Insecticide Toxicology

Augmented Susceptibility to Carbaryl Toxicity in Albino Rats Fed Purified Casein Diets

E. M. Boyd and M. A. Boulanger

The clinicopathologic syndrome of acute toxicity to carbaryl given intragastrically was determined in young male albino rats fed for 28 days after weaning on *a*, laboratory chow, *b*, a purified diet containing casein in amounts adequate for normal growth, and *c*, a purified diet containing casein in 30% of the amount present in diet *b*. The acute oral LD_{50} ± S.E. was 744 ± 40 mg. per kg. of body weight in *a*, 575 ± 51 in *b*, and 506 ± 78 in *c*. Clinical signs were similar in all three groups and consisted of immediate lacrimation, salivation, tremors and convulsive movements, followed by pallor, listlessness, anorexia, oligodipsia, loss of body weight, oliguria, aciduria, glycosuria, hematuria, proteinuria, respiratory failure, and death. Autopsy revealed hemorrhagic congestion of the brain and meninges, widespread capillary-venous dilatation, a stress reaction, and renal tubular degeneration in animals with delayed deaths in *a* and *b* plus evidence of protein deficiency in *c*. The results indicate that carbaryl is more toxic in rats fed a purified casein diet, especially if it is protein deficient.

The objective of this study was to obtain information on the safety of carbaryl as a pesticide in regions where the diet is low in protein. Best and Murray (1962) found no evidence of clinical toxicity and absorption of but minor amounts of the insecticide in workers engaged in the initial commercial production of carbaryl in the U. S. A. during 1959. Similar results were reported by Vandekar (1965) among spraymen and the inhabitants of three villages, the houses of which were sprayed with 5% carbaryl, in Africa where protein deficiency is prevalent in young children (World Health Organization, 1965). The African studies were performed with precautionary measures against contamination and no evidence is available to indicate possible influences of protein-deficient diets. As an approach to the problem, the clinicopathologic syndrome of toxicity to carbaryl was studied in kwashiorkoric rats fed from weaning on a protein-deficient diet after the technique of De Castro and Boyd (1967).

Carbaryl, 1-naphthyl-*N*-methylcarbamate, is a member of the carbamate or physostigmine series of insecticidal cholinesterase inhibitors (Casida, 1963). It is a white, crystalline, stable powder, almost insoluble in water but soluble in corn oil (Baron *et al.*, 1964). It is readily absorbed when given by mouth and is detoxified in the body by conjugation of the products of hydrolysis of the carbamic acid ester linkage and of the products of hydroxylation of the naphthyl ring (Dorough and Casida, 1964; Knaak *et al.*, 1965). While basically a cholinesterase inhibitor, it has other pharmacological effects peculiar to it (Goldberg and Johnson, 1964; Weiss and Orzel, 1967). Its extensive use as a pesticide followed its consideration in 1959 by the U. S. Department of Agriculture as a possible replacement for DDT in the gypsy moth eradication program (Gyrisco *et al.*, 1960).

Department of Pharmacology, Queen's University, Kingston, Ontario, Canada

METHODS

The experiments were performed upon weanling male Wistar-strain albino rats of 50 to 60 grams body weight obtained from Canadian Breeding Laboratories, St. Constant, Quebec, Canada. They were divided into three dietary groups: one, fed Purina laboratory chow (Ralston Purina Co., Ltd., Woodstock, Ontario, Canada); the second fed "Protein Deficient Test Diet" (General Biochemicals, Chagrin Falls, Ohio), which contained 8% casein, 75% starch, 14% hydrogenated vegetable oils, 3% salt mix (U.S.P. XIV), and adequate vitamin supplements; and the third fed "Normal Protein Test Diet" (General Biochemicals), which contained 27% casein and 56% of starch but otherwise was of the same composition as the diet fed the second group. Carbaryl was given as a single dose after the animals had been on these diets for four weeks. The numbers of animals and their body weight at the end of 4 weeks of feeding are listed in Table I.

