tympanal organ since these spikes closely follow the parameters of the stimulating pulses: that is, the spike amplitude of the sensory response is directly proportional to the intensity of the stimulus, indicating the recruitment of additional neurons; and the sensory discharge follows the pulse-repetition rate (PRR) of the stimulating sound very closely over a wide range. In addition, a long stimulus (500 msec) elicits a slightly longer response caused by an afterdischarge. If the reference electrode is placed in the abdomen, large spikes (Fig. 3, *MR*) of 4 to 8 mv amplitude (off scale in Fig. 3), each lasting 1 to 3 msec, are recorded immediately after the sensory response. These are not recorded when the reference electrode is on the wing. The onset of these spikes has a latency of about 13 msec and follows the PRR only up to three per second, above which they disappear after the initial pulse of the stimulus. We interpret these large potential changes to result from the depolarization of the nearby wing-flexing muscle after the arrival of an efferent stimulus in the muscle from the central nervous system. This conclusion follows both from the longer latency of this spike and from visual observations of the movement of the third axillary sclerite; this movement, like the large spikes, disappears when the PRR exceeds three per second.

The electrophysiological study indicates that the tympanal organ is sensitive to acoustic pulses of from 15 to 17 khz to at least 100 khz at pulse-repetition rates up to 150 per second. Below a PRR of three per second, the visual assay also indicates the ability of the insect to perceive sounds in this frequency spectrum.

A taxonomic survey by one of us (*10*) has disclosed that apparently similar tympanal organs are widely distributed among many species of the Chrysopidae, being absent from only a group of five genera which, on the basis of other structural and chromosomal characteristics, seems to be quite generalized. Adams (*11*) has recently grouped these less specialized forms together as the distinctive subfamily Nothochrysinae and has pointed out that all known fossil Chrysopidae of the Tertiary period are also referable to this subfamily. It is possible, then, that the evolution of the tympanal organ may have been a relatively recent event.

A possible function of this organ seems to be the reception of sounds generated by other chrysopids. Sound production has never been demonstrated for these insects, but, on the basis of the structure of the base of the abdomen and metathoracic femora, Adams (*12*) has suggested that males of *Meleoma schwarzi* probably stridulate, and he has speculated that such sound might play a role in courtship. Unspecified alary chordotonal organs (the tympanal organ was apparently not observed) or pedal chordotonal organs were mentioned as the possible receptors. Although stridulatory sound may have such a function in this case, as well as in other species of Chrysopidae, it seems improbable to us that the basically lower-frequency sound likely to be produced by stridulation is received by the tympanal organ of the wing. Possibly the pedal chordotonal organs, or perhaps the trichobothrial setae of the last abdominal segment (*13*), will be found to be the receptor for these sounds.

Hunting bats use ultrasonic pulses at low repetition rates to scan the environment, raising the PRR to as high as 200 per second when in pursuit of a specific target (*14*). In its sensitivity to a broad spectrum of ultrasonic frequencies and PRR's, the tympanal organ of *C. carnea* is well suited for the reception of these signals, and it seems likely that the principal adaptive role of the organ is to minimize predation by echolocating bats. Furthermore, it appears probable that a majority of the large group of species possessing a tympanal organ will be found to enjoy a similar advantage over bats, and that the apparent sudden rise to dominance of this night-flying group since the mid-Tertiary period is very likely the direct result of this advantage.

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4. Our vital staining technique was adapted from J. L. Larimer and E. A. Ashby, *Stain Tech.* **39**, 369 (1964). Zinc-free methylene blue (28 mg) was dissolved in 1 ml of an insect Ringer solution consisting of 0.75 percent NaCl in distilled water. This solution was reduced in intense light with crystalline ascorbic acid to a colorless solution, and a small amount was injected into the insect's abdomen through a glass pipette fitted over the needle of a hypodermic syringe.
5. The tissue was fixed with 3 percent glutaraldehyde in a phosphate buffer (*p*H 7.3 to 7.4), embedded in glycol methacrylate, sectioned at 1 to 3 μ on a glass knife, stained with 0.05 percent toluidine blue O in 0.02*M* benzoate buffer (*p*H 4.4), and photographed, in this case, with phase optics.
6. Culture procedures are given by E. G. MacLeod, *Nature*, in press.
7. Ultrasonic stimuli were generated in the following manner: the output of an audio oscillator was modulated into pulses by a Grason-Stadler electronic switch, with a variable rise-and-fall time, externally triggered by the stimulus pulse from a Grass stimulator. The switch output was led via an attenuator to a transducer driver [J. J. G. McCue, *Inst. Radio Eng. Int. Conv. Rec.* **6**, 310 (1961)], then to a Dukane Ionovac Duk-5 ionic loudspeaker, the sound pressure level of which was calibrated in decibels referred to 0.0002 μbar from 10 khz to 150 khz at 1 m with a 0.64-cm microphone (Brüel and Kjaer). The switch, transducer driver, and loudspeaker were modified by us. All experiments were carried out at a sound level of 78 to 88 db.
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Chlorinated Hydrocarbon Pesticides: Degradation by Microbes

