Acute Oral Toxicity of Monuron in Albino Rats Fed from Weaning on Different Diets

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Male albino rats were fed from weaning on purified diets containing 0.0 (I), 3.5 (II), 9.0 (III), and 26% (IV) protein as casein. At the end of 28 days of feeding, the acute oral LD_{50} ± S.E. of monuron was found to be 0.25 ± 0.05 gram per kg. body weight in group I, 0.95 ± 0.24 in II, 1.58 ± 0.47 in III, 2.88 ± 0.31 in IV, and 1.48 ± 0.21 in control rats fed a standard laboratory chow. The clinical syndrome of toxicity at the range of the LD_{50} was essentially the same in rats of all dietary groups. At autopsy there was found a local irritant gastroenteritis accompanied by degenerative changes in the liver and kidneys, a stress reaction and loss of weight, and insignificant alterations in water content of most body organs.

In evidence reviewed by Boyd (1969), the nature and amount of protein in diets fed to weanling albino rats were shown to have variable influences on their subsequent susceptibility to the toxic effects of pesticides. Rats fed a purified diet which contained normal amounts of protein as casein were less susceptible to the toxic effects of dicophane (DDT) and malathion and more susceptible to carbaryl and endosulfan than were rats fed a standard laboratory chow. As the percentage of casein was lowered in the purified diet, rats became increasingly more susceptible to the toxic effects of all pesticides studied but to a degree which varied from pesticide to pesticide. For example, if dietary casein were lowered to one seventh the normal requirement, the animals did not grow and became twice as susceptible to single killing doses of lindane but twenty-six times more susceptible to captan. Since these results suggested that pesticides should be selected with caution in countries where dietary protein intake is low and variable, it was decided to determine if the same precautions might apply to herbicides.

Monuron was chosen for trial. It is a substituted urea chemically described as 3-(*p*-chlorophenyl)-1,1-dimethyl urea, introduced in 1951 (Bucha and Todd, 1951), and known under various synonyms such as Karmex and Telvar. Monuron is a whitish, odorless powder which is insoluble in water but soluble in cottonseed oil. Applied to soil in a dose of 0.8 to 4.8 pounds per acre before seedlings emerge, it selectively inhibits growth of certain weeds because of differences between plants in its uptake or metabolism (Smith and Sheets, 1967). In larger doses, it may be applied to inhibit all plant growth as in railroad yards and drainage ditches (Hodge *et al.*, 1958). Ernst and Böhme (1965) reported that animals (rats) metabolize nontoxic doses of monuron by hydroxylation of the phenyl ring (*e.g.* at the alpha carbon), by dealkylation of a methyl group off the urea moiety, and partly by hydrolysis of the urea group between atoms 2 and 3. The acute oral LD_{50} in rats has been reported as 3.6 grams per kg. (Monuron Technical Data, 1968) and tolerated daily doses in rats have been noted by Hodge *et al.* (1958).

METHODS

The general technique was similar to that of a previous study on carbaryl described in detail in this Journal by Boyd and Boulanger (1968). The experiments were performed upon male weanling albino rats of a Wistar strain obtained from Canadian Breeding Laboratories, St. Constant, Quebec, Canada. They were divided into five dietary groups. Group one was fed Purina laboratory chow checkers obtained from the Ralston Purina Co. Ltd., Woodstock, Ontario, Canada. Group two was fed "Protein Test Diet, Low" obtained from General Biochemicals, Chagrin Falls, Ohio. This diet was prepared after the formula of Hegsted and Chang (1965) and contained 3.5% casein, 81.5% cornstarch, 8% hydrogenated cottonseed oil, 4% salt mix U.S.P. XIV, and 3% vitamin supplements. Group three was fed "Protein Test Diet, Normal" which contained 26% casein and 59% cornstarch and otherwise was of the same composition as the diet fed to group two. Group four was fed the same purified diet but containing 0% casein and 85% cornstarch, and group five received a diet containing 9% casein and 76% cornstarch. The diet fed to group five was similar to the protein deficient diet used in the studies of Boyd and Boulanger (1968) on carbaryl.

