Table I. Formation of Biuret from Dry Urea at 140° C.

Time, Hours	Urea Converted to Biuret, P	Rate Constant, k, Liter/Mole Hr.
0.25	0.010	1.9×10^{-3}
0.5	0.022	2.1×10^{-3}
0.75	0.028	1.8×10^{-3}
1	0.035	1.7×10^{-3}
2	0.061	1.5×10^{-3}
4	0.122	1.61×10^{-3}
5	0.150	1.63×10^{-3}
6	0.155	1.41×10^{-3}
7	0.195	1.60×10^{-3}
	Average	$(1.68 \times 0.15) \times 10^{-3}$

Table II. Rate Constant of Biuret Formation from Urea

Temp., ° C.	Rate Constant, k, Liter/Mole Hr.
50	3.98×10^{-6}
80	2.38×10^{-5}
100	6.55×10^{-5}
140	1.68×10^{-3}
150	3.34×10^{-3}
160	5.66×10^{-3}
170	8.28×10^{-3}

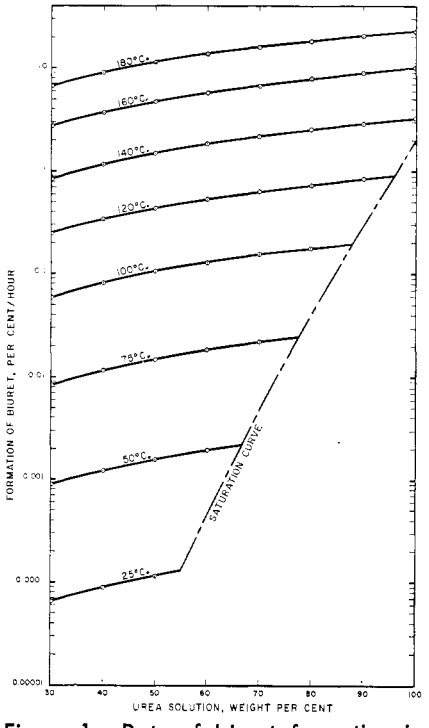

Figure 1. Rate of biuret formation in urea solution

the rates of biuret formation for the conditions shown in Figure 1 are presented on an hourly basis.

Acknowledgment

The author expresses appreciation to U. S. Industrial Chemicals Co. for permission to publish this information.

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INSECT REPELLENT TOXICITY TO ANIMALS

The Toxicology of Butoxypolypropylene Glycol 800 (Crag Fly Repellent)

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This study was undertaken to characterize the toxicity of butoxypolypropylene glycol 800 (BPG 800) by single and repeated doses to rodents and dogs, so that its acceptability for use as a fly repellent could be ascertained. Single-dose studies show that BPG 800 has only slight oral and skin penetration toxicity for rodents and does not increase toxicity when fed orally to rats with nine available insecticides. Subcutaneous and intraperitoneal injection, compared with direct intravenous injection, demonstrates that BPG 800 passes tissue barriers poorly and offers little or no hazard by the usual portals of entry to the body. It is not stored in the bodies of animals, and 50% or more of a single dose may be found in the urine unchanged. Rats tolerated 640 p.p.m. in the daily diet in chronic feeding for 2 years, while dogs tolerated 890 p.p.m. for 1 year.

THE ACUTE AND SUBACUTE toxicity studies completed prior to 1951 by Carpenter and associates (*1*) elucidated the suitability and safety of butoxypolypropylene glycols (BPG) 400 and 800 as fly repellents. This publication covered all portals of entry, as well as irritation tests and studies on absorption and excretion in rats. Granett *et al.* (*3, 4*) demonstrated the effectiveness of formulated BPG 800 as a fly repellent for livestock and in particular for dairy cattle. This compound and other members of the same series have wide solvent properties; some find use as specialty lubricants and as hydraulic fluids. Increasing uses have led to the more extensive toxicological study now reported.

BPG 800, sold as Crag Fly Repellent (Union Carbide Corp.), has a specific gravity of 0.990 and a water solubility of 0.1% at 20° C. The compound is a clear liquid, substantially nonvolatile, with a vapor pressure under 0.1 mm. of mercury at 20° C. It is soluble in most organic solvents, including alcohols, ketones, toluene, and gasoline, and is miscible with the ingredients most useful in the formulation of insecticides such as petroleum distillate and methylated naphthalene.

The present paper brings up to date the information which has been developed on acute toxicity orally, by skin penetration, and by injection of BPG into four rodent species during the period

1951 to date. To characterize the chronic toxicity of this compound, rats have received various dosages in their diets for 2 years and dogs for 1 year. Both feeding studies were conducted with a single sample of 15 gallons of BPG 800 (Union Carbide Chemicals Co., South Charleston, W. Va.) obtained early in 1955. Acute toxicity tests showed that this sample did not differ materially from previous samples.

Acute Oral, Percutaneous, and Parenteral Assays

The methods used for the 1953 to 1958 toxicity assays are substantially those reported in the series of papers by Smyth and coworkers (*6*) on the Range-Finding approach. Groups of five nonfasted Carworth Farms-Wistar rats, weighing 90 to 120 grams, were used for all the tests prior to 1957 and their Nelson specific pathogen-free stock thereafter. Rats were maintained on a Rockland rat diet (complete), and mortalities were observed for a period of 14 days, at which time the median-effective dose (LD_{50}) was calculated from the tabulation by Weil (*8*).

