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Effects of Environmentally Relevant Doses of Cyanide on Flight Times in Pigeons, *Columba livia*

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Cyanide is regularly found in mine process ponds from heap leaching. The cyanidization process of extracting gold from ore dates back to 1898, when it was first used in New Zealand and Africa. What remains as solution and effluent following the extraction of the gold is a mixture of free cyanide, metallo-cyanide complexes, cyanates, thiocyanates, and other chemical species dissolved from the initial ore (Simovic and Snodgrass 1985). Effluents from the processing of precious metals may contain concentrations ranging from 0.3 to 216 ppm of free cyanide along with metallocyanide complexes. Process ponds can average between 200 and 300 ppm and can reach as high as 1000 ppm (Clark and Hothem 1991). Although the contaminated waters do not typically affect human populations, wildlife has been poisoned after drinking from mine waste sources (Henny et al. 1994). From 1986 to 1995, mortality of several types of bird species has occurred from exposure to cyanide contaminated ponds at mines in the United States (USGS 1999). Avian species constituted 91% of 9,512 dead animals found poisoned by cyanide at Nevada gold mines between 1986 and 1991. Of the bird species that were poisoned, 73% were migratory (Henny et al. 1994). Furthermore, metal bound cyanide from heap leach ponds may be broken down to free cyanide slowly during digestion, which could result in toxicity a considerable distance from the point of exposure and potential for underreporting mortality (Clark and Hothem 1991). Since 1991 avian deaths have decreased due to measures including nets and keeping levels of cyanide in ponds at lower levels (Eisler and Wiemeyer 2004).

Aside from lethality, the effects of chronic and sub chronic relevant environmental doses of cyanide on bird populations have seldom been addressed. Henny et al. (1994) studied waterfowl exposed to cyanide in tailing ponds at Nevada mine sites including green-winged teal (*Anas crecca*) and cinnamon teal (*Anas cyanoptera*). Various degrees of toxicity were noted from no observable signs to death. Overt toxic effects were consistent with the amount of water a bird was observed drinking. Some of the birds that drank the water showed signs of cyanide intoxication including shaking their bills followed by periods of lethargy followed by what appeared to be complete recovery prior to the birds leaving. One cinnamon teal that was noted drinking more water than the others died on the spot. Recent studies have shown that cyanide administered via oral gavage to

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mallard ducks at sublethal doses from 0-2 mg/mg resulted in oxidative stress and depleted ATP levels that were dose dependent (Ma and Pritsos 1997). A reduction in ATP levels in avian species could result in compromised flight due to depressed energy metabolism. The purpose of this study is to evaluate the effects of repeated low-level and environmentally relevant doses on time of flight in pigeons. It is hypothesized that repeated low-level cyanide exposure will negatively effect flight time in these birds.

MATERIALS AND METHODS

Young homing pigeons (*Columba livia*) were obtained from two local pigeon racers who consented to the project. Pigeons were initially set free *en masse* or in groups of less than or equal to (≤) six (6) birds from several distinct liberation points at increasingly longer distances. Groups of 4 to 6 birds allowed for flock behavior in flight normally seen in both homing pigeons and migratory birds and also resulted in direct returns of the animals rather than individual releases, in which the bird would often circle the area and await the release of other pigeons.

Following the initial training period, birds were individually fitted with electronic bands to monitor their arrival times at their loft. Return time was clocked by an electronic scanner, which recorded individual bands at the loft entrance. Individual flight times were observed for experiments conducted over two years. Releases for the first year occurred at Davis Creek, CA and Valley Falls, OR, 150 and 200 air miles, respectively, to the point of return in Lemmon Valley, NV, seven miles north of Reno. For the second year of study birds were released from Susanville, CA (65 air miles), Subway Lava Cave, CA (100 air miles), Lake Britton, CA (150 air miles), and McCloud, CA (200 air miles). Distance training occurred in the same manner as in the preceding year with the same birds used previously and a new group of yearlings that were trained to at least 65 miles from their home roost prior to any dosed runs.

Treatment via oral gavage with a predetermined dose of potassium cyanide (96% purity Fisher Scientific, Pittsburgh, PA) was based on an animal's individual body weight. Birds were weighed no more than 18 hours prior to each treatment. Dosages ranged from 0.0, 1.0, 1.25, or 2.0 mg/kg KCN equal to 0, 40, 50, and 80 mg/L respectively in 10 ml dose equivalents. Control animals (0.0 mg/kg) were given an equivalent measure of filtered water. After administration by gavage, the birds were allowed to rest 15 to 20 minutes prior to release in groups of four to six. The order of administration of a given dose or control was randomized to avoid bias. Release times were separated by fifteen (15) minutes per group. On a few occasions a bird did not return from a trial release (3 times in the two years of study), and its time had to be discounted from tabulation. The three birds that failed to return consisted of one control, one dosed at1.0 mg/kg, and one dosed at 2.0 mg/kg.

