Mammalian Toxicity of 1-Naphthyl-*N*-methylcarbamate (Sevin Insecticide)

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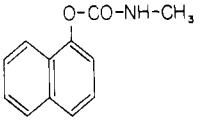
The apparent effectiveness of 1-naphthyl-*N*-methylcarbamate against a wide variety of insects encouraged the study of its mammalian toxicology. Information is presented on metabolism, cholinesterase inhibition, alleviation of symptoms with atropine sulfate and their aggravation by pyridine-2-aldoxime methiodide, and the absence of neuromuscular degenerative potential, carcinogenic activity, and sensitizing propensity. The responses of several species to single doses by oral, parenteral, percutaneous, and respiratory routes are presented. A 2-year rat feeding study demonstrates that 200 p.p.m. in the total dietary is tolerated by this species without significant deviation from suitable controls, while dogs tolerate 400 p.p.m. in their diet on the same basis.

THE INSECTICIDE 1-naphthyl-*N*-methylcarbamate (Crag Sevin, Union Carbide Corp.) is of particular interest because it is a cholinesterase inhibitor, completely free of phosphorus, but possessing greater anticholinesterase activity against insects than against mammals (*10, 11, 14*).

Sevin is a wide spectrum insecticide effective against insects attacking fruit trees, bean and cotton crop insect pests, and forest insects. Performance results give promise of wide use in substantial quantities.

Structural formula of Sevin:



Average analysis of commercial batches of Sevin:

1-Naphthyl-*N*-methylcarbamate	98.0%
1-Naphthol	0.7%
Bis(naphthylcarbonate)	1.0%
Water	0.3%

The technical material is a slightly colored, nearly odorless crystalline solid. Its melting point is 142° C., vapor pressure is less than 0.005 mm. of Hg at 26° C., and density is 1.232 at 20°/20° C. Solubility ranges from 40% in organic solvents to approximately 0.01% in water. It is sparingly soluble in hydrocarbons, somewhat more soluble in chlorinated hydrocarbons and alcohols, moderately soluble in ketones, and quite soluble in dimethylformamide, pyridine, diethanolamine, and dimethylsulfoxide. Methoxy triethylene glycol is a useful solvent for parenteral studies, as it will carry 20% of Sevin. Unless otherwise specified, the term Sevin refers to the technical grade.

Metabolism

To demonstrate the hypothesis that a substantial portion of an oral dose of Sevin would appear in the urine as free or conjugated 1-naphthol, a recovery experiment was performed. Six groups of three rats each were dosed with 0.015 gram of Sevin and urinary naphthol excretion followed 4 days after dosing. Control urine was collected during 48 hours prior to dosing. The collections from each cage were pooled to provide three sets of six samples representing 48 hours after dosing.

The naphthol analyses were made on 0.1-ml. portions of urine diluted to 1.0 ml. Conjugated naphthols were hydrolyzed by heating each sample with 0.1 ml. of 10*N* hydrochloric acid at 100° C. for 1 hour. Neutralization of the hydrolyzates was made with the dropwise addition of 0.5 ml. of 2*M* Na_2CO_3. One milliliter of 0.1*M* $Na_2B_4O_7$ and 10.0 ml. of 1-butanol were added. The color was developed by the addition of 0.2 ml. of *N*,2,6-trichloroquinoneimine (0.5% in 95% ethyl alcohol) to each tube and allowing it to stand 30 minutes. The butyl alcohol extracts were separated by centrifugation for 15 minutes and transferred to cuvettes. Colorimetric readings were made at 620 mμ against a water blank carried throughout the procedure.

There was a slight rise in free and a definite rise in conjugated naphthol in 48 hours following doses of Sevin. Concentrations fell rapidly to values near or below the controls after this period. The excess in the 24- to 48-hour concentration over the control excretion has been calculated in terms of the initial 0.015 gram per rat dose. The mean percentage and the standard error of this mean for Sevin recovered was 22.4 ± 4.4. Expressed in terms of the 1-naphthol content of Sevin, the percentage would be 31.3 ± 6.1. The 1-naphthol was excreted almost completely in the conjugated form possibly as the glucuronide. The observation that less than one third of the 1-naphthol content of Sevin was found in the urine is substantiated by work of others (*2, 3, 5*) involving the administration of 1-naphthol directly to experimental animals.

The fate of the hydrolyzed carbamate amine moiety is not known. No analytical procedure was found that would distinguish methylamine from other amines present, or that would indicate its particular metabolic fate.

Cholinesterase Inhibition

A variety of studies was undertaken to assess the extent of cholinesterase inhibition by Sevin in mammals. The work covered both in vitro and in vivo experiments on several species. In some of the work parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate), which is representative of the organic phosphate-type cholinesterase-inhibiting insecticide, was used for comparison with Sevin.

After Parenteral Doses to Dogs. The initial in vivo studies involved a determination of the inhibitory effects of Sevin. Two groups of three beagle-type dogs each were injected intravenously with 10 and 15 mg. per kg., respectively, of body weight of Sevin as an 8% solution in 95% ethyl alcohol. Ethyl alcohol is a notoriously poor vehicle for intravenous use because it damages blood vessels and denatures protein, but as propylene glycol increased plasma and erythrocyte cholinesterase levels and ethyl alcohol did

not, the latter was used. Heparinized syringes were used to collect blood samples taken several days prior to dosing and at intervals of 0.5 to 1.0, 2.0, 5.0, and 23 hours after the Sevin doses. These blood samples were immediately centrifuged to separate plasma and erythrocytes and individual determinations of the inhibition of cholinesterase were made according to the method of Frawley *et al.* (*9*).

The values expressed in this paper, in terms of percentage inhibition, were measured after a 2-hour incubation of plasma samples and 0.17*M* substrate while a 1-hour period was used with erythrocytes and 0.11*M* substrate. Varying the quantities of substrate from $^1/_4$ to 4 times the amount used for the routine determinations did not appreciably alter the apparent percentage inhibition at the 2- and 1-hour time intervals. In agreement with Burgen (*4*), percentage inhibitions with both plasma and erythrocytes changed markedly within the first 30 minutes of the in vitro incubation, but soon thereafter equilibrium was reached. Burgen points out that readings made after equilibrium may underestimate grossly the extent of cholinesterase inhibition, but results obtained prior to 30 minutes are influenced in a complex manner by both the inhibitor and substrate concentration making the quantitative significance doubtful. Because of these inherent difficulties it was decided arbitrarily to make the measurements after equilibrium was attained. Admittedly, the values obtained are not an exact measure of cholinesterase activity but they do present the relative picture of the effect of the several routes of administration.

Table I presents the results of intravenous dosing. The values are given as mean percentage of the cholinesterase activity of each subject animal prior to dosing. Intravenous administration of ethyl alcohol at levels of 0.125 or 0.1875 ml. per kg. had no measurable effect on the plasma or erythrocyte cholinesterase values. These dosage levels approximated the amount of ethyl alcohol given when the Sevin was administered as an 8% solution in ethyl alcohol. No significant effect was found on erythrocyte or plasma cholinesterase after single dosages of 10 or 15 mg. of Sevin per kg. of body weight had been injected.