The seven acute oral assays on rats yielded LD_{50} values ranging from 8.9 to 17.3 ml. per kg. for undiluted BPG (Table I). C3H/Jax mice, a highly inbred, mammary tumor-susceptible strain, used widely in carcinogenic studies, were the most susceptible species and rabbits were the most resistant. All the rodent species tested show this compound to have a slight to extremely low order of acute oral toxicity.

The joint action of BPG and each of nine insecticides, currently available on the open market, was assayed on male rats. LD_{50} were determined for each compound at a concentration in corn oil which resulted in individual rat doses of 5 ml. or less. The concentration of toxicants in corn oil was kept constant for both the individual and the joint assays. In prior assays this was found to be necessary, because a change in toxicant concentration, with dosage kept constant, radically influenced mortality.

The results were interpreted by calculating the predicted LD_{50} for each pair and establishing its ratio to the experimentally achieved or observed LD_{50}, based on the feeding of both insecticides in the corn oil vehicle simultaneously. The calculations were made as follows:

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

where P_1 = proportion of total toxicant, on "as sold" basis, that Material 1 represents in the corn oil.
P_2 = proportion of total toxicant, on "as sold" basis, that Material 2 represents in the corn oil.
LD_{50_1} = LD_{50} of Material 1.
LD_{50_2} = LD_{50} of Material 2.

Considering BPG as Material 1 and Thanite as Material 2 then,

$$\frac{1}{\text{Predicted } LD_{50}} = \frac{0.8333}{17.2} + \frac{0.1667}{2.14}$$

$$= 7.9 \text{ grams/kg.}$$

$$\frac{\text{Predicted}}{\text{Observed}} = \frac{7.9}{14.9} = 0.53$$

By comparing the predicted LD_{50} of the mixtures with the observed LD_{50} it is possible to determine whether the components act in an additive manner or whether their combined action is greater or less than one would expect from their calculated LD_{50}. The comparisons are expressed as ratios of predicted LD_{50} over the observed LD_{50}. A ratio somewhat greater than 1.00 indicates a more than additive effect (the variously described synergistic or potentiating tendency) and a ratio somewhat less than 1.00 indicates a less than additive effect (often referred to as an antidotal or antagonistic tendency).

At present, the authors consider ratios above 2.00 and below 0.50 to be on the verge of statistical significance. By this criterion BPG has a less than additive effect with toxaphene, dieldrin, chlordan, and DDT, and is additive with Thanite, Fermate, lime sulfur, Lethane 384 and lindane (Table II).

The percutaneous administration of undiluted BPG demonstrates the inability of this compound to pass the intact skin barrier in physiologically significant amounts. The 1955 sample did not kill rabbits at 20.0 ml. per kg., which is the maximum dosage that can be retained under the impervious Vinylite (Union Carbide Corp.) sheeting used to retain the compound on the clipped rabbit

Table I. Results of Single Doses of Undiluted BPG 800 to Animals

Route	Species	Sex	Year Produced	LD_{50} (1.96s Limits) 14-Day Mortality, Ml. or G./Kg.
Oral	Rat	M	1953	11.2 (9.9–12.8)
	Rat	F	1953	8.9 (7.8–10.1)
	Rat	M	1954	12.9 (8.4–19.9)
	Rat	M	1955[a]	8.9
	Rat	M	1955[a]	11.6 (10.8–12.4)
	Rat	M	1955[b]	17.2 (12.3–23.9)
	Rat	M	1956	12.9 (10.7–15.4)
	Rat	M	1957	17.3 (14.0–21.3)
	Guinea pig	M	1953	22.5 (13.9–36.4)
	Guinea pig	M	1955	9.5 (6.2–13.3)
	Rabbit	M	1953	28.3 (17.5–45.8)
	Rabbit	M	1955[a]	15 ml./kg. = 0/5
	Mouse (C3H)	M	1955[a]	6.7 (4.1–10.9)
Skin penetration	Rabbit	M	1953	21.2 (15.2–29.6)
	Rabbit	M	1954	18.8 (16.9–20.9)
	Rabbit	M	1955[a]	20 ml./kg. = 0/4
Subcutaneous	Rat	F	1954	15.8 ml./kg. = 0/5
	Rat	M	1955	25.9 (21.0–32.0)
	Rabbit	M	1953	3.6 (2.2–5.8)
Intraperitoneal	Rat	F	1953	0.85 (0.56–1.31)
	Rat	M	1954	2.2 (1.4–3.7)
	Rat	F	1955[a]	0.81 (0.62–1.07)
Intravenous	Rat	F	1953	0.22 (0.12–0.43)
	Rat	F	1954	0.19 (0.17–0.20)
	Rabbit	M	1955[a]	0.088

[a] Sample used in the 1- and 2-year oral feeding series.
[b] 50% dilution in corn oil.

Table II. Joint Action of BPG 800 and Nine Other Insecticides

Material	Active Agent in Sample, %	LD_{50} G./Kg. on "as Sold" Basis	1 Ml. = X G. "as Sold" in Corn Oil	Predicted LD_{50}, G./Kg.	Observed LD_{50}, G./Kg. (by Experiment)	Predicted / Observed
BPG	100	17.2	0.50	7.9	14.9 (10.7–20.8)	0.53
Thanite	82	2.14	0.10			
BPG	100	17.2	0.50	4.6	14.1 (no range)	0.33
Toxaphene	100	0.123	0.01			
BPG	100	17.2	0.50	10.0	18.3 (12.1–27.9)	0.55
Fermate	76	4.92	0.20			
BPG	100	17.2	0.50	5.1	16.2 (10.6–25.0)	0.32
Dieldrin	18.6	0.142	0.01			
BPG	100	17.2	0.50	10.3	24.6 (18.8–32.3)	0.42
Chlordan	45	0.49	0.01			
BPG	100	17.2	0.50	9.5	28.3 (no range)	0.34
DDT	100	0.406	0.01			
BPG	100	17.2	0.50	10.5	21.4 (15.4–29.9)	0.49
Lime sulfur	30	2.1	0.05			
BPG	100	17.2	0.50	5.3	5.4 (3.8–7.5)	0.99
Lethane 384	50	1.19	0.10			
BPG	100	17.2	0.50	4.2	4.7 (3.3–6.5)	0.89
Lindane	99	0.107	0.01			

trunk. Earlier samples had LD_{50} which demonstrated an extremely low order of toxicity for 24-hour covered applications (Table I).

