

animals fed sewage sludge-grown cabbage as compared to animals fed "normal" soil-grown plants. This highly inducible enzyme may function largely as a detoxication system, yet there may result an increased toxicity in animals and may activate procarcinogens (Parke, 1975). Induction of hepatic microsomal mixed-function oxidase activities has been recently reported in swine fed sewage sludge-fertilized corn (Hansen et al., 1976). Interestingly Cd inhibits microsomal induction (Becking, 1976), but Zn could alter this effect (Becking and Morrison, 1970).

ACKNOWLEDGMENT

The authors thank R. H. Ackermann, J. L. Anderson, L. F. Armitage, H. L. Arnold, W. Burdick, A. DeJohn, J. G. Doss, W. A. English, M. Gilbert, H. T. Greweling, R. A. Gunnip, E. J. Harris, C. L. Heffron, R. Karcher, H. G. Knight, R. Martin, W. Middleton, W. F. Miller, R. R. Ott, I. S. Pakkala, D. Pisegna, G. F. Richey, and L. E. St. John, Jr., for their assistance during this investigation.

LITERATURE CITED

- Becking, G. C., *Med. Clin. North Am.* **60**, 813 (1976).
 Becking, G. C., Morrison, A. B., *Biochem. Pharmacol.* **19**, 895 (1970).
 Browning, E., "Toxicity of Industrial Metals", 2nd ed, Butterworths, London, 1969.
 Curry, N. A., *Clearwaters N.Y. Water Pollut. Control Assoc.* **7**, 6-8 (1977).
 Evans, R. J., Bandemer, S. L., *Anal. Chem.* **26**, 595-598 (1954).
 Fisher Scientific Co., "Reagents of Choice for Arsenic in Parts per Billion", Technical Data Bulletin TD-142, Nov 1960.
 Furr, A. K., Lawrence, A. W., Tong, S. S. C., Grandolfo, M. C., Hofstadter, R. A., Bache, C. A., Gutenmann, W. H., Lisk, D. J., *Environ. Sci. Technol.* **10**, 683-687 (1976a).
 Furr, A. K., Stoewsand, G. S., Bache, C. A., Lisk, D. J., *Arch. Environ. Health* **31**, 87-91 (1976b).
 Gajan, R. J., Larry, D., *J. Assoc. Off. Anal. Chem.* **55**, 727-732 (1972).
 Greweling, H. T., "The Chemical Analysis of Plant Tissue", Mimeograph No. 6622, Agronomy Department, Cornell University, Ithaca, NY, 1966, pp 74-76.
 Hansen, L. G., Dorner, J. L., Byerly, C. S., Tarara, R. P., Hinesley, T. D., *Am. J. Vet. Res.* **37**, 711-714 (1976).
 Hinesley, T. D., Ziegler, E. L., Tyler, J. J., *Agro-Ecosystems* **3**, 11-26 (1976).
 Iwata, Y., Gunther, F. A., Westlake, W. E., *Bull. Environ. Contam. Toxicol.* **11**, 523-528 (1974).
 Olson, O. E., *J. Assoc. Off. Anal. Chem.* **52**, 627-634 (1969).
 Page, A. L., "Fate and Effects of Trace Elements in Sewage Sludge When Applied to Agricultural Lands", Report EPA-670/2-74-005, Environmental Protection Agency, Cincinnati, OH, 1974.
 Parke, D. V., in "Enzyme Induction", Plenum Press, London, 1975, pp 207-271.
 Peech, M., Olsen, R. A., Bolt, G. H., *Soil Sci. Soc. Am. Proc.* **17**, 214-218 (1953).
 Pesticide Analytical Manual, Volume 1, U.S. Department of Health, Education and Welfare, Food and Drug Administration, Washington, DC, 1971 (revised), Section 211.14d.
 Reid, M. E., Briggs, G. M., *J. Nutr.* **51**, 341-354 (1953).
 Steel, R. G. D., Torrie, J. H., "Principles and Procedures of Statistics", McGraw-Hill, New York, 1960.
 Stoewsand, G. S., Anderson, J. L., Lee, C. Y., *J. Nutr.* **103**, 419-424 (1973).
 Wattenberg, L. W., *Cancer* **20**, 99-102 (1971).
 Welch, R. M., House, W. A., Van Campen, D. R., *Nutr. Rep. Int.* **17**, 35-42 (1978).

