Table I. Recovery of EPTC from Soil as Determined by Gas Chromatography

Soil Type	Weight, Grams	EPTC, γ Added	EPTC, γ Recovered	Recovery, %
Muck	100	200	194	97 ± 2
	100	100	96	96 ± 2
Sandy Loam	100	200	190	95 ± 3
	100	100	93	93 ± 2

showed that this method gave at least equally good recoveries of EPTC from soil. In the older method, recoveries of 81% using 2000 γ of EPTC were obtained in this laboratory. These recoveries were obtained by placing the receiver in an ice bath to reduce losses; without this precaution recoveries were even lower.

The lower limit of sensitivity of this method is determined by the sensitivity of the detector system in the gas chromatograph. The thermal conductivity cell used in the instrument employed in this study requires at least 10 γ. Assuming that the lowest practical volume after dilution is 300 ml. and a sample volume of 50 ml. used for injection, one could detect as little as 60 γ of EPTC in a sample. Since 200- to 300-gram samples of soil can be as easily extracted as 100 grams, the practical sensitivity would be of the order of 0.2 p.p.m. in the soil.

Conclusions

An analytical method for EPTC in soil has been developed employing gas chromatographic techniques. This method proved to be faster than previously reported methods and gives an average recovery of 95 ± 2% on soil samples. The limits of sensitivity of this method are determined by the limitations of the instrument used.

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HERBICIDE TOXICITY

Mammalian Toxicity of Sesone Herbicide

C. P. CARPENTER, C. S. WEIL, and H. F. SMYTH, Jr.

Mellon Institute, Pittsburgh, Pa.

Sesone herbicide has proved eminently successful as a means of killing weed seedlings without harming most established plants. As it is converted in the animal body to 2,4-dichlorophenoxyacetic acid, its metabolic pathway, in all probability, is that taken by the acid. By stomach intubation the compound is moderately toxic for rats. The results of repeated oral feeding of rats and dogs are presented and the various criteria of effect are summarized. These data support the statement that rats are not injured by 200 p.p.m. in their diet and are slightly injured by 600 p.p.m. when fed during their 2-year life span. In the dog a dosage of 9 mg. per kg. per day fed by capsule 5 days a week for 1 year, approximately equivalent to 360 p.p.m. in the dry diet, was tolerated without ill effect.

IN 1950 King, Lambrech, and Finn (*9*) reported an effective new chemical, sodium 2,4-dichlorophenoxyethyl sulfate (Sesone herbicide), for use as a soil treatment. It has the unique property of not harming most established plants when sprayed or dusted directly on the foliage at concentrations that kill seedlings in the soil. This means that crops in the field being treated, or in adjacent fields, are not subject to injury by drift during application. Studies on the rate of disappearance of this herbicide from soil under greenhouse conditions indicate the time required ranges from 12 to 30 days with 1 to 10 pounds per acre.

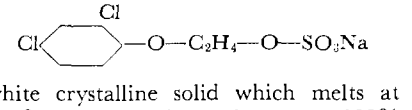
Carroll (*1*) has shown that the material is hydrolyzed readily to biologically active 2,4-dichlorophenoxyethanol and sodium bisulfate in aqueous preparations at pH 3.0 to 4.0.

Vlitos (*13*) contends that the herbicidal activity of sodium 2,4-dichlorophenoxyethyl sulfate can be attributed to a reaction pathway involving either acid or enzymic hydrolysis to 2,4-dichlorophenoxyethanol followed by oxidation of the latter compound to 2,4-dichlorophenoxyacetic acid.

Sesone herbicide is being sold commercially as a pre-emergence treatment to prevent the growth of weeds on peanuts, bulb and corm crops, established strawberries and asparagus, and a large variety of nursery stock. It is also being distributed for use on 56 different varieties of home garden plants (*4*).

Physical Properties

Sodium 2,4-dichlorophenoxyethyl sulfate marketed under the names Sesone herbicide (Union Carbide Corp.) or Crag Herbicide 1 is a stable, nonvolatile,



white crystalline solid which melts at 170° C. It is soluble to the extent of 25% by weight in distilled water at 25° C. A 4% solution can be made in hard water containing as much as 260 p.p.m. of calcium carbonate. The preferred method of estimation (*8*) is based upon the observation that sodium alkyl sulfates form chloroform-soluble complexes with methylene blue chloride, which is

also the basis for an analytical control method (*2*).

Rogoff and Reid (*10*) have isolated an organism belonging to the genus *Corynebacterium* which decomposes 2,4-dichlorophenoxyacetic acid in relatively large amounts in a synthetic medium. Indications are that complete destruction of the molecule follows ring rupture.