Twenty days later one of these dogs was started on daily repeated intravenous doses of variable size and before all accessible vessels were sclerosed by the ethyl alcohol a total of 88.3 mg. per kg. of Sevin in 11 doses was injected. Typical symptoms of cholinesterase inhibition were produced by dosages of 10 and 15 mg. per kg., but only slight reactions by 5 mg. per kg. The greatest depression over predose control values of plasma and erythrocyte cholinesterase occurred after the first four injections consisting of 9.3, 15.0, 6.7, and 15.0 mg. per kg., respectively. On the fifth day the plasma value was depressed 25% and the erythrocyte 40%; on the sixth day, after an additional 5 mg. per kg. dosage, there was a 17% depression of plasma but the erythrocyte cholinesterase level was normal. Urinary 1-naphthol excretion reached a peak on the morning of the third day. It was variable from that time on until the dog died after a rapid intravenous dose by jugular vein following anesthetization with pentobarbital sodium. The jugular vein was used because it was no longer possible to enter the sclerosed leg veins. In the depressed state of surgical anesthesia the additional shock of intravenously administered alcoholic solution of Sevin probably caused the animal to expire. Artificial respiration might have saved this animal had it been given. In our opinion Sevin alone was not responsible for this death. Pathological examination of the internal organs established the fact that no tissue damage had occurred. Cholinesterase determinations on brain tissue from the circle of Willis showed no abnormal value when compared with three parallel determinations on dogs that had never been dosed with a chemical, but had served as controls on another study.

Two more dogs were then started on intravenous injections of 8% Sevin solution in 95% ethyl alcohol. The dosage schedule, following a week of collecting control data, was 10 mg. per kg. per day. This regimen was followed until six doses had been given and all superficial vessels had become inflamed or sclerosed. Sevin was then ground in lard melting at or below 37° C. (body temperature) and injected subcutaneously into the scapular area, at a dosage of 50 mg. per kg. per week, in one injection on each subsequent Monday morning. A total of 60 mg. per kg. of Sevin in ethyl alcohol was given intravenously and 640 mg. per kg. of Sevin subcutaneously in lard, which is a total dosage of 700 mg. per kg. An unsuccessful attempt was made to collect all urine excreted so that 1-naphthol excretion could be followed. This failed because the male dog was notorious for voiding at each injection and frequently when handled otherwise. The female was not so excitable and fairly consistent trends were established for her.

Plasma and erythrocyte cholinesterase levels and blood counts were followed daily the first week, every other day the second week, and twice weekly thereafter. Means, standard deviations, and 95% fiducial limits were calculated for the predose values of each of these criteria.

Plasma cholinesterase values were below the lower fiducial limit range for the female dog on only four of 27 occasions. The inhibition was never more than 20 to 30%. These values occurred 48 hours after the first, second, and fourth subcutaneous injection and 24 hours after the third. The male dog had only one correspondingly low value, 55% inhibition, 48 hours after the third subcutaneous injection. Four other values were above the upper fiducial limit by as much as 20%. The importance of the 55% inhibition is tempered when offset by the four values which exceeded the upper limits by as much as 20%. Neither dog showed any values beyond the fiducial limits as regards erythrocyte cholinesterase, which was determined on these same blood samples.

Blood urea nitrogen was below the lower fiducial limit for the female dog on three separate occasions about a month apart with normal values intervening. They are not, therefore, interpreted as indicating any significant trend.

Total 1-naphthol in the urine of the female dog exceeded the upper fiducial

Table I. Mean and Range of Percentage of Predose Values

Intravenous administration of ethyl alcohol or Sevin in ethyl alcohol to groups of 3 dogs each

Hours, after Dose	Plasma Cholinesterase		Erythrocyte Cholinesterase	
	95% Ethyl Alcohol, Ml./Kg.			
	0.125	0.1875	0.125	0.1875
0.7	101 (100 to 101)	99 (98 to 100)	103 (98 to 106)	97 (91 to 100)
2.5	99 (96 to 102)	98 (96 to 100)	95 (92 to 99)	95 (91 to 104)
5.0	102 (100 to 103)	96 (93 to 98)	100 (94 to 107)	94 (84 to 106)
23.0	99 (96 to 102)	95 (94 to 96)	99 (98 to 100)	96 (82 to 110)
47.0	104 (101 to 106)	100 (100 to 101)	106 (94 to 113)	100 (74 to 120)
95.0	100 (99 to 101)	98 (95 to 101)	105 (98 to 111)	98 (82 to 114)
	8% Sevin in Ethyl Alcohol, Mg./Kg. of Sevin			
	10.0	15.0	10.0	15.0
0.5 to 1.0	82 (74 to 86)	96 (90 to 102)	88 (78 to 100)	97 (80 to 109)
2.0	87 (77 to 99)	87 (78 to 98)	111 (105 to 120)	102 (81 to 120)
5.0	93 (82 to 98)	97 (87 to 104)	112 (107 to 116)	104 (100 to 109)
23.0	95 (90 to 102)	98 (91 to 103)	117 (108 to 127)	113 (105 to 120)

limit 24 to 48 hours after the Monday morning 50-mg.-per-kg. subcutaneous injection of Sevin in lard in 9 of the 13 weeks it was injected. The urine collection from the male was so erratic that the highs cannot be documented, but the same pattern of increased 1-naphthol in urine 24 to 48 hours after the injection was found.

It is unlikely that Sevin had any marked effect upon the blood picture of these dogs. Both were troubled with subcutaneous abscesses at the site of these injections. This was reflected in leucocyte counts that were elevated to over 26,000 cells per cubic mm. for the female and 21,000 for the male, but hematocrits, reticulocyte enumerations, and complete differential white blood cell counts fell within the predose fiducial limits.

The fact that the female of this last pair received 12.6 grams of Sevin by injection over a 3-month period without any serious effect on cholinesterase levels in blood or in plasma and without weight loss is convincing evidence that repeated dosing with Sevin is well tolerated in the dog.

After Single Peroral Doses of Sevin and Parathion. The relative inhibition of Sevin and parathion on the in vivo cholinesterase activity in plasma, erythrocytes, and brain tissue was compared. Each insecticide was administered by stomach intubation in corn oil solution to groups of seven to 10 rats at a dosage slightly above their respective LD_{50}'s—namely, 0.56 gram per kg. of Sevin and 0.0093 gram per kg. of parathion. LD_{50} determinations made immediately prior to this study were 0.50 and 0.007 gram per kg. for a 5% suspension of Sevin and a 0.034% solution of parathion in corn oil.

The rats were anesthetized with 0.1 ml. of pentobarbital sodium given intraperitoneally immediately prior to killing, which was done at intervals of 0.5, 4, and 24 hours after dosing. Blood for plasma and erythrocyte cholinesterase determinations was withdrawn by heart puncture using heparin as an anticoagulant. The rats were then decapitated and the brain was removed and divided across the arterial circle of Willis. The anterior portion, including the optic lobes, was transferred to a small amount of distilled water, then blotted, weighed, and homogenized in 50 times its weight of water. An aliquot was analyzed for cholinesterase activity.

The cholinesterase response of rats following a single dose of Sevin or parathion is summarized in Table II. Plasma cholinesterase was not significantly depressed by these large oral doses of Sevin. On the other hand, parathion produced a 30 and 43% inhibition of this nonspecific pseudocholinesterase at 4 and 24 hours after dosing.

The pattern of inhibition of true cholinesterase in the cellular components of blood was different for Sevin and parathion. Sevin caused a 42% inhibition of erythrocyte cholinesterase in 0.5 hour which returned essentially to normal within 24 hours after dosing. Parathion resulted in a 13% inhibition in 0.5 hour, 45% in 4 hours, and 75% in 24 hours.

Similarly, brain cholinesterase values were initially depressed about 30% by Sevin at 0.5 hour, but were back to normal in rats that survived 24 hours after this dosage. While parathion depressed brain cholinesterase only 6% at 0.5 hour, a mean value of 85% inhibition was measured 24 hours after dosing.

Therefore, while the depression of all three cholinesterase systems was slower but definitely progressive after oral administration of parathion, no important depression of plasma cholinesterase was produced by Sevin, and only slight but significant and transitory inhibition of erythrocyte and brain cholinesterase was found.