Received for review August 14, 1978. Accepted November 3, 1978.

Caustic Waste Disposal on Lakeland Fine Sand. A Potential Landspreading Problem

Richard L. Coleman,* Philip E. Shaw, and Donald A. Sims

The hydroxides of sodium, potassium, and calcium, caustics used in the citrus industry, were studied in relation to their effects on percolation in Lakeland fine sand. Quantitative compositions of dried aerobic and sun-dried citrus sludges were compared with previously reported decomposition products of other plant materials. These studies show that a combination of humus substances and cations present in citrus waste water could be detrimental to percolation in Lakeland fine sand.

The water crisis in Florida (Carter, 1974), high energy costs, and increasing environmental regulation have made food processors aware of the economic and technical attractiveness of land disposal techniques (*Federal Register*, 1974). The citrus industry uses about 8.25×10^{10} gal of water (370 gal of water/box of fruit processed $\times 223 \times 10^6$ boxes of fruit processed in U.S. in 1973-1974) (Ratcliff, 1977) per production year, and liquid waste constitutes the industry's largest waste disposal problem.

U.S. Citrus and Subtropical Products Laboratory, Southern Region, U.S. Department of Agriculture, Science and Education Administration, Winter Haven, Florida 33880.

Soils receiving waste water must be capable of passing average rainfall plus the waste water. These soils and their associated flora and fauna work in a natural process that filters and decomposes waste products (McLellon, 1971). Several factors affect the permeability of soils and the capacity of associated flora and fauna to remove organic and inorganic matter. Many textbooks and other publications on soils report that certain hydrated cations, including sodium and potassium, decrease percolation in soils containing clay. Much work has been published on the extraction of aromatic acids and phenols from soil with alkaline solvents, including ethanolic sodium hydroxide at pH 11 (summarized by Haider, 1971). However, the effect on soil percolation of combinations of the humus materials and sodium hydroxide solutions has not been

explored. This effect may cause problems for the citrus industry and others who use landspreading to dispose of liquid wastes containing sodium hydroxide solutions.

Caustics used in the citrus industry (and other food industries) for cleaning extraction and canning equipment are occasionally spilled or released via waste treatment systems onto receiving soil as high pH effluent (pH ~10). The caustic most often used is sodium hydroxide. An effluent containing strong caustics "would wreak havoc with the normal flora and fauna of a balanced system...resulting in a complete loss of biological stability..." (Ratcliff, 1977). The soil-biota system could be disrupted and thereby jeopardize the effectiveness of landspreading as a final waste treatment. This is a report on a study of Lakeland fine sand, a soil commonly used by the Florida citrus industry for landspreading and its capacity to absorb high pH liquid waste solutions; and humic materials that might build up in this soil and their effects on soil percolation rates.

EXPERIMENTAL SECTION

Samples. All samples were obtained from waste treatment facilities of commercial citrus processors. The following sludge samples were dried in a vacuum oven at 105 °C under 31 in. of Hg for 2 and 1 day(s), respectively.

Aerobic sludge (ca. 1% moisture) was prepared from the gelatinous material (ca. 90% moisture) separated with a "skimmer/clarifier" from the surface of an aerobic digester.

Sun-dried citrus sludge was obtained from the waste spray field of a processor whose waste treatment system consisted of a holding tank and spray field. Residue accumulated in lower, less-permeable areas of the spray field where the sun eventually dried it to ca. 10% moisture.