Abstract. *In culture, most of the actinomycetes and filamentous fungi tested degraded PCNB; several actinomycetes dechlorinated DDT to DDD, but no microorganism degraded dieldrin.* Streptomyces aureofaciens *degraded PCNB to pentachloroaniline.*

Chlorinated hydrocarbon pesticides are very resistant to physical or microbial degradation, and remain unaltered in soil for many years after they are applied (*1*). The ecological hazards which these resistant chemicals present are well known (*2*). Reports of microbial degradation of chlorinated hydrocarbon pesticides are few. DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane] is converted to DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane] by a yeast and a few bacteria (*3*). There are no reported instances of degradation of dieldrin or pentachloronitrobenzene (PCNB) by any microbe. Our investigations were undertaken to dis-

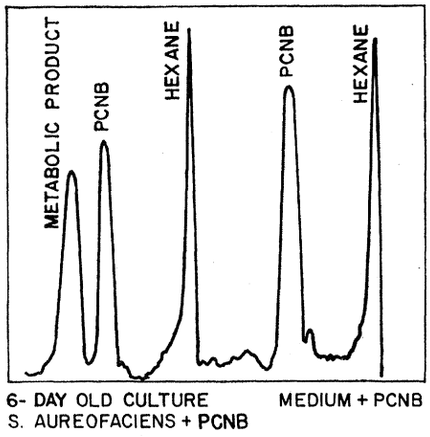

Fig. 1. Gas chromatogram of PCNB after incubation for 6 days in a medium with and without *Streptomyces aureofaciens*. PCNB was partially degraded to pentachloroaniline by *S. aureofaciens*.

cover microbes, particularly soil microbes, that degrade DDT, dieldrin, and PCNB, and also to identify the degradation products.

The ability of microorganisms to degrade these pesticides was tested in the following manner. The test organism was cultured for 6 days in a nutrient medium (glucose, 5 g; peptone, 5 g; yeast extract, 1 g; and distilled water, 1000 ml) containing 5 to 10 μg of the desired pesticide per milliliter. The culture was shaken during the growth period. Afterward it was extracted with a mixture of *n*-hexane and isopropyl alcohol (3 : 1), then the alcohol was removed from the extract by washing with water. The amount of pesticide in the extract was determined by gas chromatography and compared with the concentration originally supplied in the medium.

An Aerograph model-600D gas chromatograph (Wilkens Instrument & Research, Inc.) with an electron-capture detector was used to detect the pesticides and their degradation products. For routine determinations, we used a column (1.8 m by 0.32 cm) containing 5 percent DC 11 on Gas-chrom Q. For identification of DDD, columns containing 5 percent SE 30 and 2.5 percent QF 1 on Gas-chrom Q were also used. Injection and detection temperatures were 260° and 180°C, respectively, and the flow rate of nitrogen was maintained at 60 ml/min. The column temperature for DDT or dieldrin determinations was 190°C, and for PCNB, 155°C.

For large-scale isolation of the degradation product of PCNB, *Streptomyces aureofaciens* was grown for 6 days in the nutrient medium with 10 to 20 μg of PCNB per milliliter. Twenty liters of the culture filtrate was extracted twice with 30 liters of *n*-hexane. The extract was then concentrated to 10 ml and applied to an acid-washed silicic acid column and eluted with *n*-hexane. Fractions (100 ml) were collected, and each fraction was assayed by gas chromatography. First PCNB was eluted, and then the degradation product, which was completely separated from PCNB, was eluted. The fractions containing the degradation product were pooled, concentrated to 10 ml, and applied to an alumina column. The column was washed with *n*-hexane to remove impurities and then with anhydrous ether to elute the degradation product. Fractions (20 ml) were collected. On drying of the ether fractions containing the metabolite, white crystalline needles were formed. The compound was recrystallized from absolute ethanol.

