U.S. Department of the Interior Bureau of Land Management

North Lander Wild Horse Gather Environmental Assessment

January 2022

BLM Wyoming -Lander Field Office

DOI-BLM-WY-R050-2021-0037-EA

Lander Field Office 1335 Main Street Lander, Wyoming 82520 307-332-8400

Environmental Assessment

Introduction

Identifying Information

Project Name: North Lander Wild Horse Gather

NEPA Number: DOI-BLM-WY-R050-2021-0037-EA

Type of Project: Wild Horse gather and population control measures

Location of Proposed Action: The North Lander Complex is located in Fremont County, Wyoming within an area confined by Highway 287 on the south, the Gas Hills Highway Wyoming 136 on the north and is mostly east of Highway 135; see Appendix D, Map 1.

Name and Location of Preparing Office:

Lander Field Office

1335 Main Street

Lander, Wyoming 82520

Lease/Serial/Case File Number:

Applicant Name:

Background

This Environmental Assessment (EA) has been prepared to analyze and disclose the environmental consequences of gathering wild horses and applying wild horse population control measures in the North Lander Complex of wild horse herd management areas (HMAs) over a 10-year period, starting from the date of an initial gather event, as proposed by the Bureau of Land Management Lander Field Office. The BLM proposes to implement population control measures in conjunction with wild horse gathers and removal of excess wild horses in the Conant Creek, Dishpan Butte, Muskrat Basin, and Rock Creek Mountain Herd Management Areas, collectively known as the North Lander Complex. The four HMAs making up the North Lander Complex are shown on Map 2 in Appendix D.

НМА	BLM	State	Private	Water	Total	
	Acres	Acres	Acres	Acres	Acreage	
Conant Creek	49476	2821	5420		57717	
Dishpan Butte	92373	6089	1245	29	99736	
Muskrat Basin	176227	12113	4914	0.5	193255	
Rock Creek Mtn.	19085	3024	2475		24584	
Complex Total	337161	24047	14054	29	375292	

Figure 1 Surface Ownership Acres

The BLM protects, manages, and controls wild horses and burros under the authority of the Wild Free-Roaming Horses and Burros Act (WFRHBA) of 1971, as amended. This law ensures that healthy herds thrive on healthy rangelands. The proposed action should prevent deterioration of the rangelands and help maintain a "thriving natural ecological balance" (TNEB) and multiple-use relationships for several years.

The 2014 Lander Record of Decision and Approved Resource Management Plan (2014 RMP) identified HMAs and the appropriate management level (AML), i.e., the targeted number of horses for each HMA, given available natural resources and BLM's multiple land use mission. Table 2 shows the AMLs for the North Lander Complex, population size estimates based on aerial surveys completed in 2020, and projected herd sizes for the years 2021-2032 if no management actions are taken and herds are allowed to grow unchecked at expected rates of 20% per year.

Table 2. Population estimates for wild horses in the North Lander complex of HMAs in 2021, and projected population sizes for 2022-2032 if no population management takes place and herds are allowed to grow at 20% per year.

НМА	Low AML	High AML	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032
Conant Creek	60	100	263	316	379	454	545	654	785	942	1131	1357	1628	1954
Dishpan Butte	50	100	270	324	389	467	560	672	806	967	1161	1393	1672	2006
Muskrat Basin	160	250	952	1142	1371	1645	1974	2369	2843	3411	4093	4912	5895	7073
Rock Creek Mtn.	50	86	179	215	258	309	371	445	534	641	770	924	1108	1330
Complex Total:	320	536	1664	1997	2396	2875	3450	4141	4969	5962	7155	8586	10303	12364
*All numbers are estimates of population size on March 1 (pre-foaling)														

**2021 numbers are based on 2020 post foaling population surveys

***2022-2030 numbers are estimates based on an annual increase of 20%

Figure 3 Population Estimates 2021-2032

Purpose and Need

The BLM has determined that wild horse numbers are above the AML in these HMAs and that action is necessary to remove excess animals. Wild horse numbers above the AML constitute excess wild horses as described in the Act. The 2014 Lander RMP identified the high AML as the highest number of horses that the rangeland can accommodate and still achieve a TNEB. Current population numbers are above high AML and the HMA is not achieving TNEB, and therefore this action in necessary to achieve TNEB, consistent with the Act. Continued use of forage and water resources at the current population levels is expected to have a detrimental impact to rangeland health, and overall TNEB if actions are not taken to reduce the population in these areas.

The primary purpose for this action is to achieve the Appropriate Management Level (AML) of wild horses in the North Lander complex of HMAs and implement population control measures and management actions to maintain the population within the established AML. A secondary purpose of this action is to remove any wild horses that have strayed outside one of these HMAs but are within the immediate geographic area (north of Hwy 287 and within 10 miles of the complex boundary).

The need for the action is that wild horse populations in all HMAs within the complex are currently in excess of the high AML and at these levels, the likelihood of causing degradation of the public lands and preventing those areas from achieving a TNEB and meeting rangeland health standards is increased.

Because wild horse numbers have been in excess of the AML for many years it is imperative that any action selected not only achieve the AML but also include measures to control population growth within

the AML. The BLM must maintain a thriving natural ecological balance and multiple-use relationship on public lands consistent with the provisions of Section 3 of the 1971 Wild Free-Roaming Horses and Burros Act (WFRHBA), 16 U.S.C. § 1333, and Section 102 of the Federal Land Policy and Management Act (FLPMA), 43 U.S.C. § 1701. BLM also is responsible for preventing unnecessary or undue degradation of public lands. 43 U.S.C. § 1732. BLM must remove wild horses from private lands when requested by the affected landowners. 16 U.S.C. § 1334.

The 2014 Lander RMP incorporated the need to avoid resource damage in Decision 4121 and authorized wild horse gathers (Decision 4121 and 4123) to "maintain a thriving natural ecological balance" or as needed to maintain the herd sizes within the AML. The utilization of population control measures was approved in Decision 4122.

Decision to be Made

Based on the analysis in the EA, the authorized officer will decide how to respond to the presence of excess wild horses in the North Lander Complex. The authorized officer will decide whether to gather, remove, and/or treat and release wild horses in North Lander Complex and what population control methods, if any, will be applied.

The decision to be made would not set or adjust AMLs, which BLM set in the 2014 Lander RMP. Similarly, the decision would not adjust livestock use, which was also established in the 2014 Lander RMP.

Conformance with BLM Land Use Plans

Actions in the project area must conform with the Lander Record of Decision and Approved Resource Management Plan (RMP) for the Lander Field Office Planning Area, June 26, 2014 as updated by maintenance actions. See 43 CFR 1610.5.

The RMP provides that the planning area is open to consideration for wild horse population control. The specific management actions that apply are described below:

Record	Management Action Text
4121	Conduct regular and periodic gathers when necessary to maintain a thriving natural ecological balance or when required by emergency to maintain the initial Appropriate Management Level ranges (number of horses)
4122	Utilize chemical and other population control measures as needed to maintain Appropriate Management Level ranges
4123	Gather wild horses outside the established Herd Management Areas during routine periodic gathers. Prioritize gathers in greater sage-grouse Core Area unless removals are necessary in other areas to prevent serious issues, including herd health impacts. Utilize Required Design Features and techniques such as those in Appendix L, to promote genetic diversity and limit adverse impacts to wild horses from gathering.
4127	Manage the four North Lander Complex herds as one herd to promote good distribution and genetic mixing, but maintain separate horse Appropriate Management Levels in existing Herd Management Areas.
4021	Require the use of certified noxious-weed free forage, mulch, and other land-applied products for BLM-authorized activities on BLM-administered lands.

 Table 1. Conformance with BLM Land Use Plans

6063	Establish stocking rates in areas preferred by livestock that allow for appropriate utilization levels by livestock, adjusted for the anticipated intensity of use necessary to provide sufficient forage and cover to support and maintain healthy, diverse wildlife and wild horse populations and to achieve the Wyoming Standards for Healthy Rangelands. Utilization levels may vary based on the implementation of a comprehensive grazing strategy or as needed to achieve vegetation objectives.
4023	Require that equipment and vehicles used for BLM-authorized activities be cleaned for seeds of noxious weeds and invasive nonnative species before moving onto BLM-administered lands. Prohibit project vehicles accessing BLM-administered lands via cross-county travel from driving through infestations during access to the site. If the area on which BLM-authorized activities take place is identified as being a high risk for invasive and/or noxious weeds, require that vehicles be cleaned before leaving the worksite and include prescriptions for the disposal of wash water.
LR: 13.4	Facilitate trophy and high-quality hunting opportunities in WGFD hunt units targeted for special management criteria.
6084	Cooperatively develop mitigation measures to reduce the impact or intensity of disruptive activities in Mule Deer Hunt Area 90 and Antelope Hunt Areas 67, 68, 69, and 106.

Endangered Species Act Section 7 Consultation in accordance with Section 7 of the Endangered Species Act (ESA), the BLM consulted with the U.S. Fish and Wildlife Service (USFWS) on the 2014 RMP through a programmatic Biological Assessment (BA) prepared in 2012 and the USFWS's 2013 Biological Opinion (BO). In the BA, the BLM described wild horse management actions at a programmatic, planning level, but stated that further consultation would be needed for future site-specific actions. The BLM initiated informal ESA Section 7 consultation for this project through verbal discussions with USFWS on June 9, 2021 and a letter to USFWS on June 10, 2021. In a follow up letter on June 21, 2021, the BLM requested consultation and provided more detail on the impacts anticipated from the proposed action. In a letter dated June 25, 2021, the USFWS concurred with the BLM's conclusions, as described below.

In the 2012 BA, the BLM determined that the wild horse management program for the Lander planning area is likely to adversely affect desert yellowhead (*Yermo xanthocephalus*). The BA also stated that the wild horse program may affect, but is not likely to adversely affect, desert yellowhead critical habitat due to the BLM-committed conservation measure of not conducting gather activities such as using temporary gathering and holding facilities in critical habitat.

The BLM has determined that the proposed gather action may affect but is not likely to adversely affect desert yellowhead, for the reasons provided below in the desert yellowhead section. The BLM also has determined, consistent with the programmatic finding, that the proposed action may affect, but is not likely to adversely affect, desert yellowhead critical habitat.

In the 2012 BA, the BLM concluded that the wild horse program may affect, but is not likely to adversely affect, Ute ladies'-tresses (*Spiranthes diluvialis*). Consistent with that determination, the BLM has concluded that the proposed action may affect, but is not likely to adversely affect, Ute ladies'-tresses.

Identification of Issues and Scoping Public Involvement

Public scoping took place April 1-30, 2021. Issues identified through the scoping process have been considered in the development of this EA. Many comments from the public requested that we change

the AML and/or that we eliminate livestock grazing. Changes such as these are made through the land use planning process, and, as such, are not considered in this analysis of the proposed action and its alternatives.

The public was notified of the initiation of the NEPA process when the EA was listed on the ePlanning site. Wild Horse and Burro Program policy requires that the EA be available for public comment for 30-days prior to a final decision.

Internal Scoping

The proposed action was reviewed by an interdisciplinary team. Preliminary issues were considered in order to aid in the development of the proposed action or design features. The ID-team then determined which issues warranted further consideration.

Issues Identified for Detailed Analysis

The proposed action was reviewed by an interdisciplinary team. The following issues were identified for detailed analysis:

Wild Horse Population

How would the proposed population growth suppression activities affect wild horses?

How would gather operations affect wild horses?

How would the proposed action affect the genetic diversity of this population?

How would the proposed action affect the complex's ability to maintain a self-sustaining population?

Native Vegetation

How would the proposed action affect native vegetation within these HMAs?

Wildlife

How would the gathering of wild horses from the North Lander Complex affect wildlife resources including Threatened and Endangered Species, and special status species and their habitats?

Livestock Grazing/Range Administration

How would the reduction to AML addressed in the proposed action affect livestock operations within these HMAs?

Issues Considered and Eliminated from Further Analysis

The BLM considered the following issues, but determined that they did not warrant analysis in this EA for the reasons discussed below.

What effect will the proposed action have on properties unevaluated and eligible for the National Register of Historic Places (NRHP)?

The proposed action for this undertaking is an area of 375,292 acres. There are multiple known properties unevaluated and eligible for the NRHP in this area. Based on the project design it is not anticipated that the proposed action will have an effect on historic properties outside of potential trap sites. To help ensure that there would be no effect to known or unknown historic properties, a Class III Cultural Resource Survey will be required prior to the final selection of trap sites. This design feature should help eliminate the effects to Historic Properties. Therefore, this resource issue does not need to be carried forward for analysis.

What effect will the proposed action have on wetland/riparian areas within the HMAs?

The proposed action consists of an area containing approximately 375,292 acres. There are numerous wetland/riparian areas within this project area that wild horses, cattle, and wildlife utilize year-round. These wetlands are identified through the National Wetland Inventory (NWI). In order to ensure that there are no adverse impacts to these wetland/riparian areas, trap sites will not be located within 500 feet of any identified wetland/riparian areas. This design feature would eliminate the effects from trap sites to wetland/riparian areas throughout the proposed project location. Therefore, this resource issue does not need to be carried forward for analysis.

How many additional livestock would be placed on the range following the removal of wild horses?

None of the alternatives in this EA propose adjustments to permitted livestock use following the gather. Changes in the amount of forage allocated for livestock use are done through land use planning decisions. Information regarding the amount of forage permitted for livestock use is provided in the "Livestock Grazing" section of the EA.

Would wild horses removed from the HMAs be euthanized or sent to slaughter?

Under current policy, the BLM does not sell or send wild horses or burros to slaughter. The BLM takes measures to ensure wild horses that are sold or adopted are not sent to slaughter.

Would wild horses be treated humanely as part of this action?

In conducting all wild horse gather, removal and fertility control treatment operations, BLM follows a set of best management practices to protect the health and safety of wild horses. PIM 2021-002 establishes policy for the Comprehensive Animal Welfare Program (CAWP). BLM follows this policy in all operations to ensure wild horses are treated humanely.

How would gather operations lead to the introduction and/or spread of noxious and invasive weeds?

There are a variety of noxious weeds occurring throughout the western US that could be introduced on contractor vehicles or equipment when contractors arrive to implement gather operations or that could be introduced through hay fed to captured horses. These noxious weeds include those already in the North Lander Complex, such as black henbane, Canada thistle, cheatgrass, field bindweed, musk thistle, Russian knapweed, saltcedar, Scotch thistle, and whitetop, as well as noxious weeds that are problems elsewhere in the west that have not yet been introduced to Fremont County, such as Dyer's woad, ventenata grass, medusahead, and many others. In order to minimize the potential for new introductions of noxious weeds as a result of gather operations, the BLM has included as design features the following measures consistent with decisions in the 2014 RMP: 1. Vehicles and equipment accessing the gather area for the first time will be cleaned of mud and weed seeds prior to arrival (Decision 4023), and 2. All hay fed at trap sites or holding facilities will be certified weed-free (Decision 4021). These design features substantially minimize the potential for the gather to introduce and spread noxious weeds; therefore this resource issue was not carried forward for analysis in this EA.

How would gather operations impact desert yellowhead populations and desert yellowhead critical habitat?

Desert yellowhead is a narrowly-distributed, threatened forb species that grows in two populations areas, both of which are located within the Dishpan Butte HMA in the North Lander Complex. The species grows in dry, sparsely vegetated settings and occupies a total area of approximately 50 acres over the two populations. The desert yellowhead critical habitat is a 360-acre area surrounding the larger of the two populations.

The majority of gathers will happen in the fall. Desert yellowhead typically produces seeds, complete its life cycle, and senesces by mid September, spending the fall and winter dormant underground. Most

gathers would have no potential to impact growing and reproducing DY plants because they will be implemented after the plant has completed its growth and reproduction cycle. In addition, wild horse monitoring data show the DY populations and surrounding areas have low concentrations of wild horse use, due to substantial distance from water and horse preference for areas with greater abundance of riparian vegetation. Given the small area of occupied habitat and the large gather area (approximately 50 occupied acres in a gather area approximately 375,000 acres), it is unlikely that horses would be herded through the populations due to chance alone. If some horses did run through, it would be likely to be in relatively small numbers due to low numbers of horses typically using the areas to begin with. The very low probability that some plants could be trampled during a horse gather is not significantly higher than the probability of plants being trampled at under current conditions from wild horses utilizing the rangelands within the HMAs. The project will have a beneficial impact to native vegetation including desert yellowhead by reducing the trampling and grazing by wild horses to levels experienced when the herd is at AML. Therefore, this issue is not carried forward in this EA. The BLM consulted informally with the USFWS with a determination that the North Lander Wild Horse Gather may affect, but is not likely to adversely affect, desert yellowhead and its critical habitat. The BLM included the following conservation measure as a design feature of the project: No temporary gathering or holding facilities will be placed in desert yellowhead critical habitat or in desert yellowhead population areas.

How would gather operations impact Ute ladies'-tresses populations and habitat?

Ute ladies'-tresses is a threatened orchid species that occurs in riparian areas in scattered locations throughout the west. The gather area contains small areas of potentially suitable habitat in riparian areas throughout the North Lander Complex. Since approximately 2017, the BLM and Wyoming Natural Diversity Database have been surveying some of the highest probability habitat within the North Lander Complex for Ute ladies'-tresses, and have not found any individuals of the species. The species has never been found within either the Sweetwater River or Wind River watersheds where the project would occur. If there are Ute ladies'-tresses plants in the North Lander Complex, they would grow in riparian areas. The BLM would not use temporary gathering or holding facilities within any riparian areas, thus avoiding impacts to potential Ute ladies'-tresses habitat. Wild horses tend to congregate on riparian areas. Trampling of riparian areas due to gather activities would not be any higher than typical, daily trampling that is occurring currently. This project seeks to maintain and enhance quality riparian habitat through removing horses that trample and utilize wetland vegetation, and is therefore beneficial to Ute ladies'-tresses and its habitat. Ute ladies'-tresses completes its growth and reproductive cycle in September, so the majority of the gathers would occur outside of the growth and reproductive phase of any Ute ladies'-tresses plants. Therefore, this issue is not carried forward in this EA. The BLM consulted informally with the USFWS with a determination that the North Lander Gather may affect, but is not likely to adversely affect, Ute ladies'-tresses. The BLM included the following conservation measure as a design feature of the project: No temporary gathering or holding facilities will be placed in Ute-ladies'tresses suitable habitat, i.e., wetland and riparian areas.

How would gather operations impact BLM sensitive plant species populations?

There are several BLM sensitive species that occur within the gather area. Upland species include Beaver Rim phlox, Cedar Rim thistle, Fremont bladderpod, limber pine, Porter's sagebrush, and Rocky Mountain twinpod. These species tend to occur in small areas with specific habitat requirements, typically on steep, sparsely vegetated slopes or rocky ridgetops. BLM sensitive species occurring in wetlands include meadow milkvetch and meadow pussytoes. While BLM sensitive species habitat will not be specifically avoided for temporary gathering/holding facilities, it is unlikely that such facilities will be placed within their habitat. Meadow pussytoes and meadow milkvetch occur in wetlands, and the BLM would not place these facilities in wetland or riparian areas. The rock outcrops, rocky ridges, and steep slopes that comprise the majority of habitat for the remaining species is not a good location to put trap sites. The best locations for trap sites are on existing roads at the top of broad hills or on saddles. The project will have a beneficial impact to native vegetation including BLM sensitive species by reducing the trampling and grazing by wild horses to levels experienced when the herd is at AML. Due to the unlikelihood of trap sites being located in sensitive species habitat, this project is unlikely to affect BLM sensitive plant species, and this resource issue is not carried forward in this EA.

How would gather activities impact mule deer and pronghorn hunters in WGFD special management hunt areas?

In the Lander RMP the BLM established an objective for this area to:

Facilitate trophy and high-quality hunting opportunities in WGFD hunt units targeted for special management criteria.

In addition, the RMP identified the following action to support this objective:

Cooperatively develop mitigation measures to reduce the impact or intensity of disruptive activities in Mule Deer Hunt Area 90 and Antelope Hunt Areas 67, 68, 69, and 106.

The proposed action includes a design feature that will ensure this objective is met, and that impacts to hunting in these special management areas will be avoided or mitigated. Therefore, this issue does not require detailed analysis.

Proposed Action and Alternatives

No Action-No Gather, Removal, or Population Control

The No Action Alternative is included as a baseline for comparison with the action alternatives, as required under NEPA. It does not meet the Purpose and Need for the action since it does not address the degradation of rangeland health caused in part by high wild horse herd sizes relative to available resources, and the negative effects of that large herd size on a thriving natural ecological balance. Similarly, a no action alternative does nothing to reduce the high annual growth rate of the wild horse herds. However, in accordance with the BLM NEPA Handbook (H-1790-1), BLM can analyze the No Action Alternative to aid the analysis of other alternatives, even if it does not meet the Purpose and Need (see Handbook 6.6.2 at page 51).

Gather to the Low AML Only (No Population Control)

This alternative would include gathering and removing wild horses from the complex using a combination of helicopter drive-trapping, helicopter assisted horseback roping and bait/water trapping. Retained horses will be over the age of 5 and possess good conformation, color, and, to the extent it can be evaluated, disposition. No population growth suppression (i.e. fertility control) measures would be implemented. To meet the purpose and need for the action, gathers would have to be repeated as soon as the population exceeds the high AML, which would likely be approximately every 3 years. Over a 10-year period this would necessitate approximately 3-4 gathers. If the initial gather takes place in 2022 approximately 2076 horses would need to be removed at that time. After the initial gather, each subsequent gather would require removal of approximately 200-250 horses as long as a 3 year gather schedule is strictly adhered to. To ensure the genetic viability of the complex, as evidenced by having adequate levels of observed heterozygosity, the BLM would engage in genetic diversity monitoring of observed heterozygosity levels and one or two 1–2-year-old fillies could be exchanged between HMAs as well as introduced from external HMAs in conjunction with these regular gathers, depending on results of that genetic diversity monitoring.

Proposed Action-Gathers, Removals, and Fertility Control

The proposed action alternative is to gather wild horses from the complex as many times as needed over a ten-year period to fully implement fertility control measures analyzed in this EA and reduce the population to the AML. Removals would focus on removing young and highly adoptable animals. Older and less adoptable animals would be selected for fertility control treatments and would be returned to the range. All four HMAs within the complex would be gathered using a combination of helicopter drive-trapping, helicopter assisted horseback roping and bait/water trapping. Some horses would be removed, and some would receive fertility control treatments and be returned to the range. Fertility control measures/treatments include the following:

- Geld/vasectomize a high percentage (up to 95% or more) of captured stallions returning to the range.
- Use flexible Intrauterine Devices (IUDs) for wild horses on open (not pregnant) mares returning to the range.
- Use GonaCon-Equine vaccine on all mares returning to the range including mares receiving an IUD.
- Implement a 60:40 male:female sex ratio.

Monitoring and Adaptive Management

The population control measures proposed in this EA are intended to keep the wild horse population within the appropriate management levels. It is not the intention of this action to eliminate reproduction

within the complex; only to reduce it so that it is in balance with mortality. Because the proposed action involves multiple gathers and treatments over a 10-year period, exact numbers of treated horses would vary with each gather and be based on gather success, gather frequency, population monitoring, and response to treatment. By analyzing a suite of treatment options and utilizing population monitoring an adaptive management approach can and would be employed.

Using an adaptive approach, adjustments may be made to increase or decrease reproduction rates so that a balance between population growth and mortality can be maintained. Although four population control options are being analyzed, that does not mean that all four will necessarily be used at once.

First Gather

- All treatment options except for adjusted sex ratios would be applied.
- The initial gather would attempt to achieve an 80-90% capture rate. Assuming the gather takes place in 2022 this would equate to ~1900-2200 horses captured.
- All captured horses age 5 and under would be permanently removed.
- Remaining horses of both genders would be selected for fertility control treatments and be returned to the range, with preference going to older less adoptable mares and stallions. It is anticipated that approximately 300 of each gender would receive treatment and be returned to the range.
- Fertility control treatments would take place either at a BLM holding facility (Rock Springs, Wheatland, or Cañon City), or they could also be done at a temporary holding facility within or near the complex.
 - Animals that would be returned to the range would receive a uniquely numbered radio frequency ID (RFID) chip in the nuchal ligament of the neck.
 - While in holding mares would be checked for pregnancy via palpation or ultrasound and open mares would receive a flexible IUD that is appropriate for wild horses.
 - Pregnant mares would receive an initial dose of GonaCon-Equine vaccine and would be held for at least 60 days and given a booster dose.
 - Selected stallions would be gelded or vasectomized.
 - A few stallions and mares with exceptional conformation may be selected for retention without treatment in each HMA.
 - Animals that receive fertility control treatments would be given a unique freeze brand that indicates which treatment they received. This would aid monitoring and future treatment efforts. Once these treatments are complete and gelded males have had time to heal all treated animals will be returned to their respective HMAs.
- All other untreated, captured horses would be removed from the complex and prepped for possible private care placement (i.e., adoption), as described elsewhere in this EA.
- To ensure the genetic viability of the complex, as evidenced by having adequate levels of
 observed heterozygosity, the BLM would engage in genetic diversity monitoring of observed
 heterozygosity levels and one or two 1–2-year-old fillies could be exchanged between HMAs as
 well as introduced from external HMAs in conjunction with these regular gathers, depending on
 results of that genetic diversity monitoring.

It is anticipated that the initial gather would result in a post gather population of close to 1000 animals with approximately 60-70% having some form of fertility control treatment. Close to 1400 horses would be removed in the initial gather if it takes place in 2022. If the complex isn't gathered at that time the number of horses that would need to be gathered, removed, and treated in the initial gather will continue to increase exponentially. The 10-year duration of the management decision would begin at the time of the initial gather.

Second Gather

- Prior to a second gather, thorough aerial surveys would determine whether the population in each HMA is increasing, decreasing, or static, and determine the approximate size of the foal crop. This data would be used to approximate the foal to adult ratio which would, in turn, inform decisions about the appropriate level of future fertility control treatments. With the ultimate goal being a static or very slightly increasing population within the AML range, the BLM would aim to apply greater or lesser levels of fertility control treatments so that the number of surviving foals always slightly exceeds the expected number of animals that die due to natural mortality in the herds.
- A second gather would take place approximately 2 years after the first gather.
- Most mares treated once with GonaCon-Equine during the initial gather should be open two years after the initial gather. Re-captured previously treated mares that have not previously been given an IUD would be pregnancy checked and additional open mares could be given IUDs. The percentage of mares getting IUDs will be determined by monitoring results.
- Mares previously treated with GonaCon-Equine that are pregnant and mares not chosen for an IUD would be given a booster dose of GonaCon-Equine.
- Previously-treated geldings or vasectomized stallions would be documented and then released without additional treatment.
- As dictated by apparent foaling rates and herd size measures that result from population monitoring (i.e., aerial surveys), previously uncaptured animals would either be removed, receive fertility control treatments as described and released, or released without treatment. If aerial survey monitoring results indicate a need for additional removals, selection criteria will be age based with older horses being favored for retention and younger horses being favored for removal.

Subsequent gathers within 10-years

- The same process would be followed for subsequent gathers within the 10-year time frame of this document as is used for the second gather.
- If population monitoring shows greater mortality than reproduction, fertility control treatments
 would be reduced. If at any time monitoring indicates that the population in any particular HMA
 has dropped below the low AML or is close to doing so, young reproductively viable horses from
 within the complex could immediately be brought in to bring the population up to the AML, and /
 or new fertility control treatments for animals that are returned to the range could be suspended
 until the reproduction rate once again exceeds the mortality rate. If necessary, animals from
 other BLM-managed HMAs could also be introduced.
- Sex ratio skewing in the animals returned to the range could be implemented in the second and all subsequent gathers if population monitoring shows a need, with the population goal of no greater than 60% male and 40% female in the free-roaming herds.

Common to All Gather Events

In order to maintain relatively high levels of genetic diversity in the various individual herds within the complex area, the BLM would engage in genetic monitoring during gather events. Hair follicle samples would be taken and analyzed. In its WHB herd management handbook (2010), the BLM identified the preference for observed heterozygosity levels to be maintained at levels no lower than 1 standard deviation below the mean for feral horses, and for a relatively low rate of loss of observed heterozygosity. As a routine matter, the BLM will aim to exchange at least two 1–2 year-old fillies between HMAs within the complex in conjunction with gather events. In addition, if genetic diversity monitoring indicates that the observed heterozygosity rates are low, or that the rate of loss of heterozygosity is high, then the BLM could introduce additional fertile animals from other HMAs outside the complex. The selection of source populations could vary, but it is expected that the animals would come from a fairly closely related herd, such as one with pairwise Fst values between 0.05 and 0.15. Animals introduced from outside the complex would likely include two 1-2 year-old fillies, and may also include a small number of young fertile stallions, if appropriate. This in conjunction with normal movement between HMAs would ensure genetic viability in the herds, as demonstrated by the herds having adequate levels of observed heterozygosity so as to avoid any negative effects of inbreeding.

Design Features of the Proposed Action Alternative and Best Management Practices

Cultural Resources

Prior to the final selection of any trap site, the area must be surveyed by a Class III cultural resource survey. The survey must be conducted by an archaeologist who meets current Office of Personnel Management requirements, or who holds a current BLM Cultural Resource Use Permit.

The holder must immediately report to the authorized officer any cultural and/or paleontological resource (historic or prehistoric site, object, or fossil) discovered on Federal Land by the holder, or any person working on their behalf. The holder must suspend all operations in the immediate area of such discovery until authorization to proceed is issued by the authorized officer.

Wildlife Resources

Trap locations will be sited to avoid adverse impacts to wildlife, including occupied GRSG leks, riparian areas and other BLM sensitive species habitats.

Rangeland Management Resources

Livestock operators within the gather area would be notified prior to the gather, enabling them to take precautions and avoid conflict with gather operations.

Trap sites will not be located within 500 feet of any Wetland/Riparian areas identified by the National Wetland Inventory.

Trap locations will be located to avoid adverse impacts to established rangeland improvement projects.

Noxious Weeds

- 1. Vehicles and equipment accessing the gather area for the first time will be cleaned of mud and weed seeds prior to arrival.
- 2. All hay fed at trap sites or holding facilities will be certified weed-free.

Special Status Plant Species

1. No temporary gathering or holding facilities will be placed in desert yellowhead critical habitat or in desert yellowhead population areas.

2. No temporary gathering or holding facilities will be placed in Ute-ladies'-tresses suitable habitat, i.e., wetland and riparian areas.

Recreation and Visitor Services:

Every attempt will be made to avoid gather activities during hunting seasons. If gather activities will occur during hunting season, the BLM will work with Wyoming Game and Fish Department to mitigate those impacts, as well as notify hunters when the activity will occur.

Alternatives Considered and Eliminated from Further Analysis

Fertility Control Use in the Absence of Gathers and Removals:

The BLM considered a number of alternatives but decided not to analyze them in detail because they did not meet the Purpose and Need for the action because they did not adequately reduce the population growth and the resulting adverse impacts to rangeland health. The population growth rate of wild horses is approximately 20% per year and removing excess horses would offer only a temporary reduction in numbers. The fertility control approaches the BLM considered but did not analyze in detail were:

- Exclusive use of porcine zona pellucida (PZP) vaccines to control population growth by darting, bait trapping, helicopter gathers or a combination thereof. Darting and bait trapping are difficult to accomplish in areas with the size, remoteness, and topography of the four North Lander HMAs and have limited utility because of the low efficiency of that approach. Although PZP vaccines could be administered as part of a helicopter gather, past experience using available PZP vaccine formulations has shown them to be unsuccessful in sufficiently preventing pregnancy to control population growth when administered at intervals of three years or more. National gather needs and limited funding currently do not support a consistent three year gather cycle. PZP vaccines are also most effective when administered in the winter to very early spring. Most helicopter gathers take place late summer and fall. The BLM has utilized PZP vaccines in these and other Wyoming HMAs and it failed to reduce the population growth rate to the extent needed to economically and efficiently support the AML and rangeland health.
- Only use GonaCon-Equine on mares administered as part of a helicopter gather to control • population (not in conjunction with any removals or other forms of fertility control). The effectiveness of GonaCon-Equine for wild horse mare fertility control is largely dependent upon the ability to administer booster shots. Because darting and bait trapping would have limited effectiveness in the North Lander HMAs, the needed boosters would be dependent upon the BLM conducting additional helicopter gather/gathers within a short period of time and/or holding treated mares in temporary holding corrals long enough to administer a booster. Even if mares were held long enough to administer a booster dose, the effectiveness of GonaCon-Equine would diminish annually with little to no infertility expected after approximately 4 to 5 years (See appendix D). Because is it not expected that every mare could be treated to the extent and frequency needed to limit growth so that births equal deaths, some level of helicopter drive trap gathers would still be necessary every 3-4 years. The BLM determined that the likelihood of being able to gather this complex every three years was not good enough to justify analyzing exclusive reliance on fertility control vaccine as a stand alone alternative. Without subsequent regular boosters, a single and possibly even two applications of GonaCon-Equine would not meet the Purpose and Need for a sustainable population control solution.

Change the AML:

Public scoping led to comments, suggesting that the AMLs for the HMAs should be changed. This action would require a planning level RMP decision, and, as such, it is beyond the scope of this EA.

Alternative Capture methods:

This alternative would include only capture methods *other than* helicopter drive trapping to gather excess wild horses, which were suggested through public comment. The BLM identified bait/water trapping, chemical immobilization, net gunning, and wrangler/horseback drive trapping as potential alternative methods for gathering wild horses. The information below will demonstrate that these methods are infeasible in meeting the purpose and need for this area.

- Bait trapping is included in this EA as a possible gather method for situations where it is appropriate to employ it, but not as the only or primary gather method. Bait/water trapping as an exclusive gather method would not be effective because of the size, remoteness, and limited accessibility of the HMAs, and because forage and water in these HMAs is typically available. Bait trapping typically fails to achieve a high percentage gather in areas where water or forage are readily available, and a high percentage gather is necessary for successful implementation of any population control measures.
- Chemical immobilization would not be feasible due to the size of the HMAs and the number of horses that need to be gathered. Furthermore, chemical immobilization is a very specialized technique and is strictly regulated. The BLM does not currently have the capacity to implement this method at the scale required by this project.
- Net gunning techniques would also be infeasible due to the size of the HMA and the number of horses that need to be gathered. Net gunning techniques normally used to capture big game also rely on helicopters in close situations. Net gunning heavier animals like horses may be more dangerous to the horse compared to net gunning pronghorn and mule deer. The preparers of this EA are unaware of any previous occasion when BLM has ever used net gunning as a capture technique for wild horses. Elk & moose are net gunned, but wild horses are heavier at 900-1,000 pounds making net gunning more difficult. Net gunning also requires a capture crew to be on board of the helicopter posing additional risk to more people and to the wild horse in the event of a mishap. This alternative poses high risk to human health and safety therefore it is not under consideration as an alternative.
- Use of wranglers on horseback drive-trapping to remove excess wild horses can be fairly effective on a small scale; however, due to the number of excess wild horses to be removed and the large geographic area of the HMAs, exclusive reliance on this technique would be infeasible. Horseback drive-trapping is also very labor intensive and can be very hazardous to the domestic horses and wranglers during gather operations.

For these reasons, the identified capture method alternatives were eliminated from further consideration and are not analyzed in detail for the proposed action and alternatives.

No Horse Removal, Fertility Control Only:

An alternative considered but not carried forward for detailed analysis was the use of fertility control methods only, with no wild horse removal. As described in the gather section above, it may be possible and practical at some point to gather and treat horses with no or limited removal, but only after the AML is achieved through removals. As an exclusive management action this alternative does not meet the purpose and need to maintain the AML, as the existing population of wild horses within the HMAs is currently above the established AML and excess wild horses need to be removed to prevent degradation of rangeland resources and to be in compliance with applicable laws and regulations.

Control of Wild Horse Numbers by Natural Means:

This alternative would use natural means, such as natural predation, loss of available forage due to overgrazing by horses, and weather, to control the wild horse population. This alternative was eliminated from further consideration because it would violate the WFRHBA which requires the BLM to protect the range from deterioration associated with an overpopulation of wild horses by removing excess wild horses from the range. It is also substantially similar to the No Action alternative.

The primary "Natural Means" would be population correction based on the population reaching carrying capacity of the natural vegetation in the area. Due to the absence of natural predators for wild horses this would be limited only by vegetation and water. Furthermore, wild horses are a long-lived species with documented foal survival rates exceeding 95%. As addressed at length in the National Academies of Sciences report about wild horse and burro management (NRC 2013), wild horses are not a self-regulating species.

This alternative would allow for a steady increase in the wild horse populations which would continue to exceed the carrying capacity of the range and would cause increasing damage to the rangelands until severe range degradation or natural conditions that occur periodically – such as blizzards or extreme drought – cause a catastrophic mortality of wild horses in the HMAs. This alternative would result in severe degradation to vegetive communities upon which wildlife and livestock depend and result in unnecessary suffering and starvation of wild horse.

For these reasons this alternative would have a severe negative impact on other multiple uses (especially wildlife and livestock) and would not correspond with the multiple use mission established by the FLPMA. It also fails to maintain a TNEB, and, as such, it would not be in compliance with the WFRHBA.

Remove or Reduce Livestock within the HMAs:

Under this alternative no wild horses would be removed from these HMAs. Alternatively, livestock would be removed from these HMAs to provide adequate forage for excess wild horses. This alternative was not analyzed in detail because it does not meet the Purpose and Need to manage wild horses within the AML. Livestock grazing is an authorized use under FPLMA and the areas within the HMAs are open to livestock grazing under the Lander RMP.

While the BLM is authorized to remove livestock from HMAs, "if necessary to provide habitat for wild horses or burros, to implement herd management actions, or to protect wild horses or burros from disease, harassment or injury" (43 CFR 4710.5), this authority is usually applied in cases of emergency and not for routine management of wild horses since it cannot be applied in a manner that would be consistent with the existing land use plans (43 CFR 4710.1).

Affected Environment and Environmental Effects

General Setting and Geographic Scope of the Project Area

The area covered by this analysis is within the jurisdiction of the BLM Lander Field Office, Wyoming. The four HMAs listed in Figure 1 encompass approximately 375,292 acres of public, private and state land, all within Fremont County in central Wyoming, (see Appendix D, HMA Maps). Topography consists of rolling mesas with defined drainages with some mountains and badlands. The major topographic feature of the complex is Beaver Rim which bisects part of the complex and in other areas serves as a natural barrier and border. Elevation varies from approximately 5,300 feet to 7,548 feet. Summers are hot, and winters can range from mild to bitterly cold. Annual precipitation varies with elevation and topography throughout the complex from 6-16 inches with most of the complex falling in the 6-11 inch range. Some of this water is captured in reservoirs or pits. Flowing wells, springs, and creeks are the primary sources of water for wild horses, livestock, and wildlife within these HMAs. Snow can also provide limited seasonal water. The vegetation within these HMAs is comprised primarily of sagebrush steppe and includes a limited amount of mixed juniper and limber pine woodlands.

Resources Considered and Eliminated From Further Analysis

Resources and features not present or not affected by the proposed action or alternatives; and not discussed in this EA can be found in Appendix A.

Resources Brought Forward for Analysis Wild Horse and Burros

Issue(s) Identified

Issue 1: How would the proposed population growth suppression activities affect wild horses?

Affected Environment

The estimated wild horse population within the North Lander complex of HMAs was 1,664 on March 1st of 2021 (this was also the assumed population size in November 2020). With the addition of foals that are expected to be born in 2021 and 2022 (the 2021 and 2022 foal crops), and accounting for survival rate of foals and adults, the population is estimated to grow at an annual rate of 20% per year, and to reach 2,396 horses (see Figure 2) by the fall of 2022, if no gather takes place before then. These HMAs were last gathered in November of 2012. At that time 754 horses were gathered and an estimated 71 were not. Of the gathered horses, 194 studs were released, and 152 mares were released for an estimated post gather population size of 417 horses. 145 of the 152 mares received PZP treatments. The post gather stud to mare ratio was approximately 56% to 44%. Because the effects of PZP and mare to stud ratio skewing are not permanent, the BLM estimates that, at this time, approximately 50% of the wild horses are studs and 50% are mares, and that all breeding age wild horses are currently able to bear offspring. No other population growth suppression tactics have been used in these HMAs in the last decade. Even with the population growth suppression techniques used after the 2012 gather, the herd growth from 417 horses in November 2012 to 1,664 horses 8 years later in November 2020 indicates an approximate growth rate of 18.9% per year. This rate is very close to the assumed 20% per year that the BLM generally uses in population projections.

Environmental Effects

No Action

Since no population growth suppression strategies would be utilized under the No Action alternative, this alternative would have no direct impact on wild horses. However, there would still be excess wild horses present on these HMAs, and a thriving natural ecological balance would not be maintained. Over

time, food, water, cover and space would not be adequate to support the growing wild horse population in these HMAs. When this occurs, there would be negative impacts to wild horses, as there would be inadequate resources to sustain the population on the range.

When there is an overpopulation of wild horses on the range, there would be an overall degradation of habitat qualities for wild horses, which would negatively impact the overall health of the wild horses in the population. This alternative would result in the wild horses being more concentrated, experiencing more competition for resources, and there would be more trailing and concentrated use near water sources. This would result in more fighting among horses accessing water sources. Water quality and quantity would degrade over time to the detriment of all rangeland users, including wild horses. Wild horses would also have to travel a greater distance back and forth between water and desirable foraging areas. If an overpopulation of wild horses were to continue on the range, it would eventually lead to large-scale degradation of rangeland habitat and large-scale die-offs of wild horses and other wildlife due to starvation.

Gather Only

Under this alternative, excess wild horses would be gathered and removed from these HMAs, but no population growth suppression strategies would be implemented. Therefore, there would be no direct impacts to wild horses as a result of these strategies. However, failure to take action to control the growth rate of the wild horse population in these areas would require more frequent gathers in future years. Under this alternative, the expected future gather frequency for these HMAs would be approximately every 3 years, compared to every 4 years or more under the proposed action. Therefore, stress to wild horses as a result of future gather operations is expected to be higher.

Proposed Action

Under the Proposed Action several population growth suppression strategies would be utilized: the immunocontraceptive vaccine GonaCon, IUDs, male sterilization, and skewed sex ratios. Any combination of these tools may be used. This analysis is intended to summarize the potential effects of these treatment methods so that the right tool can be used at the right time. More detailed information, including a literature review related to all the population growth suppression strategies and their potential effects, is provided in Appendix E.

Immunocontraceptive vaccines and IUDs are administered only to breeding age mares. Because the BLM would not gather the entire herd under this alternative, there would be approximately 5 – 20% of the herd remaining that would not undergo any fertility control treatment in any given gather, and would still be able to breed normally. Additionally, not all treatments would be successful, in the sense that fertility control vaccine efficacy is less than 100%, and the vaccines under consideration can have effects that may last from one to several years, in the absence of a booster shot. Thus, some animals are still able to successfully breed after receiving an immunocontraceptive vaccine. Similarly, it is expected that IUDs will fall out of some treated mares, after which point those mares will return to fertility. However, even if only a fraction of the mares in a herd are successfully treated, that fraction of successfully treated mares can lead to a decrease in the overall fertility rate and a corresponding decrease in the realized annual growth rate for the population. In most cases, immunocontraceptive vaccines appears to be temporary and reversible, with most treated mares returning to fertility over time (see Appendix E).

Contraception has been shown to be a humane treatment to slow increases in wild horse populations or, when used with other techniques, to reduce horse population size (Bartholow 2004, de Seve and Boyles-Griffin 2013). All fertility control methods in wild animals are associated with potential risks and benefits, including effects of handling, frequency of handling, physiological effects, behavioral effects,

and reduced population growth rates (Hampton et al. 2015). Although contraceptive treatments may be associated with a number of effects, those concerns do not generally outweigh the potential benefits of using contraceptive treatments in situations where it is a management goal to reduce population growth rates (Garrott and Oli 2013).

Successful contraception would be expected to reduce the frequency and size of future wild horse gathers and their associated impacts. Under this alternative, after implementing population growth suppression strategies, the expected future gather frequency for these HMAs would be approximately every 4 years or longer.

Selectively applying contraception to older animals and returning them to the HMA could reduce the compensatory reproduction that often follows removals (Kirkpatrick and Turner 1991). On the other hand, selectively applying contraception to younger animals can slow the rate of genetic diversity loss – a process that tends to be slow in populations of long-lived animal with high levels of genetic diversity – and could reduce growth rates further by delaying the age of first parturition (Gross 2000).

Mares that undergo fertility control treatments would have increased stress from additional handling by humans. Most mares recover from the stress of capture and handling quickly once released back to the range, and none are expected to suffer long term direct effects from the fertility control treatments, other than becoming temporarily infertile.

Biological stress refers to the increased physical demands on a mare's body when pregnant and lactating. All phases of reproduction put stress on a mare. Pregnant mares have much higher energy and nutrient requirements than open mares. Foaling can be physically draining for a mare and can result in death or disease especially if mares are in poor condition leading up to foaling. Lactation also results in a higher energy demand. Mares can get bred approximately 6 days after foaling. This results in a mare needing energy for lactation, fetal development, and her own physical maintenance all at once. Consuming adequate forage of a high enough quality becomes increasingly difficult as populations increase, and range conditions deteriorate. When horses have to compete for available resources they often have to travel longer distances to get sufficient food and water, which increases their energy demands. One expected long-term, indirect effect on wild horses treated with fertility control would be a reduction in the biological stress associated with reproduction, foaling and lactation, which would lead to an improvement in their overall health (Turner and Kirkpatrick 2002). After a treated mare returns to fertility, her future foals would likely be healthier, and would benefit from improved nutritional quality in the mare's milk. This is particularly to be expected if there is an improvement in rangeland forage quality at the same time, as a result of managing wild horses within AML and maintaining a TNEB.

Following resumption of fertility, the proportion of mares that conceive and foal could be increased due to their increased fitness; this has been called a 'rebound effect.' Elevated fertility rates have been observed after horse gathers and removals (Kirkpatrick and Turner 1991). If repeated contraceptive treatment leads to a prolonged contraceptive effect, then that may minimize or delay the hypothesized rebound effect. Selectively applying contraception to older animals and returning them to the range could reduce the compensatory reproduction that often follows removals (Kirkpatrick and Turner 1991).

Contraception may change a herd's age structure, with a relative increase in the fraction of older animals in the herd (NPS 2008). Reducing the numbers of wild horses that would have to be removed in future gathers could allow for removal of younger, more easily adoptable excess wild horses, and thereby could reduce the need to send additional excess horses from this area to off-range holding corrals or pastures for long-term holding.

A principal motivation for using population growth suppression strategies is to reduce population growth rates and maintain herd sizes within AML. Where successful, this would promote improvements

in range conditions within these HMAs and achieve a thriving natural ecological balance. This would improve habitat qualities for wild horses, promoting an overall healthier wild horse population. This alternative would result in the wild horses being less concentrated, experiencing less competition for resources, and there would be less trailing and concentrated use near water sources. This would result in less fighting among horses accessing water sources. Water quality and quantity would continue to improve to the benefit of all rangeland users including wild horses. Wild horses would also have to travel less distance back and forth between water and desirable foraging areas. Among mares in the herd that remain fertile, a higher level of physical health and future reproductive success would be expected in areas where lower horse population sizes lead to increases in water and forage resources.

Potential impacts to genetic diversity associated with this alternative are discussed later in this document.

