Effects of Lindane on DNA, RNA, and Protein Synthesis in Corn Roots

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Corn roots grown in lindane-treated sand, water, or agar were stunted, had club-shaped tips, no lateral roots or root hairs, and had less weight than untreated roots. The insecticide had no effect on protein content of corn roots grown in organ culture on lindane-treated (20 ppm) and untreated agar. Although the amounts of protein per root were considerably less in lindane exposed plant material, the actual amounts of plant tissues produced were also drastically reduced. Amounts of protein per gram of root tissue produced were similar in control and lindane exposed roots. However, the synthesis of DNA in these roots was reduced due to the effects of the insecticide. At a lindane concentration of 8 ppm in water, the uptake of [^{3}H]thymidine (a DNA precursor) into corn roots and the incorporation of [^{3}H]thymidine into DNA were reduced, particularly after exposure to lindane for 120 h. Such a reduction of DNA synthesis could possibly be related to the effects of lindane in disrupting cell mitosis, which resulted in enlarged cells with multilobed nuclei. The uptake of [^{3}H]uridine (an RNA precursor) by lindane exposed corn roots was also reduced, while the incorporation of the precursor into RNA was increased. Since the amounts of actual RNA in 1 g of fresh root tissue were reduced, a more rapid turnover of RNA in lindane exposed roots was indicated.

The insecticide lindane (γ isomer of 1,2,3,4,5,6-hexachlorocyclohexane or γ-HCH), still widely used in the Eastern Hemisphere, continues to fulfill a purpose in the western world for seed treatments against fungal and wireworm attack. Its use in the United States was curtailed because of its off-flavor effect on crop plants, its persistence properties, and its effect on root growth.

The effects of lindane on higher plants have long been known. Early work revealed that lindane-treated roots exhibited c-mitosis, an inactivation of the spindle apparatus associated with delayed division of the centromeres (Nybom and Knutsson, 1947; Carpentier and Fromageot, 1950). Kostoff (1949) noted stunting, cell enlargement without differentiation, and spindle inactivation in plants exposed to lindane. Both Scholes (1953) and Zeller and Hauser (1974) reported the induction of polyploidy by lindane. Simkover and Shenefelt (1952) reported that chromosome smears of the treated root tips had diminished affinity to Feulgen stain, a stain specific to DNA, and concluded that DNA was being degraded. They also found c-mitotic nuclei, decreased number of metaphases, and chromosomes extruded from the nuclei. Lichtenstein et al. (1962) using light microscopy found accelerated cell division, irregular enlargement of scattered cells, and multiple nuclei in the root tips of treated corn and peas. Decreased respiration rates were noted in the root tips of corn and oats exposed to lindane. Using the electron microscope, Charnetski et al. (1973) reported anomalous cell wall development, cessation of mitosis, and greatly enlarged, lobed nuclei which often lay in more than one cell due to incomplete wall formation. The multiple nuclei often referred to in earlier literature were suggested to be sectioning artifacts of the large, lobed nuclei reported in Charnetski's study.

The object of this study was to investigate the effects of lindane on the synthesis of corn root protein, DNA, and RNA in an attempt to further understand the mechanism by which lindane disrupts root growth.

MATERIALS AND METHODS

Chemicals. Analytical grade lindane (1,2,3,4,5,6-hexachlorocyclohexane) was obtained from Allied Chemical and Dye Corp., New York, N.Y. The radioactive precursors 6-[^{3}H]uridine (specific activity 31 Ci/mmol) and [*methyl*-^{3}H]thymidine (specific activity 20 Ci/mmol) were purchased from New England Nuclear Corp., Boston, Mass. Solvents used were redistilled acetone, chloroform, and hexane and reagent grade methyl alcohol, ethyl alcohol, diethyl ether, and octyl alcohol.

Insecticide and Precursor Applications. Before treating with lindane, distilled water and quartz sand (1% v/w) were mixed by tumbling for 15 min to ensure uniform wetting of the sand. It was then treated with an acetone solution of lindane at 8 or 11 ppm as described by Lichtenstein and Schulz (1959). When organ cultures were used, Bonner agar (Torrey, 1954) was sterilized in cotton-plugged 500-mL Erlenmeyer flasks and cooled to about 40 °C. Methanol solutions of lindane were then added with a sterile cotton-plugged pipet in a sterile transfer chamber to yield a final insecticide concentration of 20 ppm. Just before solidifying, the agar was swirled and poured into petri plates. The concentration of methanol in the agar was 1% (v/v).

