FUNDAMENTAL AND APPLIED TOXICOLOGY 3:69-74 (1983)

# Heinz Body Production and Hematological Changes in the Hen After Administration of a Single Oral Dose of *n*-Butyl Mercaptan and *n*-Butyl Disulfide

K.M. ABDO[1], P.R. TIMMONS[2], D.G. GRAHAM[3] and M.B. ABOU-DONIA[4]

[1]National Toxicology Program, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709; [2]Union Carbide, Agricultural Products, P.O. Box 12014, Research Triangle Park, NC 27709; [3]Department of Pathology, Duke University Medical Center, Durham, NC 27710; [4]Department of Pharmacology, Duke University Medical Center, Durham, NC 27710

## ABSTRACT

**Heinz Body Production and Hematological Changes in the Hen After Administration of a Single Oral Dose of *n*-Butyl Mercaptan and *n*-Butyl Disulfide. Abdo, K.M., Timmons, P.R., Graham, D.G. and Abou-Donia, M.B. (1983). *Fundam. Appl. Toxicol.* 3:69-74. *n*-Butyl mercaptan (*n*BM) is a breakdown product of *S,S,S*-tri-*n*-butyl phosphorotrithioate (DEF) and *S,S,S*-tri-*n*-butyl phosphorotrithioite (merphos) in hens and in the environment. *n*-Butyl disulfide (*n*BD) is an oxidation product of *n*BM. A single 500 mg/kg dose of *n*BM and *n*BD was administered in gelatin capsules to groups of five 12-month old laying hens. A third group (five hens) was given gelatin capsules. One day after administration, the hens exhibited weakness which progressed to unsteadiness and inability to stand by the third day. These signs were accompanied by a pale comb 18-24 hr after dosing, which changed to dark color at 48 hr. Treated hens improved with time. Heinz bodies and extensive erythrocyte deformation and lysis were observed in blood smears taken from hens 24 and 48 hr after treatment. Hemoglobin concentration, packed cell volume, erythrocytes, and glucose-6-phosphate dehydrogenase activity were significantly lower than controls, while methemoglobin was significantly higher. As the clinical condition of these hens improved, these hematologic changes disappeared. *n*BM caused an initial increase in plasma butyrylcholinesterase activity which was dose-dependent and returned to normal by the end of the 28-day experiment. Also, brain acetylcholinesterase activity was not different from that of the control at termination.**

## INTRODUCTION

*n*-Butyl mercaptan (*n*BM) is an industrial intermediate used in the synthesis of the pesticides *S,S,S,*-tri-*n*-butyl phosphorotrithioate (DEF) and *S,S,S*-tri-*n*-butyl phosphorotrithioite (merphos). It is generated from these two pesticides in the gastrointestinal tract of animals and in the environment (Abou-Donia, 1980, 1981; Abou-Donia *et al.*, 1979a,b). It is present in technical DEF and is also the major component responsible for the offensive smell produced by skunks (Anonymous, 1975). *n*-Butyl disulfide (*n*BD) is an oxidation product of *n*BM. Poisoning related to exposure to *n*BM has been observed in humans. Signs of toxicity appear within 1 hr after exposure and include general weakness, malaise, sweating, nausea, vomiting, anxiety and drowsiness (McLeod, 1975). In hens given a single oral dose of 400 to 1000 mg/kg *n*BM, the clinical signs of toxicity appear in 6-12 hr and are identical to the late acute toxicity signs seen after high oral doses of DFP or merphos (Abou-Donia *et al.*, 1979a,b). The symptoms are characterized by weakness, malaise, loss of balance, diarrhea, disorientation, shortness of breath and salivation. Shortly before death the combs become droopy and cyanotic.

Because of the structural relationship between *n*BM and *n*BD and the known hemolytic-agents methanethiol and dimethyl disulfide (Smith, 1974) and because of the production of cyanotic comb and appearance of hemolysis in blood samples obtained from *n*BM treated hens, it has been postulated that the toxicity of *n*BM is due to some hematological changes in the orally treated hens (Abou-Donia *et al.*, 1979a, 1980). We therefore conducted this study for the purpose of identifying some of these changes.

## MATERIALS AND METHODS

### Chemicals

*n*-Butyl mercaptan (*n*BM, 98%) and *n*-butyl disulfide (*n*BD, 96%) were purchased from Aldrich Chemical Company, Milwaukee, WI.

