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Metabolism of Cyanox® and Surecide® in Bean Plants and Degradation in Soil

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Cyanox and Surecide are 4-cyanophenol esters of *O*, *O*-dimethyl phosphorothioic acid and *O*-ethyl phenylphosphonothioic acid, respectively. Metabolism of these compounds in bean plants follows the typical pathway for organophosphorus compounds and in neither case does the nitrile group seem to be involved. There is, however, a remarkable quantitative difference in the rate of metabolism between the two compounds. Cyanox is lost rapidly from treated leaves by volatilization and metabolism, and the half-life is one day or less. In contrast, Surecide is much more persistent; the half-life is estimated to be about two weeks. Moreover, cyanoxon, the toxic metabolite of Cyanox, was detected as a transient intermediate to further degradation products, whereas surecide-oxon was not detected. Perhaps the bean plant has the capacity to degrade surecide-oxon as soon as it is produced or perhaps there is no pathway from Surecide to the oxon in bean plants. Desalkyl compounds and 4-cyanophenol are the major metabolites of both compounds. In neither case does photodegradation seem to play a significant role.

Intact Cyanox is absorbed from water culture by bean plants, whereas there is practically no uptake of Surecide under the same conditions. Cyanox also dissipates from soil at a much faster rate than does Surecide, giving 4-cyanophenol and desalkyl compounds, whereas Surecide persists longer and gives practically only 4-cyanophenol as degradation products.

INTRODUCTION

Cyanox®,** *O*, *O*-dimethyl *O*-(4-cyanophenyl) phosphorothioate, and Surecide®,** *O*-ethyl *O*-(4-cyanophenyl)phenylphosphonothioate, are effective insecticides of low mammalian toxicity (Nishizawa, 1960; Nishizawa *et al.*, 1961; Tamura *et al.*, 1961; Sakamoto and Nishizawa, 1962; Nishizawa *et al.*, 1962),[2–6,8] and each has the 4-cyanophenol moiety. The present paper considers whether the cyanophenol moiety distinguishes them from other organophosphorus insecticides, and whether the metabolic and degradative patterns in plants and soil for Cyanox and Surecide differ.

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CHEMICALS

The chemical structures and names of compounds used in the present study, identified by number, and the *Rf* values on thin-layer chromatography (TLC) are shown in Fig. 1 and Table 1, respectively. The six compounds described below were synthesized in our laboratory for comparison with the metabolites. For characterization and identification, infrared (IR) spectra were determined or KBr pellets with a Shimazu IR-27S spectrophotometer and nuclear magnetic resonance (NMR) spectra were obtained on a NEVA T-60 spectrometer in deuterated water solutions.

Cyanoxon (II), *O*, *O*-dimethyl *O*-(4-cyanophenyl) phosphate, was prepared by oxidizing Cyanox (I) with nitric acid (Sakamoto *et al.*, 1962).[5] The infrared (IR) spectrum of the product was identical with that of the

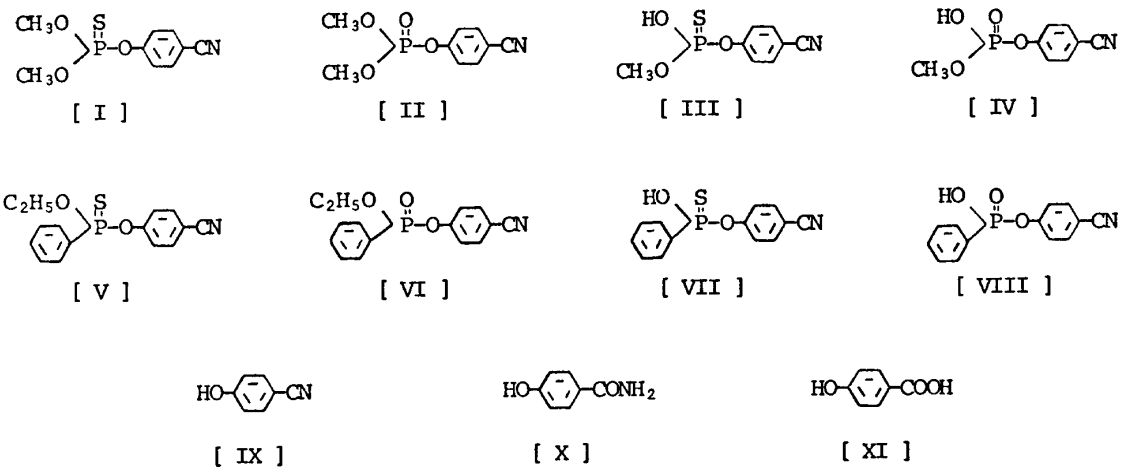

Fig. 1 Chemical structures and numbering of compounds.

authentic compound (Nishizawa *et al.*, 1962).[3] Because cyanoxon gradually decomposes on storage, it was purified before use by dissolving in chloroform and washing first with N sodium hydroxide solution and then with water.

Desmethylcyanox (III), *O*-methyl *O*-(4-cyanophenyl) phosphorothioic acid. Cyanox was demethylated by the method of Hirose *et al.* (1971).[1] 2.4 g of Cyanox was dissolved in 5 ml of ethanol; to this solution, 5 ml of an ethanol solution containing 2 g of potassium ethyl xanthate and 0.5 ml of water were slowly added while stirring. A white crystalline product (mp over 260°C), the potassium salt of compound III appeared on standing overnight, and was recovered by filtration. The salt was dissolved in water, and the solution was extracted with dichloromethane after acidification with hydrochloric acid. The IR spectrum of the liquid product obtained from the extract showed strong absorptions for OH (3520 cm^{-1}), P=S (690 cm^{-1}), CN (2290 cm^{-1}) and phenyl (1610, 1510 cm^{-1})

Table 1 TLC *Rf* values of related compounds.

