cases shows slightly more systemic activity than the methyl. This agrees with the work of Ivy, Rainwater, Scales, and Gorzycki (*7*), Magee and Gaines (*9*), and others, where contact insecticidal activity was considered.

Compounds where R = isopropyl and R = propyl present a contrast (XVII *vs.* XXII, XVIII *vs.* XXIII, etc.), with the isopropyl compounds showing much more activity. This is in contrast to the parathion series, where the propyl and isopropyl homologs are about equal in contact toxicity.

Previous work with many phosphate series has shown that when R contains more than three carbon atoms, the biological activity is lost. Therefore, only a single example where R was butyl was prepared. It was inactive in the systemic test.

It is apparent that in this series there is a direct correlation between mammalian toxicity and systemic activity. Increasing the length of R will give a safer compound, but only with considerable loss of systemic activity. Changes in R′ do not produce significant changes in mammalian toxicity until the chain length is too great for systemic activity.

The most active compounds of this series are VIII and IX. They have been identified in field tests as experimental insecticides 3911 and 12008, respectively. Compound X has also been field tested under the number 12009. It was selected on the basis of its spectrum of activity in contact toxicity tests.

One of the most interesting applications of these compounds is the treatment of cottonseed for protection of young plants against early season pests. A preliminary report of this work has been given by Ivy, Scales, and Gorzycki (*8*).

In field tests during 1954, compound 12008 applied to cottonseed as a 50% powder on activated carbon at the rate of 4 pounds of technical per 100 pounds of seed gave protection against thrips and aphids for 4 to 6 weeks. In greenhouse tests, 3911 has shown considerably longer residual effectiveness and also appears promising against the boll weevil. As foliage sprays and soil treatments, these compounds are effective against aphids, mites, certain scales, leaf hoppers, and flea beetles.

While the results given in this paper show compounds VIII (3911) and IX (12008) to be the most potent systemics, several others have a high enough level of activity to indicate that they may prove useful for specific applications.

Further extensive field testing will be required to determine the place of these compounds in the pest control picture.

Summary

A new series of phosphorodithioates has been prepared by treating an appropriate *O,O*-dialkyl hydrogen phosphorodithioate with formalin and a mercaptan to give $(RO)_2P(S)-CH_2-S-R'$. The systemic activity of these compounds was evaluated against the two-spotted spider mite using excised bean plants. Maximum activity is obtained when R is ethyl. In decreasing order of toxicity are: methyl, isopropyl, and *n*-propyl. Considering R′, highest activity is obtained when it is ethyl or isopropyl. Increasing the chain length decreases the activity, with R = dodecyl showing no toxicity.

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PESTICIDE SAFETY EVALUATION

Mammalian Investigations on *p*-Chlorophenyl Phenyl Sulfone (Sulphenone)

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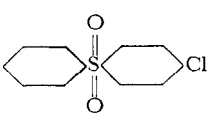
COMPREHENSIVE STUDIES were designed to evaluate the safety of Sulphenone residues which may occur following its use on raw agricultural commodities used for food. In the interest of brevity exploratory studies and those on other than commercial grade have been omitted or markedly condensed.

Materials

The material used in these studies was *p*-chlorophenyl phenyl sulfone, originally designated as Compound R-242 and later as Sulphenone. The samples were received from the Stauffer Chemical Co. at various times throughout the progress of the studies. The original investigations were conducted on a relatively less pure sample of the material than were the later experiments. The active ingredient in all the samples was *p*-monochlorophenyl phenyl sulfone.



The crude material, R-242A, contained approximately 45% of the active ingredient, the technical material, R-242-C and R-242-D, about 70%, and R-242-B was the pure material. The chief impurity is diphenyl sulfone with small quantities of bis(*p*-chlorophenyl) sulfone and of the other monochlorodiphenyl sulfone isomers.

