Meeting Report

Pesticides: Metabolism, Degradation, and Mode of Action

Continued use and development of safe and efficient pesticides are critical to maintenance of an adequate supply of food and fiber and a high standard of health for man and domestic animals. Yet there is concern about pesticide misuse, environmental contamination, potentially hazardous residues, control failures because of resistant pest populations, and related problems. Twenty-seven scientists reported on their latest research on pesticide chemical metabolism, degradation, and mode of action, in Nikko, Japan, from 16 to 19 August 1967, at the second in a series of three seminars on pest control. These seminars are part of the United States–Japan Cooperative Science Program under the joint sponsorship of the National Science Foundation and the Japan Society for the Promotion of Science [*Science* **157**, 465 (1967)]. The program encompassed the general areas of pesticide photodecomposition, herbicide metabolism and mode of action, fungicide mode of action, insecticide metabolism and mode of action, and physicochemical approaches to structure-activity relationships.

Pesticide degradation on irradiation with ultraviolet light takes place by isomerization, reduction, replacement of aromatic halogen by hydroxyl, elimination, polymerization, and other more specific reactions (D. Crosby, University of California, Davis). Sodium pentachlorophenoxide, which is extensively used for control of barnyard grass in rice paddy fields in Japan, is hazardous to exposed fish populations until it is decomposed by photochemical processes involving formation of oxidized monomers, dimers, and trimers (K. Munakata, University of Nagoya).

Philip Kearney (U.S. Department of Agriculture, Beltsville) discussed the fate of herbicides in soils, and S. Matsunaka (National Institute of Agricultural Sciences, Saitama) considered their fate in higher plants. Microbiological and chemical reactions (other than photochemical reactions) render many herbicides innocuous in soils, thereby avoiding potential problems, such as absorption into plants and entry into the food chain, and accumulation in soil to levels that might injure subsequent crops. Chemical reactions contribute to chloro-*s*-triazine and aminotriazole decomposition in soils, while microorganisms mediate dehalogenation, *N*-dealkylation, amide or ester hydrolysis, beta oxidation, ether hydrolysis, ring hydroxylation, ring cleavage, and, under anaerobic conditions, reduction of nitro groups. Certain pesticide combinations result in synergistic phytotoxic effects or in extended persistence of one of the components in soils owing to alteration by one of the chemicals of the microbial processes involved in degradation of the other chemical. The biochemical reactions associated with these processes also occur in higher plants and contribute to herbicide selective toxicity. Two important problems in the use of selective herbicides in rice culture were emphasized. One involves the activation of diphenyl ether herbicides, such as 2,4-dichlorophenyl 4′-nitrophenyl ether, by a photobiochemical process. The other concerns the unique selective herbicidal activity of 3′,4′-dichloropropionanilide.

Auxin herbicides (for example, 2,4-D) induce excessive and aberrant production of nucleic acid in the stem–tap-root axis of susceptible seedlings (J. Hanson, University of Illinois, Urbana). The tissues proliferate, forming a dominant metabolic sink, which in turn induces senescence in leaves and roots. Photosynthesis and ion accumulation fail, and the plant succumbs because it becomes nonautotrophic. The action of 2,4-D remains unclear. Although 2,4-D enhances the activity of RNA polymerase associated with chromatin in vivo, it has no effect in vitro. Its effect is possibly indirect, acting through enhanced ethylene production. It was suggested as an alternative possibility that monochloroacetic acid resulting from 2,4-D degradation may be the actual herbicide (D. Crosby). Excised leaves, isolated chloroplasts, and *Chlorella* are useful in studies of the possible mode of action of herbicides that affect the primary photosynthetic process or the subsequent electron transport. According to G. Zweig (Syracuse University Research Corporation), the substituted ureas, anilides, uracil, and benznitriles seem to inhibit the primary reaction of photosynthesis (the photolytic decomposition of water), thus stopping the one-carbon reduction cycle. The phytotoxicity of dipyridyl and quinone herbicides may result from the formation of toxic free radicals.