The animals were segregated one to a metabolism cage and given water but no food for 16 hours prior to administration of carbaryl. Analytical grade carbaryl was dissolved in cottonseed oil and given intragastrically through a cannula attached to a syringe in a fixed volume of 20 ml. per kg. of body weight. The advantages of using a fixed

Table I. Acute LD_{50} and Associated Parameters Following Oral Administration of Carbaryl[a]

	Laboratory Chow Diet	Protein-Deficient Diet	Normal Protein Diet
Number of animals	95	90	87
Body weight when carbaryl given, mean ± S.D., grams	219 ± 12	80 ± 12*	190 ± 22*
LD_{50} ± S.E., mg. per kg.	744 ± 40	506 ± 78*	575 ± 51*
Mean hours to death (±S.D.)	8.5 ± 5.1	4.5 ± 3.4	9.4 ± 8.3
Deaths within 1 hour, % incidence	56	66	60
Death at >15 hours, % incidence	38	29	26
Immediate cause of death	Respiratory failure	Respiratory failure	Respiratory failure
Gross pathology at death			
Cerebral congestion, % incidence	100	91	100
Pneumonitis, % incidence	15	11	10

[a] Asterisk indicates that results in animals fed protein-deficient or normal protein diets differed from those in rats fed laboratory chow at $P = 0.05$ or less.

volume are reviewed by Boyd (1968). Following pilot studies with doses suggested by the literature (Carpenter *et al.*, 1961; FAO, 1965), carbaryl was given in doses of from 200 to 1200 mg. per kg. of body weight to the numbers of animals indicated in Table I with 16 controls in each dietary group given cottonseed oil. Each animal was returned to its metabolism cage and offered Purina laboratory chow and water ad libitum for four days, during which time clinical measurements were recorded and after which the survivors were killed and autopsied.

Clinical signs were noted at hourly intervals during the first day and later as indicated and were semiquantitated as 1 to 4 clinical units. At 24-hour intervals during the four-day postdose period were recorded: daily growth in grams, food intake as grams of laboratory chow per kilogram of body weight per 24 hours, water intake as milliliters per kilogram per 24 hours, colonic temperature in degrees Fahrenheit, urinary volume as milliliters per kilogram per 24 hours, urinary glucose and protein output as milligrams per kilogram per 24 hours, urinary blood as clinical units per kilogram per 24 hours, and urinary pH on the 24-hour sample, using techniques which have been described (Boyd and De Castro, 1968).

Premortem signs were recorded in detail and at death the animal was autopsied and gross and microscopic observations recorded on the organs listed in Table III. Histopathology was noted on blocks of tissue fixed in Lillie's buffered formalin and sections stained with hematoxylin-phloxine-saffron. The results were analyzed by application of a t test to mean differences from respective controls and by regression analysis where indicated, using the methods of Croxton (1959). The LD_{50} ± S.E. was calculated by the linear regression method of Boyd (1965).

RESULTS

At the time of administration of carbaryl, growth was stunted in rats which had been fed the protein-deficient diet (Table I).

Data upon the LD_{50} ± S.E. of carbaryl, interval to death, immediate cause of death, and gross pathology are listed in Table I. The interval to death was inversely correlated with dose of carbaryl. Carbaryl had a lower LD_{50} in rats fed both synthetic diets than in rats fed laboratory chow but the other parameters listed in Table I were essentially the same in all three dietary groups.

Data upon immediate clinical signs in rats fed laboratory chow are illustrated in Figure 1 and upon daily measurements in Figures 2 and 3. The signs of cholinergic stimulation (lacrimation and salivation) were accompanied by tremors, convulsive movements, listlessness, and pallor. During the first 24 hours, there was marked anorexia and oligodipsia with a decline in body weight, oliguria, aciduria, hematuria, glycosuria, and proteinuria. The intensity of all these signs increased with increase in dose of carbaryl.

Differences in the intensity of clinical signs and daily clinical measurements in rats fed the protein-deficient diet are summarized in Table II. The clinical syndrome in survivors which had been fed the protein-deficient diet was less marked than in survivors fed laboratory chow and was associated with polydipsia and polyuria on days