### Birds

Adult leghorn laying hens (*Gallus gallus* domesticus), 12 months old, weighing 1.3-1.9 kg were used (Featherdown Farm, Raleigh, NC). They were housed in humidity- and temperature-controlled (21-23 °C) rooms with a 12-hr light cycle before and during the experiment. The hens were distributed in such a way so that all groups contained birds of comparable weights. Assignment of treatments to groups were made at random. Feed (Purina Layena Feed, Ralston Purina, St. Louis, MO) and water were supplied *ad libitum*.

### Treatment

In a preliminary experiment, to determine the proper dosage, groups of three hens were treated with a single oral dose ranging from 100 to 1000 mg/kg *n*BM. In the main experiment, groups of five hens were given a single 500 mg/kg oral dose of *n*BM or *n*BD in gelatin capsules. Hens were observed twice daily for untoward clinical signs. Blood samples were taken from treated and control hens at varying intervals of the experiment. These hens were killed by heart puncture 7 days after treatment. To study the effect of *n*BM on plasma butyrylcholinesterase and brain acetylcholinesterase, three groups of five hens were given a single oral 100, 400 or 500 mg/kg *n*BM. These birds were killed 28 days after the administration.

TABLE 1
Hematological Indices in Hens Treated With a Single Oral 500 mg/kg Dose of *n*-Butyl Mercaptan (*n*BM) or *n*-Butyl Disulfide (*n*BD)

| Hours After Dosing | Treatment | Hb[a] | MetHb[b] | PCV[a] | G-6-PD[a] |
|---|---|---|---|---|---|
| 24 | *n*BM | 73.4 ± 2.8[c] | 0.71 ± 0.18 | 51.1 ± 2.8[c] | 47.2 ± 2.4[c] |
| | *n*BD | 80.7 ± 4.6[d] | 0.73 ± 0.18 | N.D.[e] | 36.1 ± 3.6[c] |
| 48 | *n*BM | 62.7 ± 5.5[c] | 0.27 ± 0.09 | 60 ± 1.5[c] | 75.5 ± 8.8[c] |
| | *n*BD | 78.2 ± 2.7[d] | 0.59 ± 0.20 | 70 ± 1.5[c] | 77.4 ± 7.9[c] |
| 72 | *n*BM | 75.5 ± 7.1[c] | 0.63 ± 0.14 | 81.1 ± 3.6[d] | 97.1 ± 10.1[f] |
| | *n*BD | 90.8 ± 10.1[f] | 0.73 ± 0.15 | N.D. | 97.8 ± 9.8[f] |

[a]Results are calculated as percentage of control values measured at the same time. Each value represents mean ± SE of 10 determinations from five birds. The means ± SE of controls were: hemoglobin (Hb), 10.6 ± 0.5 g/100 mL of blood; packed cell volume (PCV), 27.2 ± 1.0 g/100 mL of blood; glucose-6-phosphate dehydrogenase (G-6-PD), 1.47 ± 0.11 U/g hemoglobin.

[b]Methemoglobin (MetHb) was expressed as g/100 mg of blood. MetHb was not detected in blood from control hens.

[c]Significant difference from control $p < 0.01$.

[d]Significant difference from control $p < 0.05$.

[e]Not determined.

[f]Not significantly different from control.

### *Collection of blood*

Blood samples were collected from the wing vein in heparinized disposable syringes. Two milliliters of blood were collected from individual hens at each time interval.

### *Hemaglobin (Hb), methemoglobin (MetHb) and packed cell volume (PCV)*

Hb and MetHb were determined spectrophotometrically using the method of Evelyn and Malloy (1938). PCV determinations were made by a microhematocrit method. Microhematocrit tubes were filled by capillary action with freshly collected heparinized blood. The tubes were sealed at both ends with plasticine, centrifuges at 5000 g for 2 min and the PCV calculated.

### *Demonstration of Heinz bodies*

#### Light microscopy

Heinz bodies in the red blood cells were demonstrated according to the method described by Dacie and Lewis (1975). The staining solution was prepared by dissolving 0.5 g of crystal violet in 100 mL of normal saline followed by filtration. One volume of heparinized blood was mixed with four volumes of staining solution, incubated for 10 min at 37 °C and diluted 40 times with normal saline prior to examination by light microscopy.

#### Electron microscopy

Red blood cells (rbcs) were separated from 1.5 mL of heparinized blood by centrifugation at 15 000 g for 5 min. The cells were covered with 0.5 mL of 0.1 M sodium cacodylate buffer (pH 7.4) containing 4% glutaraldehyde. The rbcs were then processed for electron microscopic examination. Blood samples obtained from hens injected subcutaneously with phenylhydrazine HCl (30 mg/kg) were treated similarly and used as positive controls for demonstration of the presence of Heinz bodies.

