Effect of Sterilizing Agents on Persistence of Parathion and Diazinon in Soils and Water

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Sterilizing agents, such as sodium azide, or autoclaving reduced the number of bacteria in soils, resulting in increased persistence of parathion. This persistence, however, was not affected by autoclaving prior to insecticidal application. Moreover, diazinon was no longer detectable in soil after a 2-week incubation with sodium azide. This compound also caused the disappearance of diazinon from distilled water by catalyzing its hydrolysis into diethylthiophosphoric acid, 2-isopropyl-4-methyl-6-hydroxypyrimidine, and three other breakdown products.

The study of the interrelationships between microorganisms and pesticides has increased considerably in recent years. Since control experiments must often be conducted under sterile conditions, investigators should be aware of several factors which could lead to misinterpretation of the experimental results. Thus the total disappearance of chlorinated hydrocarbon insecticides from sterile nutrient agar within 2 weeks after application of insecticides was reported by Lichtenstein *et al.* (1963, 1968). The persistence of some insecticides under sterile conditions, and the effects of autoclaving and the addition of sodium azide, an inhibitor of certain microorganisms, were investigated.

EFFECTS OF STERILIZING AGENTS ON PERSISTENCE OF SOME INSECTICIDES IN SOIL

The addition of the detergents ABS (alkyl benzene sulfonate) and LAS (linear alkyl benzene sulfonate) to parathion- or diazinon-treated soil increases the persistence of these insecticides (Lichtenstein, 1966). The presence of these detergents in soils resulted in a 5-(with ABS) to 55-(with LAS) fold increase in the total number of bacteria. Although parathion had been reported more persistent in soil whose microbiological activities were low because of dryness, or whose microbiological population was low because of autoclaving (Lichtenstein and Schulz, 1964), the persistence of parathion in soils was also increased in the presence of large numbers of bacteria owing to soil treatment with LAS. The question arises whether this increased persistence was due to a low population of certain microorganisms which usually degrade parathion but had been suppressed by the preferential development of microorganisms which do not attack parathion. Two experiments (I and II) were conducted to test the relationships between microorganisms and the stability of parathion or diazinon in soil. LAS was used to increase the number of microorganisms and sodium azide or autoclaving to decrease the microbiological population.

Experiment I. Effect of Sodium Azide and LAS in Percolating Water on Persistence of Parathion and Diazinon in Soil. The first experiment was conducted as described by Lichtenstein *et al.* (1967). Soil (Carrington silt loam) was treated with parathion at 1 p.p.m. After the removal of aliquots for initial analyses, 400-gram portions of the soil were placed in each of three glass columns (7-cm. i.d. and 60 cm. long). Afterward, water, water containing LAS (0.1%), or water containing sodium azide (0.1%) was percolated through each column until each soil was saturated. The columns were left undisturbed in the dark for 17 days, at which time water or the respective solutions were percolated through the soil until 300 ml. of each percolate had been collected. The soil was then removed from the glass columns and dried to field capacity. Extraction and analyses of soil and water were done as described by Lichtenstein *et al.* (1967). An identical experiment was conducted using soil treated with diazinon. Results are presented in Table I.

Table I. Effects of Sodium Azide and LAS on Persistence of Diazinon and Parathion in Soils and Percolated Water

	P.P.M. Recovered from Soils and Water, Percolated 17 Days after Soil Treatment at 1 P.P.M. with			
	Parathion		Diazinon	
Soil Percolated with[a]	Water	Soil	Water	Soil
H_2O	0.005	0.226	0.012	0.340
H_2O + NaN_3 (0.1%)	0.011	0.804	Trace	0.026
H_2O + LAS (0.1%)	0.006	0.385	0.016	0.285

[a] Soils saturated with water, water + NaN_3 (0.1%), or water + LAS (0.1%) immediately following insecticidal treatment.

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Although the actual amounts of LAS or sodium azide added to the soil were relatively small, their presence increased the persistence of parathion. In soils through which only water had been percolated, 77% of the applied parathion had disappeared within 17 days, compared to only 20% in soil through which water containing sodium azide had been percolated. This phenomenon was probably related to the inhibitory effect of sodium azide on microorganisms that normally degrade parathion.

In diazinon-treated soils, however, 97% of the insecticide had disappeared because of the presence of sodium azide and only traces of diazinon could be detected in the percolated water. This indicated a complete difference in behavior of these insecticides under similar conditions.

