Animal Cloning: A Risk Assessment
DRAFT Executive Summary

A. Overview

Cloning is the colloquial term used to describe the process of somatic cell nuclear transfer (SCNT), and falls on a continuum of assisted reproductive technologies (ARTs) currently used in agriculture. These are summarized in the Technology Overview (Chapter II) of the Cloning Risk Assessment. In the subsequent chapters and appendices of the Risk Assessment, the Center for Veterinary Medicine (CVM or the Center) at the US Food and Drug Administration (FDA) presents a science-based review of the risks that may arise in species traditionally used for food in which cloning has been accomplished (*e.g.,* cattle, swine, sheep, and goats). Chapter III provides a general overview of the process of risk assessment and how it has been applied to animal cloning. Chapter IV addresses potential health risks to animals involved in the process of cloning, while Chapter V addresses potential food consumption risks that may result from edible products derived from animal clones or their progeny. In order to make this process as transparent as possible, all of the information that has been used is publicly available. This limited our analysis to publications from peer-reviewed journals and to one large data set on bovine clones made available by a clone producer. The document concludes with appendices containing background information, a complete bibliography, and a glossary of terms.

B. Methodology

1. Data Analysis

Both the animal health and food consumption risk assessments evaluated information within a framework developed by CVM called the Critical Biological Systems Approach (the Systems Approach), which divides the life cycle of an animal clone into five functional developmental nodes. Developmental Node 1 incorporates the initial technical steps involved in SCNT, from cell fusion through fetal development. Developmental Node 2 encompasses the perinatal period, including late gestation, labor induction in the dam, delivery, and the critical few days after birth. The third developmental node, Juvenile Development and Function, covers the period of rapid growth between birth and the onset of puberty. The Reproductive Development and Function Node includes puberty and reproductive function throughout the reproductive life of clones. The Post-Pubertal Maturation Node consists of all non-reproductive functions of sexually maturing or mature clones, including growth, weight gain, disease frequency, aging, and, where available, lifespan.

The nature of each risk assessment (*i.e.,* animal health or food consumption) shaped the manner in which the available data were evaluated. For example, identification of adverse outcomes for animal health included both the animal clone and the surrogate dam carrying the pregnancy.

Emphasis was placed on the clones' development and probability of normal development, compared with other ARTs, such as artificial insemination (AI), *in vitro* fertilization (IVF), and blastomere nuclear transfer (BNT). For food consumption risks, however, frankly deformed and diseased clones were excluded from the analysis (because such animals would be condemned at slaughter, as currently practiced with conventional animals), and emphasis was placed on identifying subtle hazards that could have arisen as the result of the SCNT process. Because of the assumption that hazards would be subtle, data sets were evaluated on as fine a level of resolution as possible, including individual animals or even individual analytes per animal in order to have as sensitive a screen as possible for adverse outcomes (and thus potential food consumption risks). Thus, although the data sets considered by both risk assessments may have overlapped considerably, the methods by which they were evaluated differed. Most importantly, however, the conclusions of the two risk assessments may differ with respect to the amount of risk present.

One of the conditions of this risk assessment is that all of the information that CVM has used is also available to the public. Although more information was received from clone producers than is available in the Risk Assessment document, some has not been used in this iteration of the risk assessment due to time constraints. By far the greatest amount of information identified addressed bovine clones, followed by swine clones. Although the data base on goat clones was quite small, it is fairly comprehensive with respect to developmental nodes. Very little information was available for sheep clones.

The risk assessment excludes clones derived from donor cells that are transgenic (*i.e.*, cells that contain heritable DNA inserted by molecular biology techniques) from the identification of adverse outcomes. Transgenic clones are considered to occupy a different "risk space" from "just clones" because the transgenic event is accompanied by a series of construct-specific risks.