Subcutaneous injection of undiluted BPG under the loose skin of the dorsal scapular area on rats resulted in an LD_{50} of 25.9 ml. per kg. Necrosis of the skin over the injection site resulted, but the rats gained weight normally during the 2-week observation period. Rabbits tolerated less by this route, as an LD_{50} of 3.6 ml. per kg. demonstrated.

Single intraperitoneal injections of undiluted BPG to rats resulted in LD_{50} of 0.8 to 2.2 ml. per kg. Repeated injections at dosage levels of 0.5, 0.25, and 0.125 ml. per kg. for 10 days out of 16 calendar days resulted in good weight gains and no mortality among groups of four young rats weighing 122 to 169 grams. Although, the dosing pattern was asymmetrical, 3 days the first week, 4 days the second week, and 3 days the third week, the test shows that rats can tolerate, without gross symptoms, as much as 5 ml. per kg. by repeated intraperitoneal injections. This is two to six times the single injection LD_{50} by this same route.

Intravenous injection of undiluted BPG via the tail vein of the rat gave an LD_{50} of 0.22 ml. per kg. Rabbits were about twice as sensitive as rats as the LD_{50} of 0.088 ml. per kg. by ear vein demonstrated. Convulsions preceded death after parenteral administration of undiluted BPG. Capillary breakdown in the lungs, resulting in hemorrhage, was the common finding at autopsy after death imposed by the oral, percutaneous, or parenteral routes. Convulsions did not occur among orally dosed rats until the dosage reached or exceeded the LD_{50}.

Dogs under Nembutal anesthesia expired with intravenous dosages of 0.075 to 0.25 ml. per kg. under the stress of pharmacological screening tests. These tests revealed that BPG is a respiratory stimulant when given in excessive amounts—i.e., 0.15 to 0.3 ml. per kg. intravenously or 1.0 to 4.0 ml. per kg. intramuscularly. It acted on the central nervous system in a parasympathomimetic-like manner as indicated by vagal stimulation evident as a characteristic cardiac irregularity. In large doses, there were indications that BPG may cause an increase in the clotting time of blood. Symptoms of an overdose based on the observation of these dogs are as follows:

Slow steady decrease in blood pressure
Pinpoint pupils
Cardiac irregularity with increased force and decreased rate
Severe tonoclonic convulsive seizures
Respiratory volume increased and rate decreased to eventual respiratory cessation followed by cardiac arrest

These results were obtained from parenteral doses vastly larger than the amounts likely to enter the body through the topical application of BPG as a repellent.

Several facts seem to stand out upon a comparison of the various routes of administration: BPG is poorly absorbed from the gut of the four rodent species, it has a strong tendency to less than additive toxicity (reduced toxicity) when fed jointly with nine insecticides purchased on the open market. In no instance was there any greater than additive effect (increased toxicity). BPG penetrates rabbit skin slowly, if at all and it passes internal tissue barriers poorly as a comparison of parenteral results indicates. For rats, using intravenous dosage as the base line, intraperitoneal toxicity is decreased from 1/4 to 1/10 and subcutaneous toxicity to 1/80 of the intravenous dosage. In the rabbit BPG is 1/40 as toxic by the subcutaneous as by the intravenous route. The above comparisons demonstrate a low order of acute toxicity for BPG by all routes except direct intravenous injection.

Aerosol Inhalation

Six male and six female, 116- to 142-gram albino rats inhaled 0.0045 ml. per liter of BPG for 4 hours daily for five consecutive days with no apparent ill effects. The nebulizer was kept at a temperature between 40° and 47° C. to decrease the viscosity in order to achieve this concentration. The aerosol delivered by the Vaponefrin nebulizer is said to be of the order of a 2-micron diameter particle size which means that much more BPG reached the lungs of these rats than would result from the usual large particle insecticidal aerosol bomb or spray method of dispersal.

With the temperature of the nebulizer raised to 56° C., a dense fog of 0.02 ml. per liter was achieved. Six young male rats, 120 to 135 grams in weight, inhaled this concentration for 8 hours. Five of the six gained weight well during the 14-day observation period. These tests demonstrated that spraying BPG should offer no hazard under ordinary conditions of use.

Skin Irritation and Sensitization

BPG caused no reaction when 0.01-ml. amounts of the fluid were applied, uncovered, to the clipped skin of the rabbit belly and read 24 hours later.

In a 3-day repeated application test, 0.01 ml. of undiluted BPG was applied at 3-hour intervals, three times daily and the reactions were read at 24-hour intervals after the initial application. There were no reactions on four of the five rabbit bellies after nine applications and the fifth animal had only minimal capillary injection.

