Weed Science:

PRINCIPLES AND PRACTICES

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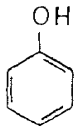
15 Phenols

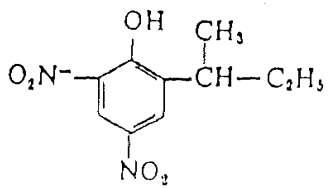


Phenol is the monohydroxy (—OH) derivative of benzene. Herbicides of this class now registered for crop use in the United States have two nitro ($—NO_2$) groups on the ring in the number 4 and number 6 positions. These are dinitrophenols or simply dinitros.

Historically, PCP (pentachlorophenol) was used as a herbicide in certain crops, but it is not registered now for this use in the United States. While it can be used as a preharvest desiccant in small-seeded legume crops grown for seed, it is largely used as a wood preservative.

Substituted phenolic herbicides are contact herbicides. Some have been used for more than 40 years. The sodium salt of DNOC (4,6-dinitro-*o*-cresol) was first used to remove broadleaf weeds from small grain in France about 1933. Soon after, it was introduced into the United States and promptly became the major material for annual broadleaf weed control in cereal crops, flax, and peas. However, it is not now registered for these uses in the United States. It is presently used as a plant growth regulator for blossom thinning in apples.

DINOSEB



dinoseb

Dinoseb is the common name for 2-*sec*-butyl-4,6-dinitrophenol. It is also often called DNBP. It has several trade names. The phenol form is usually formulated as an emulsifiable concentrate; it is also soluble in oil. It may also be formulated as water-soluble salts; the most common are the ammonium, triethanolamine, and a mixture of ethanol and isopropanolamine salts.

Dinoseb is a dark-brown solid or a dark-orange liquid, depending on temperature. As the phenol, it has a water solubility of 52 ppm, but the salts are quite soluble in water.

Toxicity to Humans and Animals

Dinoseb and its salts are dangerous poisons if taken internally, if inhaled as dusts, or if considerable quantities are absorbed through the skin. Therefore, avoid prolonged breathing of the spray drift or dusts, and avoid wearing contaminated clothing or shoes. If your skin is contaminated, wash it immediately with soap and water. Symptoms of poisoning are excessive fatigue, sweating, thirst, and fever. If these develop, send for a physician.

With normal precautions, the chemical can be applied routinely with little or no hazard to the applicators. Daily bathing and change of clothing is recommended whether the applicator thinks he is contaminated or not.

Residues on foliage normally constitute little or no hazard to livestock. In an unpublished study, a milk cow was given 1.7 g of chemical/kg body weight/day for 3 days with no ill effects. Dinoseb did not appear in the milk. If there is question, keep livestock away from sprayed foliage until a rain has removed much of the herbicide.

The acute oral LD_{50} for rats ranges from 5 to 60 mg/kg. The maximum amount tolerated in the diet for a 6-month period was 100 ppm. It is considered to be quite toxic.

Fish are sensitive to dinoseb; 1.0 ppm killed trout and sea lamprey in 14 hr, and bluegills in 5 hr (1).

Uses

Dinoseb is very toxic to growing plants, so it is used as a general contact herbicide. It is so toxic to all leaves that it lacks the selectivity of its salt derivatives. Dinoseb is valuable where mowing is impractical; for example, along fencerows, ditchbanks, and roadsides. It kills most annual weeds and removes the tops from perennial weeds. Underground parts of perennial plants are not killed except by repeated treatments. Thus, dinoseb can be used in dormant alfalfa to kill annual weeds.

TOXICOLOGICAL STUDIES ON LABORATORY ANIMALS OF CERTAIN ALKYLDINITROPHENOLS USED IN AGRICULTURE*

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THE dinitrophenols are used extensively in agriculture for the control of insects, fungi and weeds.

The older literature pertaining to the use of the dinitrophenols as insecticides has been reviewed by Kagy and Richardson (1), who point out that a preparation containing the potassium salt of dinitro-o-cresol was marketed as early as 1892. Since that time the dinitrophenols have been used extensively in dormant sprays for the control of mites, aphids, scale insects, and other pests in overwintering stages. During the last ten years 2-cyclohexyl-4,6-dinitrophenol and its compound with dicyclohexylamine have gained wide acceptance as insecticides; and especially as acaricides for use in the greenhouse, field and orchard.

The dinitrophenols, particularly dinitro-o-cresol and its derivatives, are used as eradicant fungicides for the control of fungi which are pathogenic to a number of plant species.

Dinitro-o-cresol, 2-sec-butyl-4,6-dinitrophenol, and their salts are used extensively in weed control. These materials may be applied as selective weed killers in field crops and pastures, or as contact herbicides in such locations as roadsides and right-of-ways.

The widespread use of the dinitrophenols in the agricultural field has led, naturally, to the question of the problems that may be encountered in their handling and use. The answer to this question depends, to a large extent, upon the physiological effects produced by these materials.

In the case of 2,4-dinitrophenol, there have been many reports dealing with its effect upon laboratory animals and man. This extensive literature has been reviewed by Horner (2) with special emphasis upon the role of 2,4-dinitrophenol in the production of cataracts in man. On the other hand, relatively little information is available on the toxicity of the other dinitrophenols. The report by Ambrose (3) on dinitro-o-cresol and that by Hrenoff and Leake (4) on 2-cyclohexyl-4,6-dinitrophenol essentially cover the work that has been published on these materials.

The present experimental work was undertaken in order to obtain toxicological information which would be useful in evaluating the actual hazards associated with the use of the dinitrophenols, and in formulating safe handling procedures.

*Received for publication August 21, 1947.

Compounds Investigated

The chemical and physical properties of the compounds used in this toxicological study are listed in Table 1. The solubility determinations were made by adding an excess of the material to the solvent and stirring for several hours at 25°C. The excess solid was then filtered off and the amount dissolved was determined by titration of the nitro groups with titanium chloride.

Skin Irritation and Absorption—Rabbits and Guinea Pigs

Rabbits

The effect of each of the dinitrophenols upon the skin was determined on white rabbits by a method previously described (5). The materials were all tested as 3 per cent solutions in 95 per cent ethanol. In some instances, other concentrations and vehicles were used. Heat was used when necessary to dissolve the test material. Routinely, 20 applications were made to the ear and 20 applications were bandaged onto the shaven abdomen over a period of four weeks. The absorption of lethal quantities of the test material or the development of marked skin irritation interrupted this routine in some cases.

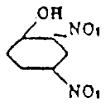
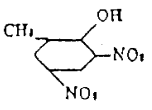
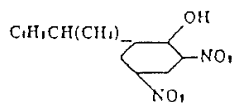
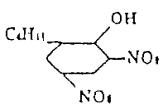
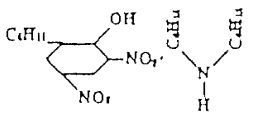
2,4-Dinitrophenol. A 3 per cent alcoholic solution of 2,4-dinitrophenol produced no irritation on the ear and only a very slight irritation on the abdomen, characterized by mild hyperemia, edema, and exfoliation. There was no indication that toxic quantities of 2,4-dinitrophenol were absorbed through the skin under the conditions of this test.

Dormant spray oil containing 4 per cent of 2,4-dinitrophenol was no more irritating to the skin than the oil alone, which produced marked irritation when bandaged onto the abdomen for two or three days. Lethal quantities of 2,4-dinitrophenol were not absorbed through the abdominal skin from three applications in the oil.

