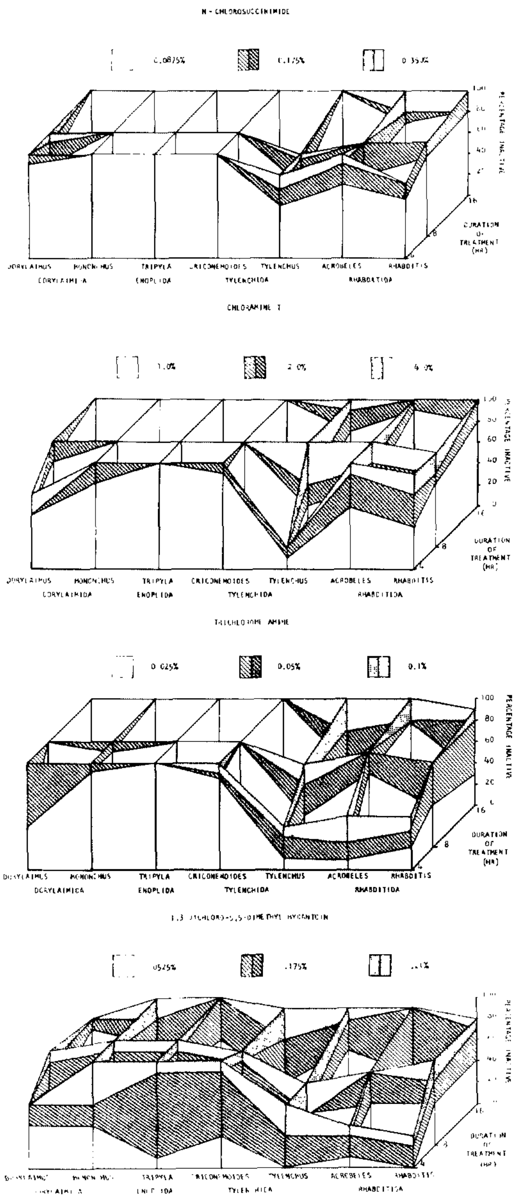

FIG. 3. A comparison of the activity of soil nematodes following treatment in various chloramines: a) N-chlorosuccinimide, b) Chloramine-T, c) trichloromelamine, e) 1,3-dichloro-5,5-dimethyl hydantoin.

DISCUSSION

The use of chloramines for surface sterilization of nematodes has been tested and rejected because of their nematocidal properties. Our data suggest the nematocidal effect is directly dependent on the length of the exposure treatment at the concentrations used. This probably reflects the period of time taken for chloramine penetration into the nematode. The nematocidal effects were both immediate and longer term, "primary and secondary kill."

The notion that the mechanism of nematode kill could be diverse and reflected in measurable terms *e.g.* rate of death has been previously noted (4). It is not surprising, therefore, to observe a dual nature of killing of nematodes by chloramine treatment. The primary kill was linear but the secondary kill was logarithmic with the time concentration product. No such dual mortality was found in treated animal parasitic nematodes.

The differential rates of kill of chloramines have been examined for a wide range of nematodes, and although toxic to all, the relative toxicity varied with different genera. The reactions of plant parasitic and soil nematodes to coal tar dyes have been grouped into three categories by Hollis (2,3): saturation reactions, basophilic reactions and resistant reactions. He concluded that the reaction of the nematodes to the dyes was a function of cuticular permeability.

Saturation reactions were characteristic in the Enoplida; we found *Dorylaimus, Tripyla,* and *Mononchus* all readily susceptible (Fig. 3). Of those treated, the most resistant forms were *Rhabditis, Acrobeles,* and *Tylenchus.* In this observation we concur with Hollis (3) who found least penetration of coal tar dyes in members of the Rhabditina and Tylenchida. Animal parasitic forms were relatively resistant and behaved in a manner similar to the Rhabditina.

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