Three synergists for the toxicants in fly sprays were incorporated in this same sample of BPG at 0.25% concentration and each combination was tested on groups of five rabbits in the repeated application test. Two produced results precisely like BPG alone and the third resulted in three negative responses and two capillary injections on the five rabbits. When the third synergist was incorporated at 0.25% in deodorized kerosine the readings, after nine applications, included marked erythema with edema on two rabbits, marked erythema and desquamation on another, moderate erythema on the fourth, and capillary injection on the fifth.

With the exquisitely sensitive skin of the rabbit belly showing no response to the repeated application of BPG no reason is seen to expect skin irritation on farm animals, or for that matter, on humans having similar contact.

Instillation of undiluted BPG into rabbit eyes demonstrated that it is harmless and bland—like castor oil, liquid petroleum, and the polyethylene glycols.

In 1946, a consulting dermatologist ran predictive patch tests with undiluted BPG on 200 human subjects with no resulting reactions. The method employed was a 1-week contact application, with frequent examinations, followed by a 2-week incubation period before the challenge test.

Absorption, Excretion, and Storage

A much greater proportion of the single oral doses of BPG given to albino rabbits was eliminated in the feces than in the urine (*1*). Total recoveries from both routes were 45, 50, and 66% of the doses administered in the three cases, respectively. The fraction recovered from the urine amounted to 16, 15, and 8% in the same order as above.

One of the potential hazards accompanying the use of pesticides on livestock is the possibility that a chemical will be stored in the animal body. In 1951, an attempt was made to determine the likelihood of this possibility with regard to BPG 400 by analysis of the livers and carcasses of the rats that had been fed a diet containing 5% of the chemical for 30 days. While the analytical method lacked sensitivity, the amount that could be determined with fair accuracy was sufficiently small in proportion to the total amount ingested to justify the conclusion that it represented only material that was passing immediately through the body, rather than an incipient accumulation. As BPG 800 is considerably less well absorbed, one would expect the same conclusions to be applicable.

Two-Year Chronic Feeding of Rats

Prior to undertaking the 2-year test on

rats, a 90-day feeding showed that the body weight gain of both sexes of rats was depressed at a dietary level of 4.0% BPG, but not at 1.0 or 0.25%. In addition, the liver weight was increased by all three levels among males, but not by the levels below 4% among females. These results guided, in part, the selection of levels of 0.256, 0.064, 0.016, 0.004, and 0.001% BPG for the 2-year test.

Carworth-Wistar albino rats (Carworth Farms, New City, N. Y.) were used for this study. They were born February 18 to 22, 1955, and received in this laboratory on March 24, 1955. After permanent identification, they were weighed at weekly intervals until April 21, 1955. Doses were started when the rats were 60 days of age, assuming a median birth date of February 20, 1955.

Only rats whose body weights at the time of randomization were within ±2 standard deviations from the mean weight of rats of their sex were accepted for the study. Any rat that lost weight or that had poor muscular tone during the preliminary observation period was rejected before separate randomization of each sex.

The basic diet in which the BPG 800 was incorporated during the entire study was this laboratory's modification of the Food Research Laboratory diet 2-C (*5*).

Ingredient	*Source*	*Parts*
Wheat, freshly ground	Locally procured	55.5
Breadlac, dried skim milk	Borden Co.	20.0
Parlac, dried whole milk	Borden Co.	10.0
Meat and bone scrap, 50% protein minimum, 5% fat minimum, and 3% fiber maximum	Buchsieb Co.	12.0
Calcium carbonate, U.S.P.		1.0
Cod liver oil	Eli Lilly (M-11)	1.0
Iodized table salt		0.5

Individual food and water containers were used for each cage of four rats and they were never transferred to any other cage unless washed and sterilized beforehand. The rats were housed in wire-bottomed cages, sexes separate, in a room that was air-conditioned during the summer months. A total of 20 males and 20 females was used for each dosage level. The diet for each cage was stored in a separate 2-quart, screw cap, glass stock jar. These jars were weighed at the start of each 28-day period, when nearly empty, when refilled, and at the end of the period to establish the amount of diet consumed by each cage of rats. The diets were presented in 16-ounce opal-glass, ointment jars that the animals could not overturn.

After 180 and 365 days of doses, or at approximately 240 and 425 days of age, some rats of each sex were killed for histological examination and to discover if there was any effect upon the weights of the livers and kidneys. These rats, maintained on 0.256 and 0.064% BPG, and on control diet, were from additional groups, randomized from the same stock as used for the primary study. They received exactly the same treatment as the other groups, but were carried along to keep the larger groups intact for the study of life span and of possible carcinogenic effects. In addition, from the 20 rats of each sex, four males and four females were sacrificed after 365 days of 0.016, 0.004, or 0.001% BPG in the diet. The rats remaining in the regular groups were kept on their respective diets until death ensued or until the second year of doses elapsed.

Rats were weighed every other week for the first year and every 4 weeks thereafter. The weighing schedule was arranged so that the end of a 28-day diet consumption period always coincided with one of the weighing periods. The rats were critically examined at the time of weighing for any outward signs of infection or abnormality. If continued or marked weight loss occurred in any rat, it was killed, and tissues were taken for histopathological study to determine the reason for the weight depression.

In each instance of death or early sacrifice, a careful autopsy was performed to determine whether the animal was diseased or was damaged by the action of the material being fed. Tissues were taken from the major organs as well as from any others observed to be abnormal or injured.

Tissue samples were taken for the microscopic examination of lung, kidney, liver, heart, spleen, pancreas, stomach, duodenum, descending colon, testes or ovary, urinary bladder, and adrenal from each of the control and exposed rats sacrificed after consuming the chemical for 6 months, 1, or 2 years. These were fixed in 8.0% formaldehyde, embedded in paraffin, and stained and counterstained with hematoxylin and eosin. Frozen sections were made for special staining techniques, when required.