Control of Symptoms by Atropine Sulfate. Six female mongrel dogs, averaging 8 kg. in weight, were force-fed dry Sevin in gelatin capsules as follows: One dog received 0.5 gram per kg., four dogs 0.375, and one dog 0.25. The dogs on the higher levels showed the classic symptoms of overstimulation of the parasympathetic nervous system. The animal that received 0.5 gram per kg. was extremely weak 24 hours after dosing and refused food for 5 days. No weight gain was made during a 14-day observation period. Cholinesterase levels were not followed on this animal. At 0.25 gram per kg. there was no adverse physiological response.

Plasma cholinesterase inhibition was insignificant in the four dogs dosed at 0.375 gram per kg., but some erythrocyte cholinesterase inhibition was seen in three of them with the greatest drop occurring in 2 to 3 hours. One dog was completely refractory. The maximum inhibition of erythrocyte cholinesterase during the first 3 hours for the other three dogs was only 33, 27, and 24%. After 7 hours these values were 30, 14, and 12%, and after 24 hours 14, 3, and 0%, respectively. A log of the symptoms is given in some detail to furnish a picture of the syndrome. For 15 to 30 minutes the dogs were quiet, then salivation and respiratory rate increased. Lacrimation, urination, defecation, and muscular twitching ensued during the interim to 90 minutes. The muscle tremor increased and one dog had a mild convulsion. All had constriction of pupils, profuse salivation, poor coordination, diarrhea, further increase in respiratory rate, and loss of bladder control after 2 to 2.5 hours. Three hours after dosing the animals vomited mucus and thick saliva and had violent intestinal movements, weakness, and considerable muscular spasm. After 5 to 5.5 hours the animals were quieter but lacrimation, salivation, slight constriction of the pupils, poor coordination, muscular twitching, and occasional vomiting of mucus persisted. After 7 hours pupils were almost normal, salivation decreased, and coordination improved. The following day their appearance was good with no adverse peripheral or central nervous symptoms notable.

About 2 months later the dog that had been most refractory to plasma and erythrocyte cholinesterase inhibition was given a second dosage of 0.375 gram per kg. of Sevin. A total dose of 10 mg. of atropine sulfate was given intramuscularly at onset of symptoms 1.5 hours after dosing, followed by 5-mg. doses given at 2.5 and 7 hours after dosing. Symptoms were controlled well by these doses.

One of the two dogs that previously gave an intermediate anticholinesterase response again was given 0.375 gram per kg. of dry Sevin in a capsule and treated with a dose of 40 mg. of pyridine-2-aldoxine methiodide (PAM) plus 10 mg. of atropine sulfate after onset of symptoms. This combination failed

Table II. Cholinesterase Response of Rats

Single peroral dose of 0.56 gram/kg. of Sevin or 0.0093 gram/kg. of parathion

Cholinesterase	Time After Dose, Hours	Sevin-Dosed Rats: No.	Sevin-Dosed Rats: Mean % of control	Sevin-Dosed Rats: Std. dev.	Parathion-Dosed Rats: No.	Parathion-Dosed Rats: Mean % of control	Parathion-Dosed Rats: Std. dev.	Sevin Mean Minus Parathion Mean	p for t Test
Plasma	0.5	9	94.9	22.8	8	91.0	14.8	+ 3.9	0.70
	4	8	86.5	19.8	8	69.6	7.8	+16.9	0.06
	24	9	92.9	15.6	9	57.1	10.3	+35.8	<0.0001
Erythrocyte	0.5	8	57.9	24.0	8	86.9	15.2	−29.0	0.01
	4	7	69.1	22.6	8	54.6	18.5	+14.5	0.20
	24	9	86.1	15.5	9	25.2	13.8	+60.9	<0.0001
Brain	0.5	9	70.0	10.0	8	93.9	8.2	−23.9	<0.0001
	4	8	80.8	12.9	7	83.6	23.3	− 2.8	0.80
	24	9	91.2	13.5	10	15.1	9.3	+76.1	<0.0001

to control symptoms, but subsequent doses of 5 and 10 mg. of atropine sulfate without PAM provided control.

Following these trials, about 2.5 months later the same two dogs were given 0.795 gram per kg. of Sevin orally and symptoms were controlled with three doses of 20, 10, and 10 mg. of atropine sulfate per animal following the same time intervals as given above. Plasma and erythrocyte cholinesterase inhibition was on the order of 25% in the former and 10 to 20% in the latter 5 hours after dosing.

Two weeks later these two dogs were dispatched 6.5 hours after a dosage of 1.0 gram per kg. of Sevin. Inhibition had reached 75 to 90% in plasma and erythrocytes in 5 hours and roughly 100% in the superior, middle, and/or inferior gyri of the brain. Brains from several undosed dogs were used to establish control values for the brain cholinesterase determinations.

Histopathological examination was made on sections of the lung, liver, gastrocnemius muscle, sciatic nerve, brain, and kidneys of these two dogs. In the kidney numerous globules of fat were diffusely distributed in the epithelial cells of the straight proximal tubules found within the medullary ray. These globules were Sudan IV positive. Hematoxylin- and eosin-stained frozen sections of the kidney showed a pronounced diffuse cloudy swelling of the proximal convoluted and loop tubules with little exudate within their lumens. All other organs were normal. The second animal treated with PAM and atropine had, in addition to the same type of kidney findings, some glycogen deposited about the central veins of the liver and a mild focal cloudy swelling of the hepatic cords about the portal veins. The gastrocnemius muscle was normal except for a single focal reaction which showed infiltration of the muscle tissue by lymphocytes.

Groups of five female, 90- to 120-gram Carworth Farms-Nelson (CF-N) rats, were given 0.4 and 0.8 gram per kg. of Sevin orally and treated 1.5 hours later with atropine sulfate in 0.9% saline intraperitoneally. A dosage of 25 mg. per kg. of atropine sulfate was required to control symptoms effectively in the rats on the lower dosage and 50 mg. per kg. on the higher lethal level for rats. All animals gained weight during the 14-day observation period.

With a lethal peroral dose of Sevin, 5 rats died after 20 mg. per kg. of PAM was given intravenously, but a comparable dosage of atropine sulfate saved a similarly dosed group of 5 rats. Five untreated controls died.

Four rabbits given 1.0 gram per kg. of Sevin as a 2% suspension in 0.25% agar were saved by the intramuscular administration of 30 mg. of atropine sulfate 1 hour after dosing and 10 mg. 3 and 7 hours after dosing. Six rabbits that received Sevin at 1.0 gram per kg. without atropine sulfate died, while two that received only 30 mg. of atropine sulfate showed no symptoms during the day of dosing but died the following night. Symptoms were comparable to the type described for the other species.

Special Studies

Neuromuscular Degenerative Potential of Sevin in Chickens. The neuromuscular degenerative potential of Sevin was compared with that of triorthocresyl phosphate (TOCP) by single subcutaneous injections in 2-year-old moulting Rhode Island Red hens. These chemicals were mixed or suspended in lard melting at or below 37° C. and administered at 3.0-, 2.0-, 1.0-, 0.50-, and 0.25-gram-per-kg. dosage levels at concentrations of 25 to 40%. In addition, undiluted TOCP was given to one chicken and undiluted lard to two others. Five control chickens received no injections. The subcutaneous route was chosen because no satisfactory vehicle was then known for the intravenous administration of Sevin.

Chickens that received 1.0 gram per kg. or less of Sevin developed no symptoms of leg weakness. At 2.0 grams per kg. weakness was observed on the first or second day following dosing and in only one case was the chicken nonambulant for 3 days. This is evidence of a transient cholinergic effect caused by the slow absorption of the Sevin from its subcutaneous locus. According to Barnes and Denz (*1*) nerve injury from the organophosphates occurs 8 to 10 days or more after dosing and permanent weakness results. TOCP, at 3.0 grams per kg., proved lethal in 4 to 10 days with leg weakness observed in three of four cases only on the day of death. At all lower dosage levels weakness was not apparent until the 13th or 14th day. One of these hens was held 8 days after weakness was observed and it showed no sign of recovery. One chicken received 1.0 ml. per kg. of TOCP undiluted, and in this case also the first leg weakness was noted after 14 days.