When corn was grown in tap water, lindane was added in methanol to give an insecticide concentration of 8 ppm. The concentration of methanol in the tap water was 0.2%.

To determine uptake and incorporation of nucleic acids by corn roots in tap water, tritiated thymidine, a precursor of DNA, and tritiated uridine, a precursor of RNA, were added at 50 μCi/400 mL with a microliter syringe at the end of the growth period, 1 to 6 h before harvest. In these experiments precautions were taken to prevent bacteria from contributing to the specific activities of the nucleic acids. Roots were grown in nutrient-poor tap water, rather than in a complete nutrient solution. The water was aerated, and all roots were washed four times with sterile buffer before extraction. Antibiotics, such as chloramphenicol, were not used since preliminary work indicated that they affected the fresh weight of the roots more than lindane.

Plant Material. Hybrid variety Funk G-4444 corn seeds, obtained through the courtesy of Funk Seeds International, Bloomington, Ill., were used in all experiments.

Plant Growth. *In Sand Culture.* Three aliquots each of 1000 g of lindane-treated (8 or 11 ppm) or 1000 g of untreated (control) sand were placed into 1 qt paper cartons. Seven corn seedlings, previously germinated between moist paper toweling for 48 h, were then inserted

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into the sand in each carton. They were watered with 100 mL of distilled water and transferred to a Percival Growth Chamber (light intensity of 1800–2000 new ft candles, 12-h day, ambient temperature 28 °C by day and night, and humidity of 70%). Plastic bags were placed over the cartons for the first 2 days to protect the young seedlings from excessive drying. On the third day, the corn seedlings were watered with Hoagland's nutrient solution (Hoagland and Arnon, 1950) and thereafter with distilled water as necessary. Plants grown in sand treated with lindane at 8 ppm were harvested after 7 days and photographed. Roots grown in sand treated with lindane at 11 ppm were harvested after 12 days, rinsed with tap water, weighed, and then extracted and analyzed for their protein content.

In Organ Culture. Corn root tips used for organ culture were obtained in the following way: corn seeds were surface sterilized by placing them in a 70% ethanol solution containing 0.1% $HgCl_2$ for 5 min and rinsing them twice with sterile water. About 25 seeds were transferred to a sterile 10-cm petri dish and germinated in 5–10 mL of water containing 0.05% streptomycin and 0.03% penicillin. The streptomycin and penicillin were necessary to control contamination. The seeds were then incubated for 48 h in darkness at a temperature of 28 °C. Since only the root tips were cultured, 5-mm tip pieces were excised aseptically. Eight tips were then transferred onto each of six petri dishes (10-cm diameter) containing sterile Bonner agar which had been treated with 20 ppm of lindane as described. Roots were grown in darkness at 28 °C for 2, 4, 8, 12, or 16 days. On each harvest date, one root was taken from each of the six petri dishes, weighed, followed by a protein analysis of each root. Results were finally expressed as the mean ± SD of the fresh weight, milligram of protein per root, and milligram of protein per gram of fresh weight of the roots. To study visible effects of lindane, roots were also grown on agar treated with the insecticide at 10 ppm and incubated for 14 days as described before being photographed.

In Tap Water. Corn seedlings, germinated for 48 h between moist paper toweling, were supported on perforated plastic trays over a 23 × 13 × 8 cm Pyrex dish with their roots immersed in 400 mL of lindane-treated (8 ppm) tap water. While air was bubbled through this water, the plants were incubated as described above in a growth chamber for various time periods as indicated below.

The total uptake of radioactive precursors was determined by combustion of roots which had been grown for 12 h in lindane-treated or in untreated tap water. At that time, [^{3}H]thymidine, or [^{3}H]uridine, were added, each at 50 μCi/400 mL to the lindane-treated or untreated water, and the roots were further incubated for 1, 2, 4, or 6 h. To avoid loss of radioactive precursors, no aeration was used after their addition to the media. After the appropriate incubation times, six roots from each treatment, weighing between 0.14 and 0.30 g, were harvested and excised from the greens. The roots incubated with [^{3}H]thymidine, were washed four times with 7 mL of sterile 0.3 M NaCl–0.1 M EDTA solution (pH 8). Roots incubated with [^{3}H] uridine were washed four times with 7 mL of sterile 0.1 M Tris buffer (pH 7.5). To assure that all surface radioactivity had been removed before extraction, the fourth and final root wash was routinely analyzed for its tritium content. This final wash, however, did not contain appreciable amounts of radioactivity. The total tritium content of the sample composed of six roots was determined by combustion to ^3H_2O as described below. These experiments were repeated three times with [^{3}H]uridine and once with [^{3}H]thymidine.