Table 1. Degradation of DDT, dieldrin, and PCNB by actinomycetes and fungi in culture solutions

Microorganisms	Extent of degradation*		
	DDT	Dieldrin	PCNB
Fungi			
Aspergillus niger	–	–	+
Fusarium solani f. *phaseoli*	–	–	+
Glomerella cingulata	–	–	+
Helminthosporium victoriae	–	–	+
Mucor ramannianus	–	–	+
Myrothecium verrucaria	–	–	+
Penicillium frequentans	–	–	+
Trichoderma viride	–	–	+
Actinomycetes			
Nocardia sp.	++	–	+
Streptomyces albus	+	–	–
S. antibioticus	+	–	++
S. aureofaciens	++	–	+++
S. cinnamoneus	++	–	+
S. griseus	–	–	+
S. lavendulae	–	–	++
S. venezuelae	–	–	+
S. viridochromogenes	++	–	+

* –, No detectable degradation; +, less than 10 percent; ++, 10 to 25 percent; +++, 25 to 50 percent.

Nine actinomycetes and eight fungi were tested for their ability to degrade DDT, dieldrin, and PCNB (Table 1). All test organisms except *Streptomyces albus* degraded PCNB to an unknown metabolic product detectable by gas chromatography (Fig. 1). From the gas-chromatography parameters referred to above, the retention time for PCNB was 2.2 minutes, and that for its degradation product was 3 minutes. None of the organisms degraded detectable quantities of dieldrin. The DDT was dechlorinated to DDD by six actinomycetes, but not by any of the fungi. The most effective dechlorinating actinomycetes were *Nocardia erythropolis*, *S. aureofaciens*, *S. viridochromogenes*, and *S. cinnamoneus*, each of which converted 20 to 25 percent of the PCNB supplied. Most of the other organisms degraded PCNB to only a

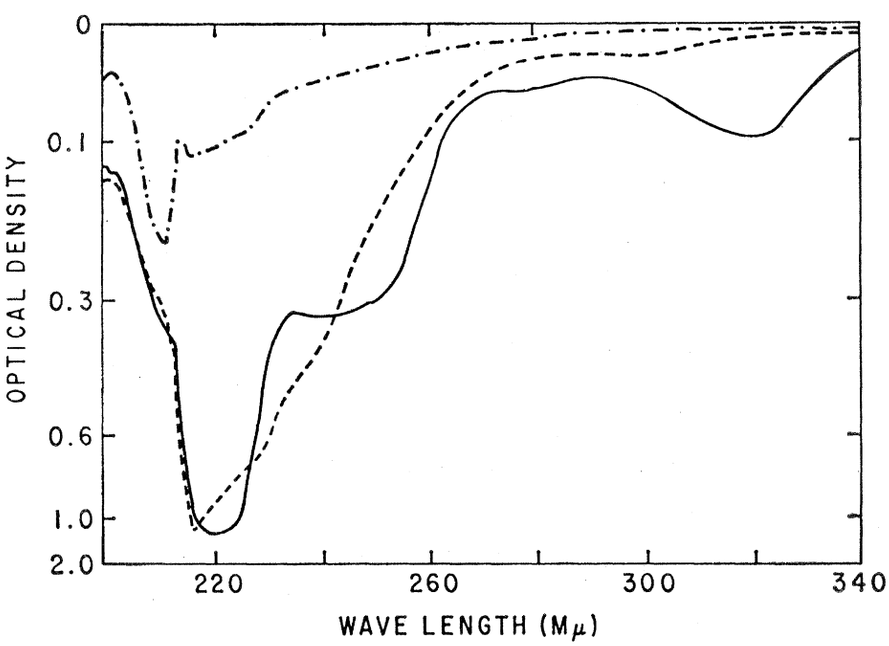

Fig. 2. Ultraviolet absorption spectra of PCNB and its degradation product (pentachloroaniline). Ether solutions contained 10 μg of either compound per milliliter. Ether, —·—·—; PCNB, — — —; degradation product, ———.

slight extent. Except for some inhibition of *S. albus* by PCNB, all test organisms were unaffected by the three pesticides at the concentrations used.

The rate of degradation of DDT and PCNB by *S. aureofaciens* in culture was determined. At 2-day intervals, duplicate cultures were harvested, and the remaining pesticides as well as the products formed were extracted and determined by gas chromatography. The concentrations of PCNB or DDT decreased concomitantly with increases in the concentrations of their respective metabolic products. Maximum degradation, that is, 25 percent of DDT and 36 percent of PCNB, was attained in both cases within about 6 days.