Compound	Solvent systems[a]									
	A		B		C		D		E	
	S[b]	U[c]	S	U	S	U	S	U	S	U
I	0.80	—	0.60	0.83	0.62	0.84	0.56	0.76	0.60	0.85
II	0.78	—	0.40	0.56	0.48	0.56	0.24	0.48	0.38	0.62
III	0.65	0.61	0	0	0	—	0	0	0	0
IV	0.54	0.52	0	0	0	—	0	0	0	0
V	0.76	—	0.57	0.76	0.60	—	0.59	0.75	0.56	0.80
VI	0.70	—	0.39	0.52	0.52	—	0.33	0.38	0.34	0.51
VII	0.53	0.54	0	—	0	—	0	—	0	0
VIII	0.46	0.38	0	—	0	—	0	—	0	0
IX	0.76	0.78	0.36	0.44	0.18	0.28	0.11	0.20	0.26	0.37
X	0.67	0.67	—	0	0	0	—	0	0	0
XI	0.69	0.69	—	0.07	0	0	—	0	0	0

a) (A) ethyl acetate-methanol-acetic acid (10 : 3 : 1 v/v)
(B) ethyl acetate-benzene (1 : 4 v/v)
(C) chloroform
(D) benzene-chloroform (1 : 1 v/v)
(E) benzene-chloroform-ethyl acetate (8 : 8 : 1 v/v)

b) Developed in a vapor-saturated tank with 10 cm development.

c) Developed in a vapor-unsaturated small jar with 5-7 cm development.

radicals. Between 1190 and 1300 cm^{-1}, there is only one strong absorption at 1255 cm^{-1} with a faint inflexion near 1240 cm^{-1}, which is believed to be P–O–aromatic bonding or overlapping of P=O with the former. The intensity of P=S absorption relative to analogues with or without the P=S bond, suggests that the product is mostly in the thiono form. The NMR spectrum for the potassium salt gave a doublet centered at 3.75 ppm for the OCH_3. Methylation of the product with diazomethane gave Cyanox and one other product in a 1 : 5 ratio as judged from TLC. The latter product had a slightly lower *Rf* value than Cyanox and is probably *O*-methyl *S*-methyl *O*-(4-cyanophenyl) phoshorothioate. To confirm the nature of the latter product, the methylation products were separated by column chromatography. Cyanox was eluted with benzene and the product of lower *Rf* value was eluted with chloroform. The latter gave doublets, each centering at 3.81 ppm (for the CH_3O) and 2.28 ppm (for the CH_3S) and corresponding to three protons. Further demethylation with potassium ethyl xanthate gave only *S*-methyl *O*-(4-cyanophenyl) phosphorothioic acid, which gave a doublet centered at 2.25 ppm for the CH_3S as the potassium salt, and gave only *O*-methyl *S*-methyl *O*-(4-cyanophenyl) phosphorothioate when methylated with diazomethane (Fig. 2). These data indicate that the product of demethylation of Cyanox is *O*-methyl *O*-(4-cyanophenyl) phosphorothioic acid (thiono form) or its mixture with the thiolo tautomer, but not *S*-methyl *O*-(4-cyanophenyl) phosphorothioic acid. Desmethylcyanox (III) thus obtained gave one spot on TLC with the solvent systems shown in Table 1, and was distinguished from *S*-methyl *O*-(4-cyanophenyl) phosphorothioic acid with system A (*Rf* values: 0.61 and 0.43, respectively) and a solvent system of toluene-ethyl formate-formic acid (5 : 7 : 1 v/v) mixture (*Rf* values: 0.22 and 0.16, respectively).

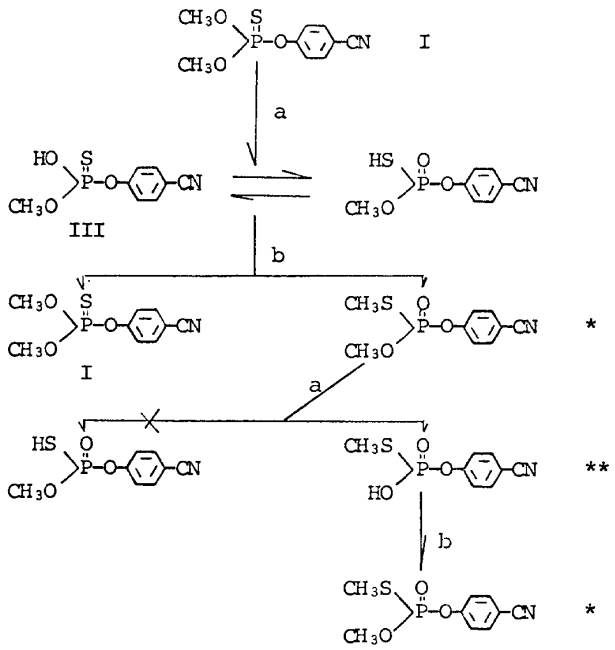

Fig. 2 Structural assignment of demethylation products from Cyanox.
a: demethylation with potassium ethyl xanthate.
b: methylation with diazomethane.
* *O*-methyl *S*-methyl *O*-(4-cyanophenyl) phosphorothioate.
** *S*-methyl *O*-(4-cyanophenyl) phosphorothioic acid.

Desmethylcyanoxon (IV), *O*-methyl *O*-(4-cyanophenyl) phosphoric acid, was prepared by reacting sodium iodide with cyanoxon (Wakimura and Miyamoto, 1971).[9] The sodium salt of compound IV thus formed, mp 140°C, was dissolved in water; the solution was acidified with hydrochloric acid and extracted with dichloromethane and after concentration yielded compound IV. The IR spectrum of the sodium salt showed the presence of P=O (1290 cm^{-1}), P–O–aromatic (1255 cm^{-1}), CN (2250 cm^{-1}), and the benzene ring (1610, 1510 cm^{-1}). Methylation of the compound yielded cyanoxon.

Surecide-oxon (VI), *O*-ethyl *O*-(4-cyanophenyl) phenylphosphonate. Surecide was oxidized with an excess of concentrated nitric acid in aqueous acetone (Sakamoto *et al.*, 1962).[6] The product gave one spot after TLC with the solvent systems shown in Table 1, and the IR spectrum was identical with that of the authentic compound. As surecide-oxon gradually decomposes on storage, it was purified before use by dissolving in chloroform and either washing with N sodium hydroxide solution and water or by eluting through a silica gel column with benzene.