Non-parametric statistical tests were employed from the various distances because the populations being sampled had the same characteristics but were non-normal in distribution (different release points, various doses, etc.). Times were relativized for all flights with liberation points ranging from sixty five (65) to (200) two hundred miles. Relativization statistic gives the value of 1.0 to the fastest time and scores all other flights based on that time. A parametric One-Way Analysis of Variance was applied to 4 flights from a one-hundred (100) mile distance in which there was a more normal distribution. All statistics were run on Prism 3.0 Statistical Software (Graph Pad Inc, San Diego).

RESULTS AND DISCUSSION

Times from experimental flights at 4 liberation points from various distances are shown in Figure 1. For the first flight in which birds were liberated from 65 miles the range was narrow and the differences in return times, although dose-dependent, were not significant. The spread in flight times increased as the distance increased and slower times were consistently recorded with increasingly higher doses.

Relativization of flight times is shown in Figure 2. Birds were relativized based on the first return time from each flight. Analysis by Mann-Whitney test indicated the difference between controls and birds dosed with 1.0 mg/kg was not significant. Every other comparison between groups, however, demonstrated significant variation ($\alpha < 0.05$) and a significant increase in flight times for concentrations of 1.25 mg and 2.0.

Results of our experiments also indicated that sub-lethal doses of cyanide slow the time of pigeon flight to the home loft and the effect is more profound at increasingly higher doses and longer distances. The distances were statistically significant between every comparison group except at the lowest dose of 1.0 mg/kg (40 mg/L) CN^- body wt (77.52% confidence level). Birds dosed with 1.25 mg/kg and 2.0 mg/kg were significantly slower than birds dosed with 1.0 and 0.0 mg/kg.

To see how the birds responded from a single liberation point, the four dosed flights from 100 miles during the second year were analyzed separately. There was a paucity of data on 1.25 mg/kg dosed flights from the 100 mile distance so Analysis of Variance was limited to Control, 1.0, and 2.0 mg/kg dosed groups (Table 1). The results showed a dose dependent increase in average flight times that approaches significance at 1.0 mg/kg and is significantly higher ($P<0.05$) than the control group at 2.0 mg/kg (Figure 3).

Cyanide affects mitochondrial function by inhibiting cytochrome c oxidase and disrupting cellular respiration, resulting in a concomitant reduction in intracellular ATP. The bioenergetic consequence is less fuel for extracellular activities such as muscular stimulation (ATSDR 1997). Despite this fact, very few studies have been done to elucidate what effects sub-chronic doses of cyanide would have on avian species. In our study homing pigeons dosed with cyanide at environmentally relevant doses were slower to return and the slower times were

