Toxicity of Endrin for Laboratory Animals

JOSEPH F. TREON, FRANK P. CLEVELAND, and JOHN CAPPEL

Kettering Laboratory, Department of Preventive Medicine and Industrial Health, College of Medicine, University of Cincinnati, Cincinnati, Ohio

When endrin was given orally in one dose, young female rats were more susceptible to its toxic effects than were young male rats. Female rats 6 months of age were more susceptible than younger females, but the reverse and more normal relationship between age and susceptibility prevailed in the case of male rats. Corresponding results were obtained with isodrin, which has the corresponding spatial configuration. Female rabbits were about as susceptible as female rats 6 months of age. Monkeys and cats were more susceptible than most groups of rats, whereas guinea pigs were more resistant than rats. The results of the oral administration of multiple doses of endrin to rats and rabbits, application of the material upon the skin of rabbits, and inhalation of the sublimed vapor of the compound by several species of animals over considerable periods of time, are described. The effects sustained by rats and dogs during and following prolonged consumption of diets contaminated with endrin are described, with particular reference to mortality, rate of growth, apparent influence upon the relative weights of specific organs, and gross and microscopic abnormalities in the viscera.

THE TOXICITY OF ENDRIN to laboratory animals has been tested in experiments which involved both single doses and multiple doses administered over periods up to 2 years.

Immediate Toxicity

The immediate toxicity (one dose) of 4-dimethanonaphthalenes (aldrin, isodrin, dieldrin, and endrin), when given orally to nonfasted rats or rabbits, has been shown (*5*) to be more closely related to the spatial configuration than to the empirical composition of these compounds. Those having the *endo, endo* configuration (isodrin and endrin) were more toxic than those which had the *endo, exo* configuration (aldrin and dieldrin).

Aldrin (Compound 118 or Octalene) is the coined name for the insecticidal product containing not less than 95% of 1,2,3,4,10,10 - hexachloro - 1,4,4a,8,8a - hexahydro-1,4 - *endo,exo* - 5,8 - dimethanonaphthalene (commonly referred to as HHDN) and not more than 5% of related compounds.

Isodrin (Compound 711) is the coined name for 1,2,3,4,10,10-hexachloro-1,4,4a,-5,8,8a - hexahydro - 1,4 - *endo,endo* - 5,8-dimethanonaphthalene.

Dieldrin (Compound 497 or Octalox) is the coined name for the insecticidal product containing not less than 85% of 1,2,3,-4,10,10 - hexachloro - *exo* - 6,7 - epoxy-1,4,4a,5,6,7,8,8a - octahydro - 1,4 - *endo,-exo*-5,8-dimethanonaphthalene (commonly referred to as HEOD) and not more than 15% of related products.

Endrin (Compound 269) is the coined name for 1,2,3,4,10,10-hexachloro-*exo*-6,7-epoxy - 1,4,4a,5,6,7,8,8a-octahydro - 1,4-*endo,endo*-5,8-dimethanonaphthalene.

Influence of Sex and Age of Rats. Young female rats, 26 to 31 days of age, appear to be slightly more susceptible than young male rats to a single oral dose of either endrin or isodrin. The lethal dosages (LD_{50}), as determined by the method of Bliss (*7*), for young female Carworth rats, each given a single oral dose of endrin or isodrin as a solution in peanut oil, are 16.8 and 16.4 mg. per kg. of body weight, respectively. The corresponding values for males of this age are 28.8 and 27.8 mg. per kg., respectively (Table I).

The greater susceptibility of female rats 6 months of age than of younger female rats to the toxic effects of endrin and isodrin, was an unusual finding which does not lend itself to ready explanation. The reverse, and more normal, relationship between age and susceptibility was found in the case of males. The lethal dosages of endrin and isodrin for Carworth female rats, at 6 months of age, were 7.3 and 11.7 mg. per kg., respectively, and the corresponding values for male rats of this age were 43.4 and 42.1 mg. per kg., respectively (Table I).

Although the dosage which is lethal to one half of a group of animals (LD_{50}) has practical significance for purposes of comparison, since its effects, numerically, are more reproducible than those of any other lethal dosage, other aspects of the data are also worthy of consideration. One of these is the slope, which measures the relationship between the change in mortality, expressed in probits, to the change in the log of the dosage (Δ mortality/Δ log of dosage).

The smaller the slope (Table I), the

Table I. Influence of Sex and Age of Rats on Susceptibility to Immediate Toxic Effects of Endrin or Isodrin

(Given orally to Carworth rats as a 0.1 to 0.5 wt. vol. % solution in peanut oil)

Compound	Age, Days	Sex	LD_{50} Mg./Kg.	Fiducial Limits 0.05	Slope	Fiducial Limits 0.05
Endrin[a]	29–31	F	16.8	13.0–21.7	3.39	2.05–4.73
Isodrin[b]	26–28	F	16.4	12.6–21.5	3.95	1.65–6.25
Endrin	29–31	M	28.8	16.2–51.2	2.05	0.66–3.42
Isodrin	26–28	M	27.8	23.2–33.3	6.71	3.32–10.12
	Months					
Endrin	6	F	7.3	6.3–8.4	7.19	3.17–11.20
Isodrin	6	F	11.7	10.2–13.5	7.05	4.15–9.95
Endrin	6	M	43.4	39.3–47.9	10.10	5.47–14.73
Isodrin	6	M	42.1	35.9–49.4	6.65	4.23–9.07

[a] 1,2,3,4,10,10-hexachloro-*exo*-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo,endo*-5,8-dimethanonaphthalene. [b] 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo,endo*-5,8-dimethanonaphthalene.