Metabolism

It is presumed that Sesone herbicide is metabolized in the animal body in a manner comparable to 2,4-dichlorophenoxyacetic acid. The hydrolysis of Sesone is known to occur in acid environment and for that reason feeding the dry compound in the diet to animals certainly would result in the immediate production of the hydrolytic products under the acid conditions existing in the stomach of mammals.

Tolerances for residues of Sesone herbicide are published in the *Federal Register* (*3*). The following tolerances are established in or on the raw agricultural commodities indicated:

6 p.p.m. in or on potatoes, peanuts, peanut hulls, peanut hay
2 p.p.m. in or on asparagus, strawberries

The studies reported in this paper formed the toxicological basis for the establishment of the tolerances mentioned. All dosages refer to grams per kilogram of the product as marketed.

Irritation

The application of 0.01 ml. of a 5% suspension of Sesone herbicide in acetone to the uncovered hair-free rabbit belly resulted in edema and necrosis, while a 1% solution caused no reaction when readings were made immediately, at 30 minutes, and 24 hours later.

In a 4-hour uncovered continuous skin contact test, eight applications of a 10% aqueous solution of Sesone herbicide were made at 30-minute intervals onto a circular area of 2-cm. diameter. Reactions read 24 hours after the application ranged from moderate to marked erythema without edema or necrosis.

Instillation of an excess of a 5% aqueous solution caused corneal necrosis of rabbit eyes, but a 1% solution was harmless.

Peroral Toxicity

Sherman, Carworth Farms–Wistar, Carworth Farms–Nelson, and Dow Wistar stock rats were used for these assays, which on the whole are in fair agreement. The LD_{50} values ranged from 0.73 to 1.09 grams per kg. for male and female rats fed concentrations of 5 or 10% in water. Changing the vehicle from water to corn oil presumably increased the rate of absorption from the rat alimentary tract and thereby increased the toxicity (Table I).

Table I. Single-Dose Peroral Toxicity of Sesone Herbicide for 90- to 120-Gram Rats

Sample Production Date	Concn., 1 Ml. = x G. in Vehicle	LD_{50} and Range, G./Kg. Male	Female
1950	0.05 in water		1.09 (0.89 to 1.33)
1951	0.10 in water	0.73 (0.64 to 0.82)	0.82 (0.70 to 0.95)
1952	0.10 in water		0.82 (0.67 to 1.00)
1956	0.10 in water	0.89 (0.76 to 1.04)	
1958	0.10 in water	0.81 (0.70 to 0.94)	
1959	0.10 in water	0.87 (0.81 to 0.93)	
1958	0.05 in corn oil[a]	0.54 (0.38 to 0.75)	
1959	0.05 in corn oil[a]	0.48 (0.39 to 0.60)	

[a] Suspension.

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). The method of moving average (*14*) for calculating the median-effective dose (LD_{50}) was applied to the 14-day mortality data.

Rats that died had lungs with varying degrees of congestion. Some survived a sufficient time for capillary breakdown and hemorrhage in the lung to occur. Livers were congested or mottled, kidneys ranged from pale to congested, and adrenals were often severely congested. There was relatively slight irritation of the gastrointestinal tract.

In less than 2 hours after a dose was given porphyrin secretion was visible on the nostrils, and when the rats were handled an invisible fine muscular tremor could be felt. There was a tendency for these animals to extend their hind feet caudally rather than to keep them in the usual position to land if dropped. Six to 8 hours after dosing some of the rats died with convulsions, but this symptom did not invariably precede death. The following morning, 20 hours after dosing, myotonus such as was reported by Hill and Carlisle (*7*) was found. The affected rats were unable to right themselves as rapidly as undosed controls when placed on their backs. In general, these symptoms are not unlike those reported by the above authors for the purified sodium salt of 2,4-dichlorophenoxyacetic acid. Rowe and Hymas (*11*) have thoroughly summarized the toxicological information on 2,4-dichlorophenoxyacetic acid and related herbicides.