The pure *p*-chlorophenyl phenyl sulfone is a white crystalline powder almost insoluble in water. It has no detectable odor or taste under the conditions of usage. *p*-Chlorophenyl phenyl sulfone

The effect of *p*-chlorophenyl phenyl sulfone (Sulphenone) upon several species of mammals was studied in order to establish a basis for evaluating the safety of Sulphenone residues in agricultural crops. Oral LD_{50}'s in mice and rats varied between 1400 and 3650 mg. per kg. The feeding of dietary levels of 10, 100, and 1000 p.p.m. to albino rats over 2 years resulted in a lowering of body weight in the high-level group only. Daily oral doses of 10, 50, and 100 mg. of Sulphenone per kg. of body weight, administered over prolonged periods to dogs, caused nonspecific toxicity at 100 mg. per kg. only. Repeated skin tests in rabbits and guinea pigs were negative. Eye tests in rabbits resulted in transient irritation. Sulphenone storage in dog and rat tissues following prolonged oral administration was insignificant. The results of these studies indicate a wide safety margin for Sulphenone.

is synonymous with 4-chlorodiphenyl sulfone.

Throughout the following report the dosages and quantities given are as of the compound in the state in which it was received, with no allowance made for the percentage of active ingredient or of impurities present. Unless otherwise indicated, the technical grade was used.

Experimental

Male Albino Mice and Rats

Acute Oral Administration. The effects of oral administration of Sulphenone to male albino mice and rats were determined following oral administration of 10% suspensions in 0.5% methylcellulose with in some cases the intervention of 0.1% Tween 80 to assist in maintenance of the suspension. The material was given directly by stomach tube and the animals were observed for approximately one week. The following calculated LD_{50}'s were determined, using the method of Wilcoxon and Litchfield (*6*):

Crude material	*Mg./Kg.*
Mice	2700
Rats	1400
Purified material	
Mice	3650
Rats	Above 2000

Acute Intraperitoneal Administration. Under the same conditions and using suspensions similar to those used for the oral administration, the following values for the LD_{50}'s were determined after intraperitoneal administration:

Crude material	*Mg./Kg.*
Mice	1000
Rats	ca 500

Rats

Chronic Oral Administration. For these studies male and female weanling albino rats obtained from Carworth Farms were used. Each rat was individually housed in wire-mesh cages with the bottoms elevated above the droppings. Water was available at all times. Diets were prepared by direct addition of technical Sulphenone to the basic laboratory diet, which was a commercial dog food specially ground to a fineness that avoids the possibility of food selection by the rat. For control groups of animals this diet with no additive was used.

In preliminary studies involving male rats only, levels of 100 and 1000 p.p.m. indicated satisfactory food acceptance and growth. At 10,000 p.p.m. (1.0%) the animals exhibited marked toxicity and this level was considered to be intolerable.

On the basis of these preliminary studies, 2-year studies were initiated according to the following design:

Level, P.P.M.	Male	Female
Control	20	10
10	20	
100	20	20
1000	20	

Individual body weights and food consumption were determined at weekly intervals throughout the period of 104 weeks; however, because an analytical method adapted to biological fluids and tissues was not available at the end of 2 years, the animals were not sacrificed simultaneously but at various intervals thereafter.

Table I summarizes at 4-week intervals the average body weight, food consumption, and survival for the male rats. With the exception of one rat at 1000 p.p.m. the distribution of mortality throughout the various groups appears to be randomized and concentrated in the last three quarters of the second year. The one rat at the level of 1000 p.p.m. died on the 28th day of feeding, having lost weight and refused food during the previous week. At autopsy there was bloody discharge from the nose, hemorrhagic intestines, and abscessed lungs. These signs are indica-

Table I. Average Weekly Body Weights and Food Consumption, in Grams, and Survival Data for Male Albino Rats

[Receiving for 2 years basic laboratory diet, or basic diet containing Sulphenone at 10, 100, or 1000 parts per million (0.001, 0.01, or 0.1%.) Surv. = survival]

