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The Toxicokinetics of Cyanide and Mandelonitrile in the Horse and Their Relevance to the Mare Reproductive Loss Syndrome

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The epidemiological association between black cherry trees and mare reproductive loss syndrome has focused attention on cyanide and environmental cyanogens. This article describes the toxicokinetics of cyanide in horses and the relationships between blood cyanide concentrations and potentially adverse responses to cyanide.

To identify safe and humane blood concentration limits for cyanide experiments, mares were infused with increasing doses (1–12 mg/min) of sodium cyanide for 1 h. Infusion at 12 mg/min produced clinical signs of cyanide toxicity at 38 min: these signs included increased heart rate, weakness, lack of coordination, loss of muscle tone, and respiratory and behavioral distress. Peak blood cyanide concentrations were about 2500 ng/mL; the clinical and biochemical signs of distress reversed when infusion stopped.

Four horses were infused with 1 mg/min of sodium cyanide for 1 h to evaluate the distribution and elimination kinetics of cyanide. Blood cyanide concentrations peaked at 1160 ng/mL and then declined rapidly, suggesting a two-compartment, open model. The distribution (alpha) phase half-life was 0.74 h, the terminal (beta phase) half-life was 16.16 h. The mean residence time was 12.4 h, the steady-state volume of distribution was 2.21 L/kg, and the mean systemic clearance was 0.182 L/h/kg. Partitioning studies showed that blood cyanide was about 98.5% associated with the red cell fraction. No clinical signs of cyanide intoxication or distress were observed during these infusion experiments.

Mandelonitrile was next administered orally at 3 mg/kg to four horses. Cyanide was rapidly available from the orally administered mandelonitrile and the C_{max} blood concentration of 1857 ng/mL was observed at 3 min after dosing; thereafter, blood cyanide again declined rapidly, reaching 100 ng/mL by 4 h postadministration. The mean oral bioavailability of cyanide from mandelonitrile was 57% ± 6.5 (SEM), and its apparent terminal half-life was 13 h ± 3 (SEM). No clinical signs of cyanide intoxication or distress were observed during these experiments.

These data show that during acute exposure to higher doses of cyanide (~600 mg/horse; 2500 ng/mL of cyanide in blood), redistribution of cyanide rapidly terminated the acute toxic responses. Similarly, mandelonitrile rapidly delivered its cyanide content, and acute cyanide intoxications following mandelonitrile administration can also be terminated by redistribution. Rapid termination of cyanide intoxication by redistribution is consistent with and explains many of the clinical and biochemical characteristics of acute, high-dose cyanide toxicity.

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On the other hand, at lower concentrations (<100 ng/mL in blood), metabolic transformation of cyanide is likely the dominant mechanism of termination of action. This process is slow, with terminal half-lives ranging from 12–16 hours. The large volume of distribution and the long terminal-phase–elimination half-life of cyanide suggest different mechanisms for toxicities and termination of toxicities associated with low-level exposure to cyanide. If environmental exposure to cyanide is a factor in the cause of MRLS, then it is likely in the more subtle effects of low concentrations of cyanide on specific metabolic processes that the associations will be found.

Keywords Cyanide, EFL, Fetal Loss, Horse, LFL, MRLS, Reproductive Loss Syndrome, Toxicity

In late April and early May 2001, horse-breeding farms in central Kentucky experienced a severe epidemic of early and late fetal losses, now called the mare reproductive loss syndrome (MRLS) (Kane and Kirby 2001). Late fetal losses (LFLs), totaling about 500 foals, began in the last days of April, peaked on May 5, and declined rapidly thereafter. Early fetal losses (EFLs), first identified on April 26, apparently followed a similar time course and ultimately totaled about 1500 cases. These episodes were followed by two apparently associated minor epidemics of pericarditis and unilateral opthalmitis, approximately 50 cases each, involving horses of all breeds, ages, and sexes.

The clinical signs of MRLS suggest that the causative agent was lethal to foals in utero at concentrations that did not affect mares, although all central Kentucky horses were apparently at risk, as suggested by the accompanying pericarditis and uveitis episodes. Epidemiological, pathological, and preliminary toxicological reports have suggested cyanide, apparently from black cherry trees or other environmental sources, as a possible causative agent (Fitzgerald et al. 2002).

Cyanide, at parts per million concentrations, can be extremely rapid-acting and very toxic in humans and most animals. Its small molecular weight (CN = 26), high diffusibility, and high lipid solubility lead to its rapid absorption, followed by rapid distribution and entry into cells and mitochondria (Sollman 1948). In the mitochondria, cyanide combines with the trivalent iron of cytochrome oxidase and blocks the final step of electron transfer to molecular oxygen (Way 1984). The end result is acute cytotoxic hypoxia, severe metabolic acidosis and, if cyanide concentrations are maintained, death of the cell and the organism. If the dose of cyanide is large and delivery is rapid, death from acute cyanide toxicity can occur within minutes (Borowitz et al. 2001).

Cyanide is found in many biological systems, including plants, where it functions as a protective toxin. In plants, cyanide may be linked to mandelonitrile, which is linked to glucosides, the specific glucoside/glucoside polymer depending on the plant species. Black cherry trees (*Prunus serotina*), which grow wild in central Kentucky, contain high concentrations of the cyanogenic glucoside prunasin in their leaves and bark (Fig. 1). Damage to black cherry leaves from any cause initiates an enzymatic cascade that releases mandelonitrile, which then spontaneously decays to yield free cyanide (Morse and Howard 1898; Smeathers et al. 1975) (see Fig. 1). This reaction occurs rapidly.

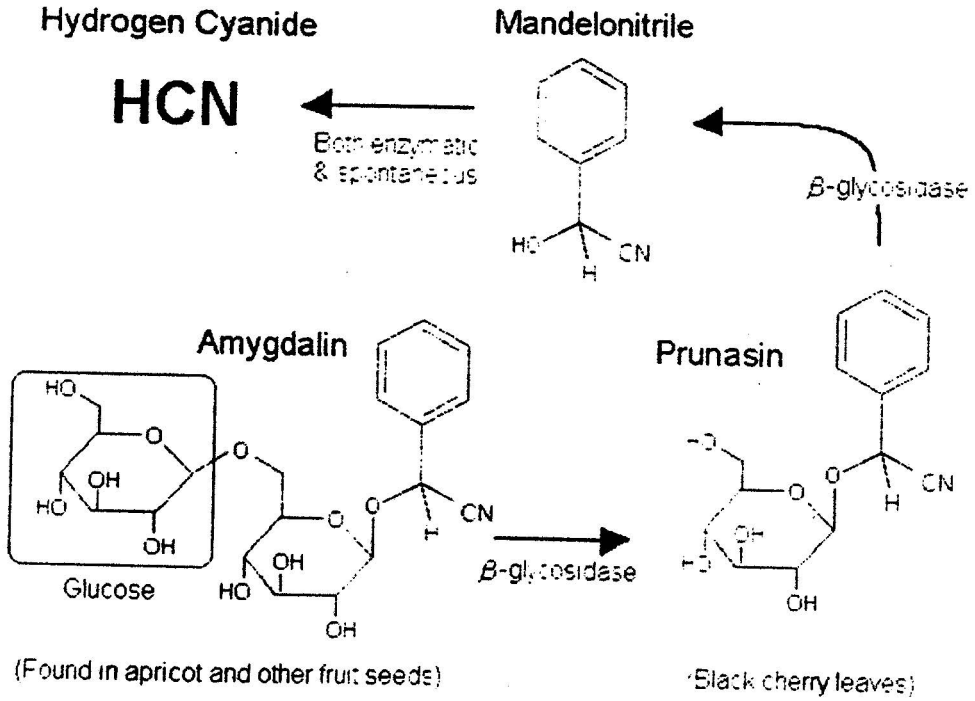

FIG. 1. Cyanogenic cascade in black cherry (prunasin) and apricot (amygdalin). Both cascades proceed through prunasin, and mandelonitrile is the proximate cyanide donor.

and under appropriate conditions mandelonitrile can deliver a large proportion of its cyanide content over a relatively short time.