Lakeland fine sand, found commonly in the citrus growing regions of Florida, was identified by John Reed of the U.S. Department of Agriculture, Soil Conservation Service, in Lakeland, FL. Two samples of this soil were altered as follows: (1) The *sterile* sample was prepared by autoclaving under 1 atm pressure at 121 °C for 1 h. (2) The *500 °C* sample was prepared by heating in a muffle furnace for 16 h to eliminate all organic matter.

Analytical Methods. *Hemicellulose Analysis.* Dried and weighed samples of citrus sludges were hydrolyzed with 2% HCl at 100 °C for 5 h. The residue from the hydrolysis was rinsed until neutral. The combined filtrate and rinse liquid was neutralized with 40% NaOH and then analyzed for reducing sugars by the method of Ting (1956). Hemicellulose, in percent, was calculated as 0.9% of the reducing sugars according to Waksman and Stevens (1928).

Cellulose Analysis. The residue from the hemicellulose hydrolysis was dried at 70 °C for 24 h and weighed. The residue was then hydrolyzed with 80% H₂SO₄ at room temperature for 2–3 h. Distilled water (150 mL) was added and the mixture was autoclaved at 121 °C for 1 h. The reducing sugars formed were analyzed by the method of Ting (1956), and cellulose was calculated.

Lignin Analysis. The residue from cellulose analysis was dried and divided into two portions, one each for determination of ash and protein. Lignin was calculated by difference, subtraction of the weight of protein plus ash from that of dried residue (Waksman, 1928).

Protein Analysis. Kjeldahl nitrogen, determined as described by Blaedel and Meloche (1963), was multiplied by 6.25 to give percent crude protein.

Ash, Volatiles (Grease), and Tannins. These materials were determined by standard procedures ("Standard Methods", 1965).

Soil Percolation Tests. Pyrex glass columns 2 cm i.d. × 35.0 cm were fitted with glass wool plugs for mea-

Table I. Analyses of Three Spray Field Soils

soil	sand, %	silt, %	colloidal material, %
A-1	98.25	0.50	1.25
A-2	97.00	2.00	1.00
A-3	99.50	0.01	0.50
B-1	96.00	2.00	2.00
B-2	98.25	1.00	0.75
C-1	98.00	1.50	0.50
C-2	98.75	0.75	0.50

surements of percolation, tannin content, and pH of effluent. All samples (100 g) of Lakeland fine sand were loaded into water filled glass columns and percolation rates were measured as milliliter/minute of effluent, using a falling head method (5 to 0 cm). Column effluent was collected in 25-mL portions for pH measurements. Where pH of effluent solution was varied, 35-mL portions of effluent were collected at each pH level. All tests were run in triplicate.

Organic and Inorganic Fractions. Soil samples were vigorously agitated in distilled water. After the inorganic fraction had settled (~20 s) the darkly colored supernate (organic suspension including some silt and clay) was centrifuged to separate water and compressed organic material. The water layer was discarded. The compressed organic material was washed and centrifuged repeatedly until pH of the supernatant was 7 to 8.

Sodium Analyses were carried out by Applied Agricultural Research, Inc., with a Perkin-Elmer Model 290 atomic absorption spectrophotometer.

Soil samples were passed through a 60-mesh screen and 1-g portions were extracted with 12 g of 1 N ammonium acetate (neutralized to pH 7.0) and filtered through No. 50 Whatman paper.

Sand, silt and colloidal material listed in Table I were determined by Applied Agricultural Research, Inc. (Lakeland, FL), using a hydrometer method of particle size analysis.

RESULTS AND DISCUSSION

Filtration and utilization of citrus waste water by the soil and associated flora and fauna involve both physical and biological activities on components of waste water. Each factor directly affects the other; physical factors help determine biological activity and biological activity affects the permeability of the landspreading system. Both the physical and biological activities associated with various soils can be affected by the ions in caustics (Lutz, 1934). The capacity of Lakeland fine sand to buffer high pH extremes was studied in the laboratory with wet-packed soil columns by adding 1925 mL of pH 11 sodium hydroxide solution to a soil column over a time period of 1 month. Eluant pH was notably stable at pH 8. Calculations indicate the volume of influent (pH 11) spread on the surface of this (2 cm) column represent about 50 days of continuous loading on a typical spray field in the Central Florida area.