Desethylsurecide (VII), *O*-(4-cyanophenyl)

phenylphosphonothioic acid. Surecide was deethylated by the method of Wakimura and Miyamoto (1971)[9] to yield compound VII. To 900 mg of Surecide dissolved in 5 ml of acetone was added 450 mg of sodium iodide (about one mole equivalent), and the mixture was refluxed for $6^1/_2$ hours. The residue, after evaporation of acetone, was partitioned between 20 ml quantities of dichloromethane and water. The organic layer was discarded, and the aqueous layer was acidified with hydrochloric acid and extracted with dichloromethane. The extract was washed with water, dried with anhydrous sodium sulphate, concentrated, and chromatographed on a silica gel column with dichloromethane-acetone (1 : 1 v/v) as the eluting solvent. 100 mg of liquid compound VII was recovered. When the compound was subjected to TLC it remained at the origin with neutral solvent systems, but moved with acidic solvent system A (Table 1), and was identified as a yellow spot after spraying with palladium chloride. This compound after methylation with diazomethane had an *Rf* value on TLC with chloroform slightly lower than Surecide. A second spot which is probably the methyl ester of the thiolo form of desethylsurecide, was also present. The NMR analysis identified only protons on the benzene ring. The IR spectrum indicated the presence of OH (3440 cm^{-1}) CN (2230 cm^{-1}) and the benzene ring (1600, 1495, 1430 cm^{-1}). A broad absorption near 1235 cm^{-1} (P=O, and P-O-aromatic) and a weak but distinct absorption at 684 cm^{-1} (P=S) suggest that the product is a tautomeric mixture of the thiolo and thiono forms.

Desethylsurecide-oxon (VIII), *O*-(4-cyanophenyl) phenylphosphonic acid was prepared by refluxing 570 mg of surecide-oxon for 4 hours with 300 mg of sodium iodide in 5 ml of acetone (Wakimura and Miyamoto, 1971).[9] After the reaction mixture stood overnight at room temperature, a crystalline product precipitated, which was filtered and washed with acetone. The melting point was above 280°C and the IR spectrum indicated the presence of P=O (1260 cm^{-1}), P-O-aromatic (1230 cm^{-1}), OH (3420 cm^{-1}), CN (2250 cm^{-1}), and the benzene ring (1605, 1510, 1438 cm^{-1}). When subjected to TLC the product moved in the acidic solvent A (Table 1), but not in the neutral solvents. It gave a blue spot with the Hanes reagent (C. W. Stanley, 1964),[10] but did not react with palladium chloride. With solvent system C *Rf* value of the methylated compound was slightly lower than that for surecide-oxon.

Cyanox (I), Surecide (V), and 4-cyanophenol (IX) were provided by Sumitomo Chemical Co. Ltd.; 4-hydroxybenzamide (X) and 4-hydroxydenzoic acid (XI) were purchased from Tokyo Kasei Co.

Radioactive compounds. ^{14}C(CN)-Cyanox (5.06 mCi/mM) and ^{14}C(CN)-Surecide (8.57 mCi/mM) free of detectable impurities were provided by Sumitomo Chemical Co. Ltd. Before use each compound was diluted with the unlabeled pure compound. 4-^{14}C(CN)-Cyanophenol was obtained by hydrolyzing ^{14}C-Cyanox with methanolic sodium hydroxide. The radiolabeled compounds, II, III, IV, and VI, were prepared from either C^{14}-Cyanox or ^{14}C-Surecide by diluting about 0.1 mCi five-fold with unlabeled compound and applying the above synthetic procedures on a semi-micro scale. Each of the products obtained gave only one spot after TLC and autoradiography.

METHODS

1. *Chromatography, Autoradiography, and Radioactivity Measurements*

Eastman Chromagram Sheet 6060 coated with silica gel and fluorescent indicator were used for thin-layer chromatography and were developed with the solvent systems shown in Table 1. Non-labeled phosphorus compounds were detected by spraying with 0.5% palladium chloride in hydrochloric acid for thiophosphorus compounds (yellow spot) and by spraying with the Hanes reagent and exposing to short wave length ultraviolet lamp or sun for several minutes for non-thiophosphorus compounds (blue spot). All phenol-moiety-containing compounds were detected under the ultraviolet lamp by viewing the quenching of gel fluorescence.

Radioactive compounds were detected on the plates by autoradiography with "Medical" X-ray Kx film (Fuji Film Co., Tokyo) exposed

for 3–7 days at room temperature.

Radioactivity in samples was measured in vials containing 10 ml of 0.4% PPO and 0.01% POPOP in toluene-ethyl cellosolve (3 : 2) mixture with an Aloka Liquid Scintillation Spectrometer (Model LSC 502). Radioactivity measurements were made on 0.2 ml volumes of aqueous preparations, organic extracts and solutions obtained from plant materials with a solubilizer (Dotite Hyamine). Radioactivity in silica gel from radioactive zones scraped from TLC plates was also measured.

To obtain the autoradiograms of intact plant tissues, the plant was first fixed on a paper sheet, covered with a polyethylene film, placed in contact with the X-ray film, and exposed for 1–14 days depending on the amount of radioactivity.

2. Treatment of the Plants

Seedlings of *Nagauzura* (*Phaseolus vulgaris* L.) at the bifoliate stage were used throughout these experiments; all were grown on a loam soil (Setagaya top soil). All the experiments were performed in duplicate and radioactive materials were administered as follows:

1) Root uptake from water culture The seedlings were removed from the soil, roots were washed free of soil particles and then inserted in 50 ml Erlenmeyer flask that contained 30 ml of water and 300 μg of radioactive compound (6.7×10^5 dpm). The stems were held upright with cotton plugs at the mouth part of the flasks and shaded from direct sunlight with aluminum foil. All plants were kept in the greenhouse for the designed period without adding any water; no symptoms of injury or abnormality were observed in the plants.

2) Dipping Leaves of the seedlings were dipped in an aqueous solution (500 ppm of the insecticide dispersed with a surfactant) for 3 seconds. Excess solution was allowed to drain off. All plants were then held in the pots until needed for analysis at appropriate intervals.

3) Topical application The radioactive compounds were dispersed in a benzene-hexane (4 : 1) mixture with a surfactant (SX-500®). 30 μl of radioactive solution (5×10^5 dpm) was applied as uniformly as possible over the upper surface of one of the two leaves. After the treatment, the plants were held in a greenhouse for a maximum of 3 days. In the photodegradation study, plants were kept in the dark for designated periods.