The epidemiological association among black cherry trees, eastern tent caterpillars (*Malacosoma americana*), and MRLS in spring 2001 has focused attention on the potential role of environmental cyanogens in the cause of the syndrome. To evaluate the role of cyanide in this syndrome, it is necessary to understand the bioavailability and toxicokinetics of cyanide and various cyanide precursors occurring in black cherry trees and also possibly in eastern tent caterpillars, whose presence in unusually high numbers in central Kentucky was associated with the spring 2001 outbreak of MRLS (Kane and Kirby 2001). This report is a preliminary evaluation of the bioavailability and toxicokinetics of cyanide and of cyanide from mandelonitrile in the horse that intends to establish a basis for the exploration of the possible roles of these agents in the spring 2001 outbreak of MRLS in central Kentucky.

MATERIALS AND METHODS

Horses and Sample Collection

Mature thoroughbred mares weighing 428 to 504 kg were used for this study. The animals were maintained on grass hay and feed (12% protein), which was a 50:50 mixture of oats and an alfalfa-based protein pellet. The horses were fed twice a day. The animals were vaccinated annually for tetanus and were dewormed quarterly with ivermectin (MSD Agvet, Rahway, NJ). A routine clinical examination was performed before each experiment to ensure that the animals were healthy and sound. Additionally, cardiac and ophthalmic evaluations were performed to ensure that those organs had no evidence of previous disease. All animals used in these experiments were managed according to the rules and regulations of the University of Kentucky Institutional Animal Care Use Committee, which also approved the experimental protocol.

Cyanide Infusion

Four mature thoroughbred mares weighing between 578 and 612 kg were used for the intravenous infusion of sodium cyanide (NaCN). The skin over the jugular vein was washed with Betadine Scrub and rinsed with methanol. An intravenous catheter (Abbocath-T, 14 g × $5^1/_2$ in, Abbott, North Chicago, IL) was inserted into the jugular vein and sutured into place. NaCN solutions were prepared by dissolving NaCN (J.T. Baker, Philipsburg, NJ) in saline and were infused at 3, 6, and 12 mg/min and 1 mg/kg × 1 h using an ambulatory withdrawal pump (Dakmed, Buffalo, NY). During infusion, the horses were monitored closely for signs of toxicity, including restlessness, anxiety, flared nostrils, rapid respiration, sweating, and increased heart rate. Heart rates (HRs) were recorded at 1-min intervals by an onboard heart-rate computer (Polar CIC, Port Washington, NY). An elastic strap with an attached receiver and transmitter was placed around the chest of the horse. The transmitter was connected to two electrodes placed on shaved areas of the sternum and left side of the anterior chest. Electrode gel was used to ensure proper conduction of the HR signal. For the 3, 6, and 12 mg/min infusions, blood samples were taken before and at various times during and after infusion for complete blood counts, chemistry panels, and blood lactates. For the 1 mg/kg/min × 1 h infusion, blood samples were obtained for analyses before infusion (0 h), during infusion (0.25, 0.5, and 1 h), and after infusion (0.08, 0.17, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, and 144 h) into Vacutainer serum tubes (Becton Dickinson, Rutherford, NJ) and Vacutainer plasma tubes (Becton Dickinson, Franklin Lakes, NJ) and analyzed immediately.

Mandelonitrile Administration

In a second series of experiments, four mature thoroughbred mares were administered oral mandelonitrile (3 mg/kg). Blood samples for cyanide analysis were obtained before (0 h) and after dosing at 0.05, 0.08, 0.17, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h into Vacutainer serum tubes and Vacutainer plasma tubes and analyzed immediately.

Safety Precautions

Two antidotes were prepared to counter any adverse effects of the cyanide infusion. A 3% solution of sodium nitrite was prepared by adding 1.8 g of sodium nitrite (J.T. Baker, Philipsburg, NJ) 08865 to 60 mL of saline. This mixture was to be administered intravenously at a rate of 10–20 mL/min. A 25% solution of sodium thiosulfate (J.T. Baker) was prepared by adding 100 g sodium thiosulfate to 400 mL of saline. This mixture was to be administered immediately after the sodium nitrate at a rate of 200 mL/min.

Analytical Detection of Cyanide

As detailed elsewhere (Hughes et al. 2003), an inexpensive, disposable alternative to the costly Warburg distillation flask has been developed, and it allowed the simultaneous running of 100 cyanide analyses. Briefly, a 10-mL plastic cup was suspended by means of Scotch tape inside a 120-mL plastic cup with a screw lid. Then 10 mL of 1 M sulfuric acid was pipetted into the larger cup. Exactly 2.5 mL of 0.25 N sodium hydroxide was pipetted into the smaller cup. The cyanide-containing sample (1–2 mL blood) was pipetted into the sulfuric acid and the cup was immediately sealed with its lid and allowed to sit overnight at room temperature while cyanide in the form of hydrogen cyanide gas was evolved from the acid solution and trapped in the sodium hydroxide. The small cup was then removed and the sodium hydroxide solution was decanted into an autoanalyzer sample cup. In the presence of chloramine-T, the cyanide ion was converted to cyanogen chloride, which reacted with pyridine-barbituric acid to form a red-blue color, the intensity of which was measured spectrophotometrically at 578 nm. Use of an autoanalyzer ensured a precise and reproducible

interval during which color developed and thus improved the detection limit to as low as 2 ng/mL in a 1-mL sample. Standard curves were linear in the range of 2 to 300 ng/mL, with a regression coefficient $r^2 > .99$.

Red Blood Cell/Serum Partitioning

Red blood cell (RBC)/serum partitioning of cyanide was determined by collecting blood and serum samples during infusion of NaCN (1 mg/kg × 1 h). The concentration in the RBCs was calculated as $C_{RBC} = [(C_{blood}-C_{serum}) \times (1\text{-hemotocrit})]/$ hematocrit, where C_{blood} and C_{serum} are the concentrations of cyanide in blood and serum, respectively. The mean serum and blood concentrations of cyanide were used to determine the RBC/serum partitioning of cyanide.