The incorporation of [^{3}H]uridine or [^{3}H]thymidine into RNA or DNA, respectively, was studied by exposing roots to lindane and one of the precursors for various lengths of time. After harvesting the roots, they were excised from the greens and washed. Roots grown for up to 48 h were washed with four 50-mL aliquots of the respective sterile buffer, while those grown for 96 and 120 h were washed with four 100-mL aliquots of the respective sterile buffer because of a larger volume of root material. After that, the nucleic acids were extracted as described, followed by analyses of the extracts by both colorimetric procedures and by radioisotope counting techniques.

Extraction and Analyses. Protein was extracted by the method of Kemp and Sutton (1971) and analyzed by a modified Lowry colorimetric procedure (Rutter, 1967).

Analyses for nucleic acids were conducted using radioactive precursors and colorimetric techniques. Thus DNA was extracted as described by Kemp and Sutton (1976) and analyzed by the Burton colorimetric method (1956). RNA was extracted by the method of Sutton and Kemp (1976) and analyzed spectrophotometrically at 257 nm (Mahler and Cordes, 1968). In both DNA and RNA extractions, all ethanol soluble material was designated the "soluble precursor pool".

In studies with radioactive precursors, [^{3}H]thymidine and [^{3}H]uridine were used. When the total uptake of the radioactive precursors was determined, the total tritium content of the roots was measured by oxidizing samples consisting of six corn roots, weighing between 0.14 and 0.30 g, in a Packard Tri-Carb Sample Oxidizer, Model 305, as described by Kaartinen (1969). The samples were burned in a stream of oxygen and the resulting ^3H_2O was collected. A scintillator consisting of 1.8 g of dimethyl-POPOP, 36 g of PPO, 180 g of naphthalene, 600 mL of methyl Cellosolve, and 3000 mL of dioxane was added to the ^3H_2O. Amounts of radioactivity were determined using a Packard Tri-Carb liquid scintillation spectrometer, Model 3320. Liquid scintillation counting (LSC) data were corrected for background and counter efficiency. Results were expressed as total disintegrations per minute (DPM)/gram of fresh weight.

The amount of radioactivity in root extracts containing nucleic acids was also determined. Data were corrected for background, counter efficiency, and adjusted to represent the total volume of the sample. In these studies, the amount of radioactivity was also determined in the tritium-treated incubation media.

Data gathered included fresh weight of root, micrograms of DNA or RNA per root, and micrograms of DNA or RNA per gram of fresh root weight. The specific activity (SA) of DNA or RNA (SA-DNA or SA-RNA) was calculated as DPM/μg of DNA or RNA. The specific activity of the soluble precursor pool (SA-DNA pool or SA-RNA pool) was calculated as DPM/total soluble precursor pool. The soluble precursor pool is represented by all the radioactivity derived from [^{3}H] thymidine or [^{3}H]uridine and their metabolites but does not include [^{3}H]DNA or [^{3}H]RNA. This includes such DNA precursors as deoxyribose thymidine monophosphate, deoxyribose thymidine diphosphate, and deoxyribose thymidine triphosphate and RNA precursors such as ribosyluridine monophosphate, ribosyluridine diphosphate, and ribosyluridine triphosphate. Finally the relative rate of incorporation was calculated as the ratio of SA-DNA to SA-DNA pool and of SA-RNA to SA-RNA pool. Since the amount of radioactivity in the DNA or in RNA is directly dependent on the amount of ^{3}H label in the soluble precursor pool, the relative rate is calculated to correct for any differences

Figure 1. Effects of lindane on corn roots grown for 7 days in a quartz sand treated with the insecticide at 8 ppm.

in concentration of radioactive precursors in the precursor pools.

EXPERIMENTAL SECTION AND DISCUSSION

Effects of Lindane on Corn Root Growth. Corn plants which had been grown in lindane-treated (8 ppm) sand for 7 days, or on lindane-treated (10 ppm) agar (organ culture) for 14 days, were stunted, club-shaped, lacked root hairs, and had no lateral root development (Figures 1 and 2).

