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**ACKNOWLEDGMENTS AND ADDRESSES**

Received October 20, 1969, from the *Department of Industrial and Physical Pharmacy, School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, IN 47907*

Accepted for publication March 3, 1970.

This study was aided partly by a grant from the American Cancer Society and partly by funds provided by the Indiana Elks.

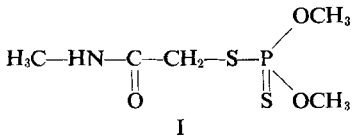
# Acute Oral Toxicity of Dimethoate in Albino Rats Fed a Protein-Deficient Diet

**ELDON M. BOYD and L. F. MUIS**

**Abstract** □ The oral $LD_{50} \pm SE$ of dimethoate was found to be 147 ± 29 mg./kg., 152 ± 22 mg./kg., and 358 ± 9 mg./kg., respectively, in male albino rats fed for 28 days from weaning on a diet containing: (*a*) 3.5% protein as casein, (*b*) normal amounts of protein as casein, and (*c*) normal amounts of protein as laboratory chow. The toxic syndrome at the range of the $LD_{50}$ was similar in animals of all three dietary groups and consisted of signs of cholinergic stimulation, depression of the central nervous system, anorexia, hypothermia, aciduria, proteinuria, an irritant gastroenteritis, widespread vascular congestion, toxic degenerative changes, dehydration, and loss of weight in body organs.

**Keyphrases** □ Pesticide toxicity study—rats □ Dimethoate—toxicity study, rats □ Protein-deficiency effect—dimethoate toxicity □ $LD_{50}$—dimethoate □ Toxic effects, rats—dimethoate

Dimethoate, an organophosphorus pesticide introduced in 1956, is effective as a systemic insecticide against pests such as houseflies, aphids, mites, and grasshoppers (1). It is a white crystalline solid with limited solubility in water but is freely soluble in cottonseed oil. The formula is shown in Structure I. It is



I
dimethoate

absorbed into plants and converted mainly to the toxic oxygen analog (2) which is taken up by insects. Insects are very sensitive to its toxic effects (3), apparently due to less effective detoxifying mechanisms (4), particularly ability to remove the methylamido group which has been termed the "weak link" in detoxification by insects (5). Dimethoate is readily absorbed from the mammalian gastrointestinal tract and biotransformed by conversion of the sulfur group to the oxygen analog, by hydrolysis of the methyl ester groups, and by removal of the methylamido group (2). Pretreatment of mice with SKF 525A lessens (3), while pretreatment with the hepatic enzyme-inducer phenobarbital increases (6), the toxicity of dimethoate. The toxic action of dimethoate is due to inhibition of various body esterases (7). The acute oral $LD_{50}$ of technical dimethoate in male rats has been reported to be between 180 and 325 mg./kg. (2). Tolerance in human foods has been set at 2 p.p.m. (8).

The World Health Organization concluded that further studies were desirable on the worldwide use of dimethoate (2) and requested the authors to investigate its acute oral toxicity in albino rats fed from weaning on diets deficient in protein. The present communication is a report of such studies.

## EXPERIMENTAL

The experiments were performed on male albino rats of a Wistar strain.[1] Group I consisted of 137 weanlings, weighing 50–60 g., fed Protein Test Diet—Low[2] which contains 3.5% casein, 81.5% cornstarch, 8% hydrogenated cottonseed oil, 4% salt mix USP XIV, and 3% of an all vitamin mixture (9). A 4-week feeding period was selected from the studies of De Castro and Boyd (10) who found that weanling rats react rather violently to a protein-deficient diet during the first 2 weeks but adjust during the 3rd and 4th weeks. After 28 days of feeding, the animals weighed 50 ± 5 g. (mean ± *SD*).

Group II was a control group of 120 weanlings fed Protein Test Diet—Normal[2] which contains 26% casein, 59% cornstarch, and other ingredients as in Protein Test Diet—Low. At the end of 28 days, their body weight was 199 ± 18 g.