Repeated Feeding of Rats

30-Day Study. Five male and five female, 100- to 150-gram, Sherman stock albino rats were distributed randomly into groups which were fed 1.0, 0.25, 0.06, 0.015, and 0.0% Sesone herbicide in a modified Food Research Laboratory diet 2C (*6*). This consisted of 60 parts of wheat, 30.6 parts of dried whole milk, 1.5 parts of USP dried liver extract, 5.0 parts of type 50-B inactive brewer's yeast, 2.0 parts of iodized salt, and 1.0 part of calcium carbonate. Rats on the 1.0% level gained almost no weight during the 30-day period and one male died. Gross autopsy findings on this group include liver congestion and increased prominence of kidney surface markings. Kidney weights were almost double those of the controls. None of the rats eating 0.25% in the diet succumbed, but kidney weights were also increased significantly above those of the control group. No adverse gross effects, whatever, were found among rats receiving 0.06 or 0.015% in the diet. This preliminary study indicated the kidney to be the target organ for injury caused by repeated small doses.

2-Year Study. Carworth Farms–Wistar rats 29 ± 3 days of age were used in this study. Only rats whose body weights were within plus or minus two standard deviations from the group mean weight were assigned to the pool for random distribution.

Sesone herbicide was fed at concentrations of 0.06, 0.02, 0.006, 0.002, and 0.00% in the Food Research Laboratory diet described above, to groups of 24 male and 24 female rats randomized separately. Four males or four females were kept together in a suspended, wire front and bottom metal cage. Feeding and watering devices were assigned to one cage and never transferred to another unless washed and sterilized between uses. Diet for each cage was stored in a separate stock jar and fed as needed. Food intake was calculated for each cage of rats at 28-day intervals.

In addition to the groups of 48 rats on each dosage level and control, extra groups of rats randomized from the same stock were assigned to the highest dosage level and control groups. At 60, 140, 220, and 364 days a number of these additional animals were weighed and killed to provide organ weights and tissues for micropathological examination. By this means the progress of injury, if any occurred, could be followed in rats free of the complications of senile changes.

All rats on the study were weighed biweekly during the first year of dosing with dates set to coincide with the 28-day diet consumption checks. In the second year weights were determined every 28 days. Rats were examined critically for any visible signs of infection at each weighing and if a marked loss occurred, the animal was killed to procure fresh tissue so that the underlying reason for this condition could be determined more accurately. Every autopsy, of course, included a search for neoplastic growths throughout the animal.

Statistical Methods. In the analysis of biological data many statistical methods must be applied to reveal the significance of differences between the dosage groups and the controls. The methods used are not unique and are described in most standard statistical treatises. When there were numerous observations, as in diet consumption and over-all growth analysis, statistics dependent on normal distribution were used. Calculations of the mean, standard deviation, standard error of the mean, standard error of the difference between the means to be compared, and the c itical ratio (difference between the means divided by the standard error of the difference) were used. With 30 observations or less the "student" *t*-distribution was used.

The number of deaths and infections and the frequency of neoplasms and of micropathological findings in tissues of the treated groups were compared to the simultaneous incidence among the controls by taking the square root of the corrected chi square (Yates' correction) and using a table of fractional parts of the total area under the normal probability curve.

To compare growth effects, the means for each sex for each biweekly weighing period were compared to the means of the control group for the same periods for evidence of a higher or lower trend. In all of the statistical analyses a fiducial limit of 0.05 has been used.

Appetite. No deleterious effect upon diet consumption was found. Females receiving Sesone herbicide in their diet ate about 8% more than did the controls, as did also the males on the lowest dosage level. On the average for the entire 2-year period the males consumed 29% more diet than did the females—an intake of 15.3 grams per rat per day *vs.* 11.6 for the females. Even though the females ate less diet than the males in grams per rat per day, their dosage level was 25% higher on a gram per kilogram basis. In general, this relationship holds where concentration in diet is held constant.

Death and Infection. The only significant increase in mortality was in the male group on 0.06% Sesone herbicide. No other male or female group differed from the respective controls. Lung infections accounted for 86% of the 149 deaths that occurred among the 240 rats on study; another 6% segment included peritonitis, parasites, intestinal hemorrhage, genitourinary tract, and alimentary tract infection. The remaining 8% were undiagnosed because of autolysis and cannibalism. Seven rats were killed because of four middle ear infections, two tumors, and one lung infection. The over-all mortality for the whole study including controls was 65%.

Life Span. At every 28-day period, the number of live rats in each dosage group was compared to the control group. The data were analyzed separately by sex, as well as in combination. A statistically verified increase in mortality was found for the males at the 0.06% level from 448 days onward. No other group, male or female, deviated significantly from the controls.

Liver and Kidney Weights. A 1959 publication by Rowe *et al.* (*12*) reaffirms the reliability of liver and kidney weights as percentage of body weights, as a sensitive criterion of stress. Although this study was initiated in 1951, liver and kidney weights as percentage of total body weight were followed at each period that rats were killed for micropathological examination—namely, after 60, 140, 220, 364, and 732 days of feeding.