4) Injection Solutions were prepared as for topical application. 30 μl solution (5×10^5 dpm) was injected into the stem of the plant. Three days after treatment, the plants were processed for analysis.

3. Extraction and Fractionation

The "organo soluble" fraction was obtained by soaking a single leaf in 10 ml of acetonitrile in a 20 ml test tube for 30 minutes with occasional shaking. The leaf was washed twice with 5 ml quantities of acetonitrile, which were added to the "organo soluble" fraction. It was then soaked in 10 ml of water for 24 hours which constituted the "water-soluble" fraction. The radioactivity left in the leaf is designated "unextracted" fraction. Because radioactivity in the unextracted fraction constituted only a small percentage of the total for the Cyanox-treated samples even after 7 days, the sum of the organo- and water-soluble fractions was regarded as the total radioactivity recovered.

The other parts of the seedling were treated in the same way, except for the roots. These were washed once with water to remove soil particles or radioactive solution in the case of those maintained in water culture, before being extracted.

4. Soil Treatment, Extraction, and Radioactivity Measurements

A loam soil (Setagaya top soil, Table 2) was adjusted to a moisture content of 57%. The radiolabeled compounds were added to the soil at the rate of 10 ppm. After thorough mixing, the soil was held at 20°C and water was added as necessary to maintain the moisture level. For radioactivity measurements 1 g of soil was taken at the surface and another at the 6 cm depth. Each sample was placed in a counting vial and 10 ml of scintillation solution was added. After thor-

Table 2 Analytical data of the soil used in this study.[a)]

Particle size analysis (%)[b)]				pH	Organic matter (%)	Air-dried moisture content (%)	Maximum water holding capacity (%)
Coarse sand (2-0.2 mm)	Fine sand (0.2-0.02 mm)	Silt (0.02-0.002 mm)	Clay (<0.002 mm)				
26.3	37.0	26.1	10.6	6.5	11.7	21.6	126.4

a) Analysed by the official method at the laboratory of soil science, Dept. of Agric. Chem., Tokyo University of Agriculture, Tokyo.

b) Type of soil: loam (Setagaya top soil).

ough shaking the suspension was allowed to settle for 24 hr before the radioactivity was determined. The moisture content of the soil was measured on 2 g sample. For a qualitative demonstration of the insecticides and their degradation products in the soil 50 g samples were extracted with 100 ml of acetone-hexane (1 : 1) mixture. For more critical study of the degradation products in a closed system, 25 g of the soil mixed with 45 μg of a radiolabeled insecticide dissolved in 0.1 ml of water and 2 drops of a surfactant solution (SX-500®, 50 mg/ml ethanol) and 10 ml of ethanolamine-ethyl cellosolve (1 : 1) mixture were placed in the main and side rooms of a biometric flask described by Bartha and Pramer (1965).[7)] The system was closed except the outlet through the solution in the side room, and kept in a room condition. Any radioactive volatiles were trapped in the ethanolamine-ethyl cellosolve (1 : 1) mixture. The soil was immersed 3 times in each 40 ml of methylene chloride-acetone (1 : 2) mixture, then, after acidifiction with hydrochloric acid to pH 1, 3 times in each 40 ml of methanol. The combined solvent extract was counted and submitted to TLC analysis.

RESULTS AND DISCUSSION

1. *Identification of Metabolites*

The diagrammatic TLC patterns (Fig. 3) show how the combination of the solvent systems can identify the metabolites, cyanoxon (II), desmethylcyanox (III), desmethylcyanoxon (IV) and 4-cyanophenol (IX), as well as Cyanox (I). All these compounds are not necessarily found in all samples; the length of the period of metabolism and the particular part of the plant examined contribute to the differences. As only the cyano-carbon of the phenol moiety is labeled, those compounds without this functional group will not be detected by autoradiography. 4-Cyanophenol was the only water-soluble metabolite found; it was present in both aqueous and organic phases.

4-Cyanophenol and desethylsurecide were

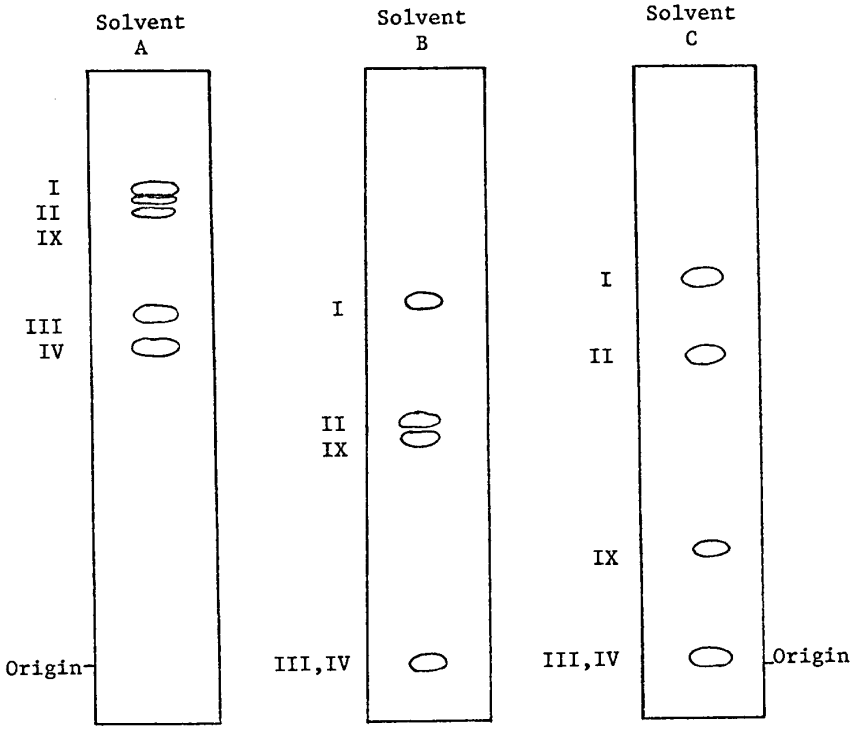

Fig. 3 Three diagrammatic TLC patterns of the metabolites in the organosoluble fraction derived from the ^{14}C-Cyanox treated leaf several days after application.