Week	Control			10 P.P.M.			100 P.P.M.			1000 P.P.M.		
No.	Wt.	Food	Surv.	Wt.	Food	Surv.	Wt.	Food	Surv.	Wt.	Food	Surv.
0	75		20/20	81		20/20	75		20/20	78		20/20
1	111	99		113	100		107	102		103	115	
4	188	129		196	137		185	128		169	133	19/20
8	274	140		284	154		264	146		247	153	
12	326	142		329	142		313	141		286	149	
16	356	128		360	133		347	130		312	130	
20	364	116		377	124		349	113		305	131	
24	383	122		392	123		364	120		337	129	
28	385	116		392	115		370	117		340	124	
32	385	106		400	107	19/20	371	108		340	116	
36	384	128		403	132		366	127		343	133	
40	389	120		414	137		374	123		348	128	
44	397	129		419	137		387	133	19/20	351	130	
48	394	127		419	137		390	135		345	126	
52	390	127		425	134		387	131		348	120	
56	402	126		434	136		397	129		356	119	
60	409	124		443	135		403	124		369	119	18/20
64	439	131	19/20	446	141		402	130		359	117	
68	426	124		446	131	18/20	401	124		369	118	17/20
72	435	120	18/20	449	127	17/20	412	121	17/20	367	114	16/20
76	410	107	17/20	448	104		402	96	16/20	358	103	
80	436	112		429	114		402	105	15/20	366	117	15/20
84	451	104	16/20	463	108		409	101	14/20	367	100	
88	438	107	14/20	451	108	16/20	389	109	13/20	355	103	
92	442	122	13/20	445	119	15/20	405	118	11/20	362	122	14/20
96	437	117		444	125	14/20	407	112		381	119	12/20
100	429	111	12/20	439	123		403	118	10/20	361	106	10/20
104	428	125	11/20	435	120		386	105	9/20	386	118	9/20

Table II. Average Weekly Body Weights and Food Consumption, in Grams, and Survival Data for Female Albino Rats

[Receiving for 2 years the basic laboratory diet, or basic diet containing Sulphenone at 100 p.p.m. (0.01%). Surv. = survival]

Week No.	Control			100 P.P.M.		
	Wt.	Food	Surv.	Wt.	Food	Surv.
0	55		10/10	54		20/20
1	84	78		79	84	
4	140	113		125	104	
8	191	120		170	119	
12	215	110		188	107	
16	229	104		206	114	
20	244	102		217	114	
24	255	111		231	118	
28	265	112		242	118	
32	270	98		249	108	
36	269	75		251	102	
40	272	76		256	78	
44	281	89		261	94	
48	286	96		269	100	
52	287	97		269	99	
56	299	94		274	97	
60	300	111		296	111	
64	306	108		288	119	
68	311	112		295	109	19/20
72	326	110		295	109	18/20
76	325	101	9/10	293	96	17/20
80	323	94		293	96	
84	327	98	8/10	294	97	
88	317	79		292	88	16/20
92	308	91		302	97	15/20
96	292	96	6/10	311	105	14/20
100	301	119	5/10	298	96	
104	330	130	4/10	307	119	

Table III. Survival and Food Consumption for Albino Rats

[Receiving for 2 years basic laboratory diet, or basic diet containing Sulphenone at 10, 100, or 1000 p.p.m. (0.001, 0.01, or 0.1%)]

Level, P.P.M.	Sex	No. of Rats		Av. Body Weight, Grams		Rat Days		
		Start	Finish	Start	Finish	Theoretical	Actual	% Survival
Control	M	20	11	75	428	14,560	13,256	91
	F	10	4	56	330	7,260	6,723	93
10	M	20	14	81	435	14,560	13,224	91
100	M	20	9	75	386	14,560	12,664	87
	F	20	14	54	307	14,520	13,522	93
1000	M	20	9	78	386	14,560	12,430	85
						13,832[a]	12,402[a]	90[a]

Level P.P.M.	Sex	Av./Rat/Day, Grams		Sulphenone Consumed
		Total diet consumed	Food consumed	
Control	M	17.4	...	...
	F	14.5		
10	M	18.2	18.2	0.18
100	M	17.5	17.5	1.75
	F	14.8	14.8	1.48
1000	M	17.9	17.8	17.8

[a] Based on 19 rats; see text.

tive of a pneumonic death and it is therefore concluded that the death was irrelevant to the feeding of Sulphenone.

Survival, average body weight, and food consumption data are summarized for the 2-year period in Table III. Statistical evaluation of the total food consumption data indicates no significant difference between any groups when evaluated at a probability of 0.05, using the Fisher Student's *t* method. The factual data indicate that the group at 10 p.p.m. averaged 0.77 gram more food consumption per day than the controls and that their weights were maintained consistently above the controls. Neither of these indications is significant. The average body weight for the group at 100 p.p.m. is intermediate between the controls and the group at 1000 p.p.m. but not significantly different from either, whereas the growth retardation for the level of 1000 p.p.m. is significant when compared to the controls. At this high level the average consumption was 17.82 mg. per rat per day for the 2-year period, on the basis of technical Sulphenone which had been added to the diet. Food consumption was normal for the lower levels; hence, the Sulphenone intake was directly proportionate. These evaluations were made at the 86- to 89-week interval for females and the 93- to 96-week interval for males, periods which represent a fully plateaued growth curve and a normal incidence of mortality.