4,6-Dinitro-o-cresol. Seven applications of a 3

TABLE 1

CHEMICAL AND PHYSICAL PROPERTIES OF COMPOUNDS INVESTIGATED

PROPERTY	COMPOUNDS				
Name*	2,4-Dinitrophenol	4,6-Dinitro-o-cresol	2-sec-Butyl 4,6-dinitrophenol	2-Cycloheryl-4,6-dinitrophenol	2-Cycloheryl-4,6-dinitrophenol compound with dicycloherylamine
Synonym	4,6-Dinitrophenol	2,4-Dinitro-o-cresol	2,4-Dinitro-o-sec-butylphenol	2,4-Dinitro-o-cycloheryl-phenol	Dicycloherylamine salt of 2,4-dinitro-o-cycloherylphenol
Molecular Formula	$C_6H_4O_5N_2$	$C_7H_6O_5N_2$	$C_{10}H_{12}O_5N_2$	$C_{12}H_{14}O_5N_2$	$C_{12}H_{27}O_5N_3$
Structural Formula					
Formula Weight	184.04	198.13	240.21	266.25	447.56
M.P., °C.	113.2–113.9	86.0–86.9	37.9–39.3	104.1–105.7	191.5–195.8
N Found, %	14.87	13.95	11.55	10.08	9.26
N Theoretical, %	15.22	14.14	11.66	10.52	9.39
Purity of Compd., %					
From N det'n.	97.7	98.7	99.1	95.8	98.6
By titration**	98.9	98.7	97.7	99.6	97.2
Crystal Form	orthorhombic	triclinic	monoclinic	monoclinic	triclinic
Appearance	yellow crystals	yellow crystals	dark amber crystals	yellow crystals	orange crystals
Solubility in grams/100 grams at 23°C					
Water	0.0597	0.075	0.0734	0.072	0.0515
95% Ethanol	3.66	3.69	23.46	1.36	1.76
Oil***	1.67	5.98	8.77	1.84	0.00

* According to Chemical Abstracts.

** Titration of nitro groups with titanium chloride.

*** Oil commonly used in Dormant Sprays.

per cent alcoholic solution of 4,6-dinitro-o-cresol resulted in death from absorption through the skin; but caused no irritation on the ear and only slight irritation of the abdominal skin.

A 5 per cent solution in olive oil was also tested and found to be similar to the alcoholic solution in irritating properties; however, the amount of 4,6-dinitro-o-cresol absorbed from the olive oil solution during the course of 20 applications was insufficient to cause death.

A 4 per cent solution of 4,6-dinitro-o-cresol in Dormant spray oil was no more irritating than the oil alone; although in the case of three different rabbits, one application of this Dormant spray oil solution bandaged onto the shaven abdomen killed the animal within twenty-four hours.

2-sec-Butyl-4,6-dinitrophenol. A 3 per cent alcoholic solution of 2-sec-butyl-4,6-dinitrophenol failed to cause any significant irritation when repeatedly applied to the rabbit ear. However, when it was bandaged onto the shaven abdomen of 3 different rabbits, death occurred after 1, 3 and 8 exposures, respectively. No irritation of the abdominal skin was observed in any instance.

A 10 per cent solution of 2-sec-butyl-4,6-dinitrophenol in butylcarbitol acetate was applied to three rabbits. In each case death occurred within twenty-four hours without evidence of any irritation to the ear or abdominal skin.

2-Cyclohexyl-4,6-dinitrophenol. Alcohol containing 2-cyclohexyl-4,6-dinitrophenol in a concentration of 3 per cent produced no irritation on the ear, but caused a moderate irritation on the abdomen, characterized by moderate hyperemia and exfoliation, and some denaturation. This irritation was severe enough to warrant the termination of the experiment after 7 applications.

A 5 per cent solution of 2-cyclohexyl-4,6-dinitrophenol in olive oil produced only slight irritation of the ear, as shown by mild hyperemia and exfoliation, and a slightly greater response on the abdomen with some blistering as well as hyperemia and exfoliation.

Dormant spray oil containing 4 per cent of the test material appeared to be only slightly more irritating than the oil alone. Lethal quantities of 2-cyclohexyl-4,6-dinitrophenol were not absorbed from 2 applications of the Dormant spray oil solution, 7 applications of the alcoholic solution, or 20 applications of the olive oil solution, bandaged onto the shaven abdomen.

2-Cyclohexyl-4,6-dinitrophenol compound with dicyclohexylamine. A 3 per cent solution of 2-cyclohexyl-4,6-dinitrophenol compound with dicyclohexylamine in 1:4 acetone-alcohol (acetone used to effect complete solubility) failed to cause any appreciable irritation when applied repeatedly to the ear or when bandaged repeatedly to the shaven abdomen.

Tested in a like manner, a 20 per cent solution of the material in olive oil produced no irritation. Lethal quantities of 2-cyclohexyl-4,6-dinitrophenol compound with dicyclohexylamine were not absorbed from either of these solutions during the course of the experiments.

TABLE 2

SUMMARY OF TOXICITY FROM SKIN ABSORPTION FOLLOWING SINGLE APPLICATION—GUINEA PIGS

COMPOUND	"SURVIVAL DOSE"*	"LETHAL DOSE"**
	g./kg.	g./kg.
2-sec-Butyl-4,6-dinitrophenol	0.1	0.5
4,6-Dinitro-o-cresol	0.2	0.5
2,4-Dinitrophenol	0.2	0.7
2-Cyclohexyl-4,6-dinitrophenol	1.0 or more	>1.0
2-Cyclohexyl-4,6-dinitrophenol compound with dicyclohexylamine	1.0 or more	>1.0

* Largest dose survived by all animals treated.

** Smallest dose causing death of all animals treated.

Guinea pigs

Guinea pigs of both sexes were used to determine the toxicity of the dinitrophenols from absorption through the intact skin. The animals used in this work were of heterogeneous stock procured from a commercial supplier and maintained on Purina Rabbit Chow (complete ration), alfalfa hay, and cabbage. A single dose of one of the dinitrophenols in alcoholic solution was applied to the clipped abdomen of each experimental animal. Each guinea pig was restrained on an animal board in such a manner that the treated area could be kept wet with ethanol during the four-hour period following the application of the test material in order to facilitate its absorption. At the end of this period, the surviving animals were removed from the boards, bandaged so as to prevent oral ingestion, caged, and observed until it was certain that they had fully recovered.

The results of this study on 108 guinea pigs are summarized in Table 2 and shown in detail in Table 3.

Summary of Skin Irritation and Absorption

These experiments on rabbits have shown that the dinitrophenols investigated are not significantly irritating to the skin. Even the most irritating one of the group, 2-cyclohexyl-4,6-dinitrophenol, required prolonged exposure to cause appreciable irritation. None of the materials caused any epithelial hyperplasia or follicular changes.

In the skin irritation tests on rabbits it was apparent that some of these materials, particularly, 2-sec-butyl-4,6-dinitrophenol and 4,6-dinitro-o-cresol, were readily absorbed through the intact skin in quantities sufficient to cause death.

from the stock colony in this laboratory, although some were purchased from the Breeding and Laboratory Institute, Brooklyn.

Aliquots of olive oil solutions of the materials were emulsified with 5–10 per cent gum arabic solution and administered by means of a stomach tube (8FS 16" all rubber catheter). The volume of oil given to each rat was always less than 3 ml. and was usually of the order of 1 ml. All of the rats that survived were observed until it was certain that they had fully recovered (usually about two weeks). Deaths that resulted from the administration of the dinitrophenols are believed to

TABLE 3

MORTALITY RESULTING FROM SINGLE APPLICATION TO ABDOMINAL SKIN—GUINEA PIGS

QUANTITY APPLIED TO SKIN	COMPOUNDS (APPLIED IN ALCOHOLIC SOLUTIONS)									
	2,4-Dinitrophenol		4,6-Dinitro-o-cresol		2-sec-Butyl-4,6-dinitrophenol		2-Cyclohexyl-4,6-dinitrophenol		2-Cyclohexyl-4,6-dinitrophenol compound with dicyclohexylamine	
	No. treated	No. died	No. treated	No. died	No. treated	No. died	No. treated	No. died	No. treated	No. died
g./kg.										
0.1	5	0	5	0	5	0	—	—	—	—
0.15	—	—	—	—	5	1	—	—	—	—
0.2	5	0	5	0	5	4	—	—	—	—
0.3	5	1	5	1	5	5	—	—	—	—
0.4	5	1	5	3	5	4	—	—	—	—
0.5	5	2	5	5	5	5	—	—	—	—
0.6	—	—	—	—	5	5	—	—	—	—
0.7	5	5	—	—	—	—	—	—	—	—
1.0	5	5	2	2	1	1	5	0	5	0
Totals....	35		27		36		5		5	

In order to obtain a better estimation of the relative toxicity of these dinitrophenols from skin absorption, the quantitative tests on guinea pigs were carried out. These experiments have demonstrated that the following compounds, listed according to decreasing toxicity, can readily be absorbed through the intact skin in lethal amounts: 2-sec-butyl-4,6-dinitrophenol; 4,6-dinitro-o-cresol; and 2,4-dinitrophenol. On the other hand, neither 2-cyclohexyl-4,6-dinitrophenol nor its compound with dicyclohexylamine are absorbed through the skin to an appreciable extent.