2. Limitations on Conclusions from Qualitative Risk Assessments

This is a qualitative, comparative risk assessment that does not attempt to assign a quantitative value to estimates of risk or safety. The strongest conclusions that can be drawn regarding positive outcomes in risk assessments of this type are "likely to be as safe as" because outcomes are weighed against comparators of known or inferred safety. Safety is the condition of absence of adverse effects; as a result, certainty of safety is approached, but never reached. Applied to the health of animal clones, the finding of "as safe as" means that the cloning process is likely to be as safe as other ARTs. Applied to the safety of edible products derived from clones, the finding means that food products derived from animal clones are likely to be as safe as corresponding products from non-clones, or as safe as foods that we eat every day. On the other hand, risks can be characterized in a qualitative, comparative risk assessment because adverse outcomes can be observed.

C. Risks to Animals Involved in Cloning

Although increased in frequency relative to other ARTs, none of the adverse outcomes noted in animals involved in the cloning process are qualitatively different from those observed in other ARTs. Risk appears to be elevated in some species (*e.g.*, cattle and sheep) relative to others (swine and goats). Adverse outcomes are noted with the highest frequency in the earliest stages of a clone's development. Most embryo clones fail to develop, and pregnancies terminate spontaneously due to fetal abnormalities or difficulties with placentation. Many of these anomalies tend to be related to fetal overgrowth, often referred to as Large Offspring Syndrome (LOS), although other frank developmental abnormalities have been noted.

1. Pregnancy through the Perinatal Developmental Period

Large Offspring Syndrome, or LOS, has been noted in many of the early reports of cloning in cattle and sheep as well as other ARTs. LOS is generally thought to consist of a nexus of clinical signs that may be directly related to fetal oversize (at least 20% heavier than the average birth weight for the breed), the size of the dam's uterus, and dystocia (difficulty giving birth) during labor. LOS thus delineates most of the risks to both animal clones and their surrogate dams. Other signs, such as under-developed respiratory, cardiovascular and renal (kidney) systems may not be due to intrauterine effects, but are often considered part of the syndrome due to the frequency with which they occur in combination with the overgrowth phenomena. Many animals born with LOS have excess fluid in their placentae or organs (hydrops), which may contribute to their large size. The causes of LOS are not known, but may be related to a combination of incomplete reprogramming of the somatic cell nucleus and the *in vitro* culture conditions that IVF and SCNT embryos experience. Large Offspring Syndrome has not been reported in swine or goats.

Cattle and sheep carrying clone pregnancies are at risk of developing hydrops and dystocia. Neither of these complications has been reported in swine or goats carrying clone pregnancies. Dystocia is an identified hazard for any pregnancy, clone or not, that goes to term. A common cause of dystocia is incompatibility between the size of the fetus and the pelvic opening through which it must pass.

Other frank abnormalities that have been noted among stillborn and neonatal clones include flexor tendon contracture, which may result from crowding in the uterus, and respiratory failure. The latter, which is one of the most commonly reported clinical signs in neonatal clones, may result from numerous causes, including inadequate surfactant and failure of the lungs to inflate. Many calf clones are born with large umbilici, often with patent blood vessels, which can increase the risk of bacterial infection. These umbilical problems are generally resolved surgically.

2. Juvenile Developmental Node

Most prepubertal cattle, sheep, swine and goat clones appear to grow and develop normally following the early neonatal period. None of the reports evaluated in this risk assessment indicated any anomalies that have not been observed in animals derived via other ARTs or natural breeding. Healthy clones appear to be physiologically similar to their conventionally bred counterparts or their genetic donor. Alterations in physiological parameters (clinical chemistry and hematology measures) that may have been noted early in the clone's life appear to resolve within a month or two of birth, and the health of bovine clones, in particular, is confirmed by veterinary records.

Some clones, although surviving the early neonatal period, died during the juvenile period due to either congenital abnormalities that did not resolve or failure to thrive. One dwarf calf that suffered skeletal defects and gastro-intestinal problems did not recover and was euthanized. Other calves in this group had no apparent health problems.