Toxicokinetic Analysis

The toxicokinetic parameters of cyanide were determined by compartmental analysis. Equations of the two-compartment model with a zero-order input rate were fitted to the individual blood concentrations versus time by least-squares nonlinear regression analysis using a nonlinear regression program (Winnonlin, version 3.1; Pharsight, Cary, NC). The quality of the fit was evaluated by the Akaike Information Criterion (AIC), residual plots, and visual inspection. The data were weighted as $1/(y_{pred})^2$, where y_{pred} was the model-predicted concentration at the actual time. The area under the curve (AUC) after intravenous administration was measured by using a linear trapezoidal approximation with extrapolation to infinity, and the slope of the terminal portion (β) of the log plasma drug concentrations versus the time curve was determined by the method of least-squares regression (Gibaldi and Perrier 1982). The rate constant of distribution (α) and of distribution half-life ($t_{1/2\alpha}$) were determined using the method of residuals (Gibaldi and Perrier 1982).

Total body clearance (Cl_s) was calculated by use of Equation 1 (Benet and Zia-Amirhosseini 2002):

$$Cl_s = \text{IV Dose}/AUC_{0\text{-inf}}. \quad [1]$$

The volume of distribution in the central compartment (Vd_c) and the volume of distribution at the steady state (Vd_{ss}) were calculated according to Equations 2 and 3, respectively (Yamaoka et al. 1978):

$$Vd_c = \text{Dose (IV)}/(A + B) \quad [2]$$

$$Vd_{ss} = \text{IV Dose}/AUC_{0\text{-inf}} \times MRT. \quad [3]$$

A and B are the Y intercepts associated with the distribution and elimination phase, respectively, and AUMC is the area under the first moment curve and is calculated by the trapezoidal method and extrapolated to infinity (Gibaldi and Perrier 1982).

The mean residence time (MRT) (Martinez 1998) was determined according to Equation 4:

$$MRT = AUMC_{0\text{-inf}}/AUC_{0\text{-inf}} - (\text{Infusion time}/2). \quad [4]$$

The pharmacokinetic variables (the elimination half-life and the area under the curve) of the cyanide after oral administration of mandelonitrile were determined using a noncompartmental approach (Gibaldi and Perrier 1982). The maximum blood concentration of the cyanide (C_{max}) and the time to reach this concentration (T_{max}) were obtained directly from the blood-concentration-versus-time curves. The absolute bioavailability (F) was calculated from the $AUC_{0\text{-inf}}$ ratio obtained following oral and iv administration according to Equation 5 (Benet and Zia-Amirhosseini 2002):

$$F = AUC_{0\text{-inf}}\,(\text{Oral})/AUC_{0\text{-inf}}\,\text{iv} \times \text{iv Dose/Oral Dose}. \quad [5]$$

Total oral clearance (Cl_o) was calculated by using Equation 6:

$$Cl_o = \text{Dose (Oral)}/AUC_{0\text{-inf}}. \quad [6]$$

RESULTS AND DISCUSSION

Identification of Safe and Humane Blood Concentration Limits for Cyanide

Cyanide is a potent and rapidly acting toxin that is capable of causing convulsions and death within minutes of administration (Borowitz et al. 2001). To determine the threshold for acute cyanide toxicity in adult horses, a series of infusion experiments were performed. An infusion rate of 1 mg/min for 1 h was the starting point, based on data concerning cyanide toxicity in mice. No apparent effects were observed at infusions of 1, 2, 3, and 6 mg/min for 1 h; however, a rate of 12 mg/min produced acute clinical signs of toxicity after 38 min (Fig. 2). Clinical signs of acute toxicity included apparent panic, flaring of the nostrils, and a sharp increase in HR from about 50 to 150 beats/min. Other signs included apparent weakness, incoordination, behavioral distress, and a sharply increased respiratory rate. Signs of distress ceased promptly when the cyanide infusion was terminated. No clinically significant changes were seen in any chemistry parameters during these experiments (Table 1), although there were small changes in blood lactate associated with clinical toxicity during the 12 mg/min infusion.

These initial experiments to establish parameters within which cyanide infusion could humanely and safely be administered were performed during the summer and early fall of 2001. At that time, serum cyanide concentrations were analyzed using a mass spectral method adapted from the literature (Kage et al. 1996; Meiser et al. 2000). It was soon concluded that this method had significant limitations, so it was abandoned as unsatisfactory. Figure 3 shows a later repetition of the 12 mg/min infusion experiment, in which clinical signs of toxicity were not seen until 70 min after of infusion had begun. Using the analytical method of determining cyanide levels in blood that was developed specifically for this project (Hughes et al. 2002), the peak cyanide level was quantified at 2500 ng/mL in blood and 36 ng/ml in serum. These data suggest that the major fraction of blood cyanide is associated with the red cell fraction. Based on this information, the working hypothesis has been that adult