Effect on Protein Accumulation. After corn roots had been grown in organ culture on lindane-treated (20 ppm) and untreated agar for 2, 4, 8, 12 or 16 days, analyses as described indicated that the insecticide had no effect on the root protein contents (Figure 3). Although the amounts of protein per root were considerably less in lindane exposed plant material (Figure 3B), this was not surprising because the actual amounts of plant tissues produced were also drastically reduced due to the presence of the insecticide (Figure 3A). However, when the amounts of protein were calculated and expressed per gram of root tissue, no differences were any more noticeable between control roots and lindane exposed roots (Figure 3C).

Identical results were obtained with roots grown for 12 days in sand treated with 11 ppm lindane. These roots had a fresh weight of 0.08 ± 0.02 g while those grown in insecticide-free sand weighed 1.22 ± 0.22 g, a difference significant at the 0.1% level (Student's t-test). Similarly, the total amount of protein per lindane exposed root was 0.44 ± 0.10 mg as compared with 6.20 ± 0.84 mg in controls. However, when expressed as milligram of protein per gram of fresh weight, roots exposed to lindane contained 5.78 ± 1.69 mg of protein per gram of fresh weight, while roots grown in insecticide-free sand contained 5.27 ± 1.44 mg of protein per gram of fresh weight.

Effects of Lindane on the Total Uptake of Radioactive Nucleic Acid Precursors in Corn Roots. Results obtained with corn roots grown for 12 h in lindane-treated (8 ppm) tap water and then for an additional 1, 2, 4, or

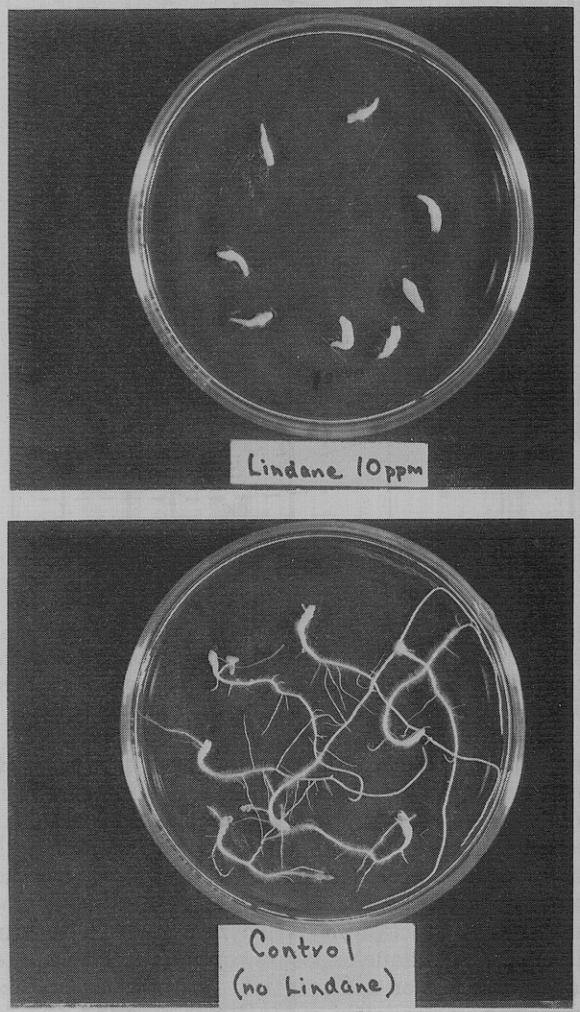

Figure 2. Effects of lindane on excised corn roots grown for 14 days under sterile conditions in organ culture on agar, treated with the insecticide at 10 ppm.

Table I. Effects of Lindane on RNA Synthesis as Measured by Incorporation of its Precursor [^{3}H]Uridine, in Corn Roots Exposed to the Insecticide for Various Lengths of Time[a]

