on the basis of the small number of animals used. The data of Martin and Beiler (*2*) cannot be subjected to analysis as they appear in their report.

The relationship of the phosphorylated hesperidins used in fertility trials to structure, antihyaluronidase potency, capillary permeability, phosphorus content, species, and mode of administration remains obscure, since significant correlations between these factors and fertility inhibition have yet to be reported.

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Manuscript received March 23, 1953.

The Toxicity of Chlordane Vapors

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The vapor toxicity to warm-blooded animals of the insecticide chlordane (1,2,4,5,6,7,8,8-octachloro-4,7-methano-3a,4,7,7a-tetrahydroindane) has been a subject of controversy for the past several years.

Experimental evidence presented in this paper offers an explanation for the variable results shown by previous authors and shows a significant lack of toxicity to mice resulting from chlordane vapors.

Lehman (*1*) was unable to maintain pigeons placed in a room which had been treated with chlordane, although it was first thoroughly scrubbed and aired. Frings and O'Tousa (*2*) reported that mice could not survive in air which had first passed through chlordane. Injury was also noted in mice that had been confined to a chamber whose sides had been treated with chlordane. On the other hand, Nickerson and Radeleff detected no injury to pigeons (*3*) or leghorn cockerels and pullets (*4*) that had been confined 30–60 days in a box whose inner surfaces had been treated with chlordane. Since the only controlled investigation indicating significant toxicity of chlordane vapors to warm-blooded animals is that of Frings and O'Tousa, the author undertook, with their cooperation, an investigation patterned exactly after theirs. The only uncontrollable variable was the source and time of manufacture of the chlordane.[1] Twenty female Swiss albino mice in a wire mesh cage were placed in a treatment chamber 12 × 20 × 36 in. and subjected to 14 days of continuous exposure to a current of air (18 ml/sec) which had first passed through 105 ml of chlordane in a saturation train. No deaths occurred nor did any mice show signs of anorexia, blindness, or loss of coordination. At autopsy, organs and tissues were normal.

1 All chlordane used in the present investigation was supplied as technical chlordane (1068 chlordane) by the Velsicol Corporation, Chicago, Illinois, and as AAEE Reference Standard chlordane by the Wisconsin Research Foundation, Madison, Wis.

The experiment was repeated 4 times, once using AAEE Reference Standard chlordane for 14 days and 3 times using three different current production batches of chlordane for 25 days each. No symptoms of toxicity were noted, and no deaths occurred. No gross pathological changes were observed, but microscopically the liver showed minimal changes such a some reticulation and oxyphilia of the cytoplasm, and the lungs showed slight congestion with some proliferation of bronchiole lining cells. Kidneys were normal. These results were at such variance with those reported by Frings and O'Tousa that further investigation was certainly indicated.

Early samples of chlordane frequently gave off irritating volatile materials but in production, this characteristic has long since been eliminated by the more complete removal of unreacted ingredients, chief among which was hexachlorocyclopentadiene. Possibly the chlordane (Octaklor, 1947 production) used by Frings and O'Tousa contained a considerable quantity of unreacted volatile material which may have been primarily responsible for the reported symptoms and high rate of mortality among the mice. In order to test this hypothesis, hexachlorocyclopentadiene[2] as utilized in chlordane production was added in varying quantities to 1068 chlordane being sold commercially. Experiments were then conducted in which female mice were subjected to air passing through these mixtures (Table 1).

TABLE 1

MORTALITY AMONG MICE SUBJECTED TO VAPORS OF CHLORDANE (CH) PLUS ADDED HEXACHLOROCYCLOPENTADIENE (HX)

		No. Mice	Mortality ratio	Comments
I	Ch, 90% Hx, 10%	20	20/20	All dead within 24 hr
II	Ch, 92.5% Hx, 7.5%	20	20/20	All dead within 48 hr
III	Ch, 95% Hx, 5%	20	20/20	Symptoms present at 4 days Deaths between 10th and 25th days
IV	Ch, 97.5% Hx, 2.5%	20	6/20	Symptoms present at 4 days Deaths between 20th and 25th days
V	Ch, 100% Control	20	0/20	No symptoms of toxicity.

In every case except that of the chlordane control, external symptoms followed the same pattern described by Frings and O'Tousa, namely, cessation of feeding and drinking, huddling together, lethargy, apparent blindness, and loss of coordination. The mixtures were also irritating to the eyes of workers in the laboratory. Onset and severity of symptoms were directly proportional to the volume of added hexachlorocyclopentadiene.