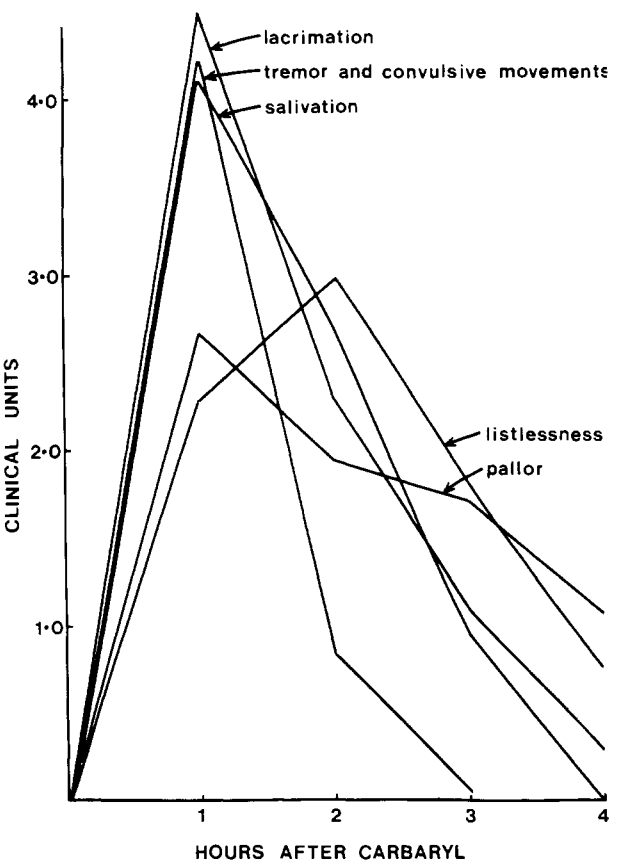

Figure 1. Regression, on hours after carbaryl administration, of signs of toxicity immediately following oral administration to albino rats fed laboratory chow

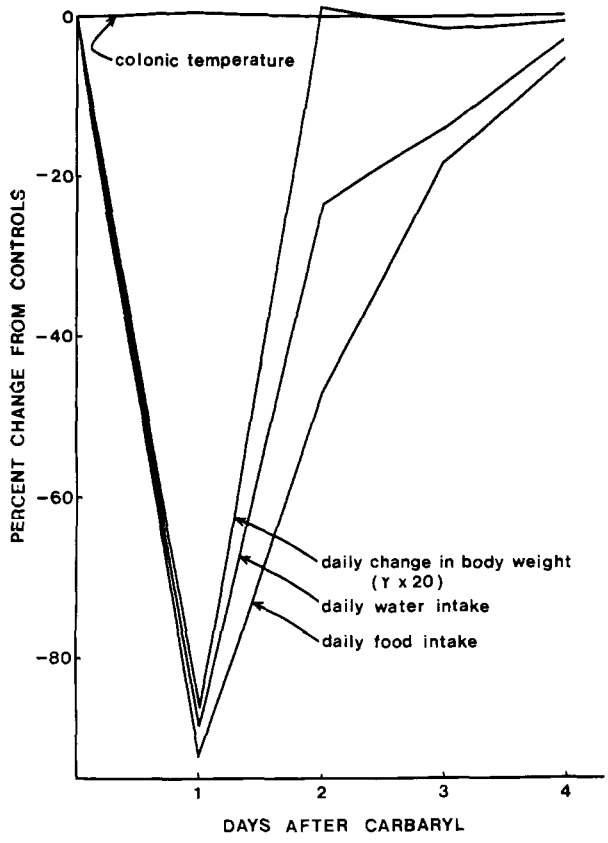

Figure 2. Regression, on days after carbaryl administration to albino rats previously fed laboratory chow, of mean per cent change from controls in colonic temperature, daily growth, daily water intake, and daily food intake

