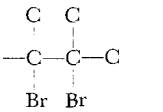
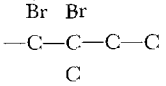
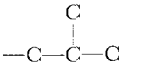
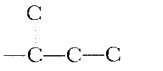
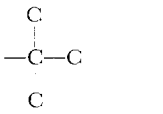
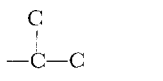
Compound Number	No. of Carbon Atoms	Carbon Skeleton Alkyl Group	I_{50}
XXIII	5		0.6
XXIV	4		0.34
XXV	4		4.0
XXVI	4		0.014
XXVII	4		0.11
XXVIII	3		0.075

Table IX. Biological Activity toward Tropical Mosquitos (6)

Compound, Alkyl Substitution	I_{50}, μG. A. stephensi	A. aegypti
2-Chloro-3-isopropyl phenyl (Hercules)	0.0018	0.0043
3-Isopropylphenyl (Union Carbide)	0.0013	0.0025
2-Isopropoxy phenyl	0.0013	0.0053
3-*sec*-Butyl phenyl XXVI (this paper)	0.0012	0.0023
1-Naphthyl (Sevin, Union Carbide)	0.0037	0.0145
DDT	0.018	0.018

Conclusions

There has been much speculation concerning physiological modes of action of the *N*-methyl phenyl carbamates other than interference with the function of the enzyme acetyl cholinesterase. This study deals only with the competition of synthesized molecules of known geometries with the normal substrate acetylcholine for a place on that enzyme. If these molecules possess modes of activity other than that provided by inhibition measurements, then the total biochemical activity is in some way different and probably greater than that provided through inhibition of AChE alone.

The actual field effectiveness of these substances is of course affected by absorption rates, detoxication in plant and animal, hydrolytic stability, solubilities, sensitivity to visible and ultraviolet radiation, etc. At this time, however, it is impossible to systematize these effects as one can do with the relations of structure to inhibition.

For the simple carbamates containing no hetero atoms on the alkyl groups, the following generalization can be drawn. Compounds of very high inhibiting character can be synthesized with relatively simple structures. Meta-alkyl substitution of the phenol nucleus provides the best opportunities for high activity.

Highest activity is derived from compounds with one α substitution on the alkyl chain. For small chains, α-ethyl substitution appears optimum; for larger chains, α-methyl substitution is optimum. β or other substitution on the side chain is noncontributory or deleterious to activity for the meta isomers. Multiple substitution on the α-position as in *tert*-alkyl groups provides compounds inferior to single substitution (*sec*-alkyl), though the compounds are still highly active. Highly active compounds with *m*-alkyl groups can have their activity enhanced when halogen is substituted on the six position. Too long a chain, even though optimally substituted, decreases the inhibition. All compounds possessing high enzymatic inhibitory properties exhibited areas of high insecticidal potency.

Literature Cited

(1) Dauterman, W. C., O'Brien, R. D., J. AGR. FOOD CHEM. **12,** 318–19 (1964).
(2) Ferguson, G. R., Alexander, C. C., *Ibid.*, **1,** 888–9 (1953).
(3) Giang, P. A., Hall, S. A., *Anal. Chem.* **23,** 1830 (1951).
(4) Grob, H., "Research on Control of Aphids with Chemicals Based on Urethanes and Phosphoric Esters," Third International Congress on Phytopharmacy, Paris, September 1952.
(5) Gysin, H., *Ibid.*, "New Group of Insecticidal Substances."
(6) Hadaway, A. B., Barlow, F., "Toxicity of Some Carbamates to Adult Mosquitoes," Tropical Pesticides Research Unit, Porton.
(7) Metcalf, R. L., "Organic Insecticides," pp. 317–29, Interscience, New York, 1955.
(8) Moore, J. E., Ospenson, J. N., Kohn, G. K., U. S. Patents **3,062,864–3,062,868; 3,066,163; 3,062,707; 3,076,741; 3,110,726** (November 1962–November 1963).
(9) Wiesmann, R., "Research on a New Insecticide Active against Resistant *Musca domestica*," XIIth International Congress of Pure and Applied Chemistry, New York, September 1961.
(10) Wiesmann, R., Gasser, R., Grob, H., *Experientia* **7** (4), 117–20 (1951).

Received for review September 18, 1964. Accepted February 1, 1965. Division of Agricultural and Food Chemistry, Symposium on Carbamate Insecticides, 148th Meeting, ACS, Chicago, Ill., September 1964.

CARBAMATE INSECTICIDES

Adaptation of Sevin Insecticide (Carbaryl) Residue Method to Various Crops

D. P. JOHNSON[1] and H. A. STANSBURY

Olefins Division, Union Carbide Corp., South Charleston, W. Va.