Table II. Immediate Toxicity of Endrin Given Orally to Animals of Several Species

(Given as dilute solution, 0.1 to 1.0 wt. vol. % in peanut oil)

Species	Sex	No. of Animals Employed at Each Dosage Level	Minimum Lethal Dosage, Mg./Kg.	LD_{50}, Mg./Kg.
Monkeys	M and F	2	1–3	3[a]
Rats[b]	F	10	<5	7.3
Cats	M and F	1	<5	...
Rats[c]	M	10	5–7	28.8
Rabbits	F	4	5–7	7–10[a]
Rats[c]	F	10	7–10	16.8
Guinea pigs	F	2	10–16	16[a]
Rats[b]	M	10	24–36	43.4
Guinea pigs	M	2	24–36	36[a]

[a] Approximate (estimated). [b] 6 months of age. [c] 26 to 31 days of age.

greater is the increase in dosage necessary to give a unit increase in mortality. A very small slope indicates that occasional instances of extreme susceptibility to a chemical may be expected, thus introducing uncertainty into the estimation of a sublethal dosage. On the other hand, a steep slope involves less variability in the dosage-mortality response, thereby providing for greater predictability as to the effects of a specific dosage. This point is illustrated by the fact that although the LD_{50} for young female rats given endrin was smaller than that for comparable males, the slope was lower in the case of the males. Thus 1 of 10 young males died when given the dosage of 7 mg. per kg., while all members of a comparable group of young females survived. The fiducial limits of the LD_{50} are wider for these males than for the females, in evidence of the lower accuracy of the former. Another example is that of the young male rats given either endrin or isodrin. The lethal dosages were almost the same but the slopes obtained were 2.05 and 6.71, respectively. One of 10 males died, following the administration of 7 mg. of endrin per kg.; 1 of 10 died when the dose of isodrin was 16 mg. per kg.

Variation with Species. The approximate lethal dosage (LD_{50}) and the minimum lethal dosage of endrin when given orally as a solution in peanut oil to several species of animals are shown in Table II. Although the values for monkeys, cats, rabbits, and guinea pigs are less accurate than those for rats, because of the smaller numbers employed for a given dosage, it appears that, in general, monkeys and cats are more susceptible than most groups of rats, and guinea pigs are more resistant. Rabbits it seems, are somewhat more resistant than monkeys or cats to a single oral dose of endrin. However, endrin is toxic for all these species.

The difference in susceptibility of rats, in relation to sex, appears to be similar to that of guinea pigs. Females were more susceptible than males (Table II), the approximate lethal dosages being 16 and 36 mg. per kg., respectively.

Table III. Immediate Toxicity of Endrin Maintained in Contact with Intact Skin of Female Rabbits by Method of Draize, Woodard, and Calvery

(Applied as recrystallized dry powder that passed 100-mesh screen. Maintained under rubber sleeve for 24 hours)

Dosage, G./Kg.	No. of Animals That Died/No. of Animals Given Dose
0.25–3.6	8/8
0.16	2/3
0.125	1/3
0.094	1/3
0.060	0/3

Female rabbits are slightly more susceptible, perhaps, to the effect of the oral administration of one dose of isodrin in peanut oil than to endrin administered correspondingly, as judged by the fact that their approximate lethal dosages were between 5 and 7 and between 7 and 10 mg. per kg., respectively. Their minimum lethal dosages, respectively, were between 3 and 5 and between 5 and 7 mg. per kg.

Animals that absorbed these compounds in sufficient quantities exhibited hyperirritability to stimuli, tremors, clonic and tonic convulsions, ataxia, dyspnea, gasping, and cyanosis.

Application upon Skin of Rabbits. When endrin, as a dry 100-mesh powder was maintained in contact with either the intact or the abraded skin of female rabbits for 24 hours, according to the sleeve method of Draize, Woodard, and Calvery (*2*), the minimum lethal dose was found to be greater than 60 and less than 94 mg. per kg. of body weight (Table III). The minimum lethal dosage of dry 100-mesh isodrin, as applied similarly to the skin of rabbits, was less than 94 mg. per kg. of body weight. Animals severely poisoned by either of these materials had convulsions. Neither gross nor microscopic evidence of damage to the skin of these animals was found.

Effects of Multiple Doses

Oral Administration. Female rats, regardless of age, appeared to be less susceptible than female rabbits but more susceptible than male rats to the cumulative action of endrin administered orally in multiple doses (Table IV).