Hours exposed to lindane[b]		Fresh weight, g	RNA, μg		Specific radioactivity		Rel rate[e]
			In one root	Per gram of root	RNA[c]	RNA pool[d]	
12	Ck[f]	0.26 ± 0.02	232.2 ± 27	883.1 ± 107	0.089 ± 0.02	46.5 ± 2	19.1
	L[g]	0.25 ± 0.03	225.6 ± 29	908.4 ± 58	0.039 ± 0.00[i]	33.3 ± 9[k]	11.7
18	Ck	0.27 ± 0.02	192.2 ± 13	704.1 ± 54	0.025 ± 0	33.7 ± 4	7.4
	L	0.27 ± 0.06	190.8 ± 28	713.7 ± 79	0.015 ± 0[j]	21.3 ± 3[i]	7.0
24	Ck	0.38 ± 0.04	233.6 ± 24	613.6 ± 23	0.115 ± 0.01	67.0 ± 6	17.2
	L	0.34 ± 0.02	212.6 ± 17	620.4 ± 48	0.097 ± 0.01[k]	50.6 ± 3[i]	19.2
48	Ck	0.36 ± 0.02	310.8 ± 39	854.8 ± 80	0.090 ± 0.01	76.4 ± 5	11.8
	L	0.35 ± 0.02	218.0 ± 22[i]	614.6 ± 49[i]	0.071 ± 0[k]	43.6 ± 5[h]	16.3
96	Ck	0.77 ± 0.07	394.2 ± 30	509.8 ± 18	0.486 ± 0.02	40.3 ± 5	120.6
	L	0.30 ± 0.01[h]	140.4 ± 11[h]	464.8 ± 44	0.966 ± 0.07[h]	7.4 ± 1[h]	1305.4
120	Ck	0.84 ± 0.08	384.4 ± 46	459.9 ± 35	0.348 ± 0.02	17.6 ± 3	197.7
	L	0.30 ± 0.02[h]	98.0 ± 6[h]	326.2 ± 14[h]	0.502 ± 0.04[h]	5.7 ± 1[h]	880.7

[a] Results are mean ± standard deviation of four replicates each of which contained six roots. Microgram of RNA was determined spectrophotometrically. [b] Roots were exposed to lindane (8 ppm) in tap water for various times. [^{3}H]Uridine at a concentration of 50 μCi/400 mL was added 6 h before the end of each experiment. [c] Specific radioactivity RNA (SA-RNA) represents DPM × 10^3 per microgram of RNA. [d] The specific radioactivity of the soluble precursor pool (SA-RNA pool) represents all the radioactivity (DPM × 10^3) due to [^{3}H]uridine and all metabolites except RNA. [e] The relative rate is the ratio of SA-RNA to SA-RNA pool × 10^{-4} [(SA DNA/SA DNA pool) × 10^{-4}]. [f] Ck = control roots. [g] L = roots grown in lindane-treated water. [h-k] Data are significantly different from the control at the [h] 0.1%, [i] 1%, [j] 2%, [k] 5% level.

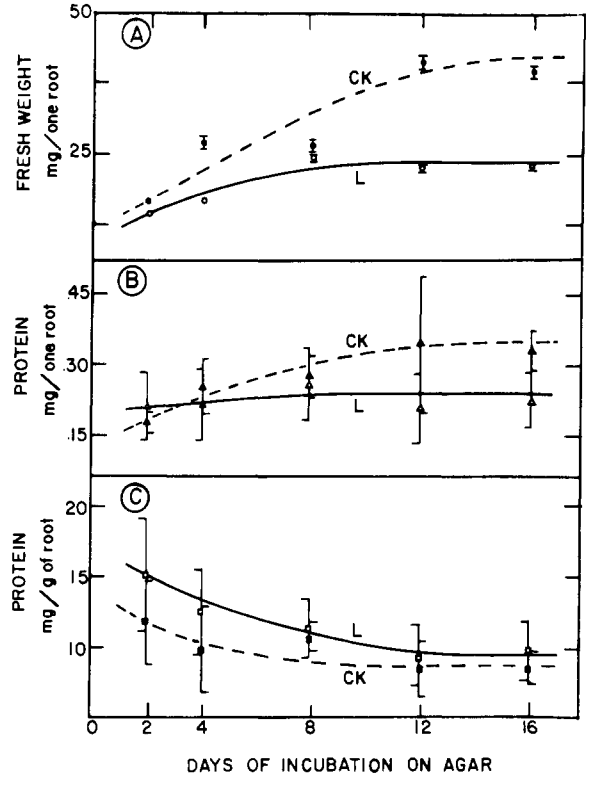

Figure 3. Effect of lindane on the fresh weight and protein content of corn roots in organ culture. L = roots grown on agar treated with lindane at 20 ppm. Ck = roots grown on agar free of lindane. Results are mean ± SD of six replicates.

