

cover slips, and the cover slips are carefully separated. The tissue adhering to both cover slips is stained with Giemsa, and the two preparations are permanently mounted for study.

The testes of five butterflies [one male each of *Speyeria cybele* (Fabricius), *S. aphrodite* (Fabricius), *Pieris rapae* (Linnaeus), *Papilio glaucus* Linnaeus, and *Papilio polyxenes asterius* Stoll] were prepared in this manner. Only one of these specimens, *S. aphrodite*, yielded dividing figures showing detailed chromosomal morphology (1).

As shown in Fig. 1 (2), karyotypes of typical elongate, beaded chromosomes, comparable with those shown by other groups of animals, can be obtained from preparations of butterfly testicular material. Certain chromosomes appear to be traceable from one cell to another.

The chromosome number of the Rocky Mountain subspecies of *S. aphrodite* (*ethne* Hemming) has been recorded as $n = 29$ (3), based on sec-

tional material. The present results indicate this number may be too high (probably $n = 27$ is correct). It is possible, though unlikely on morphologic grounds (4), that *S. aphrodite* and *S. a. ethne* may not be conspecific.

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References and Notes

1. Similar chromosomes have been obtained for eight species of Mexican butterflies during January 1966.
2. The photographs were made with a Praktica 35-mm camera mounted on a Wild M-20 microscope.
3. K. Maeki and C. L. Remington, *J. Lepidopterists' Soc.* **14**, 180 (1961).
4. C. F. dosPassos and L. P. Grey, *Am. Museum Novitates*, No. 1370 (1947).
5. We thank the following for their assistance and suggestions on the preparation of the manuscript: Drs. Richard M. Fox (Section of Insects and Spiders, Carnegie Museum, Pittsburgh, Pa.), Ross H. Arnett, Jr., Robert A. Davidson, Sergey Polivanov, and George M. Happ (Department of Biology, Catholic University of America), and Mrs. Christine M. Happ.

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Dieldrin: Extraction of Accumulations by Root Uptake

Abstract. *Certain forage crops can absorb and translocate the chlorinated hydrocarbon insecticide dieldrin from soil or sand. An extraction technique routinely used for analyses of residues does not quantitatively remove this internal chemical, but a method employing chloroform-methanol extraction leads to essentially quantitative recovery.*

Many halogenated pesticides are no longer recommended for direct application to forage crops because of their long persistence. It has recently been recognized that certain plants absorb these pesticides from the soil. Lichtenstein (1) has established that certain root crops absorb aldrin and heptachlor when grown in soils treated with these chemicals; others have demonstrated the presence of small quantities of dieldrin in alfalfa grown in soil treated with aldrin or dieldrin (2).

Most of the techniques currently used for extracting pesticides were developed to measure surface residues. The literature does not report the efficiency of extraction of residues solely within the plant. We now present data on the extraction of dieldrin absorbed through the plant-root system.

Our first experiment used Cl^{36} -labeled dieldrin (3) mixed with sand at 15 parts per million. Seeds of orchard grass, corn, and wheat and rooted alfalfa cuttings were planted in this mixture and the plants were grown for

2 weeks with the use of Hoagland's nutrient solution. Extraction and analyses by gas chromatography (4) and thin-layer chromatography confirmed the presence of radioactive dieldrin in the plant tissues.

A Research Specialties Co. model-600 gas chromatograph, equipped with an electron-capture detector, was used; flow rate of the nitrogen carrier gas was about 70 ml/min, with an inlet pressure of 1.56 atm. Two columns were used to aid identification of the compounds: one of 1.8 m, 3 percent QF-1 on Gas Chrom CLA; the other of 1.2 m, 5 percent DC-200 on Gas Chrom CLA. Temperatures of vaporizer, column, and detector were 250°, 225°, and 285°C, respectively.

It was found that extraction of radioactive plants by blending in a mixture of *n*-hexane-isopropyl alcohol (2:1) could not remove all label from tissues; this technique is used routinely in many laboratories for fresh plant materials. This failure stimulated the search for a better extraction method.

After some trial and error, it was found that almost 100 percent of the radioactivity could be extracted from labeled plants by: (i) maceration and repeated blending of the plant material with *n*-hexane-isopropyl alcohol (2:1); and (ii) 12-hour reextraction of this tissue with a 1:1 mixture of chloroform and methanol in a Soxhlet extractor.