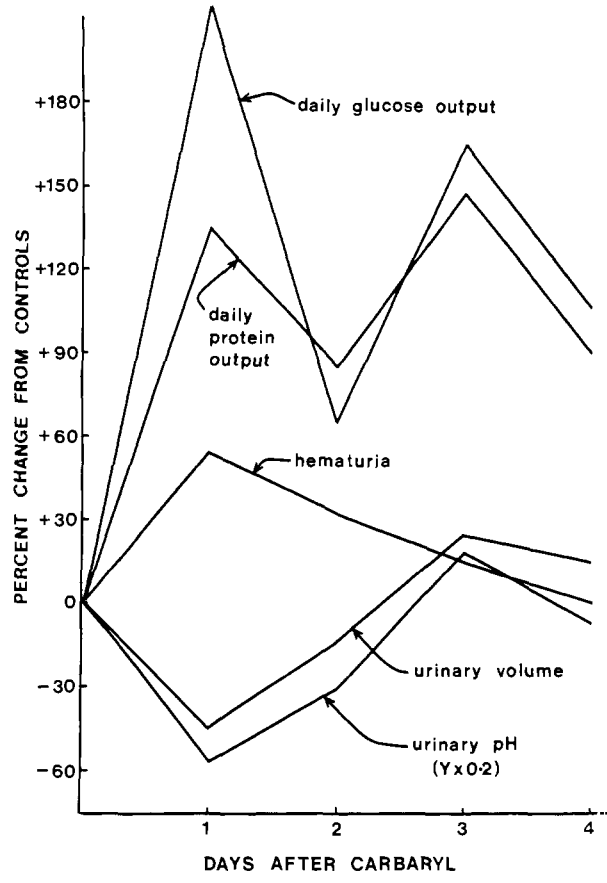

Figure 3. Regression, on days after carbaryl administration to albino rats previously fed laboratory chow, of mean per cent change from controls in urinary glucose output, urinary protein output, units of hematuria, urinary volume, and urinary pH

2 to 4. The syndrome in rats fed normal protein diet was similar to that in rats fed laboratory chow as shown in Table II.

The histopathological findings at autopsy are listed in Table III. The main observations were cerebral and meningeal congestion and hemorrhage associated with capillary-venous vasodilatation of many organs, a stress reaction in the adrenal glands, thymus gland, and spleen, and renal tubular degeneration in animals with a delayed death. Signs characteristic of kwashiorkor (Boyd and De Castro, 1968) appeared in the kidneys, liver, muscle, pancreas, salivary glands, thymus gland, and tail skin of rats fed the protein-deficient diet (Table III). Otherwise histopathology was identical in rats of the three dietary groups.

Recovery was rapid in survivors (Figures 2 and 3). Animals fed the protein-deficient diet recovered somewhat faster than those fed laboratory chow or normal protein test diet.

DISCUSSION

The acute oral LD_{50} ± S.E. of 744 ± 40 mg. per kg. of body weight found for carbaryl given to chow-fed male albino rats is somewhat higher than 390 to 670 reported by Carpenter *et al.* (1961) who used younger male rats and suspended carbaryl in 0.25% agar. The oral LD_{50} has been reported to be lower when carbaryl is dissolved in corn oil than when suspended in 0.25% agar (FAO, 1965). Corn oil has been found to inhibit liver esterase activity (Baron *et al.*, 1964). The volume of cottonseed oil solvent was maintained constant because Boyd (1968) has reported that toxicity increases with increasing volumes of solvent. Cottonseed oil produced no toxicity itself at the volume (20 ml. per kg. of body weight) used.

The clinical signs of toxicity in rats have been summarized in the abstract and were similar to those recorded in dogs (Carpenter *et al.*, 1961) except that vomiting was not seen in the rat, which has no projectile vomiting reflex. Anorexia was marked in the rat and may correspond to vomiting in dogs. Of the histopathological signs of toxicity recorded herein, renal fatty degeneration has been reported in hens (FAO, 1965). Renal fatty degeneration was not evident when death occurred within an hour at which time the main changes were cerebral congestion, widespread capillary-venous dilatation, and a stress reaction.

Carbaryl was more toxic to rats fed a protein-deficient diet only to the extent that the LD_{50} was slightly and barely significantly ($P = 0.05$) lower than in rats fed a normal

Table II. Clinical Signs of Toxicity to Carbaryl in Rats Fed a Normal Protein Diet or a Protein-Deficient Diet Compared with Those in Rats Fed Laboratory Chow

Sign	Normal Protein Diet	Protein-Deficient Diet
Tremor	More prolonged	No change
Lacrimation	No change	No change
Salivation	No change	No change
Pallor	No change	No change
Listlessness	Less marked	Less marked
Inhibition of growth	No change	Less marked during first day
Inhibition of food intake	No change	Less marked during first day
Oligodipsia	No change	Less marked during first day; marked polydipsia on days 2 to 4
Colonic temperature	No change	Sustained rise of 0.5 to 2.5%
Urinary volume	No oliguria on day 1	No oliguria on day 1 and marked diuresis on days 2 to 4
Hematuria	No change	No change
Glycosuria	More marked	No change
Proteinuria	Less marked	No change
Aciduria	No change	No change