A few frank developmental defects have been noted. Cryptorchidism (failure of testicular descent) was reported in three calves derived from the same cell line. Hyperkeratosis, a thickening and crusting of the skin that may predispose the animal to skin infections and observed in naturally bred pigs, was observed in one porcine clone. Both conditions may be related to genetics of the nuclear donor, although hyperkeratosis in swine is more often related to nutrition. Neither condition is necessarily attributed to cloning.

3. Reproductive Function

Because of the newness of the technology, very few animal clones have lived to reproductive age, and there are very few publications that specifically address reproductive function. In general, the cursory mentions of bovine reproductive capacity indicate that most clones are as fertile as their conventional counterparts, although there are reports of individual heifer clones that may fail to become pregnant after attempts at insemination. No specific studies of the reproductive capacity of bull clones were identified, although there are reports of "normal" fertility on individual bull clones in articles addressing other portions of the life cycle of clones. No adverse outcomes (*e.g.*, infertility, low sperm motility) have been reported for reproduction in male goats or sheep, but only one study in each of these species has been identified. No studies addressing the fertility of porcine clones were identified.

4. Post-Pubertal Maturation

No studies specifically addressing the health of post-pubertal clones were identified. Instead, cursory information from studies examining other portions of the clones' life cycles was reviewed. No specific reports of risks to clone health were found. This observation must be

tempered with the lack of clones that could populate this developmental node, plus the lack of impetus to publish studies addressing the health of clones of this developmental stage.

5. Conclusions for Animal Safety Based on Available Information

No reports of risks qualitatively different from those encountered by animals involved in modern agricultural practices were detected, although the frequency of the risks appears to be increased in some species during the early portions of the life cycle of animal clones.

There is an increased risk of adverse outcomes for surrogate dams bearing bovine and ovine clones relative to dams bearing non-clone pregnancies. These risks are best characterized by dystocia, likely due to LOS, and hydrops. The risk to swine and goat surrogates bearing clone pregnancies does not appear to be increased relative to dams bearing non-clone pregnancies.

Early reports of cloning in cattle and sheep indicated that most clone pregnancies failed to result in live births. As the technology improves, however, the proportion of live, normal births appears to be increasing. Most of the increased risk for the bovine and ovine clones appears to be related to LOS, although other frank developmental defects are observed. These risks do not appear to apply to porcine and caprine clones.

As clones grow and develop, they appear to become as healthy as their conventional counterparts. There do not appear to be any health risks to apparently normal clones that survive beyond a few weeks of birth. Data are very limited on the health and functionality of clones beyond the juvenile developmental period. Information on reproductive function is limited to a few studies, but appears to indicate that clones are not at increased risk of reproductive failure relative to animals produced using other ARTs or natural breeding. The information on non-reproductive maturation in animal clones is essentially limited to that provided by one clone producer. It indicates that clones in the 6-18 month cohort are virtually indistinguishable from their age- and breed-matched comparators.

D. Food Consumption Risks

1. Two-Pronged Approach to Identifying and Characterizing Food Consumption Risks

The food consumption risk assessment assumes that all clones and their products would be subject to the same local, state, and federal regulations as conventional food animals and their edible products. These rules exclude frankly malformed, diseased, and otherwise unhealthy animals from the human food supply. Therefore, this assessment focuses on clones that appear to be healthy. Because scientific and regulatory communities have traditionally assumed that it is highly unlikely that "silent" pathways producing intrinsic toxicants exist in food animals, the only hazards that could arise in animal clones would be from incomplete or inappropriate

reprogramming of the genetic information from the donor somatic nucleus (*i.e.*, epigenetic effects). These would allow a clone to develop with apparently normal appearance and functions, but with sub-clinical physiological anomalies, and are referred to as "subtle hazards."