### *Enzymatic analysis*

Heparinized blood samples were centrifuged in a Beckman Aerofuge Model B at 5000 g for 5 min. Measurement of brain acetylcholinesterase (AChE, EC 3.1.1.7) and plasma butyrylcholinesterase (BuChE, EC 3.1.1.8) activities were carried out by the method of Ellman *et al.* (1961). Brain AChE was assayed by adding 0.02 mL of a 10% brain homogenate in 0.25 M sucrose to a reaction mixture containing 0.1 mM acetylthiocholine (ATCH), 100 mM NaCl, 200 mM $MgCl_2$, 200 mM sodium phosphate buffer, pH 7.0 and 0.1 mM 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) in a final volume of 4.0 mL. Plasma BuChE was determined by adding 0.02 mL plasma to the reaction mixture which contained 0.2 mM butyrylthiocholine (BUTCH), 40 mM $MgCl_2$, 4 mM Tris buffer, pH 7.4 and 0.2 mM DTNB in a final volume of 4.0 mL. The reactions were carried out at 38 °C in a Dubnoff metabolic shaker for 5 min. Parallel blank incubations were carried out, utilizing heat-denatured brain homogenate or plasma solutions for each determination. A Varian Techtron Model 634 UV-VIS spectrophotometer was used to measure the initial hydrolysis rates of ATCH and BUTCH at 412 NM. Plasma and brain proteins were determined by the method of Lowery *et al.* (1951). Brain AChE activity was expressed as μmoles of ATCh hydrolyzed per minute per milligram protein, plasm levels of BuChE were expressed as micromoles of BUTCH hydrolyzed per minute per milligram protein.

Erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD, EC 1.1.1.49) was determined by using the standardized World Health Organization (1967) method except for the preparations of erythrocyte hemolysates. Two tenth milliliter of heparinized blood was mixed with 1.5 mL of normal saline centrifuged at 5000 in a Beckman Microfuge Model B for two minutes and the supernatant was discarded. The pellet was washed three times by the process of suspension in normal saline and centrifugation as described above. The final pellet was suspended in 1.5 mL normal saline, frozen solid in a dry ice-acetone bath then thawed at room temperature. The thawed preparation was centrifuged for 3 minutes and the

TABLE 2
Plasma Butyrylcholinesterase Activity[a] Following a Single Oral Dose of *n*-Butyl Mercaptan (*n*BM) to Hens

| *n*BM mg/kg | % of Control After (Days) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 7 | 21 | 28 |
| 500 | 145 ± 10[b] | 146 ± 13[b] | 177 ± 9[c] | 230 ± 23[c] | 92 ± 10[d] |
| 400 | 140 ± 9[b] | 141 ± 10[b] | 155 ± 11[c] | 191 ± 12[c] | 100 ± 10[d] |
| 100 | 136 ± 12[b] | 127 ± 10[b] | 120 ± 9[b] | 115 ± 11[d] | 102 ± 11[d] |

[a]Results are calculated as the percentage of enzymatic activity measured in plasma from controls taken at the same time. Each value represents mean ± SE of 10 determinations from five birds. The mean ± SE of controls was 1.50 ± 0.15 micromoles BUTCH hydrolyzed/min/mg of protein.

[b]Significant difference from control $p < 0.05$.

[c]Significant difference from control $p < 0.01$.

[d]Not significantly different from control.

supernatant saved for determination of G-6-PD activity (Bentler, 1977). The reaction mixture contained 50 mM Tris buffer (pH 7.6), 0.02 mM NADP and 0.2 mL hemolysate in a total volume of 3 mL. The mixture was placed in a 25 °C bath for 5 minutes. The reaction was started by the addition of 0.6 mM glucose-6-phosphate. The optical density (O.D.) was measured in a Varian Model 634 UV-VIS spectrophotometer at 340 nm at half minute intervals for three minutes. G-6-PD activity was calculated as follows:

$$\text{International units (IU)} = \frac{\Delta \text{O.D.} \times 10}{6.22 \times \text{Hb (g/100 mL)} \times \text{V (}\mu\text{L hemolysate/mL of assay)}}$$

### *Statistics*

The significance of differences between control and treated hens was assessed by a Student's two-tailed *t*-test. A *p*-value of 0.05 or less was considered significant.

