convoluted tubules. The liver exhibited diffuse degeneration and fatty vacuolization and, in some instances, necrosis of the liver cells. These changes were more marked in the central zone of the lobules. The hepatic cellular changes that occur in rodents (*3*) as a more or less characteristic response to the absorption of significant quantities of hydrocarbons, were not encountered in dogs. Dogs that survived, when fed on diets containing endrin at levels of 8 p.p.m., had normal viscera except for slight evidences of diffuse degeneration of the distal convoluted tubules.

In general, the female dogs fed on a diet at 4, 3, or 1 p.p.m. had normal viscera, but some of them had a renal abnormality characterized by slight tubular vacuolation; this was seen also in the female control dog. Male dogs fed on diets at these levels and male control dogs had normal viscera.

The amounts of endrin found in the tissues of dogs that survived after being fed for almost 6 months on diets containing endrin in concentrations of 4 or 8 p.p.m., were determined by Sun and Sun by microbioassay (*4*). The fat and the liver of both groups contained about 1 p.p.m., but the kidneys of those fed on the diet at the higher level contained 0.5 p.p.m. None was found in the kidneys of those fed at the lower level, nor in the brains of those fed at either level. The precision of the analytical procedure may be illustrated by the fact that when endrin was added to fat, liver, brain, and kidneys of a control dog in amounts sufficient to bring the concentration to 1 p.p.m quantities equivalent to 50, 55, 74, and 112%, respectively, of those added, were recovered.


### Summary

When ingested in one dose by rats, endrin is about three times as toxic as aldrin and 15 times as toxic as DDT. In prolonged feeding experiments, rats can consume diets containing approximately three times as much aldrin, and 12 times as much DDT, as endrin without increase in the relative weight of specific organs. Dogs can consume safely about one-half the concentration of endrin in their diet that rats can tolerate, if comparison is made on the basis of comparably prolonged periods of time. Dogs are at least ten times as susceptible to the toxic effects of endrin as to those of DDT, if judged by the influence of these insecticides on the rate of growth or the relative size (weight) of certain of the organs of these animals when fed upon diets containing one or the other of these materials in comparable concentrations.



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**PESTICIDE DETERMINATION**

# Determination of Lindane in Mushrooms


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**Lindane can be determined colorimetrically in mushrooms if interfering substances are removed by preliminary sulfonation of a methylene chloride mushroom extract. This sulfonating procedure should also be generally applicable where direct analysis or simple extractions prove inadequate.**


Trace amounts of the insecticide benzene hexachloride (the mixed isomers of 1,2,3,4,5,6-hexachlorocyclohexane) or of lindane (99% or more of the insecticidal gamma isomer of 1,2,3,-4,5,6-hexachlorocyclohexane) can be determined using the colorimetric method described by Schechter and Hornstein (*3*). This procedure is based on the dechlorination of the hexachlorocyclohexane isomers to benzene by means of zinc in acetic acid and the subsequent nitration of the benzene to *m*-dinitrobenzene.

The method is specific for benzene hexachloride with respect to interference by other insecticides. It is apparent, however, that any substance that can be reduced to benzene under the experimental conditions would yield the same color. In addition, some other aromatics (*5*) if originally present or eventually produced might distill into the nitration chamber and yield nitrated derivatives which would result in erroneously high benzene hexachloride values.

In many materials this analysis can be made directly on the sample material being examined. In others a preliminary extraction with a chlorinated hydrocarbon solvent or with glacial acetic acid is necessary (*2*). However, there have been unpublished reports from several investigators that in the analysis of mushrooms treated with lindane interfering substances were encountered that were not removable by simple extraction with solvents such as ether, carbon tetrachloride, or methylene chloride. Strong sulfuric acid has been used to purify plant and animal extracts containing DDT and hexachlorocyclohexane (*1, 4*). As the interfering substances extracted from mushrooms are probably aromatic in nature, sulfonation appeared feasible. Direct sulfonation of a methylene chloride extract of mushrooms has been found to remove all interference. To ensure sulfonation of even difficultly sulfonated aromatics, 30% fuming sulfuric acid is used. This sulfonation procedure was also found to be of value in removing traces of aromatics from chlorinated organic solvents useful in this colorimetric procedure and in analyzing hexane extracts containing lindane. Commercial hexane has far too high a concentration of aromatics to

permit its use in this analytical procedure; however, it was found possible to remove the aromatics completely from the hexane extract containing lindane by this direct sulfonation technique, as interfering substances are either sulfonated and removed by the extraction procedure or are converted to products that do not interfere in subsequent analysis.