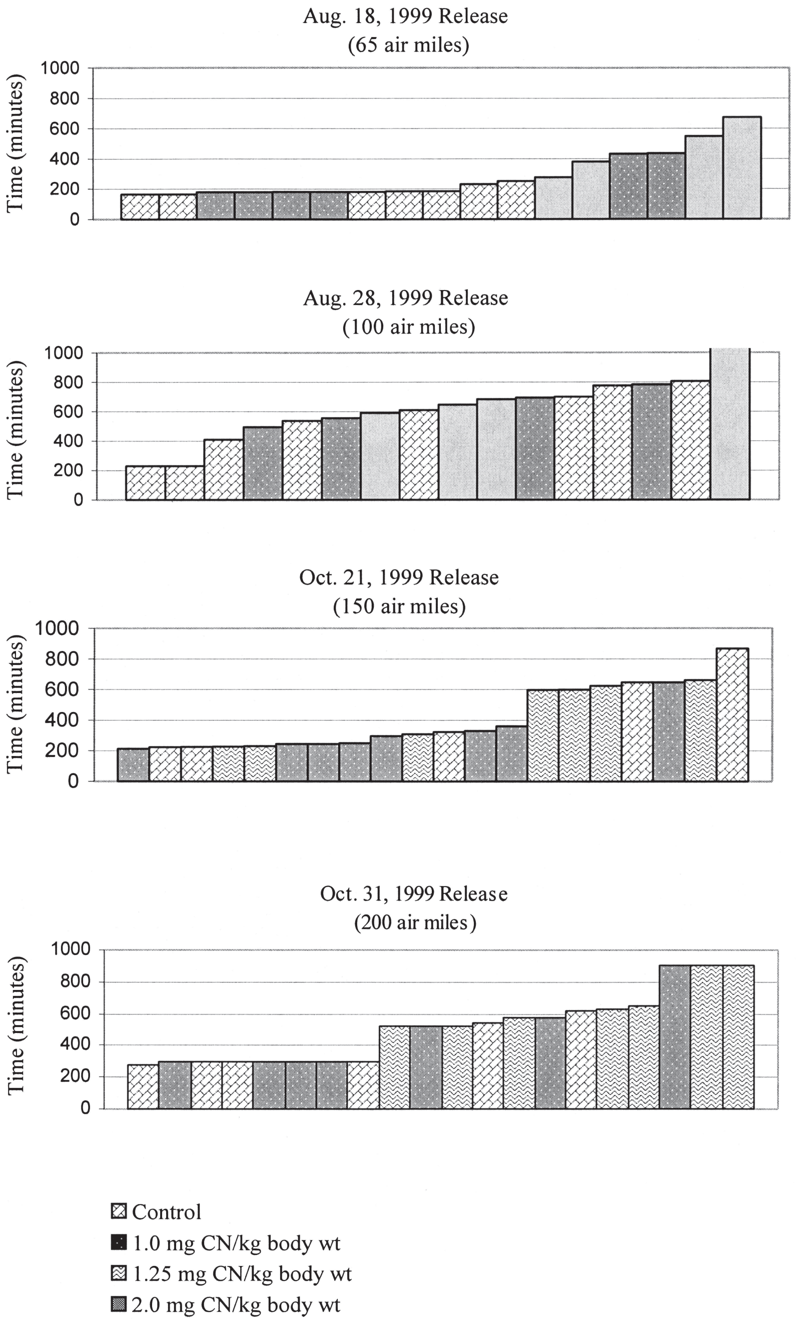

Figure 1. Flight times at various doses of cyanide from 65, 100, 150, and 200 mile. Each bar represents the flight time of an individual bird.

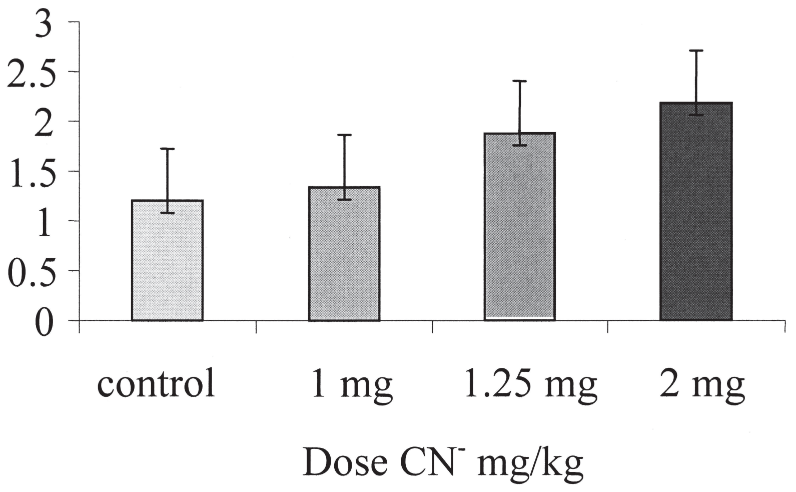

Figure 2. Relativized flight times at 0, 1.0, 1.25 and 2.0 mg/kg of cyanide for all runs. N = 14 -24 relativized individual flight times per dosage.