No statistically significant differences in mean liver weight were found during the first year of the study. Only two male rats survived the 2-year period on 0.06% Sesone herbicide and their mean liver weight was significantly higher than the controls. On the other hand, the mean liver weight of the seven females that survived 2 years did not differ from the appropriate control. No liver weight changes of significance were found at 0.02% or lower dosage levels after 2 years.

Kidney weight differed only at the 140-dose serial sacrifice of rats on the 0.06% level. However, the two male rats that survived for 2 years on the 0.06% level had increased kidney weights, but not those on the lower dosage levels. The mean kidney weights of the females were not different from their controls at any dosage level.

Body Weight. Because the male and female rats were housed separately and the complications of pregnancy thereby eliminated, it was possible to follow and analyze body weights for both sexes. At the inception of the study it was determined that the difference between the mean weight of any group and the control group was due to chance or random cause. Only rats that survived 365 doses were used in the weight comparisons. The biweekly mean weight, computed as percentage of the 60-day weight, was compared by the *t*-test to the mean of the matching control group.

The only statistically significant depressive effect upon body weight occurred among the males on the 0.06 and 0.006% dietary levels. In contrast to this finding, the females on both these levels gained significantly more than their controls. Both males and females resembled the controls at the intermediate 0.02% level, while at the lowest level the males were significantly heavier than the controls and the females were equal to their controls. This undulation is evident when one views the over-all mean weights for males, as percentage of the 60-day weights, after 365 doses. They were 242, 260, 245, 266, and 256 for the 0.06, 0.02, 0.006, 0.002, and 0.0% groups of males, respectively, and 196, 193, 197, 191, and 189% for the females.

If the above-mentioned effects upon weight were produced by the chemical in the diet, there should be a relationship between these factors. To test this hypothesis, the over-all mean weights of the rats were compared to the concentration of Sesone herbicide by means of the coefficient of correlation. For the males the probability that this relationship was due to chance and therefore not real was 25 in 100 and for the females 30 times in 100. Accordingly, these variations were judged irrelevant to the Sesone herbicide in the diet.

Hematology. Sixteen male rats were randomly selected from the 0.06% Sesone herbicide group and a similar number from the controls. Red and white blood cell enumerations, differential leukocyte counts, and hemoglobin determinations were made on surviving members of the groups when the rats were 3, 6, 9, 12, 18, and 24 months of age. Half of each group was alive at 12 months and 1/4 survived Sesone herbicide for 24 months. Statistically significant differences were found three times among the 30 comparisons of means of Sesone herbicide dosed and control groups. The number of white blood cells at 12 months was significantly higher for the Sesone herbicide group, but the highest actual single count for the Sesone herbicide group was 18,000 cells per cubic millimeter of blood *vs.* 18,150 for the controls. As both of these values fall within the normal range of white cell counts for the rats, no importance is attached to the finding.

Mean hemoglobin values were significantly lower at 6 months and higher at 18 months, with no significant differences at the other intervals. Again the extremes were numerically almost identical and such findings certainly are not indicative of a trend.

Estimation of Dichlorophenol in Fat. A possible metabolite of Sesone herbicide, following ingestion, might be dichlorophenol. Fat was removed from the carcasses of rats killed after 1 and 2 years on the 0.06% Sesone herbicide level, extracted with *n*-hexane, and

analyzed for phenolic content by the method of Gottlieb and Marsh (5). A curve obtained by analyzing known quantities of dichlorophenol added to fat from untreated rats was used to evaluate the phenol content of the fats under study. After 1 year the mean for the 0.06% Sesone herbicide group was 4.1 as compared to 2.6 μg. per gram for the control group. After 2 years the respective means were 2.8 and 1.6 μg. per gram. These differences were not statistically significant after either 1 or 2 years of survival on the diet containing 0.06% Sesone herbicide. It is concluded that metabolites of Sesone herbicide are not stored in body fat.

NEOPLASMS. Twenty-six tumors were found among the 240 rats under surveillance. These were distributed among the descending dosage groups as follows: 4, 4, 5, 9, and 4. As a large number of the rats survived 2 years of doses, it was not surprising to find tumors associated with these older rat populations. At 0.002% there were three mammary tumors and three subcutaneous tumors, and one each in the pleural cavity, neck, and kidney. Among the other 17 tumors found there were two in fallopian tubes at 0.006% and two on the gonads of the controls. All others were single occurrences per level. Not only was the frequency of tumors unrelated to dosage but type and location were similarly unrelated.