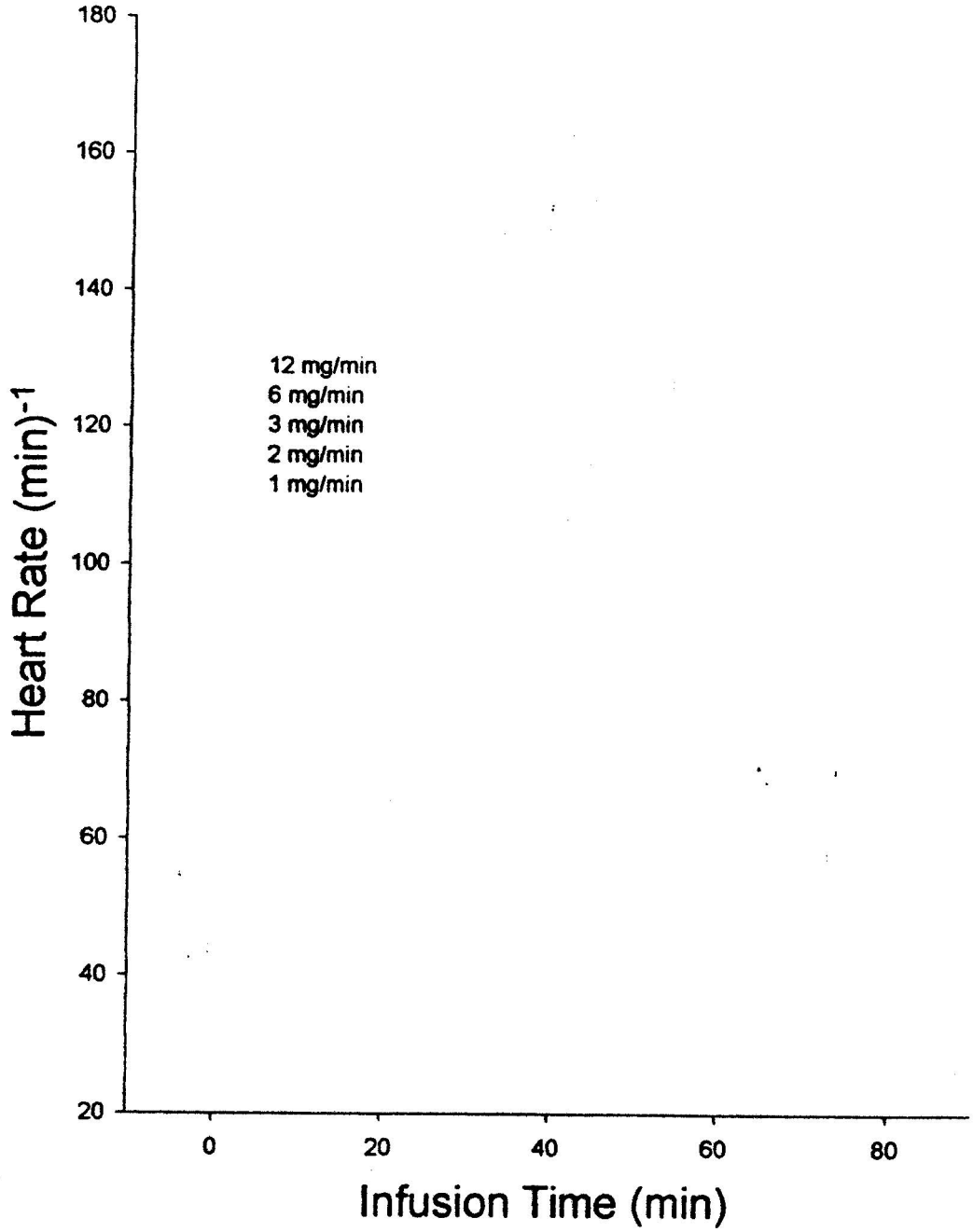

FIG. 2. Heart rates during 60-min infusion of various concentrations of NaCN. The 12 mg/min infusion was stopped at 38 min due to clinical signs of toxicity.

horses with blood cyanide concentrations above 2500 ng/mL are at risk for developing clinical signs of acute toxicity, and all precautions were taken to keep the blood cyanide concentrations below this threshold in the horses that were involved in this experiment.

A salient clinical characteristic of MRLS was the lack of apparent clinical toxicity in the affected mares. The finding that mares can maintain substantially increased blood concentrations of cyanide without clinical or biochemical signs of acute toxicity is consistent with the apparent absences of toxic signs in affected mares during the MRLS outbreak.

The Distribution and Elimination Toxicokinetics of Cyanide in the Horse

The next objective was to determine the toxicokinetics of subacutely toxic concentrations of blood cyanide in horses. The classic first experiment in a pharmacokinetic study is bolus iv administration of a suitable dose of the agent, allowing for the characterization of its distribution and elimination kinetics. Humane and safety considerations suggested a modification of this standard protocol. Therefore, four horses were infused with sodium cyanide at a rate of 1 mg/kg/h. Preliminary calculations suggested that this infusion rate would yield a peak blood cyanide

TABLE 1
Infusion of horses with sodium cyanide

Infusion rate	Sampling time (min)	Blood lactate level (mg/dL)
3 mg/min	0	6
	30	7
	60	7
6 mg/min	0	6
	60	6
	300	5
6 mg/min	0	6
	155	7
	360	5
6 mg/min	0	5
	240	7
12 mg/min	0	4
(toxic within 38 min)	20	5
	45	9
	50	8
	300	4

concentration of about 1000 ng/mL, safely below the concentrations at which acute signs of toxicity had been observed. As shown in Figure 4, blood cyanide concentrations rose in an approximately linear fashion, peaked at concentrations of about 1200 ng/mL, and declined rapidly thereafter. No clinical signs of toxicity or distress were seen in any of these animals.

Pharmacokinetic analysis of the terminal portion of the blood cyanide-concentration-versus-time curves indicated a two-compartment, open-body model following iv infusion of cyanide. The amount of cyanide administered was adjusted based on the percentage of the cyanide content of NaCN, which is 53%. Observed C_{max} of cyanide at 1 h of infusion ranged from the low of 951 ng/mL to the peak of 1313 ng/mL, with the mean C_{max} being 1160.3 ng/mL ± 88.3 (SEM) (see Fig. 4). The blood concentrations of cyanide decreased very rapidly post-iv infusion, with the mean $t_{1/2\alpha}$ 0.74 h ± 0.03 (SEM). The blood concentrations of cyanide ranged from the low of 16 ng/mL to the peak of 26 ng/mL at 24 h postinfusion, with the mean blood concentration of cyanide being 23-ng/mL ± 2.3 (SEM) (see Fig. 4). The pharmacokinetic parameters of cyanide following iv infusion of NaCN are shown in Table 2. The Vd_c and Vd_{ss} ranged from the low of 0.296 L/kg to the peak of 0.473 L/kg and from the low of 1.46 L/kg to the peak of 3.23 L/kg, respectively, with the mean Vd_c and Vd_{ss} being 0.389 L/kg ± 0.041 (SEM) and 2.21 L/kg ± 0.372 (SEM), respectively (see Table 2). The Cl_s from these four horses ranged from the low of 0.154 L/h/kg to the peak of 0.246 L/h/kg, with the mean Cl_s of 0.182 L/h/kg ± 0.02 (SEM). Table 2 sets forth in summary form the distribution and elimination parameters following iv infusion and abrupt cessation of the infusion, as set forth above.

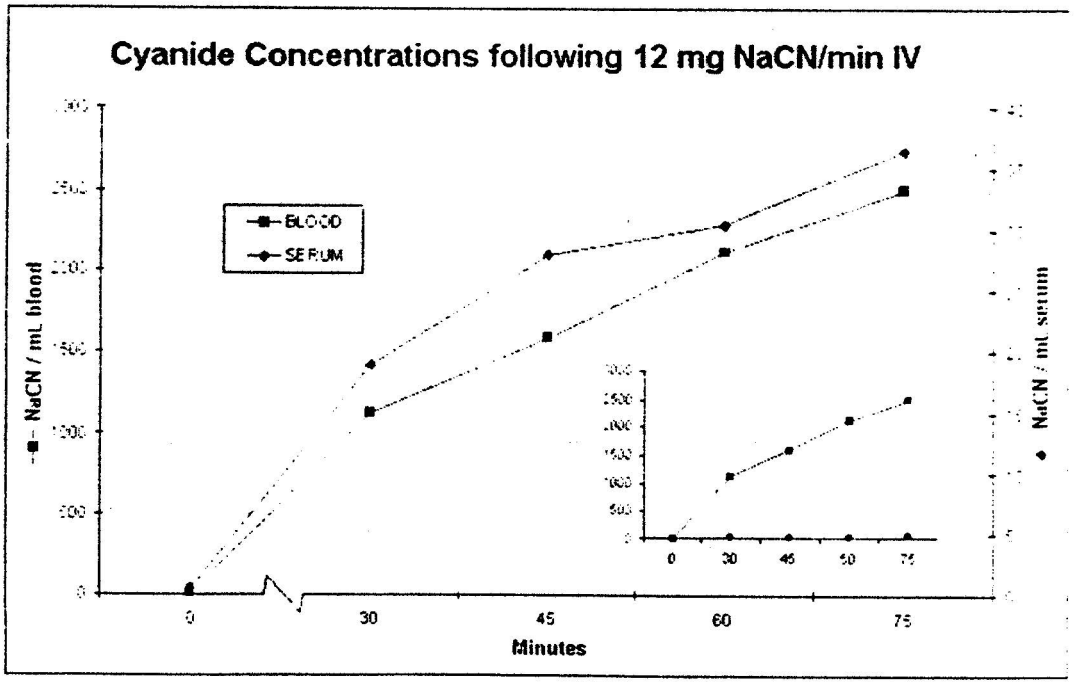

FIG. 3. Comparison of cyanide concentrations in serum and blood. Note the dual concentration range changes in the vertical axis. The insert shows the relative concentrations to scale.

