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Summary

Predators are capable of causing damage to domestic livestock throughout North America. Lethal responses for managing livestock depredations may include the use of sodium cyanide in M-44 devices. Currently, several states have banned the use of M-44s and several other states are forecast to ban these devices. Therefore, additional tools are being sought to expand the repertoire of options available for managing coyote depredations on domestic livestock. We evaluated the use of a theobromine:caf-

feine mixture delivered within a Coyote Lure Operative Device (CLOD) as an additional predacide for coyotes (*Canis latrans*). Results from six trials involving 38 captive coyotes were ambiguous. Issues related to the attractiveness of the CLOD, palatability of the compound, and absorption of the theobromine:caffeine mixture produced mortality levels below the desired >90-percent-mortality rate deemed adequate for laboratory efficacy study to the United States Environmental Protection Agency (EPA) registration and operational use. While many coyotes died from consumption of the

theobromine:caffeine mixture, several coyotes recovered with symptoms of poisoning disappearing within 12 hours in those animals that survived exposure to the toxicant. Several issues related to palatability of the mixture and compound delivery, as well as coyote behavior, sensory abilities, and physiology, indicated the use of a theobromine:caffeine mixture in a CLOD may not be an effective method for managing coyote depredations on domestic livestock.

Key Words: Caffeine, *Canis latrans*, CLOD, Coyote, Mortality, Theobromine

Introduction

Predators cause more than \$16 million in damage to sheep producers every year (United States Department of Agriculture 2000). The predator with the largest impact, by far, is the coyote (*Canis latrans*). The United States Department of Agriculture's Wildlife Services (WS) responds to requests to address livestock losses attributed to predation and removes approximately 80,000 coyotes per year to reduce losses of domestic livestock (United States Department of Agriculture 2011). Toxicants are part of an integrated pest management program that may involve both lethal and non-lethal methods to reduce predation on livestock (Knowlton et al. 1999). Sodium cyanide and sodium fluoroacetate (Compound 1080) are the only restricted use pesticides registered with the United States Environmental Protection Agency for use on coyotes. However, several states (California, Colorado, Arizona) have prohibited the use of sodium cyanide and Compound 1080. Public sentiments towards the use of toxicants for managing predators (Arthur 1981, Andelt 1987, Reiter et al. 1999) will likely lead to other states prohibiting the use of these chemicals as well. Such bans severely restrict the ability of ranchers, federal and state agencies, and pest control operators to limit livestock losses and other damage (e.g., disease transmission, irrigation system damage, crop losses, game predation, aircraft hazards, human health and safety) caused by problematic coyotes. As urban wildlife-human conflicts increase in frequency, it is likely that the need for a coyote-control device that is acceptable for use in semi-urban areas will increase. Desirable qualities for such a coyote-control device include being safe to humans and pets, as well as being safe for non-target wildlife species and the environment, and social acceptability. As such, it would be advantageous if the coyote-control compound induced mortality with minimal pre-mortality symptoms and if an antidote or reversal therapy were available for inadvertently exposed commensal dogs.

Criteria for the selection and development of a predacide include effectiveness, taste and odor, speed of action, hazard to humans, antidote/therapy, environmental safety, regulatory concerns, cost, and availability (see Fagerstone et

al. 2004 for more details). With respect to a methylxanthine (theobromine:caffeine mixture) coyote-control compound, these criteria were addressed in Fagerstone et al. (2004). Briefly:

(1) Effectiveness — Methylxanthines can induce acute toxicity in canids as the propensity for domestic dogs to overdose on methylxanthines via ingestion of chocolate is well documented (Farbman 2001, Gwaltney-Brant 2001, Pittenger, 2002). The most abundant methylxanthines in chocolate are theobromine and caffeine. The toxicity of these methylxanthines to coyotes is summarized in Johnston (2005).

(2) Taste and odor — As indicated by articles in the literature, chocolate is consumed readily by canids (Farbman 2001, Gwaltney-Brant 2001, Pittenger, 2002). Additionally, coyotes have readily ingested methylxanthine fortified dog food and lard (Johnston 2005). It appears methylxanthine can be formulated to be palatable to canids.