ORAL ADMINISTRATION—SINGLE DOSE BY STOMACH TUBE—RATS

The toxicity of the dinitrophenols when given in single oral doses was determined on young mature white rats of both sexes. Most of the animals were

be due chiefly to their pyretic effect; as a rule, death occurred an hour or two after the feeding or not at all. The detailed results obtained on the 674 rats used in this study are given in Table 5 and are summarized in Table 4.

It is evident that all of the dinitrophenols investigated are rapidly acting materials of a fairly high order of acute oral toxicity. However, the toxicity of 2-cyclohexyl-4,6-dinitrophenol or its compound with dicyclohexylamine is definitely lower than that of 2-sec-butyl-4,6-dinitrophenol or 4,6-dinitro-o-cresol.

ORAL ADMINISTRATION IN THE DIET FOR SIX MONTHS—RATS

Experimental Procedure

The modified Sherman diet (6), which has been used successfully for several years in this labora-

tory as the stock ration for rats, served as the control and basic diet in all of the experiments.

TABLE 4

SUMMARY OF ACUTE ORAL TOXICITY—RATS

COMPOUND	"SURVIVAL" DOSE*	"LETHAL" DOSE**
	g./kg.	g./kg.
2-sec-Butyl-4,6-dinitrophenol	0.005	0.060
4,6-Dinitro-o-cresol	0.010	0.050
2,4-Dinitrophenol	0.027	0.100
2-Cyclohexyl-4,6-dinitrophenol	0.030	0.180
2-Cyclohexyl-4,6-dinitrophenol compound with dicyclohexylamine	0.060	0.600

* Largest dose survived by all animals fed.

** Smallest dose causing death of all animals fed.

The following ingredients were used in the preparation of this stock diet:

Whole wheat, freshly ground	55% by weight
Dried whole milk	25% by weight
Dried meat residue (or dried extracted liver)	12% by weight
Dried brewer's yeast	5% by weight
Calcium carbonate	1% by weight
Iodized table salt	2% by weight

For several years dried meat residue was used in the stock diet, however, when this product was no longer available, dried extracted liver was substituted (March, 1944) and found to be satisfactory.

The experimental diets (see Table 6) were prepared by thoroughly mixing the dinitrophenols with the stock diet on a per cent by weight basis. The test material was added to the diet either directly or by means of a flour concentrate. In either case thorough mixing was obtained by the use of a mechanical mixer, and the actual concentration of the test material in the diet checked by chemical analysis. Each flour concentrate was prepared by adding wheat flour to an alcoholic solution of the test material to form a thick paste

TABLE 5

MORTALITY RESULTING FROM ADMINISTRATION OF SINGLE ORAL DOSE—RATS

SINGLE ORAL DOSE	COMPOUNDS (GIVEN IN OLIVE OIL EMULSIFIED IN GUM ARABIC)									
	2,4-Dinitrophenol		4,6-Dinitro-o-cresol		2-sec-Butyl-4,6-dinitrophenol		2-Cyclohexyl-4,6-dinitrophenol		2-Cyclohexyl-4,6-dinitrophenol compound with dicyclohexylamine	
	No. fed	No. died	No. fed	No. died	No. fed	No. died	No. fed	No. died	No. fed	No. died
g./kg.										
0.005	—	—	—	—	20	0	—	—	—	—
0.010	9	0	20	0	20	1	—	—	—	—
0.020	20	0	20	3	10	1	—	—	—	—
0.023	10	0	—	—	10	2	—	—	—	—
0.025	10	0	—	—	—	—	—	—	—	—
0.027	10	0	—	—	10	0	—	—	—	—
0.030	30	11	20	9	10	4	10	0	—	—
0.040	20	18	20	15	10	5	10	1	—	—
0.050	20	17	20	20	10	7	10	3	—	—
0.060	20	19	—	—	20	20	10	5	10	0
0.070	30	15	—	—	—	—	25	13	—	—
0.080	40	18	—	—	—	—	20	12	—	—
0.100	20	20	—	—	—	—	40	22	5	1
0.120	—	—	—	—	—	—	20	14	—	—
0.140	—	—	—	—	—	—	10	7	—	—
0.160	—	—	—	—	—	—	10	8	—	—
0.180	—	—	—	—	—	—	10	10	—	—
0.200	—	—	—	—	—	—	10	10	—	—
0.400	—	—	—	—	—	—	—	—	5	3
0.600	—	—	—	—	—	—	—	—	10	10
Totals	239		100		120		185		30	

TABLE 6

EXPERIMENTS IN WHICH MALE RATS RECEIVED DIETS CONTAINING DINITROPHENOLS

COMPOUND IN DIET[a]		SOURCE OF RATS	DATE STARTED ON DIET	NO. OF RATS STARTED	SURVIVAL FOR ENTIRE EXPTL. PERIOD	LENGTH OF EXPTL. PERIOD	NO. OF RATS EXAMINED AT END OF EXPT.				
Name	Conc. (wt. %)						Hematology	Bone marrow counts	Organ weights	Blood urea-N	Histopathology
					per cent	*days*					
2,4-Dinitrophenol	0.00	B—L[c]	5-22-45	20	70	179	10**	7	14	14	7
	0.01	B—L	"	15	80	178			12	12	8
	0.02	B—L	"	15	80	179			12	12	8
	0.05	B—L	"	15	66	179	9**	6	10	9	9
	0.10	B—L	"	15	93	179	14		14	14	13
	0.20	B—L	"	10	60	24			6	6	6
4,6-Dinitro-o-cresol	0.00	B—L	3-23-45	20	80	182	10**	8	16	16	8
	0.002*	B—L	"	20	90	179			18	17	10
	0.005*	B—L	"	20	100	182			20	20	10
	0.01*	B—L	"	20	90	182	10**	8	18	18	9
	0.02*	B—L	"	20	80	181			16	16	12
	0.05*	B—L	"	10	90	182	9		9	9	9
	0.05	B—L	3-31-45	12	42	77			5	5	5
	0.10	B—L	7-9-45	10	40	10			4	4	4
	0.10*	B—L	7-9-45	10	50	10			5	5	5
2-sec-Butyl-4,6-dinitrophenol	0.00	B—L	3-2-45	30	70	189	10**	9	21	21	21
	0.005*	B—L	"	20	90	189			18	17	17
	0.01*	B—L	"	20	80	189	10**	8	16	15	15
	0.02*	B—L	"	20	85	189	8	8	17	16	16
	0.05*	B—L	"	10	60	21			6	6	6
2-Cyclohexyl-4,6-dinitrophenol	0.00[b]	Dow	8-18-43	20	70	195	10***	14	14		6
	0.02	Dow	"	20	95	194	10***		19		9
	0.05	Dow	"	20	65	194	10**	12	13		7
	0.10	Dow	"	20	55	195			11		11
2-Cyclohexyl-4,6-dinitrophenol compd. with dicylcohexylamine	0.00[b]	Dow	"	20	70	195	10***	14	14		6
	0.05	Dow	"	20	65	195	10***		13		6
	0.10	Dow	"	20	80	194	10**	15	16		9
	0.20	Dow	"	20	60	196			12		12
Totals.....				472			Approx. 600 exams.	95	355	252	258

[a] Compound added directly to the control diet unless otherwise indicated.
[b] One control group for both compounds.
[c] Breeding and Laboratory Institute, Brooklyn.
* Flour concentrate containing compound added to the control diet.
** Examined bimonthly during experimental period.
*** Examined monthly during experimental period.

which was then dried, ground, passed through an 80-mesh sieve, and analyzed. The use of these concentrates facilitated the accurate addition of small quantities of the test materials to the basic diet.