Since survival is the primary interest in the long-term chronic feeding experiment, this has been calculated on the basis of individual survival time compared to a theoretical total survival. These data are presented in Table III under the heading of "rat days," where "theoretical" is the total number of rat days which would have been experienced had all rats lived for 2 years, and "actual" is the total number of days survived by the group. The "per cent of survival" is obtained from these two figures and makes possible a more direct comparison of the over-all influence of the chemical additive on the survival time of the group. For the male rats, both the control group and the group at 10 p.p.m. survived 91% of the theoretically perfect survival. This indicates that the actual survival was good and that the mortality experienced must have occurred late in the experimental period. This is a reflection of the survival data presented in Table I. At 100 p.p.m. survival was 87%, while at 1000 p.p.m. it was 85%. As indicated above, one rat at the high level died after only 28 days of feeding and this death was not related to the Sulphenone ingestion. If this animal is eliminated and the calculation based on a group of 19, the survival at the level of 1000 p.p.m. is 90%. From these data it is evident that, although the level of 1000 p.p.m. significantly retarded the growth of male rats, neither this nor the lower levels influenced survival over the 2-year period.

Comparable data for the female rats are presented in Tables II and III. In general, these data are similar to those presented for the male rats. There was no mortality during the first five quarters of the test period. During the remainder the deaths appear to be random and comparable for the two groups although, percentagewise, much higher in the controls. That this is not significant is indicated in the survival of 93% for each group. Neither the food consumption nor the average body weight of the level of 100 p.p.m. differed significantly from the control. The average Sulphenone consumption per rat for the 2-year period was 1.48 mg. per day.

A detailed study of the recorded gross observations indicates no difference between the controls and the experimental animals for either sex. Most commonly recorded variants from normal were labored respiration, bloody-appearing noses, and cutaneous lesions or nodular growths. These and other less numerous observations were less prevalent in the

level of 10 p.p.m. than in the control and levels of 100 and 1000 p.p.m.

Upon completion of the 2-year period hematological studies were conducted on five male rats of each group. The results were within normal limits. Neither the differences within nor between groups appeared to suggest any hematological abnormality.

The gross autopsy findings on animals which died during the course of the experiment, as well as those which were sacrificed after 2 years or more, were recorded. In the nonsurvivors gross autopsy revealed a uniform distribution of abnormalities, principally parasite infestation, pneumonic lungs, and abdominal nodular growths in the males. Microscopic examination of some of the nodules revealed malignancy. The male survivors were notably free of gross pathology when sacrificed after 2 years. In general, the females revealed fewer abnormalities, being free of parasite infestation and revealing abdominal nodular growths in two control animals only. These findings do not appear to indicate any grossly observable pathology peculiar to the feeding of the compound. Any evaluation must take into consideration the incidence of liver parasites in the male rats.

In the female rats sacrificed after 2 years there was no difference, either relative or absolute, in the liver and kidney weights of the control and 100 p.p.m. groups. Because as indicated above, control male rats were not sacrificed at 2 years, direct comparison on these organ weights was not possible, but when compared to values for other control rats under similar conditions the liver weights of the male rats were normal at 10 and 100 p.p.m. but significantly heavier at 1000 p.p.m. An apparent slight increase in kidney weight at this level was also observed. Livers from three of the six males at the level of 1000 p.p.m. were examined microscopically. One showed parasitic involvement but none of the three showed other abnormalities.

Dogs **Chronic Oral Administration.** In these studies male and female dogs, some of which were litter mates and some mongrels, were used. The experiments were initiated and terminated at several different intervals, the summary of which is presented in Table IV. In all cases technical Sulphenone was administered by capsule on a milligram per kilogram basis adjusted to the body weight at weekly intervals. Administration was 6 days per week. The diet consisted of a commercial dog meal supplemented by canned horse meat, and in general the quantity consisted of that amount which the animal would eat in approximately 20 minutes once per day.