Experiment II. Effect of Soil-Sterilizing Agents and LAS on Persistence of Parathion and Diazinon in Soil. Another experiment (Table II) was then conducted to investigate further the effects of sterilizing agents on the persistence of some insecticides in soil. Loam soils were treated with sodium azide (0.05% of soil weight) or autoclaved (6 hours per day for 5 days) to reduce the number of microorganisms, or they were treated with LAS (0.42% of soil weight) to increase numbers of soil microorganisms. The experiment was set up in such a way that 140-gram portions of loam soil were placed in each of sixteen 500-ml. glass jars (opening 5 cm.). To the soil in four jars, 7 ml. of water was added. To the soil in four other jars, 7 ml. of water containing sodium azide was added, so that its concentration was 0.05% of the soil weight. The soil in four additional jars was autoclaved and the soil in the four jars remaining was treated with LAS by adding 7 ml. of an aqueous solution of LAS to obtain a concentration of 0.42% of the soil weight. The soil in one jar of each particular treatment (water, water plus sodium azide, autoclaving, or water plus LAS) was also treated with diazinon, parathion, or lindane at 10 p.p.m., while the four jars remaining were left as controls (Table II). All jars were then plugged with cotton. After 7 days of incubation at 30° C., 7 ml. of water, 7 ml. of water containing 70 mg. of sodium azide, 7 ml. of sterile water, or 7 ml. of water containing LAS (0.1%) was added to the respective soils. After one additional week, the soils were extracted and analyzed (Lichtenstein, 1966). In addition, one portion of each soil sample was used for determining the numbers of several groups of microorganisms (Lichtenstein, 1966) and another portion was used for pH measurements. Results are summarized in Table II.

Control soils which had not received special treatment contained 2,000,000 to 7,000,000 bacteria per gram of dry soil. However, the addition of sodium azide to the soil or autoclaving of the soil reduced the numbers of microorganisms to such an extent that 15 days later only 270,000 to 620,000 bacteria per gram of dry soil were found in the sodium azide–treated soil and only 100 to 300 per gram of dry soil in those that had been autoclaved. Conversely, the presence of LAS resulted in an increase of bacteria whose numbers ranged from 140,000,000 to 190,000,000 per gram of dry soil. No effect on the numbers of coliforms, yeasts, molds, and actinomycetes was apparent.

As in previous experiments (Lichtenstein, 1966), the presence of LAS increased the persistence of diazinon and parathion in the soil by factors of 1.7 and 3.7, respectively

Table II. Effects of Special Soil Treatments on Populations of Microorganisms and Persistence of Insecticides[a] in Soil

	Special Soil Treatments			
	Control	NaN_3[b]	Autoclaving[c]	LAS[d]
	Recovered from Soil, 15 Days after Insecticide Application			
No insecticide				
pH	5.87	6.59	5.24	7.04
Micro[e]	5.4 M	270 T	100	150 M
Diazinon				
% of applied	31.1	[f]	28.6	52.7
% of control	100		91	169
pH	5.85	6.64	5.21	7.11
Micro	7.2 M	620 T	100	190 M
Parathion				
% of applied	25.1	45.1	65.8	91.7
% of control	100	180	262	365
pH	5.85	6.50	5.28	7.10
Micro	2 M	470 T	300	180 M
Lindane				
% of applied	76.5	78.6	90.9	94.0
% of control	100	103	119	121
pH	5.88	6.68	5.25	7.08
Micro	4 M	520 T	300	140 M

[a] Applied at 10 p.p.m. $T = 30° \pm 1°$ C.
[b] Sodium azide added to soil at time of insecticidal application at rate of 0.05% of soil weight.
[c] Soils autoclaved prior to insecticidal treatments.
[d] LAS (linear alkyl benzene sulfonate) added to soil at time of insecticidal treatment at rate of 0.42% of soil weight.
[e] Total number of bacteria per gram of dry soil (T = thousand, M = million).
[f] No measurable amount detected by gas-liquid chromatography.