Four of five female rabbits, each given an oral dosage of 1 mg. of endrin per kg. of body weight on each of 5 days per week, died following the administration of 2, 30, 35, and 50 doses, respectively. The fifth rabbit, to which 50 such doses were administered over the period of 10 weeks, survived. This level of dosage was tolerated by six female rats (three were 29 days of age, and three were 6 months of age, at the initiation of the experiment), but the level of 2 mg. per kg. was not tolerated (Table IV). Male rats survived when the level of dosage was 2 mg. of endrin per kg., but died in the early period of the experiment (fifth dose) when the level was 5 mg. per kg.

These rabbits developed abdominal distension, while some of the rats developed a hypersensitivity to stimuli. Most of the animals lost in weight or grew at retarded rates, but male rats were affected less than the other animals in this respect.

Intermittent Cutaneous Contact. Three female rabbits, the intact skin of each of which was maintained in contact with 150 mg. of dry endrin (100-mesh) for 2 hours on each of 5 days per week, died after 19, 19, and 25 such applications, respectively (Table V). One of three rabbits with intact skin died after the 40th period of contact with 75 mg. of the dry powder; the others survived 65 and 70 such periods of contact, respectively. One of four rabbits with abraded skin (Table V), subjected to

Table IV. Effects of Oral Administration of Series of Doses of Solution of Endrin in Peanut Oil to Rabbits and Rats

(Administered as 0.025 or 0.1 wt./v. % solution in peanut oil, on each of 50 days for 67 to 72 days)

Species	Sex	Age When First Dose Given	Daily Dose, Mg./Kg. 1	2	5
			No. of Animals That Died/Number Given Doses		
Rabbit	F	8–10 weeks	4/5	...	...
Rat	F	29 days	0/3	1/2	...
	F	6 months	0/3	1/3	...
	M	29 days	0/3	0/3	...
	M	6 months	...	0/3	3/3

Table V. Toxicity of Endrin Maintained upon Skin of Female Rabbits for 2 Hours on Each of 5 Days per Week over Several Weeks

(Applied as recrystallized dry powder that passed 100-mesh screen)

No. of Doses Applied	Daily Dosage G.	Daily Dosage G./kg.	Condition of Skin	No. of Animals That Died/No. Given Material
19–25	0.150	0.067–0.091	Intact	3/3
40–70	0.075	0.020–0.042	Intact	1/3
25–45	0.075	0.027–0.044	Abraded	1/4

Table VI. Fate of Animals Exposed Intermittently to Vapor of Endrin in Air

(Exposed for 7 hours per day 5 days per week. Concentration, 5.44 γ/ liter, 0.36 p.p.m.)

Species	Duration of Exposure, Hours	No. of Animals That Died/No. of Animals Exposed
Cat	130 × 7	0/1
Guinea pigs	130 × 7	0/2
Hamsters	(101 − 130) × 7	0/2
Rats	130 × 7	0/3
Rabbits[a]	118 × 7	2/4
Mice[b]	107 × 7	1/3

[a] Three additional rabbits survived 12 7-hour periods of exposure.
[b] Six additional mice, survived 18, 18, 42, 58, 64, and 64 7-hour periods of exposure.

Table VII. Mortality among Groups of Control Rats and Rats Fed 2 Years on Diets Containing Endrin

	No. That Died/No. Fed on Diet			
	Males		Females	
P.P.M.	80 weeks	106 weeks	80 weeks	106 weeks
100	18/20[a]	18/20	18/20[a]	19/20[a]
50	13/20[b]	16/20	19/20[a]	20/20[a]
25	5/20	9/20	12/20[c]	15/20
5	5/20	13/20	7/20	12/20
1	5/20	9/19	4/20	9/20
0	7/20	12/20	5/20	13/20

[a] $P. < 0.01$. [b] This value is only slightly above 0.05. [c] $P.$ 0.05 − 0.01.

similar contact with a like amount of the powder, died after 26 periods of contact, but the others survived after 25, 45, and 45 such periods, respectively. These data provide additional evidence that abrasion of the skin does not promote the percutaneous absorption of endrin to any noteworthy extent.

Convulsions, tremors, and twitching of the facial muscles were the chief signs of intoxication. The survivors grew at rates only slightly less than normal. The skin of the animals, at the site of the applications, suffered no damage.

Inhalation of Sublimed Vapor. Three animals (two rabbits and a mouse), of a group consisting of a cat, guinea pigs, hamsters, mice, rats, and rabbits, died following exposure, for 7 hours on each of 130 days over the period of 185 days, to air containing the sublimed vapor of endrin in the concentration of 5.44 γ per liter (0.36 p.p.m.). The rabbits died after 26 and 90 periods, respectively, whereas the mouse died after 22 periods (Table VI). No convulsions were observed among this group.