With but few exceptions the outline in Table IV is self-explanatory. The control dog, No. C-17, was carried throughout the course of the studies on mongrel dogs. An additional control litter mate dog, indicated as No. C-14, was originally a male control for a period of 210 days. Following this period he was placed on the level of 10 mg. per kg. per day indicated in the table for 604 days. During the interval of control feeding there were no complications. As indicated, male dog No. 2 at 10 mg. per kg. per day was sacrified in extremus after 129 days. The dog had previously had distemper but appeared to be recovering when coma ensued and during the second day the dog was sacrified. Autopsy showed internal hemorrhage throughout the entire gastrointestinal tract. There was a diverticulum of the colon approximately 5.0 cm. long. Both gall bladder and urinary bladder were distended. The near-terminal blood picture showed an increase in white blood cells and an increase in polymorphonuclear leukocytes suggestive of an infectious process. A positive diagnosis of the cause of death was not possible. The course of the other dogs receiving 10 mg. per kg. per day, which represents approximately 400 p.p.m. of the diet, was uneventful.

At the level of 50 mg. per kg. per day, corresponding to approximately 2000 p.p.m., the female dog, No. 3, followed an uneventful course throughout the entire 814 days. The male dog, No. C-22, followed an irregular course. The appetite remained good but the animal lost weight and there was progressive anemia. Although obviously thin, the dog remained alert until shortly before death, at which time depression and weakness became apparent and the appetite decreased. Autopsy revealed anemic mucous membranes and visceral organs and a heavy infestation with hookworms and ascarids in the lower intestine. There were several tapeworms also present. The veterinarian's diagnosis and the terminal blood picture suggest parasite infestation as the major cause of death. The prolonged weight loss, however, is indicative of subacute toxicity.

The two dogs at 100 mg. per kg. per day, corresponding to approximately 4000 p.p.m. or 0.4% of the diet, present a definite picture of toxicity. This appears to be a nonspecific toxicity initiated by loss of appetite followed by loss of weight, progressive emaciation, and finally a terminal state complicated by extreme inanition. The terminal phase for dog No. 4 was complicated by a 4-inch ileal intussusception which was observed at autopsy. This condition appears to have been the immediate cause of death but would not have accounted for the observed progressive effects on appetite and weight loss. The autopsy findings for dog No. 5 indicated terminal complications of pneumonia and inanition.

Throughout the course of the experiment complete blood counts, including hemoglobin, red blood cells, white blood cells, differentials, and Schilling counts, were conducted at approximately monthly intervals. In all, approximately 100 such counts were tabulated. With the exception of occasional fluctuations in white counts coinciding with infectious processes and anemia indicated in animals with definite toxicity, these counts were all within normal range.

These results indicate that dietary levels of 10 mg. per kg. per day in dogs had no detectable effects over long periods of time. At 50 mg. per kg. per day one male dog appeared to exhibit some toxic effects in the form of loss of appetite and weight, while a female dog at this level showed no ill effects. At 100 mg. per kg. per day there was a nonspecific type of progressive toxicity.

Additional Studies In addition to the acute and chronic studies described above, several other aspects of the biological activity of Sulphenone were investigated. Repeated daily application of 1.0 gram per kg. to the shaved skin of rabbits for 6 days produced no evidence of irritation nor of systemic toxicity. One group of animals received the powder dry, while another received the powder moistened with sufficient water to make a paste. In each case the material was applied to the skin, tightly covered with rubber damming, and this in turn was protected by several layers of absorbent

Table IV. Dosage Levels and Duration of Chronic Oral Administration of Technical Sulphenone to Male and Female Dogs and Fate of Each Dog

(Sulphenone was administered by capsule, 6 days per week)

Dog No.	Sex	Dose, Mg./Kg./Day	Duration, Days	Fate
C-14 (1)	M	10	604	Sacrificed
3	F	50	814	Sacrificed
2	M	10	129	Terminated[a]
4	M	100	63	Died
5	M	100	104	Died
C-17	F	Control	560	Sacrificed
C-16	F	10	567	Sacrificed
C-19	M	10	521	Sacrificed
C-22	M	50	202	Died[a]

[a] See text.

gauze. The exposure time was 6 hours daily.

