adult and is referred to as a juvenile. The cap may adhere to the juvenile for several days, marking the site of detachment and the boundary between that portion of the juvenile contributed by the parent and the newly formed portion (Fig. 1c). The body wall posterior to the boundary is notably thicker and the apex is marked externally by the cuticular thickenings of the posterior shield. Anterior to the boundary, the body wall of the newly formed anterior trunk and introvert is exceedingly thin. Immediately after its initial eversion the activity of the introvert commences; it is continually retracted to a position just above the anus and extended to reveal the terminal tentacular anlage, consisting of two long dorsal lobes and two shorter ventral lobes. As in the adult, the introvert is covered by brownish horny spines and encircled anteriorly by several rows of hooks. Within 12 days after detachment the early traces of the anterior shield appear in a dorsal position above the anus and the introvert is displaced slightly in a ventral direction.

Although asexual reproduction in sipunculans has been discovered only recently, their regenerative capabilities have been recognized for some time. Regeneration of the posterior end has been described (*5*). Experimentally induced regeneration of the introvert with the formation of anterior gut, retractor muscles, and brain, has been reported (*6*). According to these reports, regenerative cells migrate from the nerve cord to form ectodermal elements and the coelomocytes may give rise to mesodermal elements.

A comparison of the morphological aspects of asexual reproduction in *Sipunculus robustus* as related in the brief description by Rajulu and Krishnan (*1*) and in *Aspidosiphon brocki* shows that differences occur in the sequence of events relative to the separation of individuals. Whereas in *S. robustus* the introvert is formed only after separation by an elongation and subsequent inversion of the anterior portion of the new individual, in *A. brocki* the introvert is formed prior to separation by an epidermal ingrowth and the organs of the newly formed anterior end are clearly recognizable in the constricted individual before detachment.

Prior to Rajulu and Krishnan's report (*1*), sipunculans were known to reproduce only sexually (*7*). Whether sexual phases alternate with asexual phases in *Sipunculus robustus* and *Aspidosiphon brocki*, as described for some planarians and annelids (*8*), is unknown. However, the presence of gonadal tissue at the base of the retractor muscles in both the adult and the juvenile of *A. brocki* suggests that this species is capable of reproducing sexually, even though no mature gametes have been detected free in the coelom.

MARY E. RICE

Department of Invertebrate Zoology,
Smithsonian Institution,
Washington, D.C. 20560

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Enhancement of Photoalteration of Cyclodiene Insecticide Chemical Residues by Rotenone

Abstract. *When applied at low concentrations to plant foliage, rotenone catalyzes the photoisomerization of dieldrin and other cyclodiene insecticide chemical residues. This finding of a new type of interaction between pesticide chemicals suggests the possibility of controlling the persistence of residues on plants by use of certain pesticide-photosensitizer combinations and application sequences.*

Pesticide chemicals should ideally persist, on the surface to which they are applied, only for the period required for pest control. Their residues should then rapidly dissipate in order to minimize or, hopefully, obviate contamination of the agricultural commodities and the adjacent environment. It might be possible to achieve pest control for the desired period without leaving significant residues at the end of this time by the simultaneous or subsequent use of a second chemical that will regulate, as desired, the rate of dissipation of the pesticide chemical applied. For example, a photosensitizing chemical might be applied to a surface treated with a persistent pesticide to accelerate the dissipation of residue associated with the pesticide. Our finding that the presence of rotenone residues on plant foliage catalyzes the photoisomerization of dieldrin and similar insecticide chemicals indicates that the suggested idea for regulation of pesticide chemical residues is indeed possible (*1*). The structures of dieldrin and its "half birdcage" photoisomer, photodieldrin, are shown in Fig. 1.

In screening possible photosensitizers, [^{14}C]dieldrin and the candidate chemical, each at a concentration of 10 parts per million based on the weight of the fresh leaf, were separately dissolved in 50 μl of methanol and applied to the surface of growing bean leaves; the treated plants were subsequently placed in sunlight for 1 hour. The amount of [^{14}C]dieldrin, [^{14}C]photodieldrin, and other compounds containing ^{14}C that persisted on the foliage after exposure to sunlight was determined by rinsing the treated leaves with ether and analyzing the rinses by thin-layer chromatography (TLC), radioautography, and liquid scintillation counting. We used a similar procedure with certain other pesticide chemicals labeled with radioactive components, including [^{14}C]aldrin and [^{14}C]endrin, sometimes varying the time intervals, concentrations, or sequences of applying the pesticides and photosensitizers. In addition, each chlorinated hydrocarbon insecticide chemical tested was analyzed by gas-liquid chromatography (GLC) with electron capture detection, and by TLC of unlabeled material with detection by suitable chromogenic procedures (*1, 2*).