Lethal synthesis (one approach to developing selective fungicides) involves blocking the active toxophore with a chemical grouping, such as an ester, which is removed by the fungus but not the host (J. Horsfall, Connecticut Agricultural Experiment Station, New Haven). Useful fungicides also include compounds that alter the host in order to increase its resistance to fungal invasion. Antisporulants inhibit spore formation without killing the fungus; phenoxyacetic acids and other glycolic acid analogs may act in this manner through inhibition of glycolate oxidase. Blasticidin S, kasugamycin, cellocidin, and polyoxin are important agricultural antibiotics discovered and developed in Japan (T. Misato, Institute of Physical and Chemical Research, Tokyo). The probable site of action of blasticidin S is the final step of protein synthesis which occurs in the ribosomal fraction. Kasugamycin interferes with bacterial protein synthesis without affecting the synthesis of nucleic acids. Cellocidin, which is acetylenedicarboxamide, inhibits the α-ketoglutarate→succinate system. The 2:1 copper chelate of 8-quinolinol is more fungitoxic than either copper or the free quinolinol because of its lipid solubility properties and its ability to form, in the cell, a 1:1 complex, the actual fungitoxic agent released by dissociation (G. McNew, Boyce Thompson Institute for Plant Research, Yonkers, New York).

The fungus that causes rice blast can be effectively controlled by organomercuric compounds and blasticidin S, the chemicals currently in use, and by certain new candidate fungicides. T. Ishiyama (Hokko Chemical Industry Co., Atsugi, Kanagawa) reviewed phytopathological studies being made mainly in his laboratory on structure-activity relations for organomercuric compounds and on the mechanism of fungicidal action. The action of two fungicidal organophosphorus compounds useful in rice blast control, *O,O*-diethyl and *O,O*-diisopropyl *S*-benzyl phosphorothiolates, was reported by M. Kado (Ihara Chemicals Co., Shimizu, Shizuoka). Pentachlorobenzyl alcohol is highly active for rice blast control; it specifically prevents penetration of hyphae into leaves but fails to control other plant diseases. M. Ishida (Sankyo Co., Tokyo) reported that, when pentachlorobenzyl alcohol-C^{14} is administered orally to rats in metabolism studies, the unchanged compound is excreted in the feces, and pentachlorobenzoic acid and the β-glucuronide of pentachlorobenzyl alcohol appear in urine.