In any comparison of data of this type, many statistical methods and calculating procedures must be used properly to analyze the significance of differences between dosed and control animals. When large numbers of observations were made, as in the composite diet consumption and in the over-all growth analyses, statistics dependent on the normal distribution were used. Here the calculations of the mean, standard deviation, standard error of the mean, standard error of the difference between the two means to be compared, and the critical ratio were used. The latter is the difference between the means divided by the standard error of the difference.

To assay the significance of differences between the means of small samples—i.e., $N < 30$, the Student t-distribution was used. The t-test takes into account the difference between the two means that are to be compared, the dispersion of the values around their means, and the number of observations being compared in each group.

The number of deaths or infections and the frequency of occurrences of neoplasms or microscopic abnormalities in the tissues of treated rats were compared to the same criteria of effect for the controls by taking the square root of the corrected chi square (Yates' correction for continuity) (*2*) and using a table of fractional parts of the total area under the normal probability curve.

For growth effect, the means for each sex, for each biweekly weighing period, were calculated after transformation of the individual weights of each rat to percentages of their weight at 60 days of age. These means were compared to the similarly adjusted means of the control group of rats for the same periods to determine whether a significantly higher or lower trend had occurred. In these statistical tests, a fiducial limit of 0.05 was used. If the probability that the two means were not different is less than 0.05, the chances are at least 19 to 1 that the difference is real and not produced by random sampling errors.

The mean diet consumption of each group of male or female rats varied together throughout the study with depressions during the winter months and peaks during the summer. The composite means for the entire 2-year feeding were remarkably constant for the five groups of males. They varied from 17.4 to 17.8 grams per rat per day with the controls at 17.1. Three of these composite means differed statistically from their control, but all indicated an increased appetite. The composite means for the dosed females varied from 13.3 to 13.9 grams per rat per day with their control at 13.7. Only the 0.064% group differed statistically with a decrease of 0.4 gram per rat per day. As the 0.256% dosage group consumed slightly more than the controls, the lower value for the 0.064% rats is of no real significance.

Although female rats consume less diet, they attain a higher dosage level in terms of grams per kg. than the males. On the basis of composite means, the males consumed an average of 29% more diet than the females, but the average dosage of the females was 23% higher. A similar finding occurs in most, if not all, repeated dose studies.

The mean dosage for all the groups of rats decreased from a maximum at the start of the study to a fairly constant level after about 5 months. When the mean values of the six month com-

parisons for the second, third, or fourth half-year periods are divided by that of the first 6-month period, values of 74, 76, and 72%, respectively, were obtained. This indicates that the rats received approximately a 25% higher dosage during the first 6 months than during the last 18 months.

There was no significant increase in mortality among the treated groups. Extraneous lung infection accounted for 71% of all the deaths. Fifty per cent of the deaths on the highest level and 67% of those in the control group were caused by lung infection, a difference that could have occurred 64 times in 100, by chance alone. Six rats died from peritonitis, ten from debility caused by neoplasms, six were too autolyzed to determine the cause of death, and one each died with interstitial nephritis, pyelonephritis, complex ear infection, and urogenital tract infection.

Sixteen rats were killed, because of obvious infections or because they were moribund. Nine of these 16 rats had lung infection which is a proportion similar to the number of lung infections occurring among the rats which died. Of the 96 animals sacrificed after 2 years, 43% had lung infection, and 48% kidney infections. As with the apparent causes of death, no causal relationship was seen between dosage and the incidence of infections for which the rats were killed or sacrificed.

It is conceivable that daily doses of a toxic chemical might shorten the life span of an animal at a dosage level too low to produce any detectable injury to a specific body function or structure. Therefore, mean age at death, mean expectation of life at birth, and at 1 year of doses, and the mean survival of rats at monthly intervals were calculated.

No statistically significant differences were found between the treated and control groups in the mean ages at death. When the data for the rats of each sex were analyzed separately, no difference was found in the mean expectation of life at birth. On the combined data for both sexes the 0.004% BPG dosed group differed from its control. This is considered an artifact as no difference was found at 64 times the 0.004% level. Similarly, in the mean expectation of life at 1 year of doses, only the mean of the males at 0.001% was different from its control. This could not have been the result of the inclusion of BPG in the diet as the effect was seen at 0.001%, but not at the levels with 4, 16, 64, or 256 times as much repellent. In short, this material did not reduce life span; no significant effect was found in any of the three highest levels.

One of the most sensitive criteria of the effect of a chemical upon small animals, according to Smyth *et al.* (7) is the change in liver or kidney weight as a percentage of body weight. In the present study, the extirpated liver and both kidneys of all the rats killed periodically during the study and at its termination were weighed carefully on a Roller-Smith balance after section of the cervical cord and vertical tail suspension until exsanguinated.