The diets containing the dinitrophenols were

made up from freshly prepared stock diet when needed. No diet preparations over a month old were used during the course of these experiments. The rats were fed from stainless-steel hoppers which were weighed and refilled three times a week.

White male rats from the stock colony in this laboratory ("Dow") were used in the experiments on 2-cyclohexyl-4,6-dinitrophenol and its compound with dicyclohexylamine. These rats were the progeny of animals obtained from the Wistar Institute in 1938. Since sufficient "Dow" animals were not available, white male rats were purchased from the Breeding and Laboratory Institute, Brooklyn, ("B and L") for the work on the other dinitrophenols.

The "Dow" rats were maintained on the stock diet from the time of weaning until about two months of age when they were divided according to body weights into well matched groups and started on the experimental diets. The rats from the Breeding and Laboratory Institute were received when about twenty-five to thirty days old, although the exact ages were not known, maintained for three to four weeks on the stock diet, and then divided according to body weights into matched groups and started on the experimental diets.

Five rats were caged together, the "Dow" rats in solid bottom cages with wood shavings and sawdust as litter, and the "B and L" rats in wire bottom cages. The animals had free access to food and water at all times. In addition, each rat was given approximately 3 grams of cabbage twice weekly.

The general design of the experiments is presented in Table 6. The following information for each experimental diet is given: concentration of the test material in the diet, method of adding test material to the diet, source of the rats used, date started, number of rats started in each group, length of experimental period, and survival.

The experiments with "Dow" rats on 2-cyclohexyl-4, 6-dinitrophenol and its compound with dicyclohexylamine were started at the same time using only one group of controls; while the experiments with "B and L" rats on 2,4-dinitrophenol, 4,6-dinitro-o-cresol, and 2-sec-butyl-4,6-dinitrophenol were each started separately, making it necessary to use 3 groups of controls.

All of the rats were weighed twice a week throughout the experimental period. Records were kept of the body weight, general appearance, and estimated average daily food consumption of each animal. Animals that died were examined for gross pathological lesions.

During the course of the experiments, periodic hematological examinations were made on several groups of animals as indicated in Table 6. The following determinations were made routinely: erythrocyte count, hemoglobin concentration, total leucocyte count and differential count.

At the end of each experiment all of the surviving rats were starved overnight, weighed, killed by decapitation, and examined. The liver, kidneys, heart, and testes from each rat were weighed; and tissues from representative animals in each group were saved for histopathological studies. Hematoxylin and eosin stained sections of the following organs were prepared: lung, heart, liver, kidney, spleen, adrenal, pancreas, testis, stomach, and bone marrow. The number of animals examined is given in Table 6.

The concentration of urea-N in the blood was determined at the time of autopsy by the diacetyl monoxime procedure (7) on the rats that received 2,4-dinitrophenol, 4,6-dinitro-o-cresol and 2-sec-butyl-4,6-dinitrophenol.

Bone marrow counts were made on many of the rats which had been examined periodically for hematological changes (see Table 6). The number of nucleated cells per cu. mm. of bone marrow was estimated by the method described by Farrar (8) using a red-blood-cell diluting pipette and 1 per cent acetic acid as the diluting fluid. The total cell count was determined in a similar manner using Hayem's solution (9).

Experimental Results

Growth curves for each group of rats on the diets containing the dinitrophenols and on the controls are given in Figure 1. The length of time that each experimental diet was fed and the survival on each diet are given in Table 6. The average final body weights (after overnight starvation) and the average organ weights of the rats in each group that survived for a period of six months are presented in Table 7, together with the standard error of the mean (S.E.) in each case. The t-test (10) was used in comparing the mean values obtained on the experimental groups with those of the controls; probability values (P) of 0.05 or less indicating a significant difference.

No evidence of corneal opacity, cataract formation, or other pathological changes were found in

the eyes of the rats receiving any of the dinitrophenols included in these studies. The hair of all of the animals on the experimental diets was stained, the intensity of the staining being approximately proportional to the concentration of the test material in the diet. This staining probably accordance with the practice followed in this laboratory of immediately killing every animal showing definite signs of pulmonary or ear infection. In these six-month experiments there was no indication that the mortality rate was appreciably increased by the dinitrophenols in the diets.

TABLE 7

BODY WEIGHTS AND ORGAN WEIGHTS OF MALE RATS THAT SURVIVED FOR SIX MONTHS ON DIETS CONTAINING DINITROPHENOLS

COMPOUND IN DIET		NO. OF RATS	BODY WEIGHT (G.)	LIVER (G.)	KIDNEYS (G.)	HEART (G.)	TESTES (G.)
Name	Concn., (wt. %)		Mean ± S.E.[a]	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
2,4-Dinitrophenol	0.00	14	314 ± 7	7.86 ± 0.21	2.18 ± 0.05	1.08 ± 0.02	3.18 ± 0.08
	0.01	12	306 ± 7	8.13 ± 0.11	2.36 ± 0.04**	1.10 ± 0.02	3.04 ± 0.17
	0.02	12	314 ± 8	8.31 ± 0.27	2.37 ± 0.08*	1.07 ± 0.03	3.38 ± 0.13
	0.05	9	286 ± 7*	7.89 ± 0.32	2.39 ± 0.12*	1.03 ± 0.02	3.02 ± 0.17
	0.10	14	269 ± 5**	8.08 ± 0.24	2.38 ± 0.07*	1.01 ± 0.02*	3.15 ± 0.12
4,6-Dinitro-o-cresol	0.00	16	300 ± 8	7.78 ± 0.27	2.16 ± 0.09	1.05 ± 0.03	2.85 ± 0.12
	0.002	18	292 ± 8	7.08 ± 0.28	2.00 ± 0.09	1.02 ± 0.03	2.77 ± 0.08
	0.005	20	293 ± 8	7.23 ± 0.25	1.99 ± 0.05	1.00 ± 0.03	2.74 ± 0.09
	0.01	18	304 ± 8	7.55 ± 0.28	2.01 ± 0.06	1.01 ± 0.02	2.58 ± 0.15
	0.02	16	277 ± 7*	6.86 ± 0.22*	1.82 ± 0.05**	0.94 ± 0.03*	2.61 ± 0.07
	0.05	9	247 ± 7**	7.14 ± 0.19	2.09 ± 0.07	0.96 ± 0.04	2.49 ± 0.16
2-sec-Butyl-4,6-dinitrophenol	0.00	21	278 ± 4	7.13 ± 0.14	1.90 ± 0.05	0.95 ± 0.01	2.59 ± 0.06
	0.005	18	286 ± 5	6.83 ± 0.12	1.86 ± 0.04	0.95 ± 0.02	2.69 ± 0.07
	0.01	16	281 ± 5	7.47 ± 0.23	1.90 ± 0.07	0.96 ± 0.03	2.76 ± 0.10
	0.02	17	266 ± 6	7.85 ± 0.24**	2.06 ± 0.07	0.96 ± 0.03	2.67 ± 0.08
2-Cyclohexyl-4,6-dinitrophenol	0.00[b]	14	341 ± 10	9.16 ± 0.42	2.13 ± 0.06	1.01 ± 0.03	2.57 ± 0.09
	0.02	19	322 ± 5	8.88 ± 0.21	2.24 ± 0.06	1.06 ± 0.02	2.59 ± 0.09
	0.05	13	322 ± 12	9.11 ± 0.35	2.22 ± 0.08	1.05 ± 0.03	2.62 ± 0.09
	0.10	11	291 ± 14**	8.97 ± 0.40	1.98 ± 0.08	0.90 ± 0.03*	2.40 ± 0.09
2-Cyclohexyl-4,6-dinitrophenol compd. with dicyclohexylamine	0.00[b]	14	341 ± 10	9.16 ± 0.42	2.13 ± 0.06	1.01 ± 0.03	2.57 ± 0.09
	0.05	13	316 ± 8	9.19 ± 0.33	2.18 ± 0.07	0.98 ± 0.03	2.57 ± 0.08
	0.10	16	314 ± 9*	9.16 ± 0.32	2.29 ± 0.07	1.06 ± 0.02	2.53 ± 0.11
	0.20	12	289 ± 8**	8.75 ± 0.25	2.10 ± 0.05	0.96 ± 0.03	2.43 ± 0.20

[a] S.E. = Standard Error of the Mean.
[b] One control group for both compounds.
* P = 0.05 – 0.01 (as determined by the t-test (10)).
** P = < 0.01

came from contact with the food and apparently did no harm to the animals.