Suspensions of technical Sulphenone in 0.5% methylcellulose with the intervention of 0.1% Tween 80 were tested for irritation in rabbit eyes. The dose was 0.1 ml. of the suspension placed in the conjunctival sac. The vehicle produced only transient irritation and a comparable degree was seen with a Sulphenone suspension containing 1.0 mg. per ml. When the suspension contained 200 mg. per ml. there was a moderate inflammation, but all evidence of irritation disappeared by 24 hours. In view of these results, no further tests were conducted.

A group of six albino guinea pigs was used to test for skin sensitization, employing a modified Landsteiner test (*3*). The test solution contained 1.0 mg. of Sulphenone per ml. of Wesson oil. Wesson oil served as the control. Each intracutaneous injection resulted in a wheal formation until the oil was absorbed. Neither during the sensitizing series of 10 injections on alternate days nor after the challenge injection 10 days later was there any evidence of skin sensitization.

To determine the effect of food withdrawal (*7*) from rats, a diet containing 1000 p.p.m. of Sulphenone was given to male rats for 5 weeks and to female rats for 6 weeks. After this period food was withdrawn from the animals and comparable sets of controls. There was no observable difference in the reaction of the various groups to food withdrawal. There were no signs of Sulphenone intoxication which would have indicated storage and the postmortem findings were those of starvation.

Microscopic Examination of Tissues Following 2 years or more of feeding, the tissues from four surviving male rats were examined microscopically following termination by sacrifice. One rat had received Sulphenone at 10 p.p.m., two at 100 p.p.m., and one at 1000 p.p.m. in the diet over this period.

The tissues examined were lung, adrenal, bone marrow, liver, kidney, large bowel, spleen, small bowel, and heart muscle in all animals. Additionally, the brain was examined in three animals, testicles in three, skeletal muscle in two, and thyroid and stomach in one. Isolated tissues, grossly abnormal in appearance, were examined from four additional surviving animals and four which died during the experimental period. Of the eight tissues identified as tumors, four were fibrosarcomas, two were reticulum cell sarcomas, and one each was a myelosarcoma and lymphosarcoma. As the tumors were observed in the control animals in approximately the same proportion as in the experimental animals and are of a type commonly found in old animals, they did not appear to have any relationship to the Sulphenone ingestion.

Aside from the tumors, the only abnormalities were those commonly found in animals at this advanced age, and cellular reactions to parasitic infestation.

Upon completion of the 2-year experimental period the female rats were sacrificed and the tissues from two controls and three at 100 p.p.m. were examined microscopically. The tissues examined included lung, brain, adrenal, bone marrow, liver, kidney, large bowel, small bowel, spleen, heart muscle, pancreas, and bladder in all animals. In four of the animals thyroid, stomach, and uterus were also studied, as was the ovary of each of three. In these animals there was no evidence of any abnormality referrable to the feeding of Sulphenone and the tissues from the experimental animals were comparable to those of the controls. In contrast to the male rats, there was no incidence of tapeworm or other significant parasitic infestation, nor of tumorous growth.

Following sacrifice of the five surviving dogs, samples of vital organs were taken for histopathological examination. The following organs were examined from all five dogs: thyroid, adrenals, heart, brain through the cerebrum and cerebellum, kidneys, liver, and spleen. The following additional organs were also studied: pancreas in three, stomach, large, and small intestines in four, bone marrow in two, testicles in two, ovaries in two, lung in three, and ureters in one. All of these tissues, control and experimental, were within normal range. There is, therefore, no evidence of pathology following the prolonged administration of Sulphenone to dogs.

Metabolic Fate Various methods for the analysis of Sulphenone are available, but not all are applicable to body tissues. Turbidimetric methods and the total organic chloride method of Klein and Wichmann (*2*) were used in preliminary studies. These methods were analytically unsatisfactory and the results served only to indicate that no storage in animal tissues could be detected. The total organic chloride method also revealed some excretion of Sulphenone in the urine.