## RESULTS

### *Clinical observations*

A preliminary experiment demonstrated that hens treated with 400 to 1000 mg/kg *n*BM developed clinical signs of toxicity. The severity and onset of effects were dose-related. Hens given 1000 mg/kg showed general weakness, unsteadiness, loss of coordination, diarrhea and salivation. The combs of these hens were droopy and changed from bright red to white, particularly at the base of the comb, 18-24 hr after dosing. The color later changed from white to dark purple. These hens died within 48 hr of treatment. Hens given a single oral dose of 400 mg/kg showed much milder signs of intoxication, while a dose of 750 mg/kg caused an intermediate effect. Hens treated with a single oral dose of 100 mg/kg *n*BM did not develop any signs of toxicity.

Following the preliminary study, the single oral dose of 500 mg/kg was used to study in detail the toxic effects, improvement and recovery of *n*BM and *n*BD in treated hens. Treated hens showed clinical signs of toxicity within 18-24 hr after dosing. Early signs included sluggishness, reluctance to stand or walk and loss of appetite. These were followed by the severe signs of poisoning mentioned above. Improvement of the clinical condition was seen in these hens beginning of the third or fourth day. The hens were sacrificed on the seventh day after treatment and examined. No unusual gross pathological lesions were found in any of the hens.

### *Hematological indices*

The effect of treatment on the various hematological indices measured is shown in Table 1. A significant decrease in blood Hb concentration was observed in hens treated with *n*BM and *n*BD. The greatest decrease was observed 48 hr after treatment. Hb concentrations at this time period were 62.7% and 78.2% of the control for *n*BM and *n*BD, respectively. The PCV values after 24 hr for *n*BM were 51.1% and 60.0% of control, respectively. The packed cell volume of hens treated with *n*BD determined at 48 hr after dosing also showed a significant decrease as compared with that of controls. Methemoglobin concentrations were significantly higher in all treated hens at all time intervals.

### *Erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD)*

The activity of G-6-PD (an enzyme essential for the regeneration of reduced nicotinamide adenine dinucleotide phosphate and erythrocyte integrity) was significantly reduced by both *n*BM and *n*BD at 24 and 48 hr after treatment. This enzyme activity returned to levels similar to those of the controls 72 hr after treatment (Table 1).

### *Cholinesterases*

Since the clinical signs of intoxication with *n*BM closely resembled the cholinergic effect of anticholinesterase agents, the possibility that this chemical might have inhibited cholinesterase enzymes was investigated. Plasma BuChE activity of *n*BM treated hens increased significantly as compared with controls (Table 2). The effect of *n*BM on enzymatic activity was dose related; as the dose increased plasma BuChE activity increased. The maximum increase in the enzymatic activity was measured after 21 days in hens treated with a single oral 400 or 500 mg/kg *n*BM. The enzymatic activity peaked on day 2 in hens given a single 100 mg/kg oral dose of *n*BM. As time passed, plasma BuChE activity returned to normal and was not significantly different from that of the controls by day 28.

By contrast *n*BD did not have any effect on plasma BuChE activity. One measurement of the enzymatic activity in a plasma sample taken two days after the oral administration of 500 mg/kg *n*BD showed that plasma BuChE activity was comparable to that of the control (1.63 ± 0.19 for control vs. 1.3 ± 0.14 for *n*BD). Brain AChE activity was not affected by a single oral 500 mg/kg *n*BM or *n*BD treatment when measured 28 days after dosing. The enzymatic activities were: control, 45.92 ± 3.82; *n*BM, 42.03 ± 6.20; and *n*BD 45.36 ± 0.90 μmoles ATCH hydrolyzed/min/mg protein.

### *Heinz bodies*

#### Light microscopy

Wet smears of crystal violet stained blood from control and *n*BM- and *n*BD-treated hens were examined by light microscopy (Fig. 1A,B). Almost 100% of the red blood cells collected from hens 24 hr after the oral administration of 500 mg/kg *n*BM or *n*BD either contained Heinz bodies, or were lysed and fragmented. The bodies were intensely stained, some distributed in the cytoplasm and other attached to the cell wall. The erythrocytes of *n*BM- and *n*BD-treated hens were irregular in shape and showed evidence of lysis and fragmentation. Some normal erythrocytes, however, were seen in the 48 hr blood samples and by 72 hr over 90% of the red blood cells returned to normal. No Heinz bodies were detected in red blood cells obtained from the controls.