At none of the sacrifice dates were the mean kidney weights of the males or females different from their respective controls. Mean liver weight of the 0.256% BPG female group differed from its control only at the 6-month period. At this same time, the mean liver weight of the males was practically identical with the mean of its control group. Even this sensitive criterion of effect in-

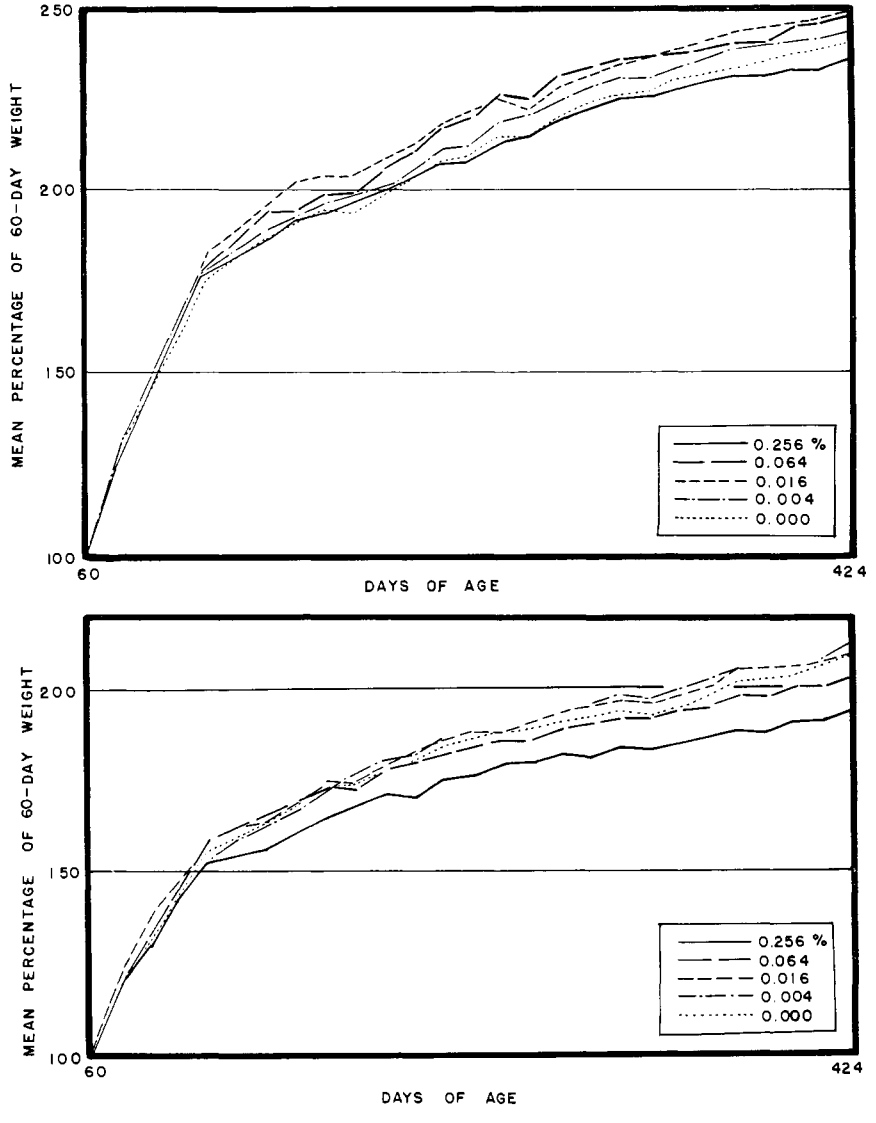

Figure 1. Weight response of male rats (top) and female rats (bottom) on diets with Crag fly repellent added

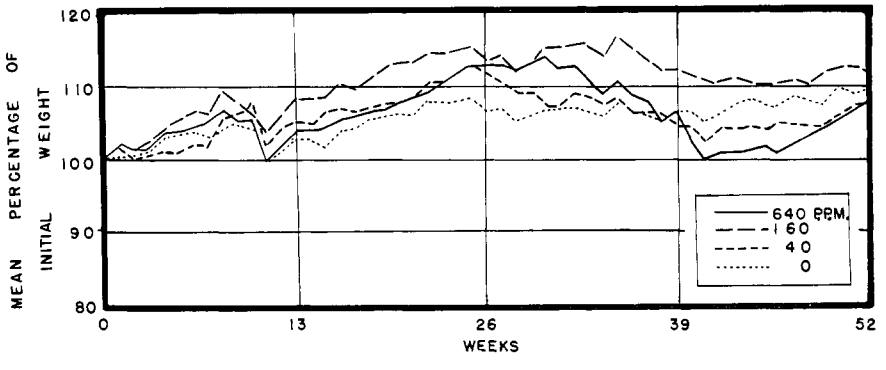

Figure 2. Mean weights of dogs receiving repeated dose of Crag fly repellent

dicated little or no difference between BPG dosed rats and their control group.

The male and female rats were weighed every other week during the first year of doses and growth analyses were performed. Weight comparisons were made only on rats that survived 365 days on BPG. The mean starting weights for the five concentration groups and their controls in descending order—i.e., 0.256, 0.064, 0.016, 0.004, 0.001, and 0.000% were for males: 190.2, 190.2, 190.2, 190.4, 190.8, and 190.2 grams; and for females: 139.4, 139.0, 138.6, 138.6, 139.0, and 138.6 grams. The odds were at least 84 in 100 that the greatest difference between the mean weight of any group of rats and their controls for that sex was produced by chance or random causes. Therefore, no significant difference between group means existed at the start of the study. Figure 1 presents graphically the mean weight response of rats for the first year of doses.

The biweekly mean weight, computed as a percentage of the 60-day weight of each group of male and female rats, was compared by the *t*-test to the mean of the control group of the same sex for the same period. Among the male rats, none of the 26 biweekly means were statistically significantly below those of the controls. In fact all 26 means for the 0.064, 0.016, and 0.004% in the diet were above those of their controls, but only four of these 78 were significantly higher.