(3) Speed of action — Following ingestion of methylxanthines, coyotes typically exhibit no symptoms for several hours. This lag time offers a margin of safety with respect to non-target pets by providing a window of opportunity for veterinary intervention to reverse the toxicity of accidentally exposed animals. Because symptoms may not be immediately apparent, the delivery system should incorporate a dye marking animals that have consumed the toxic matrix. Furthermore, signage should be used to alert pet owners to potential hazards and to provide the appropriate response to exposure, as indicated by the dye, and before onset of rapid mortality after symptoms are apparent.

(4) Antidote — The availability of an antidote or effective medical treatment to reverse the toxic effects of a predacide increases its safety. Given the frequent exposure of dogs to chocolate, veterinary supportive-therapy procedures are well documented (Hornfeldt 1987, Farbman 2001). As there is typically a significant lag time between ingestion and the onset of symptoms, inclusion of a dye in the formulation should facilitate identification and subsequent veterinary intervention of accidentally exposed dogs before the onset of toxicosis.

(5) Hazard to humans — All currently registered predacides are toxic to

humans. For theobromine, the rat oral LD₅₀ is 1,250 mg/kg (U.S. Environmental Protection Agency 2012), and humans are likely more tolerant of caffeine and theobromine. Even though humans are exposed to high amounts of caffeine and theobromine by consuming coffee, tea, cola beverages, and chocolate, there has been no documented human mortality in association with the consumption of these products.

(6) Environmental safety — Selectivity of toxicity to the target animal is desirable to minimize accidental poisoning of non-target animals. Methylxanthines appear to be selectively toxic to canids, as reports of accidental poisonings due to the consumption of methylxanthines have mainly been limited to canids.

(7) Cost and availability — Pure analytical grade methylxanthines, such as caffeine, theobromine, and theophylline are widely available through chemical supply sources. The delivery device for pest coyotes would likely need to contain approximately 6 g (see reasoning below) of active ingredient.

(8) Regulatory concerns — With the exception of 31 compounds considered by the EPA to be of negligible or minimum risk, all pesticides including predacides must be approved for use by the EPA. Acceptance criteria include efficacy, safety and environmental hazards. Methylxanthines, such as theobromine, should display high levels of efficacy and selectivity towards canid predators while being environmentally benign. The EPA's published standard for the laboratory efficacy of rodenticides is 90 percent mortality of the exposed animals (U.S. Environmental Protection Agency, 1991). There are no published standards for the laboratory efficacy of predacides, consequently, our target mortality for these trials was 90 percent.

Johnston (2005) found that caffeine was toxic to coyotes, however the symptoms accompanying toxicosis were sub-optimal because caffeine-induced mortality was preceded by severe convulsions and seizures. Theobromine was less toxic to coyotes, but symptoms, such as convulsions and seizures were mild to non-existent. Oral methylxanthine (5:1 theobromine:caffeine) administration to coyotes appeared to represent the optimal mixture of theobromine (minimal

undesirable symptoms) and caffeine (potency) (Johnston 2005). However the toxicity of this mixture dictates that about 6 g of the theobromine:caffeine would be required for an effective coyote predicide. This may limit the number of potential delivery devices available for this mixture. The Coyote Lure Operative Device (CLOD; Marsh et al. 1982, Berentsen et al. 2006a, b) can deliver a total volume of formulation containing 6 g of toxicant. It should be noted that Johnston (2005) obtained lethal doses in coyotes using oral gavage, or using a CLOD in a pan, which allowed coyotes to consume the entire contents of the mixture, including spillage of the compound. Therefore, use of the CLOD under simulated field conditions is more representative of an actual management action.

As development and required EPA registration of new toxicants typically takes 5 years to 10 years, it behooves the wildlife-management community to proactively develop a new coyote-control compound that is efficacious, cost effective, induces mortality with minimal undesirable pre-mortality symptoms, and possesses registration potential with the EPA. For this reason, we evaluated the potential of a theobromine:caffeine mixture delivered via the CLOD to induce mortality in coyotes under simulated-field conditions. The main objectives of the study were: (1) to determine the number of coyotes that will be attracted to, chew on, and consume contents of CLODs containing a theobromine:caffeine mixture, and (2) to estimate what proportion of coyotes that chew on CLODs ingest a lethal dose of the theobromine:caffeine mixture and the time interval between consumption and mortality.