Since a pH of 11 is rare in citrus waste water and the probability of such an accident occurring continuously over 50 consecutive days is remote, this experiment was discontinued. In another attempt to test the soil's capacity to buffer caustics, a stepwise increase in influent pH was used on Lakeland fine sand columns that had been altered by heat treatment. Effects of increasing pH were compared on a "sterile" soil sample, a "500 °C" sample and a "natural" control sample (Figure 1). Buffering capacities of the two altered soils were exceeded at pH 12, when their effectiveness as buffers declined rapidly. The pH of the effluent in both the 500 °C and the sterile columns in-

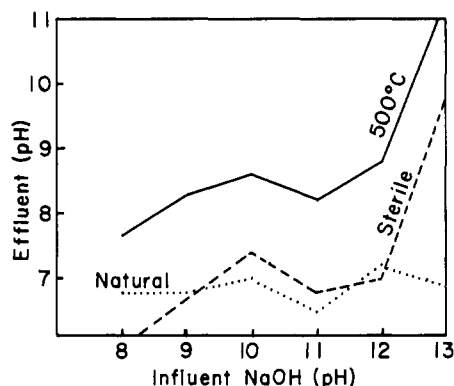


Figure 1. Cumulative effect of increased NaOH pH on the effluent pH of three soil samples.

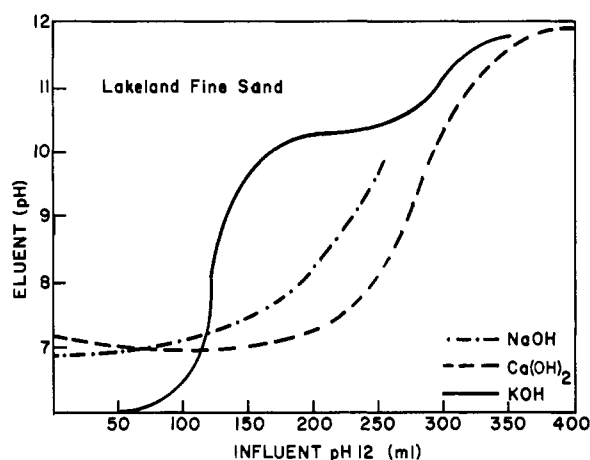


Figure 2. Cumulative effect of pH 12 influent on the effluent pH of Lakeland fine sand.

creased rapidly as influent pH increased from 12 to 13, whereas the natural control soil column continued buffering. Sterile soil maintained a slightly higher buffering capacity than the 500 °C soil. Percolation rates in the natural soil columns were drastically reduced as the influent approached pH 13, and three columns became completely clogged.

Since a pH 12 solution rapidly changed the effluent pH and percolation rate, effects of three commonly used caustics (at pH 12) on the buffering action of Lakeland fine sand were compared (Figure 2). Since 100–200 mL of pH 12 sodium and potassium hydroxide solutions severely lowered the percolation rates of these soil columns, caustics at higher pH were not tested. Of the three caustics tested, calcium hydroxide caused the least drastic reduction in percolation rate. Figure 2 illustrates the order in which the columns became plugged, i.e., sodium hydroxide caused plugging at 250 mL, potassium hydroxide at 350 mL, while the calcium hydroxide column exceeded 425 mL without plugging. Graphs in Figure 2 end when no further effluent was apparent. As sodium hydroxide was applied to the columns (Figure 2) percolation rates dropped linearly from 8.7 to 2.2 mL/min for the first 75 mL and remained constant for 200 mL of total effluent. In comparison, 500 mL of distilled water was passed through a Lakeland fine sand column without altering the average percolation rate more than 0.2 mL/min. The average distilled water column would pass 100 mL in 15 min while the same volume of a sodium hydroxide solution (pH 12) would average 76 min on the same column.