TABLE 2

The pharmacokinetic parameters of cyanide in blood following constant iv infusion of NaCN at 1 mg/kg/h for 1 h ($n = 4$)

Horse	1	2	3	4	Mean ± SEM
Weight (kg)	580	612	590	578	590 ± 7.79
$\alpha(h^{-1})$	0.8633	1.0388	0.8983	0.9741	0.9436 ± 0.0.039
$\beta(h^{-1})$	0.05513	0.05996	0.03124	0.03788	0.0461 ± 0.00684
$t_{1/2}K_{10}$ (h)	1.563	1.333	1.748	1.329	1.49 ± 0.101
$t_{1/2}\alpha$ (h)	0.803	0.667	0.772	0.712	0.739 ± 0.0304
$t_{1/2}\beta$ (h)	12.57	11.56	22.19	18.30	16.16 ± 2.5
$AUC_{0\text{-inf.}}$ (ng/mL/h)	3448.73	2154.34	3036.47	3431.50	3017.76 ± 303.15
Cl_s (L/h/kg)	0.154	0.246	0.175	0.154	0.182 ± 0.022
Vd_c(L/kg)	0.347	0.473	0.440	0.296	0.389 ± 0.041
Vd_{ss} (L/kg)	1.457	2.163	3.228	1.977	2.206 ± 0.372
R^2	0.978	0.980	0.983	0.992	0.983 ± 0.003

Note: The amount of cyanide administered was adjusted based on the cyanide content of NaCN, which is 53%

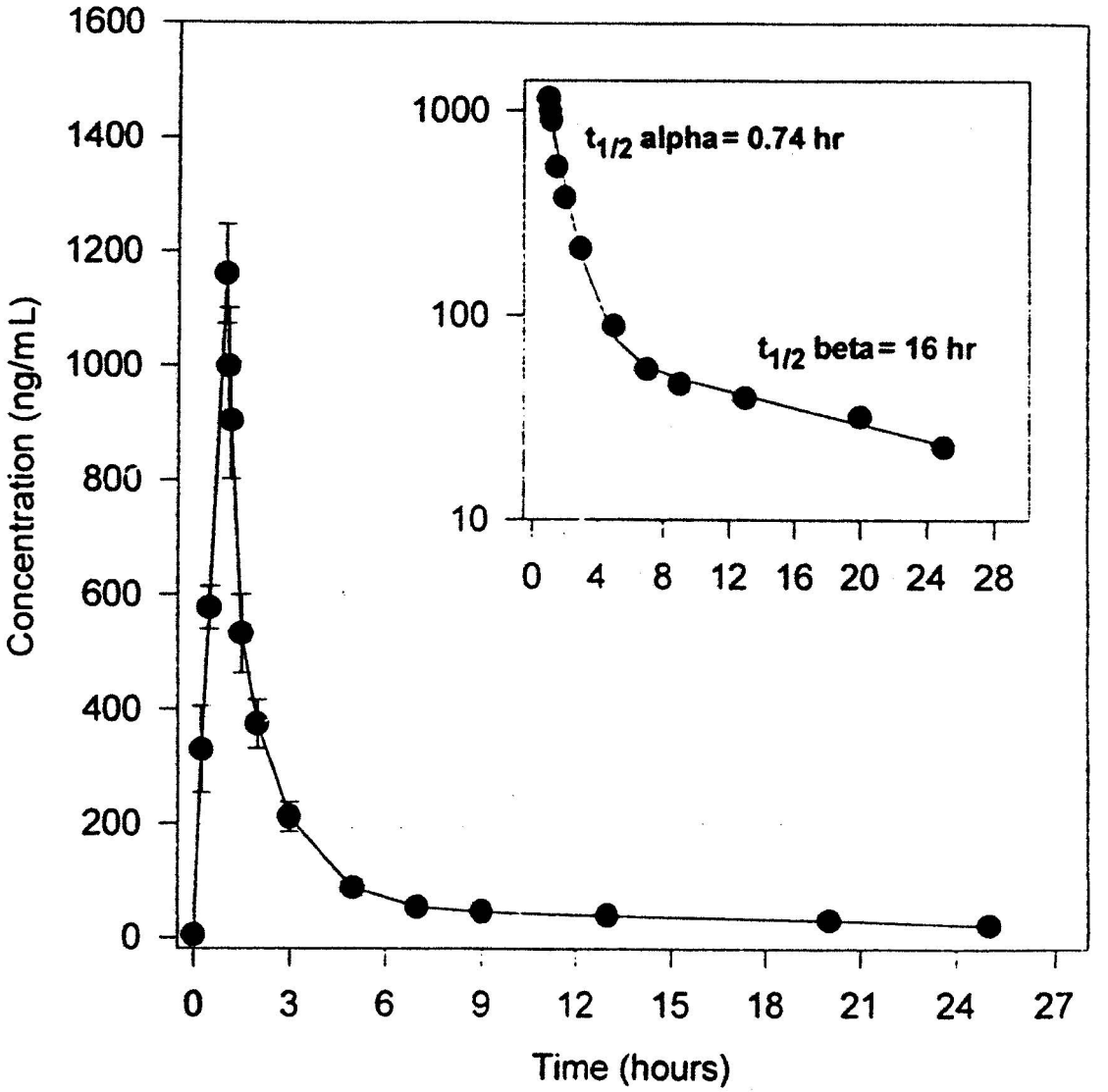

FIG. 4. The mean blood concentrations of cyanide (± SEM) following iv infusion of NaCN at 1 mg/kg/h for 1 h ($n = 4$). The insert shows logarithmic plot of concentration versus time for the postadministration portion of the data.

TABLE 3
The partitioning of cyanide between blood and serum

Time (h)	Mean blood concentrations (ng/mL) ($n = 4$)	Mean serum concentrations (ng/mL) ($n = 4$)	Blood conc./serum conc.
0.25	330	10.4	32
0.5	577	16	36
1	1160	27	43
1.08	999	24	42
1.17	904	21	43
1.5	532	12	44
2	375	6.9	54

Note: The data shown here are from the first 2 h of the experiment described by Figure 4.

Ex Vivo Red Blood Cell/Serum Partitioning of Cyanide

It has long been known that cyanide concentrations in whole blood are considerably higher than those in serum (Delaney 2001). Table 3 shows the relationship between blood and serum concentrations of cyanide during the first 2 h of the experiment that is presented in Figure 4. Analysis of these data shows that about 98.5% of the total cyanide is associated with the RBC fraction, and a very small proportion, presumably not greater than 1.5%, is associated with the serum fraction.

Analysis of these data also shows that the ratio of serum to blood cyanide remained relatively constant throughout the range of blood concentrations measured during the experiment shown in Figure 4, with no apparent suggestion of saturation of the process. Preliminary calculations also suggest that, at these concentrations, about 10–15% of the total body cyanide in the animal is held in the blood.

Because the limit of sensitivity of the analytical method was about 2 ng/mL, and "normal" background concentrations of blood cyanide in horses can be less than 10 ng/mL, blood cyanide was selected as the analytical sample and parameter of choice, and serum concentrations of cyanide were not routinely estimated because if the normal blood cyanide level in the horse is about 10 ng/mL and all but 1.5% of it is held in the blood, then the concentration of serum cyanide in the horses was not above 25 Pg/mL, a very low concentration indeed. Conversely, this means that the effective volume of distribution of cyanide in the horse is very large, leading to further complications in estimating the toxicokinetic significance of exposure to low concentrations of cyanide or cyanogens.

