gree of potentiation than previously reported. Using the criteria described earlier, the spread is 90 to 90%; thus the differential is 50% for this pair, indicating definite potentiation.

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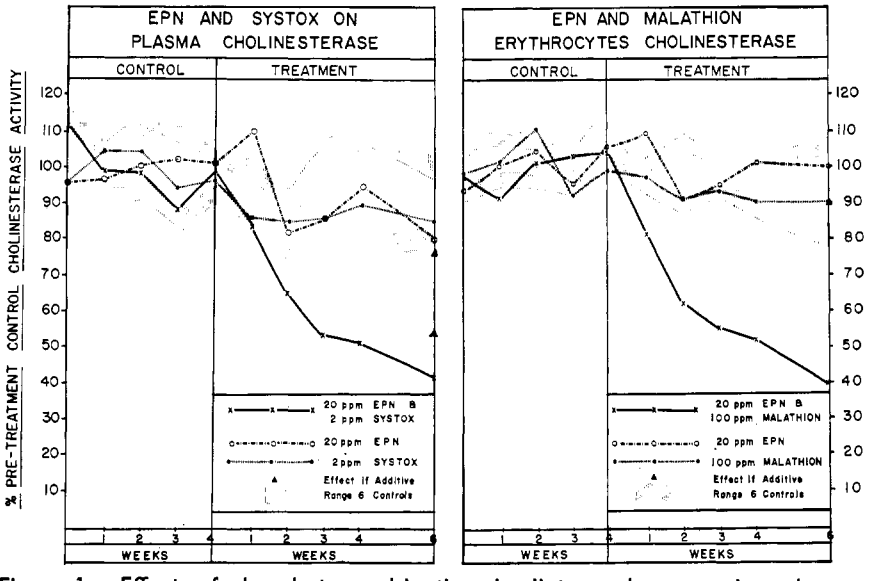

Figure 1. Effects of phosphate combinations in diet on plasma and erythrocyte cholinesterase

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INSECTICIDE RESIDUES

Endrin Content of Body Tissues of Steers, Lambs, and Hogs Receiving Endrin in Their Daily Diet

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Steers, lambs, and hogs fed endrin at dietary levels of 0.1 p.p.m. for 12 weeks showed little tendency to deposit endrin in body tissues. After 12 weeks of endrin feeding at 0.25 p.p.m., the endrin content of the fat of these animals was not higher than 0.2 p.p.m. Other tissues contained no detectable endrin at this level of intake. The analyses were performed with a method specific for endrin and sensitive to 0.1 p.p.m.

THE USE OF RESIDUAL INSECTICIDES against pests of forage crops is a practice of great potential value to agriculture. However, the problem of residues remaining when the crop is consumed by livestock and the extent of subsequent contamination of meat products must be investigated.

Claborn (*3*) measured the residues present in the fat of steers and sheep after these animals ingested feed contaminated with several chlorinated hydrocarbons. Several authors have reported the presence of DDT in animal tissues. It has been found in the tissues of sheep (*6, 13*), hogs (*1, 7*), steers (*5*), and calves (*12*) after the ingestion of DDT-treated feed. Toxaphene accumulates in the fat of steers and sheep (*2, 4*), and high levels of lindane in the diet result in the deposition of this insecticide in fat (*9*).

The present report describes analytical results obtained when the chlorinated hydrocarbon insecticide, endrin (1,2,3,4,10,10 - hexachloro - 6,7 - epoxy-1,4,4*a*,5,6,7,8,8*a* - octahydro - 1,4 *endo-endo* - 5,8 - dimethanonaphthalene) was fed to sheep, steers, and hogs, and various tissues from these animals were analyzed for endrin residues. A more complete report listing all relevant data is available in mimeograph form (*11*).

Experimental

The steers and lambs were purchased from local sources and the hogs were from the Oregon State College swine herd. The steers were stanchioned throughout the experiment. Hogs and lambs were housed in individual pens. All animals were kept under shelter.

The ration for steers and lambs was composed of barley, oats, and grass hay. The hog ration consisted of barley, oats, alfalfa meal, tankage, steamed bone meal, oyster shell flour, and iodized salt. Salt and water were available *ad libitum*. All animals were fed twice daily in individual feeders. Analyses of the rations prior to the feeding experiments indicated that they were free from endrin contamination.

The rations were fortified by distributing endrin in acetone solution over the entire ration at each feeding. A separate glass syringe was used for each level of toxicant. The endrin solutions were prepared so that the desired level of fortification could be attained by

adding 1 ml. of solution per pound of feed. The feed for each animal was weighed and fed separately.

Two steers, two lambs, and two hogs were fed control rations with no endrin added. Three animals of each species received 0.1 p.p.m. of endrin in their diets, three received 0.25 p.p.m., and three received 0.75 p.p.m. In addition, two steers received 2.00 p.p.m. of endrin in their diet. Weight gain and feed consumption records were kept throughout the experimental period.