Materials and Methods

Six different trials using a mixture of theobromine and caffeine as the predicide were designed and conducted. These trials were conducted sequentially with modifications to the compound or bait mixture made from information acquired from the previous trial. The research was conducted using captive coyotes in large pens at either the USDA Sheep Experiment Station near Dubois, Idaho (Trial 1), or the USDA/NWRC Predator Research Facil-

ity in Millville, Utah (Trials 2-6). In each pen enclosure, shade structures and natural bedding were provided. Food was provided daily, while water was provided ad libitum. This study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the National Wildlife Research Center.

Johnston (2005) demonstrated that, when a coyote received a theobromine:caffeine mixture via oral gavage, or in a pan placed in a kennel, the result was usually death of the animal. However, for application to a field setting that would mimic a management scenario, it was necessary to test the same dosage of the compound by delivering the compound in a CLOD and determine whether an appropriate lethal dose could be administered. In the current 6 trials, the CLOD consisted of a 60 ml plastic bottle and a stake that affixed the CLOD to the ground (Figure 1). A 5-part theobromine: 1-part caffeine mixture was combined with a meat matrix (water with either canned, wet dog food or hamburger), and corn syrup or hamburger), and corn syrup and placed in a CLOD. All ingredients were combined and mixed in a blender until homogeneous. The mixture of corn syrup, meat matrix, and active ingredients were then transferred to the 60 ml CLOD. In the mixture, a maximum dose of 19.6 g of active components (16.3 g of theobromine and 3.3 g of caffeine) was in each CLOD. However, doses were variable as the coyotes were self-administering the test compound. CLODs were prepared at the USDA/NWRC chemistry labs.

Trial 1

The first trial utilized a CLOD containing 25 percent active ingredient (16.3 g theobromine: 3.3 g caffeine) in a granular form, 37.5 percent dog food, and 37.5 percent corn syrup (to enhance palatability). This trial was conducted in a 65-ha enclosure with four CLODs placed within the enclosure. The coyotes were pre-baited with CLODs containing dog food and corn syrup for 1 week before the toxic CLODs were placed in the pen. These CLODs also had an attractant-infused, wax coating on them.

Trial 2

Following the results from Trial 1, there was a desire to have a marker in the CLOD to inform pet owners in the event

Figure 1. Coyote Lure Operative Device containing the theobromine-caffeine mixture that has been chewed on by a coyote (photo courtesy of P. Darrow).



of an accidental dosing. Therefore, Rhodamine B was added (0.04 percent wet weight) to the same mixture as described for Trial 1. The use of Rhodamine B would act as a marker (Evans and Griffith 1973, Marsh et al. 1982) by making the animals lips turn red upon exposure signaling to a pet owner that their animal had ingested something unusual, and thereby allowing a pet owner to get the pet to a veterinarian for treatment. Corn syrup was applied liberally to the outside of the CLOD to encourage the coyote to lick and chew the CLOD. Trial 2 was conducted in a 6-ha pen with one CLOD placed in the pen.

Trial 3

Following the lower efficacy found in Trial 2, there was concern the Rhodamine B may have limited the absorption of the compound, as well as concern that using commercial dog food would be a registration issue. Therefore, the dog food was replaced with hamburger that had been cooked in a microwave as the meat matrix, and the Rhodamine B was removed. Additionally, the amount of active ingredient was reduced to 8.15 g theobromine: 1.65 g caffeine (5:1 mixture) in granular form to determine if this lower dosage would increase palatability, yet remain effective. Corn syrup was applied liberally to the CLOD to encour-

age the coyote to lick and chew the CLOD. Trial 3 was conducted in a 6-ha pen with one CLOD placed in the pen.

Trial 4

Data from Trial 3 indicated that the lower dosage of active ingredient resulted in lower efficacy. Therefore, Trial 4 employed double (16.3 g theobromine: 3.3 g caffeine in granular form) the dosage of Trial 3. The bait formula consisted of 21 percent (wet weight) theobromine, 4 percent caffeine, ground beef (12.2 percent wet weight), water (24.4 percent wet weight), corn starch (1.8% percent wet weight), and corn syrup (36.6 percent wet weight). Corn syrup was applied liberally to the CLOD to encourage the coyote to lick and chew the CLOD. Trial 4 was conducted in a 6-ha pen with one CLOD placed in the pen.