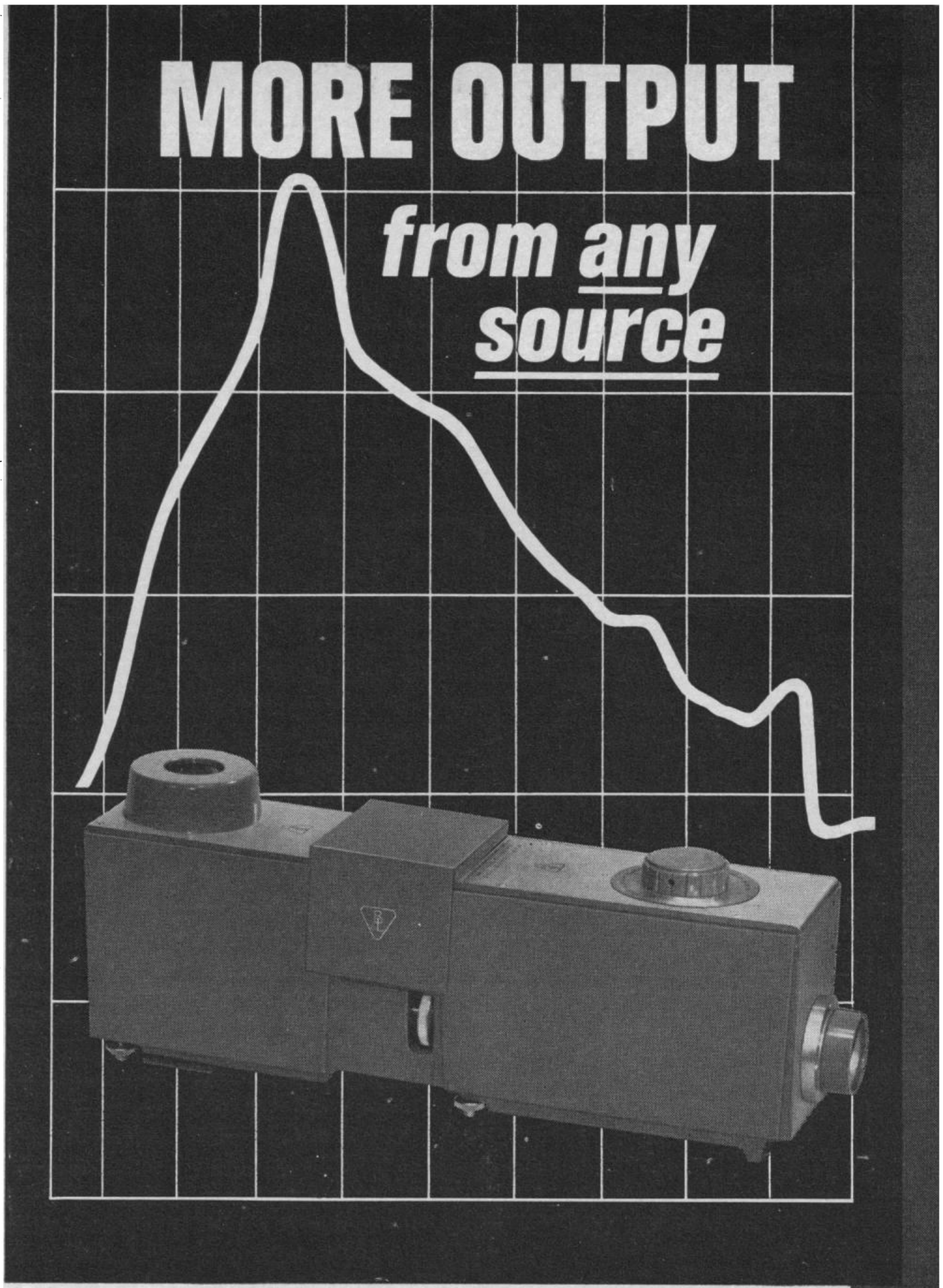
Strychnine nitrate is potentially useful for control of brown bears, which frequently are a pest in Hokkaido; it is metabolized in the bear so that toxic products do not persist in the meat (T. Inukai, Hokkaido University, Sapporo).

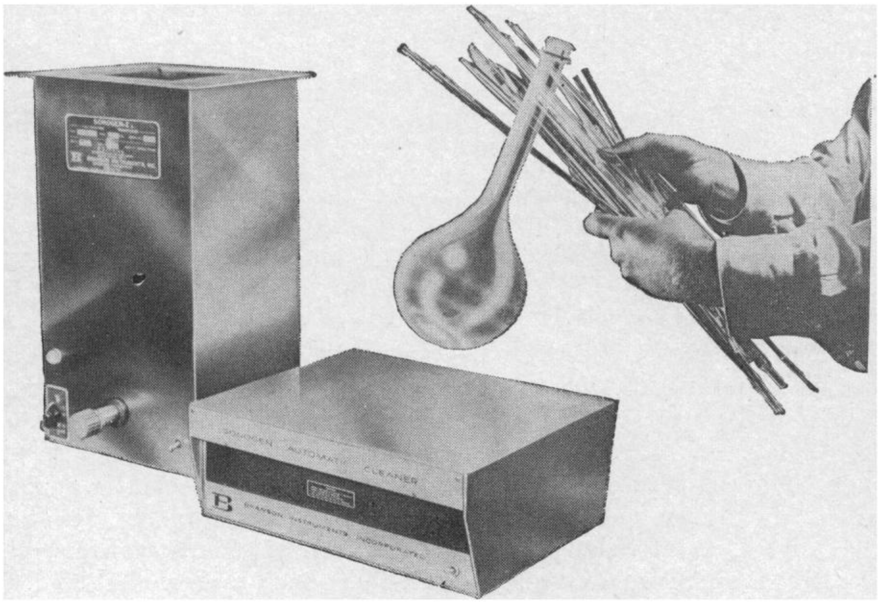
Insecticide metabolism and mode of action were the major topics of discussion. The general steps involved in radiotracer studies on insecticide action include (i) selection of the site of radiolabeling, (ii) performing the radiosynthesis, (iii) introduction of the labeled compound into an appropriate biological system or degradation situation, (iv) determination of the chemical and physical fate of the compound, and (v) interpretation of the results in relation to the mechanism, selectivity, and efficiency of action of the insecticide chemical (J. Casida, University of California, Berkeley). The toxicity of nicotinoids is conferred by the highly basic nitrogen, a carbon-atom bridge, and the pyridine ring (I. Yamamoto, Tokyo University of Agriculture, Setagaya, Tokyo). Studies on structure-activity relations and metabolism of pyrethroids and rotenoids were also reviewed. Detoxification primarily involves initial oxidation of one methyl group in the isobutenyl moiety of pyrethroids and of the isopropenyl group of rotenone.