On the other hand, it must be noted that the previous analytical method used was essentially restricted to blood serum because of interferences by whole blood and other tissues with the derivatization reaction (Hughes et al. 2002). In our hands, the blood serum data obtained by this method were unrealistically high, poorly reproducible, and made little or no analytical or toxicological sense.

The Toxicokinetics of Cyanide from Mandelonitrile in the Horse

Mandelonitrile is a labile cyanogen and is the proximal cyanide donor in the black cherry tree cyanogenic system. Based on data from preliminary ranging experiments (Hughes et al. 2002), mandelonitrile was administered orally at a dose of 3 mg/kg to four horses. Examination of the blood-cyanide-concentrations-versus-time curves indicated the very rapid absorption characteristics of cyanide: the highest blood concentrations of 1857 ng/mL were observed within 3 min after oral application of the mandelonitrile; the actual peak concentrations presumably occurred earlier. The peak blood concentrations of cyanide ranged from the low of 1443 ng/mL to the peak of 2498 ng/mL, with the mean blood concentrations of cyanide being 1857 ng/mL ± 226 (SEM) at 3 min after oral administration of mandelonitrile (Fig. 5). These data also strongly suggest that mandelonitrile is rapidly converted to cyanide in the gastrointestinal track of the horse.

Thereafter, blood concentrations of cyanide declined rapidly to ~100 ng/mL by 4 h after mandelonitrile administration, and by 24 h postadministration, the mean blood concentration of cyanide was 19 ng/ml ± 4.2 (SEM) (see Fig. 5). The pharmacokinetic parameters of cyanide following oral administration of mandelonitrile are presented in Table 4. The terminal elimination half-life of cyanide ranged from 6 to 19 h with a mean terminal elimination half-life of 13 h ± 3 (SEM) (see Table 4). The amount of cyanide administered was adjusted based on the percentage of cyanide content of mandelonitrile, which is 19.53%. For calculation of oral bioavailability of cyanide as mandelonitrile, each horse's pharmacokinetic parameters following oral mandelonitrile administration were compared with the mean pharmacokinetic parameters of cyanide when it was infused as NaCN. The oral bioavailability (F) of cyanide following oral administrations of mandelonitrile ranged from 48 to 76%, with the mean oral bioavailability of mandelonitrile being 57% ± 6.5 (SEM) (see Table 4). As previously, no clinical signs of cyanide intoxication or distress of any kind were observed in any of these sequences of mandelonitrile administration experiments.

The High-Concentration Toxicokinetics and Toxicology of Cyanide in the Horse

These toxicokinetic data suggest that when administered acutely at a sufficient dose, cyanide is a classic acute-bolus/blood-flow-distribution toxin. Its acute high-concentration toxic effects are dominated by rapid delivery of blood concentrations greater than 2.5 μg/mL (ppm) to the site of action, presumably the central nervous system (CNS) or a component thereof. Under these circumstances, rapid redistribution of cyanide is likely to be the principal factor in the termination of the acute toxic responses. Similarly, mandelonitrile rapidly delivers its cyanide content, and acute mandelonitrile toxicity, which involves similar blood concentrations of cyanide, is likely also to be terminated by the redistribution of the cyanide.

TABLE 4
Pharmacokinetic parameters of cyanide in blood following a single oral administration of mandelonitrile at 3 mg/kg ($n = 4$)

Horse	1	2	3	4	Mean ± SEM
Weight (kg)	578	590	612	605	596.25 ± 7.62
F (%)	48	53	50	76	56.8 ± 6.5
$t_{1/2}$Lambda_z (h)	19	17	6	10	13 ± 3
$AUC_{0-inf.}$ (ng/mL/h)	1579	1782	1731	2580	1918 ± 225
Cl_0 (L/h/kg)	0.371	0.329	0.339	0.227	0.317 ± 0.031
C_{max} (ng/mL)	2498	1694	1794	1443	1857 ± 226

Note: The amount of cyanide administered was adjusted based on the of cyanide content of mandelonitrile, which is 19.53%. To calculate the oral bioavailability of cyanide as mandelonitrile, each horse's pharmacokinetic parameters following oral mandelonitrile administrations were compared with the mean pharmacokinetic parameters of cyanide when it was infused as NaCN.