The animals were fed these diets for 28 days which was an interval long enough for adaptation as noted by De Castro and Boyd (1968). At this time the mean ± S.D. body weight in grams was 199 ± 11 in group I, 60 ± 5 in group 2, 177 ± 13 in group 3, 48 ± 8 in group 4, and 98 ± 12 in group 5. In the first group, 2 out of 130 animals had died, in the second group 7 out of 118, in the third group 11 out of 154, in the fourth group 23 out of 50, and in the fifth group 4 out of 50. The death rate in weanlings fed 0% casein (group 4) was significantly higher than death rates in the other 4 groups, and survivors had lost 19% of their initial weight at weaning.

At the end of 28 days on diet, the animals were placed in metabolism cages, 1 rat per cage, with water but no food for 16 hours (overnight) to empty the stomach. Next morning they were given monuron in a range of single doses estimated from pilot studies to produce from 10 to 90% mortality rates. Each dose was given to 5 to 15 animals and 15 controls received cottonseed oil. The monuron was obtained as Technical Monuron 94% from the Industrial and Biochemical Department, E. I. du Pont de Nemours and Co., Wilmington, Del. It was freshly dissolved in cottonseed oil, U.S.P., and given by intragastric cannula in a constant volume of 20 ml. per kg. body weight after evidence, reviewed by Boyd (1968), that alteration in volume may affect mortality rates.

The animal was then returned to its metabolism cage with food and water available *ad libitum*. Clinical signs of toxicity were recorded as 1+ to 4+ clinical units hourly for 8 hours and then at intervals of 24 hours with premortem signs re-

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corded in detail when possible. At intervals of 24 hours, the daily change in body weight was measured in grams, food intake as grams per kg. body weight per 24 hours, water intake as ml./kg./24 hours, colonic temperature in degrees Fahrenheit, urinary volume in ml./kg./24 hours, urinary blood in units/kg./24 hours, urinary glucose and protein output in mg./kg./24 hours, and urinary pH on 24-hour specimens.

An autopsy was performed upon all animals which died and gross pathology recorded. Histopathology and fresh weight and water content were recorded on the organs listed in Table II in rats of groups 1, 2, and 3 when autopsy could be performed within one hour of death to avoid postmortem changes described by Boyd and Knight (1963). The results were analyzed statistically by application of t tests of significance of differences between means and by regression of differences on dose or time after methods noted by Croxton (1959). The $LD_{50} \pm$ S.E. was calculated by analysis of the linear regression of dose on per cent mortality as described by Boyd (1965). Further details of methods have been reviewed by Boyd (1968).

RESULTS

Calculated values for the $LD_{50} \pm$ S.E. of monuron in animals of the several dietary groups are assembled in Table I along with the intervals to death. The LD_{50} in animals fed 9% casein diet was the same as that in animals fed laboratory chow and in this respect results with monuron were similar to those with dicophane or DDT reported by Boyd and De Castro (1968). Rats previously fed the purified diet containing 26% casein were approximately half as susceptible to the lethal action of monuron as were rats fed 9% casein or laboratory chow. Rats fed 3.5% casein were three times, and rats fed 0% casein twelve times more susceptible to monuron toxicity than were rats fed normal (26%) amounts of casein. The interval to death progressively decreased with decreasing percentages of casein in the diet.

With a few minor exceptions, clinical signs of toxicity were the same in animals of all five dietary groups. At 1 to 8 hours there appeared ataxia, drowsiness, exophthalmos, hyporeflexia, listlessness, pallor, piloerection, prostration, and tachypnea. Exophthalmos was not recorded in rats previously fed Protein Test Diet, Normal. Pallor and piloerection occurred only in rats previously fed laboratory chow. At 24 hours the dominant clinical signs were dacryorrhea, diarrhea, epistaxis, hyperreflexia, irritability, and sialorrhea, the last sign being seen only in rats previously fed laboratory chow. In rats of all five dietary groups, the premortem signs were adipsia, anorexia, anuria, dacryorrhea, dyspnea, epistaxis, hyporeflexia, hypothermia, loss of body weight, prostration, and respiratory or cardio-respiratory failure followed by death.