One or two animals, depending upon the endrin level, were slaughtered at the end of 12 weeks of endrin feeding. The remainder were fed 6 additional weeks without endrin in the ration and then slaughtered. Liver, kidney, body fat, and renal fat samples were collected at the time of slaughter. Kidney and renal fat samples were taken from both kidneys; liver samples were taken from several areas of the liver; and exterior fat was used as the source of body fat samples. Steak and roast tissue samples were obtained after the carcasses had been chilled 24 to 48 hours. Beef steaks were taken from the short-loin area and roasts from the loin and arm. Pork steaks were taken from the loin and the roasts from the loin and shoulder. Lamb steaks were taken from the loin and leg and roasts from the leg. Immediately after collection, all samples were labeled, weighed, placed in Pliofilm bags, and stored at 0° F.

Analytical Methods. All tissue samples were analyzed by a spectrophotometric method specific for endrin (*10*). This method involves three main steps: saponification of the sample to eliminate fats, chromatography of the fat-free extract to remove additional interferences, and finally, the development and measurement of the color characteristic of endrin.

As a check on the possibility that endrin was converted to a toxic metabolite not detected by the specific method, about 20% of the samples were analyzed by a nonspecific biological method using mosquito larvae. The sample preparation steps of saponification and chromatography were utilized in this method also. The mosquito larvae were then exposed to graded amounts of extracts and the resulting mortalities compared with those obtained with known concentrations of endrin.

The sensitivity of the specific method was 0.1 p.p.m. for the 50-gram samples, while the bioassay could detect 0.02 p.p.m. To validate the two analytical methods, control samples were analyzed frequently with and without fortification with endrin. Approximately 17% of the analyses performed were of this type. The average recovery under these circumstances was 100% with the chemical method and 93% with the bioassay. Representative recovery values are shown in Table I.

Table I. Recovery of Endrin from Fortified Samples by the Spectrophotometric and Bioassay Methods

Sample	Endrin, P.P.M. Added	Endrin, P.P.M. Found[a]	Recovery, %
SPECTROPHOTOMETRIC			
Fat	0.30	0.32	107
	0.30	0.26	87
	0.30	0.27	90
	0.10	0.07	70
Meat cut	0.10	0.08	80
	0.30	0.29	97
	0.10	0.13	130
	0.10	0.09	90
BIOASSAY			
Fat	0.30	0.32	107
	0.10	0.11	110
	0.10	0.09	90
Meat cut	0.30	0.32	107
	0.30	0.30	100
Liver	0.10	0.09	90

[a] Corrected for apparent endrin content of control samples.

Results and Discussion

Effect of Endrin Feeding on Experimental Animals. All steers, hogs, and lambs receiving endrin gained weight at normal rates. In the opinion of a staff veterinarian, all animals were normal in appearance throughout the trial and at slaughter.

Analytical Results. The results of the analyses of various tissues are given in Tables II and III. Data in Table II indicate that only at levels of 0.25 p.p.m. or higher were residues present in amounts that could be consistently detected by the analytical method. Steers accumulated the highest levels of endrin in their fatty tissues; hogs showed little tendency to store endrin. This may be due to differences in the relative amount of fat in steers and hogs.

During the 6 weeks of the endrin-free diet (feedoff) period, the endrin content of the fat of the steers was reduced about 60%. This is in contrast with the results obtained in an experiment with dairy cows (*8*), where all the stored endrin was dissipated during a similar feedoff period. The greater mobility of the fat of the lactating dairy cow is suggested as an explanation for this difference. The feedoff period of 6

Table II. Average Endrin Content of Fat of Treated Steers, Lambs, and Hogs[a]

No. of Animals[b]	Endrin in Diet, P.P.M.	Endrin Content, P.P.M. Steers Body Fat 12 wk.	Steers Body Fat 18 wk.	Steers Renal Fat 12 wk.	Steers Renal Fat 18 wk.	Lambs Body Fat 12 wk.	Lambs Body Fat 18 wk.	Lambs Renal Fat 12 wk.	Lambs Renal Fat 18 wk.	Hogs Body Fat 12 wk.	Hogs Body Fat 18 wk.	Hogs Renal Fat 12 wk.	Hogs Renal Fat 18 wk.
2	0.00	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
3	0.10	<0.1	<0.1	<0.1	0.2	<0.1	...	<0.1	...	<0.1	...	<0.1	...
3	0.25	0.2	0.2	<0.1	<0.1	0.1	<0.1	0.1	<0.1	0.1	...	<0.1	...
3	0.75	0.4	0.1	0.2	0.1	0.3	<0.1	0.2	0.1	0.1	<0.1	<0.1	<0.1
2	2.00	0.9	0.3	0.9	0.3	...	...	...	...	...	...	...	...