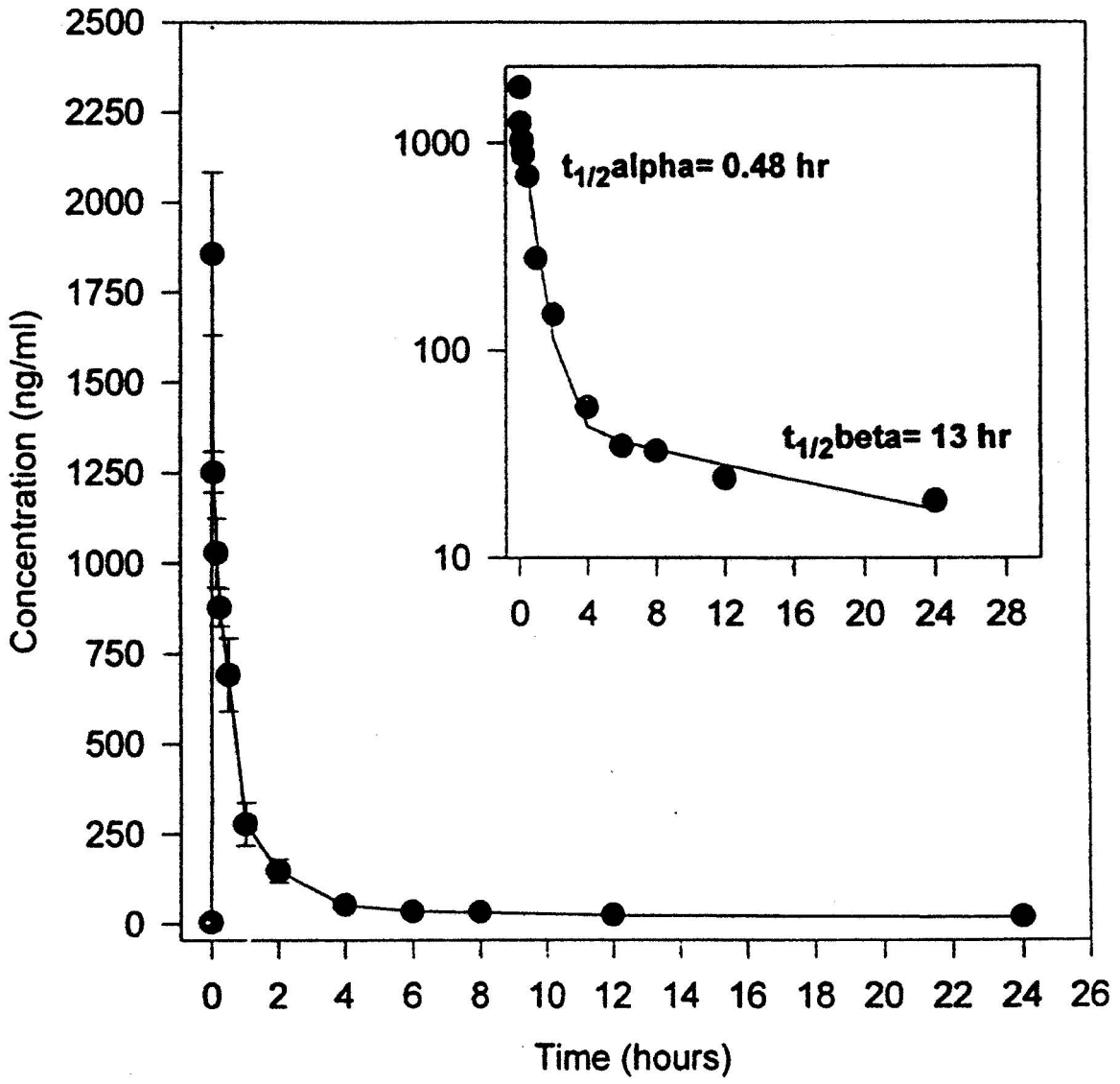

FIG. 5. The mean blood concentrations of cyanide following a single oral administration of mandelonitrile at 3 mg/kg ($n = 4$). The insert shows a logarithmic plot of concentration versus time for the postabsorption portion of the data.

However, metabolic elimination of cyanide becomes dominant at much lower blood concentrations (<100 ng/mL, ppb) suggesting that different mechanisms of toxicity and termination of action are associated with subacute and chronic exposure to cyanide or cyanogens.

These toxicokinetic observations and interpretations correlate well with a number of clinical and clinical-chemistry observations made during the course of these experiments. In the first place, during the infusion of the higher concentrations of NaCN, the infusion catheter periodically needed to be cleared to prevent clotting and kinking. To accomplish this, the infusion tubing was aseptically disconnected from the catheter, leaving approximately 0.1 mL solution in the catheter. This volume of solution contained approximately 11 mg NaCN (at an infusion rate of 6 mL/min), and it was rapidly siphoned into the bloodstream after disconnection of the infusion tubing. About 15 sec after this maneuver, and on more than one occasion, the horse would appear anxious and start to breathe rapidly, and the HR would become elevated to 70–90 bpm, which are classic signs of acute cyanide toxicosis. After about 20 sec, the HR returned to normal (~40 bpm), the anxiety subsided, and the respiratory rate and pattern returned to normal.

At first we were uncertain about how to interpret these very significant clinical signs, since they could have represented the first signs of a full-blown clinical toxicity event or the impending death of the animal. In retrospect, it is clear that these clinical signs simply represented a modest bolus of cyanide passing through the CNS or a component thereof, and that the response to this mini-bolus dose could be largely independent of the background blood level of cyanide.

Historically, small bolus administrations of cyanide were at one time used as a technique of determining circulation times. In this procedure a small amount of cyanide was administered intravenously, and the time in seconds to a respiratory "gasp" was noted. This gasp, of course, represented a minitoxic event and was rapidly followed by termination of the toxic action by redistribution. This testing procedure was, in its time, presumably nonheroic and "generally regarded as safe." The apparent safety of this historical procedure is readily understandable in view of the above-described rapid and highly effective redistribution-dependent elimination and termination of the toxicity of small acute doses of cyanide.

During these early infusion experiments, blood lactate was monitored on the assumption that cyanide was functioning as a general systemic cellular poison and that blood lactate levels would rise prior to or in parallel with the onset of toxicity. In fact, there was little evidence of substantial changes in blood lactate concentrations in the infusion experiments (see Table 1). In retrospect, the bolus effect model suggests that any biochemical changes occurring after bolus administration of cyanide would be largely restricted to the CNS and, initially at least, would be rapidly diluted in the total body pool of relatively normal lactate concentrations. This finding suggests that the greatly increased blood lactate concentrations routinely seen in acute cyanide intoxications actually represent a later stage of cellular damage and death, most likely following the initial acute stages of cyanide intoxication associated with CNS involvement. These findings are also consistent with the general clinical observation that HR is the last vital sign to be lost in acute cyanide intoxication, and that if an episode of acute cyanide toxicity is survived for as long as 1 h, then survival of the episode is very likely.

These toxicokinetic findings also suggest that there need not be a clear-cut correlation between systemic blood concentrations of cyanide and toxic events, including death. If the rate of delivery and concentration of cyanide in the CNS is an important determining factor in the onset of brain death, then the relationship between the death of the organism and its blood levels of cyanide need not be rigorously linked. This is because it will be the concentration of cyanide in the CNS, but not in the general circulation, that will be most closely linked to the toxic event. For example, inhalation of cyanide in a fire, which rapidly produces brain death, might cause death at a much lower blood cyanide concentration than, for instance, a slowly developing toxicity following ingestion and intestinal absorption of cyanide from a cyanogenic glycoside (Baud et al. 1991).

These toxicokinetic characteristics of cyanide administered acutely at high doses have not been described previously in the literature on cyanide toxicity. However, kinetically similar acute and transit responses to bolus administration of a number of high-potency narcotic analgesics have been characterized and described in the horse by our research group. It was, therefore, of great interest to us that the toxicokinetics of high doses of cyanide showed many parallels with previous pharmacokinetic and pharmacodynamic work on certain high-potency drugs used in racing horses (Combie et al. 1979).

The second clear-cut message of this work is that mandelonitrile is an extremely efficient and rapid delivery agent for cyanide. The speed with which peak blood concentrations of cyanide were attained after oral administration of mandelonitrile can only be described as exceptional. In the first ranging experiment involving mandelonitrile (Hughes et al. 2002), in which where the highest dose administered was 3 g, the horse showed classic acute, transient signs of cyanide toxicity. These signs appeared within 3 min of oral administration and then rapidly dissipated. Additionally, the transient nature of the toxic signs after mandelonitrile administration also supports the hypothesis that redistribution is the primary mechanism of termination of cyanide toxicity after an acute or bolus dose of either cyanide or mandelonitrile.

The speed and efficiency of delivery of cyanide by mandelonitrile makes it a very suitable cyanogen donor for a plant-protective mechanism such as is present in black cherry trees. Presumably, the cyanide held in the leaf as prunasin is relatively stable and unavailable. However, once hydrolysis of prunasin yields free mandelonitrile, the cyanide would seem to be extremely bioavailable (Combie et al. 1979; Vetter 2000). It is tempting to speculate that once formed in the leaf, the mandelonitrile may be held in a compartmentalized manner so as

to preserve its full cyanide potential until it is ingested by the target organism. Once ingested, which presumably ruptures the storage compartment, the mandelonitrile is released and, with its very rapid cyanide-release characteristics, rapidly delivers an acutely toxic ("rapid knock-down") dose of cyanide to the target organism.

These findings make it clear that the most economical and effective use of cyanide as a mammalian toxin is by means of rapid-bolus administration, after which cyanide is rapidly absorbed and distributed in high concentrations to its site of action. The most likely site of action is the CNS, or a part thereof, with its high oxygen demand and abundant metabolic activity. Exposure to high concentrations of cyanide for a sufficient period of time causes brain death, followed inevitably by death of the organism. This hypothesis is also consistent with observations that cardiac activity is the last vital sign to be lost during acute cyanide intoxication (Borowitz 2001).