A two-stage chromatographic separation and spectrophotometric determination method was developed in the Stauffer Chemical Co. laboratories (*5*). With this method diphenyl sulfone, 4-chlorodiphenyl sulfone, and dichlorodiphenyl sulfone can be determined. The sensitivity is 2.0 or 3.0 γ, corresponding to about 1.0 p.p.m. in the sample size used. This method was found to be applicable for animal tissues and excreta. Following completion of the chronic studies previously described, tissues from the surviving dogs and from the female rats were prepared and analyzed. All tissues were ground with anhydrous sodium sulfate and extracted with benzene in a Soxhlet extractor. Aliquots of the extracts were submitted to the Stauffer Chemical Co. laboratories for the two-stage analysis. The dog tissues were treated separately, while tissues from three rats were pooled to provide an adequate sample. The results, expressed in relation to 4-chlorodiphenyl sulfone and dichlorodiphenylsulfone, are presented in Tables V and VI.

From these data it can be concluded that Sulphenone is not stored in body

Table V. Analysis of Aliquots of Benzene Extracts of Tissues from Dogs

(Sacrificed after oral administration, for the indicated intervals, of Sulphenone at 10 or 50 mg./kg./day. Two-stage chromatographic separation and spectrophotometric determination method)

Tissue	Level of Sulphenone, Mg./Kg./Day	Dog No.	Administration Period, Days	Sample Weight, Grams	4-Chlorodiphenyl Sulfone, P.P.M.	Dichlorodiphenyl Sulfone, P.P.M.
Muscle	10	C-14	604	2.45	Nil	Nil
	10	C-16	567	2.97	Nil	Nil
	10	C-19	521	2.40	Nil	Nil
	50	3	814	3.13	17	6
Liver	10	C-14	604	2.46	Nil	Nil
	10	C-16	567	2.68	Nil	Nil
	10	C-19	521	2.72	1	Nil
	50	3	814	2.80	12	Nil
Kidney	10	C-14	604	2.68	Nil	Nil
	10	C-16	567	2.58	1	Nil
	10	C-19	521	2.58	Nil	Nil
	50	3	814	1.82	20	4
Fat	10	C-14	604	2.93	15	11
	10	C-16	567	2.92	22	11
	10	C-19	521	2.13	20	15
	50	3	814	3.08	137	23
Brain	10	C-14	604	2.07	Nil	Nil
	10	C-16	567	1.80	Nil	Nil
	10	C-19	521	1.92	Nil	Nil
	50	3	814	2.97	Nil	Nil

Table VI. Analysis of Benzene Extracts of Tissues from Female Albino Rats

(Receiving for 2 years basic laboratory diet, or basic diet containing Sulphenone at level of 100 p.p.m. Two-stage chromatographic separation and spectrophotometric determination method)

Tissue	Rat Nos.	Level, P.P.M.	Pooled Tissue Weight, Grams	4-Chloro-diphenyl Sulfone, P.P.M.	Dichloro-diphenyl Sulfone, P.P.M.
Muscle	1501, 1506, 1510	Control	26.34	Nil	Nil
	1511, 1512, 1513	100	20.42	5.1	2.3
	1514, 1517, 1518	100	19.68	8.1	3.7
	1520, 1521, 1522	100	21.97	7.9	3.8
	1523, 1525, 1528	100	18.96	2.7	2.6
Liver	1501, 1506, 1510	Contro	35.38	Nil	Nil
	1511, 1512, 1513	100	31.72	3.7	1.2
	1514, 1517, 1518	100	30.78	2.8	0.9
	1520, 1521, 1522	100	32.98	2.4	0.8
	1523, 1525, 1528	100	37.71	2.1	1.7
Kidney	1501, 1506, 1510	Control	8.92	Nil	Nil
	1511, 1512, 1513	100	8.14	1.7	3.9
	1514, 1517, 1518	100	8.19	7.9	3.1
	1520, 1521, 1522	100	8.73	5.6	2.4
	1523, 1525, 1528	100	9.87	0.9	1.4
Fat	1501, 1506, 1510	Control	28.93	Nil	Nil
	1511, 1512, 1513	100	22.01	29.0	17.0
	1514, 1517, 1518	100	22.30	37.2	19.5
	1520, 1521, 1522	100	21.46	44.6	21.6
	1523, 1525, 1528	100	21.19	21.5	26.6
Brain	1501, 1506, 1510	Control	5.18	Nil	Nil
	1511, 1512, 1513	100	5.71	0.8	Nil
	1514, 1517, 1518	100	5.69	1.1	Nil
	1520, 1521, 1522	100	5.30	1.1	Nil
	1523, 1525, 1528	100	6.54	0.9	Nil