Employing the Marchi technique, no evidence of demyelination was observed in the limited number of brain, sciatic nerve, or spinal cord sections examined microscopically from any of the 13 Sevin hens and only slight evidence of degeneration was present among three of the 10 which had been injected with TOCP. Apparently the action of Sevin may be described as cholinergic rather than demyelinating.

A nephrotoxic action was observed in fowl which received 2.0 grams per kg. or a larger injection of Sevin. This was apparent as a deposition of fine fat droplets within the epithelial cells lining the proximal tubules. Necrosis was not present. At all dosage levels tested, animals injected with TOCP showed fatty deposition of a similar but more diffuse nature involving loop as well as convoluted tubules.

Focal loss of striation and fatty infiltration of gastrocnemius muscles were observed only at the 3.0-gram-per-kg. dosage level with Sevin. Such lesions were present at all levels in hens examined after injection with TOCP with the inexplicable exception of the 2.0-gram-per-kg. dosage.

Carcinogenic Potential. Subcutaneous injections were given once a week to male mice during their third to eighth month of age after which a gross examination was made for lung tumors. A/Jax and C3H mice from Roscoe Jackson Memorial Laboratory were used. Males of the former strain have a high rate of spontaneous lung tumor while females of the latter have a high rate of spontaneous mammary tumor formation. If injections are continued for a longer interval, the appearance of spontaneous tumors clouds the picture.

Technical Sevin was suspended in 0.25% agar at a concentration of 5.0%. The weekly injection consisted of 0.2 ml. of suspension containing 10 mg. of Sevin. One control group received injections of 0.25% agar, and another was untreated. Subcutaneous injection of Sevin did not increase the incidence of tumors, lung infection, or mortality (Table III).

Studies of Single Dose Toxicity

Single Peroral Doses. Groups of five CF-N nonfasted rats, 5 to 6 weeks of age and 90 to 120 grams in weight, were dosed at levels differing by a factor of 2.0 in a geometric series. The rats were reared in our own colony and maintained from time of weaning on Rockland rat diet complete (A. E. Staley Co.). The method of moving average (*16*) for calculating the median-effective dose (LD_{50}) was applied to the 14-day mortality data for this and all other similar assays. Table IV gives the results

Table III. Results of Subcutaneous Injection of 5% Sevin in Agar to Groups of 30 Male Mice

20 Doses, One per Week, 0.2 Ml.	Mice Survived to sacrifice	Mice With tumorous lungs	Mice With lung infections
A/Jax strain			
5% suspension	26	4	3
Agar control	26	5	3
Untreated	26	6	2
C3H strain			
5% suspension	28	0	3
Agar control	29	0	4
Untreated	26	0	2

of typical LD_{50}'s based on dosing either five rats, five guinea pigs, or four rabbits per level, or in the case of dogs and cats, the number dying over number dosed when an LD_{50} cannot be calculated from data at hand.

Cats are the most sensitive species by this route, with guinea pigs, rats, and rabbits showing more resistance in that order. Sevin induces the vomiting reflex in dogs 2 to 4 hours after a capsule dose, but by this time most of the material has been absorbed and the vomitus contains only small amounts of the compound. Dogs tolerated as much as 0.5 gram per kg. without atropine sulfate and 0.795 gram per kg. with the drug. Peroral toxicity for rats may be increased slightly by the incorporation of surfactants or by the substitution of corn oil as a vehicle in place of semisolid agar.

Peroral Joint Action. Sevin was dosed simultaneously with certain organic phosphate insecticides and unrelated pesticides to determine if less than additive, simple additive, or greater than additive effects would result.

Ten organic phosphate insecticides and 14 pesticides that were currently available on the open market were given individually in single dose by stomach intubation to CF-N albino rats in corn oil to determine their LD_{50}'s. The rats weighed 90 to 120 grams and were not fasted prior to dosing. Mortality data were based on a 14-day observation period. Each of these 24 compounds then was fed jointly with Sevin. The concentration in corn oil of each constituent of each pair was kept the same as it was for the individual LD_{50} determination. This was found necessary because a change in concentration with dosage kept constant can change mortality radically. Groups of five females per dosage level were used for the organic phosphates while males were used for the unrelated pesticides.

The predicted LD_{50} for each pair was calculated as follows:

$$\text{Predicted } LD_{50} = \frac{1}{\frac{P_1}{LD_{50_1}} + \frac{P_2}{LD_{50_2}}}$$

where P_1 = proportion of material 1 in combined solution

P_2 = proportion of material 2 in combined solution

therefore, the predicted LD_{50} of the Sevin-EPN pair equals:

$$\text{Predicted } LD_{50} = \frac{1}{\frac{0.97656}{0.561} + \frac{0.02344}{0.023}} = 0.362$$

$$\frac{\text{Predicted}}{\text{Observed}} = \frac{0.362}{0.256} = 1.4$$

Parallelism of regressions is implied in the use of the above formula, but this could not be tested, as only five rats were dosed per level. Some deviation, therefore, from the similar joint action predicted value is expected

Remarkable agreement with prediction was obtained (Table V). The ratios of the predicted joint LD_{50} to the observed LD_{50} of Sevin plus each of the organic phosphate insecticides ranged from 0.7 to 1.5 and for the unrelated pesticides from 0.4 to 1.2. Arbitrarily, in this laboratory, results are considered in the range of simple additive effects if the ratio is between 0.5 and 2.0, if greater than additive the ratio will be >2.0, and if less than additive <0.5. To become important enough for consideration or concern, values must be well below a ratio of 0.5 for antagonists or above 2.0 for potentiators. In any case, there was no evidence of Sevin's causing what might be termed potentiation or antagonism.

Single Parenteral Doses. Intravenous toxicity of Sevin was determined by tail vein injection of rats with the compound in 95% ethyl alcohol, propylene glycol, or polyethylene glycol 400. This was necessitated because of the relative insolubility of Sevin in water. It is not a certainty that Sevin is completely unreactive with these vehicles, but in no case would the vehicles per se be implicated as important contributors to the intravenous toxicity, because their dosage when used as vehicles never exceeded one fourth of their undiluted LD_{50} values. Table IV contains the results which are in good agreement.

Subcutaneous injection of Sevin into the loose folds of skin over the scapular area of 90- to 120-gram rats resulted in an LD_{50} of 1.4 grams per kg. as a 25% suspension in lard. Yearling males, 450 grams or more in weight, tolerated 2.0 grams per kg. of a 40% suspension in lard. Injection of 2-year-old moulting Rhode Island Red hens with 25 to 40% suspensions in lard under the loose skin of the neck and the wing resulted in an approximate LD_{50} of 2.0 grams per kg. Intraperitoneal administration of a 5% suspension in 0.25% agar to male albino New Zealand rabbits produced an LD_{50} of 0.223 gram per kg.

The parenteral results fall in line despite the use of three different species in the assays. It was obvious from the work on chickens that the compound is poorly absorbed from a subcutaneous locus. The various routes are separated roughly by a factor of 10 in an orderly progression of decreasing toxicity from intravenous to intraperitoneal to subcutaneous.