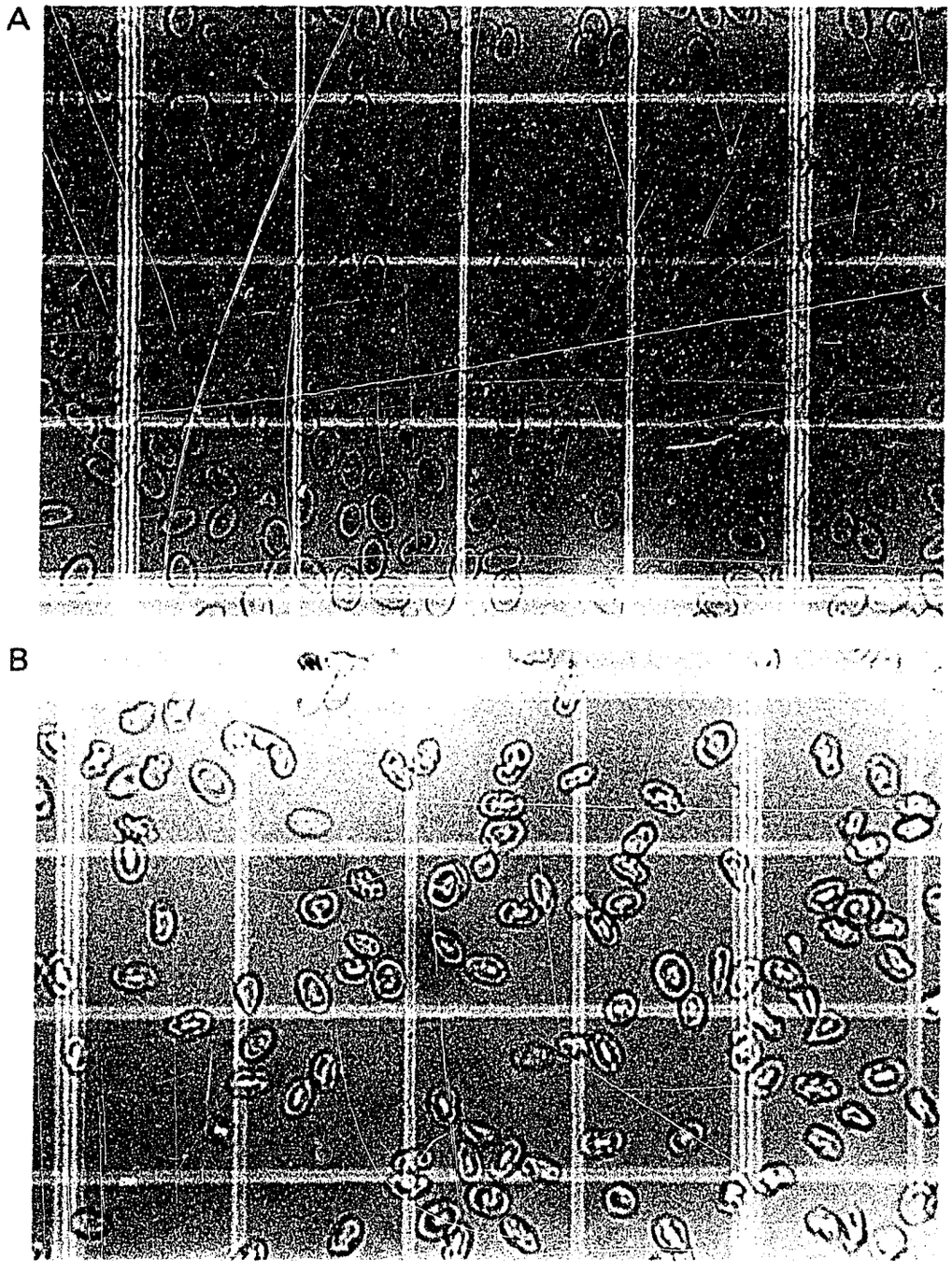


FIG. 1. Light photomicrograph of: A) normal red blood cells; B) red blood cells showing Heinz bodies stained with crystal violet × 400. Blood was sampled 24 hr after the administration of 500 mg/kg single oral dose of *n*BM.

### Electron microscopy

Red blood cells obtained from control hens (Fig. 2A), hens treated with a single i.p. injection of 30 mg/kg of phenylhydrazine (positive control) (Fig. 2B), and from those treated with a single oral dose of 500 mg/kg *n*BM (Fig. 2C) or *n*BD (Figure is not shown) were investigated by electron microscopy. Heinz bodies were present in red blood cells of phenyl hydrazine-, *n*BM- and *n*BD-treated hens. These bodies appeared as aggregates of electron dense material within the cytoplasm and within the nucleus. Heinz bodies present in the cytoplasm were much larger and concentrated particularly near the cell and nuclear membrane.