Immunocontraceptive Vaccines

Immunocontraceptive vaccines induce an immune response that causes treated animals to become temporarily infertile. Injection site reactions, including swelling, associated with immunocontraceptive treatments are possible in treated mares (Roelle and Ransom 2009, Bechert et al. 2013, French et al. 2017, Baker et al. 2018), but swelling or local reactions at the injection site are expected to be minor in nature. The primary immunocontraceptive vaccines currently utilized by the BLM include PZP vaccines and GonaCon-Equine. PZP vaccines are unlikely to be the ideal fertility control vaccine use in this complex, for reasons previously described under "Alternatives Considered and Eliminated from Detailed Analysis" (relatively short duration of effectiveness). A detailed description of PZP vaccine effects is included in Appendix E. Based on the longer-lasting effectiveness of GonaCon-Equine after a booster dose is given (Baker et al. 2018), it is more likely that that vaccine would be a good choice for this complex of HMAs. A summarized description of the direct and indirect effects of GonaCon-Equine is provided below, with a more detailed description in Appendix E:

GonaCon-Equine Vaccine

GonaCon-Equine (GonaCon) is approved for application to free-ranging wild horse herds in the United States (EPA 2013, 2015). GonaCon-Equine has been used on feral horses in the Theodore Roosevelt National Park and on wild horses managed by the BLM (BLM 2015). GonaCon-Equine would be applied to treated mares using a large gauge needle and jab-stick into the hip.

As with other contraceptives applied to wild horses, the long-term goal of GonaCon-Equine is to reduce or eliminate the need for gathers and removals (NRC 2013). GonaCon-Equine is an EPA-approved contraceptive vaccine (EPA 2013, 2015) that meets BLM requirements for safety to mares and the environment, and is produced in a USDA-APHIS laboratory. GonaCon-Equine is a pharmaceutical-grade vaccine, made with aseptic manufacturing technique to deliver a sterile vaccine product (Miller et al. 2013).

GonaCon-Equine can safely be reapplied as necessary to control the population growth rate; booster dose effects may lead to increased effectiveness of contraception (Baker et al. 2018). Even after booster treatment of GonaCon, it is expected that most mares would return to fertility at some point. Although the exact timing for the return to fertility in mares boosted more than once with GonaCon-Equine has not been quantified, a prolonged return to fertility would be consistent with the desired effect of using GonaCon-Equine (e.g., effective contraception). Females that are successfully contracepted by GonaCon-Equine enter a state similar to anestrus, have a lack of or incomplete follicle maturation, and no ovarian cycling (Botha et al. 2008, Nolan et al. 2018). The lack of estrus cycling that results from successful GonaCon-Equine vaccination has been compared to typical winter period of

anestrus in open mares. Mares treated with GonaCon-Equine would be expected to have a better overall body condition and may have a higher likelihood of survival (Goodloe 1991).

Kirkpatrick et al. (2011) raised concerns that anti-GnRH vaccines (like GonaCon) could lead to adverse effects in other organ systems outside the reproductive system. GnRH receptors have been identified in tissues outside of the pituitary system, including in the testes and placenta (Khodr and Siler-Khodr 1980), ovary (Hsueh and Erickson 1979), bladder (Coit et al. 2009), heart (Dong et al. 2011), and central nervous system, so it is plausible that reductions in circulating GnRH levels could inhibit physiological processes in those organ systems. However, anti-GnRH vaccines (like GonaCon) have been used on horses and other animals, including wildlife such as prairie dogs, elk, giraffes, goats, elephants, bison and deer, and no adverse impacts of the type hypothesized due to interactions with anti-GnRH antibodies and other organ systems have been documented in these species. Since GnRH is highly utilized across mammalian taxa, some inferences about the mechanism and effects of GonaCon-Equine in horses can be made from studies that used different anti-GnRH vaccines, in horses and other animals.

A single dose of GonaCon-Equine to wild horses would be expected to prevent pregnancy in 30%-60% of mares for one year. A smaller number of those mares would be expected to have persistent contraception for a second year, and less still for a third year. Applying one booster dose of GonaCon-Equine to previously treated mares may lead to four or more years with relatively high rates (80+%) of additional infertility expected (Baker et al. 2018).

Although fetuses are not explicitly protected under the WFRHBA, it is prudent to analyze the potential effects of fertility control vaccines on developing fetuses and foals. Any impacts identified in the literature have been found to be transient, and do not influence the future reproductive capacity of offspring born to treated females. GonaCon-Equine can be injected while a female is pregnant (Miller et al. 2008, Powers et al. 2011, Baker et al. 2013). In these cases, a successfully contracepted mare will be expected to give birth during the following foaling season, but to be infertile during the same year's breeding season. GonaCon-Equine had no apparent effect on pregnancies in progress, foaling success, or the health of offspring, in horses (Baker et al. 2013), elk (Powers et al. 2011, 2013), or deer (Miller et al. 2008). Studies have also found that anti GnRH vaccines (like GonaCon) did not affect the fertility of offspring born to treated animals (Powers et al. 2012).

It is possible that immunocontracepted mares returning to fertility late in the breeding season could give birth to foals at a time that is out of the normal range (Nuñez et al. 2010, Ransom et al 2013). However, there were no published differences in mean date of foal production in anti-GnRH vaccine trials in free-roaming horses (Goodloe 1991, Gray et al. 2010). Moreover, in PZP-treated horses that did have some degree of parturition date shift, Ransom et al. (2013) found no negative impacts on foal survival even with an extended birthing season. Similarly, we anticipate that GonaCon-Equine would not affect foal survival even with an extended birthing season.

Mares treated with GonaCon-Equine may be expected to behave similarly to pregnant mares, as a result of having suppressed estrous cycles throughout the breading season. Because of this, any concerns about PZP treated mares receiving more courting and breeding behaviors from stallions (Nuñez et al. 2009, Ransom et al. 2010) are not generally expected to be a concern for mares treated with anti-GnRH vaccines (Botha et al. 2008).

Mares treated with GonCon are likely to exhibit behavior similar to pregnant mares (Ransom et al. 2014b, Barker et al. 2018). This may lead to a reduction in reproductive behavior that may continue for a time, even after the mares resume estrus cycles (Elhay et al. 2007). GonaCon-Equine is not expected to cause an increase in harem infidelity in treated mares, because it is expected that they would behave similarly to a pregnant mare (Ransom et al. 2014b).

More detailed information regarding GonaCon-Equine is provided in Appendix E.

<u>IUDs</u>

Based on promising results from published, peer-reviewed studies in domestic mares, BLM has begun to use IUDs to control fertility as a wild horse and burro fertility control method on the range. The initial management use was in mares from the Swasey HMA, in Utah. Wild mares in several HMAs near Rock Springs, Wyoming, have been contracepted with IUDs. It is too early to know the duration of effects of that treatment procedure for those particular mares. However, IUDs have been used in domestic horses for many years. Existing scientific literature on the use of IUDs in domestic horses allows for inference about expected effects on wild horses. This literature supports that use of certain types of IUDs would be a safe and effective method of fertility control in wild horses. Overall, as with other methods of population growth suppression, use of IUDs and other fertility control measures are expected to help reduce population growth rates, extend the time interval between gathers, and reduce the total number of excess animals that will need to be removed from the range.

The 2013 National Academies of Sciences (NAS) report considered IUDs, and suggested that research should test whether IUDs cause uterine inflammation, and should also test how well IUDs stay in mares that live and breed with fertile stallions. Since that report, a recent study by Holyoak et al. (2021) indicate that a flexible, inert, y-shaped, medical-grade silicone IUD design prevented pregnancies in all the domestic mares that retained the device, even when exposed to fertile stallions. Domestic mares in that study lived in large pastures, mating with fertile stallions. Biweekly ultrasound examinations showed that IUDs stayed in 75% of treated mares over the course of two breeding seasons. The IUDs were then removed so the researchers could monitor the mares' return to fertility. In that study, uterine health, as measured in terms of inflammation, was not seriously affected by the IUDs, and most mares became pregnant within months after IUD removal. The overall results are consistent with results from an earlier study (Daels and Hughes 1995), which used O-shaped silicone IUDs. Similarly, a flexible IUD with three components connected by magnetic force (the 'iUPOD') was retained over 90 days in mares living and breeding with a fertile stallion; after IUD removal, the majority of mares became pregnant in the following breeding season (Hoopes et al. 2021).

IUDs are considered a temporary fertility control method that does not generally cause future sterility (Daels and Hughes 1995). Use of IUDs is an effective fertility control method in women, and IUDs have historically been used in livestock management, including in domestic horses. Insertion of an IUD can be a very rapid procedure, but it does require the mare to be temporarily restrained, such as in a squeeze chute. IUDs in mares may cause physiological effects including discomfort or infection. Perforation of the uterus could result if the IUD is hard and angular, but is unlikely if the IUD is soft and flexible. Endometritis, uterine edema (Killian et al. 2008), and pyometra (Klabnik-Bradford et al. 2013) could result, though studies listing those possibilities used hard IUDs.

The exact mechanism by which IUDs prevent pregnancy is uncertain, but may be related to persistent, low-grade uterine inflammation (Daels and Hughes 1995, Gradil et al. 2021, Hoopes et al. 2021), Turner et al. (2015) suggested that the presence of an IUD in the uterus may, like a pregnancy, prevent the mare from coming back into estrus. However, some domestic mares did exhibit repeated estrus cycles during the time when they had IUDs (Killian et al. 2008, Gradil et al. 2019, Lyman et al. 2021, Hoopes et al. 2021). The main cause for an IUD to not be effective at contraception is its failure to stay in the uterus (Daels and Hughes 1995, NAS 2013). As a result, one of the major challenges to using IUDs to control fertility in mares on the range is preventing the IUD from being dislodged or otherwise ejected over the course of daily activities, which could include, at times, frequent breeding.

At this time, it is thought that any IUD inserted into a pregnant mare may cause the pregnancy to terminate, which may also cause the IUD to be expelled. For that reason, IUDs would only be inserted in

non-pregnant (open) mares. Wild mares receiving IUDs would be checked for pregnancy by a veterinarian prior to insertion of an IUD. This can be accomplished by transrectal palpation and/or ultrasound performed by a veterinarian. Pregnant mares would not receive an IUD. The IUD is inserted into the uterus using a thin, tubular applicator similar to a shielded culture tube, and would be inserted in a manner similar to that routinely used to obtain uterine cultures in domestic mares. If a mare has a zygote or very small, early phase embryo, it is possible that it will fail to be detected in screening, and may develop further, but without causing the expulsion of the IUD. Wild mares with IUDs would be individually marked and identified, so that they can be monitored occasionally and examined, if necessary, in the future, consistent with other BLM management activities.

Using metallic or glass marbles as IUDs may prevent pregnancy in horses (Nie et al. 2003), but can pose health risks to domestic mares (Turner et al. 2015, Freeman and Lyle 2015). Marbles may break into shards (Turner et al. 2015), and uterine irritation that results from marble IUDs may cause chronic, intermittent colic (Freeman and Lyle 2015). Metallic IUDs may cause severe infection (Klabnik-Bradford et al. 2013).

In domestic ponies, Killian et al. (2008) explored the use of three different IUD configurations, including a silastic polymer O-ring with copper clamps, and the "380 Copper T" and "GyneFix" IUDs designed for women. The longest retention time for the three IUD models was seen in the "T" device, which stayed in the uterus of several mares for 3-5 years. Reported contraception rates for IUD-treated mares were 80%, 29%, 14%, and 0% in years 1-4, respectively. They surmised that pregnancy resulted after IUD fell out of the uterus. Killian et al. (2008) reported high levels of progesterone in non-pregnant, IUD-treated ponies.

Soft or flexible IUDs may cause relatively less discomfort than hard IUDs (Daels and Hughes 1995). Daels and Hughes (1995) tested the use of a flexible O-ring IUD, made of silastic, surgical-grade polymer, measuring 40 mm in diameter; in five of six breeding domestic mares tested, the IUD was reported to have stayed in the mare for at least 10 months. In mares with IUDs, Daels and Hughes (1995) reported some level of uterine irritation, but surmised that the level of irritation was not enough to interfere with a return to fertility after IUD removal.

More recently, several types of soft or flexible IUDs have been tested for use in breeding mares. When researchers attempted to replicate the O-ring study (Daels and Hughes 1995) in an USGS / Oklahoma State University (OSU) study with breeding domestic mares, using various configurations of silicone Oring IUDs, the IUDs fell out at unacceptably high rates over time scales of less than 2 months (Baldrighi et al. 2017, Lyman et al. 2021). Subsequently, the USGS / OSU researchers tested a Y-shaped IUD to determine retention rates and assess effects on uterine health; retention rates were greater than 75% for an 18-month period, and mares returned to good uterine health and reproductive capacity after removal of the IUDs (Holyoak et al. 2021). These Y-shaped silicone IUDs are considered a pesticide device by the EPA, in that they work by physical means (EPA 2020). The University of Massachusetts has developed a magnetic IUD that has been effective at prolonging estrus and preventing pregnancy in domestic mares (Gradil et al. 2019, Joonè et al. 2021, Gradil et al. 2021, Hoopes et al. 2021). After insertion in the uterus, the three subunits of the device are held together by magnetic forces as a flexible triangle. A metal detector can be used to determine whether the device is still present in the mare. In an early trial, two sizes of those magnetic IUDs fell out of breeding domestic mares at high rates (Holyoak et al., unpublished results), but more recent trials have shown that the magnetic IUD was retained even in the presence of breeding with a fertile stallion (Hoopes et al. 2021). The magnetic IUD was used in two trials where mares were exposed to stallions, and in one where mares were artificially inseminated; in all cases, the IUDs were reported to stay in the mares without any pregnancy (Gradil 2019, Joonè et al. 2021, Gradil et al. 2021, Hoopes et al. 2021).

More detailed information regarding IUDs is provided in Appendix E.

Male Sterilization

Gelding is the surgical removal of the testicles of a male horse. It is also commonly called castration or neutering. This procedure has been used on horses for thousands of years, in many different societies. Vasectomy involves severing or blocking the vas deferens or epididymis, to prevent sperm from being ejaculated. The procedures are fairly straight forward and have a relatively low complication rate. As noted in the review of scientific literature that follows, the expected effects of gelding and vasectomy are well understood overall, even though there is some degree of uncertainty about the exact quantitative outcomes for any given individual (as is true for any natural system).

Horses that are gelded or vasectomized will no longer be able to reproduce for the remainder of their life. The effectiveness of male sterilization in terms of reducing herd-level annual growth rates is somewhat limited, however, due to the fact that a small number of fertile studs can successfully breed most fertile mares. Therefore, for these procedures to be successfully used to reduce population growth rates, they must be paired with a strategy to also reduce the overall number of fertile females in the herd (such as spaying and/or skewing the ratio of studs to mares).

As part of BLM's SOPs, animals that are candidates for male sterilization will be screened prior to the procedure to ensure they are in adequate health to safely undergo the treatment. The surgery would be performed by a veterinarian using general anesthesia. The final determination of which specific animals would be gelded would be based on the professional opinion of the attending veterinarian in consultation with the Authorized Officer.

Though gelding males is a common surgical procedure, some level of minor complications after surgery may be expected (Getman 2009). The most common complications are almost always self-limiting, resolving with time and exercise. Individual impacts to the stallions during and following the gelding process should be minimal and would mostly involve localized swelling and bleeding. Complications may include, but are not limited to: minor bleeding, swelling, inflammation, edema, infection, peritonitis, hydrocele, penile damage, excessive hemorrhage, and eventration (Schumacher 1996, Searle et al. 1999, Getman 2009). A small amount of bleeding is normal and generally subsides guickly, within 2-4 hours following the procedure. Some degree of swelling is normal, including swelling of the prepuce and scrotum, usually peaking between 3-6 days after surgery (Searle et al. 1999). Swelling should be minimized through the daily movements (exercise) of the horse during travel to and from foraging and watering areas. Most cases of minor swelling should be back to normal within 5-7 days, more serious cases of moderate to severe swelling are also self-limiting and are expected to resolve with exercise after one to 2 weeks. In some cases, a hydrocele (accumulation of sterile fluid) may develop over months or years (Searle et al. 1999). Serious complications (eventration, anesthetic reaction, injuries during handling, etc.) that result in euthanasia or mortality during and following surgery are rare (less than 5%). Serious complications are generally noted within 3 or 4 hours of surgery but may occur any time within the first week following surgery (Searle et al. 1999). If they occur, they would be treated with surgical intervention when possible, or with euthanasia when there is a poor prognosis for recovery. Possible complications from vasectomy would be similar to gelding. Treated studs would be monitored by a veterinarian to ensure they have recovered from the surgery before the veterinarian approves them to be released back onto the range.

It is expected that testosterone levels will decline over time after gelding, though geldings may still exhibit reproductive behaviors (Rios and Houpt 1995, Schumacher 2006). Testosterone levels alone are not a predictor of masculine behavior (Line et al. 1985, Schumacher 2006). In domestic geldings, 20-30% continued to show stallion-like behavior. It is assumed that free roaming wild horse geldings would generally exhibit reduced aggression toward other horses and reduced reproductive behaviors (NRC

2013). Preliminary results from a study in the Conger HMA in Utah seem to suggest that gelded males may retain their harems for some time after gelding, but that over time they may lose harems at higher rates than intact stallions do (K. Schoenecker, USGS, unpublished data). Geldings may have a higher survival rate than fertile stallions (Jewell 1997). This is likely due to the decreased energy expenditures associated with reproduction and defending harems. Geldings may continue to behave like a harem stallion, or they may lose their harems and take on the role of a satellite male, or may join bachelor bands. All of these behaviors have been observed in geldings and seem to vary due to a number of social and environmental circumstances. However, it appears that gelded wild stallions continue to move freely throughout the environment in patterns that are similar to untreated wild horses (K. Schoenecker, USGS, unpublished data).

Testosterone levels should not change due to vasectomy. Vasectomized stallions should retain their previous levels of libido. Vasectomized males continue to attempt to defend or gain breeding access to females. It is generally expected that vasectomized WH&B will continue to behave like fertile males, given that the only physiological change in their condition is a lack of sperm in their ejaculate. If a vasectomized stallion retains a harem, the females in the harem will continue to cycle until they are fertilized by another stallion, or until the end of the breeding season. As a result, the vasectomized stallion may be involved in more aggressive behaviors to other males through the entire breeding season (Asa 1999), which may divert time from foraging and cause him to be in poorer body condition going into winter. Ultimately, this may lead to the stallion losing control of a given harem. A feral horse herd with high numbers of vasectomized stallions retained typical harem social structure (Collins and Kasbohm 2016). Again, it is worth noting that the BLM is not required to manage populations of wild horses in a manner that ensures that any given individual maintains its social standing within any given harem or band.

Sterilizing wild horses does not change their status as wild horses under the WFRHBA (as amended). In terms of whether geldings will continue to exhibit the free-roaming behavior that defines wild horses, BLM does expect that geldings would continue to roam unhindered once they are returned to the range. Wild horse movements may be motivated by a number of biological impulses, including the search for forage, water, and social companionship that is not of a sexual nature. As such, a gelded or vasectomized animal would still be expected to have a number of internal reasons for moving across a landscape and, therefore, exhibiting 'free-roaming' behavior. Despite marginal uncertainty about subtle aspects of potential changes in habitat preference, there is no expectation that gelding or vasectomizing wild horses will cause them to lose their free-roaming nature. It is worth noting that individual choices in wild horse group membership, home range, and habitat use are not protected under the WFRHBA. BLM acknowledges that geldings or vasectomized males may exhibit some behavioral differences after surgery, compared to intact stallions, but those differences are not expected to remove the geldings' or vasectomized males' rebellious and feisty nature, or their defiance of man. While it may be that a gelded or vasectomized wild horse could have a different set of behavioral priorities than an intact stallion, the expectation is that geldings and vasectomized males will choose to act upon their behavioral priorities in an unhindered way, just as is the case for an intact stallion. In this sense, a gelded or vasectomized male would be just as much 'wild' as defined by the WFRHBA as any intact stallion, even if his patterns of movement differ from those of an intact stallion. Congress specified that sterilization is an acceptable management action (16 USC § 1333.b.1).

More detailed information regarding gelding is provided in Appendix E.

Mare to Stud Ratio Skewing

Mare to stud ratio skewing (also known as sex ratio skewing) involves adjusting the ratio of mares to studs so that there are slightly more males present in the population than females. Under this

alternative, after gathering wild horses, the number returned back onto the range would consist of approximately 60% males and 40% females. Since, with wild horses, the number of actively breeding females is the primary factor determining population growth rates, reducing the number of breeding females can slow the population growth rate, and reduce the frequency of gathers, and the number of wild horses removed from the range. In the absence of other fertility control treatments, a 60:40 sex ratio can temporarily reduce population growth rates from approximately 20% to approximately 15% (Bartholow 2004). Combined with spaying, gelding and immunocontraceptive vaccines, the actual population growth rate would be expected to be less than 15% under this alternative. Because foals are born at rates with close to equal numbers of male and females born in any given year, over time the mare to stud ratio would be expected to return to approximately 50:50, with the impacts associated with this action being reduced over time.

Having a larger number of males than females is expected to lead to several demographic and behavioral changes as reviewed in the NAS report (NRC 2013). Having more fertile males than females should not alter the fecundity of fertile females. Wild mares may be distributed in a larger number of smaller harems. Increased competition and aggression between males may cause a decline in male body condition. Female foraging may be somewhat disrupted by elevated male-male aggression. With a greater number of males available to choose from, females may have opportunities to select more genetically fit sires. There would also be an increase in the genetic effective population size because more stallions would be breeding and existing females would be distributed among many more small harems. This last beneficial impact is one reason that skewing the sex ratio to favor males is listed in the BLM wild horse and burro handbook (BLM 2010) as a method to consider in herds where there may be concern about the loss of genetic diversity; having more males fosters a greater retention of genetic diversity. There are no published accounts of infanticide rates increasing as a result of having a skewed sex ratio in wild horse herds, so this is not expected to be a concern associated with this activity.

The preceeding paragraph details some of the expected results of a skewed sex ratio when both males and females are fertile. Under the proposed action it is likely that implementation of a skewed sex ratio would be done in conjunction with one or more of the previously described fertility control methods above. These treatments would most likely lessen the impacts of a skewed sex ratio. Both GonaCon-Equine and IUDs may cause mares to behave like they are pregnant during a greater portion of the breeding season, with reduced reproductive behavior. In conjunction with a high percentage of geldings in the population that should also eventually exhibit reduced sexual behavior the impacts of a skewed sex ratio are greatly lessened.

It is relatively straightforward to speed the return of skewed sex ratios back to a 50:50 ratio. The BLM wild horse and burro handbook (BLM 2010) specifies that, if post-treatment monitoring reveals negative impacts to breeding harems due to sex ratio manipulation, then mitigation measures could include removing males, not introducing additional males, or releasing a larger proportion of females during the next gather.

More detailed information regarding mare to stud ratio skewing (aka sex ratio skewing) is provided in Appendix E.

Issue 2: How would gather operations affect wild horses?

Affected Environment

Wild horses were present in the project area at the time the WFRHBA was signed in 1971. It is unknown exactly how long wild horses have populated these specific areas, but wild horses have been in Wyoming for over 100 years. The AML for these HMAs was most recently established by the 2014 Lander RMP. The AML for each of these HMAs is provided in Table 2.

There were an estimated 1664 adult wild horses present in these four HMAs as of March 1, 2021. This number is based on the most recent wild horse population surveys that took place in August of 2020. The complex was last gathered in November of 2012. At the conclusion of that gather the complex population was estimated to be 417 horses. Based on these numbers the average annual growth rate between 2012 and 2021 is approximately 19%.

Environmental Effects

The proposed action would involve gathering and removing excess wild horses, and placing them in off range corrals and pastures. The following discussion describes impacts associated with this process.

Gather Related Impacts

The BLM has been conducting wild horse gathers since the mid-1970s. During this time, methods and procedures have been identified and refined to minimize stress and effects to wild horses during gather operations. The SOPs in Appendix G would be implemented to ensure a safe and humane gather operation and would minimize potential stress and injury to wild horses.

Wild horse gathers that utilize helicopters and motorized vehicles result in gather-related mortality averages approximately 1% (Scasta 2020). Approximately six-tenths of one percent (0.6%) of the captured animals could potentially require humane euthanasia due to pre-existing conditions and in accordance with BLM policy (GAO 2008). These data confirm that the use of helicopters and motorized vehicles has proven to be a safe, humane, effective, and practical means for the gather and removal of excess wild horses (and burros) from the public lands.

As a further measure, it is BLM policy to only use helicopters to assist in the removal of wild horses from July 1 through February 28. The use of helicopters to assist in the capture of wild horses is prohibited during the six weeks before and the six weeks that follow peak foaling. The peak of foaling falls within about a two-week period during mid-April to mid-May for most wild horse herds. Therefore, the use of helicopters to capture wild horses is prohibited during March 1-June 30, except in emergencies.

Individual, direct effects to wild horses include the handling stress associated with the gathering, capture, sorting, handling, and transportation of the animals. The intensity of these effects varies by individual horse and is indicated by behaviors ranging from nervous agitation to physical distress. When being herded to trap site corrals by the helicopter, wild horses may sustain injuries bruises, scrapes, or cuts to feet, legs, face, or body from rocks, brush or tree limbs. Rarely will wild horses encounter barbed wire fences and will receive wire cuts. These injuries are very rarely fatal and are treated on-site until a veterinarian can examine the animal and determine if additional treatment is necessary.

Other injuries may occur after a wild horse has been captured and is either within the trap site corral, the temporary holding corral, during transport between facilities, or during sorting and handling. Occasionally, wild horses may sustain a spinal injury or a fractured limb but serious injuries requiring humane euthanasia occur in less than 1% of wild horses captured, on average (Scasta 2020). Similar injuries could be sustained if wild horses were captured through bait and/or water trapping, as the animals still need to be sorted, aged, transported, and otherwise handled following their capture. These injuries result from kicks and bites, or from collisions with corral panels or gates.

To minimize the potential for injuries from fighting, the animals are transported from the trap site to the temporary holding facility where they are sorted as quickly and safely as possible, then moved into large holding pens where they are provided with hay and water. On many gathers, no wild horses are injured or die. On some gathers, due to the temperament of the horses, they are not as calm and injuries are more frequent.

Indirect individual effects are those which occur to individual wild horses after the initial event. These may include miscarriages in mares, increased social displacement, and conflict in studs. These effects, like direct individual effects, are known to occur intermittently during wild horse gather operations. An example of an indirect individual impact would be the brief 1-2 minute skirmish between older studs, which ends when one stud retreats. Injuries typically involve a bite or kick with bruises which do not break the skin. Like direct individual effects, the frequency of these effects varies with the population and the individual. Observations following capture indicate the rate of miscarriage varies, but can occur in about 1% to 5% of the captured mares, particularly if the mares are in very thin body condition or in poor health.

A few foals may be orphaned during a gather. This can occur if the mare rejects the foal, the foal becomes separated from its mother and cannot be matched up following sorting, the mare dies or must be humanely euthanized during the gather, the foal is ill or weak and needs immediate care that requires removal from the mother, or the mother does not produce enough milk to support the foal. On occasion, foals are gathered that were previously orphaned on the range (prior to the gather) because the mother rejected it or died. These foals are usually in poor, unthrifty condition. Every effort is made to provide appropriate care to orphan foals. Veterinarians may be called to administer electrolyte solutions or orphan foals may be fed milk replacer as needed to support their nutritional needs. Orphan foals may be placed in a foster home in order to receive additional care. Despite these efforts, some orphan foals may die or be humanely euthanized as an act of mercy if the prognosis for survival is very poor.

Through the capture and sorting process, wild horses are examined for health, injury and other defects using the humane care and treatment methods as described in BLM PIM 2021-002. Decisions to humanely euthanize animals in field situations would be made in conformance with BLM policy. The policy described in PIM 2021-007 is used as a guide to determine if animals meet the criteria and should be euthanized. Animals that are euthanized for non-gather related reasons include those with old injuries (broken or deformed limbs) that cause lameness or prevent the animal from being able to maintain an acceptable body condition (greater than or equal to body condition score of 3); old animals that have serious dental abnormalities or severely worn teeth and are not expected to maintain an acceptable body condition, and wild horses that have serious physical defects such as club feet, severe limb deformities, or sway back. Many of these defects can cause pain to the affected animal. Some of these conditions have a causal genetic component and the animals should not be returned to the range to avoid amplifying the incidence of the problem in the population. All euthanasia activities would be conducted using methods acceptable to the American Veterinary Medical Association (AVMA).

Wild horses not captured may be temporarily disturbed and moved into another area during the gather operation. With the exception of changes to herd demographics from removals, direct population effects have proven to be temporary in nature with most, if not all, effects disappearing within hours to several days of release. No observable effects associated with these impacts would be expected within one month of release, except for a heightened awareness of human presence.

By maintaining wild horse population size within the AML, there would be a lower density of wild horses across the HMAs, reducing competition for resources and allowing wild horses to utilize their preferred habitat. Maintaining population size within the established AML would be expected to improve forage quantity and quality, and promote healthy, self-sustaining populations of wild horses in a thriving natural ecological balance and multiple use relationship on the public lands in the area. Deterioration of the range associated with wild horse overpopulation would be avoided. Managing wild horse populations in balance with the available habitat and other multiple uses would lessen the potential for individual animals or the herd to be affected by drought, and would avoid or minimize the need for emergency gathers, which would reduce stress to the animals and increase the success of these herds over the long term.

Gather and removal operations can disrupt harem structure when members of the harem are captured and removed. However, as a whole, gather and removal operations will not permanently disrupt the overall social structure of the herd. Harems will continue to form, stallions will defend their harems, and satellite males will continue to operate on the periphery of the harem.

Transport, Off Range Corrals, and Adoption (or Sale) Preparation Impacts

Total removal numbers cannot be estimated as the proposed action covers multiple gather events over a 10-year period which include multiple types of population control treatments. In the initial gather roughly 1,000 wild horses would be removed. The number of removals in subsequent gathers will be determined based on population monitoring results at or near the time of gather and will depend on the effectiveness of applied population control treatments. Captured animals would be transported from the capture/temporary holding corrals to the designated BLM off range corral (ORC, formerly short-term holding). From there, those selected for permanent removal would be made available for adoption or sale to qualified individuals or relocated to off range pastures.

Wild horses selected for removal from the range are transported to the receiving off range corral in a straight deck semi-trailers or goose-neck stock trailers. Vehicles are inspected by the BLM Contracting Officer's Representative (COR) or Project Inspector (PI) prior to use to ensure wild horses can be safely transported and that the interior of the vehicle is in a sanitary condition. Wild horses are typically segregated by age and sex and loaded into separate compartments. A small number of mares may be shipped with foals. Transportation of recently captured wild horses can include stress, as well as slipping, falling, kicking, biting, or being stepped on by another animal. Unless wild horses are in extremely poor condition, it is rare for an animal to be seriously injured or die during transport.

Upon arrival at the ORC, recently captured wild horses are off-loaded by compartment and placed in holding pens where they are fed good quality hay and water. Most wild horses begin to eat and drink immediately and adjust rapidly to their new situation. At the off range corral, a veterinarian examines each load of horses and provides recommendations to the BLM regarding care, treatment, and if necessary, euthanasia of the recently captured wild horses. Any animals affected by a chronic or incurable disease, injury, lameness or serious physical defect (such as severe tooth loss or wear, club feet, and other severe congenital abnormalities) would be humanely euthanized using methods acceptable to the AVMA. The BLM has established best management practices to ensure the health and safety of wild horses in ORCs. This includes isolating sick horses, and utilizing veterinarians to care for sick or injured horses, as well as vaccinating and deworming wild horses kept in off range facilities (PIM 2021-007).

Wild horses in very thin condition or animals with treatable injuries are sorted and placed in hospital pens, fed separately and/or treated for their injuries as indicated. Recently captured wild horses, generally mares, in very thin condition may have difficulty transitioning to feed. Some of these animals are in such poor condition that it is unlikely they would have survived if left on the range. Similarly, some mares may lose their pregnancies. Every effort is taken to help the mare make a quiet, low stress transition to captivity and domestic feed to minimize the risk of miscarriage or death.

After recently captured wild horses have transitioned to their new environment, they are prepared for adoption or sale. Preparation involves freeze-marking the animals with a unique identification number, drawing a blood sample to test for equine infections anemia, vaccination against common diseases, microchipping, castration, and de-worming. During the preparation process, potential effects to wild horses are similar to those that can occur during handling and transportation. Serious injuries and deaths from injuries during the preparation process are rare but can occur.

AtORCs, a minimum of 700 square feet is provided per animal. Mortality at ORCs averages approximately 5% per year (GAO 2008, page 51), and includes animals euthanized due to a pre-existing condition; animals in extremely poor condition; animals that are injured and would not recover; animals which are unable to transition to feed; and animals which are seriously injured or accidentally die during sorting, handling, or preparation.

Adoption or Sale with Limitations, and Off Range Pastures

Adoption applicants are required to have at least a 400-square-foot corral with panels that are at least six feet tall for horses over 18 months of age. Applicants are required to provide adequate shelter, feed, and water. The BLM retains title to the horse for one year and the horse and the facilities are inspected to assure the adopter is complying with BLM requirements. After one year, the adopter may take title to the horse, at which point the horse becomes the property of the adopter. Adoptions are conducted in accordance with 43 CFR 4750.

Potential buyers must fill out an application and be pre-approved before they may buy a wild horse. A sale-eligible wild horse is any animal that is more than 10 years old; or has been offered unsuccessfully for adoption three times. The application also specifies that all buyers are not to re-sell the animal to slaughter buyers or anyone who would sell the animal to a commercial processing plant. Sales of wild horses are conducted in accordance with IM 2019-026.

Potential effects to wild horses from transport to, adoption, sale or off range pastures (ORPs) are similar to those previously described. One difference is that when shipping wild horses for adoption, sale or ORPs, animals may be transported for a maximum of 24 hours. Immediately prior to transportation, and after every 18-24 hours of transportation, animals are offloaded and provided a minimum of 8 hours on-the-ground rest. During the rest period, each animal is provided access to unlimited amounts of clean water and approximately 25 pounds of good quality hay per horse with adequate bunk space to allow all animals to eat at one time. Most animals are not shipped more than 18 hours before they are rested. The rest period may be waived in situations where the travel time exceeds the 24-hour limit by just a few hours and the stress of offloading and reloading is likely to be greater than the stress involved in the additional period of uninterrupted travel.

ORPs are designed to provide excess wild horses with humane, life-long care in a natural setting off the public rangelands. There, wild horses are maintained in grassland pastures large enough to allow free-roaming behavior and with the forage, water, and shelter necessary to sustain them in good condition. More than 37,000 wild horses, that are in excess of the existing adoption or sale demand (because of age or other factors), are currently located on private land pastures in Iowa, Kansas, Missouri, Montana, Nebraska, Oklahoma, South Dakota, Utah, Washington, and Wyoming. Located mainly in mid or tall grass prairie regions of the United States, these ORPs are highly productive grasslands as compared to more arid western rangelands. These pastures comprise about 400,000 acres (an average of about 8-10 acres per animal). The majority of these animals are older in age.

Mares and geldings are segregated into separate pastures. Although the animals are placed in ORPs, they remain available for adoption or sale to qualified individuals who are interested in adopting or purchasing a larger number of animals. No reproduction occurs in the ORPs, but foals born to pregnant mares are gathered and weaned when they reach about 8-10 months of age and are then shipped to ORCs where they are made available for adoption. Handling by humans is minimized to the extent possible A very small percentage of the animals may be humanely euthanized if they are in very thin condition and are not expected to improve to a body condition score of 3 or greater due to age or other factors. Natural mortality of wild horses in off range pastures averages approximately 8% per year, but can be higher or lower depending on the average age of the horses pastured there (GAO 2008, page 52).

Euthanasia and Sale without Limitation

While the WFRHBA authorizes humane euthanasia and sale without limitation of healthy horses for which there is no adoption demand, Congress prohibited the use of appropriated funds between 1987 and 2004 and again starting in 2009 through the appropriations language each fiscal year through 2021 for this purpose. Sales of wild horses are conducted in accordance with IM 2019-026.

No Action

Under this alternative, no wild horses would be removed at this time. As a result, wild horses would not be subject to any individual direct or indirect impacts described in the action alternatives as a result of a gather operation. By 2022, wild horse populations would be expected to grow to about 1,997 wild horses, almost 4 times over high AML for these HMAs. Projected population increases would be expected to result in further deterioration of the range, and eventually lead to long-term impacts to both the health of the rangeland and the wild horse herds. Overall, wild horse populations under this alternative would not support a TNEB. Competition for available forage and water resources would continue to increase as the numbers of wild horses increase. Lactating mares, foals, and older animals would be affected most severely. Social stress would also be expected to increase among animals as they fight to protect their position at scarce forage and water sources. Potential for injuries to all age classes of animals would be expected to increase.

Areas closest to the water would experience severe utilization and degradation. Over time, the animals would also deteriorate in body condition as a result of declining quality and quantity of forage and increasing distances traveled to and from water to find forage. Many wild horses, especially mares with foals, would be put at risk due to a lack of forage and water, or would be expected to move outside the HMA boundaries in search of forage and water, potentially risking injury/death of animals and resulting in increasing damage to public, private, and State lands.

Gather Only

Under this alternative the BLM would gather and remove approximately 2076 wild horses. As a result, these wild horses would experience the stress associated with a helicopter gather, as described earlier in this section. These animals would also undergo the impacts associated with transportation to ORCs, adoption, purchase, and/or shipping to ORPs as described earlier in this section.

Under this alternative, long term gather related impacts are expected to be higher than the proposed action. Because no population growth suppression strategies would be implemented under this alternative, these HMAs would likely need to be gathered again in approximately 3 years (compared to a 4 year or longer gather cycle under the proposed action). This will lead to more frequent gather related impacts to wild horses in these HMAs, along with higher overall gather related stress to these animals.

This alternative will help maintain a thriving natural ecological balance, which will ensure wild horses have adequate access to forage, water, cover and space in these HMAs. However, a thriving natural ecological balance will only be maintained for approximately 3 years under this alternative. Maintaining wild horses within the AML will improve the condition of vegetation, water and soil resources within these HMAs. This in turn will ensure there are healthy wild horses, on healthy rangelands, and a TNEB will be maintained.

Proposed Action

Under this alternative the BLM would conduct wild horse gather operations in the complex multiple times during a 10-year period. Individual wild horses in the complex could be captured multiple times, once, or not at all. Captured horses would experience the stress associated with a helicopter gather, as described earlier in this section. Several hundred horses of each gender would also experience the

additional stress associated with their respective fertility control treatments. A minimum of 1000 horses would be removed from the range, transported to ORCs and eventually either be adopted, sold, or shipped to ORPs.

However, under this alternative, the impacts associated with gathers are expected to be reduced in the long term as a result of implementing population growth suppression strategies. With each successive gather a smaller number of horses will have to be captured, a smaller number will require fertility control treatments, a smaller number will need to be removed, and gather frequency will be decreased. By giving mares and stallions uniquely identifiable brands that indicate the form of population control treatment they have received it may be possible for pilots to skip over individual horses and groups of horses that are composed primarily of treated animals and focus on uncaptured/untreated horses. These reduced impacts will extend beyond the 10-year period covered by this document. As long as horses treated with fertility control make up a segment of the population there will be reduced population growth and, as a result, reduced gather impacts. Taken collectively and over the long term these proposed actions will reduce the stress placed on wild horses in these HMAs associated with gather operations.

Additionally, this alternative will help maintain a thriving natural ecological balance, which will ensure wild horses that are living on the complex have adequate access to forage, water, cover and space in these HMAs. Maintaining wild horses within AML, and slowing the population growth rate, will improve the condition of vegetation, water and soil resources within these HMAs. This in turn will ensure there are healthy wild horses, on healthy rangelands.

Issue 3: How would the proposed action affect the genetic diversity of the North Lander Complex? How would it affect the complex's ability to maintain a self-sustaining population?

Affected Environment

Most wild horses in these HMAs have mixed ancestry. BLM's wild horse handbook directs that a minimum population size of 50 effective breeding animals is recommended to maintain adequate genetic diversity (H-4700-1 Section 4.4.6.3). This is typically achieved by maintaining a total population of 150 – 200 wild horses. If the BLM cannot maintain a population of 150 – 200 animals, there are recommended management actions that can help maintain genetic diversity in the herd (H-4700-1 Section 4.4.6.4). Low AML is 50 in Dishpan Butte and Rock Creek Mtn, 60 in Conant Creek, and 160 in Muskrat Basin. At these levels, and especially with the population control methods described in this document, genetic diversity could decline without additional inputs. However, interchange between these HMAs is extensive and frequent which will help to maintain genetic diversity to some extent.

Metapopulation Considerations

Because of history, context, and periodic introductions, wild horses that live in the four HMAs analyzed here should not be considered as truly isolated populations (NRC 2013). Rather, managed herds of wild horses should be considered as components of interacting metapopulations, connected by interchange of individuals and genes due to both natural and human-facilitated movements. The 2014 RMP identified horses in the North Lander complex as being an identifiable metapopulation with a determined AML. However, these animals are also part of an even larger metapopulation (NRC 2013) that has demographic and genetic connections with other BLM-managed herds in Wyoming, Colorado, Nevada, Utah, and beyond. Wild horse herds in the larger metapopulation have a background of diverse domestic breed heritage, probably caused by natural and intentional movements of animals between herds.

The 2013 National Academies of Sciences (NAS) report included other evidence that shows that the herds in this complex are not genetically unusual, with respect to other wild horse herds. Specifically, Appendix F of the 2013 NAS report is a table showing the estimated 'fixation index' (Fst) values between

183 pairs of samples from wild horse herds. Fst is a measure of genetic differentiation, in this case as estimated by the pattern of microsatellite allelic diversity analyzed by Dr. Cothran's laboratory. Low values of Fst indicate that a given pair of sampled herds has a shared genetic background. The lower the Fst value, the more genetically similar are the two sampled herds. Values of Fst under approximately 0.05 indicate virtually no differentiation. Values of 0.10 indicate very little differentiation. Only if values are above about 0.15 are any two sampled subpopulations considered to have evidence of elevated differentiation (Frankham et al 2010). No genetic sampling has ever been collected for Rock Creek Mountain HMA but the other three HMAs have pairwise Fst values with each other ranging from 0.014 to 0.056, which would suggest that they are genetically almost identical. It is reasonable to infer that Rock Creek Mtn. would also be almost identical to the other three HMAs in the complex, considering its location in the center of the complex surrounded by these other three HMAs and the amount of observed interchange between all four HMAs. Fst values for samples from the three herds had pairwise Fst values that were less than 0.075 with several dozen other sample sets from a number of other BLMmanaged HMAs throughout the western USA. These results suggest that herds in this complex are extremely similar, genetically, to a high number of other BLM-managed herds, supporting the interpretation that these horses are components in a highly connected metapopulation that includes horse herds in many other HMAs.

Genetic Analyses of the HMAs

The BLM periodically collects hair samples from wild horses within these HMAs to test the current genetic health of the herd. Genetic variability samples were collected in 2012 for Conant Creek, Dishpan Butte, and Muskrat Basin. The genotypes of those samples were analyzed by Dr. E. Gus Cothran, Department of Veterinary Integrative Bioscience, Texas A&M University. His conclusions and recommendations regarding genetic variability in those herds are summarized below:

Conant Creek

"Genetic variability of this herd is near the average for feral herds although some measures are just below the mean with the trend for variability to be low. This herd was previously tested in 2004. Compared to 2004 the variability measures for the Conant Creek HMA have increased slightly. Sample sizes were similar so it is not likely that the differences are due to sample error. The pattern of change is not consistent with gene flow into the population as that should increase *He* relative to *Ho* however, other changes could be due to immigration. This would best fit a situation where the source population of immigration was closely related to Conant Creek. At this point it is not clear. There is a possibility that this herd has seen a recent loss of population size which would increase the risk to genetic diversity. Genetic similarity results suggest a herd with mixed ancestry.

Current variability levels are high enough that no action is needed at this point but the herd should be monitored closely due to the trend for low variability. This is especially true if it is known that the herd size has seen a recent decline. If there is known gene flow into the herd this should be allowed to continue." (Cothran 2013a)

<u>Dishpan Butte</u>

"Genetic variability of this herd is very near average overall with some measures greater than the mean and some lower. This herd was previously tested in 2004. For all measures of variation the values were greater in 2004 indicating a loss of genetic diversity. This indicates loss of genetic diversity a possibility that this herd has seen a recent loss of population size which would increase the risk to genetic diversity. Genetic similarity results suggest a herd with mixed ancestry. Current variability levels are high enough that no action is needed at this point but the herd should be monitored closely due to the loss of genetic diversity over the past eight years. This is especially true if it is known that the herd size has seen a recent decline." (Cothran 2013b)

<u>Muskrat Basin</u>

"Genetic variability of this herd in general is on the high side. This herd was previously tested in 2004. Current levels of variability for all measures except risk of loss are higher than in 2004. There is a possibility that this herd has seen recent gene flow from another population. Genetic similarity results suggest a herd with mixed ancestry with a strong indication of genes from the Thoroughbred contributing to the ancestry.

Current variability levels are high enough that no action is needed at this point. The possibility of immigration into the herd exists and if true, this will help maintain variability levels." (Cothran 2013c)

Rock Creek Mtn.

No genetic diversity monitoring analysis has been performed yet for this herd. Based on the results of the other three HMAs it is expected that the Rock Creek Herd has mixed ancestry similar to the other three herds. No action was called for in the other three herds to modify genetic variability.

Future Genetic Diversity Monitoring

Collection of genetic diversity samples at the time of the initial and subsequent gathers, and analysis of those samples, will inform the BLM about the status of genetic diversity for wild horses living on the complex. The BLM will be able to make informed decisions about the need to introduce additional wild horses, if any, to augment genetic diversity based on measures of observed heterozygosity from those analyses.

Environmental Effects

No Action

Since no gathers would occur, and no population growth suppression strategies would be implemented under this alternative, wild horse populations would continue to grow. As a result, the BLM would expect the genetic diversity of these herds to improve under this alternative, with a reduced likelihood for inbreeding over the long term.

Gather Only

Under this alternative 2,076 wild horses would be permanently removed from these HMAs. Those horses that are permanently removed from these HMAs will no longer contribute to the genetic diversity of these herds. Overall impact to genetic diversity is expected to be less than the proposed action since no population growth suppression strategies would be implemented under this alternative.

Overall, this alternative is not expected to affect the genetic diversity of the complex to the point where inbreeding depression is expected. The complex is expected to maintain an adequate number of breeding animals to maintain adequate genetic diversity and maintain a self-sustaining population. Transferring young females between the HMAs in the complex in conjunction with gathers will further ensure that the genetics within the whole complex mix thus increasing the effective breeding population.

Proposed Action

Under this alternative it is anticipated that a minimum of 1,000 wild horses would be permanently removed from these HMAs and a minimum of 600 wild horses would receive some form of population control treatment. Those horses that are permanently removed from these HMAs will no longer

contribute to the genetic diversity of these herds. Castrated or vasectomized males would no longer contribute to the genetic diversity of these herd. Mares that receive IUDs will not contribute to the genetic diversity of these herds for as long as they have the IUD. Those treated with temporary fertility control would also not contribute to the genetic diversity of these herds, until the effects of the treatments wear off. It is possible that a small portion of those treated will become permanently infertile. These animals would no longer contribute to the genetic diversity of the herd.

Once fully implemented this alternative would result in a significant reduction in the number of animals contributing to the genetics within the individual HMAs and within the complex as a whole. However, when managed at the complex level, having 50 effective breeding animals is very feasible while still limiting population growth. Furthermore, the exchange of young reproductively viable females between HMAs in conjunction with gathers as well as the normal interchange between the HMAs in the complex, will mitigate the potential for reduced genetic diversity and inbreeding depression. The ability of the complex to maintain genetic diversity would be further aided by BLM facilitated introductions of horses from outside the complex but within the larger metapopulation in the western USA. The BLM would also continue to monitor the genetic condition of these herds and take additional actions if genetic diversity drops below an acceptable level. Such actions may include adjusting the amount and type of fertility control utilized, increased infusions of outside animals, and facilitating greater interchange between HMAs within the complex.

It is true that the proposed action will cause part of the North Lander population to be non-reproducing, in the sense that sterilized animals such as gelded males will no longer be reproductive. The proposed action does not attempt to eliminate reproduction or make any HMAs within the complex completely non-reproducing. Rather it will make a segment of each HMA non-reproducing while ensuring sufficient reproduction and genetic exchange at the complex and metapopulation level to be genetically diverse and self-sustaining.