Table 3 Distribution of radioactivity in the organo-soluble fraction derived from ^{14}C-Cyanox and ^{14}C-Surecide in bean seedling through root during 9-day water culture.

Plant parts	From Cyanox[a)]		From Surecide[b)]	
	dpm/gr	Total dpm	dpm/gr	Total dpm
Leaves	300	22000	Not detected	Not detected
Young sprouts	2600	8500	Not detected	Not detected
Stem	1000	87000	130	13000
Roots	1300	65000	5800[c)]	300000

Note: 1) Recovery of total radioactivity: 75% from Cyanox; 82% from Surecide.
2) Uptake of radioactivity found in the organo-soluble fraction; 27% (from Cyanox) and 47% (from Surecide) of the total activity (670000 dpm) used in water.
3) 2200 dpm is equivalent to 1 ppm of plant parts.
a) Only part of radioactivity is represented by intact Cyanox (by TLC estimation).
b) Almost all the radioactivity is represented by intact Surecide (by TLC estimation).
c) Almost all the radioactivity is on the surface of the root.

identified by TLC from the Surecide treated leaves.

2. *Fate of Cyanox and Surecide taken up through the Roots by Bean Seedlings in Water Culture*

After 9 days in water culture 27% of the radioactivity initially present as ^{14}C-Cyanox in the water was found in the organo-soluble fraction from the bean plants. Radioactivity appeared to be distributed in all plant parts: leaves, shoot, stem, and root. Radioactivity was concentrated much more in the shoot than in the established leaves (Table 3).

The radioactivity remaining in the water after 9 days, 48% of that originally present, was about equally divided between Cyanox and its desmethyl compound based on TLC results. As Cyanox is stable in slightly acidic, neutral and slightly basic media, the root appears to be implicated in demethylating Cyanox.

Some intact Cyanox is probably taken up by the plant because, although in small amounts, it was identified by TLC in each part of the plant; the desmethyl compound and 4-cyanophenol were also present in all plant parts. Cyanoxon and its desmethyl compound were not positively confirmed. The possibility of uptake of desmethyl cyanox from water through through the root was not experimentally examined. Part of the remainder of the radioactivity (25%) was probably present in the water-soluble fraction. Loss owing to evaporation is also a possibility to account for the remainder of the unrecovered radioactivity.

Of the radioactivity derived from ^{14}C-Surecide, 47% was taken up by the plant from water. In contrast to the results with Cyanox 96% of the activity was found on the surface of the roots and little was present in the aerial parts (Table 3). Almost all the radioactivity was accounted for by the parent compound, Surecide, with just a trace amount of a polar compound, possibly desethylsurecide. Even when Surecide was taken up through the cut ends of stems only a slight amount of radioactivity was found inthe stem, petiole and vein part of the plant after 10 days.

3. *Fate of Cyanox and Surecide in Bean Seedlings after Dipping, Topical Application and Injection into Stems*

1) Cyanox

Some of the radioactivity from the leaf dipped into the radioactive solution translocated to the young sprout and stem, and slowly to the other established leaf and root within a week as shown by autoradiograms. The radioactivity recovered as the organo- and water-soluble fractions from the treated leaf and untreated parts of the plant were measured at intervals after treatment (Table 4). ^{14}C-Cyanox applied topically to the leaf,

Table 4 Dissipation and distribution of the radioactivity derived from ^{14}C-Cyanox applied by dipping the leaf.[a)]

Time after application	Radioactivity recovered %				
	Total	From treated leaf		From untreated parts[b)]	
		Organo-soluble	Water-soluble	Organo-soluble	Water-soluble
0 hr	100.0	97.20 (97.20)[c)]	2.34 (2.34)	0.26 (0.26)	0.20 (0.20)
7 hrs	67.5	61.85 (91.63)	3.87 (5.74)	1.35 (2.00)	0.43 (0.63)
1 day	45.5	39.20 (86.15)	4.02 (8.83)	1.76 (3.87)	0.52 (1.15)
3 days	43.9	27.32 (62.23)	11.10 (25.28)	3.26 (7.43)	2.22 (5.06)
7 days	28.5	12.68 (44.49)	10.22 (35.87)	4.27 (14.98)	1.33 (4.66)

a) Temperature range of 8-30°C in greenhouse conditions.
b) Amounts of radioactivity found in the untreated leaf, young sprout and stem are combined.
c) Figures in parentheses indicate the percentage of the radioactivity in each fraction of the total recovered.

Table 5 Dissipation and distribution of the radioactivity in the treated leaf after topical application of ^{14}C-Cyanox.[a)]

Time after application	Total	Radioactivity recovered %	
		Organo-soluble	Water-soluble
0 hr	100	97.5 (97.5)[b)]	2.5 (2.5)
1.5	96.6	94.4 (97.7)	2.2 (2.3)
3	93.0	91.0 (97.8)	2.0 (2.2)
1 day	90.8	82.8 (91.2)	8.0 (8.8)
3	69.6	52.4 (75.3)	17.2 (24.7)

a) Temperature range 5-20°C in greenhouse conditions.
b) Figures in parentheses indicate the percentage of the radio-activity in each fraction of the total recovered.

gave the same pattern of dissipation and distribution as was obtained by dipping the leaf (Table 5).

One week after dipping the leaf in the Cyanox solution, 12.7% of the applied radioactivity was found in the organo-soluble fraction, 10.2% in the water-soluble fraction (Table 4), and 5.6% was present in the untreated parts of the plant. As this amounted to only 28.5% of the total it appears that the rest was lost perhaps by volatilization of intact Cyanox, as it is much more volatile than its metabolites. There is also a possibility that nitrile group can be oxidized and released as carbon dioxide. However, this was not observed in this experiment and there was no appreciable radioactive carbon dioxide evolved in mammalian metabolism (Wakimura and Miyamoto, 1971).[9)]

The radioactivity in the organo-soluble fraction from the Cyanox-treated leaf was represented by Cyanox, cyanoxon, desmethylcyanox, desmethylcyanoxon and 4-cyanophenol; desmethylcyanox was the predominant metabolite. Of the aforementioned metabolites, cyanoxon is the sole toxic compound and may be considered biologically significant after the degradation of the parent compound. The identification of these metabolites was made by the use of five different solvent systems on TLC. The only metabolite found in the water-soluble fraction was 4-cyanophenol. Desmethylcyanox, desmethylcyanoxon and 4-cyanophenol were found in the untreated parts of the plant but cyanox and cyanoxon were not.