Clinical measurements at 24 hours are exemplified by data obtained in rats previously fed 3.5% casein (group 2) and the results are presented in Figure 1. The controls given no monuron lost some 5% of body weight during the overnight (16 hour) fast previous to administration of cottonseed oil; during the subsequent four days on the same diet they regained most of this loss at the average rate of 0.6 gram per rat per day. During the first two days after giving monuron, there occurred a further loss of body weight which was some fourfold the gain in body weight of the controls, yielding a daily percent change from controls of some −500% as shown in Figure 1. Similar results were recorded in animals fed 0% casein but the initial loss of body weight in animals fed 9 and 26% casein or laboratory chow was relatively less (though

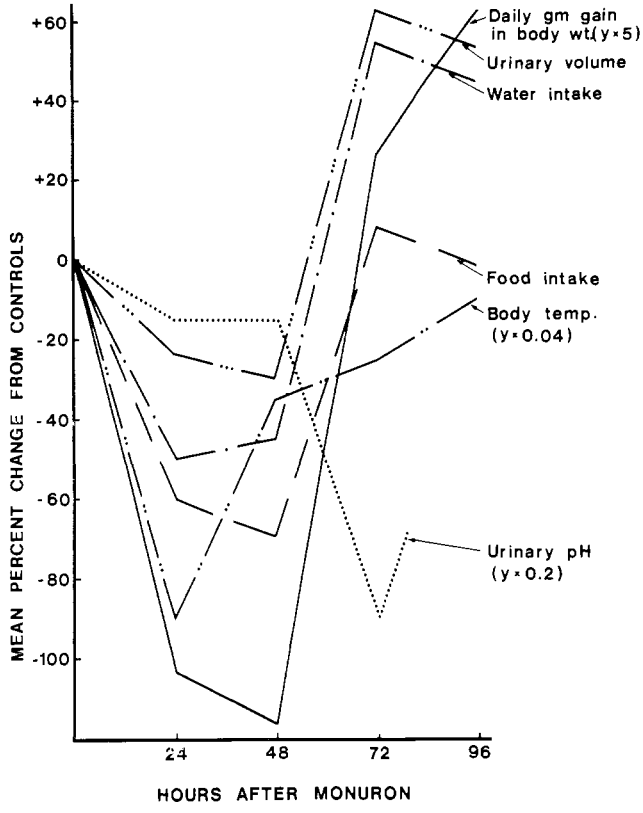

Figure 1. Regression, on days after oral administration of monuron in doses of from 0.7 to 1.2 grams per kg. body weight to albino rats previously fed a diet containing 3.5% protein, of mean percentage changes from controls (given cottonseed oil) in representative clinical measurements

Ordinate units must be multiplied by 5 to obtain percentage change in daily body weight gain, by 0.04 to obtain per cent change in body temperature, and by 0.2 for per cent change in urinary pH

Table I. $LD_{50} \pm$ S.E. in Grams per Kg., of Monuron Given Orally to Albino Rats Previously Fed Laboratory Chow or Purified Diets Containing Varying Amounts of Casein

Diet	$LD_{50} \pm$ S.E.	Hours to Death (Mean ± S.D.)
Laboratory chow	1.48 ± 0.21	27 ± 12
0% Casein	0.25 ± 0.05[a]	11 ± 5[a]
3.5% Casein	0.95 ± 0.24[a]	32 ± 14
9% Casein	1.58 ± 0.47	34 ± 18
26% Casein	2.88 ± 0.31[a]	41 ± 16[a]

[a] Significantly different by t tests (Croxton, 1959) from results in animals fed laboratory chow at P = 0.02 or less.

absolute gram loss was greater). The marked susceptibility to monuron toxicity of rats fed 0 and 3.5% casein appeared to be associated with this relatively marked initial loss of body weight in animals which had not grown beyond their weaning weight.