Samples of air from the chamber were collected 4 times daily by passing air at the rate of 5.0 liters per minute, as measured by means of a flowmeter, through an absorption train. The air was passed for 45 minutes through two fritted disks in series, each of which was immersed in 100 ml. of purified 95% ethyl alcohol in a glass cylinder (28 × 5 cm.). The transmittance of the solution contained in a 50-mm. quartz cell was then measured at 230 mμ by means of a Beckman spectrophotometer set against a corresponding cell containing redistilled alcohol.

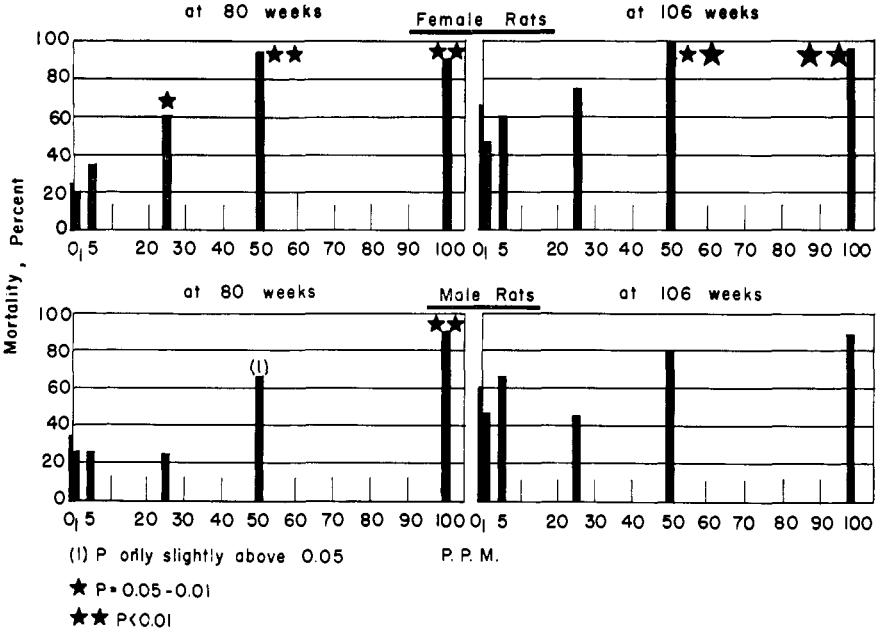
Pathological Findings

Diffuse degenerative changes were observed in the livers and kidneys of all fatally poisoned animals, regardless of the method or frequency of the administration of this material. Diffuse degenerative lesions were found in the brains of all animals that died, with the exception of the rabbits that were given multiple oral doses and the mouse that was subjected to inhalation of the sublimed vapor.

Alterations were observed in the tissues of some of the surviving animals. Two of five rabbits subjected to multiple applications on the skin exhibited severe fatty degeneration of the liver. Degeneration of the cells in the central zones of the lobules of the livers of rabbits resulted from a single application of endrin on the skin. Diffuse degeneration and fatty vacuolization of the hepatic and renal cells and degeneration of the heart were observed in a rabbit one day after it had been given the 50th of a series of oral doses. Rabbits that survived 118 periods of inhalation of the sublimed vapor of endrin were found to have developed a granulomatous type of pneumonitis.

Normal tissues were found in many of the animals that survived—in those of all species that were given one oral dose, in rats that were given multiple oral doses, and in rats, mice, hamsters, guinea pigs, a cat, and some of the rabbits that were subjected to inhalation of the vapor.

Figure 1. Mortality among control rats and rats fed for 2 years on diets containing endrin