MICROPATHOLOGY. Portions of the abdominal organs and of the lungs were taken from groups of five rats killed after 60, 140, 220, and 364 days of dosing with 0.06% Sesone herbicide. Among these rats, as well as those that died during this interval, there was no deviation in micropathology from the controls.

Of the rats that died on the 0.06% dietary level during the second year, 92% had lung infection. The type of micropathological lesion found in the treated rats that succumbed was similar to that found among the control rats that died. The importance of the finding of an increased incidence of kidney congestion and cloudy swelling and also liver congestion is open to question for this reason. A similar qualification may be made regarding the increased incidence of kidney cloudy swelling at the 0.02% level because 93% of this group had lung infection at death. Ninety-one per cent of the control rats that died during this second year had lung infection.

The livers and kidneys of the rats killed after eating 0.06% Sesone herbicide for 2 years had more pathology than the controls. Forty-four per cent of the kidneys and 67% of the livers were normal as compared to 81% of the controls. Only the highest level, 0.06%, was affected. Concentrations of 0.02, 0.006, and 0.002% in the diet resulted in no significant difference from the controls as regards pathology or any of the criteria of effect examined.

To recapitulate, in addition to an increased mortality rate the only criteria of effect that were adversely influenced at 0.06% of Sesone herbicide in the diet were liver and kidney weights of males and liver and kidney pathology of males and females after 2 years. It is concluded that the maximum dosage level tolerated would be between 0.02 and 0.06%.

One-Year Repeated Feeding to Dogs

Four dogs were fed Sesone herbicide in gelatin capsules at a dosage of 9.0 mg. per kg. per day, 5 days each week immediately prior to their daily diet of moistened Frisky meal. If converted to percentage of the dry diet, this would result in an approximate concentration of 0.036% or 360 p.p.m. This is based on a 16-kg. dog eating 400 grams of dry diet per day. The dogs were weighed each Monday morning and doses for the week were calculated. The two groups, treated and control, were each composed of two Doberman × Kerry blue terrier hybrids, one foxhound, and one beagle. The ages were 7 and 9 months for the first two breeds and 21 and 34 months for the beagles. The animals were randomly distributed between the two groups, so that sex and breed would be equally represented in the treated and control group. Hematology, and biochemical procedures including bromsulfalein retention, prothrombin time, serum urea nitrogen, and serum phosphatase, were performed prior to the first dose of Sesone herbicide and after 3, 6, 9, and 12 months of feeding.

Upon completion of the 1-year feeding the animals were weighed, anesthetized with pentobarbital sodium, and exsanguinated in order that liver and kidney weights could be determined more accurately. Portions of all abdominal and pleural viscera were taken for micropathological examination.

Results. BODY WEIGHTS. Only the 21-month old beagle failed to exceed his initial weight upon completion of 1 year on the Sesone herbicide diet. Tapeworms in the small intestine were responsible for a 6% decline from his initial weight. The differences between mean weight gains of the two groups of dogs were compared at weekly intervals and after 1 year of doses the probability was 39 times in 100 that the slight difference found could have occurred by chance. Therefore, no significant statistical effect on weight change occurred. Furthermore, there were no significant deviations between control and treated groups in erythrocyte and leukocyte counts, hemoglobin determinations, bromsulfalein retention read 15 minutes after a 5 mg. per kg. dosage, prothrombin time, and serum phosphatase. Mean values for urea nitrogen in milligrams per 100 ml. of serum differed statistically after 138 and 255 doses but not after 196, 219, 243, and 261 doses. As these differences were sporadic and within normal limits for dogs of this age, they are not considered indicative of any real or lasting effect. The highest and lowest mean serum urea nitrogen values for the Sesone herbicide dogs were 25.9 and 19.7 and for the controls 26.2 and 16.7 mg. per 100 ml. Liver and kidney weights, determined at time of autopsy, were not aberrant.

After gross examination portions of adrenal, bladder, heart, liver, kidney, lung, pancreas, small intestine, stomach, spleen, thyroid, and gonad were fixed, dehydrated, sectioned, stained, and found to be essentially normal upon microscopic examination.

The above findings lead to the conclusion that 9 mg. per kg. of Sesone herbicide fed 5 days a week in gelatin capsules for 1 year was without deleterious effect in these dogs. On the basis of the dry diet consumed this dosage resulted in a concentration of 0.036% or 360 p.p.m.

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Received for review May 23, 1960. Accepted January 27, 1961.