Table III. Summary of Histopathological Findings at Autopsy

Organ	Histopathology
Adrenal glands	Lipoid droplets prominent in cortex
Brain	Areas of capillary-venous congestion and hemorrhage in the brain and meninges
Gastrointestinal tract	
Cardiac stomach	Normal appearance
Pyloric stomach	Normal appearance
Small bowel	Hyperemia of lamina propria
Cecum	Hyperemia of lamina propria
Colon	Normal appearance
Heart	Mild hyperemia of myocardium
Kidneys	Areas of capillary-venous congestion and hemorrhage, especially in the loop region; renal tubular degeneration in late deaths; all signs more evident in rats fed the protein-deficient diet
Liver	Sinusoidal congestion in early deaths; areas of fatty degeneration and necrosis in rats fed the protein-deficient diet
Lungs	Areas of capillary-venous congestion, hemorrhage and venous thrombosis; more evident in rats fed the protein-deficient diet
Muscle (ventral abdominal wall)	Normal appearance; occasionally weak cross striation in rats fed the protein-deficient diet
Pancreas	Normal appearance; some atrophy of acinar glands in rats fed the protein-deficient diet
Salivary (submaxillary) glands	Deficiency of zymogenic granules in serous glands especially in rats fed the protein-deficient diet
Skin	Normal appearance; atrophic shedding of stratum corneum over tail in rats fed protein-deficient diet
Spleen	Red pulp contracted
Testes	Normal appearance
Thymus gland	Centrilobular loss of thymocytes especially in rats fed the protein-deficient diet

protein test diet as a purified diet control. It is apparent from Table I that factors other than protein deficiency accounted for part or most of the increased toxicity of carbaryl in rats fed the protein-deficient diet. The susceptibility of rats to drug toxicity has been found to vary when their diet is changed from laboratory chow to a purified diet apparently adequate in all nutritional ingredients. For example, on purified diets the toxicity of benzylpenicillin (Boyd *et al.*, 1965) and caffeine (Peters, 1967) increased while that of dicophane (Boyd and De Castro, 1968) decreased.

Dicophane or DDT has been reported to be no more toxic when given orally to rats fed a protein-deficient diet than when given to healthy rats fed laboratory chow (Boyd and De Castro, 1968). Since carbaryl was more toxic in rats fed both types of purified casein diets, it is apparent that diet may have variable effects on the toxicity of pesticides.

CONCLUSIONS

The acute $LD_{50} \pm$ S.E. of analytical grade carbaryl dissolved in cottonseed oil and given intragastrically to overnight-starved young male albino rats fed a diet of laboratory chow was 744 ± 40 mg. per kg. of body weight. Over half the animals died within one hour, the interval to death being inversely related to dose of drug. The clinical signs of toxicity were lacrimation, salivation, tremors, convulsive movements, listlessness, pallor, anorexia, oligodipsia, loss of body weight, oliguria, aciduria, hematuria, glycosuria, and proteinuria. The immediate cause of death was respiratory failure associated with marked vascular congestion and hemorrhage of the brain and meninges and accompanied by capillary-venous vasodilatation of many organs, a stress reaction in the adrenals, thymus gland, and spleen, and a tubular nephritis when death was delayed. The median lethal dose of carbaryl was lowered by feeding the animals an apparently nutritionally adequate synthetic diet containing casein in "normal amounts" and reduced somewhat further by feeding the same synthetic diet but with the casein content reduced to the point where growth of weanlings was markedly impaired. The results indicate that diet can affect the toxicity of carbaryl.

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Received for review February 2, 1968. Accepted July 12, 1968. This project was assisted financially by Grant F5/181/5 of the World Health Organization and by the Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada. Carbaryl was provided as analytical grade Sevin insecticide by the Olefins Division, Union Carbide Corp., South Charleston, W. Va.

Correction

DEGRADATION OF CARBAMATE HERBICIDES IN SOIL

In this article by D. D. Kaufman [J. AGR. FOOD CHEM. **15,** 582 (1967)], the following corrections should be made: Page 588, line 29 from below should read *N*-methyldithiocarbamate instead of *N*-dimethyl. . . .; page 588, line 22 from below should read methylisothiocyanate rather than methylisocyanate; page 589, line 21 from below, delete SMDC, replace with Na-dimethyldithiocarbamate; page 589, line 12 from below, delete SMDC, replace with dimethyldithiocarbamate; and page 590, Figure 8, the letters SMDC in the figure and caption should be replaced with Na-dimethyldithiocarbamate. The reference Sijpesteijn *et al.* (1962) should be Menzies (1966).