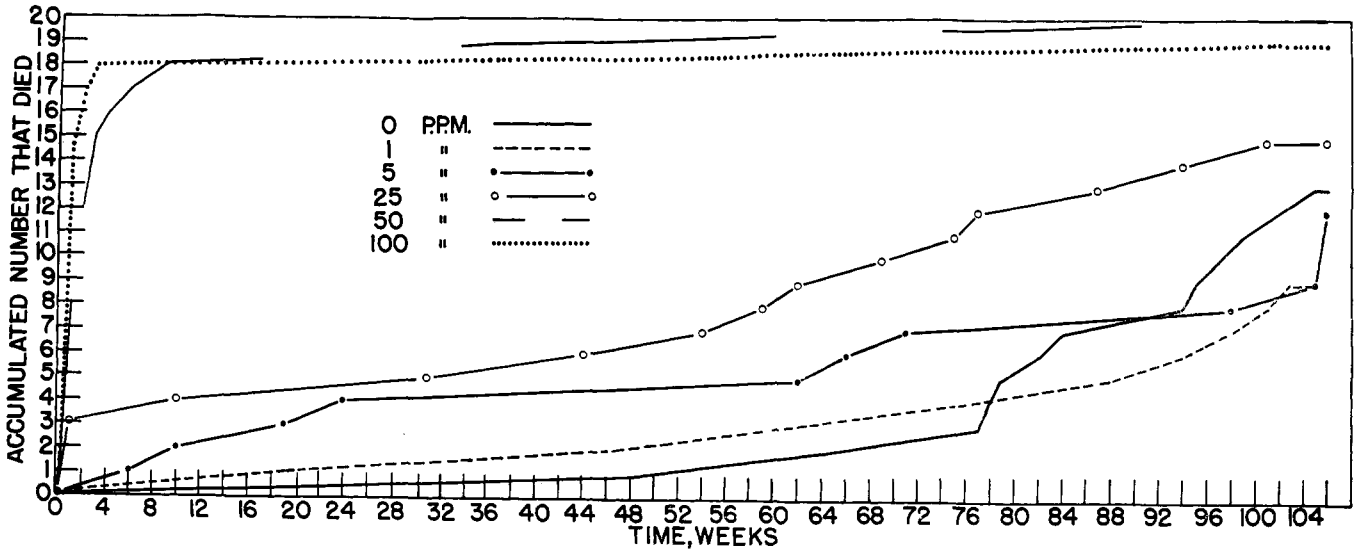


Figure 2. Accumulated deaths among female rats fed endrin

Effects of Prolonged Feeding

Rats. Groups of 20 male and 20 female Carworth rats, 28 days of age, were given diets containing endrin in the concentrations of 1, 5, 25, 50, and 100 p.p.m., respectively. Since endrin was distributed throughout Purina Fox Checkers by means of ethyl alcohol as a solvent (which then was largely evaporated away), two groups of 20 (male and female) rats were fed on this type of pellet to which ethyl alcohol, but no endrin, had been added (and largely evaporated).

The numbers of rats that had died by the 80th and 106th weeks of feeding, are shown according to sex and dietary level in Table VII. Endrin in the diets of female rats at levels of 100, 50, or 25 p.p.m. (but not at 5 or 1 p.p.m.) caused significant mortality (Figure 1). No evidence of such mortality among female rats fed at 25 p.p.m. became apparent after the 80th week. Male rats were less susceptible than female rats to the effects of endrin in their diet, levels of 1, 5, 25, or 50 p.p.m. causing no greater mortality than that encountered in the group of control males (Figure 1), but the value of *P* for the difference in the mean values for those fed at 50 p.p.m. (at 80 weeks) and their comparable controls was only slightly greater than 0.05. At the dietary level of 100 p.p.m., only 5% of the males survived beyond 2 weeks. The distribution of deaths of the female rats, as expressed accumulatively, is shown in Figure 2. Dietary levels of 50 or 100 p.p.m. resulted in the early deaths of all but a few resistant rats.

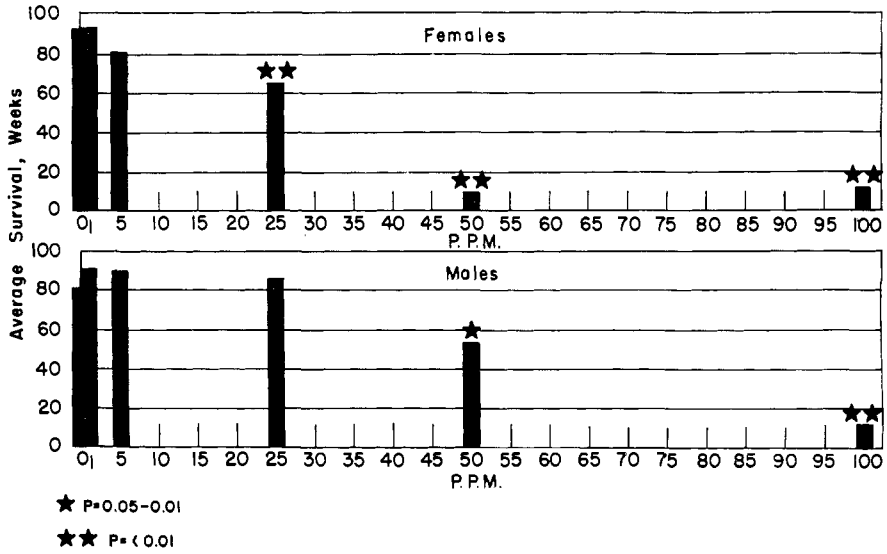


Figure 3. Average length of survival of rats fed on diets containing endrin

The average lengths of survival of rats of either sex fed on diets containing endrin at the level of either 100 or 50 p.p.m., and of female rats fed at 25 p.p.m. were significantly less than that of the corresponding controls (Figure 3). This was not the case when males were fed at 25 p.p.m., or when either sex was fed at 5 or 1 p.p.m. The average lengths of survival of the females fed on diets containing decreasing concentrations of endrin were 11.6, 8.9, 64.7, 81.4, and 93.3 weeks, respectively, in comparison with the value of 92.5 for control females. In the case of the experimental males, the corresponding values were 11.7, 53.3, 86.1, 90.1, and 90.5, and that for the control males was 80.6 weeks.