The half-life of Cyanox on the leaf under greenhouse conditions was one day or less at temperatures ranging from 5 to 40°C (Table 4, 6).

The amounts of Cyanox and its metabolites found in the organosoluble fraction of the treated leaf change with time after treatment (Table 6, Fig. 4). The original 106 ppm of Cyanox on the leaf decreased to 31, 16 and 3.5 ppm after 1, 3 and 7 days, respectively. The residual cyanoxon after 7 hours, and 1, 3, and 7 days was 0.9, 1.2, 1.3 and 0.4 ppm, respectively. The proportion of desmethylcyanox plus desmethylcyanoxon and 4-cyanophenol in the organo-soluble

Table 6 Residues (ppm)[a] of Cyanox, cyanoxon, desmethylcyanox plus desmethylcyanoxon, and 4-cyanophenol in the treated leaf.[b]

Time after application	Total residue	From organo-soluble fraction				From water-soluble fraction
		Cyanox	Cyanoxon	Desmethylcyanox & desmethylcyanoxon	4-Cyanophenol	4-Cyanophenol
0 hr	109.5	106.2	0.15	0.55	0	2.57
7	72.2	61.7	0.87	4.80	0.59	4.25
1 day	47.4	30.9	1.20	9.02	1.86	4.41
3	42.3	16.2	1.29	9.74	2.86	12.2
7	25.3	3.53	0.37	8.05	2.01	11.3

a) Residues are expressed as Cyanox equivalents based on the radioactivity; ^{14}C-Cyanox was applied by dipping the leaf.

b) Temperature range of 8-30°C in greenhouse conditions.

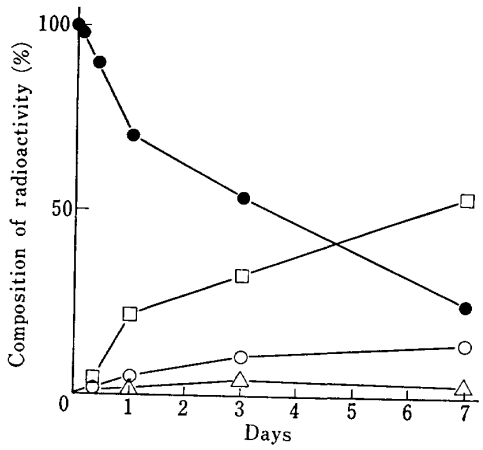

Fig. 4 Proportions of Cyanox, cyanoxon, 4-cyanophenol and desmethylcyanox plus desmethylcyanoxon in organo-soluble fraction of ^{14}C-Cyanox treated leaves in relation to time after application.
(Temperature range during the experiment; 8 to 30°C)
● parent compound (Cyanox)
△ cyanoxon, ○ 4-cyanophenol,
□ desmethylcyanox & desmethylcyanoxon.

fraction steadily increased at the expense of Cyanox. The highest metabolite level was found 3 days after treatment: cyanoxon (1.3 ppm), 4-cyanophenol (2.9 ppm) and desmethylcyanox and desmethylcyanoxon (9.7 ppm) (Table 6). The radioactive compounds in the water-soluble fraction, mostly 4-cyanophenol, also increased to more than 10 ppm after 3 days.

To establish the metabolic pathways, Cyanox, cyanoxon, and desmethylcyanox were injected into individual stems of the bean plant. After a 3 day post injection period in the greenhouse, cyanoxon, desmethyl cyanox, 4-cyanophenol and perhaps desmethyl cyanoxon were recovered from the plant injected with Cyanox. Desmethylcyanox gave 4-cyanophenol, but not desmethylcyanoxon, and cyanoxon gave both desmethylcyanoxon and 4-cyanophenol.

It is possible that Cyanox is modified by photodegradation on the leaf. However, metabolites isolated from leaves protected from sunlight after topical application of Cyanox were identical to those obtained from leaves exposed to sunlight. Cyanox exposed to sunlight or an ultraviolet light lamp (UV-lamp, Super-Light Model LS-D1, Nikko Sekiei Works, 2537Å) on TLC-plate, was converted to cyanoxon in a minute quantity, but not to 4-cyanophenol, desmethylcyanox or desmethylcyanoxon. Therefore, photodecomposition of Cyanox appears to be of little significance.

4-Hydroxybenzamide, 4-hydroxybenzoic acid, and their phosphoric acid derivatives were not found. Other possible metabolites, not detectable in the present experiment because of the labeling position, are *O*, *O*-dimethyl phosphorothioic acid, *O*, *O*-dimethyl phosphoric acid, and their further degradation products. However, they are known to be insignificant from the toxicological point of view. Results of the above experiment suggest that Cyanox is degraded according to the pathways shown in Fig. 5.

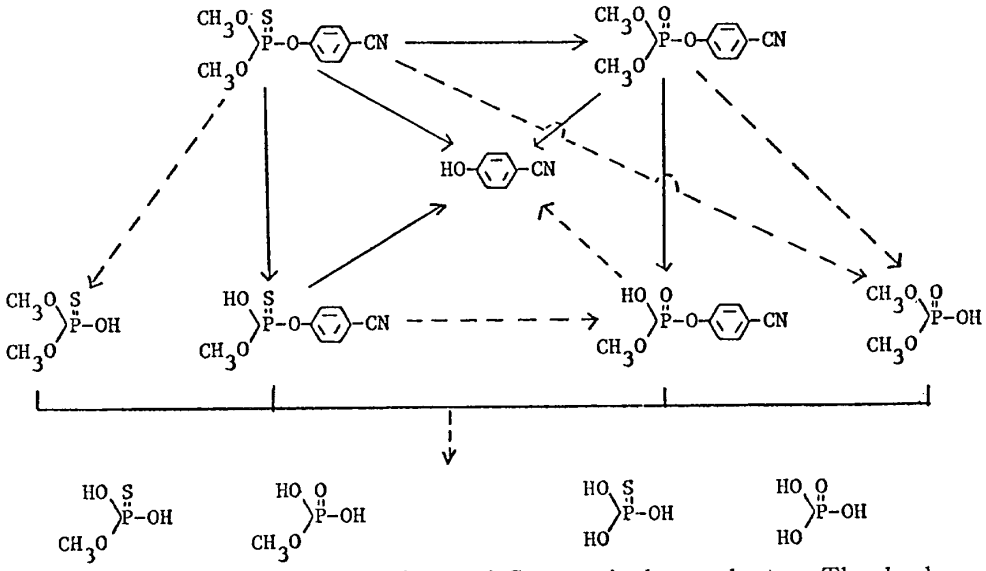

Fig. 5 *Tentative metabolic pathway* of Cyanox in bean plants. The broken line indicates the possible but experimentally not demonstrated pathway.