Vegetation (Native)

Issue(s) Identified

How would the proposed action affect native vegetation within these HMAs?

Affected Environment

There are a wide variety of ecological sites and vegetation types in the North Lander Complex. The major ecological sites that occur within these HMAs include the Loamy (Beaver Rim) 9-12" Precipitation Zone, Shallow Loamy (High Plains Southeast) 10-14" Precipitation Zone, Loamy Overflow (High Plains Southeast) 10-14" Precipitation Zone, Sandy (High Plains Southeast) 10-14" Precipitation Zone, Shallow Sandy (High Plains Southeast) 10-14" Precipitation Zone, and the Clayey (High Plains Southeast) 10-14" precipitation Zone. The main vegetation type is sagebrush/grass. Upland vegetation species include; silver sagebrush (*Artemisia cana*), big sagebrush (*Artemisia tridentata*), western wheatgrass (*Pascopyrum smithii*), thickspike wheatgrass (*Elymus lanceolatus*), Indian ricegrass (*Achnatherum hymenoides*), and needle and thread (*Hesperostipa comata*). Common forbs include phlox, buckwheat, sandwort, bearded-tongue, daisy, locoweed, lupine, paintbrush, sego lily, death-camas, goldenweed, aster, violet, buttercup, bluebells, hawksbeard, and yarrow. Native plants comprise the principle species on most sites, although cheatgrass is present in some areas, particularly on sandy soils.

Riparian habitat is rare, occupying about one percent of the landscape. Community types consist mainly of riparian grasslands. Common plant species include Nebraska sedge (Carex nebrascensis), Baltic rush (Juncus arcticus), tufted hairgrass (Deschampsia cespitosa), inland saltgrass (Distichlis spicata), Alkali sacaton (Sporobolus airoides), basin wildrye (Leymus cinereus), Kentucky bluegrass (Poa pratensis), and other sedges and rushes. Willows are rare in the North Lander Complex however there are several

species present in certain areas. Forbs are more abundant on non-saline sites, and include plantain, mint, meadow pussytoes, cinquefoil, aster, clover and native thistles.

The 375, 292 acres within these four HMAs have had continuous yearlong grazing by wild horses for decades. Wild horses generally prefer perennial grasses as forage when available. During winter conditions, wild horses may select more shrubs, primarily winterfat, and during severe winter periods it becomes the dominant plant consumed. Wild horses are known to move further from water for forage than livestock. Concentrated use and degradation in riparian areas from wild horses is common across these four HMAs. Proper Functioning Condition (PFC) monitoring has been conducted on riparian areas within these HMA's. This monitoring has indicated that the majority of riparian areas are "Functioning at Risk" or "Not Functioning", with very few riparian areas in "Proper Functioning Condition".

A land health evaluation report was completed for the Dishpan Butte allotment, within the Dishpan Butte HMA, in 2021. The data gathered for this land health evaluation report was gathered in the summer of 2020. It was determined that the 16,282 public acres that help to make up the Dishpan Butte allotment are meeting land health standards 1-4 and standards 5 and 6 are currently unknown for the Standards for Healthy Rangelands and Guidelines for Livestock Grazing Management. Livestock grazing was found to be in compliance for this allotment.

Land health evaluations for the remainder of the allotments within the proposed project area including Conant Creek Common, Big Pasture, Granite Mountain Open, Muskrat Open, and Rim Pasture allotments have not been completed with signed determinations of causal factors for any acres that are not achieving the standards of land health. These allotments are all listed in the RMP to be in the "Improve" management status.

Effects

No Action

Under the "No Action" alternative, wild horse population control measures would not be implemented, gather operations would not occur, and wild horse numbers would continue to increase within these four HMAs. Negative impacts to vegetation resources would continue to increase as the wild horse population increases due to over utilization of the native vegetation. Grazing use by wild horses would continue to overuse desirable plant species in riparian habitat, resulting in lower plant vigor and production, and increase the potential for reduced species composition and an increase in less desirable species, such as Baltic rush, alkali sacaton, and Kentucky bluegrass. At higher levels of utilization of riparian habitat, species of willow may also be overgrazed and reduced in vigor, production, and composition.

Wild horses roam much further away from water sources than cattle, so the negative impacts to plant vigor and production may occur further away, as well as close to water sources. These impacts would also extend out farther from water sources as wild horse populations increase and during years with below average precipitation during the growing season. This would also be accompanied by increased potential for the introduction and/or expansion of invasive, non-native plant species where native plant species are being overused. Without removing excess wild horses, heavy to severe utilization of native vegetation would likely occur in future years, especially during times of drought. *Gather to the Low AML Only (No Population Control)*

Under the "Gather to the low AML Only (No Population Control)" alternative, gathering would reduce the wild horse population to within its AML. Gather operations would result in trampling of vegetation at the trap sites and holding locations. The number of trap sites used during a gather can fluctuate depending on horse distribution, terrain, and seasonal limitations on horse movement (i.e. temperature, precipitation). Each trap site and holding facility varies in size but is generally less than two acres. The

trampling of two acres of vegetation at each trap site would be an immediate effect of the proposed action, however, would be short term in duration. These 2-acre trap sites each represent less than 1% of the total amount of vegetation that occurs within the 375,292 acres of the four HMAs. This would disturb 2 acres per trap site more than the "No Action" alternative. However, the "No Action" alternative would result in an overall higher amount of vegetation disturbance and loss over the long term. Desirable bunchgrasses, such as Indian ricegrass, needle-and-thread, bluebunch wheatgrass, bottlebrush squirreltail, green needlegrass, and basin wildrye, should be maintained or enhanced by reducing grazing use through all or a portion of the growing season. Key species in riparian habitat, such as Nebraska sedge and tufted hairgrass, would have a greater potential to be maintained or enhanced. Reducing the wild horse population would improve riparian habitat due to less riparian utilization and a lower number of animals congregating at these areas. Without the use of the population control described in the proposed action, gathers would need to occur more frequently.

Proposed Action

Under the proposed action wild horse fertility control and gathering would reduce the wild horse population to within its AML. Gather operations would result in trampling of vegetation at the trap sites and holding locations. The number of trap sites used during a gather can fluctuate depending on horse distribution, terrain, and seasonal limitations on horse movement (i.e. temperature, precipitation). Each trap site and holding facility varies in size but is generally less than two acres. The trampling of two acres of vegetation at each trap site would be an immediate effect of the proposed action, however, would be short term in duration. These 2-acre trap sites each represent less than 1% of the total amount of vegetation that occurs within the 375,292 acres of the four HMAs. This would disturb 2 acres per trap site more than the "No Action" alternative. However, the "No Action" alternative would result in an overall higher amount of vegetation disturbance and loss over the long term. Desirable bunchgrasses, such as Indian ricegrass, needle-and-thread, bluebunch wheatgrass, bottlebrush squirreltail, green needlegrass, and basin wildrye, should be maintained or enhanced by reducing grazing use through all or a portion of the growing season. Key species in riparian habitat, such as Nebraska sedge and tufted hairgrass, would have a greater potential to be maintained or enhanced. Reducing the wild horse population would improve riparian habitat due to less riparian utilization and a lower number of animals congregating at these areas.

Wildlife, Threatened and Endangered Species, Special Status Species, and Migratory Birds

Issue(s) Identified

How would the gathering of wild horses from the North Lander Complex affect wildlife habitat, including special status species?

Affected Environment

The mosaic of plant communities and topographic features found throughout the North Lander Complex HMAs (Conant Creek, Dishpan Butte, Muskrat Basin, and Rock Creek) supports a wide variety of wildlife species that use the various habitats for resting, courtship, foraging, travel, food and water, thermal protection, escape cover and reproduction. The Complex has been used by livestock for over 100 years and infrastructure is limited. However, in general the Complex has very low levels of other types of disturbance to wildlife habitat. These disturbances include a few improved county and BLM roads, several powerline corridors, and energy projects related to uranium mining and oil and gas development have contributed to loss and degradation of wildlife habitats within the complex.

Mule deer, elk and pronghorn, utilize the gather area year-round and approximately 68,215 acres or 18% of the area is identified as crucial winter range for mule deer, pronghorn antelope, moose, and winter

range for elk. Antelope and mule deer populations are currently below herd unit population objectives, while elk populations are at their objective.

Special Status Species:

BLM records indicate that there are 20 known Greater Sage-Grouse (GRSG) leks and associated nesting and brood-rearing habitat within the North Lander Complex HMAs. In accordance with BLM policies and guidance outlined in the RMPs, as amended, timing stipulations and surface disturbance restrictions would be used to avoid locating trap sites in sensitive habitats during the gather.

Of the approximately 375,324 acres making up the Complex, 364,756 acres (97%) is within GRSG Core/Priority Habitat Management Area (PHMA) and 10,568 acres (3%) of the Complex is within Non-core/General Habitat Management Area (GHMA).

Effects

No Action

Under the No Action alternative, there would be no temporary disturbance/disruption to wildlife as a result of gather operations. However, there would be continually increasing competition with wild horses for forage resources, space, and in some situations, limited water. Although diet overlap is highest between wild horses and elk, fecal analysis data shows higher wild horse use of winterfat during the winter, which may also increase diet overlap with antelope and mule deer. Competition for resources leads to the potential for increased stress or displacement of native wildlife species to less suitable habitats, with greater potential for reduced fitness and increased animal mortality during severe climate events. The effects would be greater in limited crucial use habitat areas such as winter habitat, nesting/brood-rearing areas, water sources, and in migration habitats. Additionally, increased competition between wild horses and wildlife species for forage resources, particularly in the spring when plants make and store carbohydrates, would impede long-term vegetation recovery, and encourage non-native or invasive plants to become established, reducing the prevalence of more desirable species used by wildlife.

Wild horse grazing has been associated with reduced plant diversity, altered soil characteristics, lower grass cover, lower grass density, and 1.6 to 2.6 times greater abundance of cheatgrass (Beever et al. 2008, pp. 180-181). GRSG need grass- and shrub-cover for protection from predators, particularly during nesting season (Connelly et al. 2000, pp. 970-971). Greater forage use by increasing wild horse populations would potentially result in lower visual security for nesting GRSG and lower nesting success. Reduction in shrub and grass cover can result in increased predation on both nests and birds, leading to lower nesting success and population. In addition to effects in sagebrush habitats, freeroaming wild horses can also degrade important meadow and spring brood-rearing habitats that provide forbs and insects for GRSG chick survival (Beever and Aldridge 2011, p. 277; Crawford et al. 2004, p. 11; Connelly et al. 2004, p. 7-37), as streams and springs within sagebrush ecosystems receive heavy use by horses (Crane et al. 1997, p. 380). The presence of wild horses is associated with a reduced degree of greater sage-grouse lekking behavior (Muñoz et al. 2020). Moreover, increasing densities of wild horses, measured as a percentage above AML, are associated with decreasing greater sage-grouse population sizes, measured by lek counts (Coates et al. 2021). The effect of expanding horse herds on wildlife and their habitats due to increasing trampling, sedimentation, and reducing aquatic or riparian vegetation negatively affects all wildlife, including aguatic species, by degrading their habitats. This Alternative would not maintain or enhance resource values supporting the designation of for GRSG or other wildlife habitat.

Gather to the Low AML Only (No Population Control)

Under the "Gather to the low AML Only (No Population Control)" alternative, gathers would occur in midsummer or later, therefore disturbance to ground nesting birds would be minimal since the chicks of all species would have fledged. Trap sites would be located to avoid trampling of sagebrush and other shrubs that provide browse for big game and habitat for other wildlife species.

Wildlife adjacent to trap sites would be temporarily displaced during capture operations by increased activity during trap setup, from helicopter noise, and vehicle traffic. Short-term stress and displacement would occur to wildlife during the gather operations, but in most cases displacement should only last 2-3 days in each trap area. Reduction of wild horse numbers inside of HMAs would result in reduced competition for forage and water resources between wild horses and wildlife. The effects of reducing wild horse numbers to the low AML would help to reduce competition for forage and water resources, as well as resulting in improved nesting habitat and hiding cover with wildlife species. More vegetation (hiding cover) and forage would be available for GRSG during critical nesting and brood-rearing periods, which may increase nesting success and populations. There would be reduced forage competition with big game that would help to maintain the numbers and health of these herds. The ability of wildlife populations to endure periods of drought or severe winter conditions would be enhanced. Riparian resources would not be used as heavily, leaving more vegetation for forage and hiding cover, as well as improving bank and stream condition and water quality.

Competition would still occur within the Complex for available forage, space, and water resources. These impacts would likely be higher for elk, than for antelope and mule deer, due to the higher diet overlap between elk and wild horses for grass species. However, reducing the wild horse population to low AML levels will decrease the level of resource competition and increase forage production, thus reducing competition for forage with other wildlife species.

Without the use of the population control described in the proposed action, long-term benefits for wildlife and wildlife habitat would be reduced and the frequency of gathers and associated wildlife disturbance would likely increase.

Proposed Action

The gathers would occur in mid-summer or later, therefore disturbance to ground nesting birds would be minimal since the chicks of all species would have fledged. Trap sites would be located to avoid trampling of sagebrush and other shrubs that provide browse for big game and habitat for other wildlife species.

Wildlife adjacent to trap sites would be temporarily displaced during capture operations by increased activity during trap setup, from helicopter noise, and vehicle traffic. Short-term stress and displacement would occur to wildlife during the gather operations, but in most cases displacement should only last 2-3 days in each trap area. Reduction of wild horse numbers inside of HMAs would result in reduced competition for forage and water resources between wild horses and wildlife. The effects of reducing wild horse numbers to the low AML would help to reduce competition for forage and water resources, as well as resulting in improved nesting habitat and hiding cover with wildlife species. More vegetation (hiding cover) and forage would be available for GRSG during critical nesting and brood-rearing periods, which may increase nesting success and populations. There would be reduced forage competition with big game that would help to maintain the numbers and health of these herds. The ability of wildlife populations to endure periods of drought or severe winter conditions would be enhanced. Riparian resources would not be used as heavily, leaving more vegetation for forage and hiding cover, as well as improving bank and stream condition and water quality.

Competition would still occur within the Complex for available forage, space, and water resources. These impacts would likely be higher for elk, than for antelope and mule deer, due to the higher diet overlap between elk and wild horses for grass species. However, reducing the wild horse population to low AML levels will decrease the level of resource competition and increase forage production, thus reducing competition for forage with other wildlife species.

Livestock Grazing

Issue(s) Identified

How would the reduction to AML addressed in the proposed action affect livestock operations within these HMA's?

Affected Environment

There are six livestock grazing allotments that are located entirely in these four HMAs. Table 4 provides a summary of number and kind of livestock permitted in each allotment, seasons of use, and permitted Animal Unit Months (AUMs) for these allotments. An AUM is the amount of forage needed to sustain one, 1000 lb cow and her calf, or five sheep for a month. An Animal Unit (AU) is an adjustment applied to an AUM depending on the animal being compared. The standard AU for wild horses is 1.2. Approximately 49,000 BLM AUMs of forage have been authorized yearly to the livestock operators (Table 4). Many livestock operators currently only utilize a portion of their permitted use. The 1664 wild horses that are currently estimated to be within these HMAs, will consume an estimated 23,960 AUMs in 2021. At their high AML (536), wild horses would use 7,719 AUMs annually.

Annual fluctuations in the use of authorized livestock AUMs are common and are the result of user demands, climatic conditions, and/or an effort to preserve or improve rangeland health. Some livestock users within these HMAs have reduced their use levels as a result of wild horse populations exceeding AML, which can negatively impact livestock operations). Livestock grazing on specific allotments is authorized during established seasons of use (Table 2). Livestock turnout in these allotments typically occurs from March to May. Livestock are typically gathered and removed from the range in late October and early November, resulting in a use period that is approximately 5 months. Most of the allotments are operated under grazing strategies incorporating rest, seasonal rotations, deferment, and prescribed use levels that provide for adequate plant recovery time to enhance rangeland health.

Numerous range improvements (such as fences or water developments) have been installed within the allotments which make up these HMAs to help manage livestock distribution and season of use, while protecting sensitive riparian habitat. Many of these range improvements benefit multiple resource values, including wild horses and wildlife. There is a limited amount of fencing within these HMAs.

Table 2. North Lander Complex Grazing Allotments						
Allotment Name and Number	НМА	Number and Kind of Livestock	Authorized Use Period	Total BLM AUMs	Exchange of Use AUMs	Number of Permits within the Allotment
Conant	Conant	1810 Sheep	03/01-04/15	7,832	0	3
Creek	Creek	1810 Sheep	12/16-02/28			
Common		1500 Sheep	05/01-06/15			
#01403		3000 Sheep	10/04-11/30			
		516 Cattle	05/01-05/30			
		900 Cattle	05/31-10/31			
		516 Cattle	11/01-11/30			

Dishpan	Dishpan	367 Cattle	05/15-06/14	1,983	0	1
Butte	Butte	387 Cattle	06/15-07/31			
#01716		387 Cattle	08/01-11/01			
Big Pasture	Dishpan	104 Cattle	05/01-11/07	11,614	0	7
#01703	Butte	1702 Cattle	05/01/08/31			
		375 Cattle	09/01-10/15			
		200 Cattle	10/25-11/07			
		753 Cattle	09/01-11/07			
		153 Cattle	05/01-06/30			
		153 Cattle	07/01-08/31			
		421 Cattle	05/15-11/07			
Granite	Muskrat	38 Cattle	05/10-10/30	13,397	813	5
Mountain	Basin	870 Cattle	06/01-10/31			
Open		1427 Cattle	05/10-10/31			
#01636		74 Cattle	5/22-10/30			
		44 Cattle	06/01-10/15			
Muskrat	Muskrat	716 Cattle	05/15-11/30	10,509	216	3
Open	Basin	587 Cattle	05/01-11/30			
#01409		420 Cattle	05/04-05/31			
		443 Cattle	06/01-11/30			
		43 Cattle	06/01-10/31			
Rim Pasture	Rock Creek	656 Cattle	06/01-10/31	3976	0	5
#01401	Mtn	1500 Sheep	06/01-07/16			
		1500 Sheep	09/01-10/08			
		1343 Sheep	06/01-10/01			

Effects

No Action

Under this alternative, wild horse population control methods would not be implemented, and wild horses would not be gathered. This would allow wild horse populations to increase by approximately 20% each year within the project area and likely expand into nearby non-HMA areas. Since livestock and wild horses compete for similar resources (food and water), livestock use would be directly impacted by an ever-growing overpopulation of wild horses, both within and outside the HMAs. In response to the overpopulation of wild horses, livestock operators may have to reduce, or remove, their livestock from the range in order to ensure their stock are adequately fed, and to prevent excessive impacts to rangeland resources.

The current wild horse population is several times above the AML set in the 2014 Lander RMP. Without removing excess wild horses, heavy to severe utilization would likely occur in future years, especially during times of drought. The indirect impacts of taking no action would include decreased rangeland health; increase competition between livestock, wild horses and wildlife for the available forage and water; reduced quantity and quality of forage and water; and impact livestock operators who utilize these grazing allotments.

Displacement of livestock under this alternative would be slow and indirect. It is possible that livestock operators would need to maintain range improvements more frequently due to the increased number of wild horses that would use them. In some cases, livestock operators may maintain their water sources,

only to find that wild horses have made full use of the water source, leaving little for livestock use. If livestock operators are forced to remove their livestock from the range, they would likely cease maintaining their range improvements altogether. As the wild horse population increases, range conditions would deteriorate. Since it can take a long time for rangelands to recover from impacts associated with overgrazing, it is likely that rangelands would continue to be in a degraded condition even if excess wild horses are removed from the range in future years.

Gather to the Low AML Only (No Population Control)

The "Gather to the low AML Only (No Population Control)" alternative would allow for the wild horse gather to be implemented in these four HMAs which would reduce the population of wild horses back to their AMLs. Reducing the current population of wild horses would directly reduce the number of AUMs utilized each year. This alternative would reduce the 29,960 AUMs that are currently being used by wild horses in 2021, to approximately 7,719 AUMs at their high AML. This would result in a reduction of approximately 22,241 AUMS if completed in 2021. This reduction of 22,241 AUMs is equivalent to 45% of the total AUMs that the BLM authorizes for livestock grazing each year. If this alternative were implemented after the year 2021, this would result in an even higher reduction of AUMs. With wild horse numbers reduced to the lower end of AML and no birth control measures implemented, future wild horse gathers would still be needed frequently.

Proposed Action

The proposed action would allow for the wild horse gather and fertility control methods to be implemented in these four HMAs which would reduce the population of wild horses back to their AMLs. Reducing the current population of wild horses would directly reduce the number of AUMs utilized each year. This alternative would reduce the 29,960 AUMs that are currently being used by wild horses in 2021, to approximately 7,719 AUMs at their high AML. This would result in a reduction of approximately 22,241 AUMS if completed in 2021. This reduction of 22,241 AUMs is equivalent to 45% of the total AUMs that the BLM authorizes for livestock grazing each year. If the proposed action were implemented after the year 2021, this would result in an even higher reduction of AUMs. With wild horse numbers reduced to the lower end of AML and birth control measures implemented, future wild horse gathers would be more infrequent, further reducing disturbance to livestock and management operations.

Mitigation

No mitigation measures are necessary. The project design features include notifying livestock operators within the gather area prior to the gather, enabling them to take precautions and avoid conflict with gather operations.

Consultation and Coordination

Tribes, Individuals, Organizations, or Agencies Consulted

Person Consulted	Title	Agency/Tribe/Organization	
Tyler Abbott	Field Supervisor	US Fish and Wildlife Service, Wyoming Field Office	
Julie Reeves	Plant and Wildlife Biologist	US Fish and Wildlife Service, Wyoming Field Office	

List of Preparers

The following Lander Field Office personnel reviewed or have been contacted with regard to this EA.

Name	Title	Responsible for
	Natural Resources	Special Status Plant Species, Noxious and
Emma Freeland	Specialist	Invasive Weeds
		Cultural Resources/Paleontology, Native
Adam T. Calkins	Archeologist	American Religious Concerns,
	Supervisory Rangeland	Livestock grazing, Wetlands/Riparian
Grant Burke	Management Specialist	Areas, Soils, Vegetation (Native)
		Recreation, Travel Management, Special
	Outdoor Recreation	Designations, and Human Health and
Jared Oakleaf	Planner	Safety.
	Wild Horse and Burro	
Clay Stott	Specialist	Wild Horses
		Liaison for: Air Quality/Climate change,
		BLM Natural Areas, Greenhouse Gas
	Planning and	emissions, Environmental Justice,
	Environmental	Prime/Unique Farmlands, Socio-economics
Holly Elliott	Coordinator	NEPA compliance review
		Fish and Wildlife, including USFWS
		designated species and BLM sensitive
Aaron Rutledge	Wildlife Biologist	species, Migratory Birds
		Geology, Floodplains, Hydrologic
Tom Sunderland	Geologist	Conditions (including Water Quality)
		Fluid Minerals (Surface), Wastes (solid,
Lindsay Aberrombie-Johler	NRS	hazardous)
Sebastien P. Guinard	Petroleum Engineer	Fluid Minerals (Subsurface)
Leta Rinker	Realty Specialist	Lands/Access
Jim Gates	Forester	Woodland/Forestry
Jim Critz	Engineer	Engineering Review

Appendix A - Resources Considered and Eliminated From Further Analysis

The following list of resource and features not present within the project area and not discussed in this EA:

- Environmental Justice,
- Prime or Unique Farmlands,
- Flood Plains,
- Native American Religious Concerns,
- Forest Resources
- Class I visual management areas,
- Class I Airsheds,
- Wild and Scenic Rivers,
- Special Designations, Wilderness values or inventoried lands with wilderness characteristics.
- ACECs
- Human Health and Safety Issues
- Air Quality
- Fire and Fuels
- Lands and Access
- Lands with Wilderness Characteristics
- Socioeconomics
- Environmental Justice
- Public Health and Safety
- Heritage Resources
- Recreation

Resources and features present in the project area but not affected by the proposed action or alternatives include:

Resource/Feature Present	Rationale for Determination
Fluid mineral resources surface	There are oil and gas activities in the area of the proposed action; however, the action is not proposed to take place in those areas and therefore there are no potentially significant environmental risks.
Hydrologic Conditions	The proposed action overlies numerous surface water drainages and aquifers. However, removal and population control of wild horses as described by the proposed action will not create erosion sedimentation or water quality issues. Therefore, the proposed action or alternatives will pose no potential impacts to hydrologic conditions or water quality.
Paleontology	The HMAs have a Potential Fossil Yield Class of low to very high (PFYC 2-5). However, since the project does not cause ground disturbance it is not anticipated the project will have an effect of paleontological resources.
Soils	The proposed action occurs over an area of approximately 375,292 acres which contains a wide variety of soil types and series. Removal and population control of wild horses as described by the proposed action will not create soil erosion, compaction, sedimentation, or other adverse impacts to the soils. Therefore, the proposed action or alternatives will pose no potential impacts to soils.

Visual Resources Travel management	The action does not result in surface disturbance that would cause contrast with the characteristic landscape. Therefore, there are no visual resource impacts. No travel management rules or restrictions are violated as a result of this action.
Threatened, Endangered, Proposed and Candidate Wildlife Species:	Potential Black-footed Ferret (Endangered; Non-Essential, Experimental Population [Federal Register October 30, 2015, 10(j) Rule)] habitat (white-tailed prairie dog towns) exists in the Complex. Past surveys conducted in relation to other development activities have not recorded the presence of black-footed ferrets. Horse trap sites and staging areas associated with gathers are never placed in prairie dog towns due to the possibility of horses breaking their legs in the burrows or degrading prairie dog habitat. This action would have no impacts to black-footed ferrets and this species will not be addressed further in the document. Areas exhibiting active white-tailed prairie dog activity would be avoided for trap sites to avoid disturbance to these and potential associated species such as burrowing owls or black footed ferrets. Some concentrated disturbance may occur during the actual gathering activity from horses falling thru/crushing shallow burrows; which also occurs as large animals naturally traverse the rangeland. There are no Threatened or Endangered wildlife species or their habitats present within the project area. No water depletions are associated with the proposed gather; therefore, there would be no effect to any federal listed aquatic species present in or downstream of the North Platte River.

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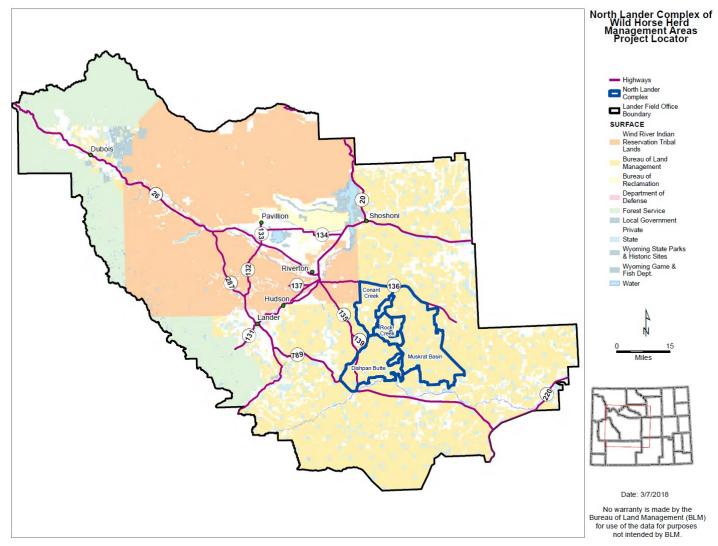
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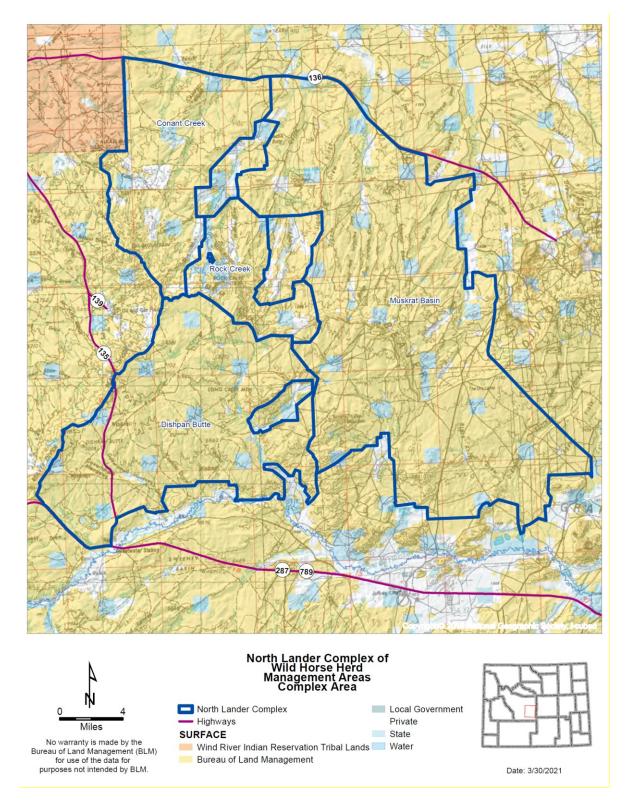
Appendix C - Acronyms and Abbreviations

- AML Appropriate Management Level AVMA - American Veterinary Medical Association **BA** – Biological Assessment **BO** – Biological Opinion **CAWP - Comprehensive Animal Welfare Program** COR - Contracting Officer Representative EA – Environmental Assessment EPA - Environmental Protection Agency ESA - Endangered Species Act FLPMA - Federal Land Policy and Management Act Fst – Fixation Index GHMA – General Habitat Management Area GRSG - Greater Sage Grouse He – Heterozygosity HMA - Herd Management Area Ho - Homozygosity **IUDs - Intrauterine Devices** NAS – National Academies of Sciences NRHP - National Register of Historic Places **NWI - National Wetland Inventory** PHMA – Priority Habitat Management Area PI - Project Inspector PZP - Porcine Zona Pellucida RMP – Resource Management Plan SOPs - Standard Operating Procedures
- TNEB Thriving Natural Ecological Balance
- USFWS U.S. Fish and Wildlife Service
- WFRHBA Wild Free-Roaming Horses and Burros Act
- WGFD Wyoming Game and Fish Department





Map 1 Location of the North Lander Comple



Map 2 The four HMAs making up the North Lander Complex

Appendix E-- Scientific Literature Review

Scientific Literature Review

This appendix includes scientific literature reviews addressing five topics: effects of gathers, effects of wild horses and burros on rangeland ecosystems, effects of fertility control vaccines and sex ratio manipulations, effects of sterilization, and effects of intrauterine devices (IUDs). This scientific literature review was compiled by the BLM's Wild Horse and Burro Program Research Coordinator (Paul Griffin, Ph.D.) and is considered current as of October 2020.

Effects of Gathers on Wild Horses and Burros

Gathering any wild animals into pens has the potential to cause impacts to individual animals. There is also the potential for impacts to individual horses and burros during transportation, short-term holding, long-term holding that take place after a gather. However, BLM follows guidelines to minimize those impacts and ensure humane animal care and high standards of welfare. The following literature review summarizes the limited number of scientific papers and government reports that have examined the effects of gathers and holding on wild horses and burros.

Two early papers, by Hansen and Mosley (2000) and Ashley and Holcomb (2001) examined limited effects of gathers, including behavioral effects and effects on foaling rates. Hansen and Mosley (2000) observed BLM gathers in Idaho and Wyoming. They monitored wild horse behaviors before and after a gather event and compared the behavioral and reproductive outcomes for animals that were gathered by helicopter against those outcomes for animals that were not. This comparison led to the conclusion that gather activities used at that time had no effect on observed wild horse foraging or social behaviors, in terms of time spent resting, feeding, vigilant, traveling, or engaged in agonistic encounters (Hansen and Mosley 2000). Similarly, the authors did not find any statistically significant difference in foaling rates in the year after the gather in comparisons between horses that were captured, those that were chased by a helicopter but evaded capture, or those that were not chased by a helicopter. The authors concluded that the gathers had no deleterious effects on behavior or reproduction. Ashley and Holcomb (2001) conducted observations of reproductive rates at Garfield Flat HMA in Nevada, where horses were gathered in 1993 and 1997, and compared those observations at Granite Range HMA in Nevada, where there was no gather. The authors found that the two gathers had a short-term effect on foaling rates; pregnant mares that were gathered had lower foaling rates than pregnant mares that were not gathered. The authors suggested that BLM make changes to the gather methods used at that time, to minimize the length of time that pregnant mares are held prior to their release back to the range. Since the publications by Hansen and Mosley (2000) and by Ashley and Holcomb (2001), BLM did make changes to reduce the stress that gathered animals, including pregnant females, may experience as a result of gather and removal activities; these measures have been formalized as policy in the comprehensive animal welfare program (BLM IM 2015-151).

A thorough review of gather practices and their effects on wild horses and burros can be found in a 2008 report from the Government Accounting Office. The report found that the BLM had controls in place to help ensure the humane treatment of wild horses and burros (GAO 2008). The controls included SOPs for gather operations, inspections, and data collection to monitor animal welfare. These procedures led to humane treatment during gathers, and in short-term and long-term holding facilities. The report found that cumulative effects associated with the capture and removal of excess wild horses include gather-related mortality averaged only about 0.5% and approximately 0.7% of the captured animals, on average, are humanely euthanized due to pre-existing conditions (such as lameness or club feet) in accordance with BLM policy. Scasta (2020) found the same overall mortality rate (1.2%) for BLM WH&B gathers in 2010-2019, with a mortality rate of 0.25% caused directly by the gather, and a mortality

rate of 0.94% attributable to euthanasia of animals with pre-existing conditions such as blindness or club-footedness. Scasta (2020) summarized mortality rates from 70 BLM WH&B gathers across nine states, from 2010-2019. Records for 28,821 horses and 2,005 burros came from helicopter and bait/water trapping. For wild burro bait / water trapping, mortality rates were 0.05% due to acute injury caused by the gather process, and death for burros with pre-existing conditions was 0.2% (Scasta 2020). For wild horse bait / water trapping, mortality rates were 0.3% due to acute injury, and the mortality rate due to pre-existing conditions was 1.4% (Scasta 2020). For wild horses gathered with the help of helicopters, mortality rates were only slightly lower than for bait / water trapping, with 0.3% due to acute causes, and 0.8% due to pre-existing conditions (Scasta 2020). Scasta (2020) noted that for other wildlife species capture operations, mortality rates above 2% are considered unacceptable and that, by that measure, BLM WH&B "…welfare is being optimized to a level acceptable across other animal handling disciplines."

The GAO report (2008) noted the precautions that BLM takes before gather operations, including screening potential gather sites for environmental and safety concerns, approving facility plans to ensure that there are no hazards to the animals there, and limiting the speeds that animals travel to trap sites. BLM used SOPs for short-term holding facilities (e.g., corrals) that included procedures to minimize excitement of the animals to prevent injury, separating horses by age, sex, and size, regular observation of the animals, and recording information about the animals in a BLM database. The GAO reported that BLM had regular inspections of short-term holding facilities and the animals held there, ensuring that the corral equipment is up to code and that animals are treated with appropriate veterinary care (including that hooves are trimmed adequately to prevent injury). Mortality was found to be about 5% per year associated with transportation, short term holding, and adoption or sale with limitations. The GAO noted that BLM also had controls in place to ensure humane care at long-term holding facilities (i.e., pastures). BLM staff monitor the number of animals, the pasture conditions, winter feeding, and animal health. Veterinarians from the USDA Animal and Plant Health Inspection Service inspect long-term facilities annually, including a full count of animals, with written reports. Contract veterinarians provide animal care at long-term facilities, when needed. Weekly counts provide an incentive for contractors that operate long-term holding facilities to maintain animal health (GAO 2008). Mortality at long-term holding was found to be about 8% per year, on average (GAO 2008). The mortality rates at short-term and long-term holding facilities are comparable to the natural annual mortality rate on the range of about 16% per year for foals (animals under age 1), about 5-10% per year for horses ages 1-10 years, and about 10-25% for animals aged 10-20 years (Ransom et al. 2016).

In 2010, the American Association of Equine Practitioners (AAEP 2011) was invited by the BLM to visit the BLM operations and facilities, spend time on WH&B gathers and evaluate the management of the wild equids. The AAEP Task Force evaluated horses in the BLM Wild Horse and Burro Program through several visits to wild horse gathers, and short- and long-term holding facilities. The task force was specifically asked to "review animal care and handling within the Wild Horse and Burro Program, and make whatever recommendations, if any, the Association feels may be indicated, and if possible, issue a public statement regarding the care and welfare of animals under BLM management." In their report (AAEP 2011), the task force concluded "that the care, handling and management practices utilized by the agency are appropriate for this population of horses and generally support the safety, health status and welfare of the animals."

In June 2010 BLM invited independent observers organized by American Horse Protection Association (AHPA) to observe BLM gathers and document their findings. AHPA engaged four independent credentialed professionals who are academia-based equine veterinarians or equine specialists. Each observer served on a team of two and was tasked specifically to observe the care and handling of the animals for a 3-4-day period during the gather process and submit their findings to AHPA. An

Evaluation Checklist was provided to each of the observers that included four sections: Gather Activities; Horse Handling During Gather; Horse Description; and Temporary Holding Facility. The independent group visited three separate gather operations and found that "BLM and contractors are responsible and concerned about the welfare of the horses before, during and after the gather process" and that "gentle and knowledgeable, used acceptable methods for moving horses... demonstrated the ability to review, assess and adapt procedures to ensure the care and well-being of the animals" (Greene et al. 2013).

BLM commissioned the Natural Resources Council of the National Academies of Sciences (NAS) to conduct an independent, technical evaluation of the science, methodology, and technical decision-making approaches of the BLM Wild Horse and Burro Management Program. Among the conclusions of their 2013 report, NAS (2013) concluded that wild horse populations grow at 15-20 percent a year, and that predation will not typically control population growth rates of free-ranging horses. The report (NAS 2013) also noted that, because there are human-created barriers to dispersal and movement (such as fences and highways) and no substantial predator pressure, maintaining a herd within an AML requires removing animals in roundups, also known as gathers, and may require management actions that limit population growth rates. The report (NAS 2013) examined a number of population growth suppression techniques, including the use of sterilization, fertility control vaccines, and sex ratio manipulation.

The effects of gathers as part of feral horse management have also been documented on National Park Service Lands. Since the 1980s, managers at Theodore Roosevelt National Park have used periodic gathers, removals, and auctions to maintain the feral horse herd size at a carrying capacity level of 50 to 90 horses (Amberg et al. 2014). In practical terms, this carrying capacity is equivalent to an AML. Horse herd sizes at those levels were determined to allow for maintenance of certain sensitive forage plant species. Gathers every 3-5 years did not prevent the herd from self-sustaining. The herd continues to grow, to the point that the NPS now uses gathers and removals along with temporary fertility control methods in its feral horse management (Amberg et al. 2014).

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Effects of Wild Horses and Burros on Rangeland Ecosystems

The presence of wild horses and wild burros can have substantial effects on rangeland ecosystems, and on the capacity for habitat restoration efforts to achieve landscape conservation and restoration goals. While wild horses and burros may have some beneficial ecological effects, such benefits are outweighed by ecological damage they cause when herds are at levels greater than supportable by allocated, available natural resources (i.e., when herds are greater than AML).

In the biological sense, all free-roaming horses and burros in North America are feral, meaning that they are descendants of domesticated animals brought to the Americas by European colonists. Horses went extinct in the Americas by the end of the Pleistocene, about 10,000 years ago (Webb 1984; MacFadden 2005). Burros evolved in Eurasia (Geigl et al. 2016). The published literature refers to free-roaming horses and burros as either feral or wild. In the ecological context the terms are interchangeable, but the terms 'wild horse' and 'wild burro' are associated with a specific legal status. The following literature review on the effects of wild horses and burros on rangeland ecosystems draws on scientific studies of feral horses and burros, some of which also have wild horse or wild burro legal status. The following literature review draws on Parts 1 and 2 of the 'Science framework for conservation and restoration of the sagebrush biome' interagency report (Chambers et al. 2017, Crist et al. 2019).

Because of the known damage that overpopulated wild horse and burro herds can cause in rangeland ecosystems, the presence of wild horses and burros is considered a threat to Greater sage-grouse habitat quality, particularly in the bird species' western range (Beever and Aldridge 2011, USFWS 2013). Wild horse population sizes on federal lands have more than doubled in the five years since the USFWS report (2013) was published (BLM 2018). On lands administered by the BLM, there were over 95,000 BLM-administered wild horses and burros as of March 1, 2020, which does not include foals born in 2020. Lands with wild horses and burros are managed for multiple uses, so it can be difficult to parse out their ecological effects. Despite this, scientific studies designed to separate out those effects, which are summarized below, point to conclusions that landscapes with greater wild horse and burro abundance will tend to have lower resilience to disturbance and lower resistance to invasive plants than similar landscapes with herds at or below target AML levels.

In contrast to managed livestock grazing, neither the seasonal timing nor the intensity of wild horse and burro grazing can be managed, except through efforts to manage their numbers and distribution. Wild horses live on the range year round, they roam freely, and wild horse populations have the potential to grow 15-20% per year (Wolfe 1980; Eberhardt et al. 1982; Garrott et al 1991; Dawson 2005; Roelle et al. 2010; Scorolli et al. 2010). Although this annual growth rate may be lower in some areas where mountain lions can take foals (Turner and Morrison 2001, Turner 2015), horses tend to favor use of more open habitats (Schoenecker 2016) that are dominated by grasses and shrubs and where ambush is less likely. Horses can compete with managed livestock in forage selected (Scasta et al. 2016).

As a result of the potential for wild horse populations to grow rapidly, impacts from wild horses on water, soil, vegetation, and native wildlife resources (Davies and Boyd 2019) can increase exponentially unless there is active management to limit their population sizes. For the majority of wild horse herds, there is little overall evidence that population growth is significantly affected by predation (NAS 2013), although wild horse herd growth rates may be somewhat reduced by predation in some localized areas, particularly where individual cougars specialize on horse predation (Turner and Morrison 2001, Roelle et al. 2010). Andreasen et al. (2021) recently found that some mountain lions (Puma concolor) prey on young horses, particularly where horses are at very high densities and native ungulates are at very low densities. The greatest rate of predation on horses was in the Virginia Range, where the state of Nevada manages a herd of feral horses that is not federally protected. Where lion predation on horses was common, Andreasen et al. (2021) found that female lions preved on horses year-round, but 13% or fewer of horses killed by lions were adults. BLM does not have the legal authority to regulate or manage mountain lion populations. Andreasen et al. (2021) concluded that "At landscape scales, cougar predation is unlikely to limit the growth of feral horse populations." Given the recent history of consistent growth in the ##### HMA wild horse herd, as documented by repeated aerial survey, the inference that predation does not limit local wild horse herd growth rates apparently applies.

The USFWS (2008), Beever and Aldridge (2011), and Chambers et al (2017) summarize much of the literature that quantifies direct ecosystem effects of wild horse presence. Beever and Aldridge (2011) present a conceptual model that illustrates the effects of wild horses on sagebrush ecosystems. In the Great Basin, areas without wild horses had greater shrub cover, plant cover, species richness, native plant cover, and overall plant biomass, and less cover percentage of grazing-tolerant, unpalatable, and invasive plant species, including cheatgrass, compared to areas with horses (Smith 1986; Beever et al. 2008; Davies et al. 2014; Zeigenfuss et al. 2014; Boyd et al. 2017). There were also measurable increases in soil penetration resistance and erosion, decreases in ant mound and granivorous small mammal densities, and changes in reptile communities (Beever et al. 2003; Beever and Brussard 2004; Beever and Herrick 2006; Ostermann-Kelm et al. 2009). Intensive grazing by horses and other ungulates can damage biological crusts (Belnap et al. 2001). In contrast to domestic livestock grazing, where postfire grazing rest and deferment can foster recovery, wild horse grazing occurs year round. These effects imply that horse presence can have broad effects on ecosystem function that could influence conservation and restoration actions.

Many studies corroborate the general conclusion that wild horses can lead to biologically significant changes in rangeland ecosystems, particularly when their populations are overabundant relative to water and forage resources, and other wildlife living on the landscape (Eldridge et al. 2020). The presence of wild horses is associated with a reduced degree of greater sage-grouse lekking behavior (Muñoz et al. 2020). Moreover, increasing densities of wild horses, measured as a percentage above AML, are associated with decreasing greater sage-grouse population sizes, measured by lek counts (Coates et al. 2021). Horses are primarily grazers (Hanley and Hanley 1982), but shrubs – including sagebrush - can represent a large part of a horse's diet, at least in summer in the Great Basin (Nordquist 2011). Grazing by wild horses can have severe impacts on water source quality, aquatic ecosystems and riparian communities as well (Beever and Brussard 2000; Barnett 2002; Nordquist 2011; USFWS 2008; Earnst et al. 2012; USFWS 2012, Kaweck et al. 2018), sometimes excluding native ungulates from water sources (Ostermann-Kelm et al. 2008; USFWS 2008; Perry et al. 2015; Hall et al. 2016; Gooch et al. 2017; Hall et al. 2018). Impacts to riparian vegetation per individual wild horse can exceed impacts per individual domestic cow (Kaweck et al. 2018, Burdick et al. 2021). Bird nest survival may be lower in areas with wild horses (Zalba and Cozzani 2004), and bird populations have recovered substantially after livestock and / or wild horses have been removed (Earnst et al. 2005; Earnst et al. 2012; Batchelor et al. 2015). Wild horses can spread nonnative plant species, including cheatgrass, and may limit the effectiveness of habitat restoration projects (Beever et al. 2003; Couvreur et al. 2004;

Jessop and Anderson 2007; Loydi and Zalba 2009). Riparian and wildlife habitat improvement projects intended to increase the availability of grasses, forbs, riparian habitats, and water will likely attract and be subject to heavy grazing and trampling by wild horses that live in the vicinity of the project. Even after domestic livestock are removed, continued wild horse grazing can cause ongoing detrimental ecosystem effects (USFWS 2008; Davies et al. 2014) which may require several decades for recovery (e.g., Anderson and Inouye 2001).

Wild horses and burros may have ecologically beneficial effects, especially when herd sizes are low relative to available natural resources, but those ecological benefits do not typically outweigh damage caused when herd sizes are high, relative to available natural resources. Under some conditions, there may not be observable competition with other ungulate species for water (e.g., Meeker 1979), but recent studies that used remote cameras have found wild horses excluding native wildlife from water sources under conditions of relative water scarcity (Perry et al. 2015, Hall et al. 2016, Hall et al. 2018). Wild burros (and, less frequently, wild horses) have been observed digging 'wells;' such digging may improve habitat conditions for some vertebrate species and, in one site, may improve tree seedling survival (Lundgren et al. 2021). This behavior has been observed in intermittent stream beds where subsurface water is within 2 meters of the surface (Lundgren et al. 2021). The BLM is not aware of published studies that document wild horses or burros in the western United States causing similar or widespread habitat amelioration on drier upland habitats such as sagebrush, grasslands, or pinyon-juniper woodlands. Lundgren et al. (2021) suggested that, due to well-digging in ephemeral streambeds, wild burros (and horses) could be considered 'ecosystem engineers;' a term for species that modify resource availability for other species (Jones et al. 1994). Rubin et al. (2021) and Bleich et al. (2021) responded by pointing out that ecological benefits from wild horse and burro presence must be weighted against ecological damage they can cause, especially at high densities. In HMAs where wild horse and burro biomass is very large relative to the biomass of native ungulates (Boyce and McLoughlin 2021), they should probably also be considered 'dominant species' (Power and Mills 1995) whose ecological influences result from their prevalence on the landscape. Wild horse densities could be maintained at high levels in part because artificial selection for early or extended reproduction may mean that wild horse population dynamics are not constrained in the same way as large herbivores that were never domesticated (Boyce and McLoughlin 2021). Another potentially positive ecological effect of wild horses and burros is that they, like all large herbivores, redistribute organic matter and nutrients in dung piles (i.e., King and Gurnell 2007), which could disperse and improve germination of undigested seeds. This could be beneficial if the animals spread viable native plant seeds, but could have negative consequences if the animals spread viable seeds of invasive plants such as cheatgrass (i.e., Loydi and Zalba 2009, King et al. 2019). Increased wild horse and burro density would be expected to increase the spatial extent and frequency of seed dispersal, whether the seeds distributed are desirable or undesirable. As is true of herbivory by any grazing animals, light grazing can increase rates of nutrient cycling (Manley et al. 1995) and foster compensatory growth in grazed plants which may stimulate root growth (Osterheld and McNaughton 1991, Schuman et al. 1999) and, potentially, an increase in carbon sequestration in the soil (i.e., Derner and Schuman 2007, He et al. 2011). However, when grazer density is high relative to available forage resources, overgrazing by any species can lead to long-term reductions in plant productivity, including decreased root biomass (Herbel 1982, Williams et al. 1968) and potential reduction of stored carbon in soil horizons. Recognizing the potential beneficial effects of low-density wild horse and burro herds, but also recognizing the totality of available published studies documented ecological effects of wild horse and burro herds, especially when above AML (as noted elsewhere), it is prudent to conclude that horse and burro herd sizes above AML may cause levels of disturbance that reduce landscapes' capacity for resilience in the face of further disturbance, such as is posed by extreme weather events and other consequences of climate change.