6 h in the lindane-treated water to which either [^{3}H]-thymidine or [^{3}H]uridine had been added indicated that the insecticide did affect the uptake of these nucleic acid precursors (Figure 4). Lindane caused a decrease in the total uptake of both [^{3}H]thymidine and [^{3}H]uridine, when expressed as total radioactivity per gram of fresh root tissue. This reduction in [^{3}H]thymidine or [^{3}H]uridine uptake due to lindane exposure could have been related to an inhibition or a disruption of tritiated precursor penetration. Charnetski et al. (1973) reported such cytological abnormalities as epidermal layer disruption and anomalous cell wall development in pea roots grown in lindane-treated sand. These morphological changes caused by lindane could have affected precursor penetration and transport.

It is interesting to note that thymidine and uridine, two similar molecules, display different kinetics of uptake. [^{3}H]Uridine was taken up after a lag period of 2 h, while [^{3}H]thymidine was taken up with no lag period. The hypothesis that the synthesis of nucleic acids, which depletes precursor pools, acts to regulate the uptake of precursors from the medium (Francki et al., 1971) may explain the difference in kinetics.

Effects of Lindane on the Incorporation of [^{3}H]-Uridine into RNA. The incorporation of [^{3}H]uridine into RNA or corn roots grown for a specific exposure time in lindane-treated (8 ppm) and untreated tap water was determined with a total of 48 roots (four replicates of six roots each for lindane exposed and control roots) which were each exposed to the insecticide for 12, 18, 24, 48, 96, or 120 h. During the last 6 h of each growing period, [^{3}H]uridine was added at a concentration of 50 μCi/400 mL to the previously lindane-treated and untreated water. After that, RNA was extracted from the roots and analyzed spectrophotometrically for RNA as described. Thus, the fresh weight, the micrograms of RNA per root, and micrograms of RNA per gram of fresh weight root were determined.

Extracts containing RNA were also tested for their radioactivity, finally resulting in data for specific activity of RNA (DPM/μg of RNA = SA-RNA) and specific activity of the soluble precursor pool (DPM/soluble precursor pool = SA-RNA pool) and the relative rate of precursor incorporation. Results are summarized in Table I. After 48, 96, and 120 h of root exposure to lindane, the actual amount of RNA per root had been significantly reduced in comparison to controls, while the amounts of RNA per gram of root tissue were only reduced after 48 and 120 h of exposure to the insecticide. The amounts of radioactivity per microgram of RNA or the specific activity of RNA were significantly reduced during the first 48 h of exposure to lindane, but were increased after 96 and 120 h in both controls and lindane exposed roots. A possible explanation for this data is that the RNA in both treated and untreated roots was being rapidly synthesized and degraded, thus resulting in a high specific radioactivity of RNA due to an increased incorporation of ^{3}H-labeled

Table II. Effects of Lindane on DNA Synthesis as Measured by Incorporation of Its Precursor [^{3}H]Thymidine, in Corn Roots Exposed to the Insecticide for Various Lengths of Time[a]

Hours exposed to lindane[b]		Fresh weight, g	DNA, μg		Specific radioactivity		Rel rate[e]
			In one root	Per gram of root	DNA[c]	DNA pool[d]	
24	Ck[f]	0.29 ± 0.01	41.8 ± 9	143.8 ± 39	0.44 ± 0.05	43.1 ± 7	102.1
	L[g]	0.31 ± 0.01	36.6 ± 13	119.2 ± 42	0.47 ± 0.06	63.0 ± 16[l]	74.6
48	Ck	0.43 ± 0.01	98.8 ± 10	241.1 ± 9	0.38 ± 0.01	83.3 ± 13	45.6
	L	0.33 ± 0.003[h]	62.1 ± 6[h]	225.8 ± 3[k]	0.27 ± 0.04[j]	36.6 ± 3[i]	73.8
96	Ck	0.88 ± 0.09	191.6 ± 27	216.3 ± 17	0.74 ± 0.07	65.6 ± 13	112.8
	L	0.35 ± 0.01[h]	59.4 ± 12[h]	168.5 ± 32[l]	0.15 ± 0.02[h]	18.9 ± 2[i]	79.4
120	Ck	1.00 ± 0.05	182.9 ± 8	182.6 ± 5	0.39 ± 0.05	47.3 ± 5	82.4
	L	0.31 ± 0.02[h]	61.8 ± 12[h]	199.6 ± 31	0.004 ± 0.0[h]	7.4 ± 1[h]	5.4