## *DISCUSSION*

Mercaptans are found in nature as products of putrefaction of sulfur-containing substances. Symptoms of acute poisoning of mercaptans' vapor in a human case was reported as coma, cyanosis, general muscular spasm, pulmonary irritation, fever and leucocytosis (Cristensen, 1941). Recovery took place gradually and was complete in about two days. *n*-Butyl mercaptan (*n*BM) was detected in the air of fields following the spraying of the cotton defoliants DEF and merphos (Maddy and Peoples, 1975). While dermal application of these two pesticides produced delayed neurotoxicity in hens, oral administra-

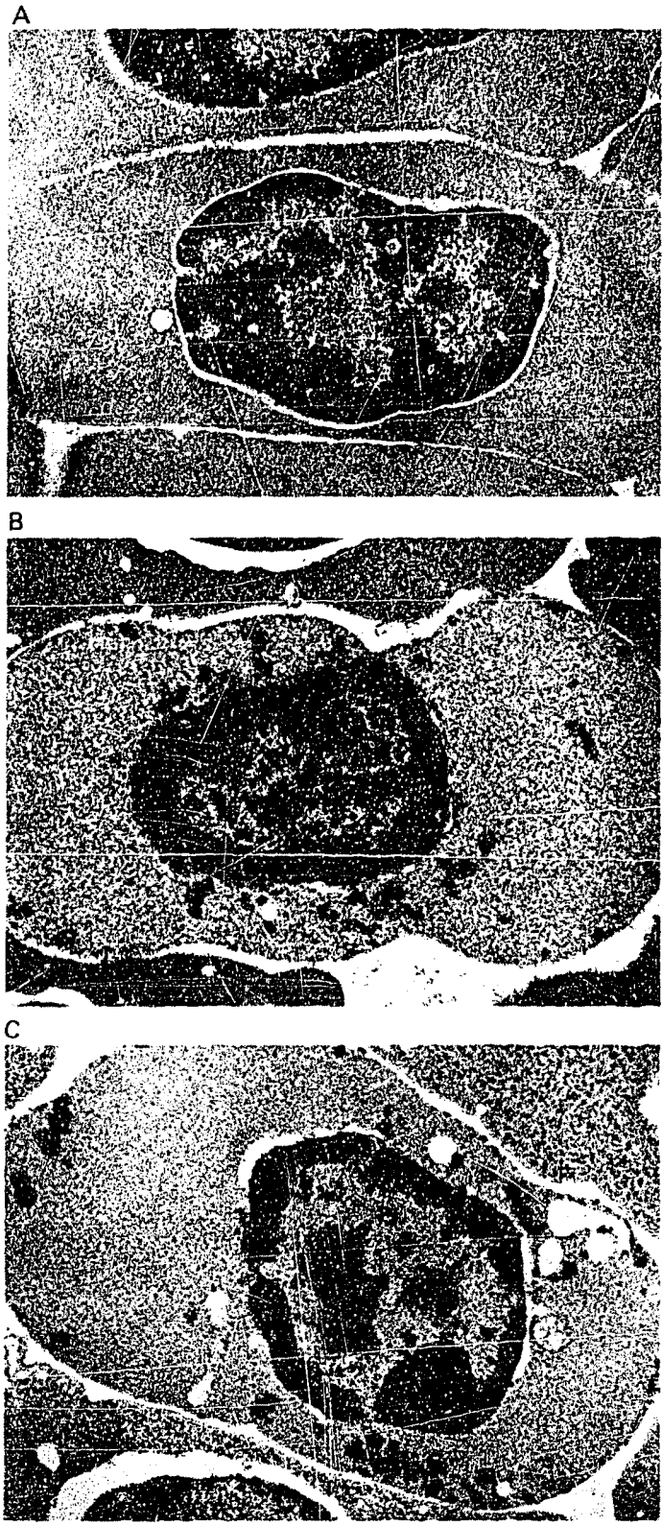

FIG. 2. A) Erythrocytes from control hens have a homogenous cytoplasm. × 34 000. B) By contrast, erythrocytes from hens five hours after subcutaneous injection of phenylhydrazine HCl at 30 mg/kg contain numerous Heinz bodies, seen as foci of increased cytoplasmic density, perticularly near cell and nuclear membranes. Similar densities are noted in nuclei. × 34 000. Sampling was 24 hr after administration. C) Heinz bodies are also present in cytoplasm of erythrocytes from hens 24 hr after a single dose of *n*-butyl mercaptan (*n*BM) at 500 mg/kg. × 34 000. Sampling was 24 hr after administration.

tion caused late acute effect (Abou-Donia *et al.*, 1979a,b, 1980). Clinical signs of the late acute effect induced by DFP and merphos were similar to those induced by *n*BM.