Single Percutaneous Doses. Male albino New Zealand rabbits, 3 to 5 months of age and averaging 2.5 kg. in weight, were immobilized during a 24-hour skin contact period. Thereafter, the Vinylite sheeting used to retain the Sevin in contact with the clipped skin of the trunk was removed and the animals were caged for the remainder

Table IV. Single Dose Toxicity of Sevin

Species	Weight Range, Grams	Concn., 1 Ml. = Gram in Vehicle	LD_{50} and Range, or Mortality Ratio as Contained Sevin, G./Kg.	
		ORAL		
Cat, female	1800 to 3000	0.02 in 0.25% agar	0.25	2 of 2
			0.125	0 of 1
Guinea pig	600 to 900	0.05 in 0.25% agar	0.28	(No range)
Dog	6750 to 9800	Powder in gelatin capsule	0.795	0 of 2[a]
			0.5	0 of 1
			0.375	0 of 4
			0.25	0 of 1
Rat	90 to 120	0.05 in 0.25% agar	0.51	(0.39 to 0.67)
Rat, female	90 to 120	0.05 in 0.25% agar	0.61	(0.49 to 0.75)
Rabbit	2400 to 3200	0.05 in 0.25% agar	0.71	(No range)
		INTRAVENOUS		
Rat, female	90 to 107	0.05 in propylene glycol	0.018	(No range)
Rat	92 to 126	0.05 in PEG 400	0.024	(0.017 to 0.033)
Rat	90 to 120	0.08 in 95% ethyl alcohol	0.033	(0.026 to 0.041)
		INTRAPERITONEAL		
Rabbit	2686 to 3544	0.05 in 0.25% agar	0.223	(0.122 to 0.407)
		SUBCUTANEOUS		
Rat	90 to 120	0.25 in lard melting below 37° C.	1.41	(1.02 to 1.95)
Rat	465 to 572	0.40 in lard melting below 37° C.	2.0	0 of 5
Chicken, female	1614 to 3300	0.25–0.40 in lard melting below 37° C.	3.0	3 of 4
			2.0	3 of 6
			1.0	0 of 1
Rabbit	2652 to 2980	0.25 in water	2.0	0 of 2

[a] Symptoms controlled with atropine sulfate.

of the 14-day observation period. The rabbits were procured locally and maintained on Rockland rabbit ration.

The precise toxicity of Sevin could not be demonstrated by dermal application because of its insolubility in suitable vehicles, and by the fact that 20 ml. per kg. is the limiting dosage for this test. A dosage of 5.0 grams per kg. of 50% wettable powder applied as a 40% aqueous suspension, which is 2.5 grams per kg. of Sevin, allowed the survival of three of four rabbits that received the dosage by skin penetration.

Sevin 85 S, a microfine wettable powder containing 85% active agent by weight, became available recently. Although 5.0 grams per kg. applied as a 25% aqueous suspension was not lethal to eight rabbits, two of four died at 2.5 grams per kg. and one of four at 1.25 grams per kg. Deaths were preceded by the typical symptoms of overstimulation of the parasympathetic nervous system. Because of the appearance of this syndrome the deaths that occurred at the lower levels must be recognized. The considerable individual variability among rabbits in skin thickness, hair pattern, and susceptibility to cholinesterase inhibitors is believed to account for these deaths. Two different samples were checked to ascertain that no chemical change was responsible.

Four rabbits received 85 S at 5.0 grams per kg. of a 50% suspension in dimethyl phthalate by 24-hour covered applications to the skin. They had no visible ill effects during the 14-day observation period. Another group of four rabbits received the maximum possible dosage of an 8% Sevin solution in 95% ethyl alcohol with resulting mortality indistinguishable from that of an equal volume of the alcohol alone—that is, one of four died at 1.4 grams per kg.

Single and Repeated Inhalation. Six guinea pigs inhaled 50% Sevin wettable powder of 15 microns average particle size, for 4 hours at a concentration of 390 (344 to 722) mg. per cubic meter and gained weight normally during the subsequent 2-week observation period. There was evidence of nasal and ocular irritation and autopsies performed after 14 days disclosed healed hemorrhagic areas in the lungs. This concentration is a dense dust cloud visible to the naked eye.

A group of six guinea pigs inhaled a mean of 230 mg. per cubic meter of Sevin 85 S, average particle size 5 microns, range <1 to 10 microns, during a 4-hour period. In the ensuing 14-day observation period the animals showed a slight weight decrease but regained their pretreatment weight by the end of this interval. Another group of five survived after 4 hours in a mean concentration of 332 mg. per cubic meter of the same dust.

Because of the enormously increased surface area presented by micronized Sevin 85 S dogs were placed in a dust concentration on the order of 75 mg. per cubic meter. Within 5 hours typical symptoms attendant upon cholinesterase inhibition were seen. Attention is called to this phenomenon even though this microfine material is not marketed for crop dusting. A much coarser material diluted with 90% of inerts will be available for dusting purposes.

Repeated inhalation of Sevin 85 S by rats results in no mortality nor grossly visible injury among rats that inhaled 10 (5 to 20) mg. per cubic meter 7 hours per day, 5 days per week, for a total of 90 inhalation periods.

Skin and Eye Effects

Skin Irritation. Primary skin irritation was evaluated by the application of 0.01 ml. of a solution to the clipped skin of the belly on five rabbits and noting the most severe reaction produced within 24 hours. Technical Sevin applied at a concentration of 10% in acetone produced no irritation whatever. Most of the Sevin went into solution but a slight turbidity remained at this concentration.

Skin Sensitization. Four of a group of 16 male albino guinea pigs were weakly sensitized by a test consisting of eight intracutaneous injections (three per week on alternate days) of 0.1 ml. of a 0.1% dispersion of Sevin in 3.3% propylene glycol made up in 0.75% NaCl in double distilled water. A 3-week incubation period was followed by a challenge dose and examinations for possible sensitization were made 24 and 48 hours thereafter.

Corneal Injury. Corneal injury was evaluated by applying a series of volumes

Table V. Peroral Joint Action of Insecticide Sevin with 10 Organic Phosphates and 14 Unrelated Pesticides

Material Trade-mark	Company	Labeled Percentage of Active Agent	LD_{50} G./Kg., Active Agent	1 Ml. = X G., Active Agent	Predicted LD_{50}	Observed LD_{50}		Predicted / Observed
			SEVIN + ORGANIC PHOSPHATES TO FEMALE RATS					
Sevin	Union Carbide	100.0	0.561	0.05	Used with first 10 entries to obtain results			
Diazinon	Geigy	95.0	0.354	0.0063	0.354	0.536	(0.384 to 0.748)	0.7
EPN	Du Pont	82.8	0.023	0.0012	0.362	0.256	(0.186 to 0.353)	1.4
Guthion	Chemagro	100.0	0.024	0.0007	0.429	0.336	(0.206 to 0.548)	1.3
Malathion		95.0	2.59	0.05	0.922	0.988	(0.708 to 1.378)	0.9
Methyl Parathion		79.8	0.011	0.0006	0.352	0.433	(0.330 to 0.569)	0.8
OMPA		100.0	0.011	0.0005	0.375	0.283	(0.205 to 0.390)	1.3
Parathion		98.0	0.0081	0.0003	0.399	0.308	(0.249 to 0.381)	1.3
Phosdrin	Reichhold	60.0	0.0065	0.0002	0.419	0.283	(0.175 to 0.457)	1.5
Systox	Chemagro	26.1	0.0092	0.0006	0.329	0.328	(0.209 to 0.514)	1.0
Trithion	Stauffer	93.2	0.032	0.006	0.202	0.187	(0.134 to 0.260)	1.1
			SEVIN + PESTICIDES TO MALE RATS					
Sevin	Union Carbide	100.0	0.31	0.05	Used with 14 entries below to obtain results			
Chlordane		45.0	0.49	0.01	0.328	0.57	(0.26 to 1.24)	0.5
Crag Fly Repellent	Union Carbide	100.0	17.2	0.50	2.65	3.54	(no range)	0.8
Crag Mylone	Union Carbide	85.0	0.325	0.01	0.312	0.500	(0.319 to 0.784)	0.6
Crag Glyodin	Union Carbide	34.0	4.23	0.50	1.86	2.68	(1.92 to 3.74)	0.7
Crag Herbicide I	Union Carbide	90.0	0.616	0.05	0.411	0.812	(0.619 to 1.07)	0.5
Crag Herbicide DCU	Union Carbide	73.0	4.76	0.10	0.823	1.87	(1.34 to 2.60)	0.4
DDT		100.0	0.406	0.01	0.321	0.268	(0.174 to 0.412)	1.2
Dieldrin		18.6	0.142	0.01	0.258	0.650	(0.495 to 0.853)	0.4
Fermate	Du Pont	76.0	4.92	0.20	1.23	2.46	(1.88 to 3.23)	0.5
Lethane 384	Rohm and Haas	50.0	1.19	0.10	0.061	1.13	(0.707 to 1.81)	0.5
Lindane		99.0	0.107	0.01	0.234	0.283	(no range)	
Lime Sulfur		30.0	2.14	0.05	0.542	1.54	(1.25 to 1.91)	0.4
Thanite	Hercules	82.0	2.14	0.10	0.721	0.933	(0.669 to 1.30)	0.8
Toxaphene	Hercules	100.0	0.123	0.01	0.246	0.373	(0.268 to 0.521)	0.7