The average gain in weight, expressed as the percentage of the initial weight, of female rats at any of the three dietary levels (1, 5, or 25 p.p.m.) was either equal to or greater than that of the corresponding controls (Table VIII). This was true also of males fed at either 1 or 50 p.p.m., but the gain in weight on the part of males fed at 25 p.p.m. was significantly reduced, and that of males fed at 5 p.p.m. was retarded during the first 20 weeks only. The curves in Figure 4 represent the average weight at

Table VIII. Average Gain in Weight by Rats Fed on Diets Containing Endrin

	Average Gain, Expressed as % of Initial Weight			
	Males		*Females*	
P.P.M.	*20 weeks*	*40 weeks*	*20 weeks*	*40 weeks*
100	550[a]	683[a]	252[a]	334[a]
50	544	659	323[a]	496[b]
25	436[c]	545[d]	317[c]	385[c]
5	474[d]	589	317[c]	392[c]
1	523	605	275	328
0	524	637	276	318

[a] Represents only 2 rats.
[b] Represents single rat.
[c] *P.* <0.01.
[d] *P.* 0.05 — 0.01.

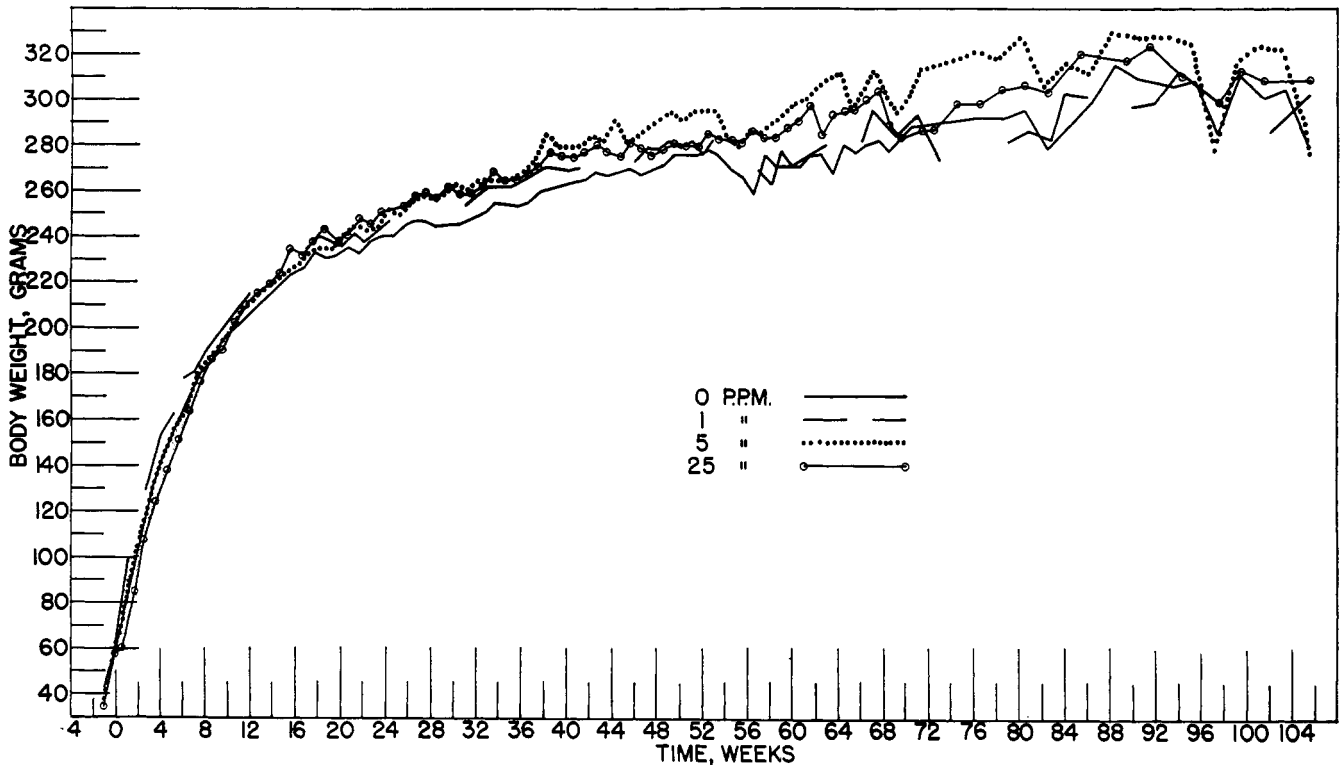


Figure 4. Average weight of female rats fed on diets containing endrin

Table IX. Ratio of Weight of Liver to Body Weight of Rats Fed 2 Years on Diets Containing Endrin

Endrin in Diet P.P.M.	Sex	n	Ratio of Weight of Liver to Body Weight, G./100 G.	P
100	M	2	3.26	[a]
50	M	4	3.08	[a]
25	M	11	3.03	0.02–0.01
5	M	7	3.14	0.05–0.01
1	M	10	2.82	>0.05
0	M	8	2.66	...
25	F	5	3.43	[a]
5	F	8	3.22	>0.05
1	F	11	3.08	>0.05
0	F	7	3.01	...

[a] Too few for statistical determination.