2) Surecide

The radioactivity levels of Surecide from the treated leaf decreased much more slowly than did those of Cyanox. The halflife of Surecide on the leaf under greenhouse conditions was about 14 days at temperature ranging 20 to 40°C. There was little or no translocation of Surecide as demonstrated by autoradiograms of treated plants and based on data in Tables 7 and 8. About 74% of the original Surecide still remained on the plants one week after treatment. Two metabolites, desethylsurecide and 4-cyanophenol, were found in the organo-soluble fraction of the treated plant; the quantities were small one day after application, but increased gradually with time. In a 7-day sample, 22% of the original activity was represented by these two compounds with desethylsurecide predominating. Surecide-oxon was not found under any conditions. When surecide-oxon was injected into the plant, it decomposed within a day to yield 4-cyanophenol and desethylsurecide-oxon, and no parent compound was found. Conversely, Surecide produced no metabolites when injected into the stem probably because it is poorly translocated to the metabolizing site.

About 10% of Surecide exposed on TLC-

Table 7 Dissipation and distribution of the radioactivity in the treated leaf after topical application of ^{14}C-Surecide.[a)]

Days after application	Total	Radioactivity recovered %			
		From treated leaf			From untreated parts
		Organo-soluble	Water-soluble	Unextracted	
0	100	99.7 (99.7)[b)]	0.32 (0.32)	0	0
1	99.1	96.4 (97.3)	1.26 (1.27)	1.42 (1.43)	0
3	98.0	93.6 (95.5)	2.18 (2.22)	2.22 (2.27)	0
7	94.4	90.2 (95.6)	0.81 (0.86)	3.41 (3.61)	0
14	87.8	80.0 (91.1)	3.82 (4.35)	3.99 (4.54)	0

a) Temperature range of 20-40°C in greenhouse conditions.

b) Figures in parentheses indicate the percentage of the radioactivity in each fraction of the total recovered.

Table 8 Residues (ppm)[a] of Surecide, desethylsurecide and 4-cyanophenol in the treated leaf.[b]

Days after application	From organo-soluble fraction			
	Surecide	Desethylsurecide	4-Cyanophenol	Total
0	103.2	1.53	0.28	105.0
1	100.2	1.80	0.63	102.6
3	92.1	4.31	4.20	100.6
7	76.0	20.6	2.43	99.0

a) Residues are expressed as Surecide equivalents based on the radioactivity; Surecide-^{14}C was applied by dipping the leaf.

b) Temperature range of 20-40°C in greenhouse conditions.

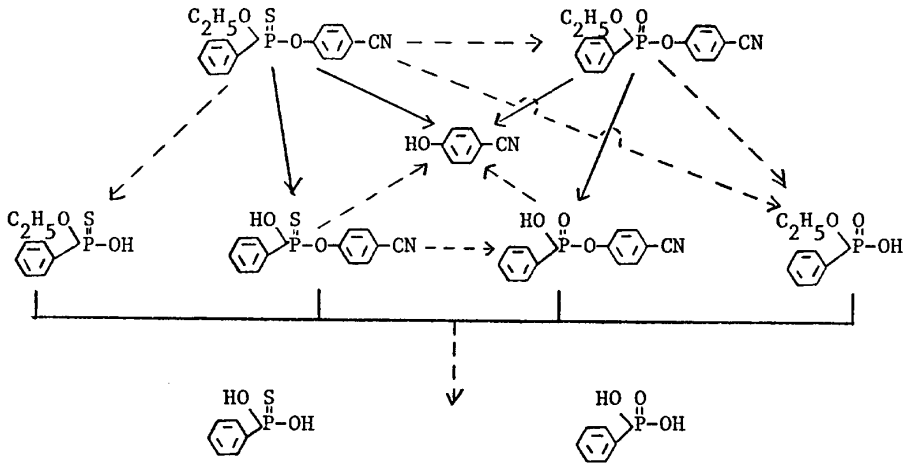

Fig. 6 *Tentative metabolic pathway* of Surecide in bean plants. The broken line indicates the possible but experimentally not demonstrated pathway.

plates to UV-lamp for several hours decomposed to surecide-oxon, 4-cyanophenol, desethylsurecide, probably desethylsurecide-oxon, and a couple of unknown products. Sunlight was less effective than UV lamp in degrading Surecide and gave only a trace of 4-cyanophenol and an unknown product with an *Rf*-value slightly lower than that for surecide-oxon on TLC (solvent system C).

These results suggest that under natural conditions Surecide will decompose to 4-cyanophenol and desethylsurecide slowly on or in the leaf, but not in the stem, and that surecide-oxon, if any is produced, will decompose rapidly to 4-cyanophenol and desethylsurecide-oxon. Or there is no pathway from Surecide to the oxon in the bean plants. A tentative metabolic pathway for Surecide degradation in bean plants is given in Fig. 6.

4. Degradation in Soil

Figure 7 shows that the measurable radio-

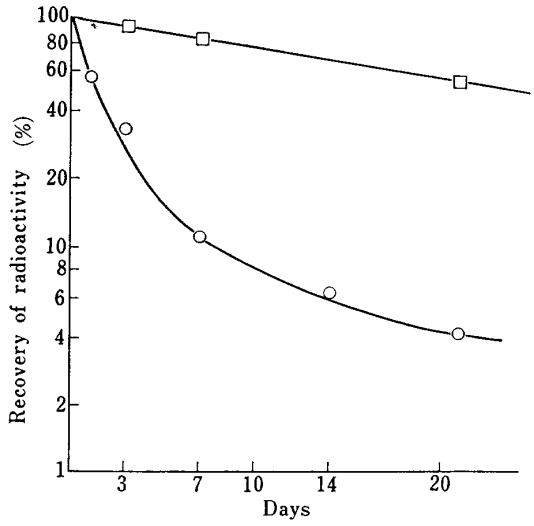

Fig. 7 Dissipation of radioactivity in soils treated with ^{14}C-Surecide or ^{14}C-Cyanox.
□ from ^{14}C-Surecide, ○ from ^{14}C-Cyanox.