Specificity in cholinesterase inhibition and in detoxification are factors contributing to the selective toxicity of *O,O*-dimethyl trichlorohydroxyethyl- and dichlorohydroxyethyl phosphonates and related compounds (T. Saito, Nagoya University). The type of biological activity of saligenin cyclic phosphorus esters is remarkably affected by the exocyclic substituent group on the phosphorus (M. Eto, Kyushu University). The inhibitor specificity for target esterases is also greatly influenced by steric factors resulting from varying the size of the exocyclic substituent group, although the reactive site with the esterase is probably, in all cases, the enolic ester portion of the hetero ring.

Toxicity of certain organophosphorothionates to mammals is antagonized by compounds that induce microsomal enzymes; these enzymes catalyze detoxification reactions, particularly phosphorothionate cleavages (S. Murphy, Harvard University School of Public Health, Boston). Increased activity of liver A-esterase and of liver and plasma aliesterase, in response to certain drugs, may result in additional sites for reaction with the esterase inhibitors, thus sparing the more vital target enzyme, acetylcholinesterase. Organophosphorus esters potentiate malathion and dimethoate if, at low doses, they inhibit carboxyesterases and carboxyamidases in species in which these pathways of detoxification are critical. According to K. Fukunaga (National Institute of Agricultural Sciences and Institute of Physical and Chemical Research, Tokyo), soluble enzymes from mammalian liver and insect fat body and midgut, which require reduced glutathione for activity, may contribute to the selective toxicity of *O,O*-dimethylphosphorothionates by virtue of their *O*-demethylation activity. Pathways for diazinon metabolism by oxidation, hydrolysis, and conjugation reactions were defined by in vitro studies with enzyme preparations from rats and American cockroaches. Studies with insect microsomal enzymes, which oxidize many insecticides, are limited by natural inhibitors of the enzymes released during homogenization. The mechanism of low mammalian toxicity of sumithion, as compared with methyl parathion, was discussed by J. Miyamoto (Sumitomo Chemical Co., Osaka). Differences in metabolism by pathways involving phosphorothionate oxidation, hydrolysis, and *O*-demethylation probably do not play a major role in the selective toxicity of the two compounds. The low toxicity of sumithion probably results, in part, from the relatively poor penetration of sumioxon into the brain compared to that of methyl paraoxon and, as a result, there is a lower degree of brain cholinesterase phosphorylation in the case of sumioxon.