Table X. Fate of Dogs Given Endrin in Diet

(Insecticide introduced into diet 6 days of each week)

Daily Dosage in Relation to Food, p.p.m.	Daily Dosage in Relation to Body weight, mg./kg.	Sex and (No. of Dogs)	Duration of Period of Feeding on Diet Containing Endrin, Months	Fate
50	2.50–4.00	M(1), F(1)	18–20 days	Both died
25	1.21–2.20	F(2)	18–30 days	Both died
5 [a]	0.25–0.36	F(1)	4.7	Died
20 [a]	0.97–1.27			
10	0.49–0.81	M(1), F(1)	24–44 days	Both died
8	0.29–0.62	M(1), F(1)	5.7	One survived
2 [a]	0.09–0.17	M(1), F(1)	9.9	One survived
8 [a]	0.31–0.65			
5	0.20–0.27	M(1)	47 days	Died
4	0.15–0.21	M(1), F(2)	5.7	All survived
3	0.12–0.25	M(2), F(2)	18.7	All survived
1	0.045–0.12	M(2), F(2)	16.4–18.7	All survived
0 [b]	0	M(1), F(1)	18.7	All survived

[a] Smaller dosage given during first portion (2.9 months) of feeding period, larger dosage during remainder of period.
[b] Three additional control dogs survived 5.7 months.

weekly intervals of groups of female rats fed on diets containing endrin at 0, 1, 5, and 25 p.p.m.

After male rats had been fed on diets containing endrin at the levels of either 25 or 5 p.p.m. for 2 years, the average ratios of the weights of their livers to their body weights were significantly greater than that of the male controls (Table IX). This effect was not observed in males fed at 1 p.p.m. or in females fed at either 1 or 5 p.p.m. Too few observations were made upon other groups to provide statistically significant data.

Among rats fed on diets containing endrin at the level of 50 or 100 p.p.m., hypersensitivity to external stimuli, and occasionally convulsions, were noted but these signs were not seen in those fed at 25 p.p.m. or less.

The rats that died while being fed at either of the three higher dietary levels exhibited diffuse degeneration of the brain, liver, kidneys, and adrenal glands. The survivors in the groups fed at the two higher levels showed degenerative changes in the livers only, while those fed at the lower levels had normal viscera. The incidence of neoplasia was no greater among the experimental animals than among the controls.

Dogs. Dogs died when fed on diets containing endrin in concentrations of 10 to 50 p.p.m. (Table X), and more than half of those that were fed 5 to 8 p.p.m. died, but all survived when their diets contained less than 5 parts of endrin per million parts of food. Groups of