Group III was a protein control group of 128 rats purchased 2 weeks from weaning and fed a standard laboratory chow for 2 weeks.[3] This diet contains 24% protein from various plant and

[1] Obtained from Woodlyn Farms Limited, Guelph, Ontario.
[2] General Biochemicals, Chagrin Falls, Ohio.
[3] The chow was Rockland Rat Diet (Complete), Teklad Inc., Monmouth, Ill.

**Table I**—Histopathological Observations at Death from Oral Administration of Dimethoate to Albino Rats

| Organ | Histopathology |
|---|---|
| Adrenal glands | Lipoid globules prominent, especially in zona fasciculata |
| Brain | Meninges and brain hyperemic and congested |
| Gastrointestinal tract: | |
| Cardiac stomach | Occasionally submucosa hyperemic |
| Pyloric stomach | Areas of ulceration of the inner half of the gastric glands |
| Small bowel | Mild capillary congestion of the lamina propria of the villi |
| Cecum | Occasionally small infiltrative ulcers of the mucosa and submucosa |
| Colon | Normal appearance |
| Heart | Occasionally mild capillary congestion of the myocardium |
| Kidneys | Congestion of the glomerulus and loop of Henle; venous stasis and thrombosis; occasionally debris beneath Bowman's capsule; cloudy swelling, fine fatty degeneration, and early necrosis of the convoluted tubules |
| Liver | Sinusoidal congestion and cloudy swelling; fatty degeneration in animals of Group I |
| Lungs | Usually capillary congestion and venous stasis and thrombosis |
| Muscle (ventral abdominal wall) | Normal appearance |
| Pancreas | Deficiency of zymogenic granules in the acinar glands |
| Salivary (submaxillary) glands | Mucous glands shrunken; serous glands shrunken, granulated, and sometimes vacuolated |
| Skin | Occasionally ischemic |
| Spleen | Red pulp contracted |
| Testes | Deficiency of normal sperm in delayed deaths; inhibition of spermatogenesis in animals of Group I |
| Thymus gland | Loss of thymocytes, particularly in animals of Group I |

animal sources. After 2 weeks of feeding the animals weighed 171 ± 5 g.

At the end of the dieting period, each rat was placed singly in a metabolism cage with water. They received no food for 16 hr. (overnight) to empty the stomach prior to oral administration of dimethoate. Technical dimethoate[4] was freshly dissolved in cottonseed oil USP and given intragastrically in a volume of 20 ml./kg. body weight. Following pilot dose studies in each dietary group, a series of definitive doses, estimated to yield mortality rates from just above 0% to just below 100%, were administered to 10 rats per dose with 15–20 controls given cottonseed oil.

The animal was then returned to its metabolism cage with an excess of diet and drinking water. Clinical signs were measured in units of 1+ to 4+ at hourly intervals during the balance of the 1st day and then at intervals of 24 hr. or as indicated. Body weight gain, food intake, water intake, colonic temperature, urinary volume, urinary blood, urinary glucose and protein output, and urinary pH were measured daily for 5 days.

An autopsy was performed upon all dead animals, the gross pathology was recorded, and a microscopic examination was made upon any organ that appeared abnormal to gross examination. Histopathology was recorded at death upon all organs listed in Table I in representative dead animals of each dietary group.

The wet weight of organs listed in Table II was measured upon animals which could be autopsied within 1 hr. of death to avoid postmortem shifts in organ weights and water levels described by Boyd and Knight (11). Water content was measured upon aliquots of the organs listed in Table III dried to constant weight in a Fisher forced draft isotemp oven at 95° and was calculated as grams water per 100 grams dry weight of tissue.

[4] Cygon, 93.3% dimethoate, Agricultural Division, American Cyanamid Co., Princeton, N. J.