The present study reports the results of an investigation into the toxicologic effects of *n*BM and its oxidation product *n*BD in hens. Large oral doses of *n*BM caused death within 48 hr most likely due to respiratory failures. These two chemicals produced changes in some hematological indices. The changes accompanied clinical signs including sluggishness, weakness, loss of appetite, loss of weight, loss of leg coordination and change in comb color from red to white to dark purple. Also, the disappearance of these changes coincided with improvement of the clinical condition of the hens that survived the treatment.

Severely affected hens showed a decrease in the hemoglobin, packed cell volume, and in erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD) activity. These hens also showed an increase in plasma BuChE activity and methemoglobin concentration. Heinz bodies and extensive erythrocyte deformation and lysis were observed 24 hr after administration of either chemical. These results were similar to those found with phenylhydrazine, a chemical known to induce Heinz body formation (Rifkind, 1965).

Although Heinz bodies have been known since 1890, their nature and disposition are not precisely known (Heinz, 1890). They are generally believed, however, to be an insoluble denaturation product of hemoglobin (Jandl *et al.*, 1960; Rifkind, 1965; Rifkind and Danon, 1965). They are composed of electron-dense units (Rifkins and Danon, 1965). Erythrocytes containing Heinz bodies are phagocytized in Kupffer cells (Rifkind, 1965; Schnitzer and Smith, 1966), where the digestion of erythrocytes occurs within phagocytic vacuoles (Essner, 1960).

The decrease in hemoglobin and hematocrit and the presence of heinz bodies reported here, are similar to those seen in cattle, sheep and hens exposed to dimethyl disulfide (Penny *et al.*, 1964; Maxwell, 1981). The decrease in hemoglobin and hematocrit of treated hens is apparently due to erythrocyte G-6-PD depression similar to that reported in subjects susceptible to drug induced hemolytic anemia (Dacie 1967; Allen and Jandl, 1961). The primary function of this enzyme is the regeneration of reduced nicotinamide adenine dinucleotide phosphate (NADPH) which is an essential factor in the reduction of glutathione. In the presence of lowered concentrations of reduced glutathione, the integrity of red blood cells is compromised, hemoglobin is denatured, surface proteins are coagulated and cells are hemolyzed and destroyed. Heinz bodies represent the end of hemoglobin denaturation where methemoglobin is an intermediate step in this process. The results of the present study suggest that Heinz body formation is due to the inhibition of erythrocyte G-6-PD as evidenced by the reduction of the activity of this essential enzyme in the red cells obtained from *n*BM- and *n*BD-treated hens. Another possible mechanism, which may have led to Heinz bodies formation, is the depletion of reduced glutathione in the red blood cells via direct reaction with the SH group of *n*BM or with the S-S bond of *n*BD. Furthermore, reactions between these two chemicals and the free thiol groups on S-S bridges present in hemoglobin may also lead to Heinz body formation.

This study demonstrated that the toxic effects of *n*BM and *n*BD are not related to the inhibition of cholinesterase enzymes. By contrast oral administration of *n*BM to hens caused an

increase in the activity of plasma BuChE. *n*BM action seems to be specific for plasma BuChE since this compound did not have a significant effect on brain AChE. The mechanism of action of this effect is being investigated.

In the present study we have demonstrated some of the adverse effects of *n*-butyl mercaptan and *n*-butyl disulfide in the hen. Among the adverse effects observed are Heinz body formation and extensive erythrocytes deformation and lysis. Hemoglobin concentration, hematocrit and erythrocyte glucose-6-phosphate dehydrogenase activity were significantly lowered in intoxicated birds. Since oral administration of DEF or merphos produced late acute effects with clinical signs similar to those produced by their metabolic product *n*BM, it is proposed that the mechanism of the late acute effects of DEF and merphos is directly related to the reported hematological changes. These hematological changes may also account for the headache occurring in persons present near cotton fields sprayed with DEF or merphos (Maddy and Peoples, 1975).

## ACKNOWLEDGEMENTS

The secretarial assistance of Erna S. Daniel is greatly appreciated. This study was supported by a grant from NIEHS, No. ES02717.

## REFERENCES

**Abou-Donia, M.D.** (1981). Organophosphorus Ester Induced Delayed Neurotoxicity. *Ann. Rev. Pharmacol. Toxicol.* 2:511-548.