of undiluted fluid samples, or an excess (0.5 ml.) of solutions of different concentrations, to the cornea of one eye on each of five rabbits. Immediate responses were observed and 24 hours later the effects were scored after the application of 5% aqueous fluorescein stain which is retained preferentially by dead corneal cells after thorough rinsing with water. An injury scoring 5.0 points or more was characterized as severe. This corresponds to dense fluorescein staining of more than $^5/_8$ of the corneal area. Technical Sevin applied in excess as a 10% suspension in propylene glycol produced only a mild injury on one of five eyes. A 25% aqueous suspension of the microfine material caused no injury and 50 mg. of the dust caused only traces of corneal necrosis.

Cataract. Cataract from 2-naphthol has been reported in rats (7) feeding on diets containing the free naphthol. Because conjugated 1-naphthol is formed by the metabolism of Sevin in the body, the eyes of rats were examined for cataracts after 419 and 719 days of the 2-year feeding study reported below. No lens abnormalities were found by means of hand slit-lamp examination of 142 rats at 419 days. After 719 days on the Sevin diet the 87 survivors were examined. One rat at 0.02% had eye inflammation; one at 0.01% had a cataract. The rats on the highest and lowest dosage levels, 0.04 and 0.005%, had no eye pathology. This single cataract is judged to be of no significance.

Chronic Feeding Studies

Two-Year Rats. Partial hydrolysis of Sevin to 1-naphthol in rat stomach contents was demonstrated before the feeding study was started. Therefore, inclusion in the diet subjected the animal to daily doses of Sevin and its hydrolysis products including 1-naphthol. Prior to starting the lifetime feeding of Sevin to rats, groups of 10 were fed 0.225% (2250 p.p.m.) or 0.15% (1500 p.p.m.) in their diet for 96 days. The 0.225% level produced a decrease in body weight of females, an increase in liver weight as percentage of body weight of males, and an increase in kidney weight of females. All of these deviations were statistically significant when compared with the appropriate controls. Appetite was not affected and only minor pathology in the form of diffuse cloudy swelling of the kidney tubules was noted in four animals at the higher level. At the 0.15% level there was no evidence of organ damage and only kidney weights of the females were significantly increased. Perhaps this is because dosage for females in terms of grams per kilogram of body weight is roughly 30% greater than for males on the same dietary levels during a 90-day study.

Procedure. For the 2-year study, CF-N rats born within a 5-day period were procured, weighed, and observed weekly until distributed randomly into groups at 60 days of age. Only rats whose body weights were within plus or minus two standard deviations from the mean for their sex were accepted. Groups of 20 males and 20 females, four of one sex per cage, were maintained on ground Purina Laboratory Chow which was intimately mixed in a Readco Vertical Batch Mixer with dry Sevin in concentrations of 0.04, 0.02, 0.01, 0.005, or 0.00%. Feed and water were furnished ad libitum in containers assigned to one specific cage in order to avoid transfer of infections. Food consumption records were kept for each cage on a 28-day basis. Rats were weighed at 2-week intervals until 1 year of age and every 4 weeks thereafter.

After periods of 6, 9, and 12 months, 4, 6, or 8 rats of each sex, randomly selected from concurrently maintained auxiliary groups, were killed to provide organ weight comparisons and tissue for histopathological examination. Mortality and life span data were not influenced because extra animals in excess of the original 40 per level were provided for this purpose. The survivors of the original groups were killed after 732 to 736 days on Sevin diets. All rats were critically examined at each weighing and animals that became moribund or that continually lost weight were killed to provide suitable tissue for microscopic diagnosis of the underlying cause of the malady. The pathologist who interpreted the micropathology performed the autopsies and collected the tissue specimens from all animals that died. The gross examination included a thorough search of the cranial, thoracic, and abdominal cav-

Table VI. Mean Results for Maximal 2-Year Feeding of Sevin in Diet of Rats

	Sevin Concentration, %									
	Male Rats					Female Rats				
	0.04	0.02	0.01	0.005	0.000	0.04	0.02	0.01	0.005	0.000
Dosage, g./kg./day	0.0156	0.0079	0.0040	0.0020	0.0000	0.0198	0.0096	0.0046	0.0024	0.0000
G. of diet eaten/rat/day	17.77	17.92	18.33	17.67	19.15	13.34	12.83	12.80	13.51	13.44
Body weight gain of rats during first year of doses	205.5	219.3	223.5	219.7	224.3	98.7	98.2	99.5	106.4	96.6
Maximum body weight[a]	543.4	545.8	567.6	547.3	557.8	318.9	316.4	332.1	347.8	318.8
Liver wt. as % of body wt.										
6 mo.	3.33	3.20	...	...	3.28	3.58	3.12	...	...	3.08
9 mo.	3.22	3.08	...	...	3.08	3.40	3.18	...	...	3.35
12 mo.	3.28	3.39	3.20	3.21	3.19	3.06	3.49	3.40	3.45	3.18
24 mo.	3.05	3.03	2.95	3.19	2.92	3.92	3.52	3.46	3.25	3.60
Kidney wt. as % of body wt.										
6 mo.	0.58	0.54	...	...	0.54	0.64	0.62	...	...	0.61
9 mo.	0.56	0.53	...	...	0.57	0.64	0.61	...	...	0.61
12 mo.	0.60	0.60	0.56	0.58	0.56	0.63	0.70	0.64	0.68	0.64
24 mo.	0.58	0.67	0.52	0.64	0.61	0.78	0.78	0.71	0.65	0.71
Days of age of rats that succumbed	654	620	500	579	580	658	653	529	587	592
	Total Results on 20 Rats per Level									
Number alive, 1 yr.	19	17	16	17	17	19	20	20	18	19
Number alive, 2 yr.	8	5	8	7	5	12	13	17	13	10
Number of rats with tumors	3	2	0	3	4	8	4	7	8	5

[a] Maximum body weights of rats that survived at least 1 year of doses, exclusive of those in auxiliary groups used for tissue studies at 6, 9, and 12 months.

ities for any and all abnormalities.

For comparisons involving large numbers of observations, statistics dependent on the normal distribution were used—namely, mean, standard deviation, standard error of the mean, standard error of the difference between two means, and the critical ratios. With fewer observations—i.e., <30—the student *t* distribution was used when the *F*-ratio was comparable. Comparison of number of deaths, neoplasms, and tissue abnormalities of treated *vs.* control rats was made by taking the square root of the corrected chi square, using Yates' (*6*) correction for continuity, and consulting a table of fractional parts of the total area under the normal probability curve.

For growth effect comparisons, the means for each biweekly weighing period were calculated after transformation of the individual weights of each rat to a gain over its weight at 60 days of age. In all statistical tests the fiducial limit of 0.05 was used.