The results were analyzed statistically by the application of *t* tests to the significance of differences between means and by the regression of differences on dose or time. The $LD_{50} \pm SE$ was calculated by linear regression analysis of dose on response. Details of the method, including statistical analysis, have been reviewed by Boyd (12).

## RESULTS

Data on calculated lethal doses of dimethoate are assembled in Table IV. The $LD_{50}$ in Group I is identical to that in Group II, and both values are about half that in Group III. The interval to death varies inversely with the dose; *i.e.*, the higher the dose of dimethoate, the more rapid is the occurrence of death. The mean interval to death is identical in all three dietary groups. Pathological lesions seen on gross examination at autopsy are similar in all three dietary groups.

The clinicopathological syndrome of toxicity is essentially the same in animals of all three dietary groups. It will be exemplified by describing changes in animals previously fed laboratory chow (Group III) and by noting significant differences in animals of the other two dietary groups.

The dominant clinical signs of toxicity during the first few hours are tremors, prostration, dacryorrhea, and exophthalmos (Fig. 1). They reach a peak of intensity during the afternoon of the 1st day and begin to disappear at 24 hr. A delayed clinical syndrome, which reaches a peak at 24 hr. (Fig. 2), consists of piloerection, listlessness, sialorrhea, and soiling of the fur. These clinical signs are accompanied by a marked inhibition of food and water intake, hypothermia, and marked loss of body weight (Fig. 3). At 24 and 48 hr., all mean changes shown in Fig. 3 are significant at $p < 0.01$.

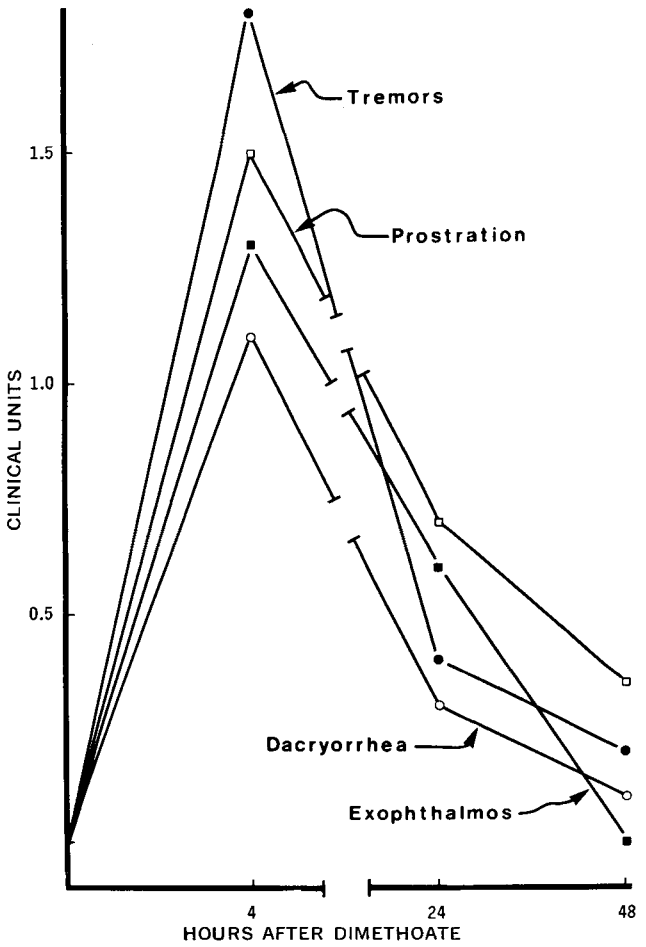



**Figure 1**—*Mean clinical units of intensity of clinical signs which were dominant during the 1st day of the toxicity syndrome to oral administration of lethal doses of dimethoate in albino rats previously fed laboratory chow.*