(Table II). The presence of sodium azide also increased the persistence of parathion, but caused the complete disappearance of diazinon during 2 weeks of incubation. At the end of that period no measurable amounts of diazinon could be detected by gas-liquid chromatography. Although autoclaving of the soils had reduced the numbers of microorganisms even more than sodium azide, the persistence of diazinon was not appreciably affected by soil autoclaving. The effect of sodium azide on the stability of diazinon in soil apparently is not directly related to microbiological activities. The persistence of lindane was slightly increased in autoclaved soils and in those that contained LAS. The varying behavior of different insecticides under identical environmental conditions is illustrated by these experiments, which indicate that it is difficult to generalize as far as the behavior of different insecticides in the environment is concerned.

EFFECT OF SODIUM AZIDE ON PERSISTENCE OF DIAZINON IN WATER

To exclude as much as possible the effect of microorganisms, distilled water was used as the medium in which the effect of sodium azide on diazinon was further investigated. For this purpose, two radioactive preparations of diazinon were obtained from the Geigy Chemical Corp. One contained C^{14} at the 2-carbon site on the pyrimidine ring and the other at the 1-carbon sites on both ethoxy groups. The specific activities of the compounds were 4.0 and 4.3 μc. per mg., respectively. Two 500-ml. Erlenmeyer flasks were filled with 250 ml. of water which had been treated with 2.5 mg. of diazinon (ring-labeled) in

acetone (10 p.p.m.). Two additional flasks were filled with 247.5 ml. of ring-labeled diazinon-treated water and with 2.5 ml. of water containing 125 mg. of sodium azide (0.05% of the water), and two more were filled with 247.5 ml. of ethoxy-labeled diazinon-treated water and with sodium azide as described. Two flasks containing only 250 ml. of 0.05% sodium azide in water were used as controls. All flasks were glass-stoppered and incubated in the dark for 14 days at 30° C.

Toxicity tests were conducted with hexane extracts of 25 ml. of each solution after 8 and 14 days of incubation Vinegar flies (*Drosophila melanogaster* Meig.) were exposed to the dry hexane residues representing 12.5 ml. of water. No mortalities were observed with a 24-hour exposure period with residues from water or from water containing only sodium azide. However, when flies were exposed to residues obtained from water treated only with diazinon, 100% mortalities were observed within a 20-minute exposure time, both 8 and 14 days after the insecticidal application. In the presence of diazinon plus sodium azide, however, toxicity effects were considerably reduced and 90% mortalities were observed only after 24-hour exposure 8 days after the insecticidal application. After an additional week of incubation, no mortality of *Drosophila* flies could be observed even during a 48-hour exposure to dry residues from the diazinon samples fortified with sodium azide. These tests clearly indicated that toxic effects of diazinon to vinegar flies had disappeared because of the presence of sodium azide.

In addition to the biological tests, pH measurements, chemical analyses by gas-liquid chromatography, and radioassays of the water samples were performed initially and after 1, 3, and 7 days of incubation. For this purpose, 25 ml. of water from each of the six diazinon-treated water samples were extracted with three 50-ml. portions of hexane. The hexane fractions from each sample were combined, dried over anhydrous sodium sulfate, and analyzed by gas-liquid chromatography (Lichtenstein, 1966). Results of these duplicated tests as presented in Figure 1

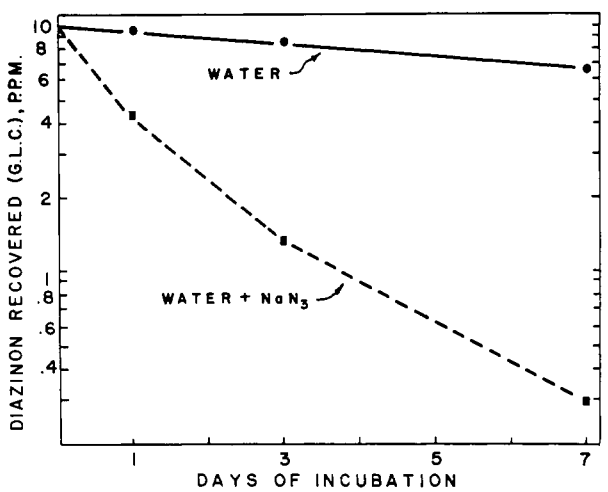

Figure 1. Effects of sodium azide (NaN_3) in water (0.05%) on stability of diazinon

Initial diazinon concentration, 10 p.p.m.
pH of water initially and after 1, 3, and 7 days, 4.9
pH of water + NaN_3 initially and after 1, 3, and 7 days, 6.1

demonstrate the rapid loss of diazinon in the presence of sodium azide. Only 3% of the originally applied diazinon could be detected in water 7 days after its treatment with the insecticide and sodium azide, while 67% of the applied diazinon was still present in water not treated with sodium azide.