The percentage decrease in food intake was of the same order in all five dietary groups. The food offered following administration of monuron was the same as that each group had been eating before receiving the herbicide. Animals fed 0% and 3.5% casein (groups 4 and 2, respectively) were particularly susceptible to decreased food intake. Animals of group 4 fed 0% casein had a death rate of some 20% during the period of overnight (16 hours) complete starvation previous to monuron administration. There was a 5% mortality in controls of group 2 and 4 following administration of

Table II. Histopathological Findings in Animals Which Died from Oral Administration of Monuron in Doses at the Range of the LD_{50}[a]

Organ	Histopathology
Adrenal glands	Edematous appearance; occasionally sinusoidal congestion
Brain	Occasionally minor meningeal congestion
Gastrointestinal tract	
Cardiac stomach	Occasionally congested with ulcers
Pyloric stomach	Hyperemic with occasional necrotic ulcer
Small bowel	Mild to moderate capillary-venous congestion of the lamina propria and submucosa, occasionally hemorrhage and edema of the villi
Cecum	Capillary-venous congestion of the lamina propria
Colon	Occasionally congested; goblet cells dilated
Heart	Occasionally capillary congestion
Kidneys	Capillary-venous congestion of the loop of Henle; cloudy swelling of the distal convoluted tubules
Liver	Areas of cloudy swelling, edema, fatty degeneration or early necrosis of the hepatic cells
Lungs	Mild congestion and edema; occasionally pneumonitis
Muscle (ventral abdominal wall)	Occasionally weak cross striation
Salivary (submaxillary) glands	Serous glands occasionally shrunken with loss of zymogenic granules
Skin	Normal appearance
Spleen	Red pulp contracted; white pulp edematous; phagocytized debris
Testes	Interstitial edema; minor inhibition of spermatogenesis
Thymus gland	Varying degrees of centrilobular edema, loss of thymocytes and capillary-venous congestion

[a] Animals fed low protein diets showed, in addition, histological signs of kwashiorkor, namely underdevelopment of many organs, especially the adrenal glands, intestinal villi, muscle, salivary glands, skin, testes, and thymus glands, plus fatty degeneration of the liver and an atrophic dermatitis.

Table III. Changes in Fresh Wet Weight of Body Organs of Albino Rats at Death from Oral Administration of Monuron[a]

Organic	Group 1 (Laboratory chow) (N = 12 plus 12 controls)	Group 2 (3.5% protein) (N = 12 plus 12 controls)	Group 3 (26% protein) (N = 12 plus 12 controls)
Adrenal glands	− 5.2	+11.8	+ 3.0
Brain	+ 4.3	− 3.3	+ 2.1
Gastrointestinal tract			
Cardiac stomach	− 7.4	−18.6[b]	0.0
Pyloric stomach	− 9.5[c]	− 3.4	− 6.2
Small bowel	−22.2[b]	− 0.9	−24.1[b]
Cecum	−20.6[b]	−22.1[b]	−18.2[b]
Colon	− 5.1	− 6.2	− 7.1
Heart	−11.6[c]	− 9.6[c]	−10.1[c]
Kidneys	− 3.1	+ 8.0	−13.1[c]
Liver	−24.4[b]	−16.2[b]	−27.4[b]
Lungs	+ 6.2	−10.3[c]	− 8.2[c]
Muscle (ventral abdominal wall)	− 7.2[c]	− 4.6	− 5.8
Salivary (submaxillary) glands	−12.0[c]	−15.8[b]	−28.8[b]
Skin	− 8.7[c]	− 6.0[c]	− 6.1[c]
Spleen	−29.4[b]	−41.7[b]	−45.8[b]
Testes	− 5.2	− 0.8	− 6.2
Thymus gland	−13.0[c]	− 2.0	−24.0[c]
Residual carcass	− 3.8	− 4.7	− 8.0[c]
Autopsy body weight	− 9.1[b]	− 9.1[b]	−10.0[b]

[a] Wet weight was measured in grams. The results are expressed as mean per cent change from controls, specifically as $[(\bar{X}_m - \bar{X}_c)/\bar{X}_c] \times 100$ where $\bar{X}_m$ is the mean in monuron-treated rats and $\bar{X}_c$ in corresponding controls given only cottonseed oil.

[b] $\bar{X}_m - \bar{X}_c$ significant at P = 0.01 or less.