**Table II**—Shifts in the Fresh Wet Weight of Body Organs at Death following Oral Administration of Dimethoate[a]

| Organ | Group I (3.5% Casein) $N = 14$ plus 14 Controls | Group II (26% Casein) $N = 15$ plus 14 Controls | Group III (Chow) $N = 15$ plus 15 Controls |
|---|---|---|---|
| Adrenal glands | −1.1 | +15.2**[c] | +13.8* |
| Brain | −5.8*[b] | −0.7 | −0.7 |
| Gastrointestinal tract: | | | |
| Cardiac stomach | −26.2** | −12.5* | −12.5* |
| Pyloric stomach | −14.7** | −13.4** | −11.1** |
| Small bowel | −4.4 | −11.1 | −18.7** |
| Cecum | −36.8** | −17.6* | −21.4** |
| Colon | −18.1** | +2.8 | −18.4** |
| Heart | −3.2 | −9.4* | +3.6 |
| Kidneys | +4.3 | −11.0* | −8.2** |
| Liver | −16.6** | −27.8** | −23.8** |
| Lungs | −17.5* | −26.0** | −0.3 |
| Muscle (ventral abdominal wall) | −13.6* | −3.3 | −18.6** |
| Salivary (submaxillary) glands | −10.3 | −23.9** | −28.5** |
| Skin | −2.3 | −13.9* | −10.4* |
| Spleen | −45.3** | −38.5** | −45.8** |
| Testes | +18.7* | −13.0* | −1.9 |
| Thymus gland | −44.4** | −33.7** | −14.3* |
| Residual carcass | −3.6 | −17.2** | −7.2* |
| Autopsy body weight | −7.0* | −13.2** | −14.0** |

[a] Weight was measured in grams. The results are expressed as mean percent change from controls fed the same diet but given cottonseed oil with no dimethoate, specifically as $[(\bar{X}d - \bar{X}c)/\bar{X}c] \times 100$, where $\bar{X}d$ is the mean in rats given dimethoate and $\bar{X}c$ in the controls. [b] * indicates that $\bar{X}d - \bar{X}c$ was significant at $p = 0.05$ to 0.02. [c] ** indicates that $\bar{X}d - \bar{X}c$ was significant at $p = 0.01$ or less.

Significant changes in urine are indicated in Fig. 4. At 24 hr., there occurs an oliguria with aciduria; at 72 hr., there is a reactive diuresis and urinary pH returns to normal. Proteinuria, glucosuria,

**Table III**—Shifts in the Water Levels of Body Organs at Death from Oral Administration of Dimethoate[a]

| Organ | Group I (3.5% Casein) $N = 14$ plus 14 Controls | Group II (26% Casein) $N = 15$ plus 14 Controls | Group III (Chow) $N = 15$ plus 15 Controls |
|---|---|---|---|
| Adrenal glands | +3.6 | +26.7**[b] | +14.3*[c] |
| Brain | −1.8 | −2.2 | −1.6 |
| Gastrointestinal tract: | | | |
| Cardiac stomach | −22.6** | −3.3 | −16.3** |
| Pyloric stomach | −6.3** | +4.2 | −5.3* |
| Small bowel | −9.0** | +1.3 | −3.5 |
| Cecum | −18.3** | −7.7* | −15.6** |
| Colon | −10.2* | −3.3 | −11.7** |
| Heart | −10.6** | −6.9** | −6.2** |
| Kidneys | −4.4* | −5.9* | −9.7** |
| Liver | −5.3* | −0.9 | −3.7* |
| Lungs | −24.1** | −18.4** | −17.3** |
| Muscle (ventral abdominal wall) | −13.4** | −16.4** | −9.4* |
| Salivary (submaxillary) glands | −11.9* | −15.0** | −22.0** |
| Skin | −14.1* | −15.0* | −11.1* |
| Spleen | −5.7 | −1.9 | −3.8 |
| Testes | +4.1 | −6.5** | −8.9** |
| Thymus gland | −23.0** | −11.4** | −15.8** |
| Residual carcass | −11.1** | −3.6 | −8.1** |

[a] Water levels were measured as grams water per 100 g. dry weight of tissue. The results are expressed as mean percent change from controls fed the same diet and given cottonseed oil without dimethoate, specifically as $[(\bar{X}d - \bar{X}c)/\bar{X}c] \times 100$, where $\bar{X}d$ is the mean in dimethoate-treated rats and $\bar{X}c$ in controls. [b] ** indicates that $\bar{X}d - \bar{X}c$ was significant at $p = 0.01$ or less. [c] * indicates that $\bar{X}d - \bar{X}c$ was significant at $p = 0.05$ to 0.02.