Trial 5

The previous trials (1-4) were still lower than the desired level of mortality (90 percent) for EPA registration and some coyotes would not consume the contents of the CLOD. Therefore the methylxanthine mixture was micro-encapsulated (50 percent) with a proprietary lipid coating (Maxx Performance, Inc., Chester, N.Y.) in an attempt to increase palatability, and therefore increase consumption of the compound. Because micro-encapsulation decreased the volume of the active ingredient that could be mixed homogeneously in the CLOD, the CLOD contained a maximum dosage of 10.4 g theobromine: 2.1 g caffeine. The meat component of the mixture was removed to maximize the amount of theobromine and caffeine that could be placed in the CLOD. Corn syrup was applied liberally to the CLOD to encourage the coyote to lick and chew the CLOD. Trial 5 was conducted in a 0.1-ha pen with one CLOD placed in the pen.

Trial 6

Due to the lower mortality demonstrated in Trial 5, there was concern that micro-encapsulation of the active ingredients had lowered absorption in the gut. Therefore, for Trial 6 a spherical form of the active ingredients was used to increase consumption without compromising absorption. A wetted-methylxanthine mixture was subjected to extrusion spheronization resulting in uniform particles of approximately 1 mm diameter.

The use of a spherical form of the compound would, in theory, limit solubility in the mouth by reducing the surface area of the compounds. The CLOD was prepared with the active ingredients in a 21:4 (wet weight) mixture of 16.3 g theobromine: 3.3 g caffeine in spherical form mixed with a corn syrup and meat (ground beef) matrix. Corn syrup was applied liberally to the CLOD to encourage the coyote to lick and chew the CLOD. Trial 6 was conducted in a 0.1-ha pen with one CLOD placed in the pen.

For each trial, a single coyote was placed in a large enclosure and allowed to acclimate to the pen for 48 hrs to 72 hrs. After the acclimation period, a CLOD containing the theobromine: caffeine mixture was placed in the pen. The coyote was observed remotely with a spotting scope, thermal imager, or remote-controlled camera. Motion-activated Internet Protocol (IP) cameras were used to monitor the CLOD. When the coyote approached the CLOD, the camera would take a picture and send a text message to the observer's phone. The observer could then view the pictures online to note when the coyote approached and consumed the CLOD. In Trials 1 through 4, the coyotes were fitted with VHF radio-collars to facilitate locating the coyote in the larger pens by the observer. Observations recorded included the time the animal approached and chewed on the CLOD, the estimated amount of the CLOD consumed, and the time of death. If the animal consumed a part, or all, of the CLOD, the behavior of the animal was observed to determine the symptoms of toxicosis. Animals consuming a part, or all, of the compound were observed for 24 hr post-consumption, or until mortality occurred. Coyotes were observed for 5 days after placement of the CLOD in the pen. As this was a simulated field test, after placing coyotes in the study pen, human-coyote interactions (including coyote monitoring before the toxicant is consumed) were minimized to reduce disturbance and allow the animal to approach and consume the contents of the CLOD.

Study Design and Statistical Analyses

The purpose of the study was to determine what proportion of coyotes interacted with the CLODS, and of those animals, what proportion suc-

cumbed to the toxicant in an acceptable manner. Thus, the experimental design was observational and statistical analyses were limited to descriptive statistics (i.e., proportions) and their associated measures of variability (range). The EPA's published standard for the laboratory efficacy of rodenticides is 90 percent mortality of the exposed animals (U.S. Environmental Protection Agency, 1991). There are no published standards for the laboratory efficacy of predicides, consequently, our target mortality for these trials was 90 percent.

Results

Trial 1 exposed 11 coyotes to the CLODs, of which 9 animals consumed some of the compound resulting in seven mortalities (Table 1), giving an overall mortality of 64 percent. While this 64-percent mortality was less than the desired 90-percent mortality, the resultant deaths of seven animals indicated that the CLOD could deliver a lethal dose of the compound, and thus the addition of a marker appeared justified to reduce the risk to non-target pets. With the addition of Rhodamine B to the compound, the results from Trial 2 indicated there might be an issue of lower absorption or palatability with the additional marker, as overall mortality was only 20 percent in Trial 2. Therefore the marker was not added in subsequent trials. In addition, the use of a commercial dog food as a bait matrix may prevent subsequent registration with the EPA, thus the matrix was changed to ground beef for subsequent trials.