[c] $\bar{X}_m - \bar{X}_c$ significant at P = 0.05 to 0.02.

cottonseed oil, but these animals ate freely while those given monuron had anorexia. Some of the monuron deaths in rats fed 0 to 3.5% protein, therefore, may have been due as much (or more) to starvation as to monuron. The anorexia at 24 to 48 hours after monuron was accompanied by oligodipsia of the same relative degree in all five dietary groups.

Body (colonic) temperature was 3 to 4° F. lower before giving monuron in rats fed 0 and 3.5% casein than in rats fed the other three diets. Lethal doses of monuron produced a decline in body temperature which was less in rats fed 0 and 3.5% casein than that in the other three dietary groups.

At 24 to 48 hours after monuron, there was an oliguria in rats fed all diets except 26% casein (group 3). In the latter group there was a diuresis at 24 hours, which presumably was due to an early onset of the diuresis of recovery seen in survivors of all dietary groups. It may, however, have been associated with hematuria, glycosuria, and proteinuria, which were marked in animals of group 3 and variable and less marked in the other dietary groups. Changes in urinary blood, glucose, and protein were not statistically significant in rats fed 3.5% casein (group 2) and hence are not shown in Figure 1. Animals of group 3 fed 26% casein also differed in that the reactive alkalinuria seen in survivors of all dietary groups appeared at 24 hours in group 3 in place of the aciduria at this time in group 2 as shown in Figure 1.

In brief, data on clinical measurements suggested that the susceptibility to monuron of rats fed 0 and 3.5% casein appeared to be due in part to their susceptibility to starvation while resistance of rats fed 26% casein appeared to be associated with the early appearance of signs of recovery such as diuresis and alkalinuria.

The gross pathological lesions seen in rats which died included gastric ulcers, gastroenteritis, renal and splenic pallor, white necrotic spots on the liver, and occasionally pneumonitis. The histopathological data are summarized in Table II. Monuron produced a local gastroenteritis, degenerative changes in the kidneys, liver, muscle, salivary glands, and testes, a stress reaction in the adrenal glands, spleen, and thymus gland, and capillary-venous congestion in the brain, heart, and lungs.

Changes in the fresh wet weight of body organs at death are summarized in Table III and in water content in Table IV. Monuron produced a significant loss of weight in many organs, particularly in the gastrointestinal tract, liver, salivary glands, and spleen. Water levels were increased in certain organs such as adrenal glands, decreased in others such as the lungs, but in most organs were insignificantly altered. The changes were in general similar in animals of all three dietary groups.

Recovery in survivors was accompanied by disappearance of clinical signs on days 3 and 4. Daily gain in body weight returned toward normal and in certain dietary groups exceeded the normal daily gain as shown in Figure 1. Food and water intake and colonic temperature returned to or toward normal values at 48 hours after monuron administration.

Table IV. Changes in Water Content of Body Organs of Albino Rats at Death from Oral Administration of Monuron[a]

Organ	Group 1 (Laboratory chow) (N = 12 plus 12 controls)	Group 2 (3.5% protein) (N = 12 plus 12 controls)	Group 3 (26% protein) (N = 12 plus 12 controls)
Adrenal glands	+39.3[b]	+40.7[b]	+ 7.9[c]
Brain	− 3.0	− 1.4	+ 0.9
Gastrointestinal tract			
Cardiac stomach	− 2.2	−15.1[c]	+ 0.6
Pyloric stomach	+ 0.9	+ 6.4	+ 2.7
Small bowel	+ 5.5	+ 6.3	+ 1.3
Cecum	− 3.9	− 6.8[c]	−16.7[b]
Colon	− 4.1	− 3.0	−14.2[b]
Heart	+ 1.7	+ 0.6	+ 1.2
Kidneys	+ 4.2	+ 7.0[c]	− 3.0
Liver	+ 8.0[c]	+ 5.7[c]	+ 0.9
Lungs	−11.2[b]	− 9.2[b]	−15.8[b]
Muscle (ventral abdominal wall)	+ 3.3	+ 2.9	−13.2[c]
Salivary (submaxillary) glands	+16.6[c]	+ 1.8	+ 6.0
Skin	− 4.1	− 5.5	− 6.0
Spleen	− 1.8	+ 0.9	− 1.7
Testes	− 2.6	+ 2.2	− 3.0
Thymus gland	− 1.7	+23.0[c]	− 1.8
Residual carcass	+ 1.3	+ 1.8	+ 4.0