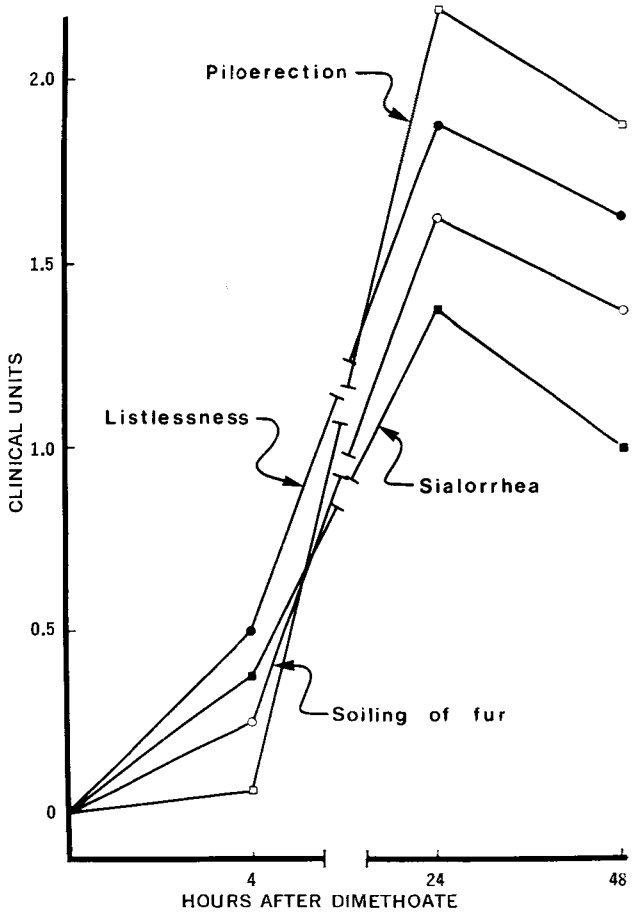



**Figure 2**—*Mean clinical units of intensity of clinical signs which were dominant at 24–48 hr. after oral administration of lethal doses of dimethoate to albino rats previously fed laboratory chow.*

and hematuria begin to appear during the first 24 hr. and are present in survivors at 48 and 72 hr.

The protein-deficient animals of Group I do not exhibit exophthalmos and have less hypothermia during the first few hours after receiving dimethoate, and piloerection is marked at this time. There is no delayed reaction at 24 hr., and recovery of survivors is rapid in Group I; growth rate, for example, returns to normal during the 2nd day.

In Group II, fed a diet of 26% casein, the initial reaction is similar to that of Group III except that listlessness, piloerection, and sialorrhea are present immediately after dimethoate. Soiling appears at 24 hr., but there is no glycosuria or hematuria. Recovery is delayed to the 3rd day as in Group III.

Microscopically (Table I), there is a moderate, local, irritant gastroenteritis with ulceration in the pyloric or glandular part of the stomach and in the cecum. Cecal ulcers are not particularly common in studies of this nature. After absorption, the most common lesion is vascular congestion. This is accompanied by degenerative changes in the kidneys, liver, pancreas, salivary glands, and testes. There is a stress reaction in the adrenal glands, spleen, and thymus gland and the skin is ischemic. There are signs of kwashiorkor due to protein deficiency in animals of Group I, as indicated by impaired development of several organs.