[a] Results are mean ± standard deviation of four replicates each consisting of seven roots. Microgram of DNA was measured colorimetrically. [b] Roots were exposed to lindane (8 ppm) in tap water for various times. [^{3}H]Thymidine at a concentration of 50 μCi/400 mL was added during the last 6 h of each experiment. [c] Specific radioactivity DNA (SA-DNA) represents DPM × 10^3 per microgram of DNA. [d] The specific radioactivity of the soluble precursor pool (SA-DNA pool) represents all the radioactivity (DPM × 10^3) due to [^{3}H]thymidine and all metabolites except DNA. [e] The relative rate is the ratio of SA-DNA to SA-DNA pool × 10^{-4} [(SA DNA/SA DNA pool) × 10^{-4}]. [f] Ck = control roots. [g] L = roots grown in lindane-treated water. [h-l] Data are significantly different from the control (Ck) at the [h]0.1%, [i]1%, [j]2%, [k]5%, [l]10% level.

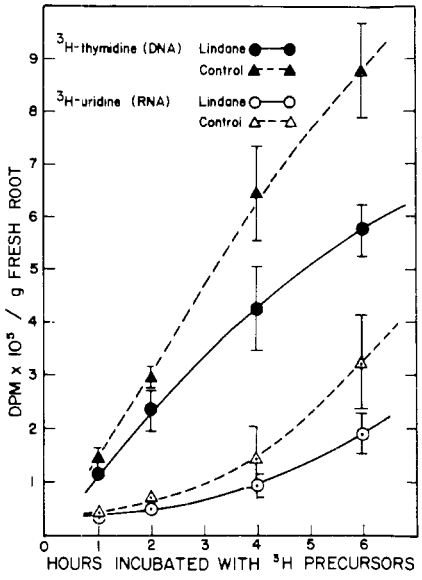

Figure 4. Effects of lindane on the total uptake of [^{3}H]thymidine, a DNA precursor, and of [^{3}H]uridine, an RNA precursor, by corn roots exposed to lindane (8 ppm) for 13, 14, 16, or 18 h in tap water. Roots were exposed to 50 μCi/400 mL of the [^{3}H]precursors during the last 1, 2, 4, or 6 h of the experiment, respectively. RNA data are the average of four experiments and DNA data are the average of two experiments.

compounds from the soluble precursor pool. RNA was being degraded at a slightly faster rate than it was being synthesized, though, which accounted for the decline in microgram of RNA/g of fresh weight values. This turnover was even more accelerated in roots which had been exposed to lindane. Alternatively, the possibility that the high specific activity of RNA was due to high specific activities in precursors immediate to RNA cannot be excluded.

Roots exposed to lindane had lower SA-RNA soluble pool values than untreated roots, indicating, as previously shown in Figure 4, that lindane decreased [^{3}H]uridine uptake. Again the reduction in [^{3}H]uridine uptake may be related to epidermal disruptions or abnormal cellular metabolism due to lindane.

Effect of Lindane on the Incorporation of [^{3}H]-Thymidine into DNA. Four experiments, each consisting of 56 corn roots (four replicates of seven roots each for lindane exposed and control roots), were conducted to study the incorporation of [^{3}H]thymidine into DNA. Corn roots were grown in lindane-treated (8 ppm) or untreated tap water for periods of 24, 48, 96, or 120 h. Six hours before the end of each growing period, [^{3}H]thymidine was added at a concentration of 50 μCi/400 mL to the previously lindane-treated and untreated water. After that, roots were extracted and analyzed colorimetrically for DNA and also for their radioactivity as described. Finally, data were obtained for fresh weight, micrograms of DNA per root, micrograms of DNA per gram of fresh weight, specific activity of DNA (DPM/μg of DNA = SA-DNA), and specific activity of the soluble precursor pool (DPM/total soluble precursor pool = SA-DNA pool) and the relative rate of precursor incorporation.

Results obtained are presented in Table II. Roots exposed to lindane had lower SA-DNA and SA-DNA pool values at all but the earliest incubation times. As previously shown in Figure 4, these results also indicate that the presence of lindane decreased [^{3}H]thymidine uptake into roots. When the SA-DNA pool values of treated roots were expressed in percent of their respective controls, values of 44% (48 h), 29% (96 h), and 16% (120 h) were obtained. In addition, the SA-DNA pool values steadily declined as incubation times with lindane increased. Thus, roots exposed to lindane for 24, 48, 96, and 120 h had SA-DNA pool values of 63.0 ± 16, 36.6 ± 3, 18.9 ± 2, and 7.4 ± 1 DPM × 10^3, respectively.