With the marker no longer added to the compound, and the bait matrix consisting of ground beef, results from Trial 3 showed 100 percent of the coyotes consumed part or all of the CLOD, but overall mortality (60 percent) was still less than the desired 90 percent threshold (Table 1). Results from Trial 3 indicated that the combination of hamburger and lower methylxanthine concentration produced 100-percent consumption. However, the new dosage was sub-lethal. Therefore in Trial 4, the dosage of the active ingredient was doubled. Trial 4 showed 100 percent of the coyotes fed on some or all of the CLOD, but overall mortality was 60 percent. Poor palatability was assumed to impact consumption. For Trial 5, the active ingredient was micro-

Table 1. Results of 6 trials involving coyotes being exposed to CLODs containing a theobromine:caffeine mixture.

Trial	#test subjects	# (%) of test subjects consuming part or all of the CLOD	(%) of test subjects that # died	Mean time [range] to death (hrs)
1	11	9 (82%)	7 (64%)	6.0 [2.0 - 12.0]
2	5	4 (80%)	1 (20%)	4.0
3	5	5 (100%)	3 (60%)	2.2 [2.0 - 2.5]
4	5	5 (100%)	3 (60%)	3.5 [2.0 - 4.5]
5	6	4 (67%)	1 (17%)	4.0
6	6	6 (100%)	4 (67%)	12.5 [2.0 - 24.0]

encapsulated with a lipid coating in an attempt to increase consumption by blocking the interaction of the bitter methylxanthines and oral taste receptors. Results from Trial 5 indicated that micro-encapsulation did not increase consumption, nor did it increase mortality as overall mortality in Trial 5 was only 17 percent. It was concluded the micro-encapsulation likely reduced absorption in the gut. The lack of meat in the mixture may have increased the motility of the compound through the gut (Kunze and Furness 1999, Olsson and Holmgren 2001), thereby lowering the absorption of the compound. Much of the issue in lower mortality was limited consumption and/or absorption of the theobromine:caffeine mixture by the coyotes. Therefore, for Trial 6 we used the spherical form of the compound in an attempt to increase consumption while not compromising absorption. Results from Trial 6 showed higher overall mortality to 67 percent, but still below the 90 percent level. In addition, the spherical form resulted in the longest average time to death, thus indicating that solubility of the methylxanthines was lower in the oral cavity and in the gut.

Of 18 coyotes in which the time was observed from consumption of the CLOD to death, the time to death varied among the trials and compounds (Figure 2). Few coyotes (28 percent) died in <2 hours, with most of the mortalities taking >2 hours (72 percent).

Coyotes consuming enough of the CLOD content to show signs of toxicosis often showed initial signs of increased excitation and sensitivity to sounds or other stimuli. As part of the increased excitation, coyotes would spend a greater amount of time running around

their pen. Coyotes also seemed to experience hypersalivation, as well as polydipsia, as they would increase their consumption of water. A few coyotes were seen vomiting within a few hours of eating the compound. As toxicosis progressed, the coyote would lose coordination and would no longer be able to walk or stand. The coyotes would then lie in a lateral recumbent position with legs, head, and tail outstretched, muscles rigid and respiration elevated. Some coyotes would pedal infrequently with their feet. Though most coyotes died after assuming the laterally recumbent position, one coyote did make a recovery after lying on the ground for several hours.