Several hundred plants grown under controlled environmental conditions in sand and soil containing unlabeled dieldrin have been extracted by this method and all extracts have been analyzed by gas chromatography. The identity of the compound, in both extracts, that was thus measured as dieldrin was confirmed by thin-layer chromatography and by mass spectrometry. In every instance the chloroform-methanol extract contained additional dieldrin.

Application of this double-extraction technique to field samples of fresh alfalfa, grown on soil containing dieldrin but not sprayed, revealed the presence of considerably more pesticide than had been detected by the usual extraction techniques. The upper portion of Table 1 shows the levels of dieldrin detected by the single- and double-extraction techniques. The hexane-isopropyl alcohol mixture removes on average only about 64 percent of the total extractable insecticide. Wheat, corn, and orchard grass grown under controlled environmental conditions have yielded similar results. Considerable variation in the efficiency of extraction was observed, depending on plant species and on concentrations of pesticides in the plants.

In the Pesticide Research Laboratory

Table 1. Dieldrin contents of three extracts from fresh alfalfa (four samples) and dry alfalfa hay (five samples); determined by use of a QF-1 column and confirmed with a DC-200 column. In parentheses are the percentages of total extractable dieldrin extracted in hexane and in 2:1 hexane-isopropyl alcohol (HI) extracts. Recoveries of dieldrin by extraction from material fortified with known quantities averaged 95 percent. CM, chloroform-methanol (1:1).

Dieldrin (ppm) in extracts		
HI	Hexane	CM
<i>Fresh alfalfa</i>		
0.025 (69)		0.011
.010 (62)		.006
.049 (67)		.024
.007 (59)		.005
<i>Dry hay</i>		
	0.020 (74)	.007
	.029 (66)	.015
	.084 (74)	.030
	.045 (70)	.019
	.078 (68)	.037

and others, samples of dry alfalfa hay are routinely milled and extracted in a Soxhlet extractor by use of a nonpolar solvent such as hexane. Dieldrin levels extracted from similar field samples by this technique and levels extracted by additional chloroform-methanol extraction in a Soxhlet apparatus are compared in the lower portion of Table 1. Approximately 70 percent of the total insecticide found was in the hexane extract.

All test data indicate that Soxhlet extraction with chloroform-methanol removes all the internal insecticide found in finely ground samples of dry hay. This single-solvent system is not effective, however, when used on fresh crop materials.

The evidence suggests that routine extraction techniques with a single-solvent system, using either hexane or hexane-isopropyl alcohol mixture, cannot quantitatively extract the compounds in question when they are present within the plant. In view of the extraction results, one may hypothesize that the chlorinated-hydrocarbon insecticides are deposited in the surfactant lipids of these plants (phospholipids, sulfolipids, and glycolipids). These lipids are quite polar in nature and are not quantitatively extracted by the *n*-hexane-isopropyl alcohol mixture. On the other hand chloroform-methanol is a far better solvent for these lipids, which fact supports the theory that the pesticides and lipids may be physically or chemically associated.

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References and Notes

1. E. P. Lichtenstein, G. R. Myrdal, K. R. Schulz, *J. Econ. Entomol.* **57**, 133 (1964); *J. Agr. Food Chem.* **13**, 126 (1965); E. P. Lichtenstein and K. R. Schulz, *ibid.*, p. 57.
2. E. P. Lichtenstein, K. R. Schulz, R. F. Skretny, P. A. Stitt, *J. Econ. Entomol.* **58**, 742 (1965); W. B. Wheeler, R. O. Mumma, D. E. H. Frear, in *Abstr. Meeting Amer. Chem. Soc. 150th* (1965), p. 12A.
3. Supplied by courtesy of Shell Development Corp.
4. The study was part of Northeastern Regional Research Project NE-53, "Transformation of insecticides by plants"; assisted by regional research funds. Paper No. 3075 in the journal series of the Pennsylvania Agricultural Experiment Station; some of the material was presented at the Amer. Chem. Soc. Meeting, Atlantic City, N.J., September 1965. We thank R. C. Cotner for technical assistance.