tissues after prolonged oral feeding to either dogs or rats. The highest values for each species were encountered in the fat. The combined total is approximately 160 p.p.m. for dog fat and 66 p.p.m. for rat fat. In view of the high intake and prolonged period of time, these values are indicative only of transient or equilibrated chemical, and not of progressively cumulative storage such as is observed with DDT, which may be stored at levels four to 10 times that of the dietary intake (*4*), or of lindane where storage is about equal to that of the concentration in the diet (*4*). It is significant that no diphenyl sulfone could be detected in either species, indicating either complete excretion of this component or metabolism to a nondetected material.

Summary

The acute oral LD_{50} for the crude Sulphenone in mice was 2700 mg. per kg. and for rats 1400 mg. per kg. The oral LD_{50} for the purified material was 3650 mg. per kg. in mice, and above 2000 mg. per kg. in rats.

The acute intraperitoneal LD_{50} of the crude material was 1000 mg. per kg. in mice, and approximately 500 mg. per kg. in rats.

Over a 2-year period levels of 10 and 100 p.p.m. in the diet of male rats, and 100 p.p.m. in females, caused no significant difference in food consumption, average body weight, or survival. At 1000 p.p.m. in males food consumption and survival were normal, while average body weight was significantly lowered. Organ weights were within normal limits, except that the liver weights were increased in the male group receiving 1000 p.p.m. in the diet. Kidney weights may have also been increased.

Hematological values for the male rats were within normal limits after completion of the 2-year feeding period.

Gross observation during the 2-year feeding period and at autopsy after sacrifice revealed no abnormality characteristic in the rats receiving Sulphenone.

Daily oral doses of 10 mg. of Sulphenone per kg. of body weight resulted in no evidence of toxicity to dogs after periods of up to 604 days. Although fluctuations occurred, there were no effects on appetite, body weight, or gross appearance, and there was no significant gross or microscopic pathology.

At oral doses of 50 mg. per kg. per day one female dog showed no evidence of toxicity after 814 days, and was sacrificed. There was no significant gross or microscopic pathology. One male dog at this dose lost weight and died after 202 days. Autopsy revealed anemic tissues and heavy infestation of hookworms and ascarids. There was no other gross pathology, and no significant microscopic pathology.

Daily oral doses of 100 mg. of Sulphenone per kg. of body weight resulted in loss of appetite and weight and a nonspecific type of toxicity when given to dogs daily over various periods of time.

Neither dry nor moistened Sulphenone, applied to rabbit skin at a dose of 1.0 gram per kg. of body weight, caused dermal irritation or systemic toxicity. Applications were repeated daily for 6 days.

Single applications of Sulphenone suspensions to rabbit eyes caused only transient irritation.

Oil solutions of Sulphenone were not sensitizing to guinea pig skin when tested by a modified Landsteiner technique.

After 6 weeks on a diet containing 1000 p.p.m. of Sulphenone, withdrawal of food from rats produced no signs of toxicity suggestive of Sulphenone storage.

Turbidimetric methods for determining Sulphenone in tissue were unsatisfactory. Total organic chloride methods while subject to considerable variation, indicated no tissue storage in rats or dogs. This method did indicate that some Sulphenone was excreted in the urine.

A two-stage chromatographic-spectrophotometric method proved satisfactory for determining the Sulphenone content of tissue. This method detects diphenyl sulfone, 4-chlorodiphenyl sulfone, and dichlorodiphenyl sulfone. After prolonged feeding to dogs and rats no diphenyl sulfone was found in the tissues analyzed. The other two constituents were present in small quantities in rat muscle, kidney, liver, and brain, but not in these tissues from dogs at 10 mg. per kg. per day. At 50 mg. per kg. per day small quantities were present in dog muscle, liver, and kidney. In both species the fat contained somewhat more than the other tissues, but did not reach high levels.

Aside from pneumonic lungs, tumorous growth, and parasites, the microscopic findings in long-term feeding male rats were essentially negative. Female rats showed even fewer abnormalities. Dog tissues revealed no pathology associated with long-term oral administration of Sulphenone.

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