On the basis of a survey of 16 known chemical photosensitizers and related compounds (*3*), and 29 pesticide chemicals and analogs having structural similarities to known photosensitizers, rotenone, of the compounds tested, is the most effective photosensitizer for enhancing the photochemical alteration of dieldrin, primarily by increasing its rate of conversion to photodieldrin (Fig. 1) (*1*). Conversion of dieldrin residues to photodieldrin is sensitized measurably when rotenone is applied to bean plants at concentrations as low as 0.3 ppm. The potency of rotenone as a sensitizer for the photoisomerization of dieldrin is approximately a hundredfold greater than that of benzophenone, which was used by Rosen and Carey in the synthesis of photodieldrin from dieldrin (*4*). Although a few pesticide chemicals other than rotenone show some photosensitizer activity against dieldrin, they are effective only at very high concentrations.

Results from the testing of 28 different rotenoids and related compounds (*5*) indicate that the *C* and *D* rings in these molecules are most critical and that the structural requirements for photosensitizer activity are met by compounds containing the moiety shown in heavy lines in Fig. 2, with aliphatic or aromatic substituents at positions 6a or 12a, or both, being necessary for activity. Hydroxylation at position 11 results in loss of potency, possibly as a result of chelation of the hydroxyl group with the ketone (*6*), and compounds with a double bond between positions 6a and 12a have greatly diminished activity.

These studies were not designed to determine the mechanism of action of the photosensitizer but only to correlate the ground-state structure of the rotenoid or other test compound with the activity in enhancing the photoalteration of dieldrin. Although the triplet energy values for active compounds, where known, were above 60 kcal/mole [xanthone, thioxanthone, benzophenone, and 4,4′-bis(dimethylamino)benzophenone], this is apparently not the only requirement for activity, because acetophenone and fluorene have high triplet energies but were inactive. The method of analyzing leaf surfaces after 1 hour of exposure to sunlight was selected to simulate conditions encountered in the use of insecticides on crops but it is not appropriate, because of possible photosensitizer volatilization and other factors, for critical studies of the mechanism.

The toxicity of photodieldrin to several insects and mammals is equal to or greater than that of dieldrin (*7, 8*), and it is more persistent on plant foliage than dieldrin, apparently because of lower volatility (*1*). Thus the enhancement of the photoalteration of dieldrin by rotenone increases rather than reduces the level and persistence of the resulting toxic residues. The enhanced conversion to photodieldrin, as was determined from an analysis of the residues by TLC, also occurs when certain commercial dieldrin and rotenone formulations are sprayed on beam foliage.

On the basis of experiments made with other chlorinated hydrocarbons, rotenone also catalyzes the photochemical alteration of aldrin, isodrin, endrin, heptachlor, and heptachlor epoxide but it is less effective or ineffective with DDT, DDD, lindane, and endosulfan (*1*). Aldrin, isodrin, and heptachlor are converted in the presence of rotenone to products which behave in the same way on the basis of analysis by GLC and TLC as their corresponding authentic photoisomers (*8, 9*). Rotenone causes the rate of isomeric cyclization to increase more than the rate of epoxidation for these three compounds. Rotenone catalyzes endrin photoconversion to the keto and aldehyde isomers (*7, 10*). The photoisomers of aldrin and heptachlor appear to be at least as toxic as their respective parent compounds, but photoisodrin and the two endrin photoisomers are less toxic than isodrin and endrin, respectively (*7–9*). The presence of rotenone favors the photoalteration of heptachlor epoxide to a major product which possibly is formed in a reaction analogous with that in endrin photoisomerization.

On the basis of results obtained with cyclodiene compounds, rotenone appears to be most effective in catalyzing photochemical isomerization; however, its photosensitizing activity is not limited to such reactions. On exposure of 14 ^{14}C-labeled pesticides, each at concentrations of 10 ppm, to sunlight for 1 hour in the presence of rotenone concentrations as high as 100 ppm, increased amounts of photodecomposition products were observed with some methylcarbamate and phosphorothionate insecticide chemicals and with the synergist, piperonyl butoxide. Although rotenone at such high concentrations reduces the volatility of some of the test compounds so that there is a greater opportunity for the accumulation of photodecomposition products, the major rotenone effect is probably the result of its action as a photosensitizer. The molecular structure of the various photoproducts separated by TLC is not known, but it is highly unlikely that photoisomerization occurs with most of the materials tested; acceleration of the photodecomposition process is more likely the result of oxidation. It is expected that, with rotenone or any other photosensitizer, the end effect of photosensitization of a given pesticide will depend on the compound and reactions involved. By comparison with the original pesticide chemical, the photodecomposition products may be more or less toxic, persistent, biodegradable, or involved in resistance phenomena.