Tannin-like humic substances began to darken the effluent as the sodium hydroxide solvent replaced distilled

Table II. Comparison of Three Decomposition Residues

	mature plant residues, %	dried aerobic citrus sludge, %	sun-dried citrus sludge, %
protein	1–15	42.0	20.9
tannins	1–8	0.4	0.7
hemicellulose	10–28	15.6	12.9
cellulose	20–50	13.7	6.3
lignins	10–30	13.9	43.7
ash	^a	0.5	11.7
grease	^b	10.4	2.7

^a Table 9 composition of various plant materials shows ash analysis whose range is 0.76–10.30% (Waksman and Stevens, 1928). ^b Mature plant residue tannins include grease.

water in the columns. As percolation decreased, tannin-like materials colored the effluent coffee black; very little light could pass through a 1-cm column of this effluent.

The decrease in percolation rates related to sodium hydroxide emphasized a problem that has been the subject of considerable interest for many years—sodium plugging of the soil. Soils are made up of primary particles, coarse matter, rocky gravel, sand, and silt. Generally, these particles are quartz or primary minerals and can be considered a skeleton of little reactive importance. Fine materials ($\leq 2 \mu\text{m}$), defined as clay, are highly reactive because of their large surface area. Organic matter in the soil is active in three ways: as the center of microbiological activity, in direct chemical reactivity, and physically as an adhesive.

The negative charges associated with clays and organic matter are partially neutralized by cations present in the solution surrounding these charged materials. These cations are electrostatically attracted by the negatively charged surfaces, while at the same time they have a tendency to diffuse away from these surfaces toward regions of lower concentration. This situation is analogous to the earth's atmosphere in which gas molecules are subject to both gravitational forces and diffusion. The concentration of cations near the negatively charged surface is high and decreases with distance from the surface. Simultaneously, anions are deficient near the negatively charged surfaces because they are electrostatically repelled. The cation and anion "atmosphere" around charged surfaces is referred to as the "diffuse" or "Gouy Layer" (VanOlphen, 1965).

The depth of the diffuse layer is important to soil–water permeability. As the cations in the soil solution decrease or as sodium ion becomes the dominate cation, the diffuse layer increases in depth, and dispersion or deflocculation of the soil matrix occurs (Grim, 1968). This phenomenon reduces the permeability of the soil either by decreasing pore size or by plugging of the soil pores by clay transport or by both methods. As tabulated in "Soil Physics" (Baver, 1956), the swelling of some colloidal clays increases eightfold when potassium is replaced with sodium and four- or fivefold when calcium is replaced with sodium. Thus, a large decrease in permeability of clay soils due to sodium would be expected, and was demonstrated by Lutz (1934).

Generally, sodium plugging is associated with soils containing clay. Soil samples from three commercial citrus waste spray fields in Florida listed in Table I contain only small amounts of clay (colloidal material). Yet, in columns of soil taken from these citrus spray fields in Central Florida, percolation rates decreased sharply as caustic

Table III. Sodium Analyses of Soil Fractions

	column numbers							
	control			experimental				
	1	2	3	1	2	3	4	5
organic fraction [Na (ppm)]	6.4	31.2	26.4	40.8	43.2	26.4	127	134
inorganic fraction [Na (ppm)]	Tr ^a	Tr	Tr	Tr	Tr	Tr	7.2	4.5

^a Tr, total amount less than 2.4 ppm.

solutions were applied. These findings suggest that an occasional industrial spill of high pH waste may decrease the percolation rates of soils used for landspreading wastes even if clay was not present.