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Hydrodynamic Performance of Porpoises (*Stenella attenuata*)

Abstract. *Two specimens of Stenella attenuata, trained to chase a winch-towed lure, reached a top speed of 11.03 meters per second (21.4 knots) in 2.0 seconds. The maximum power output, occurring 1.5 seconds after the start, was calculated from measured values of acceleration and drag coefficient. The maximum power output per unit body weight was 50 percent greater than for human athletes. The measured drag coefficient, obtained from periods of coasting, was approximately the same as that of an equivalent rigid body with a near-turbulent boundary layer.*

Research on the top speed of porpoises has been stimulated by reports of unusually high speeds relative to predicted speeds. The well-known "Gray's paradox" stems from analysis (1) of the performance of a 91-kg porpoise that was clocked at a speed of 10.3 m/sec (20 knots) for 7 seconds; drag was calculated to be severalfold lower (otherwise the power output was severalfold higher) than that expected of a torpedo-like body with a power output equivalent to that of a human being.

The top speeds of wild porpoises reported in the literature (2) generally range from 9.2 to 10.3 m/sec for durations of less than 10 seconds. Top speeds of 8.76 to 9.28 m/sec for 8 to 25 minutes have been reported by ship-board observers, and fast-moving herds have been stated to travel at 5.16 to 7.22 m/sec for somewhat longer periods. Differences in performance between species are noted, inshore species being generally slower than the pelagic species.

Some observations of high speed result from assisted locomotion (3), where the animal derives thrust from the ship's waves or from the bow pressure field. Many of the short-term high-speed bursts can be explained by the great power output of muscles that go into an oxygen debt. For example, human athletes can produce 457 kg m sec⁻¹ (6.0 horsepower) in a single movement of arms and legs, 145 kg m sec⁻¹ for 6 seconds, 69 kg m sec⁻¹ for 1 minute, and 15.2 kg m sec⁻¹ for 1 day (4). The power ratio may be 30:1, depending upon the duration of exertion. Gray's paradox can be largely resolved by consideration of duration; his analysis was based on the power output of humans for a 15-

minute period and was therefore biased by a factor of about 3.5.

Recently reported (5) are considerable differences in power output between porpoises of different species. The highly active pelagic species *Phocoenoides dalli* was credited with about 1.7 times the total blood-oxygen content of the less active pelagic species *Lagenorhynchus obliquidens* and nearly 3.0 times that of coastal *Tursiops truncatus*. The top speeds of these three species lie in the same order and generally within the range of speeds reported by Gray (1). An exception may be *Phocoenoides dalli* which is reported to be able to accelerate rapidly ahead of a 32-km/hour capture boat for 50 to 100 m after riding the bow wave for 5 minutes or more. Some of the more unusual top speeds reported in (1) and analyzed in (6) might be explained by the unusually large blood-oxygen content of the pelagic species. Speed tests with the aid of calibrated instrumentation under controlled conditions are needed for accurate measurement of speed and power.

Performance of a trained young adult female Pacific striped porpoise (*Lagenorhynchus obliquidens*) was tested in 1961 in a 96-m tank, 2 m deep (7). Top speed of the 91-kg animal, 7.76 m/sec, developed in about 2 seconds; maximum-acceleration power output after about 1 second was 160 kg m sec⁻¹ at 4.59 m/sec, and the best estimate of the measured drag-area coefficient (drag/dynamic pressure) was 0.0056 m². No unusual performance was found.

In 1964 a trained, 89-kg 3-year-old, male, Pacific bottlenose porpoise (*Tursiops gilli*) was tested for speed along a racecourse in a 300-m lagoon, 3 m deep, at Coconut Island, Oahu, Hawaii (8), and in the open sea near Rabbit Island and in Kaneohe Bay, Oahu (9), where the animal was trained to pursue a speedboat. Maximum speeds were 8.30 m/sec for 7.5 seconds, 7.01 m/sec for 10 seconds, and 6.09 m/sec for 50 seconds; the results generally compared closely with predictions. Only the 7.5-second top speed was somewhat unusual, indicating either about 40-percent greater power per unit body weight than the power of athletes or equivalent reduction in drag.

In March 1965 a new kind of speed-run training was initiated with two subadult male specimens of *Stenella attenuata*, a pelagic species believed capable of unusually high speed. The smaller animal weighed 40.5 kg and was 1.69