Most analyses of wild horse effects have contrasted areas with wild horses to areas without, which is a study design that should control for effects of other grazers, but historical or ongoing effects of livestock grazing may be difficult to separate from horse effects in some cases (Davies et al. 2014). Analyses have generally not included horse density as a continuous covariate; therefore, ecosystem effects have not been quantified as a linear function of increasing wild horse density. One exception is an analysis of satellite imagery confirming that varied levels of feral horse biomass were negatively correlated with average plant biomass growth (Ziegenfuss et al. 2014).

Horses require access to large amounts of water; an individual can drink an average of 7.4 gallons of water per day (Groenendyk et al. 1988). Despite a general preference for habitats near water (e.g., Crane et al. 1997), wild horses will routinely commute long distances (e.g., 10+ miles per day) between water sources and palatable vegetation (Hampson et al. 2010).

Wild burros can also substantially affect riparian habitats (e.g., Tiller 1997), native wildlife (e.g., Seegmiller and Ohmart 1981), and have grazing and trampling impacts that are similar to wild horses (Carothers et al. 1976; Hanley and Brady 1977; Douglas and Hurst 1983). Where wild burros and Greater sage-grouse co-occur, burros' year-round use of low-elevation habitats may lead to a high degree of overlap between burros and Greater sage-grouse (Beever and Aldridge 2011).

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Effects of Fertility Control Vaccines and Sex Ratio Manipulations

Various forms of fertility control can be used in wild horses and wild burros, with the goals of maintaining herds at or near AML, reducing fertility rates, and reducing the frequency of gathers and removals. The WFRHBA of 1971 specifically provides for contraception and sterilization (16 U.S.C. 1333 section 3.b.1). Fertility control measures have been shown to be a cost-effective and humane treatment to slow increases in wild horse populations or, when used in combination with gathers, to reduce horse population size (Bartholow 2004, de Seve and Boyles-Griffin 2013, Fonner and Bohara 2017). Although fertility control treatments may be associated with a number of potential physiological, behavioral, demographic, and genetic effects, those impacts are generally minor and transient, do not prevent overall maintenance of a self-sustaining population, and do not generally outweigh the potential benefits of using contraceptive treatments in situations where it is a management goal to reduce population growth rates (Garrott and Oli 2013).

An extensive body of peer-reviewed scientific literature details the impacts of fertility control methods on wild horses and burros. No finding of excess animals is required for BLM to pursue contraception in wild horses or wild burros, but NEPA analysis has been required. This review focuses on peer-reviewed scientific literature. The summary that follows first examines effects of fertility control vaccine use in mares, then of sex ratio manipulation. This review does not examine effects of spaying and neutering. Cited studies are generally limited to those involving horses and burros, except where including studies on other species helps in making inferences about physiological or behavioral questions not yet addressed in horses or burros specifically. While most studies reviewed here refer to horses, burros are extremely similar in terms of physiology, such that expected effects are comparable, except where differences between the species are noted.

On the whole, the identified impacts are generally transient and affect primarily the individuals treated. Fertility control that affects individual horses and burros does not prevent BLM from ensuring that there will be self-sustaining populations of wild horses and burros in single herd management areas (HMAs), in complexes of HMAs, and at regional scales of multiple HMAs and complexes. Under the WFRHBA of 1971, BLM is charged with maintaining self-reproducing populations of wild horses and burros. The National Academies of Sciences (2013) encouraged BLM to manage wild horses and burros at the spatial scale of "metapopulations" – that is, across multiple HMAs and complexes in a region. In fact, many HMAs have historical and ongoing genetic and demographic connections with other HMAs, and BLM routinely moves animals from one to another to improve local herd traits and maintain high genetic diversity. The NAS report (2013) includes information (pairwise genetic 'fixation index' values for sampled WH&B herds) confirming that WH&B in the vast majority of HMAs are genetically similar to animals in multiple other HMAs.

All fertility control methods affect the behavior and physiology of treated animals (NAS 2013), and are associated with potential risks and benefits, including effects of handling, frequency of handling, physiological effects, behavioral effects, and reduced population growth rates (Hampton et al. 2015). Contraception alone does not remove excess horses from an HMA's population, so one or more gathers are usually needed in order to bring the herd down to a level close to AML. Horses are long-lived, potentially reaching 20 years of age or more in the wild. Except in cases where extremely high fractions of mares are rendered infertile over long time periods of (i.e., 10 or more years), fertility control methods such as immunocontraceptive vaccines and sex ratio manipulation are not very effective at reducing population growth rates to the point where births equal deaths in a herd. However, even more modest fertility control activities can reduce the frequency of horse gather activities, and costs to taxpayers. Bartholow (2007) concluded that the application of 2-year or 3-year contraceptives to wild mares could reduce operational costs in a project area by 12-20%, or up to 30% in carefully planned population management programs. Because applying contraception to horses requires capturing and handling, the risks and costs associated with capture and handling of horses may be comparable to those of gathering for removal, but with expectedly lower adoption and long-term holding costs. Population growth suppression becomes less expensive if fertility control is long-lasting (Hobbs et al. 2000).

In the context of BLM wild horse and burro management, fertility control vaccines and sex ratio manipulation rely on reducing the number of reproducing females. Taking into consideration available literature on the subject, the National Academies of Sciences concluded in their 2013 report that forms of fertility control vaccines were two of the three 'most promising' available methods for contraception in wild horses and burros (NAS 2013). That report also noted that sex ratio manipulations where herds have approximately 60% males and 40% females can expect lower annual growth rates, simply as a result of having a lower number of reproducing females.

Fertility Control Vaccines

Fertility control vaccines (also known as (immunocontraceptives) meet BLM requirements for safety to mares and the environment (EPA 2009a, 2012). Because they work by causing an immune response in treated animals, there is no risk of hormones or toxins being taken into the food chain when a treated mare dies. The BLM and other land managers have mainly used three fertility control vaccine formulations for fertility control of wild horse mares on the range: ZonaStat-H, PZP-22, and GonaCon-Equine. As other formulations become available they may be applied in the future.

In any vaccine, the antigen is the stimulant to which the body responds by making antigen-specific antibodies. Those antibodies then signal to the body that a foreign molecule is present, initiating an immune response that removes the molecule or cell. Adjuvants are additional substances that are included in vaccines to elevate the level of immune response. Adjuvants help to incite recruitment of lymphocytes and other immune cells which foster a long-lasting immune response that is specific to the antigen.

Liquid emulsion vaccines can be injected by hand or remotely administered in the field using a pneumatic dart (Roelle and Ransom 2009, Rutberg et al. 2017, McCann et al. 2017) in cases where

mares are relatively approachable. Use of remotely delivered (dart-delivered) vaccine is generally limited to populations where individual animals can be accurately identified and repeatedly approached within 50 m (BLM 2010). Booster doses can be safely administered by hand or by dart. Even with repeated booster treatments of the vaccines, it is expected that most mares would eventually return to fertility, though some individual mares treated repeatedly may remain infertile. Once the herd size in a project area is at AML and population growth seems to be stabilized, BLM can make adaptive determinations as to the required frequency of new and booster treatments.

BLM has followed SOPs for fertility control vaccine application (BLM IM 2009-090). Herds selected for fertility control vaccine use should have annual growth rates over 5%, have a herd size over 50 animals, and have a target rate of treatment of between 50% and 90% of female wild horses or burros. The IM requires that treated mares be identifiable via a visible freeze brand or individual color markings, so that their vaccination history can be known. The IM calls for follow-up population surveys to determine the realized annual growth rate in herds treated with fertility control vaccines.

Vaccine Formulations: Porcine Zona Pellucida (PZP)

PZP vaccines have been used on dozens of horse herds by the National Park Service, US Forest Service, Bureau of Land Management, and Native American tribes and PZP vaccine use is approved for freeranging wild and feral horse herds in the United States (EPA 2012). PZP use can reduce or eliminate the need for gathers and removals, if very high fractions of mares are treated over a very long time period (Turner et al. 1997). PZP vaccines have been used extensively in wild horses (NAS 2013), and in feral burros on Caribbean islands (Turner et al. 1996, French et al. 2017). PZP vaccine formulations are produced as ZonaStat-H, an EPA-registered commercial product (EPA 2012, SCC 2015), as PZP-22, which is a formulation of PZP in polymer pellets that can lead to a longer immune response (Turner et al. 2002, Rutberg et al. 2017), and as Spayvac, where the PZP protein is enveloped in liposomes (Killian et al. 2008, Roelle et al. 2017, Bechert and Fraker 2018). 'Native' PZP proteins can be purified from pig ovaries (Liu et al. 1989). Recombinant ZP proteins may be produced with molecular techniques (Gupta and Minhas 2017, Joonè et al. 2017a, Nolan et al. 2018a).

When advisories on the product label (EPA 2015) are followed, the product is safe for users and the environment (EPA 2012). In keeping with the EPA registration for ZonaStat-H (EPA 2012; reg. no. 86833-1), certification through the Science and Conservation Center in Billings Montana is required to apply that vaccine to equids.

For maximum effectiveness, PZP is administered within the December to February timeframe. When applying ZonaStat-H, first the primer with modified Freund's Complete adjuvant is given and then the booster with Freund's Incomplete adjuvant is given 2-6 weeks later. Preferably, the timing of the booster dose is at least 1-2 weeks prior to the onset of breeding activity. Following the initial 2 inoculations, only annual boosters are required. For the PZP-22 formulation, each released mare would receive a single dose of the two-year PZP contraceptive vaccine at the same time as a dose of the liquid PZP vaccine with modified Freund's Complete adjuvant. The pellets are applied to the mare with a large gauge needle and jab-stick into the hip. Although PZP-22 pellets have been delivered via darting in trial studies (Rutberg et al 2017, Carey et al. 2019), BLM does not plan to use darting for PZP-22 delivery until there is more demonstration that PZP-22 can be reliably delivered via dart.

Vaccine Formulations: Gonadotropin Releasing Hormone (GnRH)

GonaCon (which is produced under the trade name GonaCon-Equine for use in feral horses and burros) is approved for use by authorized federal, state, tribal, public and private personnel, for application to free-ranging wild horse and burro herds in the United States (EPA 2013, 2015). GonaCon has been used on feral horses in Theodore Roosevelt National Park and on wild horses administered by BLM (BLM

2015). GonaCon has been produced by USDA-APHIS (Fort Collins, Colorado) in several different formulations, the history of which is reviewed by Miller et al. (2013). GonaCon vaccines present the recipient with hundreds of copies of GnRH as peptides on the surface of a linked protein that is naturally antigenic because it comes from invertebrate hemocyanin (Miller et al 2013). Early GonaCon formulations linked many copies of GnRH to a protein from the keyhole limpet (GonaCon-KHL), but more recently produced formulations where the GnRH antigen is linked to a protein from the blue mussel (GonaCon-B) proved less expensive and more effective (Miller et al. 2008). GonaCon-Equine is in the category of GonaCon-B vaccines.

As with other contraceptives applied to wild horses, the long-term goal of GonaCon-Equine use is to reduce or eliminate the need for gathers and removals (NAS 2013). GonaCon-Equine contraceptive vaccine is an EPA-approved pesticide (EPA, 2009a) that is relatively inexpensive, meets BLM requirements for safety to mares and the environment, and is produced in a USDA-APHIS laboratory. GonaCon is a pharmaceutical-grade vaccine, including aseptic manufacturing technique to deliver a sterile vaccine product (Miller et al. 2013). If stored at 4° C, the shelf life is 6 months (Miller et al 2013).

Miller et al. (2013) reviewed the vaccine environmental safety and toxicity. When advisories on the product label (EPA 2015) are followed, the product is safe for users and the environment (EPA 2009b). EPA waived a number of tests prior to registering the vaccine, because GonaCon was deemed to pose low risks to the environment, so long as the product label is followed (Wang-Cahill et al., *in press*).

GonaCon-Equine can safely be reapplied as necessary to control the population growth rate; booster dose effects may lead to increased effectiveness of contraception, which is generally the intent. Even after booster treatment of GonaCon-Equine, it is expected that most, if not all, mares would return to fertility at some point. Although the exact timing for the return to fertility in mares boosted more than once with GonaCon-Equine has not been quantified, a prolonged return to fertility would be consistent with the desired effect of using GonaCon (e.g., effective contraception).

The adjuvant used in GonaCon, Adjuvac, generally leads to a milder reaction than Freund's Complete Adjuvant (Powers et al. 2011). Adjuvac contains a small number of killed *Mycobacterium avium* cells (Miller et al. 2008, Miller et al. 2013). The antigen and adjuvant are emulsified in mineral oil, such that they are not all presented to the immune system right after injection. It is thought that the mineral oil emulsion leads to a 'depot effect' that is associated with slow or sustained release of the antigen, and a resulting longer-lasting immune response (Miller et al. 2013). Miller et al. (2008, 2013) have speculated that, in cases where memory-B leukocytes are protected in immune complexes in the lymphatic system, it can lead to years of immune response. Increased doses of vaccine may lead to stronger immune reactions, but only to a certain point; when Yoder and Miller (2010) tested varying doses of GonaCon in prairie dogs, antibody responses to the 200µg and 400µg doses were equal to each other but were both higher than in response to a 100µg dose.

Direct Effects: PZP Vaccines

The historically accepted hypothesis explaining PZP vaccine effectiveness posits that when injected as an antigen in vaccines, PZP causes the mare's immune system to produce antibodies that are specific to zona pellucida proteins on the surface of that mare's eggs. The antibodies bind to the mare's eggs surface proteins (Liu et al. 1989), and effectively block sperm binding and fertilization (Zoo Montana, 2000). Because treated mares do not become pregnant but other ovarian functions remain generally unchanged, PZP can cause a mare to continue having regular estrus cycles throughout the breeding season. More recent observations support a complementary hypothesis, which posits that PZP vaccination causes reductions in ovary size and function (Mask et al. 2015, Joonè et al. 2017b, Joonè et al. 2017c, Nolan et al. 2018b, 2018c). PZP vaccines do not appear to interact with other organ systems, as antibodies specific to PZP protein do not crossreact with tissues outside of the reproductive system (Barber and Fayrer-Hosken 2000).

Research has demonstrated that contraceptive efficacy of an injected liquid PZP vaccine, such as ZonaStat-H, is approximately 90% or more for mares treated twice in the first year (Turner and Kirkpatrick 2002, Turner et al. 2008). The highest success for fertility control has been reported when the vaccine has been applied November through February. High contraceptive rates of 90% or more can be maintained in horses that are given a booster dose annually (Kirkpatrick et al. 1992). Approximately 60% to 85% of mares are successfully contracepted for one year when treated simultaneously with a liquid primer and PZP-22 pellets (Rutberg et al. 2017, Carey et al. 2019). Application of PZP for fertility control would reduce fertility in a large percentage of mares for at least one year (Ransom et al. 2011). The contraceptive result for a single application of the liquid PZP vaccine primer dose along with PZP vaccine pellets (PZP-22), based on winter applications, can be expected to fall in the approximate efficacy is measured as the relative decrease in foaling rate for treated mares, compared to control mares:

Year 1	Year 2	Year 3
0 (developing fetuses come to term)	~30-75%	~20-50%

If mares that have been treated with PZP-22 vaccine pellets subsequently receive a booster dose of either the liquid PZP vaccine or the PZP-22 vaccine pellets, the subsequent contraceptive effect is apparently more pronounced and long-lasting. The approximate efficacy following a booster dose can be expected to be in the following ranges (based on figure 3 in Rutberg et al. 2017).

Year 1	Year 2	Year 3	Year 4
0 (developing fetuses come to term)	~50-90%	~55-75%	~40-75%

The fraction of mares treated in a herd can have a large effect on the realized change in growth rate due to PZP contraception, with an extremely high portion of mares required over many years to be treated to totally prevent population-level growth (e.g., Turner and Kirkpatrick 2002). Gather efficiency does not usually exceed 85% via helicopter, and may be less with bait and water trapping, so there will almost always be a portion of the female population uncaptured that is not treated in any given year. Additionally, some mares may not respond to the fertility control vaccine, but instead will continue to foal normally.

Direct Effects: GnRH Vaccines

GonaCon-Equine is one of several vaccines that have been engineered to create an immune response to the gonadotropin releasing hormone peptide (GnRH). GnRH is a small peptide that plays an important role in signaling the production of other hormones involved in reproduction in both sexes. When combined with an adjuvant, a GnRH vaccine stimulates a persistent immune response resulting in

prolonged antibody production against GnRH, the carrier protein, and the adjuvant (Miller et al., 2008). The most direct result of successful GnRH vaccination is that it has the effect of decreasing the level of GnRH signaling in the body, as evidenced by a drop in luteinizing hormone levels, and a cessation of ovulation.

GnRH is highly conserved across mammalian taxa, so some inferences about the mechanism and effects of GonaCon-Equine in horses can be made from studies that used different anti-GnRH vaccines, in horses and other taxa. Other commercially available anti-GnRH vaccines include: Improvac (Imboden et al. 2006, Botha et al. 2008, Janett et al. 2009a, Janett et al. 2009b, Schulman et al. 2013, Dalmau et al. 2015, Nolan et al. 2018c), made in South Africa; Equity (Elhay et al. 2007), made in Australia; Improvest, for use in swine (Bohrer et al. 2014); Repro-BLOC (Boedeker et al. 2011); and Bopriva, for use in cows (Balet et al. 2014). Of these, GonaCon-Equine, Improvac, and Equity are specifically intended for horses. Other anti-GnRH vaccine formulations have also been tested, but did not become trademarked products (e.g., Goodloe 1991, Dalin et al 2002, Stout et al. 2003, Donovan et al. 2013, Schaut et al. 2018, Yao et al. 2018). The effectiveness and side-effects of these various anti-GnRH vaccines may not be the same as would be expected from GonaCon-Equine use in horses. Results could differ as a result of differences in the preparation of the GnRH antigen, and the choice of adjuvant used to stimulate the immune response. For some formulations of anti-GnRH vaccines, a booster dose is required to elicit a contraceptive response, though GonaCon can cause short-term contraception in a fraction of treated animals from one dose (Powers et al. 2011, Gionfriddo et al. 2011a, Baker et al. 2013, Miller et al 2013).

GonaCon can provide multiple years of infertility in several wild ungulate species, including horses (Killian et al., 2008; Gray et al., 2010). The lack of estrus cycling that results from successful GonaCon vaccination has been compared to typical winter period of anoestrus in open mares. As anti-GnRH antibodies decline over time, concentrations of available endogenous GnRH increase and treated animals usually regain fertility (Power et al., 2011).

Females that are successfully contracepted by GnRH vaccination enter a state similar to anestrus, have a lack of or incomplete follicle maturation, and no ovarian cycling (Botha et al. 2008, Nolan et al. 2018c). A leading hypothesis is that anti-GnRH antibodies bind GnRH in the hypothalamus – pituitary 'portal vessels,' preventing GnRH from binding to GnRH-specific binding sites on gonadotroph cells in the pituitary, thereby limiting the production of gonadotropin hormones, particularly luteinizing hormone (LH) and, to a lesser degree, follicle-stimulating hormone (FSH) (Powers et al. 2011, NAS 2013). This reduction in LH (and FSH), and a corresponding lack of ovulation, has been measured in response to treatment with anti-GnRH vaccines (Boedeker et al. 2011, Garza et al. 1986).

Females successfully treated with anti-GnRH vaccines have reduced progesterone levels (Garza et al. 1986, Stout et al. 2003, Imboden et al. 2006, Elhay 2007, Botha et al. 2008, Killian et al. 2008, Miller et al. 2008, Janett et al. 2009, Schulman et al. 2013, Balet et al 2014, Dalmau et al. 2015) and β -17 estradiol levels (Elhay et al. 2007), but no great decrease in estrogen levels (Balet et al. 2014). Reductions in progesterone do not occur immediately after the primer dose, but can take several weeks or months to develop (Elhay et al. 2007, Botha et al. 2008, Schulman et al. 2013, Dalmau et al. 2015). This indicates that ovulation is not occurring and corpora lutea, formed from post-ovulation follicular tissue, are not being established.

Antibody titer measurements are proximate measures of the antibody concentration in the blood specific to a given antigen. Anti-GnRH titers generally correlate with a suppressed reproduction system (Gionfriddo et al. 2011a, Powers et al. 2011). Various studies have attempted to identify a relationship between anti-GnRH titer levels and infertility, but that relationship has not been universally predictable or consistent. The time length that titer levels stay high appears to correlate with the length of suppressed reproduction (Dalin et al. 2002, Levy et al. 2011, Donovan et al. 2013, Powers et al. 2011).

For example, Goodloe (1991) noted that mares did produce elevated titers and had suppressed follicular development for 11-13 weeks after treatment, but that all treated mares ovulated after the titer levels declined. Similarly, Elhay (2007) found that high initial titers correlated with longer-lasting ovarian and behavioral anoestrus. However, Powers et al. (2011) did not identify a threshold level of titer that was consistently indicative of suppressed reproduction despite seeing a strong correlation between antibody concentration and infertility, nor did Schulman et al. (2013) find a clear relationship between titer levels and mare acyclicity.

In many cases, young animals appear to have higher immune responses, and stronger contraceptive effects of anti-GnRH vaccines than older animals (Brown et al. 1994, Curtis et al. 2001, Stout et al. 2003, Schulman et al. 2013). Vaccinating with GonaCon at too young an age, though, may prevent effectiveness; Gionfriddo et al. (2011a) observed weak effects in 3-4 month old fawns. It has not been possible to predict which individuals of a given age class will have long-lasting immune responses to the GonaCon vaccine. Gray (2010) noted that mares in poor body condition tended to have lower contraceptive efficacy in response to GonaCon-B. Miller et al. (2013) suggested that higher parasite loads might have explained a lower immune response in free-roaming horses than had been observed in a captive trial. At this time it is unclear what the most important factors affecting efficacy are.

Several studies have monitored animal health after immunization against GnRH. GonaCon treated mares did not have any measurable difference in uterine edema (Killian 2006, 2008). Powers et al. (2011, 2013) noted no differences in blood chemistry except a mildly elevated fibrinogen level in some GonaCon treated elk. In that study, one sham-treated elk and one GonaCon treated elk each developed leukocytosis, suggesting that there may have been a causal link between the adjuvant and the effect. Curtis et al. (2008) found persistent granulomas at GonaCon-KHL injection sites three years after injection, and reduced ovary weights in treated females. Yoder and Miller (2010) found no difference in blood chemistry between GonaCon treated and control prairie dogs. One of 15 GonaCon treated cats died without explanation, and with no determination about cause of death possible based on necropsy or histology (Levy et al. 2011). Other anti-GnRH vaccine formulations have led to no detectable adverse effects (in elephants; Boedeker et al. 2011), though Imboden et al. (2006) speculated that young treated animals might conceivably have impaired hypothalamic or pituitary function.

Kirkpatrick et al. (2011) raised concerns that anti-GnRH vaccines could lead to adverse effects in other organ systems outside the reproductive system. GnRH receptors have been identified in tissues outside of the pituitary system, including in the testes and placenta (Khodr and Siler-Khodr 1980), ovary (Hsueh and Erickson 1979), bladder (Coit et al. 2009), heart (Dong et al. 2011), and central nervous system, so it is plausible that reductions in circulating GnRH levels could inhibit physiological processes in those organ systems. Kirkpatrick et al. (2011) noted elevated cardiological risks to human patients taking GnRH agonists (such as leuprolide), but the National Academy of Sciences (2013) concluded that the mechanism and results of GnRH agonists would be expected to be different from that of anti-GnRH antibodies; the former flood GnRH receptors, while the latter deprive receptors of GnRH.

Reversibility and Effects on Ovaries: PZP Vaccines

In most cases, PZP contraception appears to be temporary and reversible, with most treated mares returning to fertility over time (Kirkpatrick and Turner 2002). The ZonaStat-H formulation of the vaccine tends to confer only one year of efficacy per dose. Some studies have found that a PZP vaccine in long-lasting pellets (PZP-22) can confer multiple years of contraception (Turner et al. 2007), particularly when boostered with subsequent PZP vaccination (Rutberg et al. 2017). Other trial data, though, indicate that the pelleted vaccine may only be effective for one year (J. Turner, University of Toledo, Personal Communication to BLM).

The purpose of applying PZP vaccine treatment is to prevent mares from conceiving foals, but BLM acknowledges that long-term infertility, or permanent sterility, could be a result for some number of individual wild horses receiving PZP vaccinations. The rate of long-term or permanent sterility following vaccinations with PZP is hard to predict for individual horses, but that outcome appears to increase in likelihood as the number of doses increases (Kirkpatrick and Turner 2002). Permanent sterility for mares treated consecutively in each of 5-7 years was observed by Nuñez et al. (2010, 2017). In a graduate thesis, Knight (2014) suggested that repeated treatment with as few as three to four years of PZP treatment may lead to longer-term sterility, and that sterility may result from PZP treatment before puberty. Repeated treatment with PZP led long-term infertility in Przewalski's horses receiving as few as one PZP booster dose (Feh 2012). However, even if some number of mares become sterile as a result of PZP treatment, that potential result would be consistent with the contraceptive purpose that motivates BLM's potential use of the vaccine.

In some number of individual mares, PZP vaccination may cause direct effects on ovaries (Gray and Cameron 2010, Joonè et al. 2017b, Joonè et al. 2017c, Joonè et al. 2017d, Nolan et al. 2018b). Joonè et al. (2017a) noted reversible effects on ovaries in mares treated with one primer dose and booster dose. Joonè et al. (2017c) and Nolan et al. (2018b) documented decreased anti-Mullerian hormone (AMH) levels in mares treated with native or recombinant PZP vaccines; AMH levels are thought to be an indicator of ovarian function. Bechert et al. (2013) found that ovarian function was affected by the SpayVac PZP vaccination, but that there were no effects on other organ systems. Mask et al. (2015) demonstrated that equine antibodies that resulted from SpayVac immunization could bind to oocytes, ZP proteins, follicular tissues, and ovarian tissues. It is possible that result is specific to the immune response to SpayVac, which may have lower PZP purity than ZonaStat or PZP-22 (Hall et al. 2016). However, in studies with native ZP proteins and recombinant ZP proteins, Joonè et al. (2017a) found transient effects on ovaries after PZP vaccination in some treated mares; normal estrus cycling had resumed 10 months after the last treatment. SpayVac is a patented formulation of PZP in liposomes that led to multiple years of infertility in some breeding trials (Killian et al. 2008, Roelle et al. 2017, Bechert and Fraker 2018), but unacceptably poor efficacy in a subsequent trial (Kane 2018). Kirkpatrick et al. (1992) noted effects on horse ovaries after three years of treatment with PZP. Observations at Assateague Island National Seashore indicated that the more times a mare is consecutively treated, the longer the time lag before fertility returns, but that even mares treated 7 consecutive years did eventually return to ovulation (Kirkpatrick and Turner 2002). Other studies have reported that continued PZP vaccine applications may result in decreased estrogen levels (Kirkpatrick et al. 1992) but that decrease was not biologically significant, as ovulation remained similar between treated and untreated mares (Powell and Monfort 2001). Bagavant et al. (2003) demonstrated T-cell clusters on ovaries, but no loss of ovarian function after ZP protein immunization in macaques.

Reversibility and Effects on Ovaries: GnRH Vaccines

The NAS (2013) review pointed out that single doses of GonaCon-Equine do not lead to high rates of initial effectiveness, or long duration. Initial effectiveness of one dose of GonaCon-Equine vaccine appears to be lower than for a combined primer plus booster dose of the PZP vaccine Zonastat-H (Kirkpatrick et al. 2011), and the initial effect of a single GonaCon dose can be limited to as little as one breeding season. However, preliminary results on the effects of boostered doses of GonaCon-Equine indicate that it can have high efficacy and longer-lasting effects in free-roaming horses (Baker et al. 2017, 2018) than the one-year effect that is generally expected from a single booster of Zonastat-H.

Too few studies have reported on the various formulations of anti-GnRH vaccines to make generalizations about differences between products, but GonaCon formulations were consistently good at causing loss of fertility in a statistically significant fraction of treated mares for at least one year (Killian et al. 2009, Gray et al. 2010, Baker et al. 2013, 2017, 2018). With few exceptions (e.g., Goodloe

1991), anti-GnRH treated mares gave birth to fewer foals in the first season when there would be an expected contraceptive effect (Botha et al. 2008, Killian et al. 2009, Gray et al. 2010, Baker et al. 2013, 2018). Goodloe (1991) used an anti-GnRH-KHL vaccine with a triple adjuvant, in some cases attempting to deliver the vaccine to horses with a hollow-tipped 'biobullet, 'but concluded that the vaccine was not an effective immunocontraceptive in that study.

Not all mares should be expected to respond to the GonaCon-equine vaccine; some number should be expected to continue to become pregnant and give birth to foals. In studies where mares were exposed to stallions, the fraction of treated mares that are effectively contracepted in the year after anti-GnRH vaccination varied from study to study, ranging from ~50% (Baker et al. 2017), to 61% (Gray et al. 2010), to ~90% (Killian et al. 2006, 2008, 2009). Miller et al. (2013) noted lower effectiveness in free-ranging mares (Gray et al. 2010) than captive mares (Killian et al. 2009). Some of these rates are lower than the high rate of effectiveness typically reported for the first year after PZP vaccine treatment (Kirkpatrick et al. 2011). In the one study that tested for a difference, darts and hand-injected GonaCon doses were equally effective in terms of fertility outcome (McCann et al. 2017).

In studies where mares were not exposed to stallions, the duration of effectiveness also varied. A primer and booster dose of Equity led to anoestrus for at least 3 months (Elhay et al. 2007). A primer and booster dose of Improvac also led to loss of ovarian cycling for all mares in the short term (Imboden et al. 2006, Nolan et al. 2018c). It is worth repeating that those vaccines do not have the same formulation as GonaCon.

Results from horses (Baker et al. 2017, 2018) and other species (Curtis et al. 2001) suggest that providing a booster dose of GonaCon-Equine will increase the fraction of temporarily infertile animals to higher levels than would a single vaccine dose alone.

Longer-term infertility has been observed in some mares treated with anti-GnRH vaccines, including GonaCon-Equine. In a single-dose mare captive trial with an initial year effectiveness of 94%, Killian et al. (2008) noted infertility rates of 64%, 57%, and 43% in treated mares during the following three years, while control mares in those years had infertility rates of 25%, 12%, and 0% in those years. GonaCon effectiveness in free-roaming populations was lower, with infertility rates consistently near 60% for three years after a single dose in one study (Gray et al. 2010) and annual infertility rates decreasing over time from 55% to 30% to 0% in another study with one dose (Baker et al. 2017, 2018). Similarly, gradually increasing fertility rates were observed after single dose treatment with GonaCon in elk (Powers et al. 2011) and deer (Gionfriddo et al. 2011a).

Baker et al. (2017, 2018) observed a return to fertility over 4 years in mares treated once with GonaCon, but then noted extremely low fertility rates of 0% and 16% in the two years after the same mares were given a booster dose four years after the primer dose. Four of nine mares treated with primer and booster doses of Improvac did not return to ovulation within 2 years of the primer dose (Imboden et al. 2006), though one should probably not make conclusions about the long-term effects of GonaCon-Equine based on results from Improvac.

It is difficult to predict which females will exhibit strong or long-term immune responses to anti-GnRH vaccines (Killian et al. 2006, Miller et al. 2008, Levy et al. 2011). A number of factors may influence responses to vaccination, including age, body condition, nutrition, prior immune responses, and genetics (Cooper and Herbert 2001, Curtis et al. 2001, Powers et al. 2011). One apparent trend is that animals that are treated at a younger age, especially before puberty, may have stronger and longer-lasting responses (Brown et al. 1994, Curtis et al. 2001, Stout et al. 2003, Schulman et al. 2013). It is plausible that giving ConaGon-Equine to prepubertal mares will lead to long-lasting infertility, but that has not yet been tested.

To date, short term evaluation of anti-GnRH vaccines, show contraception appears to be temporary and reversible. Killian et al. noted long-term effects of GonaCon in some captive mares (2009). However, Baker et al. (2017) observed horses treated with GonaCon-B return to fertility after they were treated with a single primer dose; after four years, the fertility rate was indistinguishable between treated and control mares. It appears that a single dose of GonaCon results in reversible infertility. If long-term treatment resulted in permanent infertility for some treated mares, such permanent infertility fertility would be consistent with the desired effect of using GonaCon (e.g., effective contraception).

Other anti-GnRH vaccines also have had reversible effects in mares. Elhay (2007) noted a return to ovary functioning over the course of 34 weeks for 10 of 16 mares treated with Equity. That study ended at 34 weeks, so it is not clear when the other six mares would have returned to fertility. Donovan et al. (2013) found that half of mares treated with an anti-GnRH vaccine intended for dogs had returned to fertility after 40 weeks, at which point the study ended. In a study of mares treated with a primer and booster dose of Improvac, 47 of 51 treated mares had returned to ovarian cyclicity within 2 years; younger mares appeared to have longer-lasting effects than older mares (Schulman et al. 2013). Joonè et al. (2017) analyzed samples from the Schulman et al. (2013) study, and found no significant decrease in anti-Mullerian hormone (AMH) levels in mares treated with GnRH vaccine. AMH levels are thought to be an indicator of ovarian function, so results from Joonè et al. (2017) support the general view that the anoestrus resulting from GnRH vaccination is physiologically similar to typical winter anoestrus. In a small study with a non-commercial anti-GnRH vaccine (Stout et al. 2003), three of seven treated mares had returned to cyclicity within 8 weeks after delivery of the primer dose, while four others were still suppressed for 12 or more weeks. In elk, Powers et al. (2011) noted that contraception after one dose of GonaCon was reversible. In white-tailed deer, single doses of GonaCon appeared to confer two years of contraception (Miller et al. 2000). Ten of 30 domestic cows treated became pregnant within 30 weeks after the first dose of Bopriva (Balet et al. 2014).

Permanent sterility as a result of single-dose or boostered GonaCon-Equine vaccine, or other anti-GnRH vaccines, has not been recorded, but that may be because no long-term studies have tested for that effect. It is conceivable that some fraction of mares could become sterile after receiving one or more booster doses of GonaCon-Equine. If some fraction of mares treated with GonaCon-Equine were to become sterile, though, that result would be consistent with text of the WFRHBA of 1971, as amended, which allows for sterilization to achieve population goals.

In summary, based on the above results related to fertility effects of GonaCon and other anti-GnRH vaccines, application of a single dose of GonaCon-Equine to gathered or remotely-darted wild horses could be expected to prevent pregnancy in perhaps 30%-60% of mares for one year. Some smaller number of wild mares should be expected to have persistent contraception for a second year, and less still for a third year. Applying one booster dose of GonaCon to previously-treated mares may lead to four or more years with relatively high rates (80+%) of additional infertility expected (Baker et al. 2018). There is no data to support speculation regarding efficacy of multiple boosters of GonaCon-Equine; however, given it is formulated as a highly immunogenic long-lasting vaccine, it is reasonable to hypothesize that additional boosters would increase the effectiveness and duration of the vaccine.

GonaCon-Equine only affects the fertility of treated animals; untreated animals will still be expected to give birth. Even under favorable circumstances for population growth suppression, gather efficiency might not exceed 85% via helicopter, and may be less with bait and water trapping. Similarly, not all animals may be approachable for darting. The uncaptured or undarted portion of the female population would still be expected to have normally high fertility rates in any given year, though those rates could go up slightly if contraception in other mares increases forage and water availability.

Changes in hormones associated with anti-GnRH vaccination lead to measurable changes in ovarian structure and function. The volume of ovaries reduced in response to treatment (Garza et al. 1986, Dalin et al. 2002, Imboden et al. 2006, Elhay et al. 2007, Botha et al. 2008, Gionfriddo 2011a, Dalmau et al. 2015). Treatment with an anti-GnRH vaccine changes follicle development (Garza et al. 1986, Stout et al. 2003, Imboden et al. 2006, Elhay et al. 2007, Donovan et al. 2013, Powers et al. 2011, Balet et al. 2014), with the result that ovulation does not occur. A related result is that the ovaries can exhibit less activity and cycle with less regularity or not at all in anti-GnRH vaccine treated females (Goodloe 1991, Dalin et al. 2002, Imboden et al. 2006, Elhay et al. 2007, Janett et al. 2009a, Powers et al. 2011, Donovan et al. 2013). In studies where the vaccine required a booster, hormonal and associated results were generally observed within several weeks after delivery of the booster dose.

Effects on Existing Pregnancies, Foals, and Birth Phenology: PZP Vaccines

Although fetuses are not explicitly protected under the WFRHBA of 1971, as amended, it is prudent to analyze the potential effects of fertility control vaccines on developing fetuses and foals. Any impacts identified in the literature have been found to be transient, and do not influence the future reproductive capacity of offspring born to treated females.

If a mare is already pregnant, the PZP vaccine has not been shown to affect normal development of the fetus or foal, or the hormonal health of the mare with relation to pregnancy (Kirkpatrick and Turner 2003). Studies on Assateague Island (Kirkpatrick and Turner 2002) showed that once female offspring born to mares treated with PZP during pregnancy eventually breed, they produce healthy, viable foals. It is possible that there may be transitory effects on foals born to mares or jennies treated with PZP. For example, in mice, Sacco et al. (1981) found that antibodies specific to PZP can pass from mother mouse to pup via the placenta or colostrum, but that did not apparently cause any innate immune response in the offspring: the level of those antibodies were undetectable by 116 days after birth. There was no indication in that study that the fertility or ovarian function of those mouse pups was compromised, nor is BLM aware of any such results in horses or burros. Unsubstantiated, speculative connections between PZP treatment and 'foal stealing' has not been published in a peer-reviewed study and thus cannot be verified. 'Foal stealing,' where a near-term pregnant mare steals a neonate foal from a weaker mare, is unlikely to be a common behavioral result of including spayed mares in a wild horse herd. McDonnell (2012) noted that "foal stealing is rarely observed in horses, except under crowded conditions and synchronization of foaling," such as in horse feed lots. Those conditions are not likely in the wild, where pregnant mares will be widely distributed across the landscape, and where the expectation is that parturition dates would be distributed across the normal foaling season. Similarly, although Nettles (1997) noted reported stillbirths after PZP treatments in cynomolgus monkeys, those results have not been observed in equids despite extensive use in horses and burros.

On-range observations from 20 years of application to wild horses indicate that PZP application in wild mares does not generally cause mares to give birth to foals out of season or late in the year (Kirkpatrick and Turner 2003). Nuñez's (2010) research showed that a small number of mares that had previously been treated with PZP foaled later than untreated mares and expressed the concern that this late foaling "may" impact foal survivorship and decrease band stability, or that higher levels of attention from stallions on PZP-treated mares might harm those mares. However, that paper provided no evidence that such impacts on foal survival or mare well-being actually occurred. Rubenstein (1981) called attention to a number of unique ecological features of horse herds on Atlantic barrier islands, such as where Nuñez made observations, which calls into question whether inferences drawn from island herds can be applied to western wild horse herds. Ransom et al. (2013), though, did identify a potential shift in reproductive timing as a possible drawback to prolonged treatment with PZP, stating that treated mares foaled on average 31 days later than non-treated mares. Results from Ransom et al.

(2013), however, showed that over 81% of the documented births in that study were between March 1 and June 21, i.e., within the normal, peak, spring foaling season. Ransom et al. (2013) pointedly advised that managers should consider carefully before using fertility control vaccines in small refugia or rare species. Wild horses and burros managed by BLM do not generally occur in isolated refugia, nor are they at all rare species. The US Fish and Wildlife Service denied a petition to list wild horses as endangered (USFWS 2015). Moreover, any effect of shifting birth phenology was not observed uniformly: in two of three PZP-treated wild horse populations studied by Ransom et al. (2013), foaling season of treated mares extended three weeks and 3.5 months, respectively, beyond that of untreated mares. In the other population, the treated mares foaled within the same time period as the untreated mares. Furthermore, Ransom et al. (2013) found no negative impacts on foal survival even with an extended birthing season. If there are shifts in birth phenology, though, it is reasonable to assume that some negative effects on foal survival for a small number of foals might result from particularly severe weather events (Nuñez et al. 2018).

Effects on Existing Pregnancies, Foals, and Birth Phenology: GnRH Vaccines

Although fetuses are not explicitly protected under the WFRHBA of 1971, as amended, it is prudent to analyze the potential effects of fertility control vaccines on developing fetuses and foals. Any impacts identified in the literature have been found to be transient, and do not influence the future reproductive capacity of offspring born to treated females.

GonaCon and other anti-GnRH vaccines can be injected while a female is pregnant (Miller et al. 2000, Powers et al. 2011, Baker et al. 2013) – in such a case, a successfully contracepted mare will be expected to give birth during the following foaling season, but to be infertile during the same year's breeding season. Thus, a mare injected in November of 2018 would not show the contraceptive effect (i.e., no new foal) until spring of 2020.

GonaCon had no apparent effect on pregnancies in progress, foaling success, or the health of offspring, in horses that were immunized in October (Baker et al. 2013), elk immunized 80-100 days into gestation (Powers et al. 2011, 2013), or deer immunized in February (Miller et al. 2000). Kirkpatrick et al. (2011) noted that anti-GnRH immunization is not expected to cause hormonal changes that would lead to abortion in the horse, but this may not be true for the first 6 weeks of pregnancy (NAS 2013). Curtis et al. (2011) noted that GonaCon-KHL treated white tailed deer had lower twinning rates than controls, but speculated that the difference could be due to poorer sperm quality late in the breeding season, when the treated does did become pregnant. Goodloe (1991) found no difference in foal production between treated and control animals.

Offspring of anti-GnRH vaccine treated mothers could exhibit an immune response to GnRH (Khodr and Siler-Khodr 1980), as antibodies from the mother could pass to the offspring through the placenta or colostrum. In the most extensive study of long-term effects of GonaCon immunization on offspring, Powers et al. (2012) monitored 15 elk fawns born to GonaCon treated cows. Of those, 5 had low titers at birth and 10 had high titer levels at birth. All 15 were of normal weight at birth, and developed normal endocrine profiles, hypothalamic GnRH content, pituitary gonadotropin content, gonad structure, and gametogenesis. All the females became pregnant in their second reproductive season, as is typical. All males showed normal development of secondary sexual characteristics. Powers et al. (2012) concluded that suppressing GnRH in the neonatal period did not alter long-term reproductive function in either male or female offspring. Miller et al. (2013) report elevated anti-GnRH antibody titers in fawns born to treated white tailed deer, but those dropped to normal levels in 11 of 12 of those fawns, which came into breeding condition; the remaining fawn was infertile for three years.

Direct effects on foal survival are equivocal in the literature. Goodloe (1991), reported lower foal survival for a small sample of foals born to anti-GnRH treated mares, but she did not assess other possible

explanatory factors such as mare social status, age, body condition, or habitat in her analysis (NAS 2013). Gray et al. (2010) found no difference in foal survival in foals born to free-roaming mares treated with GonaCon.

There is little empirical information available to evaluate the effects of GnRH vaccination on foaling phenology, but those effects are likely to be similar to those for PZP vaccine treated mares in which the effects of the vaccine wear off. It is possible that immunocontracepted mares returning to fertility late in the breeding season could give birth to foals at a time that is out of the normal range (Nuñez et al. 2010, Ransom et al 2013). Curtis et al. (2001) did observe a slightly later fawning date for GonaCon treated deer in the second year after treatment, when some does regained fertility late in the breeding season. In anti-GnRH vaccine trials in free-roaming horses, there were no published differences in mean date of foal production (Goodloe 1991, Gray et al. 2010). Unpublished results from an ongoing study of GonaCon treated free-roaming mares indicate that some degree of seasonal foaling is possible (D. Baker, Colorado State University, personal communication to Paul Griffin, BLM WH&B Research Coordinator). Because of the concern that contraception could lead to shifts in the timing of parturitions for some treated animals, Ransom et al. (2013) advised that managers should consider carefully before using PZP immunocontraception in small refugia or rare species; the same considerations could be advised for use of GonaCon, but wild horses and burros in most areas do not generally occur in isolated refugia, they are not a rare species at the regional, national, or international level, and genetically they represent descendants of domestic livestock with most populations containing few if any unique alleles (NAS 2013). Moreover, in PZP-treated horses that did have some degree of parturition date shift, Ransom et al. (2013) found no negative impacts on foal survival even with an extended birthing season; however, this may be more related to stochastic, inclement weather events than extended foaling seasons. If there were to be a shift in foaling date for some treated mares, the effect on foal survival may depend on weather severity and local conditions; for example, Ransom et al. (2013) did not find consistent effects across study sites.

Effects of Marking and Injection

Standard practices require that immunocontraceptive-treated animals be readily identifiable, either via brand marks or unique coloration (BLM 2010). Some level of transient stress is likely to result in newly captured mares that do not have markings associated with previous fertility control treatments. It is difficult to compare that level of temporary stress with the long-term stress that can result from food and water limitation on the range (e.g., Creel et al. 2013). Handling may include freeze-marking, for the purpose of identifying that mare and identifying her vaccine treatment history. Under past management practices, captured mares experienced increased stress levels from handling (Ashley and Holcombe 2001), but BLM has instituted guidelines to reduce the sources of handling stress in captured animals (BLM 2015).

Most mares recover from the stress of capture and handling quickly once released back to the range, and none are expected to suffer serious long term effects from the fertility control injections, other than the direct consequence of becoming temporarily infertile. Injection site reactions associated with fertility control treatments are possible in treated mares (Roelle and Ransom 2009, Bechert et al. 2013, French et al. 2017, Baker et al. 2018), but swelling or local reactions at the injection site are expected to be minor in nature. Roelle and Ransom (2009) found that the most time-efficient method for applying PZP is by hand-delivered injection of 2-year pellets when horses are gathered. They observed only two instances of swelling from that technique. Whether injection is by hand or via darting, GonaCon-Equine is associated with some degree of inflammation, swelling, and the potential for abscesses at the injection site (Baker et al. 2013). Swelling or local reactions at the injection site are generally expected to be minor in nature, but some may develop into draining abscesses. Use of remotely delivered vaccine is generally limited to populations where individual animals can be accurately identified and repeatedly approached. The dart-delivered PZP formulation produced injection-site reactions of varying intensity, though none of the observed reactions appeared debilitating to the animals (Roelle and Ransom 2009) but that was not observed with dart-delivered GonaCon (McCann et al. 2017). Joonè et al. (2017a) found that injection site reactions had healed in most mares within 3 months after the booster dose, and that they did not affect movement or cause fever.