The physical and chemical properties of the degradation product of PCNB closely resemble those of pentachloroaniline. Its ultraviolet-absorption spectrum, which differs from that of PCNB, is identical with that of pentachloroaniline, with peaks at 320, 240, and 220 mμ (Fig. 2). The pentachloroaniline and the metabolite had the same retention time, 3 minutes, with a gas chromatograph with a DC 11 column. The infrared-absorption spectrum of the degradation product corresponds exactly with that of PCA. The mass weight of the compound determined by mass spectrophotometry is 263 ± 1, and that of pentachloroaniline, 263. The melting point of each compound determined separately was 230° to 232°C; the mixed melting point was 230.5° to 232.5°C. Therefore, we conclude that the microbial-degradation product of PCNB is indeed pentachloroaniline.

This is the first report of soil microbes degrading DDT or of any microorganism degrading PCNB. Degradation of the pesticides in culture occurred only during the active growth phase of the actinomycetes or fungi, and stopped completely when growth ceased. Thus, the chlorinated hydrocarbon compounds were not utilized by these microorganisms as a sole source of carbon. Since microbes in soil tend to be largely inactive because of a deficiency of available carbon (*4*), these compounds persist in soil in spite of the presence of microorganisms that can partially degrade them.

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Molybdenum Diselenide: Rhombohedral High Pressure–High Temperature Polymorph

Abstract. *A three-layered rhombohedral form of molybdenum diselenide has been produced by subjecting the normal two-layered hexagonal form to pressures of 40 kilobars and temperatures of 1500°C. The new form is isostructural with rhombohedral molybdenum disulfide.*

A three-layer rhombohedral (designated 3R) form of molybdenum diselenide ($MoSe_2$) isostructural with rhombohedral molybdenum disulfide (MoS_2) (*1*) has been produced by subjecting the common two-layer hexagonal (designated 2H) form of $MoSe_2$ to high pressures and temperatures. The temperature-pressure conditions necessary for the transformation from the 2H to the 3R form are illustrated in Fig. 1, in which the phase boundary has been approximately located. The extent of conversion under various conditions was judged from x-ray powder photographs, where the new phase distinguished itself by lines indexable on the basis of a three-layer rhombohedral cell. In Fig. 1 solid shading indicates the production of a relatively pure 3R form, partial shading indicates a 2H and 3R mixture, and the open symbols represent a relatively pure 2H form. The reaction is sluggish and is not complete in a 1-hour heat. In the patterns from 6-hour heats we observed lines from either the 3R form or the 2H form, rather than mixtures. Two samples of the 3R form slowly reverted to the hexagonal form when held at temperatures about 200°C below the indicated phase boundary.

These experiments were performed with a girdle type of high-pressure system. The sample was contained in a boron nitride capsule ⅛ inch (about ⅓ cm) in diameter and ⅜ inch (about 1 cm) long. This was surrounded by a concentric graphite heater and pyrophylite pressure-transmitting medium. The overall dimensions of the high-pressure cell were ½ inch (about 1¼ cm) in diameter by ⅝ inch (about 1½ cm) long.

The pressure calibration of the apparatus was obtained at room temperature by using the resistance discontinuities in bismuth and thallium wires, encased in silver chloride, as fiducial points (*2*). The sample temperatures were calculated from the power consumption in the graphite heater. The temperature-power relationship was established in calibration tests in which platinum–platinum rhodium thermocouples were located in the center of the sample cell. The accuracy of the calibrations was checked by observing the melting point of germanium as a function of pressure. The results agreed with published data within about 5 percent (*3*). Corrections were not made for the effect of pressure on the thermocouple electromotive force or for the error in the pressure scale used by Hall (*3*), since these errors were less than the typical scatter expected in the experimental conditions (±50°C and ±1 kb).

The physical properties of the new rhombohedral form of $MoSe_2$ have not been measured. Outwardly it is nearly indistinguishable from the normal hexagonal form, having a pronounced graphitic appearance. It is expected that

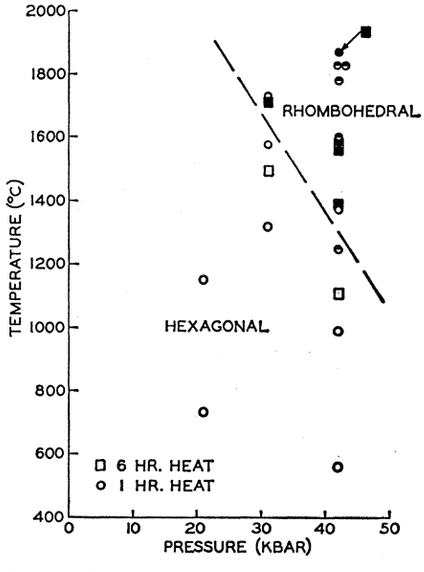

Fig. 1. Tentative phase boundary between the rhombohedral and hexagonal forms of MoSe (see text).