In order to determine whether these subtle hazards pose food consumption risks, CVM has developed a two-pronged approach. The previously described first component, the *Critical Biological Systems Approach*, is based on the hypothesis that a healthy animal is likely to produce safe food products, and incorporates a systematic review of the health of the animal clone or its progeny. It accepts that at this time, SCNT is a biologically imprecise and inefficient process, but recognizes that animals are capable of biological repair or adaptation. The cumulative nature of the Critical Biological Systems Approach allows for the incorporation of both favorable and unfavorable outcomes. The former, provided that all other measures appear to be normal, will result in the finding that the clone is likely to produce food that is safe for consumption; the latter implies that clones with anomalies are likely to be considered unsuitable for food.

The second component, or the *Compositional Analysis Method*, assumes that food products from healthy animal clones and their progeny that are not materially different from corresponding products from conventional animals are as safe to consume as their conventional counterparts. It relies on the comparison of individual components of edible products, and the identification of the appropriate comparators.

Assessing the safety of food products from animal clones and their progeny, at least during these early stages of the development of the technology, is best accomplished by using both approaches: prospectively drawing on our knowledge of biological systems in development and maturation, and in retrograde, from an analysis of food products. Subtle hazards and potential risks that may be posed by animal clones must, however, be considered in the context of other mutations and epigenetic changes that occur in all food animal populations.

At this time, CVM anticipates that for economic reasons (*i.e.*, a cost of tens of thousands of dollars per clone), "founder" animal clones are not likely to be slaughtered for meat, and that most of the food products from cloned lineages will enter the food chain as first progeny[1] of animal clones, or their subsequent offspring. If, however, the technology would become sufficiently cost-effective, it is possible that clones themselves could be consumed for food.

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[1] For the purposes of this analysis, an animal clone is one arising directly from a somatic cell nuclear transfer event. A progeny animal is one derived from sexual reproduction that has at least one cloned animal as a parent (but could result from two cloned animals mating). Clones of clones would be considered as clones (*i.e.*, directly arising from an SCNT process).

Animal clones could also be introduced into the food supply as meat if conditions outside the producer's control forced herd culling (*e.g.,* loss of funding), if clones suffered non-recoverable injuries, or if older clones had reached the end of their functional utility (*e.g.,* breeders). Milk from clones, however, could enter the food stream. Information on the composition of clone meat or milk is extremely limited. Very few of the bovine clones are old enough to have been bred, given birth, and begun lactating. One study has been identified on the composition of milk from clone cows; no studies on the composition of meat from clones have been identified.

2. Preliminary Conclusions Regarding Potential Food Consumption Risks

a. Bovine Clones

i. Perinatal Bovine Clones

The underlying biological assumption for this age cohort is that clones may be fragile at birth possibly due to residual incomplete or inappropriate reprogramming of the donor nucleus. Many of these animals adjust to life outside the womb within a relatively short period of time, either on their own, or with assistance from caregivers. The data on the overall health of the clones are consistent with such a hypothesis. Nonetheless, because of the consistently reported relatively poor condition of clones at birth, and variability of their physiological parameters, it is difficult to prove that these animals do not differ materially from their age-matched conventional cohorts. Given that live neonatal clones are unlikely to enter the food supply, they pose an extremely limited risk for consumption as food. Moribund or euthanized clones might enter the food supply via rendering; if they met all of the conditions imposed for euthanized non-clone neonates, they would not likely pose risks greater than the non-clones.

ii. Juvenile Bovine Clones

The underlying biological assumption for this developmental node is that if any anomalies were to be found in the youngest clones, and those animals were to survive to be healthy adults, the juvenile developmental node would be a period of equilibration and normalization. The data are consistent with such a hypothesis: juvenile bovine clones are largely healthy and normal. The results from the information supplied by the clone producer and the peer-reviewed literature indicate that clones show the appropriate physiological responses to developmental signals. The data set from the clone producer indicates the overall health of these animals is comparable to their age-matched comparators, with the exception of the sequellae of umbilical problems and cryptorchidism. Although these outcomes pose risk to the animals, if appropriately managed, they do not appear to pose any food safety concerns. The peer-reviewed literature indicates that although some animals at this developmental node have alterations in physiological parameters such as body temperature, some hormone and cytokine levels, these discrepancies are resolved relatively rapidly (by approximately 50 days of life). Thus, even for physiological parameters in which differences were detected between clones and controls, most resolved soon after birth in

apparently healthy animals. Juvenile bovine clones are likely to be as safe to eat as their contemporary conventional comparators.