SINCE its discovery in 1953, Sevin insecticide (carbaryl) has become established as a broad spectrum pesticide of relatively low mammalian toxicity. This product, chemically 1-naphthyl *N*-methylcarbamate, has been registered for use on approximately 85 field, vegetable, and fruit crops as well as for dermal treatment of most domestic and livestock animals. In addition, it is being investigated for use in oyster beds to control oyster predators. To obtain such broad registration, thousands of samples had to be analyzed to determine residue concentrations in and on the products or certain fractions of products involved. In addition, many related analytical investigations have been performed, including the differentiation between Sevin and 1-naphthol, stability tests in crops and soil, and concentration

[1] Present address, Research Department, R. J. Reynolds Tobacco Co., Winston-Salem, N. C.

Sevin insecticide (carbaryl) has been registered for use on over 85 food and feed products, requiring analyses of thousands of samples for residue data. Approximately 20 residue methods have been developed, using *p*-nitrobenzenediazonium fluoborate as the chromagenic detection agent. Sensitivities range from 0.02 to 0.2 p.p.m. A modified method based on nitrozation of 1-naphthol has been developed for determining Sevin in animal dip-tank fluid. Crop samples are extracted with a Waring Blendor, using methylene chloride as the principal solvent. Cleanup is achieved by a variety of techniques, including coagulation and filtration, chromatography with Florisil, aqueous sodium hydroxide extraction, and liquid-liquid partitioning involving petroleum ether and acetonitrile. Accumulated analytical data show the normal half life of Sevin on growing crops to be 3 days in spinach, 2 days in berries, 4 days in apples, and in soil, approximately 8 days under normal conditions.

requirements associated with the use of Sevin in animal dip tanks.

The wide variety of products encountered has intensified the problem of providing reliable and specific analytical methods for Sevin residues. Although a fairly general chemical procedure can be used for most succulent fruit and vegetable crops, numerous modifications are necessary for others. Cottonseed, cocoa beans, nuts, and dried corn grain, for example, must be treated differently because of the high oil content. Products of high aromatic content may require special treatment to remove interferences. Still other modifications are necessary to adapt the method to products of animal origin such as meat, milk, eggs, and oysters (*1*, *3*, *5*). As a result, approximately 20 procedures of varying degrees of similarity and sensitivities ranging from 0.02 to 0.2 p.p.m. have been developed.

End Determination

The end determination generally used for Sevin is based on hydrolysis to 1-naphthol, followed by colorimetric measurement of 1-naphthol. Although several methods have been applied for naphthol determination, coupling with *p*-nitrobenzenediazonium fluoborate has proved most satisfactory. This technique was first used for Sevin by Miskus, Gordon, and George (*4*), who conducted the coupling reaction in basic medium. Under these conditions, a purple color with maximum absorption at 575 mμ is obtained.

To achieve greater specificity and reduce the cleanup requirements, the naphthol coupling procedure was modified to permit color development in acetic acid. In this medium, 1-naphthol forms an orange-yellow color with maximum absorption at 475 mμ, while many natural phenolic substances which react in basic medium produce no color. Sodium hydroxide is added to the sample prior to the addition of acetic acid to catalyze the coupling reaction. Thus, maximum color is produced within 1 to 2 minutes, whereas, without the base, a reaction period of up to 10 minutes is required.

Extraction

Sevin may be extracted from the various products in a number of ways, depending on the nature of the substrate. The most general procedure is to homogenize the sample in a Waring Blendor with the appropriate solvent. This not only removes surface residues but also extracts any Sevin that may be present in the tissue as a result of systemic translocation. Methylene chloride is usually the solvent of choice, because it has excellent solubility toward Sevin, and its low boiling point allows rapid concentration of the extract.

In some cases methylene chloride is not entirely satisfactory. For example, in tests involving the identification of pesticide residues in honeybees, it consistently gave somewhat low recoveries of Sevin. It was found that, for this particular application, benzene is superior, probably because of its greater solubility toward beeswax. In other cases involving products of low water content, such as soil, acetone has proved very efficient for extraction.

Dehydrated fibrous products generally present greater extraction difficulties than fresh, succulent samples. In such cases, the extractability of Sevin can usually be enhanced simply by soaking the sample in water for 6 to 24 hours prior to extraction. No significant loss of Sevin occurs during the soaking period.

When extracting watery crops with methylene chloride, benzene, or other water-immiscible solvents, anhydrous sodium sulfate is added to the blender to prevent troublesome emulsions.