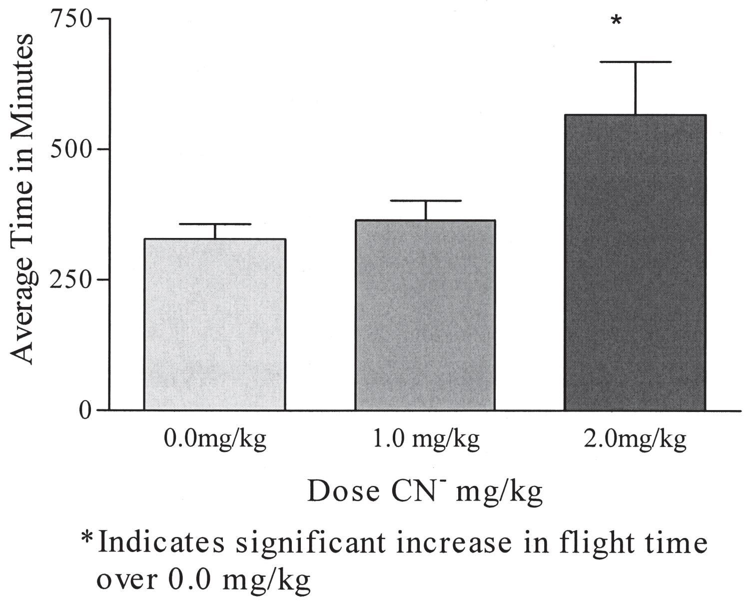

Figure 3. Average flight times in minutes from 100 miles by CN^- dose.

Table 1. Flight times in minutes at 0.0, 1.0, and 2.0 mg CN^- /kg from 100 miles

8/28/1999			**9/5/1999**		
0.0 mg/kg	**1.0 mg/kg**	**2.0 mg/kg**	**0.0 mg/kg**	**1.0 mg/kg**	**2.0 mg/kg**
229.5	494.0	591.6	182.3	190.5	371.2
230.4	555.2	646.4	185.2	217.5	371.3
409.0	695.2	684.0	185.2	338.3	371.4
536.5	782.6	2176.6	185.3	339.2	372.1
610.0			185.4	652.3	373.2
700.4			189.0		1284.5
775.1			191.0		
806.3			191.1		
			191.1		
			266.1		
			377.6		
9/30/1999			**10/16/1999**		
0.0 mg/kg	**1.0 mg/kg**	**2.0 mg/kg**	**0.0 mg/kg**	**1.0 mg/kg**	**2.0 mg/kg**
140.3	142.2	170.3	202.5	174.3	197.2
140.3	168.4	196.4	203.5	198.5	222.6
140.3	168.4	196.6	292.2	233.2	245.0
141.5	170.2	199.0	302.2	233.2	272.3
246.3	392.3	274.0	486.0	281.4	310.4
247.3	565.4	418.6	538.1	483.2	562+
486.5		741.3		562+	562+
		776.1			562+

dose dependent. Birds were randomized for each trial and therefore exposures varied widely among the treated birds over the 2 years of the study. Each trial provided information interdependently with the other trials within the study population considering a short half-life and clearance of cyanide.

The dose-dependent effect on flight times in our study with cyanide was observed by increased relative time of flight in homing pigeons from a 65 to 200 mile distance and increased average time of flight from 100 miles. The studies also indicated that on flights from 100 miles, birds dosed at 2.0 mg/kg had significantly slower return times. Therefore, with the understanding of primary mitochondrial disruption and secondary oxidative stress mechanisms of cyanide in birds we can speculate from our results that repeated environmentally relevant doses of cyanide can slow migration in individual birds and small flocks of birds and the effect is possibly due to mitochondrial dysfunction, oxidative stress, or both.

Based on our study, the first dose at which cyanide affected relative flight times was at 1.25 mg/kg for the various distances ranging from 65 to 200 miles (Figure 2). This dose (1.25 mg/kg) was administered to the birds at a concentration of 50 ppm which represents a concentration regarded as "safe" for tailings ponds by the mining industry. At a dose of 2.0 mg/kg and a distance of 100 miles for both relativized and flight variance (ANOVA analysis) the results were significant. In prior studies mallard ducks showed compromised mitochondrial function and oxidative stress in brain, heart and liver tissue at similar low concentrations (Pritsos and Ma 1997). Furthermore, several antioxidant enzymes have been shown to be bound by cyanide including xanthine oxidase, superoxide dismutase, catalase, and glutathione peroxidase (Ardelt et al. 1994; Kanthasamy et al. 1997) which would also result in oxidative stress to exposed birds. A potential manifestation of the biochemical effects would be inability to fly long distances. Another consequence of slower flight in birds such as pigeons and migratory birds is the likely predation by raptors or, in some cases, terrestrial predators.

The results of the study provide useful information on flight time and thus flight speed following environmentally relevant doses of cyanide administered pre-flight to homing pigeons. Further research is needed to determine the utility and application of homing pigeons on the population level effects of environmental contaminants on migratory birds and other avian species. Chemicals such as cyanide, arsenic, and various pesticides are among toxins that are commonly encountered in the wild by birds. In alternative scenarios, individual chemical and synergistic effects of two or more toxins could be evaluated under similar experimental conditions. Longitudinal studies including developmental toxicities and generational effects utilizing homing pigeons may also be studied.

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