The mean weights of the females, at 0.256% in the diet only, were below their controls. All 26 means were numerically lower with 11 of these statistically significantly lower. At the 0.064% concentration, only 16 of the 26 were below their controls. At 0.016% 25 of the 26 means were higher than their controls. This weight effect, apparent in Figure 2, was present only at the highest level or 0.256% BPG in the diet.

The above test is statistically valid in every respect, but it does not show the over-all effect of a chemical upon the growth of rats over the whole period. To estimate this, the mean weights of each group of rats for the entire first year of doses were calculated. These over-all means for the five groups were 204, 212, 213, 208, and 205% of the 60-day weights, respectively, for the males, with none significantly lower than the control mean of 205. In fact, those for 0.064 and 0.016% were statistically significantly higher than their controls. The over-all means for the females showed agreement with the biweekly mean *t*-test previously described. The 0.256% group was significantly depressed, but the others were statistically similar to their control. Means of 171, 179, 182, 181, 180, and 180 were obtained for the five groups and the control in descending order.

If an effect on body weight was produced by the inclusion of the chemical being tested, a relationship would be expected between the amount in the diet and the weight of the rats. To test for this, the relationship between the over-all mean weights of the rats and concentrations of BPG in the diet were compared by the calculations of the coefficient of correlation. This coefficient was −0.331 for the males and −0.974 for the females. These could have occurred 52 times in 100 and two times in 1000, respectively, by chance or random causes alone. The value for the males, therefore, is not indicative of a quantitative relationship between dosage and body weight, but that for the females is highly so. Even this latter was produced solely by the mean of the high level group—omission of it results in a nonsignificant correlation coefficient.

The mean maximum weights attained by the rats alive at 365 days of doses showed no significant effect on this criterion of growth in any of the groups of male or female rats. In summary, no significant weight depression was found in any of the criteria examined for any of the groups of males and only the weights of the 0.256% female rats were depressed.

To follow the hematological picture, 10 male rats were randomly selected for the first hematocrit determination at 55 days of age and at least five of the 10 were alive for the 730-day determination. In all, hematocrits were read six times during the 2 years on the two highest levels and the controls at 90, 180, 270, 360, 540, and 730 days of age. The individual and mean results indicated no statistically significant deviations in the 0.256% dosage level at any time.

Neither incidence of occurrence nor type of tumor was associated with the inclusion of BPG in the diet. There was a total of 24 tumors found among the five dosage and the control groups distributed, respectively, as follows: 6, 3, 6, 4, 2, and 3 in descending order of dosage. These were placed in 11 classes by the pathologist with 16 having abdominal or mammary origin. The probability was 28 in 100 that the difference between the group with the highest incidence and the control was a chance occurrence.

A microscopic examination of organs, enumerated previously, was made on 44 of the 106 animals which succumbed during the course of the 2-year experiment. These 44 included all animals with neoplasms as well as those for which the primary cause of death or sickness was not positively determined at autopsy. Because of the absence of BPG induced pathology among animals at the 0.016 and 0.004% levels, the 0.001% level tissues were not examined microscopically.

Various lung and kidney infections were present but were not related to dosage. An incidence of 43% lung infection and 48% kidney infection occurred in the 96 animals sacrificed after 2 years. 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 representative animals fed 180 doses of the 0.256 and 0.064% diets revealed a pronounced tendency at both levels for cloudy swelling of the kidney convoluted tubules. The probability that the kidney conditions of sacrificed rats at these levels differed by chance from that of their controls was 0.025. The liver and other organs examined histologically appeared unaffected. Subsequent tissue examination after 365 doses showed probabilities of 0.90 and 0.43 for the difference between the incidence of cloudy swelling of the kidney and liver of the 0.256% and their control groups. Therefore, any slight numerical differences which occurred probably were of a random nature.

An evaluation of the observed micropathology for each diet level of BPG after 2 years of doses similarly indicated that none of the tissues examined showed permanent, degenerative changes which could be charged to BPG toxicity. However, transitory changes in the kidney, characterized by diffuse cloudy swelling of the epithelial lining of the proximal convoluted and loop tubules were seen at 0.256 and 0.064% as well as cloudy swelling of the hepatic cords principally located about the central veins in the 0.256% group. At 0.016 and 0.004% microscopic examination revealed no pathology significantly different from that of their controls.

In summary, the 2-year feeding of BPG 800 at five dosage levels separated by a geometric factor of four in quantities ranging from 0.001 to 0.256% in the diet of rats resulted in no deleterious effects in any of the criteria examined among the 0.016, 0.004, or 0.001% groups. These criteria were: amount of diet eaten, body weight gain, liver and kidney weights, incidence of neoplasms, major organ pathology, mortality, and life span.

A synopsis of the toxicological interpretation of the statistically significant effects at 0.256% showed a positive, but transitory effect upon the liver weight of females occurring at 6 months and upon the body weight during the entire 2-year period. Both sexes had cloudy swelling of the kidney convoluted tubules at 6 months, but not at 1 or 2 years. The only criterion that was positive at 0.064% was this same type of minor kidney pathology at 6 months. With the exception of body weight gains of females, all of these significant effects were transitory in nature.

No deleterious effect was found at the highest level, 0.256% in (1) mortality, (2) body weight gain of the males, (3)

incidence of micropathology after 1 or 2 years, (4) kidney weight throughout the study, (5) liver weight at 1 or 2 years, (6) incidence of neoplasms, (7) life span, and (8) hematocrit values. Therefore, the level of BPG 800 that was without permanent significant effect on the health of the rats when included in their diet for 2 years was between 0.064 and 0.256%.