Statistical Evaluation of Results (Table VI). APPETITE. The mean diet consumption curves for each group of rats, regardless of sex, paralleled one another throughout the study. Consumption of diet fell off during the winter months and increased during the summertime. The composite means were compared each 6 months and in none of these periods were the means of the highest dosage level, 0.04%, for females statistically significantly different from the controls. Therefore, although slight differences were found in the next two lower levels, 0.02 and 0.01%, these were held to be of no importance. Furthermore, the correlations between dietary concentration and amount of feed eaten per day were not significant.

Control groups, for some unexplained reason, ate more per rat than any other group throughout the study. No relationship between dosage and the diet consumption was found in any of the groups fed Sevin and the coefficients of correlations for the males were not significant. The only correlation that approached significance was a coefficient of −0.824 for the first 6-month period and this could have occurred by chance 18 times in 100. Therefore, it was concluded that the male control group was divergent.

Results on males and females were analyzed separately. This is because females consume less diet than males but obtain a higher dosage level in terms of Sevin per kg. of body weight per rat per day. The composite means show that the males consumed 38% more diet than the females yet the average dosage for females was 21% higher than for males. Mean dosage for all groups decreased from a maximum at the start to a fairly constant level after 6 months. Actually, dosage was 30% higher for the first six-month period than for the last 18 months. These variations in dosage occur in most repeated oral studies and it is not to be inferred that they are peculiar to this study.

DEATH AND INFECTIONS. The mortality, calculated by the life table technique (*12*) for all rats during each six-month period of the study are given in Table VII. No group differed significantly from its control.

Lung infections accounted for 88% of all deaths or specifically 95, 86, 85, 88, and 86% of the deaths of the rats that received 0.04, 0.02, 0.01, 0.005, and 0.0% of Sevin in their diets. The other causes of death were as follows: peritonitis 6.5%, debility from neoplasm 3.3%, anuria 1.1%, and nephritis 1.1%. Apparent cause of death was not related to dosage of Sevin. In fact, mortality in the control group exceeded that in any treated group.

LIFE SPAN. No statistically significant differences were found between the Sevin-treated rats and their controls as regards mean age at death, mean expectation of life at birth, and mean expectation of life after 1 year of doses. The mean days of age at death for both sexes at the 0.04 and 0.02% levels was 656 and 630 days and for the controls 585 days, demonstrating greater longevity for the Sevin-dosed rats.

LIVER AND KIDNEY WEIGHTS. Liver and kidney weights after exsanguination were calculated as percentage of live body weight for rats killed for examination at 6, 9, 12, and 24 months. At none of these periods were the means for the Sevin-dosed animals different from the controls. Increased kidney weights were reported after the 96-day feeding of female rats on 0.15% Sevin which make the absence of any such effect at 0.04% for 2 years noteworthy.

BODY WEIGHT. The mean weights at 60 days of age for the four groups and the controls were within 3 grams of each other. Each biweekly mean weight gain was compared by the *t* test to the mean of the appropriate control group. For the female rats, none of the 26 biweekly mean weight gains were statistically significantly below their controls.

The coefficient of correlation between the over-all mean weights and the concentrations of Sevin in the diets was −0.928 for the males and −0.217 for the females, values which would have occurred two and 72 times in 100 by chance alone. This indicates a direct relationship between dosage and body weight for the males but not for females. A single or several divergent values may force a significant correlation among otherwise uncorrelated variables. All other tests indicated that only the highest dosage level or the 0.04% group of male rats differed from the controls. In an attempt to verify this fact, Rao's (*15*) new method of growth curve analysis was applied to body weight gains of all groups excluding the 0.04% level. The method yielded absolutely no indication of a different growth rate among the three lower dosage levels and the controls for the first four 84-day periods of this study.

The mean maximum weights attained by the Sevin-fed rats alive at 365 days of doses were not statistically significantly different from the controls. In summation, no significant weight depression was found in any group except that of the males eating 0.04% Sevin in their diet.

HEMATOLOGY. Ten male rats were selected randomly at 54 days of age from each of the 0.04, 0.02, and 0.00% Sevin groups for predose hematocrit determinations and for subsequent study at 89, 180, 269, 358, 543, and 724 days of age. Seven of the 10 were alive in each group at 543 days and three, five, and five of 10 after 729 days. None of the means of the 0.04% group differed at any interval from the controls and only the 358-day mean for the 0.02% group was different. No significance is attached to this isolated deviation.

NEOPLASMS. Tumors were sought in all animals that died or were killed during the study as well as at its termination. No single type of tumor or site of origin was associated with the inclusion of Sevin in the diet. The total number of tumors seen in the 0.04, 0.02, 0.01, 0.005, and 0.00% groups was 12, 9, 8, 12, and 10 occurring in 11, 6, 7, 11, and 9 rats. The incidence of tumors in the Sevin-fed groups was not different from the control group which

Table VII. Mortality during Each 6-Month Period of 2-Year Rat Feeding Study

% Sevin in Diet	Percentage Mortality during Days of Dosing[a]			
	0 to 196	197 to 364	365 to 560	561 to 736
0.040	0.0	5.0	10.5	41.2
0.020	0.0	7.5	13.5	43.8
0.010	5.0	5.3	22.2	10.7
0.005	2.5	10.2	14.3	33.3
0.000	2.5	7.7	22.2	46.4

[a] Percentage mortality = $\frac{\text{no. dead within period}}{\text{no. alive at start of period}} \times 100$.

bears out the negative results of the carcinogenic study on C3H mice.

PATHOLOGY. Portions of tissue were taken for micropathological examination from lung, kidney, liver, heart, spleen, pancreas, stomach, duodenum, descending colon, testis or ovary, fallopian tube, esophagus, trachea, thyroid, urinary bladder, and adrenal from each of the 98 control and treated rats killed at the termination of the study. Samples of gastrocnemius muscle and sciatic nerve were taken from representative animals on each of the various levels. A similar procedure was used after the animals had received Sevin for 180, 270, and 365 days. A microscopic examination of most of these organs was made on 61 of the 106 animals which succumbed during the course of the 2-year study. These 61 included all animals with neoplasms as well as those for whom the primary cause of death or morbidity was not recorded at autopsy. Tissue sections were fixed in 8.0% neutral buffered formalin solution, embedded in paraffin, stained, and counterstained in the conventional manner with hematoxylin and eosin. On occasion, liver, kidney, and spleen were sectioned using the freezing technique and stained with Sudan IV and Turnbull blue for identification of intracellular lipoid deposits and hemosiderin.

Lung and kidney infections were present in various forms, but were not related to chemical dosage. Of the 98 animals sacrificed after feeding for 2 years, 49% showed lung infections while 40% had kidney infections. The effects of these lesions were reflected throughout the various organs, and the resultant pathology had to be considered in the light of this influence.

Microscopic examination of tissues from randomly selected animals fed 180 and 270 doses of the respective diets revealed no significant differences from their controls. After 365 doses, kidney changes of a mild and transitory nature were observed and characterized as cloudy swelling of the convoluted and loop tubules. The reaction, which was primarily localized in the proximal tubules, was observed to be present to a greater degree in animals of the 0.04% (p = 0.004) than in the control rats. Its distribution within the organ was more diffuse in those animals receiving the higher dosage levels. A tendency, not of statistical significance, toward central cloudy swelling of the hepatic cords was found occasionally in the liver at the 0.04% dietary level.

After 2 years none of the tissues examined on 0.04% Sevin showed permanent degenerative changes which could be charged to toxicity of the insecticide. However, there was a suggestion of transitory change in the kidney, characterized by diffuse cloudy swelling of the epithelial lining of the convoluted, primarily proximal, and loop tubules. These changes were similar to those observed after 1-year feeding, but the incidence at 2 years was no longer statistically significant (p = 0.40). More noteworthy was the cloudy swelling of the hepatic cords, principally located about the central veins (p = 0.002). At the lower levels, 0.02, 0.01, and 0.005%, microscopic examination revealed no pathology significantly different from that of the controls.

