Carbaryl Effects on Growth and Development in Suspension Cultures of Wild Carrot

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Carbaryl is a common contact poison insecticide used for protection of various crops. Considerable research has been done on measuring the toxicity of this compound on whole organisms. In recent years attention has been given to use of various plant and mammalian cell culture systems for measuring pesticide toxicity (GABLIKS and FRIEDMAN, 1969; LITTEREST et al, 1969) and metabolism (BARON and LOCKE, 1970; LOCKE et al, 1971; LOCKE and BARON, 1972). Plant tissue culture systems have been used primarily as a tool to study pesticide metabolism while little attention has been given to the affects of these chemicals on normal growth and developmental processes. Tobacco cell suspensions have been used successfully to study carbaryl metabolism (LOCKE et al, 1971) but no information is available regarding developmental effects of the pesticide.

Suspension cultures of Daucus carota, wild carrot, represent a sensitive system for the analysis of various compounds and their effect on the growth and development of these cultured tissues. The purpose of these studies was to determine the effect of carbaryl and 1-naphthol on adventive embryo development, tissue growth, macromolecular synthesis and metabolism of carbaryl in vitro.

MATERIALS AND METHODS

The pesticide used was carbaryl (1-naphthyl methylcarbamate) (99.7%) supplied by Union Carbide Corporation. The ^{14}C-naphthyl-labeled carbaryl was a gift from R. K. Locke and was obtained from Union Carbide Corporation. The specific activity was 4.4 μCi/μmole and was diluted to a final concentration of 10 ppm in growth medium. Labeled and unlabeled carbaryl was tested for purity by thin layer chromatography. The solvent systems used were ether-hexane (4:1), benzene-ethanol (19:1), chloroform-acetonitrile (4:1) and chloroform-methanol (49:1).

Bulletin of Environmental Contamination & Toxicology,
Vol. 16, No. 3 © 1976 by Springer-Verlag New York Inc.

The tissue used was a stock liquid culture of wild carrot which has been actively growing in liquid medium for five years. The culture medium has been described elsewhere (WETHERELL and HALPERIN, 1963). In the presence of medium supplemented with 5 μM 2, 4-dichlorophenoxyacetic acid, (2,4-D), a synthetic auxin, the tissue grows as tissue aggregates without any organ differentiation. Transfer of tissue to auxin-free medium results in vegetative embryo development from the tissue clumps. This feature permits the use of this system as a tool for measuring the growth inhibition or teratogenic effects of various chemical compounds. Carbaryl and 1-naphthol were added to presterilized liquid medium prior to tissue transfer. Both compounds were prepared in 5% acetone and sterilized through a 0.45 μm Millipore filter using a Swinney apparatus. The solutions were then added aseptically in 0.5 ml aliquots to 4.5 ml of presterilized culture medium at room temperature. Control tubes contained 4.5 ml of medium and 0.5 ml of a 5% acetone solution with no additives. All transfers were performed in a Baker Edgeguard laminar flow hood. Test tubes containing tissue in 5 ml of medium were placed on a rotating tissue culture drum in a Hotpack incubator and maintained in continuous light at 25°C.

Protein was analyzed by the method of Lowry (LOWRY et al, 1951), RNA by an orcinol test, and DNA with diphenylamine (PACKER, 1967). Radioactivity in TLC plates and tissue extracts was counted with a Packard Tricarb liquid scintillation spectrometer. The scintillation fluid used was Liquifluor-Triton X-100.

Embryo numer was determined by dispensing the tissue fragments in a tightly packed monolayer and counting the number of embryos per unit of area which had reached the heart stage of development (WOCHOK and WETHERELL, 1972).

RESULTS AND DISCUSSION

Growth of cultured carrot tissues was inhibited to 33% of controls when maintained in a medium supplemented with 20 ppm carbaryl, and 67% of control values in 10 ppm. With increasing concentrations of carbaryl there resulted an increase in the specific activities of RNA and protein. This was partially due to the decrease in fresh weights with increasing concentrations of carbaryl. At 20 ppm carbaryl the specific activity of RNA increased to almost four times that of the controls. The increase in protein was less dramatic and was not evidenced at any concentration