There is a marked loss of weight in most organs (Table II). Loss of weight is particularly marked in the tissues of the gastrointestinal tract, liver, lungs, muscle, spleen, and thymus gland. Loss of weight is due in part to dehydration as shown by data summarized in Table III. The changes are, in general, similar in animals of all three dietary groups, and the few differences which are found may be seen from values quoted in Tables II and III.

## DISCUSSION

The results of this investigation indicated that dimethoate was no more toxic to rats fed a protein-deficient diet than to controls

**Table IV**—Lethal Doses, Interval to Death, and Gross Pathology following Oral Administration of Lethal Doses of Dimethoate

| Measurement | Group I, Protein Test Diet—Low (3.5% Casein) | Group II, Protein Test Diet—Normal (26% Casein) | Group III, Laboratory Chow (24% Protein) |
|---|---|---|---|
| Estimated maximal $LD_0$, mg./kg. | 92†[a] | 74† | 345 |
| $LD_{50} \pm SE$, mg./kg. | 147 ± 29† | 152 ± 22† | 358 ± 9 |
| Estimated minimal $LD_{100}$, mg./kg. | 202† | 230† | 371 |
| Hours to death, mean ± $SD$*[b] | 17 ± 4 | 21 ± 14 | 22 ± 9 |
| Gross pathology | Congested brain<br>Acute gastritis<br>Renal pallor<br>Hepatitis<br>Pneumonitis | Congested brain<br>Acute gastritis<br>Renal pallor<br>Hepatitis<br>Pneumonitis | Congested brain<br>Acute gastritis<br>Hepatitis<br>Pneumonitis |

[a] †, significantly different from results in Group III at $p = 0.02$ or less.
[b] *, see text for variation with dose.

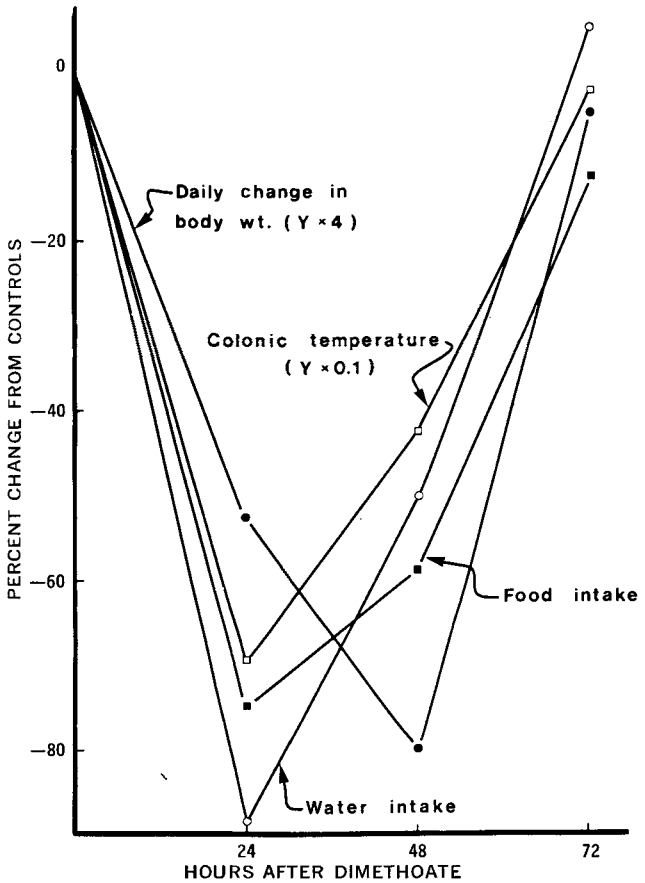



**Figure 3**—*Significant mean differences in daily body weight gain (Y × 4), colonic temperature (Y × 0.1), food intake (Y × 1), and water intake (Y × 1) following oral administration of lethal doses of dimethoate to albino rats previously fed laboratory chow. Differences were calculated as mean percent change from controls given cottonseed oil, specifically as* $[(\bar{X}_d - \bar{X}_c)/\bar{X}_c] \times 100$, *where* $\bar{X}_d$ *is the mean in dimethoate-treated animals and* $\bar{X}_c$ *the corresponding mean in controls. Note that the ordinate units must be multiplied by the factors indicated in parenthesis to obtain the mean percentage change for daily gain in body weight and for colonic temperature.*