2 Supplied by the Velsicol Corporation.

Gross examination of the organs of mice in Expts. I–IV revealed extensive hemorrhagic areas in the lungs and lesions in the livers. Microscopically, the lungs showed in addition to hemorrhagic areas, congestion of capillaries, edema, and some consolidation. The liver showed extensive areas of coagulative necrosis, hyalinization, bile duct proliferation, congestion, cytoplasmic oxyphilia, and disruption of the normal architecture. Kidney damage included evidence of protein leakage, degeneration of tubular epithelium, and capillary engorgement in glomerular tufts. The extent of injury was proportional to the volume of added hexachlorocyclopentadiene.

The results of the above experiments point clearly to the explanation for variance in results between currently produced chlordane and that used by Frings and O'Tousa. When, and only when, hexachlorocyclopentadiene is added to chlordane, the results are in entire agreement with those obtained by Frings and O'Tousa.

Another type of experiment similar to that performed by Frings and O'Tousa, wherein mice were confined to a poorly ventilated box, the inner surface of which had been treated with 5 g of chlordane and renewed every 3 weeks failed to produce any signs of intoxication during a 4-month test. Gross findings at autopsy were negative and histological changes, confined to the liver, were minimal.

It can be concluded from the above investigation that the reported vapor toxicity to mice should not have been attributed to chlordane, but rather to an unreacted intermediate. The intermediate was undoubtedly present in chlordane as manufactured at one time, but has since been reduced to a point where it is no longer present in quantity sufficient to produce significant vapor toxicity to mice.

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Manuscript received March 16, 1953.

An Improved Holder for Seedlings in the Avena Test[1]

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The glass holders and wooden racks used in the standard Avena test method for plant growth regulators (*1*) can be replaced by convenient and easily constructed Lucite racks (Fig. 1). These racks hold the seedlings more firmly, allow straighter growth, hold twice the number of plants in the same space, save considerable time in the selection of uniform rows for testing, and are easily cleaned.

One-quarter inch Lucite is cut into $2 \times 7\frac{3}{8}$-in. strips, for use in standard $10 \times 16\frac{1}{2} \times 2\frac{1}{2}$-in. enamelware pans. Five or six racks will fit in a pan this size. Twenty-four holes are drilled through each strip,

[1] Published with the approval of the Director as Technical Paper No. 212 of the Pineapple Research Institute of Hawaii.

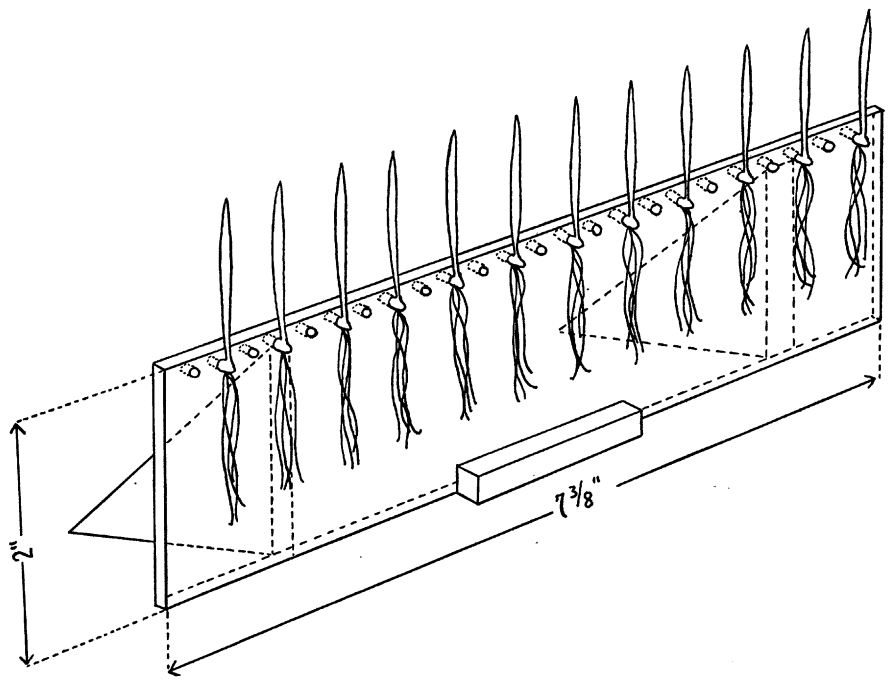

FIG. 1.