These findings led to an exploration of the organic fraction in a given soil as another possible factor in sodium plugging. The organic fraction of soil is derived from decomposing plant and animal matter. Table II shows components of mature plant residues representative of this process. Waste from cultural sources (paper, oil) becomes part of the decomposing organic fraction. Citrus processors refer to this waste from fruit parts, juice, plant debris, and their decomposition products as "heavy waste". The concentration of heavy waste is measured as milligram of biological oxygen demand (BOD) per liter. Ratcliff calculated an annual average (BOD) for this heavy waste of 590 mg of BOD/L (1977). This carbonaceous material eventually becomes the energy source for microorganisms in both soil and water. Various flora and fauna associated with the decomposition process use the more readily oxidizable material, leaving a residue of less oxidizable humic matter including tannins and lignins (Waksman, 1938).

Sludges formed as a byproduct of the waste treatment system in a citrus processing plant reflect the body composition of life forms in the waste waters, viz., protozoa, alga, fungus, plankton, as well as decomposition products of effluent added to the waste treatment system. Comparison of dried aerobic and sun-dried citrus sludge with mature plant residues (Table II) show qualitative similarity and quantitative differences among the major components. Amino acid analyses of the protein from these dried citrus sludges have been reported (Coleman and Shaw, 1977). Dougherty and McNary (1958) reported a crude protein content ranging from 29 to 44%. Their protein was generated, under varied conditions, by a laboratory-scale activated citrus sludge system. The range of crude protein found in dried citrus sludges generated under commercial waste treatment conditions (Table II) are in agreement with that found in these laboratory-prepared samples.

Organic fractions from citrus sludges and mature plant residues could function like clay particles since they are negatively charged and attract cations. According to "Soil, The 1957 Yearbook of Agriculture", "on a basis of unit weight, the retentive capacity of organic matter (for cations) is greater than that of the most reactive clays" (USDA, 1957).

The affinity of sodium for the organic fraction in Lakeland fine sand is demonstrated in Table III. Original samples of Lakeland fine sand contained $3.9 \pm 0.2\%$ (standard deviation) of volatiles, mostly organic materials and small amounts of silt and clay (see Table I). After separation of organic and inorganic fractions the quantity of volatile components in the organic fraction was $29.9 \pm 1.0\%$. After this extraction procedure, $1.1 \pm 0.04\%$ volatiles remained in the inorganic fraction. Table III compares parts per million of sodium in both organic

(including some silt and clay) and inorganic fractions for the control and experimental columns of Lakeland fine sand. The experimental columns were subjected to a sodium hydroxide solution (pH 12) until percolation had been drastically reduced or stopped. Even though this Lakeland fine sand had been exposed to sodium cations in the processor's waste water, there was a notable retention of sodium in the organic fraction of the experimental soil. Soil samples 1-3 were collected at the beginning of a citrus processing year before heavy waste had begun to be released. Soil samples 4 and 5 were taken at the end of the processing season and may reflect a buildup of sodium in the organic material of that spray field. Over the summer when citrus production is low, organic matter accumulating during the production year may oxidize and release accumulated sodium.

As described previously, when three caustics [NaOH, KOH, Ca(OH)₂] used by food and other industries were passed through columns of Lakeland fine sand, the permeability of this soil decreased markedly as effluent pH increased. Decreasing permeability would be expected in the presence of clays, but very little clay was found in this soil. Organic materials are known to adsorb larger quantities of cations than clay and may, thereby, contribute to the reduction of permeability observed in the column tests. Quantitative and qualitative comparisons of citrus wastes and decomposition products from other plant and animal matter revealed qualitative similarities but quantitative differences. This study emphasizes the importance to citrus processors of careful monitoring of effluents to spray fields to avoid even brief periods of application of high pH effluent to the soil. Even in soils with low clay content commonly found in Florida, the buildup of humus materials present in citrus waste can lead to problems in landspreading and drainage when high pH effluent is applied. In this study, the capacity of Lakeland fine sand to maintain an adequate percolation rate for extended periods of time was demonstrated, provided these precautions were taken.