Long-lasting nodules observed did not appear to change any animal's range of movement or locomotor patterns and in most cases did not appear to differ in magnitude from naturally occurring injuries or scars. Mares treated with one formulation of GnRH-KHL vaccine developed pyogenic abscesses (Goodloe 1991). Miller et al. (2008) noted that the water and oil emulsion in GonaCon will often cause cysts, granulomas, or sterile abscesses at injection sites; in some cases, a sterile abscess may develop into a draining abscess. In elk treated with GonaCon, Powers et al. (2011) noted up to 35% of treated elk had an abscess form, despite the injection sites first being clipped and swabbed with alcohol. Even in studies where swelling and visible abscesses followed GonaCon immunization, the longer term nodules observed did not appear to change any animal's range of movement or locomotor patterns (Powers et al. 2013, Baker et al. 2017, 2018). The result that other formulations of anti-GnRH vaccine may be associated with less notable injection site reactions in horses may indicate that the adjuvant formulation in GonaCon leads a single dose to cause a stronger immune reaction than the adjuvants used in other anti-GnRH vaccines. Despite that, a booster dose of GonaCon-Equine appears to be more effective than a primer dose alone (Baker et al. 2017). Horses injected in the hip with Improvac showed only transient reactions that disappeared within 6 days in one study (Botha et al. 2008), but stiffness and swelling that lasted 5 days were noted in another study where horses received Improvac in the neck (Imboden et al. 2006). Equity led to transient reactions that resolved within a week in some treated animals (Elhay et al. 2007). Donovan et al. noted no reactions to the canine anti-GnRH vaccine (2013). In cows treated with Bopriva there was a mildly elevated body temperature and mild swelling at injection sites that subsided within 2 weeks (Balet et al. 2014).

Indirect Effects: PZP Vaccines

One expected long-term, indirect effect on wild horses treated with fertility control would be an improvement in their overall health (Turner and Kirkpatrick 2002). Many treated mares would not experience the biological stress of reproduction, foaling and lactation as frequently as untreated mares. The observable measure of improved health is higher body condition scores (Nuñez et al. 2010). After a treated mare returns to fertility, her future foals would be expected to be healthier overall, and would benefit from improved nutritional quality in the mare's milk. This is particularly to be expected if there is an improvement in rangeland forage quality at the same time, due to reduced wild horse population size. Past application of fertility control has shown that mares' overall health and body condition remains improved even after fertility resumes. PZP treatment may increase mare survival rates, leading to longer potential lifespan (Turner and Kirkpatrick 2002, Ransom et al. 2014a) that may be as much as 5-10 years (NPS 2008). To the extent that this happens, changes in lifespan and decreased foaling rates could combine to cause changes in overall age structure in a treated herd (i.e., Turner and Kirkpatrick 2002, Roelle et al. 2010), with a greater prevalence of older mares in the herd (Gross 2000, NPS 2008). Observations of mares treated in past gathers showed that many of the treated mares were larger than, maintained higher body condition than, and had larger healthy foals than untreated mares (BLM, anecdotal observations).

Following resumption of fertility, the proportion of mares that conceive and foal could be increased due to their increased fitness; this has been called a 'rebound effect.' Elevated fertility rates have been observed after horse gathers and removals (Kirkpatrick and Turner 1991). If repeated contraceptive treatment leads to a prolonged contraceptive effect, then that may minimize or delay the hypothesized rebound effect. Selectively applying contraception to older animals and returning them to the range

could reduce long-term holding costs for such horses, which are difficult to adopt, and may reduce the compensatory reproduction that often follows removals (Kirkpatrick and Turner 1991).

Because successful fertility control in a given herd reduces foaling rates and population growth rates, another indirect effect should be to reduce the number of wild horses that have to be removed over time to achieve and maintain the established AML. Contraception may change a herd's age structure, with a relative increase in the fraction of older animals in the herd (NPS 2008). Reducing the numbers of wild horses that would have to be removed in future gathers could allow for removal of younger, more easily adoptable excess wild horses, and thereby could eliminate the need to send additional excess horses from this area to off-range holding corrals or pastures for long-term holding.

A principle motivation for use of contraceptive vaccines or sex ratio manipulation is to reduce population growth rates and maintain herd sizes at AML. Where successful, this should allow for continued and increased environmental improvements to range conditions within the project area, which would have long-term benefits to wild horse and burro habitat guality, and well-being of animals living on the range. As the population nears or is maintained at the level necessary to achieve a thriving natural ecological balance, vegetation resources would be expected to recover, improving the forage available. With rangeland conditions more closely approaching a thriving natural ecological balance, and with a less concentrated distribution of wild horses and burros, there should also be less trailing and concentrated use of water sources. Lower population density should lead to reduced competition among wild horses using the water sources, and less fighting among horses accessing water sources. Water quality and quantity would continue to improve to the benefit of all rangeland users including wild horses. Wild horses would also have to travel less distance back and forth between water and desirable foraging areas. Among mares in the herd that remain fertile, a higher level of physical health and future reproductive success would be expected in areas where lower horse and burro population sizes lead to increases in water and forage resources. While it is conceivable that widespread and continued treatment with fertility control vaccines could reduce the birth rates of the population to such a point that birth is consistently below mortality, that outcome is not likely unless a very high fraction of the mares present are all treated in almost every year.

Indirect Effects: GnRH Vaccines

As noted above to PZP vaccines, an expected long-term, indirect effect on wild horses treated with fertility control would be an improvement in their overall health. Body condition of anti-GnRH-treated females was equal to or better than that of control females in published studies. Ransom et al. (2014b) observed no difference in mean body condition between GonaCon-B treated mares and controls. Goodloe (1991) found that GnRH-KHL treated mares had higher survival rates than untreated controls. In other species, treated deer had better body condition than controls (Gionfriddo et al. 2011b), treated cats gained more weight than controls (Levy et al. 2011), as did treated young female pigs (Bohrer et al. 2014).

Following resumption of fertility, the proportion of mares that conceive and foal could be increased due to their increased fitness; this has been called by some a 'rebound effect.' Elevated fertility rates have been observed after horse gathers and removals (Kirkpatrick and Turner 1991). If repeated contraceptive treatment leads to a prolonged contraceptive effect, then that may minimize or delay the hypothesized rebound effect. Selectively applying contraception to older animals and returning them to the range could reduce long-term holding costs for such horses, which are difficult to adopt, and could negate the compensatory reproduction that can follow removals (Kirkpatrick and Turner 1991).

Because successful fertility control would reduce foaling rates and population growth rates, another indirect effect would be to reduce the number of wild horses that have to be removed over time to achieve and maintain the established AML. Contraception would be expected to lead to a relative

increase in the fraction of older animals in the herd. Reducing the numbers of wild horses that would have to be removed in future gathers could allow for removal of younger, more easily adoptable excess wild horses, and thereby could eliminate the need to send additional excess horses from this area to off-range holding corrals or pastures for long-term holding. Among mares in the herd that remain fertile, a high level of physical health and future reproductive success would be expected because reduced population sizes should lead to more availability of water and forage resources per capita.

Reduced population growth rates and smaller population sizes could also allow for continued and increased environmental improvements to range conditions within the project area, which would have long-term benefits to wild horse habitat quality. As the local horse abundance nears or is maintained at the level necessary to achieve a thriving natural ecological balance, vegetation resources would be expected to recover, improving the forage available to wild horses and wildlife throughout the area. With rangeland conditions more closely approaching a thriving natural ecological balance, and with a less concentrated distribution of wild horses across the range, there should also be less trailing and concentrated use of water sources. Lower population density would be expected to lead to reduced competition among wild horses using the water sources, and less fighting among horses accessing water sources. Water quality and quantity would continue to improve to the benefit of all rangeland users including wild horses. Wild horses would also have to travel less distance back and forth between water and desirable foraging areas. Should GonaCon-Equine treatment, including booster doses, continue into the future, with treatments given on a schedule to maintain a lowered level of fertility in the herd, the chronic cycle of overpopulation and large gathers and removals might no longer occur, but instead a consistent abundance of wild horses could be maintained, resulting in continued improvement of overall habitat conditions and animal health. While it is conceivable that widespread and continued treatment with GonaCon-Equine could reduce the birth rates of the population to such a point that birth is consistently below mortality, that outcome is not likely unless a very high fraction of the mares present are all treated with primer and booster doses, and perhaps repeated booster doses.

Behavioral Effects: PZP Vaccines

Behavioral difference, compared to mares that are fertile, should be considered as potential results of successful contraception. The NAS report (2013) noted that all forms of fertility suppression have effects on mare behavior, mostly because of the lack of pregnancy and foaling, and concluded that fertility control vaccines were among the most promising fertility control methods for wild horses and burros. The resulting impacts may be seen as neutral in the sense that a wide range of natural behaviors is already observable in untreated wild horses, or mildly adverse in the sense that effects are expected to be transient and to not affect all treated animals.

PZP vaccine-treated mares may continue estrus cycles throughout the breeding season. Ransom and Cade (2009) delineated wild horse behaviors. Ransom et al. (2010) found no differences in how PZP-treated and untreated mares allocated their time between feeding, resting, travel, maintenance, and most social behaviors in three populations of wild horses, which is consistent with Powell's (1999) findings in another population. Likewise, body condition of PZP-treated and control mares did not differ between treatment groups in Ransom et al.'s (2010) study. Nuñez (2010) found that PZP-treated mares had higher body condition than control mares in another population, presumably because energy expenditure was reduced by the absence of pregnancy and lactation. Knight (2014) found that PZP-treated mares that foaled spent more time concentrating on grazing and lactation and had lower overall body condition.

In two studies involving a total of four wild horse populations, both Nuñez et al. (2009) and Ransom et al. (2010) found that PZP vaccine treated mares were involved in reproductive interactions with stallions

more often than control mares, which is not surprising given the evidence that PZP-treated females of other mammal species can regularly demonstrate estrus behavior while contracepted (Shumake and Killian 1997, Heilmann et al. 1998, Curtis et al. 2001, Duncan et al. 2017). There was no evidence, though, that mare welfare was affected by the increased level of herding by stallions noted in Ransom et al. (2010). Nuñez's later analysis (2017) noted no difference in mare reproductive behavior as a function of contraception history

Ransom et al. (2010) found that control mares were herded by stallions more frequently than PZPtreated mares, and Nuñez et al. (2009, 2014, 2017, 2018) found that PZP-treated mares exhibited higher infidelity to their band stallion during the non-breeding season than control mares. Madosky et al. (2010) and Knight (2014) found this infidelity was also evident during the breeding season in the same population that Nuñez et al. (2009, 2010, 2014, 2017, 2018) studied. Nuñez et al. (2014, 2017, 2018) concluded that PZP-treated mares changing bands more frequently than control mares could lead to band instability. Nuñez et al. (2009), though, cautioned against generalizing from that island population to other herds. Also, despite any potential changes in band infidelity due to PZP vaccination, horses continued to live in social groups with dominant stallions and one or more mares. Nuñez et al. (2014) found elevated levels of fecal cortisol, a marker of physiological stress, in mares that changed bands. The research is inconclusive as to whether all the mares' movements between bands were related to the PZP treatments themselves or the fact that the mares were not nursing a foal, and did not demonstrate any long-term negative consequence of the transiently elevated cortisol levels. In separate work in a long-term study of semi-feral Konik ponies, Jaworska et al. (2020) showed that neither infanticide nor feticide resulted for mares and their foals after a change in dominant stallion. Nuñez et al. 2014 wrote that these effects "...may be of limited concern when population reduction is an urgent priority." Nuñez (2018) and Jones et al. (2019, 2020) noted that band stallions of mares that have received PZP treatment can exhibit changes in behavior and physiology. Nuñez (2018) cautioned that PZP use may limit the ability of mares to return to fertility, but also noted that, "such aggressive treatments may be necessary when rapid reductions in animal numbers are of paramount importance...If the primary management goal is to reduce population size, it is unlikely (and perhaps less important) that managers achieve a balance between population control and the maintenance of more typical feral horse behavior and physiology."

In contrast to transient stresses, Creel et al. (2013) highlight that variation in population density is one of the most well-established causal factors of chronic activation of the hypothalamic-pituitary-adrenal axis, which mediates stress hormones; high population densities and competition for resources can cause chronic stress. Creel et al. (2013) also state that "...there is little consistent evidence for a negative association between elevated baseline glucocorticoids and fitness." Band fidelity is not an aspect of wild horse biology that is specifically protected by the WFRHBA of 1971. It is also notable that Ransom et al. (2014b) found higher group fidelity after a herd had been gathered and treated with a contraceptive vaccine; in that case, the researchers postulated that higher fidelity may have been facilitated by the decreased competition for forage after excess horses were removed. At the population level, available research does not provide evidence of the loss of harem structure among any herds treated with PZP. No biologically significant negative impacts on the overall animals or populations overall, long-term welfare or well-being have been established in these studies.

The National Research Council (2013) found that harem changing was not likely to result in serious adverse effects for treated mares:

"The studies on Shackleford Banks (Nuñez et al., 2009; Madosky et al., 2010) suggest that there is an interaction between pregnancy and social cohesion. The importance of harem stability to mare well-being is not clear, but considering the relatively large number of free-ranging mares that have been treated with liquid PZP in a variety of ecological settings, the likelihood of serious adverse effects seem low."

Nuñez (2010) stated that not all populations will respond similarly to PZP treatment. Differences in habitat, resource availability, and demography among conspecific populations will undoubtedly affect their physiological and behavioral responses to PZP contraception, and may be considered. Kirkpatrick et al. (2010) concluded that: "the larger question is, even if subtle alterations in behavior may occur, this is still far better than the alternative," and that the "...other victory for horses is that every mare prevented from being removed, by virtue of contraception, is a mare that will only be delaying her reproduction rather than being eliminated permanently from the range. This preserves herd genetics, while gathers and adoption do not."

The NAS report (2013) provides a comprehensive review of the literature on the behavioral effects of contraception that puts research up to that date by Nuñez et al. (2009, 2010) into the broader context of all of the available scientific literature, and cautions, based on its extensive review of the literature that:

"... in no case can the committee conclude from the published research that the behavior differences observed are due to a particular compound rather than to the fact that treated animals had no offspring during the study. That must be borne in mind particularly in interpreting long-term impacts of contraception (e.g., repeated years of reproductive "failure" due to contraception)."

Behavioral Effects: GnRH Vaccines

The result that GonaCon treated mares may have suppressed estrous cycles throughout the breeding season can lead treated mares to behave in ways that are functionally similar to pregnant mares. Where it is successful in mares, GonaCon and other anti-GnRH vaccines are expected to induce fewer estrous cycles when compared to non-pregnant control mares. This has been observed in many studies (Garza et al. 1986, Curtis et al. 2001, Dalin et al. 2002, Killian et al. 2006, Dalmau et al. 2015). Females treated with GonaCon had fewer estrous cycles than control or PZP-treated mares (Killian et al. 2006) or deer (Curtis et al. 2001). Thus, any concerns about PZP treated mares receiving more courting and breeding behaviors from stallions (Nuñez et al. 2009, Ransom et al. 2010) are not generally expected to be a concern for mares treated with anti-GnRH vaccines (Botha et al. 2008).

Ransom et al. (2014b) and Baker et al. (2018) found that GonaCon treated mares had similar rates of reproductive behaviors that were similar to those of pregnant mares. Among other potential causes, the reduction in progesterone levels in treated females may lead to a reduction in behaviors associated with reproduction. Despite this, some females treated with GonaCon or other anti-GnRH vaccines did continue to exhibit reproductive behaviors, albeit at irregular intervals and durations (Dalin et al. 2002, Stout et al. 2003, Imboden et al. 2006), which is a result that is similar to spayed (ovariectomized) mares (Asa et al. 1980). Gray et al. (2009a) and Baker et al. (2018) found no difference in sexual behaviors in mares treated with GonaCon and untreated mares. When progesterone levels are low, small changes in estradiol concentration can foster reproductive estrous behaviors (Imboden et al. 2006). Owners of anti-GnRH vaccine treated mares reported a reduced number of estrous-related behaviors under saddle (Donovan et al. 2013). Treated mares may refrain from reproductive behavior even after ovaries return to cyclicity (Elhay et al. 2007). Studies in elk found that GonaCon treated cows had equal levels of precopulatory behaviors as controls (Powers et al. 2011), though bull elk paid more attention to treated cows late in the breeding season, after control cows were already pregnant (Powers et al. 2011).

Stallion herding of mares, and harem switching by mares are two behaviors related to reproduction that might change as a result of contraception. Ransom et al. (2014b) observed a 50% decrease in herding behavior by stallions after the free-roaming horse population at Theodore Roosevelt National Park was reduced via a gather, and mares there were treated with GonaCon-B. The increased harem tending

behaviors by stallions were directed to both treated and control mores. It is difficult to separate any effect of GonaCon in this study from changes in horse density and forage following horse removals.

With respect to treatment with GonaCon or other anti-GnRH vaccines, it is probably less likely that treated mares will switch harems at higher rates than untreated animals, because treated mares are similar to pregnant mares in their behaviors (Ransom et al. 2014b). Indeed, Gray et al. (2009a) found no difference in band fidelity in a free-roaming population of horses with GonaCon treated mares, despite differences in foal production between treated and untreated mares. Ransom et al. (2014b) actually found increased levels of band fidelity after treatment, though this may have been partially a result of changes in overall horse density and forage availability.

Gray et al. (2009) and Ransom et al. (2014b) monitored non-reproductive behaviors in GonaCon treated populations of free-roaming horses. Gray et al. (2009a) found no difference between treated and untreated mares in terms of activity budget, sexual behavior, proximity of mares to stallions, or aggression. Ransom et al. (2014b) found only minimal differences between treated and untreated mare time budgets, but those differences were consistent with differences in the metabolic demands of pregnancy and lactation in untreated mares, as opposed to non-pregnant treated mares.

Genetic Effects of Fertility Control Vaccines

In HMAs where large numbers of wild horses have recent and / or an ongoing influx of breeding animals from other areas with wild or feral horses, contraception is not expected to cause an unacceptable loss of genetic diversity or an unacceptable increase in the inbreeding coefficient. In any diploid population, the loss of genetic diversity through inbreeding or drift can be prevented by large effective breeding population sizes (Wright 1931) or by introducing new potential breeding animals (Mills and Allendorf 1996). The NAS report (2013) recommended that single HMAs should not be considered as isolated genetic populations. Rather, managed herds of wild horses should be considered as components of interacting metapopulations, with the potential for interchange of individuals and genes taking place as a result of both natural and human-facilitated movements. Introducing 1-2 mares every generation (about every 10 years) is a standard management technique that can alleviated potential inbreeding concerns (BLM 2010).

In the last 10 years, there has been a high realized growth rate of wild horses in most areas administered by the BLM, such that most alleles that are present in any given mare are likely to already be well represented in her siblings, cousins, and more distant relatives. With the exception of horses in a small number of well-known HMAs that contain a relatively high fraction of alleles associated with old Spanish horse breeds (NAS 2013), the genetic composition of wild horses in lands administered by the BLM is consistent with admixtures from domestic breeds. As a result, in most HMAs, applying fertility control to a subset of mares is not expected to cause irreparable loss of genetic diversity. Improved longevity and an aging population are expected results of contraceptive treatment that can provide for lengthening generation time; this result would be expected to slow the rate of genetic diversity loss (Hailer et al. 2006). Based on a population model, Gross (2000) found that a strategy to preferentially treat young animals with a contraceptive led to more genetic diversity being retained than either a strategy that preferentially treats older animals, or a strategy with periodic gathers and removals.

Even if it is the case that repeated treatment with a fertility control vaccine may lead to prolonged infertility, or even sterility in some mares, most HMAs have only a low risk of loss of genetic diversity if logistically realistic rates of contraception are applied to mares. Wild horses in most herd management areas are descendants of a diverse range of ancestors coming from many breeds of domestic horses. As such, the existing genetic diversity in the majority of HMAs does not contain unique or historically unusual genetic markers. Past interchange between HMAs, either through natural dispersal or through assisted migration (i.e., human movement of horses) means that many HMAs are effectively

indistinguishable and interchangeable in terms of their genetic composition (i.e., see the table of Fst vales *in* NAS 2013). Roelle and Oyler-McCance (2015) used the VORTEX population model to simulate how different rates of mare sterility would influence population persistence and genetic diversity, in populations with high or low starting levels of genetic diversity, various starting population sizes, and various annual population growth rates. Their results show that the risk of the loss of genetic heterozygosity is extremely low except in case where all of the following conditions are met: starting levels of genetic diversity are low, initial population size is 100 or less, the intrinsic population growth rate is low (5% per year), and very large fractions of the female population are permanently sterilized.

It is worth noting that, although maintenance of genetic diversity at the scale of the overall population of wild horses is an intuitive management goal, there are no existing laws or policies that require BLM to maintain genetic diversity at the scale of the individual herd management area or complex. Also, there is no Bureau-wide policy that requires BLM to allow each female in a herd to reproduce before she is treated with contraceptives.

One concern that has been raised with regards to genetic diversity is that treatment with immunocontraceptives could possibly lead to an evolutionary increase in the frequency of individuals whose genetic composition fosters weak immune responses (Cooper and Larson 2006, Ransom et al. 2014a). Many factors influence the strength of a vaccinated individual's immune response, potentially including genetics, but also nutrition, body condition, and prior immune responses to pathogens or other antigens (Powers et al. 2013). This premise is based on an assumption that lack of response to any given fertility control vaccine is a heritable trait, and that the frequency of that trait will increase over time in a population of vaccine-treated animals. Cooper and Herbert (2001) reviewed the topic, in the context of concerns about the long-term effectiveness of immunocontraceptives as a control agent for exotic species in Australia. They argue that imunocontraception could be a strong selective pressure, and that selecting for reproduction in individuals with poor immune response could lead to a general decline in immune function in populations where such evolution takes place. Other authors have also speculated that differences in antibody titer responses could be partially due to genetic differences between animals (Curtis et al. 2001, Herbert and Trigg 2005). However, Magiafolou et al. (2013) clarify that if the variation in immune response is due to environmental factors (i.e., body condition, social rank) and not due to genetic factors, then there will be no expected effect of the immune phenotype on future generations. It is possible that general health, as measured by body condition, can have a causal role in determining immune response, with animals in poor condition demonstrating poor immune reactions (NAS 2013).

Correlations between physical factors and immune response would not preclude, though, that there could also be a heritable response to immunocontraception. In studies not directly related to immunocontraception, immune response has been shown to be heritable (Kean et al. 1994, Sarker et al. 1999). Unfortunately, predictions about the long-term, population-level evolutionary response to immunocontraceptive treatments are speculative at this point, with results likely to depend on several factors, including: the strength of the genetic predisposition to not respond to the fertility control vaccine; the heritability of that gene or genes; the initial prevalence of that gene or genes; the number of mares treated with a primer dose of the vaccine (which generally has a short-acting effect); the number of mares treated with one or more booster doses of the vaccine; and the actual size of the genetically-interacting metapopulation of horses within which the vaccine treatment takes place.

BLM is not aware of any studies that have quantified the heritability of a lack of response to immunocontraception such as PZP vaccine or GonaCon-Equine in horses or burros. At this point, there are no studies available from which one could make conclusions about the long-term effects of sustained and widespread immunocontraception treatments on population-wide immune function. Although a few, generally isolated, feral horse populations have been treated with high fractions of

mares receiving PZP immunocontraception for long-term population control (e.g., Assateague Island National Park, and Pryor Mountains Herd Management Area), no studies have tested for changes in immune competence in those areas. Relative to the large number of free-roaming feral horses in the western United States, immunocontraception has not been, and is not expected to be used in the type of widespread or prolonged manner that might be required to cause a detectable evolutionary response.

Sex Ratio Manipulation

Skewing the sex ratio of a herd so that there are more males than females is an established BLM management technique for reducing population growth rates. As part of a wild horse and burro gather process, the number of animals returned to the range may include more males, the number removed from the range may include more females, or both. By reducing the proportion of breeding females in a population (as a fraction of the total number of animals present), the technique leads to fewer foals being born, relative to the total herd size.

Sex ratio is typically adjusted in such a way that 60 percent of the horses are male. In the absence of other fertility control treatments, this 60:40 sex ratio can temporarily reduce population growth rates from approximately 20% to approximately 15% (Bartholow 2004). While such a decrease in growth rate may not appear to be large or long-lasting, the net result can be that fewer foals being born, at least for a few years – this can extend the time between gathers, and reduce impacts on-range, and costs off-range. Any impacts of sex ratio manipulation are expected to be temporary because the sex ratio of wild horse and burro foals at birth is approximately equal between males and females (NAS 2013), and it is common for female foals to reproduce by their second year (NAS 2013). Thus, within a few years after a gather and selective removal that leads to more males than females, the sex ratio of reproducing wild horses and burros will be returning toward a 50:50 ratio.

Having a larger number of males than females is expected to lead to several demographic and behavioral changes as noted in the NAS report (2013), including the following. Having more fertile males than females should not alter the fecundity of fertile females. Wild mares may be distributed in a larger number of smaller harems. Competition and aggression between males may cause a decline in male body condition. Female foraging may be somewhat disrupted by elevated male-male aggression. With a greater number of males available to choose from, females may have opportunities to select more genetically fit sires. There would also be an increase the genetic effective population size because more stallions would be breeding and existing females would be distributed among many more small harems. This last beneficial impact is one reason that skewing the sex ratio to favor males is listed in the BLM wild horse and burro handbook (BLM 2010) as a method to consider in herds where there may be concern about the loss of genetic diversity; having more males fosters a greater retention of genetic diversity.

Infanticide is a natural behavior that has been observed in wild equids (Feh and Munktuya 2008, Gray 2009), but there are no published accounts of infanticide rates increasing as a result of having a skewed sex ratio in wild horse or wild burro herds. Any comment that implies such an impact would be speculative.

The BLM wild horse and burro management handbook (BLM 2010) discusses this method. The handbook acknowledges that there may be some behavioral impacts of having more males than females. The handbook includes guidelines for when the method should be applied, specifying that this method should be considered where the low end of the AML is 150 animals or greater, and with the result that males comprise 60-70 percent of the herd. Having more than 70 percent males may result in unacceptable impacts in terms of elevated male-male aggression. In NEPA analyses, BLM has chosen to follow these guidelines in some cases, for example:

- In the 2015 Cold Springs HMA Population Management Plan EA (DOI-BLM-V040-2015-022), the low end of AML was 75. Under the preferred alternative, 37 mares and 38 stallions would remain on the HMA. This is well below the 150 head threshold noted above.
- In the 2017 Hog Creek HMA Population Management Plan EA (DOI-BLM-ORWA-V000-2017-0026-EA), BLM clearly identified that maintaining a 50:50 sex ratio was appropriate because the herd size at the low end of AML was only 30 animals.

It is relatively straightforward to speed the return of skewed sex ratios back to a 50:50 ratio. The BLM wild horse and burro handbook (BLM 2010) specifies that, if post-treatment monitoring reveals negative impacts to breeding harems due to sex ratio manipulation, then mitigation measures could include removing males, not introducing additional males, or releasing a larger proportion of females during the next gather.

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Effects of Sterilization, Including Spaying and Neutering

Various forms of fertility control can be used in wild horses and wild burros, with the goals of maintaining herds at or near AML, reducing fertility rates, and reducing the frequency of gathers and removals. The WFRHBA of 1971 specifically provides for contraception and sterilization (16 U.S.C. 1333 section 3.b.1). Fertility control measures have been shown to be a cost-effective and humane treatment to slow increases in wild horse populations or, when used in combination with gathers, to reduce horse

population size (Bartholow 2004, de Seve and Boyles-Griffin 2013, Fonner and Bohara 2017). Population growth suppression becomes less expensive if fertility control is long-lasting (Hobbs et al. 2000), such as with sterilization methods. Sterilizing a female horse (mare) or burro (jenny) can be accomplished by several methods, some of which are minimally invasive, and others of which are surgical. In this review, 'spaying' is defined to be surgical sterilization, usually accomplished by removal of the ovaries, but other surgical methods such as tubal ligation that lead to sterilization, such as trans-cervical methods that occlude the oviduct, are not labeled as spaying in this review, but may have similar physiological outcomes as surgical methods that leave the ovaries intact. In this review, 'neutering' is defined to be the sterilization) or burro (jack), either by removal of the testicles (castration, also known as gelding) or by vasectomy, where the testicles are retained but no sperm leave the body by severing or blocking the vas deferens or epididymis.

In the context of BLM wild horse and burro management, sterilization is expected to be successful to the extent that it reduces the number of reproducing females. By definition, sterilizing a given female is 100% effective as a fertility control method for that female. Neutering males may be effective in one of two ways. First, neutered males may continue to guard fertile females, preventing the females from breeding with fertile males. Second, if neutered males are included in a herd that has a high male-to-female sex ratio, then the neutered males may comprise some of the animals within the appropriate management level (AML) of that herd, which would effectively reduce the number of females in the herd. Although these and other fertility control treatments may be associated with a number of potential physiological, behavioral, demographic, and genetic effects, those impacts are generally minor and transient, do not prevent overall maintenance of a self-sustaining population, and do not generally outweigh the potential benefits of using contraceptive treatments in situations where it is a management goal to reduce population growth rates (Garrott and Oli 2013).

Peer-reviewed scientific literature details the expected impacts of sterilization methods on wild horses and burros. No finding of excess animals is required for BLM to pursue sterilization in wild horses or wild burros, but NEPA analysis has been required. This review focuses on peer-reviewed scientific literature. The summary that follows first examines effects of female sterilization, then neuter use in males. This review does not examine effects of fertility control vaccines. Cited studies are generally limited to those involving horses and burros, except where including studies on other species helps in making inferences about physiological or behavioral questions not exhaustively addressed in horses or burros specifically. While most studies reviewed here refer to horses, burros are extremely similar in terms of physiology, such that expected effects are comparable, except where differences between the species are noted.

On the whole, the identified impacts at the herd level are generally transient. The principle impact to individuals treated is sterility, which is the intended outcome. Sterilization that affects individual horses and burros does not prevent BLM from ensuring that there will be self-sustaining populations of wild horses and burros in single HMAs, in complexes of HMAs, and at regional scales of multiple HMAs and complexes. Under the WFRHBA of 1971, BLM is charged with maintaining self-reproducing populations of wild horses and burros. The WFRHBA makes clear that BLM is not explicitly charged with ensuring the fertility of any given individual wild horse or burro. The National Academies of Sciences (2013) encouraged BLM to manage wild horses and burros at the spatial scale of "metapopulations" – that is, across multiple HMAs and complexes in a region. In fact, many HMAs have historical and ongoing genetic and demographic connections with other HMAs, and BLM routinely moves animals from one to another to improve local herd traits and maintain high genetic diversity.

Discussions about herds that include some 'non-reproducing' individuals, or even those that are entirely non-reproducing, should be considered in the context of this 'metapopulation' structure, where the 'self-

sustaining' nature of herds is not necessarily to be measured at the scale of single HMAs. So long as the definition of what constitutes a self-sustaining herd includes the larger set of HMAs that have past or ongoing demographic and genetic connections – as is recommended by the NAS 2013 report – it is clear that particular HMAs can be managed as non-reproducing in whole or in part while still allowing for a self-sustaining population of wild horses or burros at the broader spatial scale. Wild horses are not an endangered species (USFWS 2015), nor are they rare. Over 79,000 adult wild horses and over 15,000 adult wild burros roamed BLM lands as of March 1, 2020, and those numbers do not include at least 10,000 WHB on US Forest Service lands, nor at least 50,000 feral horses on tribal lands in the Western United States.

All fertility control methods affect the behavior and physiology of treated animals (NAS 2013), and are associated with potential risks and benefits, including effects of handling, frequency of handling, physiological effects, behavioral effects, and reduced population growth rates (Hampton et al. 2015). Contraception methods alone do not remove excess horses from an HMA's population, so one or more gathers are usually needed in order to bring the herd down to a level close to AML. Horses are long-lived, potentially reaching 20 years of age or more in the wild. Except in cases where extremely high fractions of mares are rendered infertile over long time periods of (i.e., 10 or more years), spaying and neutering are not very effective at reducing population growth rates to the point where births equal deaths in a herd. However, even modest levels of fertility control activities can reduce the frequency of horse gather activities, and costs to taxpayers. Population growth suppression becomes less expensive if fertility control is long-lasting (Hobbs et al. 2000), such as with sterilization. Because sterilizing animals requires capturing and handling, the risks and costs associated with capture and handling of horses may be comparable to those of gathering for removal, but with expectedly lower adoption and long-term holding costs.

Effects of handling and marking

Sterilization techniques, while not reversible, may control horse reproduction without the kind of additional handling or darting that can be needed to administer contraceptive vaccines. In this sense, sterilization can be used to achieve herd management objectives with a relative minimum level of animal handling and management over the long term. The WFRHBA (as amended) indicates that management should be at the minimum level necessary to achieve management objectives (CFR 4710.4), and if sterilizing mares or neutering some stallions can lead to a reduced number of handling occasions and removals of excess horses from the range, then that is consistent with legal guidelines. Other fertility control options that may be temporarily effective on male horses, such as the injection of GonaCon-Equine immunocontraceptive vaccine, apparently require multiple handling occasions to achieve longer-term male infertility. Similarly, some formulations of PZP immunocontraception that is currently available for use in female wild horses and burros require handling or darting every year (though longer-term effects may result after 4 or more treatments; Nuñez et al. 2017). By some measures, any management activities that require multiple capture operations to treat a given individual could be seen as more intrusive for wild horses and potentially less sustainable than an activity that requires only one handling occasion.

It is prudent for sterilized animals to be readily identifiable, either via freeze brand marks or unique coloration, and uniquely numbered RFID chips inserted in the nuchal ligament, so that their treatment history is easily recognized (e.g., BLM 2010). Markings may also be useful into the future to determine the approximate fraction of geldings in a herd, and could provide additional insights about gather efficiency. BLM has instituted capture and animal welfare program guidelines to reduce the sources of handling stress in captured animals (BLM 2015, 2021). Handling may include freeze-marking, for the purpose of identifying an individual. Some level of transient stress is likely to result in newly captured horses that are not previously marked. Under past management practices, captured horses experienced

increased, transient stress levels from handling (Ashley and Holcombe 2001). It is difficult to compare that level of temporary stress with long-term stress that can result from food and water limitation on the range (e.g., Creel et al. 2013), which could occur in the absence of herd management.

Most horses recover from the stress of capture and handling quickly once released back to the range, and none are expected to suffer serious long term effects from gelding, other than the direct consequence of becoming infertile.

Observations of the long term outcomes of sterilization may be recorded during routine resource monitoring work. Such observations could include but not be limited to band size, social interactions with other geldings and harem bands, distribution within their habitat, forage utilization and activities around key water sources. Periodic population inventories and future gather statistics could provide additional anecdotal information.

Neutering Males

Whether or not stallion sterilization methods are considered in any of the action alternatives in this EA, they are included here for comparison and for the sake of completeness in the review. Castration (the surgical removal of the testicles, also called gelding or neutering) is a surgical procedure for the horse sterilization that has been used for millennia. Vasectomy involves severing or blocking the vas deferens or epididymis, to prevent sperm from being ejaculated. The procedures are fairly straight forward, and has a relatively low complication rate. As noted in the review of scientific literature that follows, the expected effects of gelding and vasectomy are well understood overall, even though there is some degree of uncertainty about the exact quantitative outcomes for any given individual (as is true for any natural system).

Including a portion of neutered males in a herd can lead to a reduced population-level per-capita growth rate if they cause a marginal decrease in female fertility or if the neutered males take some of the places that would otherwise be occupied by fertile females. By having a skewed sex ratio with fewer females than males (fertile stallions plus neutered males), the result will be that there will be a lower number of breeding females in the population. Including neutered males in herd management is not new for BLM and federal land management. Geldings have been released on BLM lands as a part of herd management in the Barren Valley complex in Oregon (BLM 2011), the Challis HMA in Idaho (BLM 2012), and the Conger HMA in Utah (BLM 2016). Vasectomized males and geldings were also included in US Fish and Wildlife Service management plans for the Sheldon National Wildlife Refuge that relied on sterilization and removals (Collins and Kasbohm 2016). Taking into consideration the literature available at the time, the National Academies of Sciences concluded in their 2013 report that a form of vasectomy was one of the three most promising methods for WH&B fertility control (NAS 2013). However, BLM is not pursuing the chemical vasectomy method. The NAS panel noted that, even though chemical vasectomy had been used in dogs and cats up to that time, "There are no published reports on chemical vasectomy in horses..." and that, "Only surgical vasectomy has been studied in horses, so side effects of the chemical agent are unknown." The only known use of chemical vasectomy in horses was published by Scully et al. (2015); this was part of a study cited in the EA (Collins and Kasbohm 2016). They injected chlorhexidine into the stallions' epididymis. That is the same chemical agent as had been used to chemically vasectomize dogs. Scully et al. (2015) found that the chemical vasectomy method failed to prevent fertile sperm from being located in the vas deferens seminal fluid. Stallions treated with the chemical vasectomy method still had viable sperm and were still potentially as fertile as untreated 'control' stallions in that study. Thus, the method did was not effective.

Nelson (1980) and Garrott and Siniff (1992) modeled potential efficacy of male-oriented contraception as a population management tool, and both studies agreed that while slowing growth, sterilizing only dominant males (i.e., harem-holding stallions) would result in only marginal reduction in female fertility

rates. Eagle et al. (1993) and Asa (1999) tested this hypothesis on HMAs where dominant males were vasectomized. Their findings agreed with modeling results from previous studies, and they also concluded that sterilizing only dominant males would not provide the desired reduction in female fertility and overall population growth rate, assuming that the numbers of fertile females is not changed. While bands with vasectomized harem stallions tended to have fewer foals, breeding by bachelors and subordinate stallions meant that population growth still occurred – female fertility was not dramatically reduced. Collins and Kasbohm (2016) demonstrated that there was a reduced fertility rate in a feral horse herd with both spayed and vasectomized horses – some geldings were also present in that herd. Garrott and Siniff (1992) concluded from their modeling that male sterilization would effectively cause there to be zero population growth (the point where births roughly equal deaths) only if a large proportion of males (i.e., >85%) could be sterilized. In cases where the goal of harem stallion sterilization is to reduce population growth rates, success appears to be dependent on a stable group structure, as strong bonds between a stallion and mares reduce the probability of a mare mating an extra-group stallion (Nelson 1980, Garrott and Siniff 1992, Eagle et al. 1993, Asa 1999). Unpublished USGS results from a study at Conger HMA indicate that a non-zero fraction of geldings that were returned to the range with their social band did continue with females, apparently excluding fertile stallions, for at least 2 years.

Despite these studies, neutered males can be used to reduce overall growth rates in a management strategy that does not rely on any expectation that geldings will retain harems or lead to a reduction in per-female fertility rates. The primary goal of including neutered males in a herd need not necessarily be to reduce female fertility (although that may be one result). Rather, by including some neutered males in a herd that also has fertile mares and stallions, the neutered males would take some of the spaces toward AML that would otherwise be taken by fertile females. If the total number of horses is constant but neutered males are included in the herd, this can reduce the number of fertile mares, therefore reducing the absolute number of foals produced. Put another way, if neutered males occupy spaces toward AML that would otherwise be filled by fertile mares, that will reduce growth rates merely by the fact of causing there to be a lower starting number of fertile mares.

Direct Effects of Neutering

No animals which appear to be distressed, injured, or in poor health or condition would be selected for gelding. Stallions would not typically be neutered within 72 hours of capture. The surgery would be performed by a veterinarian using general anesthesia and appropriate surgical techniques. The final determination of which specific animals would be gelded would be based on the professional opinion of the attending veterinarian in consultation with the Authorized Officer (i.e., See the SOPs for neutering in the Antelope / Triple B gather EA, DOI-BLM-NV-E030-2017-010-EA).

Though neutering males is a common surgical procedure, especially gelding, some level of minor complications after surgery may be expected (Getman 2009), and it is not always possible to predict when postoperative complications would occur. Fortunately, the most common complications are almost always self-limiting, resolving with time and exercise. Individual impacts to the stallions during and following the gelding process should be minimal and would mostly involve localized swelling and bleeding. Complications may include, but are not limited to: minor bleeding, swelling, inflammation, edema, infection, peritonitis, hydrocele, penile damage, excessive hemorrhage, and eventration (Schumacher 1996, Searle et al. 1999, Getman 2009). A small amount of bleeding is normal and generally subsides quickly, within 2-4 hours following the procedure. Some degree of swelling is normal, including swelling of the prepuce and scrotum, usually peaking between 3-6 days after surgery (Searle et al. 1999). Swelling should be minimized through the daily movements (exercise) of the horse during travel to and from foraging and watering areas. Most cases of minor swelling should be back to normal within 5-7 days, more serious cases of moderate to severe swelling are also self-limiting and are

expected to resolve with exercise after one to 2 weeks. Older horses are reported to be at greater risk of post-operative edema, but daily exercise can prevent premature closure of the incision, and prevent fluid buildup (Getman 2009). In some cases, a hydrocele (accumulation of sterile fluid) may develop over months or years (Searle et al. 1999). Serious complications (eventration, anesthetic reaction, injuries during handling, etc.) that result in euthanasia or mortality during and following surgery are rare (e.g., eventration rate of 0.2% to 2.6% noted in Getman 2009, but eventration rate of 4.8% noted in Shoemaker et al. 2004) and vary according to the population of horses being treated (Getman 2009). Normally one would expect serious complications in less than 5% of horses operated under general anesthesia, but in some populations these rates have been as high as 12% (Shoemaker 2004). Serious complications are generally noted within 3 or 4 hours of surgery but may occur any time within the first week following surgery (Searle et al. 1999). If they occur, they would be treated with surgical intervention when possible, or with euthanasia when there is a poor prognosis for recovery. Vasectomized stallions may remain fertile for up to 6 weeks after surgery, so it is optimal if that treatment occurs well in advance of the season of mare fertility starting in the spring (NAS 2013). The NAS report (2013) suggested that chemical vasectomy, which has been developed for dogs and cats, may be appropriate for wild horses and burros.

For intact stallions, testosterone levels appear to vary as a function of age, season, and harem size (Khalil et al 1998). It is expected that testosterone levels will decline over time after castration. Testosterone levels should not change due to vasectomy. Vasectomized stallions should retain their previous levels of libido. Domestic geldings had a significant prolactin response to sexual stimulation, but lacked the cortisol response present in stallions (Colborn et al. 1991). Although libido and the ability to ejaculate tends to be gradually lost after castration (Thompson et al. 1980), some geldings continue to mount mares and intromit (Rios and Houpt 1995, Schumacher 2006).

Indirect Effects of Neutering

Other than the short-term outcomes of surgery, neutering is not expected to reduce males' survival rates. Castration is actually thought to increase survival as males are released from the cost of reproduction (Jewell 1997). In Soay sheep castrates survived longer than rams in the same cohort (Jewell 1997), and Misaki horse geldings lived longer than intact males (Kaseda et al. 1997, Khalil and Murakami 1999). Moreover, it is unlikely that a reduced testosterone level will compromise gelding survival in the wild, considering that wild mares survive with low levels of testosterone. Consistent with geldings not expending as much energy toward in attempts to obtain or defend a harem, it is expected that wild geldings may have a better body condition that wild, fertile stallions. In contrast, vasectomized males may continue to defend or compete for harems in the way that fertile males do, so they are not expected to experience an increase in health or body condition due to surgery.

Depending on whether an HMA is non-reproducing in whole or in part, reproductive stallions may or may not still be a component of the population's age and sex structure. The question of whether or not a given neutered male would or would not attempt to maintain a harem is not germane to population-level management. It is worth noting, though, that the BLM is not required to manage populations of wild horses in a manner that ensures that any given individual maintains its social standing within any given harem or band. Neutering a subset of stallions would not prevent other fertile stallions and mares from continuing with the typical range of social behaviors for sexually active adults. For fertility control strategies where gelding is intended to reduce growth rates by virtue of sterile males defending harems, the NAS (2013) suggested that the effectiveness of gelding on overall reproductive rates may depend on the pre-castration social roles of those animals. Having a post-gather herd with some neutered males and a lower fraction of fertile mares necessarily reduces the absolute number of foals born per year, compared to a herd that includes more fertile mares. An additional benefit is that geldings that would otherwise be permanently removed from the range (for adoption, sale or other disposition) may be released back onto the range where they can engage in free-roaming behaviors.

Behavioral Effects of Neutering

Feral horses typically form bands composed of an adult male with 1 to 3 adult females and their immature offspring (Feist and McCullough 1976, Berger 1986, Roelle et al. 2010). In many populations subordinate 'satellite' stallions have been observed associating with the band, although the function of these males continues to be debated (see Feh 1999, and Linklater and Cameron 2000). Juvenile offspring of both sexes leave the band at sexual maturity (normally around two or three years of age (Berger 1986), but adult females may remain with the same band over a span of years. Group stability and cohesion is maintained through positive social interactions and agonistic behaviors among all members, and herding and reproductive behaviors from the stallion (Ransom and Cade 2009). Group movements and consortship of a stallion with mares is advertised to other males through the group stallion marking dung piles as they are encountered, and over-marking mare eliminations as they occur (King and Gurnell 2006).

In horses, males play a variety of roles during their lives (Deniston 1979): after dispersal from their natal band they generally live as bachelors with other young males, before associating with mares and developing their own breeding group as a harem stallion or satellite stallion. In any population of horses not all males will achieve harem stallion status, so all males do not have an equal chance of breeding (Asa 1999). Stallion behavior is thought to be related to androgen levels, with breeding stallions having higher androgen concentrations than bachelors (Angle et al. 1979, Chaudhuri and Ginsberg 1990, Khalil et al. 1998). A bachelor with low libido had lower levels of androgens, and two-year-old bachelors had higher testosterone levels than two year olds with undescended testicles who remained with their natal band (Angle et al. 1979).

Vasectomized males continue to attempt to defend or gain breeding access to females. It is generally expected that vasectomized WH&B will continue to behave like fertile males, given that the only physiological change in their condition is a lack of sperm in their ejaculate. If a vasectomized stallion retains a harem, the females in the harem will continue to cycle until they are fertilized by another stallion, or until the end of the breeding season. As a result, the vasectomized stallion may be involved in more aggressive behaviors to other males through the entire breeding season (Asa 1999), which may divert time from foraging and cause him to be in poorer body condition going into winter. Ultimately, this may lead to the stallion losing control of a given harem. A feral horse herd with high numbers of vasectomized stallions retained typical harem social structure (Collins and Kasbohm 2016). Again it is worth noting that the BLM is not required to manage populations of wild horses in a manner that ensures that any given individual maintains its social standing within any given harem or band.

Neutering males by gelding adult male horses is expected to result in reduced testosterone production, which is expected to directly influence reproductive behaviors (NAS 2013). However, testosterone levels alone are not a predictor of masculine behavior (Line et al. 1985, Schumacher 2006). In domestic geldings, 20-30% continued to show stallion-like behavior, whether castrated pre- or post-puberty (Line et al. 1985). Gelding of domestic horses most commonly takes place before or shortly after sexual maturity, and age-at-gelding can affect the degree to which stallion-like behavior is expressed later in life. In intact stallions, testosterone levels peak increase up to an age of ~4-6 years, and can be higher in harem stallions than bachelors (Khalil et al 1998). It is assumed that free roaming wild horse geldings would generally exhibit reduced aggression toward other horses, and reduced reproductive behaviors (NAS 2013). The behavior of wild horse geldings in the presence of intact stallions has not been well documented, but the literature review below can be used to make reasonable inferences about their likely behaviors.

Despite livestock being managed by neutering males for millennia, there is relatively little published research on castrates' behaviors (Hart and Jones 1975). Stallion behaviors in wild or pasture settings are better documented than gelding behaviors, but it inferences about how the behaviors of geldings will change, how quickly any change will occur after surgery, or what effect gelding an adult stallion and releasing him back in to a wild horse population will have on his behavior and that of the wider population must be surmised from the existing literature. There is an ongoing BLM study in Utah focused on the individual and population-level effects of including some geldings in a free-roaming horse population (BLM 2016), but results from that study are not yet available. However, inferences about likely behavioral outcomes of gelding can be made based on available literature.