[a] Water content was measured as grams water per 100 grams dry weight of tissue. The results are expressed as mean per cent change from controls, specifically as $[(\bar{X}_m - \bar{X}_c)/\bar{X}_c] \times 100$ where $\bar{X}_m$ is the mean in monuron-treated rats and $\bar{X}_c$ in corresponding controls given only cottonseed oil.
[b] $\bar{X}_m - \bar{X}_c$ significant at P = 0.01 or less.
[c] $\bar{X}_m - \bar{X}_c$ significant at P = 0.05 to 0.02.

Recovery was accompanied by a diuresis and a tendency toward a reactive alkalinuria.

DISCUSSION

The results of the present study indicate that susceptibility of rats to the lethal action of monuron varies with the amount and nature of protein in the diet. It should be emphasized that these differences were recorded in male albino rats of about 2 months of age fed different diets from the time of weaning. The results could have been different had the several diets been fed to adult rats or to animals fed laboratory chow for some time after weaning and before being transferred to the special diets. Kato *et al.* (1968) fed rats weighing 150 to 170 grams diets containing from 0 to 50% of protein (casein) and found that the mortality rate to strychnine, pentobarbital, and zoxazolamine increased with decrease in dietary protein. As the percentage of dietary casein was reduced, the percentage of glucose was increased in the studies of Kato *et al.* (1968). Boyd, Covert, and Pitman (1966) have demonstrated that feeding diets high in glucose or sucrose but normal in protein augment susceptibility to the toxic effect of certain drugs because of synergism with the toxic effects of the osmotically active dietary carbohydrates. The dietary carbohydrate in groups 2 to 5 of the present study was cornstarch which is nontoxic except in amounts approaching 50% of body weight and these produce death by bowel obstruction (Boyd and Liu, 1968).

Kato *et al.* (1968) presented evidence indicating that feeding diets low in protein leads to a deficiency in the synthesis of hepatic enzymes concerned with detoxification of foreign chemicals. A similar idea was advanced by Boyd and De Castro (1968). It would appear from the present studies that animals fed from weaning on very small amounts of dietary protein, and particularly no dietary protein, are very susceptible to the effects of starvation. If such animals are given a lethal dose of a drug which inhibits food intake, death appears to be due to a combination of drug effect and of starvation. Rats previously fed laboratory chow do not begin to die until a week after food has been withdrawn completely (Peters, 1967); some rats previously fed a diet containing no protein died during an overnight starvation period of 16 hours in the present study. Protein binding of orally administered lethal doses of the chemical agent may also be a factor in toxicity.

The susceptibility of rats fed from weaning on a purified diet containing 3.5% of protein as casein varies considerably from one chemical agent to another as noted in evidence reviewed by Boyd (1969). Compared with results in rats fed a normal (26% casein) diet and using the acute oral LD_{50} as a criterion, the rats fed diets containing 3.5% casein are twice as susceptible to the lethal effects of diazinon, lindane, and malathion, three times as susceptible to monuron, four times as susceptible to endosulfan and 26 times as susceptible to captan. The clinicopathologic syndrome of toxicity to doses of these agents in the range of the oral LD_{50} was essentially similar no matter what diet had been previously fed—within the range of the diets actually used.

Toxicity varies with the nature of dietary protein as well as with its amount. The LD_{50} of dicophane or DDT (Boyd and De Castro, 1968) as well as of monuron, is higher in rats fed from weaning on a purified diet containing normal amounts of protein as casein than in rats fed normal amounts of protein as laboratory chow. Corresponding values for the LD_{50} of carbaryl and endosulfan are lower in rats fed purified diets containing normal amounts of protein as casein than in rats fed laboratory chow. The effects of the nature of dietary protein, as well as of its amount, on the response to lethal doses of chemical agents appear to vary from agent to agent. This is probably due to quantitative differences in the inhibition of hepatic detoxifying mechanisms concerned in the metabolism of each agent but other factors may be involved.

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