TABLE 1. EFFECT OF CARBARYL ON GROWTH, RNA AND PROTEIN

ppm	FRESH WEIGHT (mgms)	SPECIFIC ACTIVITY*	
		RNA	PROTEINS
0.0	25.7	2.4	18.5
0.1	22.4	2.8	18.9
1.0	22.6	2.7	23.2
10.0	17.2	3.3	20.5
20.0	8.6	8.0	32.9

*Specific activity is equivalent to mg (RNA or protein) /mg fresh weight. Data represent an average of no fewer than 4 experiments, each with 6-10 replicates. The growth period was 10 days.

except 20 ppm (Table 1). It should be noted that there was little change in total RNA in the differently treated populations of cells while the total protein in 20 ppm carbaryl was reduced to 60% of control tissue. The decrease in total protein is apparently an indication of reaction by the system to the physiological stress imposed on it by the carbaryl.

The effects of carbaryl on embryo development in cultured carrot tissues was dramatic. An average of almost 13 embryos per field was observed in control medium lacking auxin. Development of adventive embryos was diminished by over 50% of controls even at 0.1 ppm. At 20 ppm there was an 85% reduction in embryo development. Teratogenic effects were observed in embryos that did develop at high concentrations and these deviated morphologically from control embryos. Growth was determined by fresh weight analysis in the embryonic cultures (Table 2) and followed the same pattern as in Table 1.

When carrot tissues were incubated in radioactively labeled carbaryl (10 ppm) for 14 days it was found that only about 0.5 - 1% of the ^{14}C radioactivity was recovered in the cells. The remainder of radioactivity was in the medium and cell washes. The amount of radioactivity in cells after a two week incubation was considerably lower than that reported by LOCKE et al (1971). We also found that after this period of culture 91% of ^{14}C radioactivity in the medium was concentrated in the 1-naphthol fraction as determined by thin layer chromatography with an ether-hexane solvent (4:1). A number of other metabolites were also evident

TABLE 2. EFFECT OF CARBARYL ON EMBRYOGENESIS AND FRESH WEIGHT

CONCENTRATION (ppm)	0	0.1	1.0	10	20
EMBRYO NUMBER/FIELD	12.9	6.3	4.8	2.6	1.9
AVE. FRESH WEIGHT (mgms)	25.6	21.6	20.2	14.2	7.2

in the chromatograms but no attempt was made to identify them. Metabolism of carbaryl by cultures of Aspergillus terreus to 1-naphthol through a series of intermediate reactions has been reported (LIU and BOLLAG, 1971).

Our results with radioactively labeled carbaryl suggest that very little is incorporated by carrot cells, yet there is unquestionably an effect of this compound on growth. Since a significant percentage of carbaryl apparently breaks down to 1-naphthol it occurred to us that the developmental effects we were observing might be due to 1-naphthol. A series of experiments were done in which carrot cultures were maintained on a medium supplemented with carbaryl and another with 1-naphthol. The results are shown in Table 3. The effect of these compounds on growth as measured by dry weight was identical.

While a considerable amount of work on the metabolism of carbaryl by carrot cultures still needs to be done, our results suggest that the inhibition of growth and interference with developmental processes

TABLE 3. EFFECT OF CARBARYL AND 1-NAPHTHOL ON GROWTH OF CARROT TISSUES IN VITRO

ppm	AVERAGE DRY WEIGHT (mgms)	
	Carbaryl	1-Napththol
0.00	7.05	7.05
0.01	4.9	4.4
0.10	4.7	4.2
1.00	3.8	4.0
10.00	3.2	3.1

The growth period was 11 days.

is not necessarily a direct effect of carbaryl. That similar results may be obtained with 1-naphthol suggests that this compound, an important breakdown product of carbaryl, may be primarily responsible for interfering with normal regulatory mechanisms in this system.

SUMMARY

Carbaryl has been demonstrated to inhibit growth of carrot suspension cultures at 10 and 20 ppm. The specific activities of RNA and protein increased at 20 ppm. Total RNA was relatively unchanged at any concentration while total protein was reduced considerably at 20 ppm. Embryo development was inhibited by increasing concentrations of carbaryl. Thin layer chromatography indicated considerable degradation of carbaryl to 1-naphthol. When 1-naphthol was tested in this system it was shown to elicit the same inhibitory effects on growth as carbaryl, suggesting that the 1-naphthol residue is itself a potent inhibitor of tissue growth and development.

ACKNOWLEDGMENT

This investigation was supported by a grant from the National Science Foundation GY-10724.

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