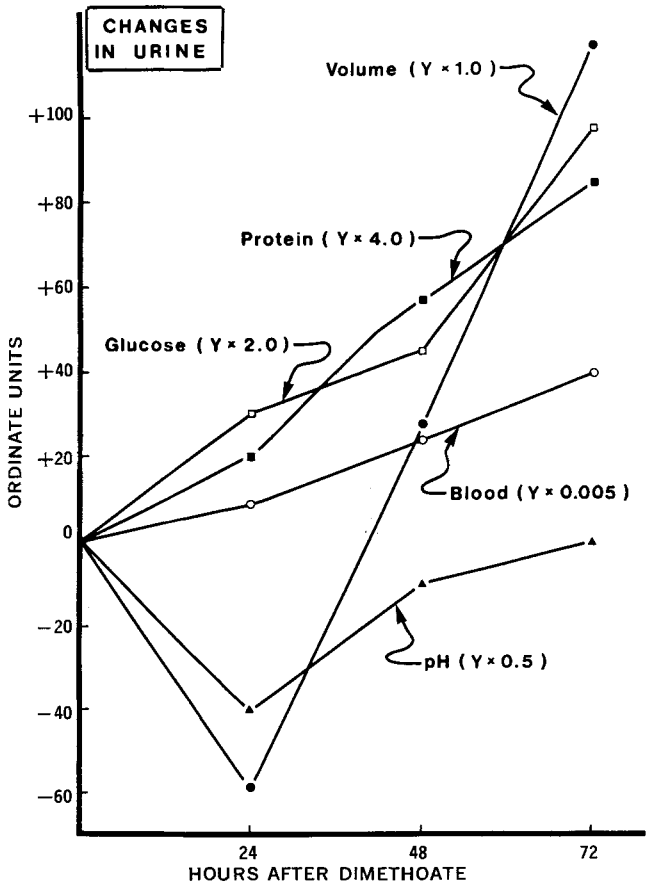



**Figure 4**—*Significant mean changes in the urine of albino rats previously fed laboratory chow following oral administration of lethal doses of dimethoate. The ordinate units for volume, protein output, and pH represent mean percent change from controls calculated as in Fig. 3. The ordinate units for glucose output indicate milligrams per kilogram body weight per day since there was no glucose in the urine of controls given cottonseed oil. The ordinate for urinary blood indicates units of blood. The ordinate units must be multiplied by the factors indicated in parenthesis to obtain the mean change in each parameter.*

fed adequate amounts of protein as casein. Protein deficiency causes degenerative changes and inhibition of growth in the liver (10) and could be expected to limit production of hepatic detoxifying enzymes. This, in turn, could lessen production of the highly toxic oxygen analog of dimethoate. A decreased production of the oxygen analog could account for no increase in the toxicity of dimethoate in the protein-deficient rat, which is generally more susceptible to pesticide toxicity.

Available data on pesticide toxicity in protein-deficient rats have been summarized in Table V. Dimethoate is the only pesticide studied which is not more toxic to the protein-deficient rat. Five pesticides were twice as toxic to the protein-deficient rat; the five were diazinon and malathion, which are organic phosphorothioates like dimethoate, and chlordane, endrin, and lindane, which are chlorinated organic insecticides. Dicophane or DDT and monuron, an herbicidal chloro compound, were three times as toxic. Four compounds were four times as toxic: chlorpropham, which is an herbicide related to monuron, demeton (an organic phosphorothioate), and two chlorinated insecticides (endosulfan and toxaphene). Carbaryl is a physostigminelike cholinesterase inhibitor of the naphthyl carbamate series and was six times as toxic. Parathion is a *p*-nitrophenyl organic phosphorothioate and was eight times as toxic. Casterline and Williams (29) have reported parathion to be more toxic in young adult rats fed for 30 days on a diet containing no protein than in animals fed a diet containing 15% of protein as casein. Captan is a mercapto-phthalidimide related structurally to thalidomide and was 26 times as toxic.