In summary, 2-year feeding of Sevin in concentrations ranging from 0.005 to 0.04% of the diet of rats resulted in no deleterious effects in any of the criteria examined in the 0.02, 0.01, or 0.005% groups. The criteria of effect under scrutiny were: mortality or life span, appetite as measured by diet eaten, body weight gain, liver and kidney weights, incidence of neoplasms, slit-lamp examination of eyes for cataract formation, hematocrits at 3- and 6-month intervals, and micropathology of lung, liver, kidney, heart, spleen, pancreas, stomach, duodenum, descending colon, testis or ovary, urinary bladder, and adrenal.

One-Year Feeding of Dogs. PROCEDURE. Fourteen Basenji-Cocker hybrids, born between 12–12–53 and 12–30–55, were immunized against distemper and hepatitis, freed of worms, and then fed Sevin between 7–24–57 and 7–25–58. The dogs were randomly distributed by sex, litter, and breed among the treated and control groups. Sevin was fed in gelatin capsules, 5 days per week, at dosage levels approximating 414, 95, 24, and 0.00 p.p.m. on a dry diet basis by holding dosage to 0.0072, 0.0018, 0.00045, and 0.00000 gram per kg. of body weight by weekly adjustment. Their daily basic diet consisted of Fiskies Dog Food Meal with water ad libitum.

Hematocrit, hemoglobin, erythrocyte fragility, and differential leucocyte evaluations were made prior to first dose and after 3, 6, 7.5, 9, and 12 months of doses. Except at 7.5 months, sulfobromophthalein retention, alkaline phosphatase, urea nitrogen, and bilirubin determinations were made concurrently. Sulfobromophthalein retention was determined in blood drawn from the saphenous vein 15 minutes after a 5-mg.-per-kg. intravenous dosage by antecubital vein. The means for the above hematological and biochemical tests were evaluated statistically by comparing results of the Sevin groups to those of their control by the t test. Body weight data were compared on the basis of the weekly deviation from starting weight by the t test.

Plasma and erythrocyte cholinesterase determinations were made five times within the 3 weeks prior to initiation of dosing. Twenty additional assays were then made: weekly for 9 weeks, bimonthly for 1 month, and approximately monthly thereafter using the electrometric method of Michel (*13*) as modified by Frawley and Fuyat (*8*).

At the termination of 1 year, the dogs were anesthetized with pentobarbital sodium, exsanguinated by section of the vena cava, and thoroughly searched for any abnormalities. The examination included contents of the skull, thorax, and abdomen as well as gastrocnemius muscle and sciatic nerve.

RESULTS. The final body weight of each of the dogs that received Sevin approximately equivalent to 400, 100, 25, or 0 p.p.m. of their dry diet was higher than the predose weight as shown in Table VIII. Mean weights, as gains over weights on the first day of dose, showed no statistically significant deviations between the Sevin-fed dogs and the controls. The single female control dog gained 5.3 kg. which was 2.5 to 44 times as much as the individual gains of the male controls. This, of course, affected the mean weight for the controls so that it seems inordinately high.

No important statistically significant differences were found at any interval between the means of the Sevin-dosed group and the control group as regards hematocrits, hemoglobin determinations,

Table VIII. Mean Values after 1 Year of Feeding Sevin to Dogs

Calcd. concn. in dry diet, p.p.m.	414.1	95.1	23.8	0.0
Dosage in g./kg./day	0.0072	0.0018	0.00045	0.00000
Liver wt. as % of body wt.	2.29	2.35	2.54	2.00
Kidney wt. as % of body wt.	0.457	0.440	0.440	0.486
Hematocrit	49.8	51.2	52.0	50.7
Hemoglobin, g./100 ml. of blood	16.8	16.4	16.6	16.3
Sulfobromophthalein retention, %	6.16	6.33	5.92	8.16
Serum urea nitrogen, mg./100 ml.	7.66	10.85	6.93	8.14
Total bilirubin, mg./100 ml.	0.79	0.47	0.61	0.89
Alkaline phosphatase, mg./100 ml.	1.77	1.89	1.56	1.54
Plasma cholinesterase, mean % of predose mean	107.8	108.7	116.7	111.8
Eyrthrocyte cholinesterase, mean % of predose mean	115.0	108.6	111.0	109.7
Body weight gain, kg.	+0.79	+1.25	+0.33	+1.99

and differential leucocyte counts. Sulfobromophthalein retention, serum urea nitrogen, total serum bilirubin, and serum alkaline phosphatase never deviated significantly. At each of the 20 periods when cholinesterase was determined the values were calculated as percentages of their predose values. The mean percentages for the Sevin-dosed dogs then were compared to those of the control dogs similarly handled. None of these was significantly different.

The weights of livers and kidneys of the Sevin-dosed dogs, individually or collectively by group, were not significantly higher than those of their controls. The obese female had organs of normal weight but on the basis of percentage of body weight they were about one half the usual value.

Microscopic examination of sections of the kidney revealed diffuse cloudy swelling of the proximal convoluted and loop tubules and focal Sudanophilic dust in the glomeruli of dogs which received 400 p.p.m. of Sevin. These are interpreted as transitory conditions and not as early stages of toxic degeneration, because the same conditions were present in the control dogs but to a lesser extent. Considerable intracellular fat was observed in the proximal kidney tubules of females. Its presence apparently does not represent a significant pathological lesion resulting from specific action of the compound but rather variability within the normal range.

One of the two female dogs that received 25 p.p.m. of Sevin had a transient hind leg weakness after the 189th dose of Sevin. Dosing was continued unremittingly and before 21 days elapsed the dog appeared normal. At the end of the study there was no microscopically detectable difference between the tissues of this dog and those of the other animals which had demonstrated no such weakness even though many of them received 16 times as much insecticide.

The tissues from the 14 dogs killed after 1 year of oral doses of 400 p.p.m. or less of Sevin showed no permanent degenerative changes. Neither was there any statistically significant deviation from the controls in the other criteria of effect studied—namely, body weight change, organ weights, hematological studies, biochemical tests, cholinesterase levels, and mortality.

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ANIMAL METABOLISM OF INSECTICIDES

The Metabolism of Orally Administered Malathion by a Lactating Cow

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Malathion was rapidly excreted by a lactating cow, principally via the urine, which accounted for 90% of the excreted material. About 23% of the dose was not excreted over 3 weeks. As in nonruminants, the major metabolite was produced by carboxyester hydrolysis; the principal fecal metabolite, however, was dimethyl phosphate. Milk contained no malathion or malaoxon, but had 0.11 p.p.m. of radioactive materials, most of which could not be identified. Blood metabolites were also examined.

WHEN malathion, *O,O*-dimethyl *S*-1,2-bis(carboethoxy)ethyl phosphorodithioate, is injected into mice, rats, or dogs, it is degraded rapidly, the principal reaction being hydrolysis of the ethyl ester bonds (*8, 9*). In insects the degradation is slower, and ethyl ester hydrolysis is somewhat less important, cleavage of a phosphate thioester bond being correspondingly more significant (*9*). Tissue residues following spray application of P^{32}-labeled malathion to calves were reported by March *et al.*, but no metabolites were identified (*10*). Milk and fat residues after spraying cows were reported by Claborn *et al.* (*6*); colorimetric assays on milk showed small residues which disappeared after 1 day.

Malathion is degraded to unknown products by cow rumen juice (*1*). Therefore, when malathion was fed to the cow, the metabolites produced might be unlike those found in nonruminant mammals. The extent of degradation might also be increased.

In the present study, malathion was fed at moderate levels to a lactating cow for 3 days, and the nature and extent of the metabolites produced were examined for 3 weeks.

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