In all of the experiments which lasted for six months, the survival was essentially the same in all groups of rats, controls as well as those on the diets containing the dinitrophenols (Table 6). Most of the deaths, that occurred during the course of these experiments, were shown by autopsy to be due to pulmonary infection. Other rats were sacrificed in

The daily food intake of the control rats and of the rats in the experimental groups that grew equally as well as the controls was found to be from 10 to 20 grams per rat. It may be calculated that these rats, weighing from 200 to 350 grams, ingested quantities of the dinitrophenols of the order of 0.002–0.00054, 0.005–0.00135, 0.01–0.0027, 0.02–0.0054, 0.05–0.0135, 0.10–0.027, and 0.20–0.054 g./kg./day when maintained on diets con-

taining 0.002, 0.005, 0.01, 0.02, 0.05, 0.10, and 0.20 per cent of the test materials, respectively.

No doubt there was considerable individual variation in the quantity ingested depending upon the body weight and food intake of each rat. During the first few weeks on the experimental diets the young rats actually received greater quantities of the dinitrophenols on a g./kg./day basis than later when they were approaching maturity.

The animals that made poor weight gains usually showed an obvious dislike for the experimental diet, ate rather sparingly, and wasted a great deal of the food by pawing and scratching at the hopper; in these cases it was impossible to obtain an accurate estimation of the food consumption.

The hematological examinations which were made during the course of these experiments are indicated in Table 6. In every case, the erythrocyte counts, hemoglobin concentrations, leucocyte counts, and differential counts obtained on the animals receiving the dinitrophenols agreed satisfactorily with those obtained at the same time on their own controls. Similarly, there was satisfactory agreement between the bone marrow counts obtained at autopsy on the rats that had been maintained for six months on diets containing the dinitrophenols and the values obtained on their own controls. Thus, there was no indication that the feeding of diets containing 2,4-dinitrophenol, 4,6-dinitro-o-cresol, 2-sec-butyl-4,6-dinitrophenol, 2-cyclohexyl-4,6-dinitrophenol, or its compound with dicyclohexylamine at the concentrations shown in Table 6 for a period of six months produced any change in the bone marrow or blood picture of male rats. For the sake of clarity, further discussion of the experimental results is given separately for each of the dinitrophenols included in this investigation.

2,4-Dinitrophenol. Rats in the group of 10 animals on the diet containing 0.20 per cent 2,4-dinitrophenol lost weight rapidly and four of them died after seven, twelve, sixteen, and twenty-one days, respectively. The six survivors were killed and examined after twenty-four days on the diet, at which time they were very thin and weak. Gross examination of each animal revealed marked emaciation, an empty gastrointestinal tract, a slightly enlarged, dark spleen and small testes. Microscopic examination showed slight congestion and cloudy swelling of the liver, very slight parenchymatous degeneration of the epithelium of the renal tubules, slight congestion and hemosiderosis of the spleen, and testicular atrophy. No significant pathological changes were observed in the lung, heart, adrenal, pancreas, or stomach. The concentration of urea-N in the blood of these animals averaged 31.7 mg. per cent. It is difficult to distinguish clearly between the ill effects produced in these animals by dinitrophenol and those due to decreased food consumption.

The growth curve of the group of rats that received the diet containing 0.1 per cent 2,4-dinitrophenol fell 10 to 15 per cent below that of the controls throughout the experimental period of six months (Figure 1). The difference between the average final body weight of these animals and that of their controls was highly significant ($P = (<0.001)$). During the course of the experiment these animals showed no discernible ill effects other than slight emaciation. At autopsy, the only changes noted in the experimental group as compared with the controls was a slight depletion of the body fat, a very slight increase in the average weight of the kidneys and a very slight decrease in the weight of the heart (Table 7). Two of the 14 animals examined gave blood urea-N values of 32.8 and 36.6 mg. per cent; the other animals in this group had an average blood urea-N of 21.6 as compared with 19.4 mg. per cent for the controls. No appreciable changes were found upon microscopic examination of sections of the lung, liver, kidney, heart, spleen, adrenal, pancreas, testis, stomach, and bone marrow from these animals.

The growth curve of the group of rats that received the diet containing 0.05 per cent 2,4-dinitrophenol fell 5 to 10 per cent below that of the controls throughout the six-month experimental period (Figure 1). The difference between the average final body weight of these animals and that of their own controls was quite significant ($P = 0.01$). At autopsy the only changes observed in the experimental animals as compared with the controls was a very slight depletion of body fat, and a very slight increase in the average weight of the kidneys (Table 7). Blood urea-N values of 38.2 and 44.8 mg. per cent were found in two of the nine experimental animals. The other rats in this group had an average blood urea-N of 19.5 as compared with 19.4 mg. per cent for the controls. No significant pathological changes were found upon microscopic examination of the tissues.

In the case of the groups that received the diets containing 0.02 and 0.01 per cent 2,4-dinitrophenol, the growth curves were essentially the same as that of the controls throughout the six-month experimental period (Figure 1). During the course of these experiments no discernible ill effects were noted in these animals; and at autopsy the only change observed in the experimental groups as compared with the controls was a very slight increase in the average weight of the kidneys.