LITERATURE CITED

- Baver, L. D., "Soil Physics", Wiley, New York, 1956, p 38.
- Blaedel, W. J., Meloche, V. W., "Elementary Quantitative Analysis Theory and Practice", Harper & Row, New York, 1963, p 378.
- Carter, L. J., "The Florida Experience", Johns Hopkins University Press, Baltimore, MD., 1974.
- Coleman, R. L., Shaw, P. E., *J. Agric. Food Chem.* **25**, 971 (1977).
- Dougherty, M. H., McNary, R. R., *Sewage Ind. Wastes*, 1151 (1958).
- Federal Register*, Vol. 39, No. 56, Chapter I, Part 407, Sec. 6, Thursday, March 21, 1974.
- Grim, R. E., "Clay Mineralogy", McGraw-Hill, New York, 1968, p 215.
- Haider, K., Martin, J. P., Filip, Z., "Soil Biochemistry", Vol. 4, Paul, E. A., McLaren, A. D., Ed., Marcel Dekker, New York, 1975, p 202.
- Lutz, J. F., *Mo. Agric. Exp. Sta. Res. Bull.* **No. 212** (1934).
- McLellan, W. M., Proceedings of the Landspreading Conference, East Central Florida Regional Planning Council, Orlando, FL, July 15, 1971, pp 2-7.

Ratcliff, M. W., "Citrus Science and Technology", Vol. 2, Nagy, S., Shaw, P. E., Veldhuis, M. K., Ed., Avi Publishing Co., Westport, CT, 1977, p 546.
 "Standard Methods", 12th ed, American Public Health Association, Inc., New York, 1965, pp 304, 383, 534.
 Ting, S. V., *J. Agric. Food Chem.* **4**, 262 (1956).
 U.S. Department of Agriculture, "Soil, The Yearbook of Agriculture", U.S. Government Printing Office, Washington, DC., 1957, p 155.
 VanOlephen, H., "An Introduction to Clay Colloid Chemistry", Wiley, New York, 1965, p 31.

Waksman, S. A., "Humus Origin, Chemical Composition, and Importance in Nature", Williams & Wilkins, Co., Baltimore, MD, 1938, pp 95, 421.

Waksman, S. A., Stevens, K. R., *Soil Sci.* **26**, 113 (1928).

Received for review March 14, 1978. Accepted November 15, 1978. Mention of a brand name or proprietary product is for identification only and does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of others which may also be suitable.

1-Phenylcarbamoyl-2-pyrazolines, a New Class of Insecticides. 3. Synthesis and Insecticidal Properties of 3,4-Diphenyl-1-phenylcarbamoyl-2-pyrazolines

Arnold C. Grosscurt, Roelof van Hes,* and Kobus Wellinga

The syntheses and biological activities of 3,4-diphenyl-1-phenylcarbamoyl-2-pyrazolines are discussed. The structures of these compounds have been confirmed by nuclear magnetic resonance. The insecticidal properties were evaluated with the larval stages of *Aedes aegypti* L., *Pieris brassicae* L., and *Leptinotarsa decemlineata* Say. The compounds out of this group of pyrazolines have a much higher insecticidal activity than those of the 3-phenyl-1-phenylcarbamoyl-2-pyrazolines and the 3,5-diphenyl-1-phenylcarbamoyl-2-pyrazolines, which were discussed in part 1 and part 2, respectively.

In this article we report the synthesis and biological evaluation of the 3,4-diphenyl-1-phenylcarbamoyl-2-pyrazolines. These 3,4-diphenyl derivatives give rise to products with much better insecticidal properties than those with phenyl substitution at only the 3 position mentioned in part 1 (Wellinga et al., 1977) or even those with 3,5-diphenyl substitution mentioned in part 2 (van Hes et al., 1978). Although the 3,4-diphenyl-1-phenylcarbamoyl-2-pyrazolines also are active on adult insects, just as the 3-phenyl-substituted compounds and the 3,5-diphenyl-substituted compounds, only the larvicidal activities on *Aedes aegypti* L., *Pieris brassicae* L., and *Leptinotarsa decemlineata* Say are discussed. The phenomena observed after intoxication are similar to those described for the 3-phenyl-substituted pyrazolines.