### Apparatus

A specially designed all-glass digestion and nitrating apparatus (*3*). A suitable photometer.

### Reagents

Fuming sulfuric acid, 30%. Reagents as described by Schechter and Hornstein (*3*).

### Procedure

One hundred grams of whole mushrooms are washed under tap water, cut into small pieces, and added to 200 ml. of methylene chloride in a 500-ml. Erlenmeyer flask equipped with a reflux condenser. The mixture is refluxed gently for 3 to 4 hours, then allowed to stand overnight. The extract is decanted through a filter paper into a separatory funnel, where small amounts of water either extracted or introduced in the rinse step separate to the top. The methylene chloride phase is then drained into an Erlenmeyer flask and its volume reduced on a steam bath to approximately 50 ml. The solution is poured into a 250-ml. separatory funnel and is extracted with a 10-ml. portion of 30% fuming sulfuric acid. The methylene chloride–sulfuric acid mixture is shaken vigorously for 2 minutes with frequent venting of the separatory funnel. (Precautions should be used in handling fuming sulfuric acid.) The layers are allowed to separate, and the bottom acid layer is drained off and discarded. To ensure complete sulfonation, the methylene chloride layer is extracted in a similar fashion with two more 10-ml. portions of 30% fuming sulfuric acid.

After the third 10-ml. portion of the acid has been discarded, 25 ml. of cold water is cautiously added to the methylene chloride. The mixture is thoroughly shaken, again with frequent venting of the separatory funnel, and the two phases are allowed to separate. The water layer is discarded, and the methylene chloride fraction is drained into a second 250-ml. separatory funnel and washed with another 25 ml. of cold water. The methylene chloride phase is returned to the first separatory funnel and washed with a third 25-ml. portion of water. After phase separation the methylene chloride is emptied into a 250-ml. Erlenmeyer flask having a ⅋ 24/40 joint (traces of water will cause no harm), a boiling chip is added, and the methylene chloride is evaporated on a steam bath to a volume of 1 to 2 ml. One hundred and twenty milliliters of glacial acetic acid is added and 20 ml. of the acid is distilled out by heating in an oil bath at 130° to 140° C. to remove the last traces of volatile solvent. The glacial acetic acid solution is then analyzed (*3*).

**Table I. Recoveries of Lindane Added to Untreated Mushrooms**

(Weight of each sample 100 grams. Analysis run on methylene chloride extracts after sulfonation.[a])

| Added, γ | Recovered, γ | Recovery, % |
|---|---|---|
| 50 | 47 | 94 |
| 75 | 73 | 97 |
| 100 | 95 | 95 |

[a] Apparent lindane blank on methylene chloride extract of 100 grams of mushrooms (no lindane added) without sulfonation was 35 γ, or 0.35 p.p.m. Sulfonation reduced this to 2 γ, or 0.02 p.p.m.

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**HERBICIDAL ACTION**

# Activities and Residues of Sulfur-35–Labeled Bis(ethyl Xanthic) Disulfide


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Sulfur-35–containing bis(ethyl xanthic) disulfide, known commercially as Herbisan, was synthesized and evaluated as a pre- and postemergence herbicide. Herbicidal concentrations of bis(ethyl xanthic) disulfide as formulated were not absorbed in sufficient quantities to be detected by regular radioisotopic counting procedures in any of the vegetables studied, when it was applied as a pre-emergence spray on muck soil. Foliage applications of bis(ethyl xanthic) disulfide showed that of the 16 vegetable crops studied, only in cabbage and cauliflower was translocation ascertained by autoradiographic procedures and verified by Geiger-Müller counting. These studies indicate that bis(ethyl xanthic) disulfide is primarily a contact herbicide.


BIS(ETHYL XANTHIC) DISULFIDE, known commercially as Herbisan and Sulfasan, shows promise as both a pre- and postemergence contact herbicide (*1*, *3*, *4*). The literature contains no information on its uptake, transport, accumulation, or residual qualities in vegetable crops.

Herbisan, like most herbicides, is required in relatively minute quantities for effective weed control. Regular chemical methods for its detection are not of sufficient sensitivity to allow a critical evaluation of its action and its residues. Consequently, it appeared that labeling with sulfur-35 and studying with isotope techniques would prove of great value, as such techniques are several hundred