Cleanup

Usually, the cleanup operation is the most versatile phase of the Sevin method and provides the greatest flexibility. Many substances which otherwise would interfere in the end determination can be eliminated by proper selection of a cleanup procedure. The most general technique for fruit and vegetable crops involves the use of an aqueous coagulation solution. In this operation, the residue from the initial extract is dissolved in acetone or methanol; then a solution of 0.1% ammonium chloride in 0.2% phosphoric acid is added to precipitate plant waxes and other water-insoluble extractives. After a few minutes of standing, Hyflo-Supercel or some other appropriate filter aid is added and the slurry filtered under vacuum through a Büchner funnel. A clear filtrate containing the Sevin residues is obtained. The Sevin is then extracted from the filtrate into methylene chloride.

From this point, a number of choices is available for additional cleanup, depending on the crop under test. Some crops, including apples and lettuce, require no further treatment, in which case the methylene chloride is evaporated and the residue processed directly for color. This is the basis for the general method recently adopted as official by the Association of Official Agricultural Chemists (*2*).

Many products require chromatographic treatment of the methylene chloride extract before color development. Florisil has proved to be a very satisfactory adsorbent for this purpose, since Sevin can be eluted with water-saturated methylene chloride, leaving most extraneous materials on the column. However, the water content of the Florisil influences the recovery of Sevin and should be adjusted on each new batch of material prior to use. The optimum concentration of water in the adsorbent is 3.5% as determined by titration with Karl Fischer reagent. In some instances, the coagulation procedure may be eliminated and the residue applied directly to a Florisil column. However, additional cleanup is usually required.

As an alternative to the Florisil treatment, extraction of the methylene chloride solution with aqueous sodium hydroxide often provides the additional cleanup needed. This removes some pigments and other acidic materials which interfere in the color development. One disadvantage to the base treatment, however, is that free 1-naphthol, if present as a degradation product of

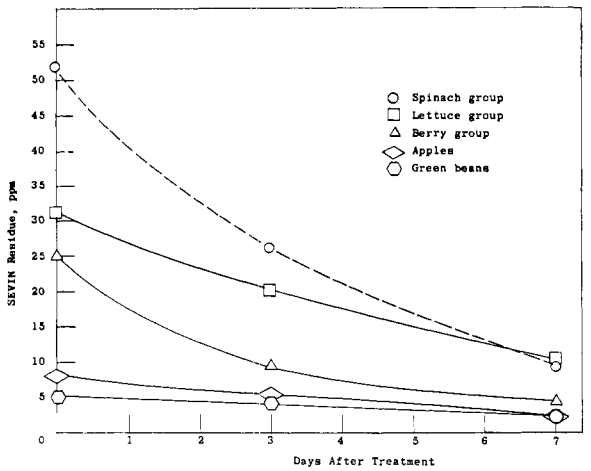


Figure 1. Disappearance of Sevin residues from vegetable and fruit crops

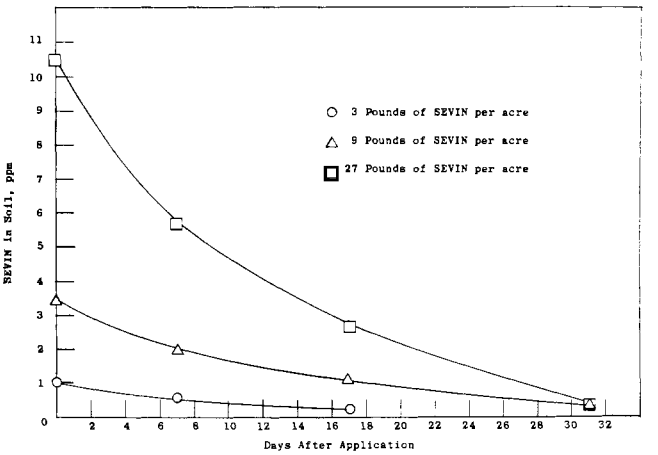


Figure 2. Degradation of Sevin in soil

Sevin, would also be eliminated. However, free 1-naphthol has never been found on crops following treatment with Sevin.

Products such as nuts, cottonseed, and dried corn grain, which contain large amounts of oil, cannot be processed directly by coagulation. Neither direct sodium hydroxide extraction nor chromatography on Florisil will provide the necessary cleanup. Consequently, the oil must first be eliminated. The most satisfactory means of separating the oil from Sevin is to partition the residue from the initial extraction between petroleum ether and acetonitrile. The oils are removed by the hydrocarbon, while Sevin is recovered in the acetonitrile. The acetonitrile can then be evaporated and the residue either processed through the coagulation treatment or applied directly to a Florisil column, depending on the requirements.