The effect of castration on aggression in horses has not often been quantified. One report has noted that high levels of aggression continued to be observed in domestic horse geldings who also exhibited sexual behaviors (Rios and Houpt 1995). Stallion-like behavior in domestic horse geldings is relatively common (Smith 1974, Schumacher 1996), being shown in 20-33% of cases whether the horse was castrated pre- or post-puberty (Line et al. 1985, Rios and Houpt 1995, Schumacher 2006). While some of these cases may be due to cryptorchidism or incomplete surgery, it appears that horses are less dependent on hormones than other mechanisms for the maintenance of sexual behaviors (Smith 1974). Domestic geldings exhibiting masculine behavior had no difference in testosterone concentrations than other geldings (Line et al. 1985, Schumacher 2006), and in some instances the behavior appeared context dependent (Borsberry 1980, Pearce 1980).

Dogs and cats are commonly neutered, and it is also common for them to continue to exhibit reproductive behaviors several years after castration (Dunbar 1975). Dogs, ferrets, hamsters, and marmosets continued to show sexually motivated behaviors after castration, regardless of whether they had previous experience or not, although in beagles and ferrets there was a reduction in motivation post-operatively (Hart 1968, Dunbar 1975, Dixson 1993, Costantini et al. 2007, Vinke et al. 2008). Ungulates continued to show reproductive behaviors after castration, with goats and llamas continuing to respond to females even a year later in the case of goats, although mating time and the ejaculatory response was reduced (Hart and Jones 1975, Nickolmann et al. 2008).

The likely effects of castration on geldings' social interactions and group membership can be inferred from available literature. In a pasture study of domestic horses, Van Dierendonk et al. (1995) found that social rank among geldings was directly correlated to the age at which the horse was castrated, suggesting that social experiences prior to sterilization may influence behavior afterward. Of the two geldings present in a study of semi-feral horses in England, one was dominant over the mares whereas a younger gelding was subordinate to older mares; stallions were only present in this population during a short breeding season (Tyler 1972). A study of domestic geldings in Iceland held in a large pasture with mares and sub-adults of both sexes, but no mature stallions, found that geldings and sub-adults formed associations amongst each other that included interactions such as allo-grooming and play, and were defined by close proximity (Sigurjónsdóttir et al. 2003). These geldings and sub-adults tended to remain in a separate group from mares with foals, similar to castrated Soay sheep rams (Ovis aries) behaving like bachelors and grouping together, or remaining in their mother's group (Jewell 1997). In Japan, Kaseda et al. (1997) reported that young males dispersing from their natal harem and geldings moved to a different area than stallions and mares during the non-breeding season. Although the situation in Japan may be the equivalent of a bachelor group in natural populations, in Iceland this division between mares and the rest of the horses in the herd contradicts the dynamics typically observed in a population containing mature stallions. Sigurjónsdóttir et al. (2003) also noted that in the absence of a stallion, allo-grooming between adult females increased drastically. Other findings included increased social interaction among yearlings, display of stallion-like behaviors such as mounting by the adult females, and decreased association between females and their yearling offspring (Sigurjónsdóttir et al. 2003). In

the same population in Iceland Van Dierendonck et al. (2004) concluded that the presence of geldings did not appear to affect the social behavior of mares or negatively influence parturition, mare-foal bonding, or subsequent maternal activities. Additionally, the welfare of broodmares and their foals was not affected by the presence of geldings in the herd (Van Dierendonck et al. 2004). These findings are important because treated geldings will be returned to the range in the presence of pregnant mares and mares with foals of the year.

The likely effects of castration on geldings' home range and habitat use can also be surmised from available literature. Bands of horses tend to have distinct home ranges, varying in size depending on the habitat and varying by season, but always including a water source, forage, and places where horses can shelter from inclement weather or insects (King and Gurnell 2005). By comparison, bachelor groups tend to be more transient, and can potentially use areas of good forage further from water sources, as they are not constrained by the needs of lactating mares in a group. The number of observations of gelded wild stallion behavior are still too few to make general predictions about whether a particular gelded stallion individual will behave like a harem stallion, a bachelor, or form a group with geldings that may forage and water differently from fertile wild horses.

Sterilizing wild horses does not change their status as wild horses under the WFRHBA (as amended). In terms of whether geldings will continue to exhibit the free-roaming behavior that defines wild horses, BLM does expect that geldings would continue to roam unhindered once they are returned to the range. Wild horse movements may be motivated by a number of biological impulses, including the search for forage, water, and social companionship that is not of a sexual nature. As such, a gelded animal would still be expected to have a number of internal reasons for moving across a landscape and, therefore, exhibiting 'free-roaming' behavior. Despite marginal uncertainty about subtle aspects of potential changes in habitat preference, there is no expectation that gelding wild horses will cause them to lose their free-roaming nature. It is worth noting that individual choices in wild horse group membership, home range, and habitat use are not protected under the WFRHBA. BLM acknowledges that geldings may exhibit some behavioral differences after surgery, compared to intact stallions, but those differences are not be expected to remove the geldings' rebellious and feisty nature, or their defiance of man. While it may be that a gelded horse could have a different set of behavioral priorities than an intact stallion, the expectation is that geldings will choose to act upon their behavioral priorities in an unhindered way, just as is the case for an intact stallion. In this sense, a gelded male would be just as much 'wild' as defined by the WFRHBA as any intact stallion, even if his patterns of movement differ from those of an intact stallion. Unpublished USGS results from the Conger study herd indicate that geldings' movement patterns were not qualitatively different from those of fertile stallions, when controlling for social status as bachelor or harem stallion. Congress specified that sterilization is an acceptable management action (16 USC §1333.b.1). Sterilization is not one of the clearly defined events that cause an animal to lose its status as a wild free-roaming horse (16 USC §1333.2.C.d). Several academics have offered their opinions about whether gelding a given stallion would lead to that individual effectively losing its status as a wild horse (Rutberg 2011, Kirkpatrick 2012, Nock 2017). Those opinions are based on a semantic and subjective definition of 'wild,' while BLM must adhere to the legal definition of what constitutes a wild horse, based on the WFRHBA (as amended). Those individuals have not conducted any studies that would test the speculative opinion that gelding wild stallions will cause them to become docile. BLM is not obliged to base management decisions on such opinions, which do not meet the BLM's principle and practice to "Use the best available scientific knowledge relevant to the problem or decision being addressed, relying on peer reviewed literature when it exists" (Kitchell et al. 2015).

Mare Sterilization

Sterilizing mares has already been shown to be an effective part of feral horse management that reduced herd growth rates on federal lands (Collins and Kasbohm 2016). Herd-level birth rate is expected to decline in direct proportion to the fraction of spayed mares in the herd because spayed mares cannot become pregnant. A number of methods are available, with potentially differing effects.

Current Methods of Sterilization

This literature review of mare sterilization impacts focuses on 4 methods: pharmacological or immunocontraceptive methods, minimally invasive physical sterilization, ovariectomy via colpotomy, and ovariectomy via flank laparoscopy. The range of anticipated effects may be both physical and behavioral. Whether or not surgical mare sterilization methods are considered in any of the action alternatives in this EA, they are included here for comparison and for the sake of completeness in the review.

Pharmacological or immunocontraceptive sterilization methods would use a drug or vaccine to cause sterilization. BLM has not yet identified a pharmacological or immunocontraceptive method to sterilize mares that has been proven to reliably and humanely sterilize wild horse mares. However, there is the possibility that current or future development and testing of new methods could make an injectable sterilant available for wild horse mares. An oocyte growth factor OGF vaccine is currently under testing, for its ability to cause long-term infertility or, potentially, sterility (BLM 2020). Mares that received 5 or more doses of ZonaStat-H vaccine have been shown to have reduced ovarian function, and to be effectively infertile for life (Nuñez et al. 2017), and it is conceivable that the contraceptive effects of repeated treatment with GonaCon-Equine may last longer than a mare's lifespan, depending on her age at treatment and the number of doses received (Baker et al. 2018). While the physiological effects of various potential methods may differ, the herd-level effects of having sterile mares as a part of a wild horse herd would be expected to be similar for minimally invasive and surgical methods. Salient differences in individual breeding behavior that result from either retaining functioning ovaries, or having no or reduce ovarian function, are discussed below.

Minimally invasive, physical sterilization procedure could include any physical form of sterilization that does not involve removal of the ovaries, and entail only minimal or no incisions. Such procedures could include any form of physical procedure that leads a mare to be unable to become pregnant, or to maintain a pregnancy. For example, in endoscopic oviduct ablation, minimally invasive sterilization causes a long-term blockage of the oviduct by infusion of a surgical-grade glue into the oviducts, so that fertile eggs cannot go from the ovaries to the uterus (i.e., Bigolin et al. 2009). Or, in endoscopic laser ablation of the oviduct papilla, scarring caused by heat applied at the uterotubal junction prevents eggs from reaching the uterus. These two procedures use trans-cervical endoscopy, so any treated mares would first need to have been screened by a veterinarian (e.g., using trans-rectal ultrasonography) to ensure they are not pregnant. Endoscopic approaches also require temporary insufflation of the uterus, to allow the veterinarian to fully visualize the internal structures. The result of such minimally invasive procedures that prevent pregnancy but do not harm the ovaries is that the mare would be sterile, although she would continue to have estrus cycles.

Ovariectomy via colpotomy is a surgical technique in which there is no external incision, reducing susceptibility to infection. Ovariectomy via colpotomy, has been an established veterinary technique since 1903 (Loesch and Rodgerson 2003, NAS 2013). Spaying via colpotomy has the advantage of not leaving any external wound that could become infected. For this reason, it has been identified as a good choice for sterilization of feral or wild mares (Rowland et al. 2018). The procedure has a relatively low complication rate, although post-surgical mortality and morbidity are possible, as with any surgery. For this reason, ovariectomy via colpotomy has been identified as a good choice for feral or wild horses

(Rowland et al. 2018). Ovariectomy via colpotomy is a relatively short surgery, with a relatively quick expected recovery time. In 1903, Williams first described a vaginal approach, or colpotomy, using an ecraseur to ovariectomize mares (Loesch and Rodgerson 2003). The ovariectomy via colpotomy procedure has been conducted for over 100 years, normally on open (non-pregnant), domestic mares. It is expected that the surgeon should be able to access ovaries with ease in mares that are in the early- or mid-stage of pregnancy. The anticipated risks associated with the pregnancy are described below. When wild horses are gathered or trapped for fertility control treatment there would likely be mares in various stages of gestation. Removal of the ovaries is permanent and 100 percent effective, however the procedure is not without risk.

Ovariectomy via flank laparoscopy (Lee and Hendrickson 2008, Devick et al. 2018, Easley et al. 2018) is commonly used in domestic horses for application in mares due to its minimal invasiveness and full observation of the operative field. Ovariectomy via flank laparoscopy was seen as the lowest risk method considered by a panel of expert reviewers convened by USGS (Bowen 2015). In a review of unilateral and bilateral laparoscopic ovariectomy on 157 mares, Röcken et al. (2011) found that 10.8% of mares had minor post-surgical complications, and recorded no mortality. Mortality due to this type of surgery, or post-surgical complications, is not expected, but is a possibility. In two studies, ovariectomy by laparoscopy or endoscope-assisted colpotomy did not cause mares to lose weight, and there was no need for rescue analgesia following surgery (Pader et al. 2011, Bertin et al. 2013). This surgical approach entails three small incisions on the animal's flank, through which three cannulae (tubes) allow entry of narrow devices to enter the body cavity: these are the insufflator, endoscope, and surgical instrument. The surgical procedure involves the use of narrow instruments introduced into the abdomen via cannulas for the purpose of transecting or sealing (Easley 2018) the ovarian pedicle, but the insufflation should allow the veterinarian to navigate inside the abdomen without damaging other internal organs. The insufflator blows air into the cavity to increase the operating space between organs, and the endoscope provides a video feed to visualize the operation of the surgical instrument. This procedure can require a relatively long duration of surgery, but tends to lead to the lowest postoperative rates of complications. Flank laparoscopy may leave three small (<5 cm) visible scars on one side of the horse's flank, but even in performance horses these scars are considered minimal. It is expected that the tissues and musculature under the skin at the site of the incisions in the flank will heal quickly, leaving no long-lasting effects on horse health. Monitoring for up to two weeks at the facility where surgeries take place will allow for veterinary inspection of wound healing. The ovaries may be dropped into the abdomen, but this is not expected to cause any health problem; it is usually done in ovariectomies in cattle (e.g., the Willis Dropped Ovary Technique) and Shoemaker et al. (2014) found no problems with revascularization or necrosis in a study of young horses using this method.

Effects of Sterilization on Pregnancy and Foal

The physical, behavioral, and herd-level effects of immunocontraceptives have been addressed elsewhere in this review. In the case of repeated PZP vaccine or GonaCon applications that cause infertility through the duration of a given mare's life, that effects of that form of treatment have been discussed previously; neither vaccine appears to disrupt pregnancy or foal development. OGF vaccine effects on fetal development are unknown, as no pregnant mares have been injected with this vaccine to date; use on pregnant mares may be limited until further information is available.

Trans-cervical, minimally-invasive sterilization methods are not suitable for pregnant mares, because disruption of the cervix may lead to termination of the pregnancy. Therefore, any mares under consideration for such methods must first be screened for pregnancy, such as via transrectal ultrasound.

The average mare gestation period ranges from 335 to 340 days (Evans et al. 1977, p. 373). There are few peer reviewed studies documenting the effects of surgical ovariectomy on the success of pregnancy in a mare. A National Research Council of the National Academies of Sciences (NAS) committee that reviewed research proposals in 2015 explained, "The mare's ovaries and their production of progesterone are required during the first 70 days of pregnancy to maintain the pregnancy" (NAS 2015). In female mammals, less progesterone is produced when ovaries are removed, but production does not cease (Webley and Johnson 1982). In 1977, Evans et al. stated that by 200 days, the secretion of progesterone by the corpora lutea is insignificant because removal of the ovaries does not result in abortion (p. 376). "If this procedure were performed in the first 120 days of pregnancy, the fetus would be resorbed or aborted by the mother. If performed after 120 days, the pregnancy should be maintained. The effect of ovary removal on a pregnancy at 90-120 days of gestation is unpredictable because it is during this stage of gestation that the transition from corpus luteum to placental support typically occurs" (NAS 2015). In 1979, Holtan et al. evaluated the effects of bilateral ovariectomy at selected times between 25 and 210 days of gestation on 50 mature pony mares. Their results show that abortion (resorption) of the conceptus (fetus) occurred in all 14 mares ovariectomized before day 50 of gestation, that pregnancy was maintained in 11 of 20 mares after ovariectomy between days 50 and 70, and that pregnancy was not interrupted in any of 12 mares ovariectomized on days 140 to 210. Those results are similar to the suggestions of the NAS committee (2015). For those pregnancies that are maintained following an ovariectomy procedure, likely those past approximately 120 days, the development of the foal is not expected to be affected. However, because this procedure is not commonly conducted on pregnant mares the rate of complications to the fetus has not yet been quantified. There is the possibility that entry to the abdominal cavity could cause premature births related to inflammation. However, after five months the placenta should hormonally support the pregnancy regardless of the presence or absence of ovaries. Gestation length was similar between ovariectomized and control mares (Holtan et al. 1979).

Direct Effects of Sterilization

The direct effects of immunocontraceptive PZP vaccines and GonaCon-Equine have been discussed previously. In cases where PZP vaccines have been administered enough times to cause effective sterility, the mechanism of action may be related to long-term reduction in ovarian activity (i.e., Nolan et al 2018c). The direct effects of OGF vaccine treatment were discussed by BLM (2020) and may include an injection site reaction that is comparable to that of GonaCon-Equine; a brief period of heightened inflammation and mild fever that is characteristic of a successful immune response; development of an immune response against GDF9 and BMP15, with related reductions in the concentration of those proteins; and a reduction in estrus activity.

The direct effects of successful minimally invasive mare sterilization procedures are sterility, for example through occlusion of the oviduct with surgical glue and associated tissue damage, or creation of scar tissue in part of the oviduct. Hysteroscopy is a common procedure in humans (i.e., WebMD 2014). Because such minimally invasive procedures do not involve major incisions or removal of ovaries, there is no risk of hemorrhage, failure of sutures, or prolonged discomfort. There is the potential for mild, transient colic (abnormal cramping) after the procedure due to temporary inflation and expansion of the uterus. Use of analgesics prior to any procedure should minimize this incidence. Side effects of minimally invasive sterilization procedures may include mild discomfort in the short term, for example at the location where the oviduct is blocked. For example, if surgical grade glue is placed in the oviduct or if a laser is used to ablate the oviduct papilla, that may cause transient irritation. For this reason, systemic and / or topical analgesics are generally provided before or during the procedure. An NAS review of the endoscopic laser ablation of the oviduct papilla technique concluded that the method is relatively non-invasive, with a relatively low risk of complications (NAS 2015); the expected severe

complication rate for the laser ablation procedure may be lower than 1 percent. Ablation of the oviduct via cyanoacrylate glue has been performed successfully in mares at UC Davis, and laser ablation of the oviduct papilla has been performed successfully in burros and horses, in California and Georgia. In addition, other transcervical endoscopic procedures (including the use of a laser diode) are not uncommon in mares (Blikslager et al. 1993, Griffin and Bennet 2002, Ley et al. 2002, Brinsko 2014).

Between 2009 and 2011, the Sheldon NWR in Nevada conducted ovariectomy via colpotomy surgeries (August through October) on 114 feral mares and released them back to the range with a mixture of sterilized stallions and untreated mares and stallions (Collins and Kasbohm 2016). Gestational stage was not recorded, but a majority of the mares were pregnant (Gail Collins, US Fish and Wildlife Service (USFWS), pers. comm.). Only a small number of mares were very close to full term. Those mares with late term pregnancies did not receive surgery as the veterinarian could not get good access to the ovaries due to the position of the foal (Gail Collins, USFWS, pers. comm.). After holding the mares for an average of 8 days after surgery for observation, they were returned to the range with other treated and untreated mares and stallions (Collins and Kasbohm 2016). During holding the only complications were observed within 2 days of surgery. The observed mortality rate for ovariectomized mares following the procedure was less than 2 percent (Collins and Kasbohm 2016, Pielstick pers. comm.). During the Sheldon NWR ovariectomy study, mares generally walked out of the chute and started to eat; some would raise their tail and act as if they were defecating; however, in most mares one could not notice signs of discomfort (Bowen 2015). In their discussion of ovariectomy via colpotomy, McKinnon and Vasey (2007) considered the procedure safe and efficacious in many instances, able to be performed expediently by personnel experienced with examination of the female reproductive tract, and associated with a complication rate that is similar to or less than male castration. Nevertheless, all surgery is associated with some risk. Loesch et al. (2003) lists that following potential risks with colpotomy: pain and discomfort; injuries to the cervix, bladder, or a segment of bowel; delayed vaginal healing; eventration of the bowel; incisional site hematoma; intraabdominal adhesions to the vagina; and chronic lumbar or bilateral hind limb pain. Most horses, however, tolerate ovariectomy via colpotomy with very few complications, including feral horses (Collins and Kasbohm 2016). Evisceration is also a possibility, but these complications are considered rare (Prado and Schumacher, 2017). Mortality due to surgery or post-surgical complications is not anticipated, but it is a possibility and therefore every effort would be made to mitigate risks.

In September 2015, the BLM solicited the USGS to convene a panel of veterinary experts to assess the relative merits and drawbacks of several surgical ovariectomy techniques that are commonly used in domestic horses for potential application in wild horses. A table summarizing the various methods was sent to the BLM (Bowen 2015) and provides a concise comparison of several methods. Of these, ovariectomy via colpotomy was found to be relatively safe when practiced by an experienced surgeon and was associated with the shortest duration of potential complications after the operation. The panel discussed the potential for evisceration through the vaginal incision with this procedure. In marked contrast to a suggestion by the NAS report (2013), this panel of veterinarians identified evisceration as not being a probable risk associated with ovariectomy via colpotomy and "none of the panel participants had had this occur nor had heard of it actually occurring" (Bowen 2015).

Most ovariectomy surgeries on mares have low morbidity¹ and with the help of medications, pain and discomfort can be mitigated. Pain management is an important aspect of any ovariectomy (Rowland et

¹ Morbidity is defined as the frequency of the appearance of complications following a surgical procedure or other treatment. In contrast, mortality is defined as an outcome of death due to the procedure.

al. 2018); according to surgical protocols that would be used, a long-lasting direct anesthetic would be applied to the ovarian pedicle, and systemic analgesics in the form of butorphanol and flunixin meglumine would be administered, as is compatible with accepted animal husbandry practices. In a study of the effects of bilateral ovariectomy via colpotomy on 23 mares, Hooper and others (1993) reported that postoperative problems were minimal (1 in 23, or 4%). Hooper et al. (1993) noted that four other mares were reported by owners as having some problems after surgery, but that evidence as to the role the surgery played in those subsequent problems was inconclusive. In contrast Röcken et al. (2011) noted a morbidity of 10.8% for mares that were ovariectomized via a flank laparoscopy. "Although 5 mares in our study had problems (repeated colic in 2 mares, signs of lumbar pain in 1 mare, and clinical signs of peritonitis in 1 mare) after surgery, evidence is inconclusive in each as to the role played by surgery" (Hooper et al. 1993). A recent study showed a 2.5% complication rate where one mare of 39 showed signs of moderate colic after laparoscopic ovariectomy (Devick 2018 personal communication).

Behavioral Effects of Mare Sterilization

All fertility control methods affect physiology or behavior of a mare (NAS 2013). Any action taken to alter the reproductive capacity of an individual has the potential to affect hormone production and therefore behavioral interactions and ultimately population dynamics in unforeseen ways (Ransom et al. 2014). The health and behavioral effects of sterilizing wild horse mares that live with other fertile and infertile wild horses has not been well documented, but the literature review below can be used to make reasonable inferences about their likely behaviors.

The behavioral effects of PZP vaccines and GonaCon-Equine have been discussed previously. For the OGF vaccine, a paired immune reaction to two proteins (GDF9 and BMP15) can prevent the completion of oocyte development, with the result being that successfully treated mares do not exhibit estrus cycles. As a result, the behavioral and herd-level effects of OGF vaccine treatment are expected to be similar to those documented for GonaCon-Equine; namely, a reduced incidence of breeding behaviors, but a continuation of affiliative behaviors within the social band (see previous discussion of effects of GonaCon-Equine).

Horses are anovulatory (do not ovulate/express estrous behavior) during the short days of late fall and early winter, beginning to ovulate as days lengthen and then cycling roughly every 21 days during the warmer months, with about 5 days of estrus (Asa et al. 1979, Crowell-Davis 2007). Estrus in mares is shown by increased frequency of proceptive behaviors: approaching and following the stallion, urinating, presenting the rear end, clitoral winking, and raising the tail towards the stallion (Asa et al. 1979, Crowell-Davis 2007). In most mammal species other than primates estrus behavior is not shown during the anovulatory period, and reproductive behavior is considered extinguished following spaying (Hart and Eckstein 1997). However mares may continue to demonstrate estrus behavior during the anovulatory period (Asa et al. 1980).

The behavioral effects of minimally invasive mare sterilization methods that cause no change in ovarian functionality would be expected to be similar to those observed in mares treated with a small number of doses of PZP vaccine (i.e., those in which ovarian functionality is not impaired). Those behavioral outcomes are discussed previously, but include a continuation of estrus cycling, and associated proceptive and breeding behaviors, including copulation. As a result of the expectation that the minimally invasive procedures would have similar behavioral effects as treatment with PZP, BLM does not anticipate any need to study the behavioral effects of minimally invasive mare sterilization, in which functional ovaries are retained. Sterile mares with functional ovaries would be expected to continue to engage in breeding activities, although they would not become pregnant. There is the possibility that such mares may change social bands at a greater rate than fertile mares (e.g., Nuñez et al. 2017).

Ovariectomized mares may continue to exhibit estrous behavior (Scott and Kunze 1977, Kamm and Hendrickson 2007, Crabtree 2016), with one study finding that 30% of mares showed estrus signs at least once after surgery (Roessner et al 2015) and only 60 percent of ovariectomized mares cease estrous behavior following surgery (Loesch and Rodgerson 2003). Mares continue to show reproductive behavior following ovariectomy due to non-endocrine support of estrus behavior, specifically steroids from the adrenal cortex. Continuation of this behavior during the non-breeding season has the function of maintaining social cohesion within a horse group (Asa et al. 1980, Asa et al. 1984, NAS 2013). This may be a unique response of the horse (Bertin et al. 2013), as spaying usually greatly reduces female sexual behavior in companion animals (Hart and Eckstein 1997). In six ponies, mean monthly plasma luteinizing hormone² levels in ovariectomized mares were similar to intact mares during the anestrous season, and during the breeding season were similar to levels in intact mares at mid-estrus (Garcia and Ginther 1976).

The likely effects of spaying on mares' social interactions and group membership can be inferred from available literature, even though wild horses have rarely been spayed and released back into the wild, resulting in few studies that have investigated their behavior in free-roaming populations. Wild horses and burros are instinctually herd-bound and this behavior is expected to continue. Overall the BLM anticipates that some spayed mares may continue to exhibit estrus behavior which could foster band cohesion. If free-ranging ovariectomized mares show estrous behavior and occasionally allow copulation, interest of the stallion may be maintained, which could foster band cohesion (NAS 2013). This last statement could be validated by the observations of group associations on the Sheldon NWR where feral mares were ovariectomized via colpotomy and released back on to the range with untreated horses of both sexes (Collins and Kasbohm 2016). No data were collected on inter- or intra-band behavior (e.g. estrous display, increased tending by stallions, etc.), during multiple aerial surveys in years following treatment, all treated individuals appeared to maintain group associations, and there were no groups consisting only of treated males or only of treated females (Collins and Kasbohm 2016). In addition, of solitary animals documented during surveys, there were no observations of solitary treated females (Collins and Kasbohm 2016). These data help support the expectation that ovariectomized mares would not lose interest in or be cast out of the social dynamics of a wild horse herd. As noted by the NAS (2013), the ideal fertility control method would not eliminate sexual behavior or change social structure substantially.

A study conducted for 15 days in January 1978 (Asa et al. 1980), compared the sexual behavior in ovariectomized and seasonally anovulatory (intact) pony mares and found that there were no statistical differences between the two conditions for any measure of proceptivity or copulatory behavior, or days in estrous. This may explain why treated mares at Sheldon NWR continued to be accepted into harem bands; they may have been acting the same as a non-pregnant mare. Five to ten percent of pregnant mares exhibit estrous behavior (Crowell-Davis 2007). Although the physiological cause of this phenomenon is not fully understood (Crowell-Davis 2007), it is thought to be a bonding mechanism that assists in the maintenance of stable social groups of horses year round (Ransom et al. 2014b). The complexity of social behaviors among free-roaming horses is not entirely centered on reproductive receptivity, and fertility control treatments that suppress the reproductive system and reproductive behaviors should contribute to minimal changes to social behavior (Ransom et al. 2014b, Collins and Kasbohm 2016).

² Luteinizing hormone (LH) is a glycoprotein hormone produced in the pituitary gland. In females, a sharp rise of LH triggers ovulation and development of the corpus luteum. LH concentrations can be measured in blood plasma.

BLM expects that wild horse harem structures would continue to exist under the proposed action because fertile mares, stallions, and their foals would continue to be a component of the herd. It is not expected that spaying a subset of mares would significantly change the social structure or herd demographics (age and sex ratios) of fertile wild horses.

'Foal stealing,' where a near-term pregnant mare steals a neonate foal from a weaker mare, is unlikely to be a common behavioral result of including sterilized mares in a wild horse herd, no matter the method of sterilization. McDonnell (2012) noted that "foal stealing is rarely observed in horses, except under crowded conditions and synchronization of foaling," such as in horse feed lots. Those conditions are not likely in the wild, where pregnant mares will be widely distributed across the landscape, and where the expectation is that parturition dates would be distributed across the normal foaling season.

Indirect Effects of Mare Sterilization

The free-roaming behavior of wild horses is not anticipated to be affected by mare sterilization, as the definition of free-roaming is the ability to move without restriction by fences or other barriers within a HMA (BLM H-4700-1, 2010) and there are no permanent physical barriers being proposed.

In domestic animals, sterilization is often associated with weight gain and associated increase in body fat (Fettman et al 1997, Becket et al 2002, Jeusette et al. 2006, Belsito et al 2009, Reichler 2009, Camara et al. 2014). Spayed cats had a decrease in fasting metabolic rate, and spayed dogs had a decreased daily energy requirement, but both had increased appetite (O'Farrell & Peachey 1990, Hart and Eckstein 1997, Fettman et al. 1997, Jeusette et al. 2004). In wild horses, contracepted mares tend to be in better body condition that mares that are pregnant or that are nursing foals (Nuñez et al. 2010); the same improvement in body condition is likely to take place in spayed mares. In horses, surgical sterilization through ovariectomy has the potential to increase risk of equine metabolic syndrome (leading to obesity and laminitis), but both blood glucose and insulin levels were similar in mares before and after ovariectomy over the short-term (Bertin et al. 2013). In wild horses the quality and quantity of forage, and frequent exercise, is unlikely to be sufficient to promote over-eating and obesity.

Coit et al. (2009) demonstrated that spayed dogs have elevated levels of LH-receptor and GnRHreceptor mRNA in the bladder tissue, and lower contractile strength of muscles. They noted that urinary incontinence occurs at elevated levels in spayed dogs and in post-menopausal women. Thus, it is reasonable to suppose that some ovariectomized mares could also suffer from elevated levels of urinary incontinence.

Sterilization had no effect on movements and space use of feral cats or brushtail possums (Ramsey 2007, Guttilla & Stapp 2010), or greyhound racing performance (Payne 2013). Rice field rats (*Rattus argentiventer*) tend to have a smaller home range in the breeding season, as they remain close to their litters to protect and nurse them. When surgically sterilized, rice field rats had larger home ranges and moved further from their burrows than hormonally sterilized or fertile rats (Jacob et al. 2004). Spayed possums and foxes (*Vulpes vulpes*) had a similar core range area after spay surgery compared to before, and were no more likely to shift their range than intact females (Saunders et al. 2002, Ramsey 2007).

The likely effects of sterilization on mares' home range and habitat use can also be surmised from available literature. Bands of horses tend to have distinct home ranges, varying in size depending on the habitat and varying by season, but always including a water source, forage, and places where horses can shelter from inclement weather or insects (King and Gurnell 2005). It is unlikely that sterilized mares will change their spatial ecology, but being emancipated from constraints of gestation and lactation may mean they can spend more time away from water sources and increase their home range size. Lactating mares need to drink every day, but during the winter when snow can fulfill water needs or when not lactating, horses can traverse a wider area (Feist & McCullough 1976, Salter 1979). During

multiple aerial surveys in years following the mare ovariectomy study at the Sheldon NWR, it was documented that all treated individuals appeared to maintain group associations, no groups consisted only of treated females, and none of the solitary animals observed were treated females (Collins and Kasbohm 2016). Given that treated females maintained group associations, this indicates that their movement patterns and distances may be unchanged.

Sterilizing wild horses does not change their status as wild horses under the WFRHBA (as amended). In terms of whether sterile mares would continue to exhibit the free-roaming behavior that defines wild horses, BLM does expect that sterile mares would continue to roam unhindered. Wild horse movements may be motivated by a number of biological impulses, including the search for forage, water, and social companionship that is not of a sexual nature. As such, a sterilized animal would still be expected to have a number of internal reasons for moving across a landscape and, therefore, exhibiting 'free-roaming' behavior. Despite marginal uncertainty about subtle aspects of potential changes in habitat preference, there is no expectation that spaying wild horses will cause them to lose their free-roaming nature.

In this sense, a sterilized wild mare would be just as much 'wild' as defined by the WFRHBA as any fertile wild mare, even if her patterns of movement differ slightly. Congress specified that sterilization is an acceptable management action (16 USC §1333.b.1). Sterilization is not one of the clearly defined events that cause an animal to lose its status as a wild free-roaming horse (16 USC §1333.2.C.d). As noted in the discussion of neutering, any opinions based on a semantic and subjective definition of what constitutes a 'wild' horse are not legally binding for BLM, which must adhere to the legal definition of what constitutes a wild free-roaming horse³, based on the WFRHBA (as amended). BLM is not obliged to base management decisions on personal opinions, which do not meet the BLM's principle and practice to "Use the best available scientific knowledge relevant to the problem or decision being addressed, relying on peer reviewed literature when it exists" (Kitchell et al. 2015).

Sterilization is not expected to reduce mare survival rates on public rangelands. Individuals receiving fertility control often have reduced mortality and *increased* longevity due to being released from the costs of reproduction (Kirkpatrick and Turner 2008). Similar to contraception studies, in other wildlife species a common trend has been higher survival of sterilized females (Twigg et al. 2000, Saunders et al. 2002, Ramsey 2005, Jacob et al. 2008, Seidler and Gese 2012). Observations from the Sheldon NWR provide some insight into long-term effects of ovariectomy on feral horse survival rates. The sterilized mares in Sheldon NWR were returned to the range along with untreated mares. Between 2007 and 2014, mares were captured, a portion treated, and then recaptured. There was a minimum of 1 year between treatment and recapture; some mares were recaptured a year later and some were recaptured several years later. The long-term survival rate of treated wild mares appears to be the same as that of untreated mares (Collins and Kasbohm 2016). Recapture rates for released mares were similar for treated mares.

Effects on Bone Histology

There is no known mechanism by which bone development would change in mares treated with pharmacological or immunological sterilization methods, or with minimally invasive sterilization methods. The BLM knows of no scientific, peer-reviewed literature that documents bone density loss in mares following ovariectomy. A concern has been raised in an opinion article (Nock 2013) that ovary removal in mares could lead to bone density loss. That opinion article was not peer reviewed nor was it

^{3 &}quot;wild free-roaming horses and burros" means all unbranded and unclaimed horses and burros on public lands of the United States.

based on research in wild or domestic horses, so it does not meet the BLM's standard for "best available science" on which to base decisions (Kitchell et al. 2015). Hypotheses that are forwarded in Nock (2013) appear to be based on analogies from modern humans leading sedentary lives. Post-menopausal women appear to have a greater chance of developing osteoporosis (Scholz-Ahrens et al. 1996), but BLM is not aware of any research examining bone loss in horses following ovariectomy. Bone loss in humans has been linked to reduced circulating estrogen. There have been conflicting results when researchers have attempted to test for an effect of reduced estrogen on animal bone loss rates in animal models; all experiments have been on laboratory animals, rather than free-ranging wild animals. While some studies found changes in bone cell activity after ovariectomy leading to decreased bone strength (Jerome et al. 1997, Baldock et al. 1998, Huang et al. 2002, Sigrist et al. 2007), others found that changes were moderate and transient or minimal (Scholz-Ahrens et al. 1996, Lundon et al. 1994, Zhang et al. 2007), and even returned to normal after 4 months (Sigrist et al. 2007).

Consistent and strenuous use of bones, for instance using jaw bones by eating hard feed, or using leg bones by travelling large distances, may limit the negative effects of estrogen deficiency on microarchitecture (Mavropoulos et al. 2014). The effect of exercise on bone strength in animals has been known for many years and has been shown experimentally (Rubin et al. 2001). Dr. Simon Turner, Professor Emeritus of the Small Ruminant Comparative Orthopaedic Laboratory at Colorado State University, conducted extensive bone density studies on ovariectomized sheep, as a model for human osteoporosis. During these studies, he did observe bone density loss on ovariectomized sheep, but those sheep were confined in captive conditions, fed twice a day, had shelter from inclement weather, and had very little distance to travel to get food and water (Simon Turner, Colorado State University Emeritus, written comm., 2015). Dr. Turner indicated that an estrogen deficiency (no ovaries) could potentially affect a horse's bone metabolism, just as it does in sheep and human females when they lead a sedentary lifestyle, but indicated that the constant weight bearing exercise, coupled with high exposure to sunlight ensuring high vitamin D levels, are expected to prevent bone density loss (Simon Turner, Colorado State University Emeritus, written comm., 2015)

Home range size of horses in the wild has been described as 4.2 to 30.2 square miles (Green and Green 1977) and 28.1 to 117 square miles (Miller 1983). A study of distances travelled by feral horses in "outback" Australia shows horses travelling between 5 and 17.5 miles per 24-hour period (Hampson et al. 2010a), travelling about 11 miles a day even in a very large paddock (Hampson et al. 2010b). Thus extensive movement patterns of wild horses are expected to help prevent bone loss. The expected daily movement distance would be far greater in the context of larger pastures typical of BLM long-term holding facilities in off-range pastures. A horse would have to stay on stall rest for years after removal of the ovaries in order to develop osteoporosis (Simon Turner, Colorado State University Emeritus, written comm., 2015) and that condition does not apply to any wild horses turned back to the range or any wild horses that go into off-range pastures.

Genetic Effects of Mare Sterilization and Neutering

It is true that spayed females and neutered males are unable to contribute to the genetic diversity of the herd. BLM is not obligated to ensure that any given individual in a herd has the chance to sire a foal and pass on genetic material. Management practices in the BLM Wild Horse and Burro Handbook (2010) include measures to increase population genetic diversity in reproducing herds where monitoring reveals a cause for concern about low levels of observed heterozygosity. These measures include increasing the sex ratio to a greater percentage of fertile males than fertile females (and thereby increasing the number of males siring foals), and bringing new animals into a herd from elsewhere.

In a hypothetical herd that is managed to be entirely non-reproducing, it would not be a concern to maintain genetic diversity because the management goal would be that animals in such a herd would not breed.

In reproducing herds where large numbers of wild horses have recent and / or an ongoing influx of breeding animals from other areas with wild or feral horses, spaying and neutering is not expected to cause an unacceptable loss of genetic diversity or an unacceptable increase in the inbreeding coefficient. In any diploid population, the loss of genetic diversity through inbreeding or drift can be prevented by large effective breeding population sizes (Wright 1931) or by introducing new potential breeding animals (Mills and Allendorf 1996). The NAS report (2013) recommended that single HMAs should not be considered as isolated genetic populations. Rather, managed herds of wild horses should be considered as components of interacting metapopulations, with the potential for interchange of individuals and genes taking place as a result of both natural and human-facilitated movements. It is worth noting that, although maintenance of genetic diversity at the scale of the overall population of wild horses is an intuitive management goal, there are no existing laws or policies that require BLM to maintain genetic diversity at the scale of the individual herd management area or complex. Also, there is no Bureau-wide policy that requires BLM to allow each female in a herd to reproduce before she is treated with contraceptives. Introducing 1-2 mares every generation (about every 10 years) is a standard management technique that can alleviated potential inbreeding concerns (BLM 2010). The NAS report (2013) recommended that managed herds of wild horses would be better viewed as components of interacting metapopulations, with the potential for interchange of individuals and genes taking place as a result of both natural and human-facilitated movements.

In the last 10 years, there has been a high realized growth rate of wild horses in most areas administered by the BLM. As a result, most alleles that are present in any given mare are likely to already be well represented in her siblings, cousins, and more distant relatives on the HMA. With the exception of horses in a small number of well-known HMAs that contain a relatively high fraction of alleles associated with old Spanish horse breeds (NAS 2013), the genetic composition of wild horses in lands administered by the BLM is consistent with admixtures from domestic breeds. The NAS report (2013) includes information (pairwise genetic 'fixation index' values for sampled WH&B herds) confirming that WH&B in the vast majority of HMAs are genetically similar to animals in multiple other HMAs. As a result, in most HMAs, applying fertility control to a subset of mares is not expected to cause irreparable loss of genetic diversity. Improved longevity and an aging population are expected results of contraceptive treatment that can provide for lengthening generation time; this result would be expected to slow the rate of genetic diversity loss (Hailer et al. 2006). Based on a population model, Gross (2000) found that a strategy to preferentially treat young animals with a contraceptive led to more genetic diversity being retained than either a strategy that preferentially treats older animals, or a strategy with periodic gathers and removals.

Roelle and Oyler-McCance (2015) used the VORTEX population model to simulate how different rates of mare sterility would influence population persistence and genetic diversity, in populations with high or low starting levels of genetic diversity, various starting population sizes, and various annual population growth rates. Although those results are specific to mares, some inferences about potential effects of stallion sterilization may also be made from their results. Roelle and Oyler-McCance (2015) showed that the risk of the loss of genetic heterozygosity is extremely low except in cases where all of the following conditions are met: starting levels of genetic diversity are low, initial population size is 100 or less, the intrinsic population growth rate is low (5% per year), and very large fractions of the population are permanently sterilized. Given that 94 of 102 wild horse herds sampled for genetic diversity did not meet a threshold for concern (NAS 2013), the starting level of genetic diversity in most wild-horse herds is relatively high.

In a breeding herd where more than 85% of males in a population are sterile, there could be genetic consequences of reduced heterozygosity and increased inbreeding coefficients, as it would potentially allow a very small group of males to dominate the breeding (e.g., Saltz et al. 2000). Such genetic consequences could be mitigated by natural movements or human-facilitated translocations (BLM 2010). Garrott and Siniff's (1992) model predicts that gelding 50-80% of mature males in the population would result in reduced, but not halted, mare fertility rates. However, neutering males tends to have short-lived effects, because within a few years after any male sterilization treatment, a number of fertile male colts would become sexually mature stallions who could contribute genetically to the herd.

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Intrauterine Devices (IUDs)

Based on promising results from published, peer-reviewed studies in domestic mares, BLM has begun to use IUDs to control fertility as a wild horse and burro fertility control method on the range. The initial management use was in mares from the Swasey HMA, in Utah. The BLM has supported and continues to support research into the development and testing of effective and safe IUDs for use in wild horse mares (Baldrighi et al. 2017, Holyoak et al. 2021). However, existing literature on the use of IUDs in horses allows for inferences about expected effects of any management alternatives that might include use of IUDs, and support the apparent safety and efficacy of some types of IUDs for use in horses. Overall, as with other methods of population growth suppression, use of IUDs and other fertility control measures are expected to help reduce population growth rates, extend the time interval between gathers, and reduce the total number of excess animals that will need to be removed from the range.

The 2013 National Academies of Sciences (NAS) report considered IUDs, and suggested that research should test whether IUDs cause uterine inflammation, and should also test how well IUDs stay in mares that live and breed with fertile stallions. Since that report, a recent study by Holyoak et al. (2021) indicate that a flexible, inert, y-shaped, medical-grade silicone IUD design prevented pregnancies in all the domestic mares that retained the device, even when exposed to fertile stallions. Domestic mares in that study lived in large pastures, mating with fertile stallions. Biweekly ultrasound examinations showed that IUDs stayed in 75% of treated mares over the course of two breeding seasons. The IUDs were then removed so the researchers could monitor the mares' return to fertility. In that study, uterine health, as measured in terms of inflammation, was not seriously affected by the IUDs, and most mares became pregnant within months after IUD removal. The overall results are consistent with results from an earlier study (Daels and Hughes 1995), which used O-shaped silicone IUDs. Similarly, a flexible IUD with three components connected by magnetic force (the 'iUPOD') was retained over 90 days in mares

living and breeding with a fertile stallion; after IUD removal, the majority of mares became pregnant in the following breeding season (Hoopes et al. 2021).

IUDs are considered a temporary fertility control method that does not generally cause future sterility (Daels and Hughes 1995). Use of IUDs is an effective fertility control method in women, and IUDs have historically been used in livestock management, including in domestic horses. Insertion of an IUD can be a very rapid procedure, but it does require the mare to be temporarily restrained, such as in a squeeze chute. IUDs in mares may cause physiological effects including discomfort, infection, perforation of the uterus if the IUD is hard and angular, endometritis, uterine edema (Killian et al. 2008), and pyometra (Klabnik-Bradford et al. 2013). In women, deaths attributable to IUD use may be as low as 1.06 per million (Daels and Hughes 1995). The effects of IUD use on genetic diversity in a given herd should be comparable to those of other temporary fertility control methods; use should reduce the fraction of mares breeding at any one time, but does not necessarily preclude treated mares from breeding in the future, as they survive and regain fertility.

The exact mechanism by which IUDs prevent pregnancy is uncertain, but may be related to persistent, low-grade uterine inflammation (Daels and Hughes 1995, Gradil et al. 2021, Hoopes et al. 2021), Turner et al. (2015) suggested that the presence of an IUD in the uterus may, like a pregnancy, prevent the mare from coming back into estrus. However, some domestic mares did exhibit repeated estrus cycles during the time when they had IUDs (Killian et al. 2008, Gradil et al. 2019, Lyman et al. 2021, Hoopes et al. 2021). The main cause for an IUD to not be effective at contraception is its failure to stay in the uterus (Daels and Hughes 1995, NAS 2013). As a result, one of the major challenges to using IUDs to control fertility in mares on the range is preventing the IUD from being dislodged or otherwise ejected over the course of daily activities, which could include, at times, frequent breeding.

At this time, it is thought that any IUD inserted into a pregnant mare may cause the pregnancy to terminate, which may also cause the IUD to be expelled. For that reason, it is expected that IUDs would only be inserted in non-pregnant (open) mares. Wild mares receiving IUDs would be checked for pregnancy by a veterinarian prior to insertion of an IUD. This can be accomplished by transrectal palpation and/or ultrasound performed by a veterinarian. Pregnant mares would not receive an IUD. Only a veterinarian would apply IUDs in any BLM management action. The IUD is inserted into the uterus using a thin, tubular applicator similar to a shielded culture tube, and would be inserted in a manner similar to that routinely used to obtain uterine cultures in domestic mares. If a mare has a zygote or very small, early phase embryo, it is possible that it will fail to be detected in screening, and may develop further, but without causing the expulsion of the IUD. Wild mares with IUDs would be individually marked and identified, so that they can be monitored occasionally and examined, if necessary, in the future, consistent with other BLM management activities.

Using metallic or glass marbles as IUDs may prevent pregnancy in horses (Nie et al. 2003), but can pose health risks to domestic mares (Turner et al. 2015, Freeman and Lyle 2015). Marbles may break into shards (Turner et al. 2015), and uterine irritation that results from marble IUDs may cause chronic, intermittent colic (Freeman and Lyle 2015). Metallic IUDs may cause severe infection (Klabnik-Bradford et al. 2013).

In domestic ponies, Killian et al. (2008) explored the use of three different IUD configurations, including a silastic polymer O-ring with copper clamps, and the "380 Copper T" and "GyneFix" IUDs designed for women. The longest retention time for the three IUD models was seen in the "T" device, which stayed in the uterus of several mares for 3-5 years. Reported contraception rates for IUD-treated mares were 80%, 29%, 14%, and 0% in years 1-4, respectively. They surmised that pregnancy resulted after IUD fell out of the uterus. Killian et al. (2008) reported high levels of progesterone in non-pregnant, IUD-treated ponies.

Soft or flexible IUDs may cause relatively less discomfort than hard IUDs (Daels and Hughes 1995). Daels and Hughes (1995) tested the use of a flexible O-ring IUD, made of silastic, surgical-grade polymer, measuring 40 mm in diameter; in five of six breeding domestic mares tested, the IUD was reported to have stayed in the mare for at least 10 months. In mares with IUDs, Daels and Hughes (1995) reported some level of uterine irritation, but surmised that the level of irritation was not enough to interfere with a return to fertility after IUD removal.