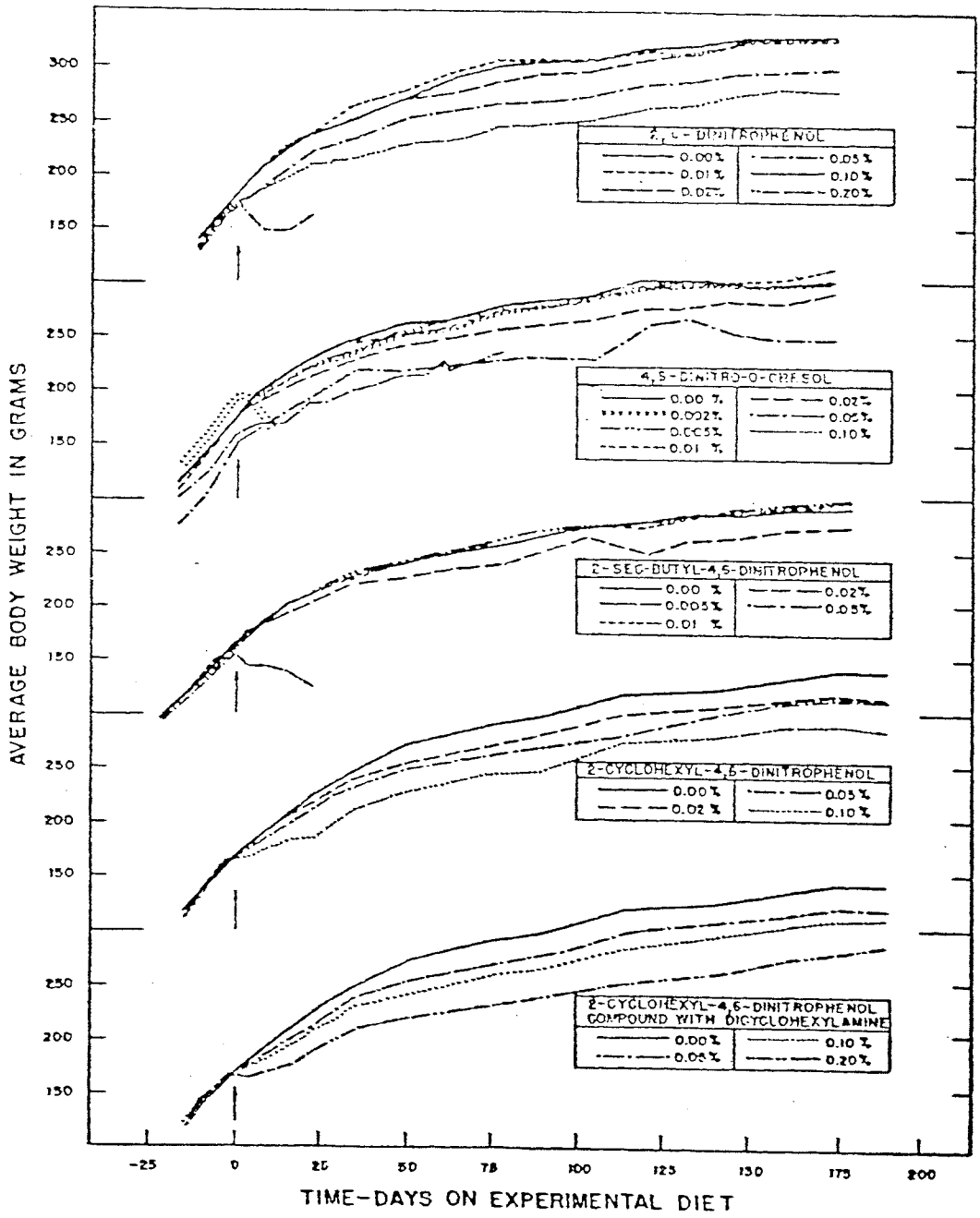


FIG. 1. Growth curves of male rats on diets containing dinitrophenols.

The average blood urea-N was 22.8 and 18.2 mg. per cent for the groups on the 0.02 and 0.01 per cent diets, respectively, as compared with 19.4 mg. per cent for the controls. No pathological changes were found upon microscopic examination of sections of the lung, liver, kidney, heart, spleen, adrenal, pancreas, and testis from these animals.

4,6-Dinitro-o-cresol. The two groups of rats on the diets containing 0.10 per cent 4,6-dinitro-o-cresol, one prepared from the flour concentrate and the other by direct mixing, lost weight rapidly (Figure 1), and appeared weak, hungry, thin and unkempt. The surviving rats were killed on the tenth day of the experiment, since about half of the rats in each group had died by this time (Table 6). Gross examination of each animal revealed marked emaciation, an empty gastrointestinal tract, and a slightly enlarged dark spleen. The average blood urea-N concentration in these rats was 44.4 mg. per cent. Microscopic examination showed cloudy swelling of the liver, very slight degenerative changes in the renal tubules, and slight congestion of the spleen. No appreciable changes were observed in the lungs, heart, adrenals, pancreas or testes. In an experiment such as this, it is difficult to differentiate between the effects produced by the test material and those due to decreased food intake.

Both groups of rats on the diets containing 0.05 per cent 4,6-dinitro-o-cresol made poor weight gains (Figure 1) and appeared hungry, thin and unkempt throughout the course of the experiment. The surviving rats on the diet prepared by direct mixing with 4,6-dinitro-o-cresol were killed and examined after seventy-seven days (Table 6). No appreciable change in the organ weights was noted in these animals. Two of the five rats gave blood urea-N values of 34.4 and 35.0 mg. per cent, the other three an average value of 24.4 per cent. Congestion and hemosiderosis of the spleen was noted in three animals. No appreciable changes were observed upon microscopic examination of the lung, heart, kidney, liver, adrenal, pancreas, testis and stomach. The group receiving the diet prepared from the flour concentrate were allowed to continue on the experiment until the end of the six-month period. At autopsy depletion of the body fat was observed, but no gross changes were evident in the viscera. The average weights of the liver, kidneys, heart, and testes for the experimental animals were essentially the same as those for the controls even though the average body weight of the experimental group was much lower than that of the controls (Table 7). Four of the nine experimental animals showed an average blood urea-N of 30.4 mg. per cent, the other 5 an average of 21.9 mg. per cent. No significant changes were observed upon microscopic examination of the tissues from these animals.

The growth curve of the group of rats on the diet containing 0.02 per cent 4,6-dinitro-o-cresol fell 7 to 9 per cent below that of the controls throughout the experimental period of six months (Figure 1). This depression in growth is probably significant, since a comparison, by means of the t-test (10), of the body weights of the experimental animals with those of the controls at frequent intervals during the 6-month period yielded P values of 0.05 or less in most instances. The general appearance of these animals was good during the course of the experiment and at autopsy there were no evident ill effects; although the average organ weights in the case of the liver, kidneys, and heart were slightly lower than those for the controls (Table 7). The average blood urea-N in this group was 16.1 as compared with 15.8 mg. per cent for the controls. No pathological changes were observed upon microscopic examination of the tissues from these animals.

In the case of the groups that received the diets containing 0.01, 0.005, and 0.002 per cent 4,6-dinitro-o-cresol, the growth curves were essentially superimposed on that of the controls throughout the six-month experimental period (Figure 1). No ill effects were observed in these animals during the course of the experiment or at autopsy. The organ weights obtained on these experimental animals compared favorably with those of the controls (Table 6). The average blood urea-N concentration was 14.0, 17.8 and 18.1 mg. per cent for the rats on the 0.01, 0.005, and 0.002 per cent diets, respectively, as compared with 15.8 mg. per cent for the controls. No histopathological changes were found upon examination of the tissues from these animals.

2-sec-Butyl-4,6-dinitrophenol. The group of 10 rats on the diet containing 0.05 per cent 2-sec-butyl-4,6-dinitrophenol lost weight rapidly and four of the animals died after five, ten, and thirteen days, respectively. The six survivors were killed and examined after twenty-one days on the diet. Examination of these animals showed marked emaciation, an empty gastrointestinal tract and an average blood urea-N concentration of 55.0 mg. per

cent. Microscopic examination of their tissues revealed slight degenerative changes in the renal tubules, and slight cloudy swelling of the liver, but no appreciable changes in the lung, heart, spleen, adrenal, pancreas, or testis. Here again, it is difficult to distinguish between the effects due to the test material and those associated with inanition.

The growth curve of the group of rats on the diet containing 0.02 per cent 2-sec-butyl-4,6-dinitrophenol fell 3 to 8 per cent below that of the controls during the experimental period of six months (Figure 1). This depression in growth is probably significant since a comparison of the body weights of the experimental rats with those of the controls at frequent intervals during the course of the experiment yielded P values slightly below 0.05 in most cases. No discernible ill effects were observed in these animals, either during the course of the experiment or at autopsy. The average blood urea-N was 20.3 as compared with 17.5 mg. per cent for the controls. The organ weights did not vary appreciably from that of the controls except for a slight increase in the weight of the liver (Table 7). Microscopic examination of the tissues from these animals failed to reveal any appreciable changes as compared with the controls.

The growth curves of the groups of rats that received the diets containing 0.01 and 0.005 per cent 2-sec-butyl-4,6-dinitrophenol were superimposed on that of the controls throughout the six-month experimental period (Figure 1). These animals showed no evidence of ill effects either during the course of the experiment or at autopsy. The average blood urea-N concentration in the animals on the 0.01 and 0.005 per cent diets were 20.9 and 17.3 mg. per cent, respectively, as compared with 17.5 mg. per cent for the controls. The organ weights of the experimental animals compared favorably with those of the controls (Table 7); and no histopathological changes were found upon examination of their tissues.