Although none of the photosensitizers tested is as potent as rotenone (under the short-exposure periods employed), it is possible that less active compounds, if stable and persistent, might be considerably more effective

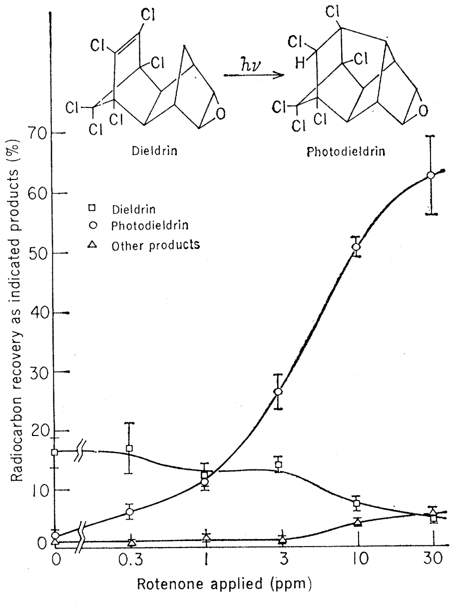

Fig. 1. Residual radioactive products on bean leaves that were exposed to sunlight for 1 hour after application of 10 ppm of [^{14}C]dieldrin and various concentrations of rotenone.

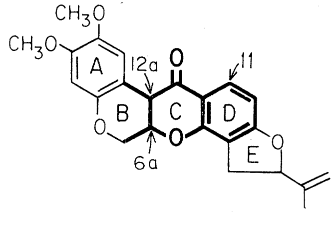

Fig. 2. Rotenone. The chromanone moiety (shown in heavy lines) is apparently necessary for a high degree of photosensitizer activity. Positions 6a *or* 12a require aliphatic or aromatic substituents, and activity is destroyed or greatly diminished on hydroxylation at position 11 or on the introduction of unsaturation between positions 6a and 12a.

as photosensitizers if longer exposure conditions were employed. This is so because rotenone itself is highly unstable to sunlight, its half-life on foliage under the conditions of study being no longer than 30 minutes *(1)*. It is known that the majority of rotenone photodecomposition products are less active than rotenone or are inactive as photosensitizers *(1)*; thus the effective sensitizing period resulting from rotenone applications is expected to be a short one.

The fact that rotenone is a natural plant product suggests that other naturally occurring sensitizers might be used for pesticide chemical photodecomposition. Except for earlier reports that pyrethrins are more susceptible to photodecomposition when plant pigments are present in crude pyrethrum extracts *(11)*, there are apparently no indications that such sensitizers exist. However, their existence is confirmed by the results of our preliminary studies which show that aqueous suspensions of spinach chloroplasts are potent sensitizers for the decomposition of certain ^{14}C-labeled carbamate and pyrethroid insecticides in sunlight but not in darkness; this is probably the result of the chlorophyll content of these chloroplasts. Whether or not chloroplasts sensitize the photodecomposition of other pesticide compounds is, as yet, unknown. Although an interesting speculation, it may be difficult to assess the possible *in situ* significance of chloroplast pigments in the alteration of pesticide residues in plants.

The present studies are not definitely applicable to the field use of pesticides, although they show that very low concentrations of a photosensitizing compound can greatly alter the persistence pattern of a pesticide chemical. Further, they show that rotenone, which is currently being used for insect control on food crops and which degrades quickly by photodecomposition to nontoxic products, is quite effective as a photosensitizer for certain chlorinated cyclodienes. It is likely that other interesting pesticide-photosensitizer mixtures exist in addition to the combinations reported here. This means that one pesticide chemical can greatly affect the residual persistence of a second pesticide chemical on a crop, not only by altering its rate of biotransformation *(12)* but also by sensitizing its photoalteration. It is important to determine such interactions because pest control frequently involves the application of more than one type of pesticide chemical to the plants. Perhaps the most intriguing possibility is that of "managing" residues or pesticide persistence by applying an appropriate photosensitizer to cause, at will, an increased rate of residue alteration, including dissipation, when this is desirable from the standpoint of efficient pest control procedure, good agricultural practice, or minimum environmental contamination.

GLEN WAYNE IVIE
JOHN E. CASIDA
Division of Entomology, University of California, Berkeley 94720

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Oncogenic Purine Derivatives: Evidence for a Possible Proximate Oncogen

Abstract. *Two additional urinary metabolites of the chemical oncogen 3-hydroxyxanthine are now identified as 8-chloroxanthine and 8-methylmercaptoxanthine. Such products are thought to be derived from a reactive intermediate which can be tentatively considered to be a proximate oncogen. Since each of these 8-substituted xanthines has also been obtained in vitro by reactions of 3-acetoxyxanthine with chloride ion or methionine, their production in vivo can be explained as resulting through the metabolic formation of an activated ester with a reactivity similar to that of the chemical model.*

Guanine 3-*N*-oxide and 3-hydroxyxanthine are chemical oncogens *(1)* with potencies comparable to those of the most oncogenic arylamines and many oncogenic hydrocarbons *(2)*. We have now identified two additional urinary metabolites of 3-[8-^{14}C]hydroxyxanthine as 8-chloroxanthine and 8-methylmercaptoxanthine. Each has been separated and characterized by ion-exchange and subsequent paper chromatography (discussed below). Samples of each so purified were reduced to xanthine with no loss of specific activity. The identity of 8-chloroxanthine was confirmed by the isolation of 8-[^{36}Cl]chloroxanthine when Na^{36}Cl was administered with unlabeled 3-hydroxyxanthine. The results in Table