More recently, several types of soft or flexible IUDs have been tested for use in breeding mares. When researchers attempted to replicate the O-ring study (Daels and Hughes 1995) in an USGS / Oklahoma State University (OSU) study with breeding domestic mares, using various configurations of silicone Oring IUDs, the IUDs fell out at unacceptably high rates over time scales of less than 2 months (Baldrighi et al. 2017, Lyman et al. 2021). Subsequently, the USGS / OSU researchers tested a Y-shaped IUD to determine retention rates and assess effects on uterine health; retention rates were greater than 75% for an 18-month period, and mares returned to good uterine health and reproductive capacity after removal of the IUDs (Holyoak et al. 2021). These Y-shaped silicone IUDs are considered a pesticide device by the EPA, in that they work by physical means (EPA 2020). The University of Massachusetts has developed a magnetic IUD that has been effective at prolonging estrus and preventing pregnancy in domestic mares (Gradil et al. 2019, Joonè et al. 2021, Gradil et al. 2021, Hoopes et al. 2021). After insertion in the uterus, the three subunits of the device are held together by magnetic forces as a flexible triangle. A metal detector can be used to determine whether the device is still present in the mare. In an early trial, two sizes of those magnetic IUDs fell out of breeding domestic mares at high rates (Holyoak et al., unpublished results), but more recent trials have shown that the magnetic IUD was retained even in the presence of breeding with a fertile stallion (Hoopes et al. 2021). The magnetic IUD was used in two trials where mares were exposed to stallions, and in one where mares were artificially inseminated; in all cases, the IUDs were reported to stay in the mares without any pregnancy (Gradil 2019, Joonè et al. 2021, Gradil et al. 2021, Hoopes et al. 2021).

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Appendix F- Genetic Reports

Genetic Analysis of the

Conant Creek HMA, WY

E. Gus Cothran

August 19, 2013

Department of Veterinary Integrative Bioscience Texas A&M University College Station, TX 77843-4458 The following is a report of the genetic analysis of the Conant Creek HMA, WY.

A few general comments about the genetic variability analysis based upon DNA microsatellites compared to blood typing. The DNA systems are more variable than blood typing systems, thus variation levels will be higher. Variation at microsatellite loci is strongly influenced by allelic diversity and changes in variation will be seen in allelic measures more quickly that at heterozygosity, which is why more allelic diversity measures are calculated. For mean values, there are a greater proportion of rare domestic breeds included in the estimates than for blood typing so relative values for the measures are lower compared to the feral horse values. As well, feral values are relatively higher because the majority of herds tested are of mixed ancestry which results in a relatively greater increase in heterozygosity values based upon the microsatellite data. There are no specific variants related to breed type so similarity is based upon the total data set.

METHODS

A total of 28 samples were received by Texas A&M University, Equine Genetics Lab on January 15, 2013. DNA was extracted from the samples and tested for variation at 12 equine microsatellite (mSat) systems. These were *AHT4*, *AHT5 ASB2*, *ASB17*, *ASB23*, *HMS3*, *HMS6*, *HMS7*, *HTG4*, *HTG10*, *LEX33*, and *VHL20*. These systems were tested using an automated DNA sequencer to separate Polymerase Chain Reaction (PCR) products.

A variety of genetic variability measures were calculated from the gene marker data. The measures were observed heterozygosity (*Ho*) which is the actual number of loci heterozygous per individual; expected heterozygosity (*He*), which is the predicted number of heterozygous loci based upon gene frequencies; effective number of alleles (*Ae*) which is a measure of marker system diversity; total number of variants (*TNV*); mean number of alleles per locus (*MNA*); the number of rare alleles observed which are alleles that occur with a frequency of 0.05 or less (*RA*); the percent of rare alleles (*%RA*); and estimated inbreeding level (*Fis*) which is calculated as 1-*Ho*/*He*.

Genetic markers also can provide information about ancestry in some cases. Genetic resemblance to domestic horse breeds was calculated using Rogers' genetic similarity coefficient, *S*. This resemblance was summarized by use of a restricted maximum likelihood (RML) procedure.

RESULTS AND DISCUSSION

Variants present and allele frequencies are given in Table 1. No variants were observed which have not been seen in horse breeds. Table 2 gives the values for the genetic variability measures of the Conant Creek HMA herd. Also shown in Table 2 are values from a representative group of domestic horse breeds. The breeds were selected to cover the range of variability measures in domestic horse populations. Mean values for feral herds (based upon data from 126 herds) and mean values for domestic breeds (based upon 80 domestic horse populations) also are shown.

Mean genetic similarity of the Conant Creek HMA herd to domestic horse breed types are shown in Table 3. A dendrogram of relationship of the Conant Creek HMA herd to a standard set of domestic breeds is shown in Figure 1.

Genetic Variants: A total of 72 variants were seen in the Conant Creek HMA herd which is very slightly below the mean for feral herds and well below the mean for domestic breeds. Of these, 15 had frequencies below 0.05 which is about the average percentage of variants at risk of future loss. Allelic diversity as represented by *Ae* is lower than the average for feral herds while *MNA* is slightly lower than the mean for the feral horses.

Genetic Variation: Observed heterozygosity was just below the feral herd mean while expected heterozygosity was well below the feral mean. *Ho* was a good deal greater than *He* which could indicate a recent decline in population size. More information is needed to confirm this possibility.

Genetic Similarity: Overall similarity of the Conant Creek HMA herd to domestic breeds was about average for feral herds. Highest mean genetic similarity of the Conant Creek HMA herd was with Light Racing and Riding breeds, followed by the Old World Iberian breeds. As seen in Fig. 1, however, the Conant Creek HMA herd clusters with Garanno. This breed is one that feral herds frequently cluster with when the herd is of mixed origins with no clear indication of ancestral breed type. As with most trees involving feral herds, the tree is somewhat distorted.

SUMMARY

Genetic variability of this herd is near the average for feral herds although some measures are just below the mean with the trend for variability to be low. This herd was previously tested in 2004. Compared to 2004 the variability measures for the Conant Creek HMA have increased slightly. Sample sizes were similar so it is not likely that the differences are due to sample error. The pattern of change is not consistent with gene flow into the population as that should increase He relative to Ho however, other changes could be due to immigration. This would best fit a situation where the source population of immigration was closely related to Conant Creek. At this point it is not clear. There is a possibility that this herd has seen a recent loss of population size which would increase the risk to genetic diversity. Genetic similarity results suggest a herd with mixed ancestry.

RECOMMENDATIONS

Current variability levels are high enough that no action is needed at this point but the herd should be monitored closely due to the trend for low variability. This is especially true if it is known that the herd size has seen a recent decline. If there is know gene flow into the herd this should be allowed to continue.

VHL20															
I	J	К	L	М	Ν	0	Р	Q	R	S					
0.089	0.000	0.000	0.018	0.071	0.196	0.179	0.429	0.000	0.018	0.000					
HTG4															
I	J	К	L	М	Ν	0	Р	Q	R						
0.000	0.000	0.089	0.018	0.804	0.000	0.071	0.018	0.000	0.000						
AHT4															
Н	I	J	К	L	М	Ν	0	Ρ	Q	R					
0.107	0.000	0.143	0.250	0.054	0.000	0.000	0.375	0.071	0.000	0.000					
HMS7															
I	J	К	L	М	Ν	0	Р	Q	R						
0.000	0.000	0.018	0.393	0.018	0.392	0.179	0.000	0.000	0.000						
AHT5															
I	J	К	L	М	Ν	0	Р	Q	R						
0.072	0.000	0.214	0.143	0.143	0.214	0.214	0.000	0.000	0.000						
HMS6															
I	J	К	L	М	Ν	0	Р	Q	R						
0.000	0.000	0.089	0.071	0.108	0.000	0.143	0.589	0.000	0.000						
ASB2															
В	I	J	К	L	М	Ν	0	Р	Q	R					
0.000	0.000	0.000	0.285	0.000	0.071	0.411	0.018	0.000	0.161	0.054					
HTG10															
Н	I	J	К	L	М	Ν	0	Р	Q	R	S	Т			
0.000	0.054	0.000	0.161	0.018	0.000	0.054	0.285	0.000	0.178	0.250	0.000	0.000			
HMS3															
Н	I	J	К	L	М	Ν	0	Р	Q	R	S				
0.000	0.000	0.000	0.000	0.000	0.304	0.339	0.143	0.196	0.018	0.000	0.000				
ASB17															
D	F	G	Н	I	J	К	L	М	Ν	0	Р	Q	R	S	Т
0.000	0.054	0.036	0.000	0.089	0.000	0.000	0.036	0.071	0.357	0.232	0.071	0.018	0.036	0.000	0.000
ASB23															
G	Н	I	J	К	L	М	Ν	0	Ρ	Q	R	S	Т	U	V
0.000	0.000	0.071	0.000	0.571	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.197	0.000
LEX33															
F	G	К	L	М	Ν	0	Р	Q	R	S	Т				
0.000	0.018	0.000	0.054	0.000	0.000	0.214	0.000	0.357	0.357	0.000	0.000				

 Table 1. Allele frequencies of genetic variants observed in Conant Creek HMA feral horse herd.

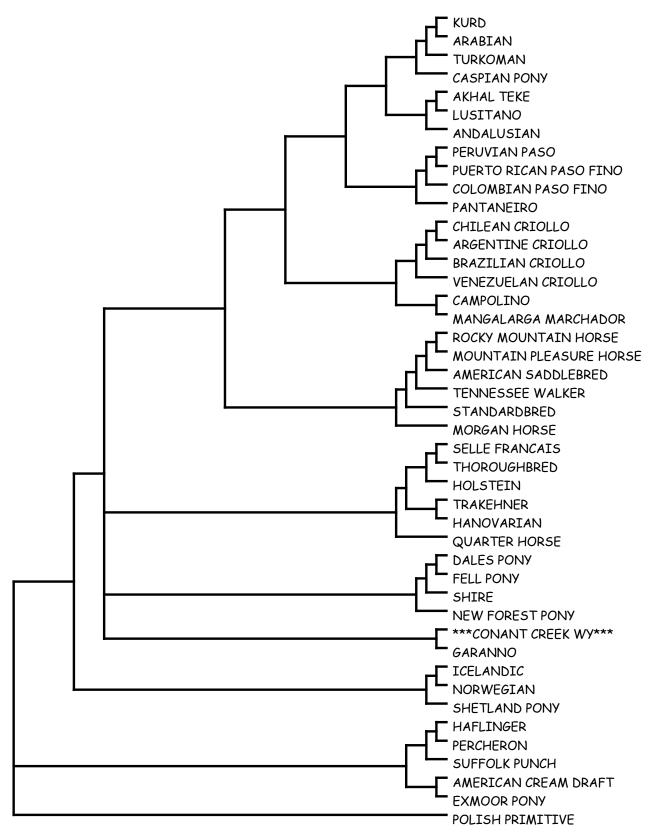
	N	Но	He	Fis	Ae	TNV	MNA	Ra	%Ra
CONANT CREEK WY	28	0.714	0.688	-0.038	3.590	72	6.00	15	0.208
Cleveland Bay	47	0.610	0.627	0.027	2.934	59	4.92	16	0.271
American Saddlebred	576	0.740	0.745	0.007	4.25	102	8.50	42	0.412
Andalusian	52	0.722	0.753	0.041	4.259	79	6.58	21	0.266
Arabian	47	0.660	0.727	0.092	3.814	86	7.17	30	0.349
Exmoor Pony	98	0.535	0.627	0.146	2.871	66	5.50	21	0.318
Friesian	304	0.545	0.539	-0.011	2.561	70	5.83	28	0.400
Irish Draught	135	0.802	0.799	-0.003	5.194	102	8.50	28	0.275
Morgan Horse	64	0.715	0.746	0.041	4.192	92	7.67	33	0.359
Suffolk Punch	57	0.683	0.711	0.038	3.878	71	5.92	13	0.183
Tennessee Walker	60	0.666	0.693	0.038	3.662	87	7.25	34	0.391
Thoroughbred	1195	0.734	0.726	-0.011	3.918	69	5.75	18	0.261
Feral Horse Mean	126	0.716	0.710	-0.012	3.866	72.68	6.06	16.96	0.222
Standard Deviation	120	0.056	0.059	0.071	0.657	13.02	1.09	7.98	0.222
Minimum		0.030	0.039	-0.284	2.148	37	3.08	0	0.088
Maximum		0.490	0.489	0.133	5.253	<u> </u>	8.00	33	0.400
Domestic Horse Mean	80	0.710	0.720	0.012	4.012	80.88	6.74	23.79	0.283
Standard Deviation		0.078	0.071	0.086	0.735	16.79	1.40	10.11	0.082
Minimum		0.347	0.394	-0.312	1.779	26	2.17	0	0
Maximum		0.822	0.799	0.211	5.30	119	9.92	55	0.462

 Table 2. Genetic variability measures.

Table 3. Rogers' genetic similarity of the Conant Creek HMA feral horse herd to major groups of domestic horses.

	Mean S	Std	Minimum	Maximum
Light Racing and Riding Breeds	0.736	0.027	0.692	0.771
Oriental and Arabian Breeds	0.690	0.031	0.647	0.741
Old World Iberian Breeds	0.724	0.035	0.696	0.782
New World Iberian Breeds	0.706	0.030	0.645	0.739
North American Gaited Breeds	0.719	0.026	0.688	0.745
Heavy Draft Breeds	0.678	0.042	0.620	0.730
True Pony Breeds	0.705	0.028	0.672	0.739

Figure 1. Partial RML tree of genetic similarity to domestic horse breeds.



AID	VHL20	HTG4	AHT4	HMS7	AHT5	HMS6	ASB2	HTG10	HMS3	ASB17	ASB23	LEX33	LEX3
63272	MN	MO	КО	NO	MO	MM	NN	OR	NN	IM	KU	QR	IP
63273	PP	MM	HO	LN	LO	PP	KN	KQ	NN	MN	КК	LQ	ΗМ
63274	MR	MO	HO	LO	LO	PP	KK	NR	NP	GM	IK	OR	MP
63275	OP	MM	КО	LN	MN	KM	KN	00	NP	NO	КК	OQ	IM
63276	PP	MP	KK	LN	KM	MP	KN	OQ	NP	NO	КК	QR	LM
63277	IN	MM	KP	LN	NN	OP	MQ	QR	MN	NO	KS	QR	MM
63278	PP	MM	КО	LL	KN	KP	NN	OQ	PP	FO	KS	00	FM
63279	NP	КО	JO	NO	MO	LP	KN	OR	NO	IP	IK	RR	MP
63280	MN	MO	HJ	LO	LO	OP	KR	KN	MN	GP	IM	RR	ΗМ
63281	IP	MM	HP	LN	KL	PP	NQ	NQ	MN	NN	KS	QR	ΗМ
63282	00	MM	00	LO	KL	OP	MQ	00	MN	NO	KU	OQ	LM
63283	NO	MM	JO	NN	IK	KP	NO	КК	MP	OP	MU	OQ	FH
63284	OP	KM	JJ	NN	10	KP	KN	00	NP	NP	IK	OQ	MP
63285	PP	MM	КО	LL	NN	OP	KN	QR	NP	NR	KS	OQ	LM
63286	NN	MM	LO	LL	MN	OP	NQ	RR	MN	NO	KS	RR	LO
63287	MN	MM	КО	NO	00	MP	NQ	КО	MN	IN	KU	OQ	IN
63288	IP	MM	КО	NO	KK	PP	QR	IK	MN	NO	KU	QR	LM
63289	PP	MM	HO	00	KL	PP	KQ	OQ	MO	NN	KU	OQ	NO
63290	PP	KM	JO	LN	IN	PP	KN	KR	00	NN	КК	QR	MP
63291	NP	MM	LP	LL	MN	OP	KN	KR	MO	NQ	КК	OR	MO
63292	IP	LM	OP	MN	MO	LP	MN	IR	MO	IM	KU	LR	LL
63293	OP	KM	00	NN	00	LL	NN	RR	OQ	10	UU	QR	LM
63294	00	MM	КО	LO	KK	PP	KQ	10	MM	OR	KU	QQ	LM
63295	LP	MM	HJ	NN	LN	KP	NN	OQ	NP	FN	КК	QR	FO
63296	IP	MM	КО	LL	KN	PP	KQ	КО	NP	LN	SU	LQ	MO
63297	OP	MM	KL	LN	KM	MO	КM	OQ	MM	00	KS	QR	LO
63298	NP	KM	КО	LN	IN	OP	NN	RR	MO	FN	КК	RR	OP
63299	NO	MM	JK	KN	LO	PP	KR	LQ	MP	LO	КК	GO	LM

Appendix 1. DNA data for the Conant Creek HMA, WY herd.

Dishpan Butte HMA, WY

E. Gus Cothran

August 19, 2013

Department of Veterinary Integrative Bioscience Texas A&M University College Station, TX 77843-4458 The following is a report of the genetic analysis of the Dishpan ButteHMA, WY.

A few general comments about the genetic variability analysis based upon DNA microsatellites compared to blood typing. The DNA systems are more variable than blood typing systems, thus variation levels will be higher. Variation at microsatellite loci is strongly influenced by allelic diversity and changes in variation will be seen in allelic measures more quickly that at heterozygosity, which is why more allelic diversity measures are calculated. For mean values, there are a greater proportion of rare domestic breeds included in the estimates than for blood typing so relative values for the measures are lower compared to the feral horse values. As well, feral values are relatively higher because the majority of herds tested are of mixed ancestry which results in a relatively greater increase in heterozygosity values based upon the microsatellite data. There are no specific variants related to breed type so similarity is based upon the total data set.

METHODS

A total of 30 samples were received by Texas A&M University, Equine Genetics Lab on January 15, 2013. DNA was extracted from the samples and tested for variation at 12 equine microsatellite (mSat) systems. These were *AHT4*, *AHT5 ASB2*, *ASB17*, *ASB23*, *HMS3*, *HMS6*, *HMS7*, *HTG4*, *HTG10*, *LEX33*, and *VHL20*. These systems were tested using an automated DNA sequencer to separate Polymerase Chain Reaction (PCR) products.

A variety of genetic variability measures were calculated from the gene marker data. The measures were observed heterozygosity (*Ho*) which is the actual number of loci heterozygous per individual; expected heterozygosity (*He*), which is the predicted number of heterozygous loci based upon gene frequencies; effective number of alleles (*Ae*) which is a measure of marker system diversity; total number of variants (*TNV*); mean number of alleles per locus (*MNA*); the number of rare alleles observed which are alleles that occur with a frequency of 0.05 or less (*RA*); the percent of rare alleles (*%RA*); and estimated inbreeding level (*Fis*) which is calculated as 1-*Ho*/*He*.

Genetic markers also can provide information about ancestry in some cases. Genetic resemblance to domestic horse breeds was calculated using Rogers' genetic similarity coefficient, *S*. This resemblance was summarized by use of a restricted maximum likelihood (RML) procedure.

RESULTS AND DISCUSSION

Variants present and allele frequencies are given in Table 1. No variants were observed which have not been seen in horse breeds. Table 2 gives the values for the genetic variability measures of the Dishpan Butte HMA herd. Also shown in Table 2 are values from a representative group of domestic horse breeds. The breeds were selected to cover the range of variability measures in domestic horse populations. Mean values for feral herds (based upon data from 126 herds) and mean values for domestic breeds (based upon 80 domestic horse populations) also are shown.

Mean genetic similarity of the Dishpan Butte HMA herd to domestic horse breed types are shown in Table 3. A dendrogram of relationship of the Dishpan Butte HMA herd to a standard set of domestic breeds is shown in Figure 1.

Genetic Variants: A total of 78 variants were seen in the Dishpan Butte HMA herd which is above the mean for feral herds and slightly below the mean for domestic breeds. Of these, 19 had frequencies below 0.05 which is a percentage of variants at risk of future loss that is lower than the average. Allelic diversity as represented by *Ae* is somewhat higher than the average for feral herds while *MNA* is slightly lower than the mean for the feral horses.

Genetic Variation: Both observed heterozygosity and expected heterozygosity in the Dishpan Butte HMA herd are well above the feral mean and *Ho* is a good bit greater than *He*. This pattern of variation suggests a recent decline in population size.

Genetic Similarity: Overall similarity of the Dishpan Butte HMA herd to domestic breeds was about average for feral herds. Highest mean genetic similarity of the Dishpan Butte HMA herd was with Old World Iberian breeds, followed by the New World Iberian and Light Racing and Riding breeds. As seen in Fig. 1, the Dishpan Butte HMA herd clusters within a draft horse and pony group. This pattern is indicative of mixed heritage with no clear primary breed type. As with most trees involving feral herds, the tree is somewhat distorted.

SUMMARY

Genetic variability of this herd is very near average overall with some measures greater than the mean and some lower. This herd was previously tested in 2004. For all measures of variation the values were greater in 2004 indicating a loss of genetic diversity. This indicates loss of genetic diversity a possibility that this herd has seen a recent loss of population size which would increase the risk to genetic diversity. Genetic similarity results suggest a herd with mixed ancestry.

RECOMMENDATIONS

Current variability levels are high enough that no action is needed at this point but the herd should be monitored closely due to the loss of genetic diversity over the past eight years. This is especially true if it is known that the herd size has seen a recent decline.

VHL20															
I	J	К	L	м	Ν	0	Р	Q	R	S					
0.167	0.000	0.000	0.167	0.083	0.300	0.017	0.266	0.000	0.000	0.000					
HTG4															
1	J	к	L	м	N	ο	Р	Q	R						
0.000	0.000	0.067	0.000		0.000	0.233	0.017	-	0.000						
AHT4															
н	I	J	к	L	м	N	0	Р	Q	R					
0.000		-	0.150												
HMS7															
1	J	к	L	м	N	ο	Р	Q	R						
0.000	0.167	0.000	0.283			0.117	0.000								
AHT5															
1	J	к	L	м	N	ο	Р	Q	R						
0.100	-	0.033	0.117			-									
HMS6															
1	J	к	L	м	N	ο	Р	Q	R						
0.000	0.000	0.017	0.217	0.433	0.000	0.150	0.183		0.000						
ASB2															
В	I	J	к	L	м	N	0	Р	Q	R					
0.000	0.000	0.000	0.300	0.000		0.133	0.167	0.000		0.167					
HTG10															
н	I	J	к	L	м	N	0	Р	Q	R	S	т			
0.000	0.133	0.000	0.050	0.033	0.083	0.017	0.551	0.000	0.000	0.133	0.000	0.000			
HMS3															
н	I	J	к	L	м	N	0	Р	Q	R	S				
0.000	0.000	0.000	0.000	0.000	0.217	0.417	0.183	0.083		0.000	0.000				
ASB17															
D	F	G	н	I	J	к	L	М	N	ο	Р	Q	R	S	т
0.000	0.050	0.000	0.000	0.133	0.000	0.000	0.000	0.183	0.100	0.184	0.150	0.067	0.133	0.000	0.000
ASB23															
G	н	I	J	К	L	м	Ν	Ο	Р	Q	R	S	т	U	v
0.000	0.000	0.317	0.000	0.333	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.133	0.033	0.151	0.000
LEX33															
F	G	к	L	М	N	0	Р	Q	R	S	т				
0.000	0.000	0.100	0.133	0.000	0.000	0.150	0.000	-	0.267	0.000	0.000				

 Table 1. Allele frequencies of genetic variants observed in Dishpan Butte HMA feral horse herd.

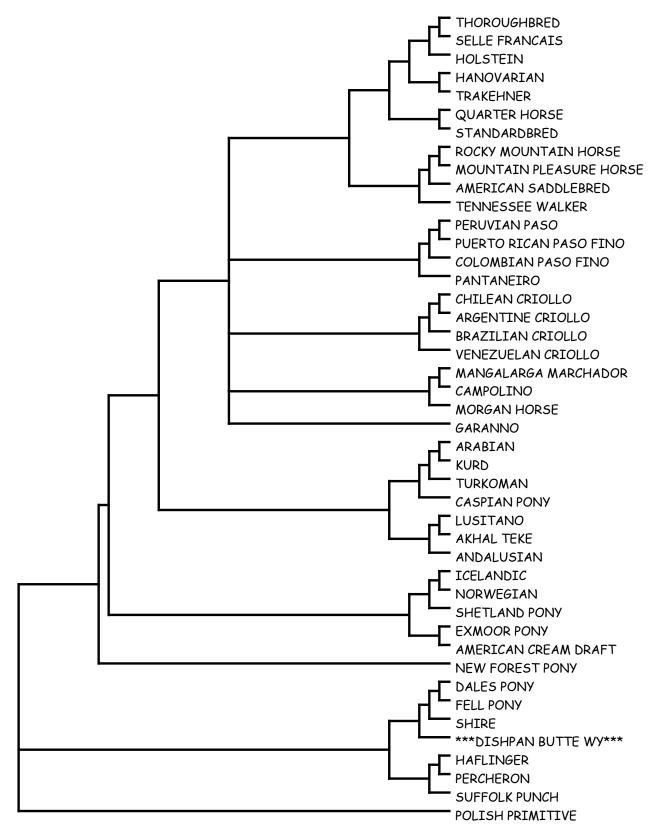
	N	Но	Не	Fis	Ae	TNV	MNA	Ra	%Ra
DISHPAN BUTTE WY	30	0.756	0.726	-0.041	4.002	70	5.83	14	0.200
Cleveland Bay	47	0.610	0.627	0.027	2.934	59	4.92	16	0.271
American Saddlebred	576	0.740	0.745	0.007	4.25	102	8.50	42	0.412
Andalusian	52	0.722	0.753	0.041	4.259	79	6.58	21	0.266
Arabian	47	0.660	0.727	0.092	3.814	86	7.17	30	0.349
Exmoor Pony	98	0.535	0.627	0.146	2.871	66	5.50	21	0.318
Friesian	304	0.545	0.539	-0.011	2.561	70	5.83	28	0.400
Irish Draught	135	0.802	0.799	-0.003	5.194	102	8.50	28	0.275
Morgan Horse	64	0.715	0.746	0.041	4.192	92	7.67	33	0.359
Suffolk Punch	57	0.683	0.711	0.038	3.878	71	5.92	13	0.183
Tennessee Walker	60	0.666	0.693	0.038	3.662	87	7.25	34	0.391
Thoroughbred	1195	0.734	0.726	-0.011	3.918	69	5.75	18	0.261
Feral Horse Mean	126	0.716	0.710	-0.012	3.866	72.68	6.06	16.96	0.222
Standard Deviation		0.056	0.059	0.071	0.657	13.02	1.09	7.98	0.088
Minimum		0.496	0.489	-0.284	2.148	37	3.08	0	0
Maximum		0.815	0.798	0.133	5.253	96	8.00	33	0.400
Domestic Horse Mean	80	0.710	0.720	0.012	4.012	80.88	6.74	23.79	0.283
Standard Deviation		0.078	0.071	0.086	0.735	16.79	1.40	10.11	0.082
Minimum		0.347	0.394	-0.312	1.779	26	2.17	0	0
Maximum		0.822	0.799	0.211	5.30	119	9.92	55	0.462

 Table 2. Genetic variability measures.

Table 3. Rogers' genetic similarity of the Dishpan Butte HMA feral horse herd to major groups of domestic horses.

	Mean S	Std	Minimum	Maximum
Light Racing and Riding Breeds	0.738	0.029	0.692	0.780
Oriental and Arabian Breeds	0.712	0.024	0.690	0.757
Old World Iberian Breeds	0.712	0.026	0.683	0.753
New World Iberian Breeds	0.689	0.021	0.650	0.709
North American Gaited Breeds	0.713	0.028	0.667	0.742
Heavy Draft Breeds	0.681	0.043	0.609	0.727
True Pony Breeds	0.710	0.022	0.688	0.747

Figure 1. Partial RML tree of genetic similarity to domestic horse breeds.



AID	VHL20	HTG4	AHT4	HMS7	AHT5	HMS6	ASB2	HTG10	HMS3	ASB17	ASB23	LEX33	LEX3
63300	LN	MO	JO	MN	LN	LP	00	КО	MM	MM	KU	LR	LM
63301	IL	MO	MO	11	00	MM	OR	OR	00	OR	IU	QQ	MM
63302	MN	MM	JN	NN	LO	MO	MR	MO	NN	IN	IK	OQ	MM
63303	NN	MO	КО	NO	00	MM	КО	MO	OP	OP	KS	KQ	IM
63304	MN	MM	JO	LN	LO	MO	MR	00	NQ	NO	IK	OR	MM
63305	IP	MO	JO	LN	NO	LP	KN	OR	NN	FP	IK	OQ	MM
63306	NN	MM	JN	NN	IL	MP	RR	MO	NQ	10	IK	RR	MM
63307	П	MO	КО	LN	00	MM	KQ	00	OQ	OQ	KS	QR	MM
63308	LP	MO	JP	LN	MM	LO	MQ	NO	MM	OR	IK	LO	FH
63309	LM	MM	KK	LN	MM	MO	KQ	10	MN	IM	IU	LR	FL
63310	10	KM	JP	MN	JO	MO	NO	00	MM	MO	IK	LR	IL
63311	IP	MM	OP	JN	00	LM	KN	10	NN	MR	IK	KR	MM
63312	LN	MO	OP	LN	LN	LP	OR	КО	MM	MP	LU	LQ	MM
63313	PP	MO	JO	JN	MO	LM	KN	10	NN	OR	IS	KR	MM
63314	LP	MM	JP	JN	MO	OP	NQ	00	MP	MO	TU	LL	IL
63315	IN	MM	КО	LO	10	MP	KK	00	00	PQ	КК	QQ	MM
63316	NP	MM	JO	JO	00	MM	KK	10	NP	FP	КК	OQ	MM
63317	LP	MM	IJ	JL	MO	MM	KN	IM	NN	OR	KS	KR	MM
63318	PP	MM]]	LN	IM	LL	КО	10	NO	MR	Ш	OR	MM
63319	LN	MM	JO	JL	MO	MP	MQ	00	MN	MN	KU	LQ	LM
63320	LP	MO	JO	JN	MO	LM	KR	IR	OQ	MR	IS	OQ	MM
63321	PP	KM	JK	LO	JN	OP	NO	LR	NP	NP	П	QR	MP
63322	IN	КО	JO	LN	IN	KM	КО	KR	NN	PR	IL	OR	MM
63323	NP	KM	JK	LO	NO	LP	KQ	OR	NO	MQ	SU	QQ	LM
63324	IN	00	JO	LN	IM	LO	OR	OR	NQ		IT	OR	LM
63325	NN	MO	00	JL	10	LM	КM	10	NQ	PP	KS	KQ	IL
63326	LM	MP	JO	LN	KL	LP	MQ	MO	MN	NO	SU	QR	LN
63327	PP	MM	KO	00	JM	MO	KN	LR	MP	FN	IU	QR	PP
63328	NN	MM	JN	NN	LO	MP	RR	00	NN	11	KK	QQ	MM
63329	IM	MO	КО	LN	КО	MM	KQ	00	00	IQ	IK	KQ	MM

Appendix 1. DNA data for the Dishpan Butte HMA, WY herd.

Genetic Analysis of the

Muskrat Basin HMA, WY

E. Gus Cothran

August 16, 2013

Department of Veterinary Integrative Bioscience Texas A&M University College Station, TX 77843-4458 The following is a report of the genetic analysis of the Muskrat Basin HMA, WY.

A few general comments about the genetic variability analysis based upon DNA microsatellites compared to blood typing. The DNA systems are more variable than blood typing systems, thus variation levels will be higher. Variation at microsatellite loci is strongly influenced by allelic diversity and changes in variation will be seen in allelic measures more quickly that at heterozygosity, which is why more allelic diversity measures are calculated. For mean values, there are a greater proportion of rare domestic breeds included in the estimates than for blood typing so relative values for the measures are lower compared to the feral horse values. As well, feral values are relatively higher because the majority of herds tested are of mixed ancestry which results in a relatively greater increase in heterozygosity values based upon the microsatellite data. There are no specific variants related to breed type so similarity is based upon the total data set.

METHODS

A total of 77 samples were received by Texas A&M University, Equine Genetics Lab on January 15, 2013. DNA was extracted from the samples and tested for variation at 12 equine microsatellite (mSat) systems. These were *AHT4*, *AHT5 ASB2*, *ASB17*, *ASB23*, *HMS3*, *HMS6*, *HMS7*, *HTG4*, *HTG10*, *LEX33*, and *VHL20*. These systems were tested using an automated DNA sequencer to separate Polymerase Chain Reaction (PCR) products.

A variety of genetic variability measures were calculated from the gene marker data. The measures were observed heterozygosity (*Ho*) which is the actual number of loci heterozygous per individual; expected heterozygosity (*He*), which is the predicted number of heterozygous loci based upon gene frequencies; effective number of alleles (*Ae*) which is a measure of marker system diversity; total number of variants (*TNV*); mean number of alleles per locus (*MNA*); the number of rare alleles observed which are alleles that occur with a frequency of 0.05 or less (*RA*); the percent of rare alleles (*%RA*); and estimated inbreeding level (*Fis*) which is calculated as 1-*Ho*/*He*.

Genetic markers also can provide information about ancestry in some cases. Genetic resemblance to domestic horse breeds was calculated using Rogers' genetic similarity coefficient, *S.* This resemblance was summarized by use of a restricted maximum likelihood (RML) procedure.

RESULTS AND DISCUSSION

Variants present and allele frequencies are given in Table 1. No variants were observed which have not been seen in horse breeds. Table 2 gives the values for the genetic variability measures of the Muskrat Basin HMA herd. Also shown in Table 2 are values from a representative group of domestic horse breeds. The breeds were selected to cover the range of variability measures in domestic horse populations. Mean values for feral herds (based upon data from 126 herds) and mean values for domestic breeds (based upon 80 domestic horse populations) also are shown.

Mean genetic similarity of the Muskrat Basin HMA herd to domestic horse breed types are shown in Table 3. A dendrogram of relationship of the Muskrat Basin HMA herd to a standard set of domestic breeds is shown in Figure 1.

Genetic Variants: A total of 81 variants were seen in the Muskrat Basin HMA herd which is well above the mean for feral herds and slightly above the mean for domestic breeds. Of these, 18 had frequencies below 0.05 which is the average percentage of variants at risk of future loss. Allelic diversity as represented by *Ae* is somewhat higher than the average for feral herds as is MNA.

Genetic Variation: Both observed heterozygosity and expected heterozygosity in the Muskrat Basin HMA herd were well above the feral mean. *He* is not significantly higher than *Ho*. This suggests the herd is in genetic equilibrium.

Genetic Similarity: Overall similarity of the Muskrat Basin HMA herd to domestic breeds was about average for feral herds. Highest mean genetic similarity of the Muskrat Basin HMA herd was with Light Racing and Riding followed by Old World Iberian breeds North American Gaited breeds with the same value of *S*. As seen in Fig. 1, the Muskrat Basin HMA herd clusters with the same group of horse breeds that it had the greatest similarity to. This likely means that the Throughbred or Quarter Horse figured in the herd's ancestry. As with most trees involving feral herds, the tree is somewhat distorted.

SUMMARY

Genetic variability of this herd in general is on the high side. TThis herd was previously tested in 2004. Current levels of variability for all measures except risk of loss are higher than in 2004. There is a possibility that this herd has seen recent gene flow from another population. Genetic similarity results suggest a herd with mixed ancestry with a strong indication of genes from the Thoroughbred contributing to the ancestry.

RECOMMENDATIONS

Current variability levels are high enough that no action is needed at this point. The possibility of immigration into the herd exists and if true, this will help maintain variability levels.

VHL20															
I	J	к	L	м	Ν	0	Р	Q	R	S					
0.110	0.000	0.000	0.143	0.227	0.248	0.104	0.162	0.000	0.006	0.000					
HTG4															
I	J	к	L	м	N	0	Р	Q	R						
0.000	0.000	0.201	0.013	0.494	0.000	0.273	0.019	0.000	0.000						
AHT4															
н	I	J	к	L	м	Ν	0	Р	Q	R					
0.006	0.000	0.331	0.143	0.039	0.065	0.039	0.351	0.026	0.000	0.000					
HMS7															
I	J	к	L	м	Ν	0	Р	Q	R						
0.000	0.130	0.000	0.318	0.013	0.357	0.156	0.000	0.026	0.000						
AHT5															
I	J	к	L	м	Ν	0	Р	Q	R						
0.058	0.195	0.078	0.045	0.188	0.123	0.313	0.000	0.000	0.000						
HMS6															
I	J	к	L	м	Ν	0	Р	Q	R						
0.000	0.000	0.071	0.188	0.325	0.000	0.169	0.247	0.000	0.000						
ASB2															
В	I	J	К	L	М	Ν	0	Р	Q	R					
0.000	0.000	0.000	0.149	0.000	0.071	0.403	0.195	0.000	0.091	0.091					
HTG10															
н	I	J	к	L	м	Ν	0	Р	Q	R	S	т			
0.000	0.091	0.000	0.058	0.019	0.065	0.110	0.398	0.006	0.039	0.214	0.000	0.000			
HMS3															
н	Т	J	К	L	М	Ν	0	Р	Q	R	S				
0.000	0.000	0.000	0.000	0.000	0.273	0.396	0.156	0.071	0.104	0.000	0.000				
ASB17															
D	F	G	н	I	J	К	L	М	Ν	0	Р	Q	R	S	Т
0.000	0.065	0.007	0.000	0.092	0.000	0.000	0.000	0.190	0.131	0.298	0.118	0.007	0.092	0.000	0.000
ASB23															
G	н	I	J	к	L	м	Ν	Ο	Р	Q	R	S	т	U	v
0.000	0.000	0.195	0.045	0.240	0.143	0.026	0.000	0.000	0.000	0.000	0.000	0.162	0.065	0.124	0.000
LEX33															
F	G	К	L	м	Ν	0	Р	Q	R	S	т				
0.000	0.000	0.143	0.143	0.045	0.000	0.071	0.000	0.293	0.305	0.000	0.000				

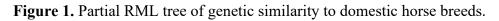
 Table 1. Allele frequencies of genetic variants observed in Muskrat Basin HMA feral horse herd.

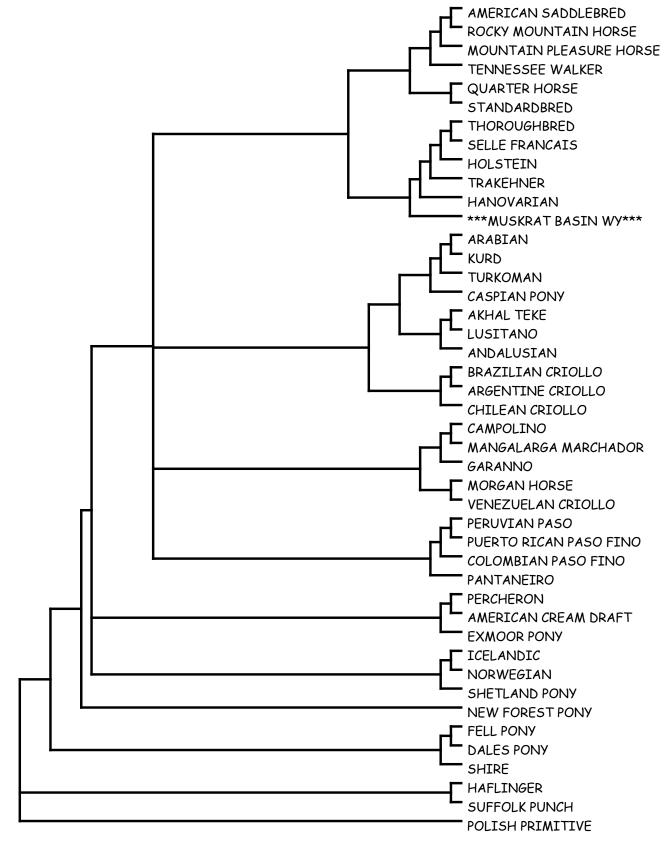
 Table 2. Genetic variability measures.

	N	Но	He	Fis	Ae	TNV	MNA	Ra	%Ra
MUSKRAT BASIN WY	77	0.759	0.765	0.008	4.440	81	6.75	18	0.222
Cleveland Bay	47	0.610	0.627	0.027	2.934	59	4.92	16	0.271
American Saddlebred	576	0.740	0.745	0.007	4.25	102	8.50	42	0.412
Andalusian	52	0.722	0.753	0.041	4.259	79	6.58	21	0.266
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Morgan Horse	64	0.715	0.746	0.041	4.192	92	7.67	33	0.359
Suffolk Punch	57	0.683	0.711	0.038	3.878	71	5.92	13	0.183
Tennessee Walker	60	0.666	0.693	0.038	3.662	87	7.25	34	0.391
Thoroughbred	1195	0.734	0.726	-0.011	3.918	69	5.75	18	0.261
Feral Horse Mean	126	0.716	0.710	-0.012	3.866	72.68	6.06	16.96	0.222
Standard Deviation		0.056	0.059	0.071	0.657	13.02	1.09	7.98	0.088
Minimum		0.496	0.489	-0.284	2.148	37	3.08	0	0
Maximum		0.815	0.798	0.133	5.253	96	8.00	33	0.400
Domestic Horse Mean	80	0.710	0.720	0.012	4.012	80.88	6.74	23.79	0.283
Standard Deviation		0.078	0.071	0.086	0.735	16.79	1.40	10.11	0.082
Minimum		0.347	0.394	-0.312	1.779	26	2.17	0	0
Maximum		0.822	0.799	0.211	5.30	119	9.92	55	0.462

Table 3. Rogers' genetic similarity of the Muskrat Basin HMA feral horse herd to major groups of domestic horses.

	Mean S	Std	Minimum	Maximum
Light Racing and Riding Breeds	0.771	0.029	0.730	0.813
Oriental and Arabian Breeds	0.738	0.033	0.699	0.790
Old World Iberian Breeds	0.748	0.025	0.718	0.788
New World Iberian Breeds	0.720	0.017	0.688	0.740
North American Gaited Breeds	0.748	0.022	0.711	0.769
Heavy Draft Breeds	0.702	0.047	0.651	0.782
True Pony Breeds	0.717	0.031	0.671	0.752





vht	oena				-								<u> </u>
AID				HMS7				HTG10					
3330	IM	MM	KN	JN	NO	MP	MR	10	00	10		RR	LM
3331	MN	MP	JO	NN	JL	MP	OR	00	NO	IO	IK	QQ	MM
3332	OR	KM	HO	NN	JO	PP	KK	NR	MM	NN	IK	QR	MM
3333 3334	MM NP	MO KK	1N JO	NO JJ	JN JM	M0 MM	N0 00	OP LO	NO NN	NO IM	LL IL	QR KM	MN MM
3335	IM	MM	NO		LO	MM	ко	KQ	NQ	NR	JL	QR	HL
3336	IP	MO	KK	JQ NN	MO	LP	MN	IR	MO	IN	II	LR	
3337	IM	KM	LN	LL	JO	KL	RR	KO	MN	OR	ST	RR	
			NP										
3338	NN	00		LL	MO	MM	OQ	0Q	NO	IM	KK	КО	HP
3339	MP	KL	KP	NN	NO	LM	NN	IR	MM	MM	KU	LL	FL
3340	NO	MO	KO	LN	MO	PP	NR	QR	MN	IP	IK	LL	OP
3341	LL	KM	JK	JN	KK	MO	KN	NN	MQ	NO	MS	QR	PP
3342	MN	MO	00	NO	IN	MP	NO	MR	MN	FF	KK	LQ	LM
3343	NP	MO	JO	NQ	11	LM	MN	00	NQ	00	JK	MQ	HP
3344	MM	MM	JO	LN	11	LO	NO	OR	NP	OR	KL	RR	FN
3345	NP	KM	JK	LO	MN	OP	NQ	NR	NO	MM	KS	QQ	LM
3346	NP	LM	JK	NO	MO	LP	KR	IR	NP	MQ	KT	QR	MM
3347	MP	MM	JO	LN	NO	00	NN	RR	NO	NO	IJ	QQ	NP
3348	MM	MO	КО	NN	JN	MP	NO	OR	NQ	FO	IS	LR	LL
3349	LM	ко	KK	NN	LN	MO	NQ	IR	MN	FR	IT	KQ	FF
3350	MN	MO	00	NO	MO	LP	NO	OR	NQ	OP	TU	KQ	LM
3351	LP	КM	00	JN	MO	LO	NQ	LO	MN	PR	LS	LR	LM
3352	MP	MM	JO	LN	MN	LP	OQ	IM	NN	FF	SS	LR	LM
3353	LO	КM	00	JL	IJ	MP	NN	NO	NP	IM	KK	LR	NP
3354	PP	MO	LM	JL	NO	KL	NN	IK	MN	PP	LS	OQ	LM
3355	00	MO	JK	LO	JO	OP	KN	KN	NO	MM	LL	OR	LP
3356	IM	00	JO	NO	KN	LM	OQ	00	00	NO	IK	QR	NP
3357	IM	MM	11	LO	IM	MP	KK	KO	OP	NO	IL	QR	MP
3358	MN	MO	JL	LL	IN	MM	NO	OR	NP	OR	IK	QR	NP
3359	IM	MM]]	LL	MO	OP	NQ	00	MP	00	KU	LQ	HL
3360	NO	КО]]	LN	Ш	MP	MN	КО	MO	MO	IS	KK	LL
3361	NP	MO	JK	JO	JM	MO	MO	OR	MN	MO	IL	KR	LM
3362	IN	MO	KM	NO	JM	OP	KM	00	MP	MO	KL	KR	FL
3363	LM	MP	11	LL	MO	LP	KQ	MR	MN	FO	IS	QR	FM
3364	MM	КО	JM	LL	00	MM	NO	KМ	NQ	IR	TU	KR	LP
3365	IN	MO	JO	LN	КО	MP	NO	OR	NN	OP	SU	KL	MM
3366	OP	MO	JO	LN	LL	PP	KN	KN	MQ	IN	KS	00	IM
3367	LN	MO	КО	MO	MO	KP	KN	10	NO	MP	KS	КQ	LM
3368	NN	MO	LO	JN	KL	LP	KK	NO	OQ	G	IK	QR	MO
3369	LM	MO	MO	LO	КM	KL	NO	00	00	NO	IU	QR	LL
3370	NO	MO	LO	NN	JO	LP	NQ	00	MN	MM	KS	KL	MO
3371	NN	KM	JO	NN	MO	MP	NR	IR	NN	MO	IU	KL	HM
3372	IL	MM	JK	NO	10	LO	NN	OR	MQ	IR	TU	OQ	FL
3373	NP	00	JO	LO	MO	MO	КM	IM	NO	PR	SU	QR	IN
3374	LM	MM	JO	LL	11	OP	NO	NO	NP	MO	LU	LR	NP
3375	00	KM	00	JL	IJ	MO	NO	NQ	MP	MM	KK	LR	MP
3376	MP	MM	JM	LO	00	00	NN	MR	NN	NN	IJ	OR	HN
3377	NP	KM	11	10	JO	LM	NN	OR	NN	00	LL	KM	LP
3378	NO	MO	JO	LM	ко	MP	NQ	10	PQ	OP	IL	ко	FM
3379	LN	KM	10	JL	00	MP	NR	NR	MN	PP	IM	QR	LM
3380	LP	КК	MM	NN	MO	KM	NN	MO	NO	00	IS	QR	HM
3381	MN	MO	JO	NO	NN	KP	OR	MO	NN	FM	KL	QQ	IM
3382	NN	MO	00	NN	00	LO	QR	QR	MN	MN	KK	LQ	NO
3383	MP	MO	JO	LL	NN	KP	NO	MO	MN	FM	SS	LR	MM
3384	LN	MO	10	LO	JO	MM	NQ	00	NN	IR	IK	QQ	LN
3385	IN	MO	11	NO	00	00	OR	LO	OQ	MN	JS	QR	IL
3386	NN	00	11	NQ	11	LO	MN	OR	MO	OR	JK	MQ	LP
3387	MP	MO	10	LN	MO	MO	NQ	OR	NN	NP	KU	QR	MN
3388	IL	MM	KO	NO	10	LL	KN	OR	MQ	IR	TU	OR	FM
3389	LO	KO	OP	LN	IM	KP	MN	IQ	MM	MO	КК	LM	MM
3390	MN	MO	JO	0Q	11/1	LO	MM	00	MQ	OR	KL	KM	HL
3390 3391	NO	MP	OP	JL	MN	MP	OR	NO	NN	MO	IT	LO	IL
	LL		JO	LN		MP							LP
3392 3393		KO			MO		KK	NR	MQ	NO	IM	QR	
	IM	KO	00	LL	MM	MM	NN	MN	MQ	OP	SU	KK	LM LL
394	LM	MM	MO	JN	KM	KL	NO	00	NO	OP	TU	QR	
3395	LO	MM	00	JL	11	MP	OQ	NO	NP	MR	KU	QR	LP
3396	PP	KK	MO	JN	KO	MM	KN	00	MM	00	LS	KM	LM
3397	LN	KM	KK	JL 	MO	LO	NN	NR	NN	IP	IU	QR	LM
3398	IP	MM	JL	JL	00	KL	NO	KO	MN	OP	SS	QR	FL
3399	MP	MM	NO	LN	JN	LP	NN	00	MN	FO	SU	RR	LM
3400	MO	КК	NO	JO	JM	KM	NO	NO	MM	00	LU	КК	IL
3401	11	MO	JO	NN	КО	OP	KR	00	NQ	NO	IJ	QR	IP
	NO	KM	JK	NN	КM	MM	KR	00	MN	PP	КM	RR	HМ
		ĸм	JM	LO	LO	LM	ко	IR	MO	NN	KL	QR	MM
3402 3403	NN					n 4n 4	NO	IR	MO	MO	KL	LL	FM
	MP	КО	JO	LN	NO	MM	NO	IN	1410		INE.	LL	1 101
403		KO KM	10 10	LN LN	KO	LM	KN	OR	MN	MO	IS	KQ	FF

Appendix 1. DNA data for the Muskrat Basin HMA, WY herd.