2-Cyclohexyl-4,6-dinitrophenol. The growth curve of the group of rats on the diet containing 0.10 per cent 2-cyclohexyl-4,6-dinitrophenol fell 10 to 15 per cent below that of the controls throughout the six-month experimental period (Figure 1). This depression in growth is quite significant as shown by frequent comparisons of body weights during the course of the experiment which consistently yielded P values much below 0.05. The general appearance of these animals was good and at autopsy there were no evident ill effects other than a slight loss in body fat. The organ weights of the experimental animals agreed well with those of the controls except for heart weights which were slightly lower (Table 7). Slight cloudy swelling of the liver was the only change observed upon microscopic examination of the tissues.

In the case of the groups of rats on the diets containing 0.05 and 0.02 per cent 2-cyclohexyl-4,6-dinitrophenol the growth curves fell 3 to 10 per cent below that of the controls throughout the experimental period (Figure 1). It is very doubtful that this slight depression in growth is significant, since analyses of the body weights at frequent intervals during the course of the experiment yielded values of P below 0.05 in only a few instances; in no case was a P value of less that 0.014 encountered. The organ weights obtained on these animals compared favorably with those of the controls (Table 7). No histopathological changes were found upon examination of the tissues from these animals.

2-Cyclohexyl-4,6-dinitrophenol compound with dicyclohexylamine. The group of rats receiving the diet containing 0.20 per cent 2-cyclohexyl-4,6-dinitrophenol compound with dicyclohexylamine showed a definite depression in growth throughout the experimental period (Figure 1); their final average body weight falling 15 per cent below that of the controls ($P = <0.001$). Except for slight emaciation, these experimental animals showed no evident ill effects, either during the course of the experiment or at autopsy. Their organ weights compared favorably with those of the controls (Table 7), and no histopathological changes were observed other than slight cloudy swelling of the liver.

The growth curves of the groups of rats on the 0.10 and 0.05 per cent diets fell 5 to 9 and 7 to 11 per cent respectively, below that of the controls during the experimental period of six months (Figure 1). The significance of this depression in growth is questionable since comparisons at frequent intervals of the body weights of the rats on the 0.05 per cent diet with those of the controls gave values of P slightly above 0.05 in most instances; while similar studies on the group receiving the 0.10 per cent diet yielded P values only slightly below 0.05 in most cases, with an occasional value above 0.05. No discernible ill effects were apparent in these experimental animals either during the course of the experiment or from organ weight and

histopathological studies conducted at its termination.

Summary of Feeding Experiments with Rats

Male rats maintained for six months on diets containing 0.01 per cent 2-sec-butyl-4,6-dinitrophenol, 0.01 per cent 4,6-dinitro-o-cresol, 0.02 per cent 2,4-dinitrophenol, 0.05 per cent 2-cyclohexyl-4,6-dinitrophenol, and 0.05 per cent 2-cyclohexyl-4,6-dinitrophenol compound with dicyclohexylamine showed no appreciable ill effects as determined by frequent gross observations, growth curves, periodic blood counts, analyses for blood urea-N, organ weights, and histopathological examinations.

Effects that may be attributed to the action of the dinitrophenols as metabolic stimulants were observed in rats that received diets containing greater quantities of these materials. Thus, depression in body weight, chiefly at the expense of body fat, was the characteristic finding rather than appreciable organic injury. No cataracts were produced in any of the rats receiving the dinitrophenols, nor were there any changes in the bone marrow or blood picture.

In the case of each of the dinitrophenols investigated, it is evident that there is very little summation of toxic effects upon prolonged ingestion, as judged by a comparison of the quantity that caused death after a single oral dose with the quantity that produced no appreciable ill effects when administered daily in the diet for a period of six months.

Oral Administration in the Diet for Short Time—Ducklings

In view of the unfortunate human experience with 2,4-dinitrophenol, there have been many attempts to produce cataracts in laboratory animals by the administration of this material. However, all of these were unsuccessful until Robbins (11) found that cataracts formed very quickly in ducklings or chicks following the feeding of a diet containing the sodium salt of 2,4-dinitrophenol.

In order to obtain comparative information concerning the production of cataracts by 2,4-dinitrophenol, 4,6-dinitro-o-cresol, 2-sec-butyl-4,6-dinitrophenol, and 2-cyclohexyl-4,6-dinitrophenol, the following experimental work was undertaken.

Experimental Procedure

Five-day old white Pekin ducklings were purchased from a commercial hatchery, maintained on Purina Duck Startena for about a week, and then started on diets prepared by thoroughly mixing definite quantities of the dinitrophenols with the Startena. The 8 to 10 ducklings in each of the experimental groups were examined frequently for body weight changes, observable ill effects, and particularly cataract formation.

Diets containing the following concentrations of the dinitrophenols were used: 0.25 per cent sodium salt of 2,4-dinitrophenol; 0.25 per cent 4,6-dinitro-o-cresol; 0.25, 0.10, and 0.03 per cent 2-sec-butyl-4,6-dinitrophenol; and 0.25 and 0.10 per cent 2-cyclohexyl-4,6-dinitrophenol.

Experimental Results

The per cent mortality in each experimental group and the incidence of cataracts are tabulated in Table 8.

Within twenty-four hours all of the ducklings receiving the diet containing 0.25 per cent sodium salt of 2,4-dinitrophenol showed bilateral cataracts. This concentration was sufficient to cause considerable retardation in growth and even a few deaths within the experimental period of thirty-five days. Since the appearance of the cataracts and the changes occurring in the eye during the course of the experiment were similar to those reported by Robbins (11), a detailed description is unnecessary.

Similarly, cataracts were produced within twenty-four hours in the ducklings on the diet containing 0.25 per cent 4,6-dinitro-o-cresol. This concentration proved rapidly fatal to the birds.

The ducklings on the diet containing 0.25 per cent 2-sec-butyl-4,6-dinitrophenol died within three days without showing cataracts. Those on the 0.10 per cent diet died within four days with the appearance of cataracts in one animal on the third day. Even at the level of 0.03 per cent in the diet, 2-sec-butyl-4,6-dinitrophenol was not well tolerated, 50 per cent of the ducklings dying within five days; nevertheless cataracts were observed in one bird on the fifth day and in another on the eighth, when the experimental birds were accidentally killed.

No cataracts were observed in the ducklings that received diets containing 2-cyclohexyl-4,6-dinitrophenol. The birds on the diet containing 0.25 per cent of the material died within four days; while

of the ten ducklings that received the 0.10 per cent diet one died on the second day, one on the third, two on the fourth, two on the fifth, two on the ninth, and one on the thirty-eighth, at which time the surviving bird was killed.

Summary of Feeding Experiments with Ducklings

The production of cataracts in ducklings by the administration of the sodium salt of 2,4-dinitrophenol, first reported by Robbins (11), has been substantiated. Furthermore, it has been demonstrated that cataracts can be produced in this species as readily by 4,6-dinitro-o-cresol as by 2,4-dinitrophenol, and only slightly less readily by 2-sec-butyl-4,6-dinitrophenol. On the other hand, cataracts were not produced in ducklings by the administration of 2-cyclohexyl-4,6-dinitrophenol in the diet for as long as thirty-eight days.

TABLE 8

PER CENT MORTALITY AND INCIDENCE OF CATARACTS IN YOUNG DUCKLINGS ON DIETS CONTAINING DINITROPHENOLS

NO. OF DAYS ON DIET	CONTROL DIET		SODIUM SALT OF DINITROPHENOL		4,6-DINITRO-O-CRESOL		2-SEC-BUTYL-4,6-DINITROPHENOL						2-CYCLOHEXYL-4,6-DINITROPHENOL			
	0.00%		0.25%		0.25%		0.25%		0.10%		0.03%		0.25%		0.10%	
	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C
1	0	0	0	100	56	100	56	0	0	0	0	0	11	0	0	0
2	0	0	0	100	100		56	0	63	0	20	0	11	0	10	0
3	0	0	0	100			100		88	100	40	0	56	0	20	0
4	0	0	0	100					100		50	0	100	0	40	0
5	0	0	0	100							50	20			60	0
6	0	0	10	100							50	20			60	0
7	0	0	10	100							50	20			60	0
8	0	0	10	100							50**	40			60	0
9	0	0	10	100											60	0
10	0	0	10	100											80	0
15	0	0	10	100											80	0
18	0*	0	30	100											80	0
25			30	100											80	0
30			30	100											80	0
35			40*	100											80	0
38															90*	0

M—Per cent mortality.
C—Per cent of living ducklings with cataracts.
* All surviving ducklings killed.
**Surviving ducklings accidentally killed.