Relevance of the High-Concentration Toxicokinetics and Toxicology of Cyanide to MRLS

As described in this article the high-concentration toxicokinetics of cyanide, which are generally applicable in acute forensic situations, are not likely to be directly involved in the pathogenesis of MRLS. For acute cyanide toxicity to develop, the concentrations of cyanide must be relatively high—in the range of low ppm—and bolus administration achieves these concentrations locally in the CNS by virtue of the high local blood flows. On the other hand, the transfer of cyanide to the fetus from the maternal circulation is likely to be a slower process, and local high concentrations are unlikely to be attained in the fetus in association with local acute high blood concentrations in the circulatory system of the mother.

Furthermore, it must be kept in mind that the fetus exists in a relatively low-oxygen environment and, as such, the fetus is likely to be significantly more sensitive to hypoxic insult than the mare. Equine fetuses are known to be particularly sensitive to red maple poisoning, in which hemolysis and loss of oxygen-carrying capacity in the mare is associated with an increased rate of fetal loss (Witonsky 2001). Similarly, the fetuses in pregnant sows have been shown to be highly sensitive to low-level exposure to carbon monoxide, concentrations that cause no apparent clinical signs in the sows (Dominick and Carson 1983). These clinical circumstances support the hypothesis that the fetus in general, including that of the foal, is likely to be significantly more sensitive than the mare to low concentrations of cyanide. Consistent with this hypothesis, ongoing or steady-state administration of cyanide to Syrian golden hamsters has resulted in a fetal reabsorption rate as high as 83%, which is reminiscent of the pattern observed in MRLS (Doherty et al. 1982).

In keeping with these findings, work with sheep has shown that infusion of nitroprusside, a significant source of cyanide, has caused fetal death in sheep concomitant with a significantly lower incidence of maternal toxicity (Naulty et al. 1981). Additionally, micropathological analysis suggests that close to full-term fetal losses had characteristic respiratory micropathological changes, including lungs that were darker and firmer than normal. Consistent with this finding, a majority of the LFLs had amniotic fluid in their lungs, suggesting that these foals may have been gasping or struggling to breathe in utero, consistent with exposure to a hypoxic insult (Powell 2001; Riddle 2001). Therefore, there are good scientific precedents for suggesting that the fetus may be particularly sensitive to interference with its oxygen supply and utilization, and there are also precedents that indicate that exposure to cyanide results in fetal reabsorption. Cases of LFL involved mares with symptoms that included agalactia, dystocia, and stillbirth. The placentas of the fetuses were edematous, having an excess of serous fluid in connective tissue or serous cavities. In cases of EFL, ultrasound revealed abnormal conditions of the fluid surrounding the fetuses (Powell 2001; Riddle 2001).

The Low-Concentration Toxicokinetics and Toxicology of Cyanide in the Horse

In contrast with the generally transient nature of the effects of the high-concentration bolus of cyanide, cyanide at lower concentrations is handled entirely differently. In the first place, the volume of distribution of cyanide is large, corresponding kinetically at a steady state to 2.21 L/kg. Additionally, this volume of distribution is estimated based on total blood cyanide. If the volume of distribution were to be estimated based on the actual concentration in serum of cyanide, which is low (on the order of parts per trillion), the effective volume of distribution becomes very large indeed. This large effective volume of distribution is consistent with the prolonged plasma half-life of cyanide, on the order of 12 to 16 h.

The serum cyanide level is held at such extremely low concentrations by virtue of the ability of the RBCs to bind or concentrate cyanide. This property of the RBC is unlikely to be accidental, and the ability of the RBC and hemoglobin to serve as a cyanide "sink" is presumed to be biologically important to the organism.

Relevance of the Low-Concentration Toxicokinetics and Toxicology of Cyanide to MRLS

There are a number of possible mechanisms by which increased exposure to cyanide could influence the viability of the equine fetus. Working in the field of equine grass sickness, McGorum and Kirk (2001) showed that horses in high, white-clover pastures associated with grass sickness had significantly higher blood concentrations of cyanide and substantially lower blood concentrations of sulfur-containing amino acids. This apparent "drawing down" of the sulfur-containing amino acid pool could have two possible effects. In the first place, by reducing the concentration of sulfane sulfur available to metabolize cyanide into thiocyanate, it could reduce the rate of detoxification of cyanide. This would have the effect of increasing the terminal-elimination half-life of cyanide (dose-dependent kinetics) and result in increased blood concentrations of cyanide, thereby

interfering with the animal's defenses against cyanide toxicity. Second, by drawing down the sulfur-containing amino acid pool, exposure to cyanide could potentially weaken the growing fetus by depriving it of access to important sulfur-containing amino acids. Either of these mechanisms could interfere with the viability of the fetus, ultimately resulting in death and reabsorption of the fetus.

A second and relatively less widely explored aspect of cyanide toxicity is the toxicity associated with thiocyanate. Although much less toxic than cyanide, thiocyanate itself carries with it a significant potential for toxicity, particularly with reference to its effects on thyroid function.

Another mechanism by which lower concentrations of cyanide could contribute to the cause of MRLS involves bacterial proliferation. Macrophages kill phagocytosed bacteria by utilizing oxidative bursts; higher blood cyanide concentrations may have the ability to interfere with the generation of oxidative bursts by macrophages and thus with the ability of macrophages to control bacterial proliferation (Suhonen et al. 2000). Beyond this, simply by selecting against the proliferation of aerobic bacteria, the presence of low concentrations of cyanide in the gastrointestinal tract or elsewhere may favor the proliferation of microaerophilic bacteria, two of which, *Actinobacillus* species and alpha *Streptococcus* species, have been uniquely and consistently identified with MRLS.

CONCLUSIONS

The toxicokinetics of cyanide have been characterized in the horse with a view to experimentally defining the possible role of cyanide in MRLS. The toxicokinetics of cyanide in the horse seem to divide into two very distinct phases—the toxicokinetics associated with acutely administered high doses of this agent and the toxicokinetics associated with lower, chronic exposures to this agent.

At higher doses of cyanide, consistent with those found in most human forensic situations, cyanide appears to be rapidly distributed to the CNS, where it produces its primary toxic effects. In horses, and apparently in other species, the toxic actions of cyanide administered as a high-bolus dose can be very rapidly terminated by redistribution.

At lower, or subacute, exposures to cyanide, the metabolic phase of detoxification appears to be dominant. Under these circumstances, the toxicokinetics of cyanide are likely to be dominated by its large volume of distribution and long plasma half-life, on the order of 12 to 16 h. Under these circumstances, it is clear that the steady-state blood concentrations will take relatively longer (up to 60 h) to develop; therefore, changes in blood cyanide concentrations are likely to be relatively gradual. Questions that remain to be answered include the following: What is the extent to which increased exposure to environmental cyanide and moderately increased blood concentrations of cyanide can influence factors like blood concentrations of sulfur-containing amino acids? What is the availability of sulfane sulfur to fuel the cyanide-to-thiocyanate detoxification reaction? And what are the effects of high concentrations of cyanide in the blood on oxidative bursts and other equine cellular functions and on intestinal and other bacterial populations in the horse?

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