CHEMICAL METHODS

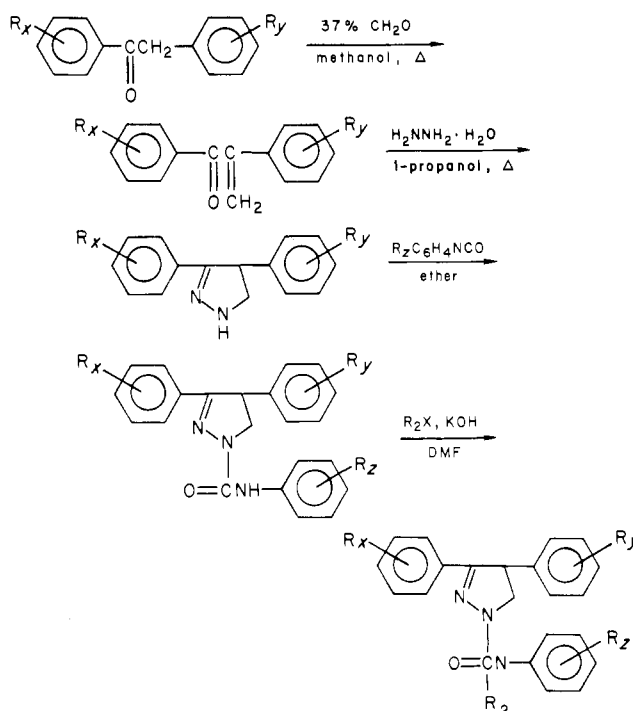
Microanalyses were carried out in the Analytical Department of the Institute for Organic Chemistry TNO, Utrecht, Netherlands, under the supervision of W. J. Buis. Nuclear magnetic resonance spectra were recorded on a Varian HA 100 spectrometer with tetramethylsilane as the internal reference. The melting points are uncorrected.

The compounds mentioned in Tables I and II were prepared by one general method, which is outlined in Scheme I.

4'-Chloro-2-phenylacrylophenone. To a solution of 41.5 g of 4'-chloro-2-phenylacetophenone (0.18 mol) (Curtin et al., 1954) in 450 mL of methanol, 2 mL of piperidine, 2 mL of acetic acid, and 54 mL of formalin (37%) were added. After refluxing for 3 h, 200 mL of methanol was distilled off. After cooling, the reaction mixture was poured into 500 mL of ice-water and the resulting solid was collected and dried. Yield 42.7 g (98%), mp 45–47 °C.

3-(4-Chlorophenyl)-4-phenyl-2-pyrazoline. A mixture of 121.3 g of 4'-chloro-2-phenylacrylophenone (0.5 mol)

Scheme I. Preparation of Substituted 3,4-Diphenyl-1-phenylcarbamoyl-2-pyrazolines



and 50 mL of hydrazine hydrate in 280 mL of 1-propanol was refluxed for 3 h. After cooling, 200 mL of ice-cold methanol was added. The precipitate was collected and washed with cold methanol and ether. Yield 96.5 g (75%), mp 164–167 °C (dec).

Anal. Calcd for $C_{15}H_{13}ClN_2$ (M_r , 256.74): C, 70.17; H, 5.11; Cl, 13.81; N, 10.91. Found: C, 70.2; H, 5.2; Cl, 13.7; N, 11.0.

3-(4-Chlorophenyl)-1-(4-chlorophenylcarbamoyl)-4-phenyl-2-pyrazoline [Table I, Compound 13 (I,13)]. To

Research Laboratories, Philips-Duphar B.V., Weesp, 1230, Netherlands.