One-Year Feeding of Dogs

After 1 year of the 2-year rat study it became evident that the most probable level of acceptability would lie between 0.256 and 0.064% BPG in the diet. Based on this deduction levels of 640, 160, and 40 p.p.m. were chosen to feed dogs for 1 year in order to broaden the knowledge of toxicity by observing reactions of a nonrodent species. To achieve these levels 0.0128, 0.0032, and 0.0008 gram per kg. per day of BPG were fed in the diet. Because of the individual idiosyncrasies of dogs in their eating habits, mean concentrations of 890, 190, and 47 p.p.m. resulted at the three levels.

The dogs on this study were cocker × basenji or three-quarter basenji hybrids. They were immunized against distemper and hepatitis before delivery from the Roscoe Jackson Memorial Laboratories. All were 2 to 3 years of age, in good health, free of external and internal parasites.

To eliminate any question concerning the intake of BPG the material was fed in size 000 gelatin capsules 5 days per week. Measurement was facilitated by the preparation of dilutions of BPG in Mazola corn oil so that in actual practice the dosages were 0.025 ml. per kg. of a 51.2% dilution for 0.0128 gram per kg., 0.02 ml. per kg. of a 16% dilution for 0.0032 gram per kg. and 0.016 ml. per kg. per day of a 5% dilution for the 0.0008 gram per kg. The largest single dose never exceeded 0.5 ml. of any dilution. The controls received 0.3 ml. of corn oil in similar capsules.

The basic diet for the dogs during the first 42 weeks of the study was Friskies dog food meal. The guaranteed analysis was as follows:

Crude protein minimum	24.0%
Crude fat, minimum	5.0%
Crude fiber, maximum	4.0%
Nitrogen-free extract, minimum	42.0%
Ash, maximum	11.9%
Moisture, maximum	10.0%

The listed ingredients were: meat meal and bone meal, processed wheat, corn, hulled barley and oat groats, soybean oil meal, wheat bran, dried skimmed milk, animal liver and glandular meal, dried beet pulp and molasses, fish meal, wheat germ oil, fish liver oil 0.4%, charcoal 0.2%, iodized salt 0.2%, iron oxide 0.5%, and irradiated yeast 0.03%. In addition, to maintain interest in the diet, cooked hog liver and broth were added sparingly to the meal during the last 10 weeks.

Dogs were randomly distributed by sex, litter, and breed among the treated and control groups. Red and white blood cell counts, differentials, and hematocrits were made prior to the first dose and after 63, 128, 160, 197, and 256 doses. Red cell counts were not made for the last three periods and white cell counts were omitted for the next to the last. At each period hematocrit determinations, fragility, sedimentation rate, bromsulfalein retention, phosphatase, urea nitrogen, and bilirubin determinations were performed. Bromsulfalein retention was determined by dosing the dogs intravenously with 5 mg. per kg. and drawing blood 15 minutes later. Total and direct bilirubin were determined.

The means of the biochemical and hematological values were compared to those of the control group by the *t*-test, using a fiducial limit of significance of 0.05.

The body weight of each dog, for each weekly period, was divided by its weight on the day prior to the first day of doses and this quotient was multiplied by 100. Then, the mean percentages of the original weights of the treated and control groups were compared by the *t*-test.

At the termination of the study the dogs were weighed, anaesthetized with Nembutal, and exsanguinated. Liver and kidneys were extirpated and weighed. Portions of organs were fixed in 8.0% formaldehyde, sectioned, stained with hematoxylin and eosin, and examined microscopically. Included in the examination were: lung, kidney, liver, heart, spleen, pancreas, stomach, small and large intestine, testis or ovary, aorta, prostate, adrenal, thyroid, parathyroid, urinary bladder, tracheo-bronchial lymph nodes, and vastus lateralis muscle. Where required, frozen sections were made for special staining techniques.

The mean weights of the three groups of dogs treated were compared at weekly intervals to those of the controls throughout the study (Figure 2). After 1 year of doses the dogs on 0.0128 gram per kg. were 108% of their first day weight, those on 0.0032 gram per kg. 112%, those on 0.0008 gram per kg. 108% and the controls 110%. None of the mean weights of any group at any week in the year of doses were statistically significantly below those of the controls. The curve shows the results of the temporary loss of appetite and the response to the addition of liver to the basal diet during the last 10 weeks.

No trend of significant difference occurred between the BPG and the control groups, in the means of following criteria of effect: hematocrit values, amount of hemoglobin, total number of white blood cells, percentage composition of the differential white cell counts, blood fragility, and sedimentation rate.

At like intervals in which blood counts were made, no significant differences were found between the means of the treated and the control groups for bromsulfalein retention, serum urea nitrogen, direct and total bilirubin, and alkaline phosphatase.

The weights of the livers of the BPG dogs were significantly different on only one level. The mean for the 0.0032 gram per kg. group was 2.51% of body weight as compared to 3.14% for the controls with values of 2.77% and 2.72% for the highest and lowest levels, respectively. This is not considered a deleterious toxic effect, because it is definitely not related to dosage. The kidney weights were not different.

A dog on the lowest of the three levels died of acute bronchopneumonia after 160 doses. None of the tissue sections of the other animals indicated degenerative changes which could have been produced by the toxicity of BPG. The incidence of each type of micropathological finding was similar among the treated and control groups.

In summary, no detectable deleterious effects were produced in dogs by 0.0128, 0.0032, or 0.0008 grams of BPG 800 per kg. of body weight in their diet 5 days each week during 1 year.

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