In addition, lindane could have affected the rate of DNA synthesis. After 96 and 120 h of incubation with lindane, roots showed decreased relative rates as compared with untreated root values. Also, roots exposed to lindane for 48 and 96 h had lower quantities of DNA on a per gram of fresh weight basis than untreated roots. Furthermore, the average quantity of DNA per root increased about 4.4 times in untreated roots from 24 to 120 h of growing time. During the same period, however, the average quantity of DNA per root increased only about 1.7 times in roots exposed to lindane. While the SA-DNA maintained a steady level in untreated roots, that in roots exposed to lindane steadily decreased, resulting after 120 h of exposure to lindane in only 1% of the radioactivity in the DNA of control roots. This reduced precursor incorporation cannot be interpreted as reduced DNA turnover, since DNA does not usually turn over (Novikoff and Holtzman, 1970). Furthermore, the low SA-DNA of roots exposed to lindane for 120 h indicates that bacterial contamination was not a significant factor. If it had been, one would expect to see the highest specific activities at the longer incubation times, when bacterial contamination was apparently at its peak.

Such a reduction of DNA synthesis could be a factor in the inhibition of mitotic processes in the cell. It has long

been known that the inhibition of nuclear DNA synthesis by certain chemicals invariably blocks mitosis (DuPraw, 1968). Charnetski et al. (1973) reported a cessation of mitotic divisions in the apical meristems of treated pea roots resulting in enlarged cells with multilobed nuclei. Certainly a reduction of DNA synthesis and subsequent inhibition of mitosis could explain the stunted, dormant character of roots exposed to lindane. On the other hand, the reduction of DNA synthesis and mitotic abnormalities due to lindane may be related to disruptions of membrane or energy supply systems. Lichtenstein et al. (1962) found that lindane decreased the respiration rate in corn and oat root tips. Evidence of membrane abnormalities were found both in our study and that of Charnetski et al. (1973).

Data described above indicate that the insecticide lindane affected the synthesis of DNA in corn roots, possibly disrupting normal cell mitosis. This then seems to explain findings by others (Charnetski et al., 1973) who used microscopy and electron microscopy and showed the presence of enlarged root cells with greatly enlarged and lobed nuclei.

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Received for review November 15, 1976. Accepted March 8, 1977. Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison and by grants from the National Science Foundation (BMS 72-02179) and the Environmental Protection Agency (R804920). Contribution from the Wisconsin Agricultural Experiment Station as a collaborator under North Central Cooperative Research Project 96, "Environmental Implications of Pesticide Usage".

Effects of Graded Levels of Toxaphene on Poultry Residue Accumulation, Egg Production, Shell Quality, and Hatchability in White Leghorns

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Graded levels of toxaphene (0, 0.5, 5, 50, and 100 ppm) were added to the diets of female (White Leghorn) chicks from 1 day of age. Each treatment consisted of 90 randomly selected birds (30 birds in each of three replicates). On the sixth week, 20 birds in each replicate were transferred to floor pens and continued into the egg laying phase. Two sexually mature White Leghorn roosters were placed in each pen during the 23rd week. The equations which best describe the dissipation of toxaphene from adipose and egg tissue are $\hat{Y} = e^{-0.0346 \times +4.70}$ and $\hat{Y} = e^{-0.0441 \times +2.498}$, respectively ($\hat{Y}$ = toxaphene level in ppm and X = withdrawal period in days). Birds fed 100 ppm toxaphene in their diet did not have significantly weaker shells as measured by Instron stress analysis. Birds fed 5, 50, and 100 ppm toxaphene exhibited sternal or keel deformation at 30 weeks of age. Histopathological examination revealed renal lesions in birds fed toxaphene at 50 and 100 ppm. Toxaphene at 100 ppm did not significantly alter egg production, hatchability, or fertility.

Toxaphene has been extensively used for insect pest control on crops and livestock for the past 15–20 years. In 1971, 37 million pounds of toxaphene (Andrilenas, 1970) was used by agriculture for control of insect pests. Despite the fact that toxaphene is used in larger amounts than other chlorinated hydrocarbon insecticides in the United States (Andrilenas, 1970), there is little information on several aspects of the chemistry, persistence, and environmental fate of this group of compounds.

With the banning of DDT and dieldrin, toxaphene–methyl parathion combinations will be used much more extensively. Toxaphene is registered for the use on agronomic commodities such as alfalfa, barley, corn, cotton, cow peas, sorghum, rice, rye, soybeans, wheat, and a variety of horticultural crops (EPA Summary, 1976). Since

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