[a] Data corrected for apparent endrin content of control samples and rounded to nearest 0.1 p.p.m. Values below this level are indicated.
[b] One animal at each level was retained for feedoff studies.

Table III. Average Endrin Content of Various Samples from Experimental Animals Fed Endrin Daily for 12 Weeks

No. of Animals[a]	Endrin in Diet, P.P.M.	Endrin Content, P.P.M.[b] Steers Steaks	Steers Roasts	Steers Liver	Steers Kidney	Lambs Steaks	Lambs Roasts	Lambs Liver	Lambs Kidney	Hogs Steaks	Hogs Roasts	Hogs Liver	Hogs Kidney
2	0.00	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
3	0.10	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
3	0.25	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
3	0.75	<0.1	<0.1	0.2	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.1	<0.1	<0.1
2	2.00	0.3	0.2	0.2	<0.1	...	...	...	...	...	...	...	...

[a] One animal at each endrin level was retained for feedoff studies. Analyses of samples from these animals showed endrin residues of less than 0.1 p.p.m.
[b] These data have been corrected for apparent endrin content of control samples and rounded to the nearest 0.1 p.p.m. Values below this level are indicated.

Table IV. Agreement in Endrin Content of Fat Samples Analyzed by Spectrophotometric and Bioassay Methods

Sample	Endrin in Diet, P.P.M.	Endrin Content, P.P.M. Bio-assay	Spectrophotometric
Steer, body fat	0.00	<0.02	<0.1
	0.10	0.17	<0.1
	0.25	0.20	0.2
	0.75	0.34	0.4
Lamb, body fat	0.00	<0.02	<0.1
	0.10	0.04	<0.1
	0.25	0.08	0.1
Hog, body fat	0.10	0.02	<0.1
	0.25	0.09	0.1
	0.75	0.22	0.1
Steer, body fat, after feedoff	2.00	0.47	0.3
Lamb, body fat, after feedoff	0.75	0.07	<0.1

weeks was sufficient to allow elimination of the endrin to levels below those detectable by the specific method in the case of hogs and lambs.

Limited data obtained with the confirmatory bioassay indicated that small amounts—less than 0.1 p.p.m.—of endrin were deposited in the tissues at all levels of endrin intake.

The endrin content of roasts and steaks is given in Table III. Only the higher levels of endrin intake resulted in a deposition in this type of tissue in the case of steers and hogs. No detectable endrin was present in steaks and roasts of lambs. Fat determination made on steer steaks and roasts showed that steaks had a higher fat content which may account for the higher residue shown in Table III. In confirmation of this, a steak and a roast from a steer receiving 2.00 p.p.m. of endrin were divided into gross fat and protein fractions and analyzed separately. Practically all of the endrin—nearly 1.0 p.p.m.—was located in the fat.

All meat cuts which appeared to contain significant amounts of endrin at the end of the 12-week feeding period were analyzed again after cooking. The results indicated little or no decrease in endrin content. An increase was actually noted in several cases, possibly owing to the loss of moisture during cooking.

Of all liver and kidney tissues examined, only the liver tissue of steers receiving the two higher levels of endrin showed definite amounts of endrin at the end of the 12-week feeding period (Table III).

The analyses by the biological method were included in the study as a means of detecting any toxic materials produced by the metabolism of the endrin. Data in Table IV reveal that there are no consistent differences between the two sets of analytical results—good evidence that no metabolites more toxic than endrin are produced.

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INSECTICIDE RESIDUES

Endrin Content of Milk and Body Tissues of Dairy Cows Receiving Endrin Daily in Their Diet

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Dairy cows were given daily doses of endrin ranging from 0.1 to 2.00 p.p.m. total dietary concentration for 12 weeks. During and after the endrin intake period, milk samples were analyzed for endrin residues. Various tissue samples were also analyzed for endrin content at the end of the 12-week period. Small amounts of endrin were secreted in milk at all levels of intake. Concentrations of endrin up to 1.00 p.p.m. were found in the body fat.

SPRAY RESIDUES remaining on forage crops at the time they are eaten by livestock constitute a potential hazard to the consumer of milk and meat products. Before any material can be considered for such uses, information on its storage in animal tissues and its secretion in milk must be obtained.

Bateman and coworkers (*3*) studied the fate of toxaphene consumed by dairy cows and found residues in milk in concentrations ranging from 2 to 13 p.p.m. Biddulph *et al.* (*4*) and Shepard *et al.* (*11*) found that cows eating DDT-treated forage secrete DDT in milk and store it in fatty tissues. Harris *et al.* (*8*) and Ely *et al.* (*6*) obtained similar results with dieldrin. Davidow, Radomski, and Ely (*5*), Harris *et al.* (*8*), and Ely *et al.* (*7*) demonstrated the presence of heptachlor epoxide, a toxic metabolite of heptachlor, in the milk of cows receiving this insecticide. The following report describes experiments