The pH of water treated only with diazinon was 4.83 ± 0.02, 4.90 ± 0.01, 4.90 ± 0.01, and 4.92 ± 0.03 initially and after 1, 3, or 7 days of incubation, respectively. The pH of water treated with diazinon plus sodium azide, however, was 6.13 ± 0.02, 6.14 ± 0.01, 6.14 ± 0.01, and 6.22 ± 0.08 initially and after 1, 3, and 7 days, respectively. The hydrolysis of diazinon in an acid environment progresses rather rapidly (Margot and Gysin, 1957). The differences observed in pH measurements of water and water containing sodium azide, therefore, cannot account for the disappearance of diazinon from water.

Radioassays were performed initially and 1, 3, and 7 days after water treatment by liquid scintillation counting in a Packard Tri-Carb liquid scintillation spectrometer, Model 3003. The analyses were performed with aliquots of the unextracted water samples, the hexane fraction of the water extracts, and the water fraction after extraction with hexane.

The hexane and hexane-extracted water fractions from the 7-day samples were also analyzed by thin-layer chromatography and compared with six potential metabolites of diazinon obtained from the Geigy Agricultural Chemicals Corp.: 2-isopropyl-4-methyl-6-hydroxypyrimidine, 2-isopropyl-4-methyl-6-mercaptopyrimidine, 2-isopropyl-4-methyl-6-ethoxypyrimidine, diazoxon, dithionotetraethyl pyrophosphate, and diethylthiophosphoric acid.

The hexane fraction was concentrated and spotted on silica gel–coated TLC plates (silica gel G, containing calcium sulfate as a binder, DESAGA, Heidelberg), 2.5 cm. above the lower edge. The chromatogram was developed with benzene-chloroform–ethyl acetate (2:2:1) (Geigy Chemical Corp., 1963), followed by spraying with 0.01% Rhodamine B in ethyl alcohol (95%), exposure to ultraviolet light, and additional successive sprays with 0.5% palladium chloride and 5*N* sodium hydroxide.

The water fraction, obtained after the separation of the hexane and water, was acidified to pH 2 with concentrated hydrochloric acid, and then re-extracted with two 25-ml. portions of diethyl ether. These portions were combined and concentrated. The concentrate was then divided into two equal portions, which were spotted onto separate silica gel–coated thin-layer plates. One plate was developed as described and the other with a mixture of 2-propanol and ammonium hydroxide (8 to 2). Both plates were then sprayed and handled as described for the hexane fraction. All plates were autoradiographed with Kodak x-ray film for 60 hours.

The hexane fractions of the diazinon- and sodium azide–treated samples contained four compounds (R_f 0.02, 0.37, 0.49, and 0.75) as indicated after spraying of the thin-layer plate with chromogenic agents. One spot (R_f 0.02) was identical to *O',O*-diethylthiophosphoric acid or 2-isopropyl-4-methyl-6-hydroxypyrimidine, another (R_f 0.37) to 2-isopropyl-4-methyl-6-ethoxypyrimidine, and the third (R_f 0.49) to diazinon. Exposure of this thin-layer plate to x-ray film, however, revealed that with ethoxy-labeled

diazinon only the originally applied diazinon was detected, indicating the absence of both the *O,O*-diethylthiophosphoric acid and 2-isopropyl-4-methyl-6-ethoxypyrimidine. With ring-labeled diazinon, a spot was revealed that had the same R_f value as 2-isopropyl-4-methyl-6-ethoxypyrimidine. If this spot had been due to the presence of the latter compound, it should also have been detected with ethoxy-labeled diazinon. This did not occur, thus indicating the formation of an unknown hexane-soluble pyrimidine ring-containing breakdown product of diazinon. This compound was formed only in the presence of sodium azide in water.