Conclusions

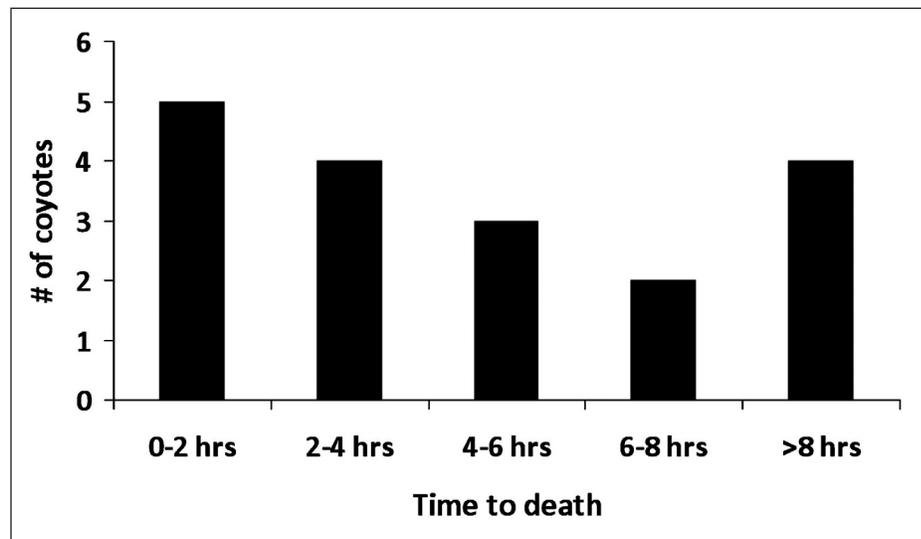
None of the trials resulted in the 90-percent mortality desired for EPA registration of the theobromine:caffeine mix-

ture as a predace for coyotes. Reasons for the below-par efficacy are many. In the early trials, many coyotes would not chew on the CLOD, suggesting an issue of attractiveness of the CLOD to precipitate chewing by the coyotes. Subsequent trials incorporated corn syrup to make the CLOD more attractive to consumption. Generally, coyotes desire compounds that contain sugar (Marsh et al. 1982, Mason and McConnell 1997). Another problem was spillage of the mixture from the CLOD after the coyote had bitten the plastic container, thereby preventing complete consumption.

The theobromine:caffeine mixture appeared to have low palatability as the coyotes would often cease consumption once they chewed on the CLOD. Many times the coyotes would bite the CLOD, cease consumption, and the compound would then leak out and spill onto the ground. Therefore, palatability of the compound was in question during the earlier trials, with subsequent trials using either the micro-encapsulated or spherical form of the compound in an attempt to increase palatability. However, these forms appeared to affect absorption of the compound and hence lower morbidity following consumption of the theobromine:caffeine mixture.

Observations of coyotes that survived consuming the contents of the CLOD also indicated other issues in whether the theobromine:caffeine mixture could provide a lethal dosage to the coyote. Some animals would drink copi-

Figure 2. Frequency of time to death for coyotes exposed to the theobromine:caffeine mixture contained in a Coyote Lure Operative Device.



ous amounts of water after showing signs of toxicosis and they would survive. Fluid uptake may increase excretion and prevent reabsorption through the urinary bladder, and administering fluids to domestic dogs accidentally ingesting chocolate is recommended as treatment (Farbman 2001). Also, some animals regurgitated the compound and subsequently survived. Coyotes may have recognized the onset of symptoms and induced regurgitation. Because the pH level of the animal stomach contents could greatly influence absorption and uptake of the theobromine:caffeine mixture, insufficient amounts of the mixture may have been absorbed before regurgitation began.

While the time to death is not a standard used for registration, the coyote's time to death in these trials was lengthy and may not be acceptable to the general public (Andelt 1987). Surveys of the general public have repeatedly shown the use of toxicants to be the least favored method for managing predators (Arthur 1981, Andelt 1987, Reiter et al. 1999). However, this long-time period is desirable for it allows for treatment of domestic dogs that may be accidentally dosed. Use of some form of marker that does not interfere with either palatability or absorption of the mixture is desirable to alert pet owners in the event of an accidental dosing and the need for subsequent veterinary treatment of their dog.

It is realized that many of these variables (i.e., access to water, amount of food in the stomach, coyote physiology, and a coyote's ability to regurgitate the compound) are all beyond the control of wildlife managers, particularly in a field setting. However, the use of the theobromine:caffeine mixture in a CLOD, as administered in this experiment, may not be an effective toxicant for managing coyote depredation events. At a minimum, future research needs to be performed that will identify coyote sensory sensitivities, as they appear more than capable of detecting the theobromine:caffeine mixture. The sensory sensitivity of coyotes to bitter-tasting compounds has received limited research (e.g., Mason and McConnell 1997). Equally important is whether the CLOD is the proper delivery device for administering a lethal dose of a toxicant, particularly for coyotes, which are extremely

wary of novel objects (Windberg and Knowlton 1990, Windberg 1996, Harris and Knowlton 2001, Séquin et al. 2003).

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