In some cases, nonacidic interferences have been encountered which cannot be removed by any of the techniques so far discussed. This problem often can be solved by hydrolyzing the Sevin to 1-naphthol, then extracting an aqueous sodium hydroxide solution of the latter with methylene chloride. Interferences are removed by the methylene chloride, while 1-naphthol remains in the aqueous phase as sodium naphthoxide. The alkaline solution is then acidified and the naphthol extracted into methylene chloride. The latter treatment probably also prevents interference from some of the more recent carbamates derived from aminophenols.

Still another cleanup approach is to apply the coagulation technique after hydrolysis of Sevin. In this case, the residue from the initial extract is dissolved in alcoholic sodium hydroxide and allowed to stand at room temperature for approximately 10 minutes. Coagulation solution is then added and the sample processed in the usual manner.

Considering the diversity of the cleanup techniques, the recovery of Sevin by the various procedures is remarkably good. In most cases, absorbances of calibration standards are at least 90% of the theoretical values, while recovery from crops usually exceeds 90%. Furthermore, Sevin is sufficiently stable under all the cleanup conditions to prevent any critical timing problems. The only process in which time may be considered an important factor is the sodium hydroxide extraction of the methylene chloride solution. Even in this operation, Sevin has been shown to survive at least 60 seconds of shaking, while only 10 seconds are needed for effective cleanup.

Water Analysis

In contrast to the complex analysis of biological products, Sevin can usually be determined in water with relative ease. In many cases, including both fresh and sea water, the only requirement is to extract the sample with methylene chloride, followed by solvent evaporation and color development on the residue. In other cases, involving water contaminated with organic materials, the methylene chloride extract is passed through Florisil before color development.

Dip-Tank Fluid Analysis

A more complicated problem involving water is the determination of Sevin in animal dip-tank fluid. These analyses are necessary for programming topping-up schedules to maintain the proper chemical concentration and liquid level in the tanks. To be of maximum benefit, the analysis must be performed at tank side, requiring that the method be applicable in the hands of nontechnical ranch personnel.

From the standpoint of Sevin concentration, the dip-tank problem does not fall exactly into the category of residue analysis. However, the cleanup problems are closely related. In normal practice, dip-tank fluid is used almost indefinitely, resulting in buildup of excreta, hair, mud, and other contaminants which interfere in the analysis. The problem is further complicated by the slow degradation of Sevin, with a resultant slight buildup of free 1-naphthol. These interferences must be eliminated before Sevin can be accurately determined.

Interference from 1-naphthol is prevented by adding potassium permanganate solution to the fluid prior to extraction. Naphthol is oxidized, leaving the Sevin unaffected. Sevin is then extracted into chloroform and the extract is treated with activated carbon to remove pigments and other extraneous materials. Then alcoholic sodium hydroxide is added to the chloroform to saponify the Sevin, followed by addition of acetic acid and sodium nitrite. The yellow nitrosonaphthol color thus formed is matched with a varnish color standard, using a Hellige Comparator.

Stability of Sevin Residues

From the thousands of Sevin analyses performed to date, several residue patterns have evolved (6). For example, the initial residues on leafy vegetable and forage crops which have high surface to weight ratios are significantly higher than on fruits. The residues on various crops rapidly disappear, making it possible, in many cases, to continue use of Sevin close to harvest. Primary factors influencing the disappearance rate seem to be weathering and dilution by plant growth rather than chemical (or photochemical) degradation or systemic effects. The disappearance of Sevin from several crops and crop groups is illustrated in Figure 1. The residues, which represent averages of numerous determinations, are plotted against the number of days after treatment. The spinach group shows an initial residue of 52 p.p.m., but the concentration rapidly decreases and only 9 p.p.m. remain after 7 days. For this crop group, Sevin is shown to have a half life of 3 days.

The berry group showed a sixfold decrease in residues over a 7-day period, indicating a half life of 2 days. Similar curves are shown for apples, green beans, and the lettuce group.

Another pattern demonstrated by the analytical program is the rapid rate at which Sevin degrades in soil. Tests involving Sevin at three different concentrations were conducted in Norfolk sandy loam soil at the Union Carbide Agricultural Research Station at Clayton, N. C. The chemical was tilled into the top 6 inches of soil, which was then sampled periodically for analysis. The results are shown graphically in Figure 2. The half life of Sevin is demonstrated to be approximately 8 days at all concentrations, and in every case the residue appeared to be completely degraded within 40 days.