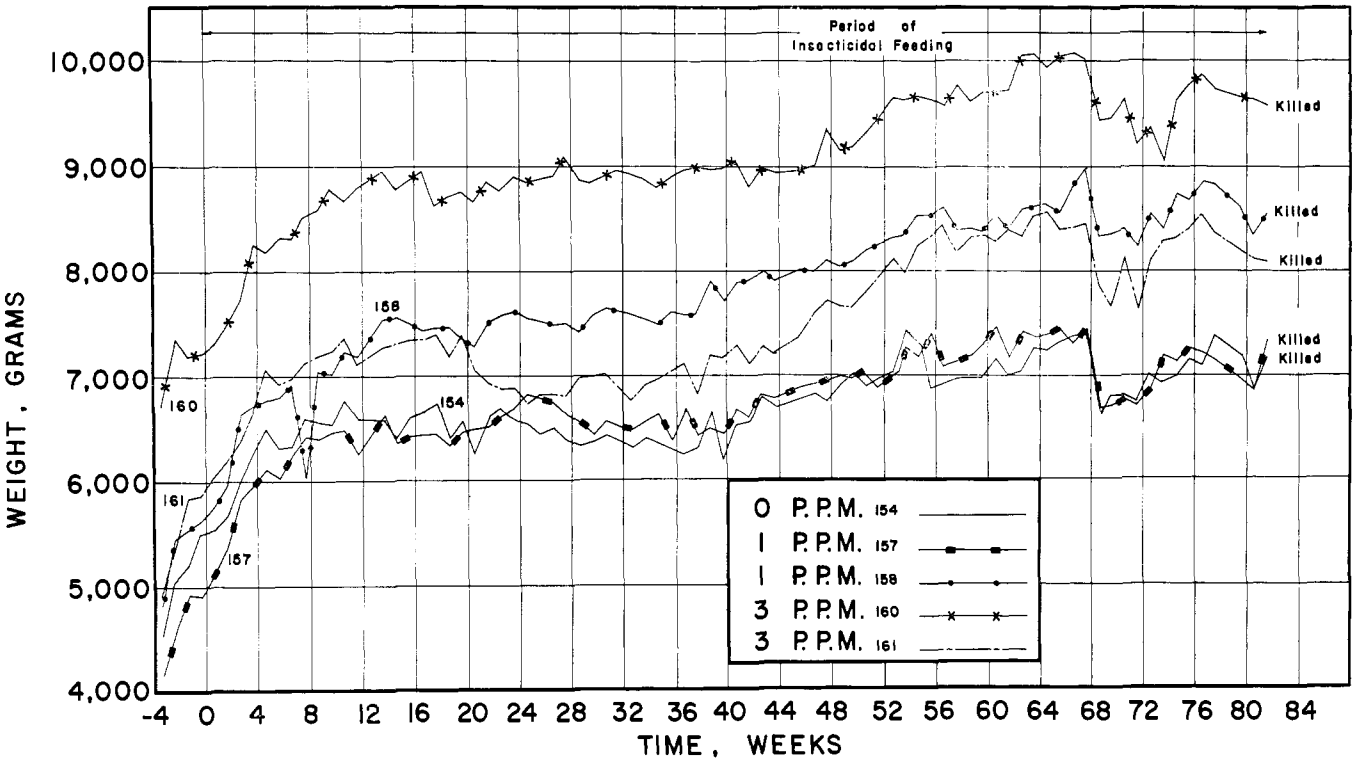


Figure 5. Average weight of male dogs fed on diets containing endrin

four beagles (two males and two females, eligible for registration with the American Kennel Club), survived over periods of 16.4 to 18.7 months, during which they were fed on diets containing endrin at either 3 or 1 p.p.m.

All the dogs that were fed on diets that contained endrin in the concentration of 10 p.p.m. or more suffered extensive losses in weight; those fed at 8 p.p.m. gained during the early period of feeding but eventually lost much of their weight; those fed at 4 p.p.m. did not grow normally, but those at either 3 or 1 p.p.m. grew as well as the control dog.

The average gain in weight (expressed as percentage of the initial weight over the entire period) of male beagles fed at 3 and 1 p.p.m. was 39.8, that of the male control being 23.6%. All the male beagles were 4.7 months of age at the initiation of the feeding; at that time the average weight of the experimental dogs was 6.01 kg., and that of the control male beagle was 5.59. The experimental females fed on similar diets gained 63.2% and the female control gained 20.3%. At the start of the feeding, the average age of the experimental female dogs was 5.0 months (4.1 to 5.6 months), and the female control was 6.7 months of age. Their respective weights at that time were 4.86 and 7.28 kg. The curves in Figure 5 present the weekly changes in weight over the period of feeding of the control male beagle, and of the male fed on endrin at levels of either 3 or 1 p.p.m.

Dogs that died when fed on diets containing toxic concentrations of endrin regurgitated their food, became lethargic, salivated, and later refused to eat. They became emaciated and developed respiratory distress and signs of irritation of the central nervous system (hypersensitivity to stimulation, tremors, twitching, and severe convulsions). Dogs fed at levels of 4, 3, or 1 p.p.m. exhibited no signs of intoxication.

Diets containing endrin in the concentration of 8 p.p.m., when fed for almost 6 months, induced enlargement of the liver, kidneys, and brain and reduction in the deposition of free peritoneal and omental fat of dogs (Table XI). Moreover, after the dogs had been fed for almost 19 months on diets containing endrin at the level of 3 p.p.m., their kidneys and hearts were significantly enlarged (Table XII). On the other hand, the ratios of the weights of the livers, brains, spleens, and fat to the body weight of dogs fed at either 3 or 1 p.p.m. were not significantly different from those of control beagles.

No change occurred in the relative numbers or in the types of the formed elements in the peripheral blood of the dogs fed on diets containing endrin in the concentration of either 3 or 1 p.p.m. over the period of 18.7 months.

Dogs that were fatally poisoned by being fed on diets containing endrin were found to have diffuse degenerative lesions in the brain, heart, liver, and kidneys, together with pulmonary hyperemia and edema. The renal damage was severe and was characterized by diffuse degeneration and necrosis of the

Table XI. Ratio of Weight of Fat and Organs to Body Weight of Dogs Fed Almost 6 Months on Diets Containing Endrin

Endrin in Diet, P.P.M.	n	Grams per 100 Grams Body Weight			
		Liver	Kidneys	Brain	Fat
8	3	3.16	0.52	1.16	0.20
4	3	3.06	0.36	0.92	0.30
0	3	2.66	0.37	0.85	0.43

Table XII. Ratio of Weight of Fat and Organs to Body Weight of Dogs Fed Almost 19 Months on Diets Containing Endrin

Endrin in Diet, P.P.M.	n	Grams per 100 Grams Body Weight					
		Liver	Kidneys	Brain	Fat	Heart	Spleen
3	4	2.92	0.52[a]	0.91	0.45	0.84[a]	0.41
1	4	3.07	0.39	0.91	0.46	0.76	0.33
0	6[b]	2.86	0.42	0.92	0.52	0.67	0.30

[a] P. 0.05 — 0.01.

[b] Includes 2 additional male beagles and 2 additional female beagles, less than 2 months older and also eligible for AKC registration, employed as controls in overlapping experiments.

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

IRWIN HORNSTEIN

Entomology Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md.

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