Acetylcholinesterase from housefly heads differs from that purified from vertebrate sources in hydrolyzing butyrylcholine as well as acetylcholine





(C. Kearns, University of Illinois, Urbana). Chromatography of diethylaminoethyl-cellulose partially resolves the fly preparation into two fractions, which differ in extent of activation by butanol and in the ratio of activity on acetyl- and butyrylcholine. Nerve components from insects and mammals were also considered with respect to the binding of various insecticides. Data were presented on the marked differences in binding of dieldrin, DDT, BHC, phthalthrin, and nicotine to nerve components which were prepared and separated centrifugally from rat brain, and from axonic and ganglionic portions of cockroach nerve cords (F. Matsumura, University of Wisconsin, Madison). Electrophysiological approaches (involving arthropod nerve preparations) to the mode of action of insecticides were discussed by T. Narahashi (Duke University Medical School, Durham, North Carolina). The voltage-clamp method proved highly successful in analyzing the actions of DDT and allethrin. DDT delays the turning-off process of peak sodium current and suppresses steady-state potassium current. Allethrin suppresses both peak sodium and steady-state potassium currents from either side of the nerve membrane and it also delays the turning-off process of peak sodium current when applied internally.

Insecticide resistance mechanisms in houseflies in relation to biochemical genetics were reviewed by M. Tsukamoto (Nagasaki University). The linkage-group distribution of major insecticide resistance factors was defined and the importance of factors on each of the 2nd, 3rd, 4th, and 5th chromosomes was emphasized. Enzymes involved in insecticide metabolism, including DDT-dehydrochlorinase, organophosphate-detoxifying esterases, and mixed functional oxidases of microsomes depend on factors present on the 5th chromosome. A factor on the 2nd chromosome confers low nerve sensitivity to DDT.

A relation was reported between electronic and hydrophobic characters of substituents and the activity of substituted phenols causing chlorosis in *Lemna minor* (T. Fujita and M. Nakajima, Kyoto University). The results suggest action or factors limiting the action, such as a one-step partitioning involving adsorption of the molecule to the cell surface. With regard to organophosphorus insecticides, there is good correlation between anticholinesterase activity and reactivity of the phosphorus ester, as determined by such parameters as hydrolysis and solvolysis, Hammett's sigma constants, and shifts in infrared absorption (T. Fukuto, University of California, Riverside). Improved correlations result from taking steric factors into account. Additional effects are: (i) the stereospecificity in cholinesterase inactivation associated with an asymmetric center, and (ii) the possible contribution to selective toxicity of the significant structural difference between insect and mammalian enzymes. Absorption phenomena on the surface of insects and considerations of insecticide chemical transport into the body were discussed by M. Suwanai (Tokyo University of Agriculture and Technology, Fuchu, Tokyo). A method for calculating the rate of insecticide action based on kinetic and diffusion equations was illustrated.

The papers presented at the Nikko seminar are scheduled for publication as a separate volume of the international book series, *Residue Reviews*, edited by F. Gunther and published by Springer-Verlag New York, Inc.

Both Japan and the United States were among the first to recognize certain serious problems resulting from the adverse effects on animals and plants arising from the rapidly increasing use of pesticides. Cooperation between the two countries can accelerate a solution to problems arising from contamination by pesticides.

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Calendar of Events

May

National Meetings

3–4. **Physical Medicine and Rehabilitation**, Northwest Assoc., Palo Alto, Calif. (J. C. Montero, Div. of Rehabilitation Medicine, Stanford Univ. School of Medicine, Palo Alto 94394)

3–4. Society for **Pediatric Research**, Atlantic City, N.J. (Secretary, The Society, % Dept. of Pediatrics, J. H. Miller Health Center, Univ. of Florida, Gainesville, Fla.)

3–5. **Parts, Materials, and Packaging** Technical Conf., Washington, D.C. (W. Hepner, Electronic Industries Assoc., 2001 Eye St., NW, Washington, D.C. 20006)

4. American Society for **Clinical Nutrition**, Atlantic City, N.J. (A. B. Eisenstein, Secretary-Treasurer, The Society, 818 S. Meramec Ave., St. Louis, Mo. 63105)

4–5. **Economics and Evaluation** Symp.,