Literature Cited

(1) Claborn, H. V., Roberts R. H., Mann, H. D., Bowman, M. C., Ivey, M. C., Weidenbach, C. P., Radeleff, R. D., J. AGR. FOOD CHEM. **11,** 74 (1963).
(2) Johnson, D. P., *J. Assoc. Offic. Agr. Chemists* **47,** 283 (1964).
(3) Johnson, D. P., Critchfield, F. E., Arthur, B. W., J. AGR. FOOD CHEM. **11,** 77 (1963).
(4) Miskus, R., Gordon, H. T., George, D. A , *Ibid.*, **7,** 613 (1959).
(5) Whitehurst, W. E., Bishop, E. T., Critchfield, F. E., Gyrisco, G. G., Huddleston, E. W., Arnold, H., Lisk, D. J., *Ibid.*, **11,** 167 (1963).
(6) Yoder, D. M., Back, R. C., Union Carbide Chemicals Division, private communication, 1963.

Received for review August 18, 1964. Accepted December 21, 1964. Division of Agricultural and Food Chemistry, Symposium on Carbamate Insecticides, 148th Meeting, ACS, Chicago, Ill., September 1964.

END OF SYMPOSIUM

HERBICIDE DEGRADATION

Microbial Degradation of Simazine

D. D. KAUFMAN, P. C. KEARNEY, and T. J. SHEETS

Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md.

Soil microorganisms effective in the degradation of the herbicide simazine were isolated by the enrichment method. Bioassays of simazine culture solutions of *Aspergillus fumigatus* Fres. indicated that this organism degraded simazine to nonphytotoxic or at least less phytotoxic compounds. This organism utilized simetone as a sole source of both carbon and nitrogen. Decreases in radioactivity in culture solutions containing chain-labeled simazine were greater than those observed from solutions containing ring-labeled simazine. C^{14} from chain-labeled simazine was found largely in lipids, proteins, and $C^{14}O_2$, whereas little C^{14} from ring-labeled simazine was incorporated and no $C^{14}O_2$ evolved. Chromatographic analysis of both ring- and chain-labeled simazine culture solutions indicated that simazine was transformed into several compounds not previously reported. Data obtained indicate that degradation of simazine by *A. fumigatus* occurs by a dealkylation, deamination, or both, of the side chains, and that hydroxysimazine is not an intermediate in this mechanism.

MICROBIAL degradation is an important factor in the behavior of pesticides applied to soil. The decomposition of *s*-triazine herbicides by soil microorganisms has been studied by several investigators (*1–4, 9, 10, 15, 20–23*). Until recently, few investigators have been able to demonstrate clearly that soil microorganisms can utilize 2 - chloro - 4,6 - bis(ethylamino) - *s* - triazine (simazine) as a source of carbon, nitrogen, or both. Guillemat *et al.* (*10*) and Waeffler (*25*) concluded that the carbon of the simazine molecule was unavailable to soil fungi. Guillemat *et al.* (*10*) also concluded that the nitrogen of simazine could be utilized in the presence of an adequate carbon source, whereas Waeffler (*25*) concluded that simazine nitrogen could be utilized by soil fungi, but not as a sole source of nitrogen. Burnside (*3*) isolated five microorganisms which were able to subsist for 3 months in media containing simazine as the sole source of nitrogen and nearly sole source of carbon. Bioassay of the growth medium, however, revealed no significant decrease in toxicity during a 30-day incubation period. Kaufman, Kearney, and Sheets (*15*) reported the isolation of several organisms capable of degrading simazine. Although one organism, *Aspergillus fumigatus* Fres., utilized simazine as a sole source of carbon, degradation was more rapid in the presence of a supplemental carbon source. The purpose of this study was to investigate further the degradation of simazine by soil microorganisms.

Materials and Methods

A population of soil microorganisms effective in the degradation of simazine was developed by an enrichment technique.

This technique consisted of placing 10 grams of a Hagerstown silty clay loam in a 500-ml. flask containing 250 ml. of distilled water. Sufficient simazine was added (1.25 grams of wettable powder formulation per 1000 ml.; Simazine 80W) to establish an active ingredient concentration of 1000 p.p.m. A duplicate set of flasks which contained no simazine was used as a blank. Four replications were used. The flasks were maintained on a reciprocating shaker at 24° C. Simazine degradation was determined by measuring chloride ion liberation by the method of Iwasaki, Utsumi, and Ozawa (*13*) at 3-day intervals. A soil dilution–plate method (*14*) was used for the final isolation of effective organisms from enriched culture solutions. A 5-ml. sample of solution from simazine-treated flasks served as the starting point for dilution series. The plating medium consisted of 0.2 gram of K_2HPO_4, 0.3 gram of NH_4NO_3, 0.2 gram of $CaSO_4$, 0.2 gram of $MgSO_4$.-