The water fraction of sodium azide–treated samples, previously extracted with hexane, contained three compounds (R_f 0.00, 0.01, and 0.69) as evidenced by thin-layer chromatography and development with chloroform–benzene–ethyl acetate (2:2:1). The lower spots were identical in color and R_f values to diethylthiophosphoric acid and 2-isopropyl-4-methyl-6-hydroxypyrimidine. Autoradiography showed the presence of these same spots, with both the ethoxy-labeled diazinon (indicating the formation of diethylthiophosphoric acid) and ring-labeled diazinon (indicating the formation of the water-soluble hydroxypyrimidine compound). Water treated with ring-labeled diazinon only also revealed the presence of three compounds with chromogenic agents (R_f 0.00, 0.01, and 0.69) and a pyrimidine ring compound (R_f 0.01) that had formed from the ring-labeled diazinon.

After the spotting of ether extracts of water samples on thin-layer plates and their development with 2-propanol and ammonium hydroxide, a clear separation of *O,O*-diethylthiophosphoric acid (R_f 0.43) and 2-isopropyl-4-methyl-6-hydroxypyrimidine (R_f 0.61) was obtained. These two compounds were present in diazinon- and sodium azide–treated water samples, whereas in the absence of sodium azide only *O,O*-diethylthiophosphoric acid could be detected. Two additional radioactive water-soluble products were revealed in the ethoxy-labeled samples (R_f 0.28 and 0.73). No attempt was made to identify any of the three unknown products.

Results obtained by liquid scintillation counting of the water and hexane phases are presented in Figure 1. With ethoxy-labeled diazinon, results obtained from investigations of the hexane fraction were nearly identical to those obtained by gas-liquid chromatography (Figure 2). This was not surprising, since only the intact diazinon molecule could be accounted for with the ethoxy label of the insecticide in the hexane fraction. The water fraction, however, contained increasing amounts of radioactivity owing to the presence of diethylthiophosphoric acid and the other ethoxy-derived products through hydrolysis.

When ring-labeled diazinon had been used, the radioactivity within the hexane fraction was high and declined only slightly with time. After 7 days of incubation, 88% of the total radioactivity was still found in the hexane fraction. Since by that time most of the diazinon had been hydrolyzed, the high amount of radioactivity was due to the presence of the unidentified hexane-soluble pyrimidine compound. The radioactivity detected in the water fraction of the ring-labeled diazinon-treated water samples was due to the formation of 2-isopropyl-4-methyl-6-hydroxypyrimidine, as indicated by thin-layer chromatography.

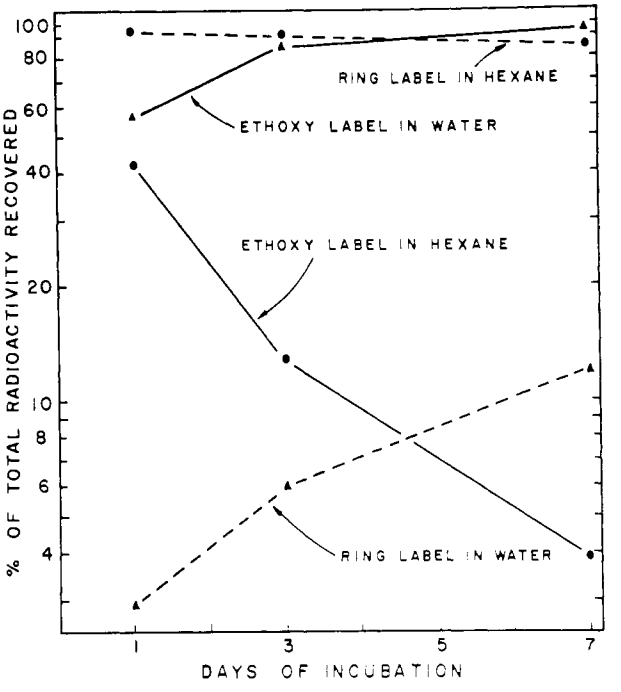

Figure 2. Effect of sodium azide in water on persistence of diazinon

Water treatment, C-14 ethoxy-labeled diazinon or C-14 ring-labeled diazinon

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Received for review April 15, 1968. Accepted June 24, 1968. Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. Research supported in part by a grant from the U. S. Public Health Service Communicable Disease Center, CC-297 (formerly EF-168). Contribution from the Wisconsin Agricultural Experiment Station as a collaborator under North Central Regional Cooperative Research Project 85, entitled "Reduction of Hazards Associated with the Presence of Residues of Insecticidal Chemicals in the Environment."