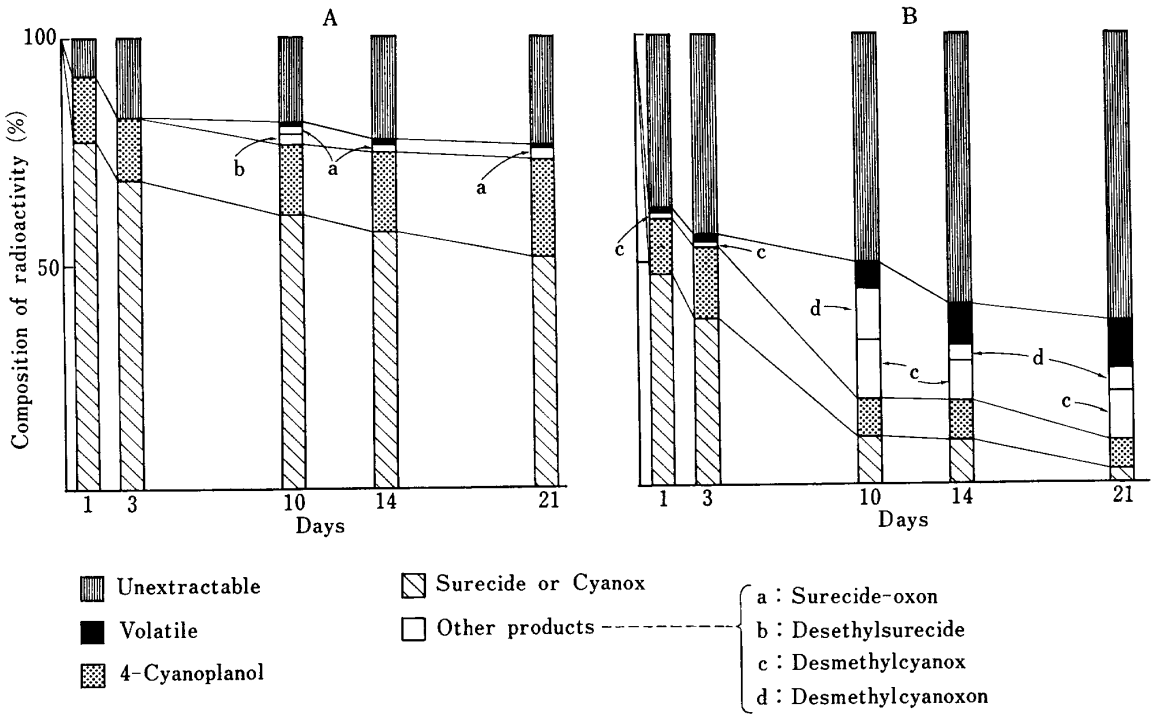

Fig. 8 Degradation of ^{14}C-Surecide (A) and ^{14}C-Cyanox (B) in soil.

activity found in the Cyanox treated soil decreased rapidly; only 6% remained after two weeks. Under similar conditions, 53% of the radioactivity derived from Surecide remained after 3 weeks. No differences in amounts of residue were observed between soil samples from the surface and the 6 cm depth. By TLC of organic solvent extract, Cpanox, Surecide, 4-cyanophenol, and other products were detected. Many factors may be involved to produce the dissipation curves shown in Fig. 7: evaporation of the insecticide or the products from the soil; and efficiency of radioactivity counting in the presence of soil, particularly for substances which are bound to the soil.

More critical study in a closed system showed that the recovery of radioactivity to the organic solvent extract from ^{14}C-Surecide treated soil dropped to 73% after 2 or 3 weeks (Fig. 8-A). During that period, the trapped volatile was very small in amount, only 0.9% even after 3 weeks, indicating the formation of bound residue not easily recovered from the soil. Degradation of Surecide was slow, 53% still remaining after 3 weeks. The balance of radioactivity recovered was mostly 4-cyanophenol, 21% after 3 weeks, and a very small amount of surecide-oxon (2% after 3 weeks) and desethylsurecide (2% on 10 days, but not shown on other days).

The recovery of radioactivity from ^{14}C-Cyanox treated soil decreased rapidly, partly due to the volatiles (increased to 11% after 3 weeks), but mostly due to the unextractable form (Fig. 8-B). Cyanox degraded rapidly, 47% remaining after one day, and only 3% after 3 weeks. Initially 4-cyanophenol was practically the sole product, but the formation of desmethylcyanox and desmethylcyanoxon became remarkable after 10 days (13 and 11%, respectively). 4-Cyanophenol was produced from Cyanox and most probably so from the further degradation of the desmethyl compounds. Nevertheless, 4-cyanophenol formation decreased after a maximum (15%) on 3 day. In view of the results, probably 4-cyanophenol and/or its further degradation products were gradually bound firmly to the soil.

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要　　約

Cyanox, Surecide は，それぞれ *O*, *O*-dimethylphosphorothioic acid と *O*-ethyl phenylphosphonothioic acid の 4-cyanophenol エステルであるが，^{14}C-ニトリル標識物による研究によれば，両者ともナガウズラ植物で有機リン剤に典型的な経路を通り代謝され，この際ニトリル基上の変化は認められなかった．両化合物の代謝速度は異なり半減期は Cyanox で１日以下，Surecide で約２週間であった．Cyanoxon はわずかに認められたが，surecide-oxon は認められなかった．主代謝物はいずれも，脱アルキル体と 4-cyanophenol であった．Cyanox は，水耕液から植物体に入るに対し，Surecide は入らない．Cyanox の土壌中での分解は早く，4-cyanophenol と脱アルキル化合物を与えるに対し，Surecide は残留性長く，また 4-cyanophenol しか与えなかった．

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