Appendix G – Standard Operating Procedures

Wild Horse Gathers

Gathers are conducted by utilizing contractors from the Wild Horse Gathers-Western States Contract or BLM personnel. The following standard operating procedures (SOPs) for gathering and handling wild horses apply whether a contractor or BLM personnel conduct a gather. For helicopter gathers conducted by BLM personnel, gather operations would be conducted in conformance with the *Wild Horse Aviation Management Handbook* (January 2009).

Prior to any gathering operation, the BLM would provide for a pre-gather evaluation of existing conditions in the gather area(s). The evaluation would include animal conditions, prevailing temperatures, drought conditions, soil conditions, road conditions, and a topographic map with WSA boundaries, the location of fences, other physical barriers, and acceptable gather locations in relation to animal distribution. The evaluation would determine whether the proposed activities would necessitate the presence of a veterinarian during operations. If it is determined that a large number of animals may need to be euthanized or gather operations could be facilitated by a veterinarian, these services would be arranged before the gather would proceed. The contractor would be apprised of all conditions and would be given instructions regarding the gather and handling of animals to ensure their health and welfare is protected.

Gather sites and temporary holding sites would be located to reduce the likelihood of injury and stress to the animals, and to minimize potential damage to the natural resources of the area. These sites would be located on or near existing roads whenever possible.

The primary gather methods used in the performance of gather operations include:

- 1. Helicopter Drive Gathering. This gather method involves utilizing a helicopter to herd wild horses into a temporary gather site.
- 2. Helicopter Assisted Roping. This gather method involves utilizing a helicopter to herd wild horses to ropers.
- 3. Bait Trapping. This gather method involves utilizing bait (e.g., water or feed) to lure wild horses into a temporary gather site.

The following procedures and stipulations would be followed to ensure the welfare, safety and humane treatment of wild horses in accordance with the provisions of 43 CFR 4700.

A. Gather Methods used in the Performance of Gather Contract Operations

The primary concern of the contractor is the safe and humane handling of all animals gathered. All gather attempts shall incorporate the following:

- All gather sites and holding facilities locations must be approved by the Contracting Officer's Representative (COR) and/or the Project Inspector (PI) prior to construction. The Contractor may also be required to change or move gather locations as determined by the COR/PI. All gather sites and holding facilities not located on public land must have prior written approval of the landowner.
- 2. The rate of movement and distance the animals travel shall not exceed limitations set by the COR who would consider terrain, physical barriers, access limitations, weather, extreme temperature (high and low), condition of the animals, urgency of the operation (animals facing drought, starvation, fire rehabilitation, etc.) and other factors. In consultation with the contractor

the distance the animals travel would account for the different factors listed above and concerns with each HMA.

- 3. All gather sites, wings, and holding facilities shall be constructed, maintained and operated to handle the animals in a safe and humane manner and be in accordance with the following:
 - a. Gather sites and holding facilities shall be constructed of portable panels, the top of which shall not be less than 72 inches high for horses and 60 inches high for burros, and the bottom rail of which shall not be more than 12 inches from ground level. All gather sites and holding facilities shall be oval or round in design.
 - b. All loading chute sides shall be a minimum of 6 feet high and shall be fully covered with plywood or metal without holes.
 - c. All runways shall be a minimum of 30 feet long and a minimum of 6 feet high for horses, and 5 feet high for burros, and shall be covered with plywood, burlap, plastic snow fence or like material a minimum of 1 foot to 5 feet above ground level for burros and 1 foot to 6 feet for horses. The location of the government furnished portable fly chute to restrain, age, or provide additional care for the animals shall be placed in the runway in a manner as instructed by or in concurrence with the COR/PI.
 - d. All crowding pens including the gates leading to the runways shall be covered with a material which prevents the animals from seeing out (plywood, burlap, plastic snow fence, etc.) and shall be covered a minimum of 1 foot to 5 feet above ground level for burros and 2 feet to 6 feet for horses.
 - e. All pens and runways used for the movement and handling of animals shall be connected with hinged self-locking gates.
- 4. No modification of existing fences would be made without authorization from the COR/PI. The Contractor shall be responsible for restoration of any fence modification which he has made.
- 5. When dust conditions occur within or adjacent to the gather site or holding facility, the Contractor shall be required to wet down the ground with water.
- 6. Alternate pens, within the holding facility shall be furnished by the Contractor to separate mares or jennies with small foals, sick and injured animals, estrays, or other animals the COR determines need to be housed in a separate pen from the other animals. Animals shall be sorted as to age, number, size, temperament, sex, and condition when in the holding facility so as to minimize, to the extent possible, injury due to fighting and trampling. Under normal conditions, the government would require that animals be restrained for the purpose of determining an animal's age, sex, or other necessary procedures. In these instances, a portable restraining chute may be necessary and would be provided by the government. Alternate pens shall be furnished by the Contractor to hold animals if the specific gathering requires that animals be released back into the gather area(s). In areas requiring one or more satellite gather site, and where a centralized holding facility is utilized, the contractor may be required to provide additional holding pens to segregate animals transported from remote locations so they may be returned to their traditional ranges. Either segregation or temporary marking and later segregation would be at the discretion of the COR.
- 7. The Contractor shall provide animals held in the gather sites and/or holding facilities with a continuous supply of fresh clean water at a minimum rate of 10 gallons per animal per day. Animals held for 10 hours or more in the gather site or holding facilities shall be provided good quality hay at the rate of not less than two pounds of hay per 100 pounds of estimated body

weight per day. The contractor would supply certified weed free hay if required by State, County, and Federal regulation.

- 8. An animal that is held at a temporary holding facility through the night is defined as a horse/burro feed day. An animal that is held for only a portion of a day and is shipped or released does not constitute a feed day.
- 9. It is the responsibility of the Contractor to provide security to prevent loss, injury or death of gathered animals until delivery to final destination.
- 10. The Contractor shall restrain sick or injured animals if treatment is necessary. The COR/PI would determine if animals must be euthanized and provide for the destruction of such animals. The Contractor may be required to humanely euthanize animals in the field and to dispose of the carcasses as directed by the COR/PI.
- 11. Animals shall be transported to their final destination from temporary holding facilities as quickly as possible after gather unless prior approval is granted by the COR for unusual circumstances. Animals to be released back into the HMA following gather operations may be held up to 21 days or as directed by the COR. Animals shall not be held in gather sites and/or temporary holding facilities on days when there is no work being conducted except as specified by the COR. The Contractor shall schedule shipments of animals to arrive at final destination between 7:00 a.m. and 4:00 p.m. No shipments shall be scheduled to arrive at final destination on Sunday and Federal holidays; unless prior approval has been obtained by the COR. Animals shall not be allowed to remain standing on trucks while not in transport for a combined period of greater than three (3) hours in any 24 hour period. Animals that are to be released back into the gather area may need to be transported back to the original gather site. This determination would be at the discretion of the COR or Field Office Wild Horse & Burro Specialist.

B. Gather Methods That May Be Used in the Performance of a Gather

- 1. Gather attempts may be accomplished by utilizing bait (feed, water, mineral licks) to lure animals into a temporary gather site. If this gather method is selected, the following applies:
 - a. Finger gates shall not be constructed of materials such as "T" posts, sharpened willows, etc., that may be injurious to animals.
 - b. All trigger and/or trip gate devices must be approved by the COR/PI prior to gather of animals.
 - c. Gather sites shall be checked a minimum of once every 10 hours.
- 2. Gather attempts may be accomplished by utilizing a helicopter to drive animals into a temporary gather site. If the contractor selects this method the following applies:
 - a. A minimum of two saddle-horses shall be immediately available at the gather site to accomplish roping if necessary. Roping shall be done as determined by the COR/PI. Under no circumstances shall animals be tied down for more than one-half hour.
 - b. The contractor shall assure that foals shall not be left behind, and orphaned.
- 3. Gather attempts may be accomplished by utilizing a helicopter to drive animals to ropers. If the contractor, with the approval of the COR/PI, selects this method the following applies:
 - a. Under no circumstances shall animals be tied down for more than one hour.
 - b. The contractor shall assure that foals shall not be left behind, or orphaned.

c. The rate of movement and distance the animals travel shall not exceed limitations set by the COR/PI who would consider terrain, physical barriers, weather, condition of the animals and other factors.

C. Use of Motorized Equipment

- 1. All motorized equipment employed in the transportation of gathered animals shall be in compliance with appropriate State and Federal laws and regulations applicable to the humane transportation of animals. The Contractor shall provide the COR/PI, if requested, with a current safety inspection (less than one year old) for all motorized equipment and tractor-trailers used to transport animals to final destination.
- 2. All motorized equipment, tractor-trailers, and stock trailers shall be in good repair, of adequate rated capacity, and operated so as to ensure that gathered animals are transported without undue risk or injury.
- 3. Only tractor-trailers or stock trailers with a covered top shall be allowed for transporting animals from gather site(s) to temporary holding facilities, and from temporary holding facilities to final destination(s). Sides or stock racks of all trailers used for transporting animals shall be a minimum height of 6 feet 6 inches from the floor. Single deck tractor-trailers 40 feet or longer shall have at least two (2) partition gates providing at least three (3) compartments within the trailer to separate animals. Tractor-trailers less than 40 feet shall have at least one partition gate providing at least two (2) compartments within the trailer to separate the animals. Compartments in all tractor-trailers shall be of equal size plus or minus 10 percent. Each partition shall be a minimum of 6 feet high and shall have a minimum 5-foot-wide swinging gate. The use of double deck tractor-trailers is unacceptable and shall not be allowed.
- 4. All tractor-trailers used to transport animals to final destination(s) shall be equipped with at least one (1) door at the rear end of the trailer which is capable of sliding either horizontally or vertically. The rear door(s) of tractor-trailers and stock trailers must be capable of opening the full width of the trailer. Panels facing the inside of all trailers must be free of sharp edges or holes that could cause injury to the animals. The material facing the inside of all trailers must be strong enough so that the animals cannot push their hooves through the side. Final approval of tractor-trailers used to transport animals shall be held by the COR/PI.
- 5. Floors of tractor-trailers, stock trailers and loading chutes shall be covered and maintained with wood shavings to prevent the animals from slipping as much as possible during transport.
- 6. Animals to be loaded and transported in any trailer shall be as directed by the COR/PI and may include limitations on numbers according to age, size, sex, temperament and animal condition. The following minimum square feet per animal shall be allowed in all trailers:
 - 11 square feet per adult horse (1.4 linear foot in an 8 foot wide trailer);
 - 8 square feet per adult burro (1.0 linear foot in an 8 foot wide trailer);
 - 6 square feet per horse foal (0.75 linear feet in an 8-foot-wide trailer);
 - 4 square feet per burro foal (0.5 linear feet in an 8-foot-wide trailer).
- 7. The COR/PI shall consider the condition and size of the animals, weather conditions, distance to be transported, or other factors when planning for the movement of gathered animals. The COR/PI shall provide for any brand and/or inspection services required for the gathered animals.

8. If the COR/PI determines that dust conditions are such that the animals could be endangered during transportation, the Contractor would be instructed to adjust speed.

D. Safety and Communications

- 1. The Contractor shall have the means to communicate with the COR/PI and all contractor personnel engaged in the gather of wild horses utilizing a VHF/FM Transceiver or VHF/FM portable Two-Way radio. If communications are ineffective the government would take steps necessary to protect the welfare of the animals.
- 2. The proper operation, service and maintenance of all contractor furnished property is the responsibility of the Contractor. The BLM reserves the right to remove from service any contractor personnel or contractor furnished equipment which, in the opinion of the contracting officer or COR/PI violate contract rules, are unsafe or otherwise unsatisfactory. In this event, the Contractor would be notified in writing to furnish replacement personnel or equipment within 48 hours of notification. All such replacements must be approved in advance of operation by the Contracting Officer or his/her representative.
- 3. The Contractor shall obtain the necessary FCC licenses for the radio system.
- 4. All accidents occurring during the performance of any task order shall be immediately reported to the COR/PI.
- 5. Should the contractor choose to utilize a helicopter the following would apply:
 - 1. The Contractor must operate in compliance with Federal Aviation Regulations, Part 91. Pilots provided by the Contractor shall comply with the Contractor's Federal Aviation Certificates, applicable regulations of the State in which the gather is located.
 - 2. Fueling operations shall not take place within 1,000 feet of animals.

E. Site Clearances

- 1. No Personnel working at gather sites may excavate, remove, damage, or otherwise alter or deface or attempt to excavate, remove, damage or otherwise alter or deface any archaeological resource located on public lands or Indian lands.
- Prior to setting up a gather site or temporary holding facility, the BLM would conduct all necessary clearances (archaeological, T&E, etc.). All proposed site(s) must be approved by a government archaeologist and wildlife biologist. Once clearance has been obtained, the gather site or temporary holding facility may be set up. Said clearance shall be arranged for by the COR, PI, or other BLM employees.
- 3. Gather sites and temporary holding facilities would not be constructed on wetlands or riparian zones.

F. Animal Characteristics and Behavior

Releases of wild horses would be near available water when possible. If the area is new to them, a short-term adjustment period may be required while the wild horses become familiar with the new area.

G. Public Participation

Opportunities for public viewing (i.e. media, interested public) of gather operations would be made available to the extent possible; however, the primary considerations would be to protect the health, safety and welfare of the animals being gathered and the personnel involved. The public must adhere to guidance from the on-site BLM representative. It is BLM policy that the public would not be allowed to

come into direct contact with wild horses being held in BLM facilities. Only authorized BLM personnel or contractors may enter the corrals or directly handle the animals. The general public may not enter the corrals or directly handle the animals at any time or for any reason during BLM operations.

H. Responsibility and Lines of Communication

- Lander Field Office Contracting Officer's Representative/Project Inspector: Clay Stott
- Alternate Contracting Officer's Representative/Project Inspector:
 - Patricia Hatle June Wendlandt Benjamin Smith
 - Jay D'Ewart
- Wyoming State Office Contracting Officer's Representative/Project Inspector: N/A

The Contracting Officer's Representatives (CORs) and the project inspectors (PIs) have the direct responsibility to ensure the Contractor's compliance with the contract stipulations. The Lander Assistant Field Managers for Renewable Resources and the Field Manager will take an active role to ensure the appropriate lines of communication are established between the field, Field Office, District Office, State Office, National Program Office, and BLM Holding Facility offices. All employees involved in the gathering operations would keep the best interests of the animals at the forefront at all times.

All publicity, formal public contact and inquiries would be handled through the Assistant Field Manager for Renewable Resources and District Public Affairs Officer. These individuals would be the primary contact and would coordinate with the COR/PI on any inquiries.

The COR would coordinate with the contractor and the BLM Corrals to ensure animals are being transported from the gather site in a safe and humane manner and are arriving in good condition.

The contract specifications require humane treatment and care of the animals during removal operations. These specifications are designed to minimize the risk of injury and death during and after gather of the animals. The specifications would be vigorously enforced.

Should the Contractor show negligence and/or not perform according to contract stipulations, he would be issued written instructions, stop work orders, or defaulted.

Fertility Control Treatments

Standard operating procedures for use of fertility control vaccines, and insertion of Y-shaped silicone lud for feral horses

SOPs common to all vaccine types:

Animal Identification

Animals intended for treatment must be clearly, individually identifiable to allow for positive identification during subsequent management activities. For captured animals, marking for identification may be accomplished by marking each individual with a freeze mark on the hip or neck and a microchip in the nuchal ligament. In some cases, identification may be accomplished based by cataloguing markings that make animals uniquely identifiable. Such animals may be photographed using a telephoto lens and high quality digital camera as a record of treated individuals.

Safety

Safety for both humans and animals is the primary consideration in all elements of fertility control vaccine use. Administration of any vaccine must follow all safety guidance and label guidelines on applicable EPA labeling.

Injection Site

For hand-injection, delivery of the vaccine should be by intramuscular injection, while the animal is standing still, into the left or right side, above the imaginary line that connects the point of the hip (hook bone) and the point of the buttocks (pin bone): this is the hip / upper gluteal area. For dart-based injection, delivery of the vaccine should be by intramuscular injection, while the animal is standing still, into the left or right areas (lower gluteal / biceps femoralis).

Monitoring and Tracking of Treatments

Estimation of population size and growth rates (in most cases, using aerial surveys) should be conducted periodically after treatments.

Population growth rates of some herds selected for intensive monitoring may be estimated every year post-treatment using aerial surveys. If, during routine HMA field monitoring (on-the-ground), data describing adult to foal ratios can be collected, these data should also be shared with HQ-261.

Field applicators should record all pertinent data relating to identification of treated animals (including photographs if animals are not freeze-marked) and date of treatment, lot number(s) of the vaccine, quantity of vaccine issued, the quantity used, the date of vaccination, disposition of any unused vaccine, the date disposed, the number of treated mares by HMA, field office, and State along with the microchip numbers and freeze-mark(s) applied by HMA and date. A summary narrative and data sheets will be forwarded to HQ-261 annually (Reno, Nevada). A copy of the form and data sheets and any photos taken should be maintained at the field office.

HQ-261 will maintain records sent from field offices, on the quantity of PZP issued, the quantity used, disposition of any unused PZP, the number of treated mares by HMA, field office, and State along with the freeze-mark(s) applied by HMA and date.

SOPs for GonaCon-Equine Vaccine Treatments

GonaCon-Equine vaccine (USDA Pocatello Storage Depot, Pocatello, ID; Spay First!, Inc., Oklahoma City, OK) is distributed as preloaded doses (2 mL) in labeled syringes. Upon receipt, the vaccine should be kept refrigerated (4° C) until use. <u>Do not freeze GonaCon-Equine</u>. The vaccine has a 6-month shelf-life from the time of production and the expiration date will be noted on each syringe that is provided.

For initial and booster treatments, mares would ideally receive 2.0 ml of GonaCon-Equine.

Administering GonaCon-Equine Vaccine by Hand-Injection

Experience has demonstrated that only 1.8 ml of vaccine can typically be loaded into 2 cc syringes and darts, and this dose has proven successful. Calculations below reflect a 1.8 ml dose.

For hand-injection, delivery of the vaccine should be by intramuscular injection, while the animal is standing still, into the left or right side, above the imaginary line that connects the point of the hip (hook bone) and the point of the buttocks (pin bone): this is the hip / upper gluteal area.

A booster vaccine may be administered after the first injection to improve efficacy of the product over subsequent years.

Application of GonaCon-Equine via Darting

General practice guidelines for darting operations, as noted above for dart-delivery of ZonaStat-H, should be followed for dart-delivery of GonaCon-Equine.

Wearing latex gloves, the applicator numbers darts, and loads numbered darts with vaccine by attaching a loading needle (7.62 cm; provided by dart manufacturer) to the syringe containing vaccine and placing the needle into the cannula of the dart to the fullest depth possible. Slowly depress the syringe plunger and begin filling the dart. Periodically, tap the dart on a hard surface to dislodge air bubbles trapped within the vaccine. Due to the viscous nature of the fluid, air entrapment typically results in a maximum of approximately 1.8 ml of vaccine being loaded in the dart. The dart is filled to max once a small amount of the vaccine can be seen at the tri-ports.

Important! Do not load and refrigerate darts the night before application. When exposed to moisture and condensation, the edges of gel barbs soften, begin to dissolve, and will not hold the dart in the muscle tissue long enough for full injection of the vaccine. The dart needs to remain in the muscle tissue for a minimum of 1 minute to achieve dependable full injection. Sharp gel barbs are critical.

Darts should be weighed to the nearest hundredth gram by electronic scale when empty, when loaded with vaccine, and after discharge, to ensure that 90% (1.62 ml) of the vaccine has been injected. GonaCon-Equine weighs 0.95 grams/mL, so animals should receive 1.54 grams of vaccine to be considered treated. Animals receiving <50% should be darted with another full dose; those receiving <50% but <90% should receive a half dose (1 ml). All darts should be weighed to verify a combination of ≥1.62 ml has been administered. Therefore, every effort should be made to recover darts after they have fallen from animals.

Although infrequent, dart injections can result in partial injections of the vaccine, and shots are missed. As a precaution, it is recommended that extra doses of the vaccine be ordered to accommodate failed delivery (which may be as high as ~15 %). To determine the amount of vaccine delivered, the dart must be weighed before loading, and before and after delivery in the field. The scale should be sensitive to 0.01 grams or less, and accurate to 0.05 g or less.

For best results, darts with a gel barb should be used. (i.e. 2 cc Pneu-Dart brand darts configured with Slow-inject technology, 3.81 cm long 14 ga.tri-port needles, and gel collars positioned 1.27 cm ahead of the ferrule). One can expect updates in optimal dart configuration, pending results of research and field applications.

Darts (configured specifically as described above) can be loaded in the field and stored in a cooler prior to application. Darts loaded, but not used can be maintained in dry conditions at about 4° C and used the next day, but do not store in any refrigerator or container likely to cause condensation, which can compromise the gel barbs.

SOPs for one-year liquid PZP vaccine (ZonaStat-H)

ZonaStat-H vaccine (Science and Conservation Center, Billings, MT) would be administered through hand-injection or darting by trained BLM personnel or collaborating partners only. At present, the only PZP vaccine for dart-based delivery in BLM-managed wild horses or burros is ZonaStat-H. For any darting operation, the designated personnel must have successfully completed a nationally recognized wildlife darting course and who have documented and successful experience darting wildlife under field conditions.

Until the day of its use, ZonaStat-H must be kept frozen.

Animals that have never been treated with a PZP vaccine would receive 0.5 cc of PZP vaccine emulsified with 0.5 cc of Freund's Modified Adjuvant (FMA). Animals identified for re-treatment receive 0.5 cc of the PZP vaccine emulsified with 0.5 cc of Freund's Incomplete Adjuvant (FIA).

Hand-injection of liquid PZP vaccine would be by intramuscular injection into the gluteal muscles while the animal is restrained in a working chute. The vaccine would be injected into the left hind quarters of the animal, above the imaginary line that connects the point of the hip (hook bone) and the point of the buttocks (pin bone).

For Hand-injection, delivery of the vaccine would be by intramuscular injection into the left or right buttocks and thigh muscles (gluteals, biceps femoris) while the animal is standing still.

Application of ZonaStat-H via Darting

Only designated darters would prepare the emulsion. Vaccine-adjuvant emulsion would be loaded into darts at the darting site and delivered by means of a projector gun.

No attempt to dart should be taken when other persons are within a 100-m radius of the target animal. The Dan Inject gun should not be used at ranges in excess of 30 m while the Pneu-Dart gun should not be used over 50 m.

No attempts would be taken in high wind (greater than 15 mph) or when the animal is standing at an angle where the dart could miss the target area and hit the flank or rib cage. The ideal is when the dart would strike the skin of the animal at a 90° angle.

If a loaded dart is not used within two hours of the time of loading, the contents would be transferred to a new dart before attempting another animal. If the dart is not used before the end of the day, it would be stored under refrigeration and the contents transferred to another dart the next day, for a maximum of one transfer (discard contents if not used on the second day). Refrigerated darts would not be used in the field.

A darting team should include two people. The second person is responsible for locating fired darts. The second person should also be responsible for identifying the animal and keeping onlookers at a safe distance.

To the extent possible, all darting would be carried out in a discrete manner. However, if darting is to be done within view of non-participants or members of the public, an explanation of the nature of the project would be carried out either immediately before or after the darting.

Attempts will be made to recover all darts. To the extent possible, all darts which are discharged and drop from the target animal at the darting site would be recovered before another darting occurs. In exceptional situations, the site of a lost dart may be noted and marked, and recovery efforts made at a later time. All discharged darts would be examined after recovery in order to determine if the charge fired and the plunger fully expelled the vaccine. Personnel conducting darting operations should be equipped with a two-way radio or cell phone to provide a communications link with a project veterinarian for advice and/or assistance. In the event of a veterinary emergency, darting personnel would immediately contact the project veterinarian, providing all available information concerning the nature and location of the incident.

In the event that a dart strikes a bone or imbeds in soft tissue and does not dislodge, the darter would follow the affected animal until the dart falls out or the animal can no longer be found. The darter would be responsible for daily observation of the animal until the situation is resolved. eSOPs for application of PZP-22 pelleted vaccine:

PZP-22 pelleted vaccine treatment would be administered only by trained BLM personnel or designated partners.

A treatment of PZP-22 is comprised of two separate injections: (1) a liquid dose ee

Until the day of its use, the liquid portion of PZP-22 must be kept frozen.

At this time, delivery of PZP-22 treatment would only be by intramuscular injection into the gluteal muscles while the animal is restrained in a working chute. The primer would consist of 0.5 cc of liquid PZP emulsified with 0.5 cc of adjuvant. Animals that have never been treated with a PZP vaccine would receive 0.5 cc of PZP vaccine emulsified with 0.5 cc of Freund's Modified Adjuvant (FMA). Animals identified for re-treatment receive 0.5 cc of the PZP vaccine emulsified with 0.5 cc of Freund's Incomplete Adjuvant (FIA). The syringe with PZP vaccine pellets would be loaded into the jabstick for the second injection. With each injection, the liquid or pellets would be injected into the left hind quarters of the animal, above the imaginary line that connects the point of the hip (hook bone) and the point of the buttocks (pin bone).

In the future, the PZP-22 treatment may be administered remotely using an approved long range darting protocol and delivery system if and when BLM has determined that the technology has been proven safe and effective for use.

Insertion of Y-shaped Silicone IUD for Feral Horses

Background

Mares must be open. A veterinarian must determine pregnancy status via palpation or ultrasound. Ultrasound should be used as necessary to confirm open status of mares down to at least 14 days for those that have recently been with stallions. For mares segregated from stallions, this determination may be made at an earlier time when mares are identified as candidates for treatment, or immediately prior to IUD insertion. Pregnant mares should not receive an IUD. Preparation

IUDs must be clean and sterile. Sterilize IUDs with a low-temperature sterilization system, such as Sterrad.

The Introducer is two PVC pipes. The exterior pipe is a 29" length of ½" diameter pipe, sanded smooth at one end, then heat-treated to smooth its curvature further (Figure 1). The IUD will be placed into this smoothed end of the exterior pipe. The interior pipe is a 29 ½" long, ¼" riser tube (of the kind used to connect water lines to sinks), with one end slightly flared out to fit more snugly inside the exterior pipe (Figure 1), and a plastic stopper attached to the other end (Figure 2).



Figure 1. Interior and exterior pipes (unassembled), showing the ends that go into the mare



Figure 2. Interior pipe shown within exterior pipe. After the introducer is 4" beyond the os, the stopper is pushed forward (outside the mare), causing the IUD to be pushed out from the exterior pipe.

Introducers should be sterilized in Benz-all cold steriliant, or similar. Do not use iodine-based sterilant solution. A suitable container for sterilant can be a large diameter (i.e., 2") PVC pipe with one end sealed and one end removable.

Prepare the IUD: Lubricate with sterile veterinary lube, and insert into the introducer. The central stem of the IUD goes in first (Figure 3).



Figure 3. Insert the stem end of the IUD into the exterior pipe.

Fold the two 'legs' of the IUD, and push the IUD further into the introducer, until just the bulbous ends are showing (Figure 4).

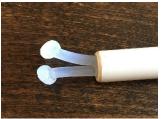


Figure 4. Insert the IUD until just the tips of the 'legs' are showing.

Restraint and Medication: The mare should be restrained in a padded squeeze chute to provide access to the rear end of the animal, but with a solid lower back door, or thick wood panel, for veterinarian safety.

Only a veterinarian shall oversee this procedure and insert IUDs. Some practitioners may choose to provide sedation. If so, when the mare's head starts to droop, it may be advisable to tie the tail up to

prevent risk of the animal sitting down on the veterinarian's arm (i.e., double half hitch, then tie tail to the bar above the animal).

Some practitioners may choose to provide a dose of long-acting progesterone to aid in IUD retention. Example dosage: 5mL of BioRelease LA Progesterone 300 mg/mL (BET labs, Lexington KY), or longacting Altrenogest). No other intrauterine treatments of any kind should be administered at the time of IUD insertion.

Insertion Procedure:

- Prep clean the perineal area.
- Lubricate the veterinarian's sleeved arm and the Introducer+IUD.
- Carry the introducer (IUD-end-first) into the vagina.
- Dilate the cervix and gently move the tip of the introducer past the cervix.
- Advance the end of the 1/2" PVC pipe about 4 inches past the internal os of the cervix.
- Hold the exterior pipe in place, but push the stopper of the interior pipe forward, causing the IUD to be pushed out of the exterior pipe, into the uterus.
- Placing a finger into the cervical lumen just as the introducer tube is removed from the external os allows the veterinarian to know that the IUD is left in the uterus, and not dragged back into or past the cervix.
- Remove the introducer from the animal, untie the tail.

Mares that have received an IUD should be observed closely for signs of discharge or discomfort for at least 24 hours following insertion after which they may be released back to the range.

Label for Y-Shaped Silicone IUD for Feral Horses

Y-Shaped Silicone IUD for Feral Horses

The *Y-Shaped Silicone IUD for Feral Horses* is an intrauterine device (IUD) comprised solely of medical-grade, inert, silicone that is suitable for use in female feral horses (free-roaming or "wild" *Equus caballus*). Intended users include government agencies with feral horses in their management purview, Native American tribes that have management authority over feral horses, and authorized feral horse care or rescue sanctuaries that manage feral horses in a free ranging environment. The *Y-Shaped Silicone IUD for Feral Horses* can mitigate or reduce feral horse population growth rates because these IUDs can provide potentially reversible fertility control for female feral horses. This IUD prevents pregnancy by its physical presence in the mare's uterus as long as the IUD stays in place. In trials, approximately 75% of mares living and breeding with fertile stallions retained the *Y-Shaped Silicone IUD for Feral Horses* over two breeding seasons. None of the mares that kept their IUDs became pregnant during an experimental trial. After IUD removal, the majority of mares returned to fertility.

Directions for Use:

The *Y-Shaped Silicone IUD for Feral Horses* is to be placed in the uterus of feral horse mares by a veterinarian. The *Y-Shaped Silicone IUD for Feral Horses* is intended for use in feral mares that are at least approximately 1 year old, where age is determined based on available evidence, such as tooth eruption pattern.

IUDs must be sterilized before use. The IUD is inserted into the uterus using a thin, tubular applicator, similar to a shielded culture tube commonly used in equine reproductive veterinary medicine, in a manner similar to methods used for uterine culture of domestic mares. Feral mares with IUDs should be individually marked and identified (i.e., with an RFID microchip, or via visible freeze-brand on the hip or neck).

Caution:

These IUDs are only to be used in mares that are confirmed to be not pregnant. Checking pregnancy status can be accomplished by methods such as a transrectal palpation and/or ultrasound performed by a veterinarian. If a *Y-Shaped Silicone IUD for Feral Horses* is inserted in the uterus of a pregnant mare, it may cause the pregnancy to terminate, and the IUD to be expelled.

Manufactured for:

U.S. Bureau of Land Management (97949)

1340 Financial Blvd., Reno, NV 89052

EPA Est .: 97628-MI-1

Gelding and Vasectomy Procedure:

All practices that apply to gelding will be the same for vasectomy unless noted. For simplicity, the following text will only refer to "gelding". The specific procedure for vasectomy will be at the discretion of the attending veterinarian, but will be consistent with standard veterinary care in terms of providing humane care of any treated animals.

Gelding will be performed with general anesthesia and by a veterinarian. The combination of

pharmaceutical compounds used for anesthesia, method of physical restraint, and the specific surgical technique used will be at the discretion of the attending veterinarian with the approval of the authorized officer (I.M. 2009-063).

Pre-surgery Animal Selection, Handling and Care

- 1. Stallions selected for gelding will be greater than 6 months of age and less than 20 years of age.
- 2. All stallions selected for gelding will have a Henneke body condition score of 3 or greater. No animals which appear distressed, injured or in failing health or condition will be selected for gelding.

- 3. Stallions will not be gelded within 36 hours of capture and no animals that were roped during capture will be gelded at the temporary holding corrals for rerelease.
- 4. Gelding will either take place at a BLM prep facility or at a temporary holding facility within or near the gather area. When performed at a temporary holding facility to the extent possible, a separate holding corral system will be constructed on site to accommodate the stallions that will be gelded. These gelding pens will include a minimum of 3 pens to serve as a working pen, recovery pen(s), and holding pen(s). An alley and squeeze chute built to the same specifications as the alley and squeeze chutes used in temporary holding corrals (solid sides in alley, minimum 30 feet in length, squeeze chute with non-slip floor) will be connected to the gelding pens.
- 5. When possible, stallions selected for gelding will be separated from the general population in the temporary holding corral into the gelding pens, prior to castration.
- 6. When it is not possible or practical to build a separate set of pens for gelding, the gelding operation will only proceed when adequate space is available to allow segregation of gelded animals from the general population of stallions following surgery. At no time will recently anesthetized animals be returned to the general population in a holding corral before they are fully recovered from anesthesia.
- 7. All animals in holding pens will have free access to water at all times. Water troughs will be removed from working and recovery pens prior to use.
- 8. Prior to surgery, animals in holding pens may be held off feed for a period of time (typically 12-24 hours) at the recommendation and direction of the attending veterinarian.
- 9. The final determination of which specific animals will be gelded will be based on the professional opinion of the attending veterinarian in consultation with the Authorized Officer.
- 10. Whether the procedure will proceed on a given day will be based on the discretion of the attending veterinarian in consultation with the Authorized Officer taking into consideration the prevailing weather, temperature, ground conditions and pen set up. If these field situations can't be remedied, the procedure will be delayed until they can be, the stallions will be transferred to a prepfacility, gelded, and later returned, or they will be released to back to the range as intact stallions.

Gelding Procedure

- 1. All gelding operations will be performed under a general anesthetic administered by a qualified and experienced veterinarian. Stallions will be restrained in a portable squeeze chute to allow the veterinarian to administer the anesthesia.
- 2. The anesthetics used will be based on a xylazine/ketamine combination protocol. Drug dosages and combinations of additional drugs will be at the discretion of the attending veterinarian.
- 3. Animals may be held in the squeeze chute until the anesthetic takes effect or may be released into the working pen to allow the anesthesia to take effect. If recumbency and adequate anesthesia is not achieved following the initial dose of anesthetics, the animal will either be redosed or the surgery will not be performed on that animal at the discretion of the attending veterinarian.
- 4. Once recumbent, rope restraints or hobbles will be applied for the safety of the animal, the handlers and the veterinarian.
- 5. The specific surgical technique used will be at the discretion of the attending veterinarian. The same applies to vasectomy technique.

- 6. Flunixin meglamine or an alternative analgesic medication will be administered prior to recovery from anesthesia at the professional discretion of the attending veterinarian.
- 7. Tetanus prophylaxis will be administered at the time of surgery.
- 8. Other medications may also be administered at the time of surgery at the professional discretion of the attending veterinarian.
- 9. All geldings will be allowed to recover from anesthesia within the working pen or the adjacent recovery pen. Once, fully recovered each gelding will be transferred to the gelding holding pen(s). Animals will remain segregated from intact stallions for at least 24 hours following surgery or until their release.
- 10. Any stallions determined or believed to be a cryptorchid will be allowed to recover from the anesthesia, marked for later recognition, and shipped to a BLM prep facility for appropriate surgery or euthanasia if it is determined that they cannot be fully castrated. At no time will a partial castration be performed. Because cryptorchidism is an inherited condition, cryptorchid stallions should never be released back into an HMA.
- 11. Gelded animals will be given a unique freeze marked on with an identifying mark to minimize the potential for future recapture and to facilitate post-treatment monitoring. Each State will establish its own marking system in compliance with their State Brand Board.

Post-operative handling, care and monitoring

- 1. All animals that have fully recovered from anesthesia will have free access to water and hay prior to subsequent release.
- 2. All geldings will be held at least overnight for observation. Animals will not be left unattended for at least 3 hours following the procedure.
- 3. The attending veterinarian will observe all animals 12-24 hours after the procedure or again prior to release. Geldings will be released no later than 48 hours following surgery near a water source in their home range when possible.
- 4. Any gelding observed to have complications will be held at the gather site until his condition improves or be shipped to a holding facility until he is able to be returned to the range.
- 5. Gelded animals would be monitored periodically for complications for approximately 7-10 days post-surgery. In a prep-facility this will occur multiple times a day. On the range this monitoring will be completed either through aerial recon if available or field observations from major roads and trails. It is not anticipated that all the geldings will be observed but the goal is to detect complications if they are occurring and determine if the horses are freely moving about the HMA.
- 6. Animals found on the range with serious gelding complications will either be recaptured for treatment, if possible or euthanized as an act of mercy if necessary.
- 7. Observations of the long term outcomes of gelding will be recorded during routine resource monitoring work. Such observations will include but may not limited to band size, social interactions with other geldings and harem bands, distribution within their habitat, forage utilization and activities around key water sources.

Appendix H Win Equus Population Modeling Results

Population Model Overview

WinEquus is a program used to simulate the population dynamics and management of wild horses created by Stephen H. Jenkins of the Department of Biology, University of Nevada at Reno.

Detailed information is provided within the WinEquus program available at <u>http://unr.edu/homepage/jenkins</u>, and will provide background about the use of the model, the management options that may be used, and the types of output that may be generated.

The population model for wild horses was designed to help the BLM evaluate various management strategies that might be considered for a particular area. The model uses data on average survival probabilities and foaling rates of horses to project population growth for up to 20 years. The model accounts for year-to-year variation in these demographic parameters by using a randomization process to select survival probabilities and foaling rates for each age class from a distribution of values based on these averages. This aspect of population dynamics is called environmental stochasticity and reflects the fact that future environmental conditions that may affect wild horse population's demographics can't be established in advance. Therefore, each trial with the model will give a different pattern of population growth. Some trials may include mostly "good" years, when the population grows rapidly; other trials may include a series of several "bad" years in succession. The stochastic approach to population modeling uses repeated trials to project a range of possible population trajectories over a period of years, which is more realistic than predicting a single specific trajectory.

The model incorporates both selective removal and fertility control treatment as management strategies. A simulation may include no management, selective removal, fertility control treatment, or both removal and fertility control treatment. Wild horse and burro specialists can specify many different options for these management strategies such as the schedule of gathers for removal or fertility control treatment, the threshold population size which triggers a gather, the target population size following a removal, the ages and sexes of horses to be removed, and the effectiveness of fertility control treatment.

To run the program, one must supply an initial age distribution (or have the program calculate one), annual survival probabilities for each age-sex class of horses, foaling rates for each age class of females, and the sex ratio at birth. Sample data are available for all of these parameters. Basic management options must also be specified.

Population Modeling – North Lander Complex

To complete the population modeling for the North Lander Complex, version 1.40 of the WinEquus program, created April 2, 2002, was utilized.

Objectives of Population Modeling

Review of the data output for each of the simulations provided many useful comparisons of the possible outcomes for each alternative. Some of the questions that need to be answered through the modeling include:

- Do any of the Alternatives "crash" the population?
- What effect does fertility control have on population growth rate?
- What effects do the different alternatives have on the average population size?
- What effects do the different alternatives have on the genetic health of the herd?

Population Data, Criteria, and Parameters utilized for Population Modeling

Initial age structure for the 2021 herd was created using the WinEquus population model for the Garfield HMA magnified to North Lander complex population levels. The following table shows the proposed age structure that was utilized in the population model for the Proposed Action and Alternatives:

Initial Age Structure			
Age Class	Females	Males	
Foal	213	104	
1	185	114	
2	117	95	
3	125	83	
4	109	74	
5	44	23	
6	38	42	
7	50	40	
8	7	62	
9	14	42	
10-14	43	132	
15-19	43	100	
20+	14	84	
Total	1002	995	

All simulations used the survival probabilities, foaling rates, and sex ratio at birth that was supplied with the WinEquus population model for the Garfield HMA:

Sex ratio at Birth: 50% Females; 50% Males

The following percent effectiveness of fertility control was utilized in the population modeling for the proposed action:

Year 1: 80%, Year 2: 80% Year 3: 80%

The following table displays the removal parameters utilized in the population model for the proposed action:

Removal Criteria			
Age	Percentages for Removals		
	Females Males		
Foal	100%	100%	
1	100%	100%	
2	100%	100%	
3	100%	100%	
4	100%	100%	
5	0%	0%	

Removal Criteria		
Age	Percentages for Removals	
6	0%	0%
7	0%	0%
8	0%	0%
9	0%	0%
10-14	0%	0%
15-19	0%	0%
20+	0%	0%

The following table displays the contraception parameters utilized in the population model for the proposed action:

Contraception Criteria		
	Percentages for	
Age	Fertility Control	
	Treatment	
Foal	0%	
1	100%	
2	100%	
3	100%	
4	100%	
5	100%	
6	100%	
7	100%	
8	100%	
9	100%	
10-14	100%	
15-19	100%	
20+	100%	

Population Modeling Criteria

The following summarizes the population modeling criteria for the proposed action:

- Starting Year: 2021
- Initial gather year: 2021
- Gather interval: 2023, 2027, 2031
- Gather for fertility control treatment regardless of population size: yes
- Continue to gather after reduction to treat females: Yes

- Sex ratio at birth: 50:50
- Percent of the population that can be gathered: 85%
- Minimum age for long-term holding facility horses: Not Applicable
- Foals are included in the AML
- Simulations were run for 10 years with 100 trials each

The following table displays the population modeling parameters utilized in the model:

Population Modeling Parameters Results of WinEquus Population Modeling

Interpretation of the Model

The estimated population of 1,997 wild horses in the North Lander Complex was based on an August 2020 population survey and was used in the population modeling. Year one is the baseline starting point for the model that reflects wild horse numbers immediately prior to the gather action and reflects an almost 50:50 sex ratio. The same sex ratio was entered into the model for the post gather action population. In this population modeling, year one would be 2021. Year two would be exactly one year in time from the original action, and so forth for years three, four, and five, etc. Consequently, at year eleven in the model, exactly ten years in time would have passed. In this model, year eleven is 2031. This is reflected in the Population Size Modeling Table by "Population sizes in ten years" and in the Growth Rate Modeling Table by "Average growth rate in 10 years." Growth rate is averaged over ten years in time, while the population is predicted out the same ten years to the end point of year eleven. The Full Modeling Summaries contain tables and graphs directly from the modeling program.

The initial herd size, sex ratio and age distribution for 2021 was structured by the WinEquus population model for the Garfield HMA. This initial population data was then entered into the model and the model was used to predict various outcomes of the different alternatives, including the No Action Alternative for comparison purposes.

The parameters for the population modeling were:

- 1. Gather when population exceeds 536 wild horses in the complex.
- 2. Foals are included in AML.
- 3. Percent to gather 85%
- 4. Gather specific years: 2021, 2023, 2027, 2031
- 5. Number of trials 100
- 6. Number of years 10
- 7. Initial calendar year 2021
- 8. Initial population size: 1,997
- 9. Population size after gather would be 320 wild horses in the complex.
- 10. Implement selective removal criteria.
- 11. Fertility control Yes for proposed action

Results:

No Action Alternative: - No Gather or Removal in the North Lander Complex

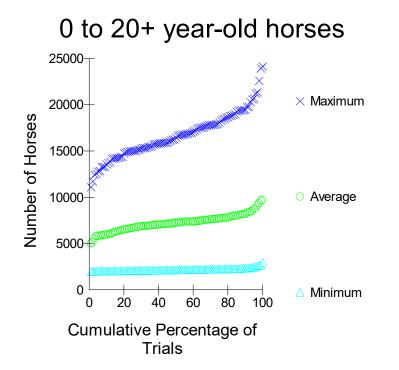
The parameters for the population modeling were:

Do not gather in 2021

Foals are included in AML

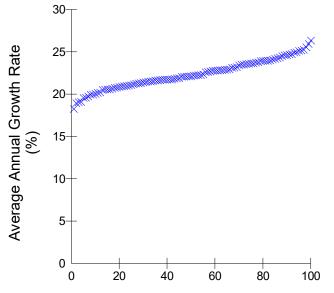
Percent to gather 0

Population Size and Modeling Graph and Table (Gather and Fertility Control)



POPULATION SIZES IN 11 YEARS*			
	MINIMUM	AVERAGE	MAXIMUM
Lowest Trial	1999	5000	11110
10 th Percentile	2057	5981	13658
25 th Percentile	2096	6694	15040
Median Trial	2176	7238	16572
75 th Percentile	2270	7723	18202
90 th Percentile	2356	8253	19622
Highest Trial	2992	9695	24159

* 0 to 20+ year-old horses



Growth Rate Modeling Graph and Table (Gather and Fertility Control)

Cumulative Percentage of Trials

AVERAGE GROWTH RATE IN 10 YEARS		
Lowest Trial	18.3%	
10 th Percentile	20.1%	
25 th Percentile	21.1%	
Median Trial	22.2%	
75 th Percentile	23.7%	
90 th Percentile	24.7%	
Highest Trial	26.4%	

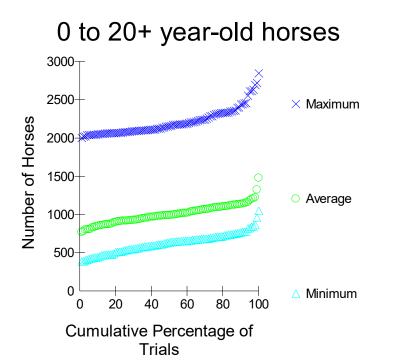
Proposed Action: – Removal of Excess Animals to the Lower Limit of AML range (320) with Fertility Control in the North Lander Complex HMA.

The parameters for the population modeling were:

1-10, The same as parameters listed above.

11, Yes, treat all mares released with fertility control.

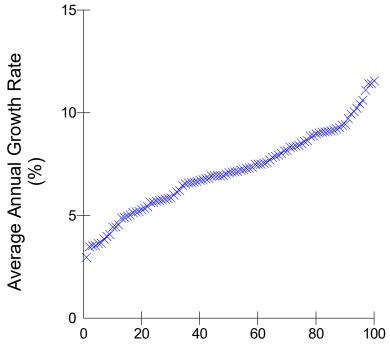
Population Size and Modeling Graph and Table (Gather and Fertility Control)



	POPULATION SIZES IN 11 YEARS*		
	MINIMUM	AVERAGE	MAXIMUM
Lowest Trial	390	772	2003
10 th Percentile	448	854	2053
25 th Percentile	534	921	2080
Median Trial	639	994	2160
75 th Percentile	706	1093	2311
90 th Percentile	771	1140	2443
Highest Trial	1052	1481	2849

* 0 to 20+ year-old horses





Cumulative Percentage of Trials

AVERAGE GROWTH RATE IN 10 YEARS		
Lowest Trial	3.0%	
10 th Percentile	4.5%	
25 th Percentile	5.7%	
Median Trial	7.1%	
75 th Percentile	8.6%	
90 th Percentile	9.6%	
Highest Trial	11.6%	