**Abou-Donia, M.B., Graham, D.G., Abdo, K.M. and Komeil, A.A.** (1979a). Delayed Neurotoxic, Late Acute and Cholinergic Effects of *S,S,S*-Tributyl Phosphorotrithioate (DEF): Subchronic (90 Days) Administration in Hens. *Toxicol.* 14:229-243.

**Abou-Donia, M.B., Graham, D.G., Timmons, P.R. and Reichert, B.L.** (1979b). Delayed Neurotoxic and Late Acute Effects of *S,S,S*-Tributyl Phosphorotrithioate on the Hen: Effect of Route of Administration. *Neurotoxicol.* 1:425-447.

**Abou-Donia, M.B., Graham, D.G., Timmons, P.R. and Reichert, B.L.** (1980). Late Acute, Delayed Neurotoxic and Cholinergic Effects of *S,S,S*-Tributyl Phosphorotrithioite (Merphos) in Hens. *Toxicol. Appl. Pharmacol.* 53:439-457.

**Allen, D.W. and Jandel, J.H.** (1961). The Role of the Thiols in Oxidant Drug Action. *J. Clin. Invest.* 40:454-475.

**Anonymous** (1976). *The Merk Index*, Ninth Ed., p. 201. Rahway, NY.

**Bentler, E.** (1977). Erythrocyte Enzyme Assays. In *Hematology*, 2nd Ed., W.J. Williams, E. Bentler, A.J. Erslev and R.W. Rundels, editors, p. 1605-1608. McGraw-Hill Book Co., New York, NY

**Cristensen, V.** (1941). A Case of Poisoning with Mercaptans. *Standard Oil Co.* (New Jersey) *Medical Bulletin* 5:78.

**Dacie, J.V.C.** (1967). *The Hemotolytic Anemia: Congenital and Acquired, Part IV*, 2nd ed., p. 1022. J.A. Churchill, Ltd., London.

**Dacie, J.V. and S.M. Lewis** (1975). *Practical Hematology*, 5th ed., p. 140. Churchill Livingston, Edenburgh.

**Ellman, G.L., Courtney, K.D., Andres, V., Jr. and Featherstone, K.M.** (1961). A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. *Biochem. Pharmacol.* 7:88-95.

**Essner, E.** (1960). An Electron Microscopic Study of Erythrophagocytosis. *J. Biophys. Biochem. Cytol.* 7:329-334.

**Evelyn, K.A. and Malloy, H.T.** (1938). Microdetermination of Oxyhemoglobin and Sulfhemoglobin in a Single Sample of Blood. *J. Biol. Chem.* 126:655-662.

**Heinz, R.** (1890). Morphologische Veranderungen der Rothen Blutkorperchen Durch Gifte. *Virchows Arch. Path. Anat.* 122:112-116.

**Jandl, J.H., Engle, L.K. and Allen, D.W.** (1960). Oxidative Hemolysis and Precipitation of Hemoglobin. I. Heinz Body Anemias as an Acceleration of Red Cell Aging. *J. Clin. Invest.* 39:1818-1836.

**Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J.** (1951). Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* 193:265-275.

**Maxwell, M.H.** (1981). Production of a Heinz Body Anemia in the Domestic Fowl After Ingestion of Dimethyl Disulfide: A Hematological and Ultrastructural Study. *Res. Vet. Sci.* 30:233-238.

**McLeod, W.R.** (1975). Merphos Poisoning or Mass Panic. *New Zea. J. Psychiatry* 9:225-229.

**Penny, R.H.C., Davis, J.S.E. and Wright, A.I.** (1964). Observation on the Blood Picture of Cattle, Sheep and Rabbits Fed on Kale. *Vet. Record* 76:1053-1059.

**Rifkind, R.A.** (1965). Heinz Body Anemia: An Ultrastructural Study. II. Red Cell Sequestration and Destruction. *Blood* 26:433-448.

**Rifkind, R.W. and Danon, D.** (1965). Heinz Body Anemia — An Ultrastructural Study. I. Heinz Body Formation. *Blood* 25:885-896.

**Schnitzer, B. and Smith, E.B.** (1966). Observations of Phagocytized Red Cells Containing Heinz Bodies. A Light and Electron Microscopic Study. *Am. J. Clin. Path.* 46:538-545.

**Smith, R.H.** (1974). Kale Poisoning. *Rep. Rowett. Inst.* 30:112-131.

**World Health Organization** (1967). Standardization of Procedures for the Study of Glucose-6-phosphate Dehydrogenase. *WHO Tech. Rep.* Ser. No. 366.