**Table V**—Acute Oral $LD_{50}$ of Pesticides in Male Albino Rats Fed for 28 Days from Weaning on a Protein-Deficient Diet Containing 3.5% Casein Compared with Values in Controls Fed a Normal Protein Diet Containing 26% Casein[a]

| Pesticide | Protein-Deficient Diet, Group I | Normal Protein Diet, Group II | Quotient: II/I | Reference |
|---|---|---|---|---|
| Dimethoate | 147 ± 29 | 152 ± 22 | 1 | This paper |
| Chlordane | 137 ± 30 | 267 ± 44 | 2 | 13 |
| Diazinon | 215 ± 26 | 415 ± 39 | 2 | 14 |
| Endrin | 6.69 ± 0.80 | 16.6 ± 3.0 | 2 | 15 |
| Lindane | 95 ± 33 | 184 ± 16 | 2 | 16 |
| Malathion | 599 ± 138 | 1401 ± 99 | 2 | 17 |
| Dicophane (DDT) | 165 ± 34 | 481 ± 13 | 3 | 18, 19 |
| Monuron | 950 ± 240 | 2880 ± 310 | 3 | 20 |
| Chlorpropham | 2590 ± 480 | 10390 ± 1580 | 4 | 21 |
| Demeton | 2.13 ± 0.37 | 7.62 ± 0.22 | 4 | 22 |
| Endosulfan | 24 ± 10 | 102 ± 16 | 4 | 23 |
| Toxaphene | 80 ± 19 | 293 ± 31 | 4 | 24 |
| Carbaryl | 89 ± 11 | 575 ± 51 | 6 | 25, 26 |
| Parathion | 4.86 ± 1.31 | 37.1 ± 4.9 | 8 | 27 |
| Captan | 480 ± 110 | 12600 ± 2100 | 26 | 28 |

[a] The $LD_{50}$ is expressed as mg./kg. ± *SE*.

The results of these investigations indicate that further studies, including clinical toxicology trials, should be done upon pesticides listed toward the bottom of Table V to determine if they present a particular hazard when used in countries where the diet is low in protein. No evidence is present in these investigations to indicate that such a hazard exists for pesticides listed toward the top of Table V.

The type of dietary protein does not appear to be as important a factor contributing to augmented susceptibility to pesticides as is deficiency of protein. Of the pesticides listed in Table V, chlorpropham and monuron were twice as toxic in rats fed laboratory chow as in rats fed normal amounts of protein as casein, while dimethoate was half as toxic. Dietary soy protein in normal amounts has been found, in current studies, to augment the susceptibility of rats to certain drugs such as phenacetin. There may be significant differences depending upon the type of protein in a protein-deficient diet, a problem which has not been studied in this laboratory.

Variation in the concentration of dietary protein involves alteration in the concentration of other dietary ingredients and is usually accomplished by changes in the amount of carbohydrate. The type of carbohydrate selected is of considerable importance. Soluble sugars such as glucose and sucrose should not be used in large amounts, because they can produce dehydration and death (30), and, in sublethal doses, they can add to the toxicity of drugs (31). Starch, on the other hand, produces death only by bowel obstruction from huge doses (32) and is nontoxic in large amounts in the diet. In the present study, therefore, cornstarch was used, rather than a sugar, to replace a lowered concentration of dietary protein.

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## ACKNOWLEDGMENTS AND ADDRESSES


Received November 3, 1969, from the *Department of Pharmacology, Queen's University, Kingston, Ontario, Canada.*

Accepted for publication April 10, 1970.

This project was assisted by a grant from the World Health Organization.