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Low Erythrocyte Glucose-6-Phosphate Dehydrogenase (G-6-PD) Activity and Susceptibility to Carbaryl-Induced Methemoglobin Formation and Glutathione Depletion

Edward J. Calabrese and Christopher P. Geiger

Division of Public Health, University of Massachusetts, Amherst, MA 01003

The identification and quantification of subsegments of the population that may be at increased risk to experience enhanced toxicity and/or carcinogenicity from environmental agents is of considerable regulatory interest. One such potential high risk group involves persons with an erythrocyte G-6-PD deficiency. This condition is known to enhance hemolytic susceptibility to numerous oxidant drugs and industrial chemicals (Beutler 1978; Calabrese 1984). That G-6-PD deficient persons may be at enhanced hemolytic risk is of considerable social concern since 13% of the American Black male population have this deficiency (Beutler 1978). Since ethical considerations may preclude *in vivo* experimental exposure of G-6-PD deficients to environmental oxidant stressor agents, it is important that an animal model be developed which accurately predicts the response of human G-6-PD deficient humans to oxidant stressor agents.

The intention of this paper is to evaluate the *in vitro* effects of the carbamate insecticide carbaryl, which is widely used in aerial spraying for the control of gypsy moths in some affected states in the Northeast (e.g., New Jersey) and Northwest (e.g., Oregon) (USDA 1985) on the responses of erythrocytes of the Dorset sheep which also exhibit a similar enzyme activity as those humans with a G-6-PD deficiency (Calabrese 1984). That carbaryl may pose an oxidative stress to G-6-PD deficient red cells is suggested by previous studies with α-napthol, a metabolite of carbaryl, which causes hemolysis in human G-6-PD deficient erythrocytes (Bloom et al. 1983).

MATERIALS AND METHODS

Blood was collected in heparin from five non-pregnant female sheep. Transported samples were maintained in ice packs until testing. All samples were tested

within one hour of collection. The blood sample from each sheep was divided for control and test purposes. Blood was aliquoted into 1 ml samples. Each sample received 20 µL of technical grade carbaryl dissolved in ethyl alcohol. The concentrations of carbaryl employed in the 20 µL additions were 1 ppm, 10 ppm and 100 ppm along with an ethyl alcohol control and an untreated incubated control. The blood was incubated for a period of 2 hours in a water bath at 37°.

The hematological parameters measured were selected primarily on the basis of their being widely accepted indicators of oxidative stress. The parameters included: methemoglobin (MetHb), levels of reduced glutathione (GSH). MetHb was measured according to the methods by Brown (1973) using potassium ferricyanide and potassium cyanide as reagents and measuring changes in optical density at 630 nm. A colorimetric reaction employing 5,5'-dithiobis nitrobenzoic acid (DTNB) was used to measure the amount of GSH in blood according to Prins and Loos (1969) at 412 nm. Measurments were made with a spectrophotometer with a temperature-controlled flow cell and automatic printer, calculator. All hematological parameters were measured within 3 hours after incubation.

Data were computer analyzed by multiple variant analysis of variance (MANOVA) with the significance level set at 0.05. If MANOVA showed a difference in effect among blood samples, Tukey's HSD multiple comparisons test was carried out.

RESULTS AND DISCUSSION

The results indicate the carbaryl incubation produces a dose-dependent and highly significant ($p < 0.01$) increase in MetHb formation at 10 and 100 ppm as well as a dose-dependent and highly significant decreases in GSH levels at 10 ppm ($p < 0.05$) and 100 ppm ($p < 0.01$). No statistically significant differences occurred between the ethanol control and the 1 ppm exposure group with respect to either MetHb formation or GSH levels.

Table 1. The Effects of Carbaryl Treatment on Methemoglobin Formation (%)

Control	ETOH Control	Carbaryl Levels		
		1 ppm	10 ppm	100 ppm
1.7±.54	2.1±.63	2.5±.47	9.1±1.55	53.3±8.35**

Table 2. The Effects of Carbaryl Treatment on GSH Levels (mg%)

Control	ETOH Control	Carbaryl Levels 1 ppm	10 ppm	100 ppm
84.7±6.0	73.6±6.4	72.6±8.2	53.1±7.3*	9.0±2.7**

*Differs from the ETOH control at $p < 0.05$.
** Differs from the ETOH control at $p < 0.01$.

This study represents the first report of carbaryl on erythrocytes with low G-6-PD activity. Previous in vivo 90-day feeding studies with rats gave a no adverse effects dosage of 66 mg/kg/day (Union Carbide Corp. 1956, cited by USEPA 1975) while a later study (Union Carbide Corp. 1958, cited by USEPA 1975) showed no adverse effects in rats at 104 mg/kg/day while 167 mg/kg/day reduced growth, increased liver weight and slightly decreased cholinesterase activity.

It would not be unexpected that rats would show no adverse effects on red blood cells since they have a high antioxidant enzymatic defense as reflected in erythrocyte activity levels of G-6-PD, catalase, glutathione reductase, superoxide dismutase and methemoglobin reductase (Calabrese 1983).

The present studies suggest that the G-6-PD deficient red cells of the sheep are at increased risk to the oxidative stress caused by carbaryl. These findings support the need for an in vivo assessment of the effects of carbaryl on Dorset sheep using both dermal and oral routes. It should be noted that a recent USDA study estimated aerial spraying of carbaryl could provide from 0.29 (realistic) to 2.88 (worse case) mg/kg/day carbaryl exposure to livestock (i.e., goats) (USDA 1985).

While the mechanism by which carbaryl causes an increase in MetHb and a decrease in GSH levels remains to be more fully assessed, it is likely that it may be caused by α-napthol, a well-established hydrolysis metabolite of carbaryl (O'Brien 1967). Analysis of urine for α-napthol of persons occupationally exposed to carbaryl is common practice (Schulze et al. 1979; USDA 1985). Approximately one-third of the carbaryl dose to humans is excreted in the urine as α-napthol (USDA 1985). α-napthol is a known potent hemolysing agent in G-6-PD deficient humans but not in normal humans (Bloom et al. 1983).

The concordance of the response of sheep erythrocytes

with low G-6-PD activity and humans with a G-6-PD deficiency suggests that human G-6-PD deficients may also be at potentially increased hemolytic risk to carbaryl exposure. However, this hypothesis remains to be assessed along with the relative sensitivity of the hemolytic versus anticholinesterase effects of carbaryl.

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