DISCUSSION OF PRACTICAL HANDLING PROBLEMS

The following discussion of the health problems associated with the handling and use of the dinitrophenols is based upon information obtained from experimental work on animals and from extensive field experience in the agricultural use of these materials.

Physiological Action

The predominant physiological action of this class of compounds is that of a metabolic stimulant. This action is the same whether the material is absorbed through the skin or is absorbed following inhalation or oral ingestion. This characteristic physiological effect makes it possible to use the increase in basal metabolic rate as an indication of absorption following exposure. Increased body temperature, profuse sweating, and nausea may also be observed in some cases. A serious effect attributed to 2,4-dinitrophenol following its clinical use was the production of cataracts in some individuals.

Skin Contact

Contact with the dinitrophenols incident to their use in agriculture does not present a significant problem from skin irritation. The rare individual

who is hypersensitive to this class of compounds should not work with these materials.

Neither 2-cyclohexyl-4,6-dinitrophenol nor its compound with dicyclohexylamine are absorbed through the skin to an appreciable extent and, consequently, there is no significant problem of skin absorption associated with the handling and use of these materials.

On the other hand, both 4,6-dinitro-o-cresol and 2-sec-butyl-4,6-dinitrophenol are very readily absorbed through the skin and, therefore, present many problems in safe handling. Strict precautions must be observed in order to avoid contact of the skin with liquid concentrates (greater than 5 per cent) of 4,6-dinitro-o-cresol, 2-sec-butyl-4,6-dinitrophenol, or their salts. Should some of the concentrate come in contact with the skin, it must be removed immediately by very thorough washing of the contaminated area with soap and water. Clothing contaminated with the liquid concentrate must be removed immediately and washed before it is worn again. In some cases, it may be necessary to wear suitable protective clothing in order to avoid skin contact with liquid concentrates of these materials.

The low concentrations (usually less that 1 per cent) of 4,6-dinitro-o-cresol and 2-sec-butyl-4,6-dinitrophenol that are commonly found in diluted sprays ready for field use, should not present a significant handling problem from skin absorption; nevertheless, it is advisable to avoid excessive contamination of the clothing and prolonged wetting of the skin with sprays containing these materials.

Appreciable skin absorption should not result from contact with dusts or dry mixes containing the dinitrophenols, except in the case of preparations containing very high concentrations (above 30 per cent) of 4,6-dinitro-o-cresol, 2-sec-butyl-4,6-dinitrophenol, or their salts. In the handling of such preparations reasonable care should be taken to avoid unnecessary skin contact.

It should be remembered that the staining of the skin by a dinitrophenol compound gives no indication of the quantity absorbed or of the actual hazard involved, but merely shows that there has been some contact with the material.

Inhalation

The systemic toxicity of the dinitrophenols is sufficiently high that any one of them, under certain conditions, may present a health hazard from the inhalation of dusts or sprays. Nevertheless, the practical handling problems presented by preparations containing either 2-cyclohexyl-4,6-dinitrophenol or its compound with dicyclohexylamine are much less serious than those encountered with preparations containing 4,6-dinitro-o-cresol or 2-sec-butyl-4,6-dinitrophenol.

The inhalation of either liquid or dry mix concentrates (greater than 5 per cent) of any of these compounds should be avoided. In some cases suitable respirators may be necessary to afford adequate protection. The low concentrations of the dinitrophenols commonly found in preparations ready for field use should not present a significant problem, although reasonable care should be taken to avoid the breathing of excessive amounts of the dust or spray.

Ingestion

The oral intake of toxic quantities of the dinitrophenols should not present a significant practical handling problem. Persons handling these materials should exercise reasonable care to avoid the contamination of food or anything else that may be taken into the mouth. Care should be taken to wash well before eating.

Disposal of Wastes

The improper disposal of the sludge or residue left after a spraying operation may lead to serious consequences. The ingestion of these wastes by cattle or other livestock must be prevented by adequate safeguards. The careless dumping of such wastes in ponds, streams, or irrigation ditches may be deleterious to aquatic life as well as contaminate the water for other uses.

Residues on Fruits and Vegetables

During the many years that dusts and sprays containing dinitrophenols have been used, no significant health problems have arisen from residues remaining on fruits and vegetables. Under the usual conditions of use, there is ample time for the removal of the residue from the fruit before harvest by volatilization and by the leaching action of rain. In addition, the experimental results reported in this paper indicate clearly that a much greater margin of safety is provided by the use of 2-cyclohexyl-4,6-dinitrophenol or its compound with dicyclohexylamine than by 4,6-dinitro-o-cresol or 2-sec-butyl-4,6-dinitrophenol.

Foliage Treated with Weed-killers

Field experience with the selective weed-killers containing dinitrophenols has shown that when these sprays are used as recommended, livestock may be pastured upon recently treated foliage without evident ill effects. On the other hand, foliage that has been heavily or excessively sprayed with either selective or contact weed-killers may be dangerous; livestock should be kept away from even small areas treated in such a manner.

Summary and Conclusions

Studies on rabbits have shown that none of the dinitrophenols investigated are appreciably irritating to the skin.

Experiments on rabbits and guinea pigs have demonstrated that the following compounds, listed according to decreasing toxicity, can readily be absorbed through the skin in lethal amounts: 2-sec-butyl-4,6-dinitrophenol, 4,6-dinitro-o-cresol, and 2,4-dinitrophenol. On the other hand, neither 2-cyclohexyl-4,6-dinitrophenol nor its compound with dicyclohexylamine is absorbed through the skin to an appreciable extent.

It has been determined by experimental work on rats that all of the dinitrophenols investigated are rapidly acting materials of a fairly high order of acute oral toxicity. The following values for "Survival Dose" and "Lethal Dose," respectively, were obtained for each of the compounds: 2-sec-butyl-4,6-dinitrophenol, 0.005 and 0.060 g./kg.; 4,6-dinitro-o-cresol, 0.010 and 0.050 g./kg.; 2,4-dinitrophenol, 0.027 and 0.100 g./kg.; 2-cyclohexyl-4,6-dinitrophenol, 0.030 and 0.180 g./kg.; and 2-cyclohexyl-4,6-dinitrophenol compound with dicyclohexylamine, 0.060 and 0.600 g./kg.

Male rats maintained for six months on diets containing 0.01 per cent 2-sec-butyl-4,6-dinitrophenol, 0.01 per cent 4,6-dinitro-o-cresol, 0.02 per cent 2,4-dinitrophenol, 0.05 per cent 2-cyclohexyl-4,6-dinitrophenol, and 0.05 per cent 2-cyclohexyl-4,6-dinitrophenol compound with dicyclohexylamine exhibited no appreciable ill effects as determined by gross observations, growth curves, periodic blood counts, analyses for blood urea-N, organ weights, and histopathological examinations. Higher concentrations in the diet produced effects that may be attributed to the action of these materials as metabolic stimulants.

In the case of each of the dinitrophenols studied, there is very little summation of toxic effects upon prolonged ingestion, as indicated by a comparison of the quantity that caused death after a single oral dose with the quantity that produced no appreciable ill effects when administered daily in the diet for a period of six months.

Cataracts were readily produced in ducklings by the administration of 2,4-dinitrophenol, 4,6-dinitro-o-cresol, or 2-sec-butyl-4,6-dinitrophenol in the diet; but not by 2-cyclohexyl-4,6-dinitrophenol when fed for as long as thirty-eight days.

A discussion is given of the practical handling problems associated with the use of the dinitrophenols, based upon information obtained from experimental work on animals and from extensive field experience in agriculture. Consideration is given to the health problems that may arise from skin contact, inhalation, or ingestion.

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