iii. Adult Bovine Clones

The underlying biological assumption for this developmental cohort is that there is no fundamental reason to suspect that animals derived via SCNT would produce toxins, there are no introduced genes from other sources, and any biological changes that are not immediately apparent (*e.g.*, gross malformations) would, at most, tend to present subtle changes that do not pose food consumption concerns. The weight of the evidence is consistent with this biological prediction. Non-healthy clones are immediately apparent, and culled from the herd. Data from the clone producer indicate that healthy clones of the oldest (6-18 months) cohort evaluated are virtually indistinguishable from their comparators even at the level of clinical chemistry and hematology. The available information on reproductive function in cows or bulls of this age cohort is quite limited, but appears to indicate that clones have normal reproductive function and give birth to healthy offspring. These observations provide a high degree of confidence to the Center's judgment regarding the health of bovine clones.

Edible products from healthy adult bovine clones are therefore likely to be as safe to eat as those from non-clone adult cattle. The consistency of the observations provide the Center with a high degree of confidence in judgments regarding the health of (and likely food safety of edible products derived from) bovine clones. The Center notes that although these animals may be used for food, given the economic considerations involved it is not likely that large numbers of clones will enter the food supply. The most likely scenarios for these animals to be introduced into the food supply are as the result of non-treatable injury or old age.

There is one study presenting a compositional analysis of the milk from cow clones. Although the sample size is small, and the differences in animal diet and management between clones and comparator animals may confound the interpretation of the results presented, this study indicates that the milk of clones and the study comparators is very similar, and that there are no apparent food safety concerns.

b. Swine Clones

The same underlying biological assumptions were employed in the assessment of porcine clones as for bovine clones. The body of evidence on the health of swine clones is considerably more limited than for bovine clones. Although generating swine clones appears to pose more technical difficulties than bovine clones, once piglets are born, they appear to be healthy. The health status of perinatal animals is generally presented as "normal" or "healthy" in peer-reviewed publications. The most compelling argument for the normal health status of swine clones has been in the evaluation of the behavior and physiological status of a small cohort of relatively

young (15 weeks) and approximately market age (27 weeks) swine clones relative to closely related conventionally-bred pigs. No significant differences were observed in either behavior or physiological measurements, indicating that these animals were not materially different from the comparators. No information was available for review on the reproductive status of swine clones or their non-reproductive maturation. Based on the available information, edible products from swine clones are likely to be as safe to eat as corresponding products from non-clone swine. Confidence in this judgment is tempered by the relatively small data set for these animals, although the consistent responses across species tend to increase our overall confidence level.

c. Sheep Clones

Except by relying on underlying biological assumptions, and by inference from other species, there is insufficient information on the health status of ovine clones to draw conclusions with respect to potential risks that could be posed from the consumption of food products from these clones. CVM was unable to find any publicly available reports on the health status of ovine clones. There are several studies addressing methodological issues for optimizing the generation of clones, but these do not address post-natal health.

d. Goat Clones

This conclusion is based on the same underlying biological assumption cited for the other livestock species, and a small but compelling data set that indicates that goats appear to be particularly "cloning friendly." Once embryo clones are transferred to surrogate dams and pregnancies are confirmed, the "success rate" for live births is quite high. The only anomaly noted was that approximately half of the cohort of goats on which data are available appeared to have poor suckling response immediately after birth, but by the second day were responding normally and nursing from their surrogate dams. Clones appear to have developed well through reproductive age, and the available data indicate their physiological responses are appropriate for age and breed. Reports on the reproductive development and function of male goat clones indicate that they functioned appropriately relative to age- and breed matched comparators. One male progeny goat was derived from the buck clones; this animal also appeared to function in an age- and breed-appropriate manner.

Edible products from goat clones are likely to be as safe to eat as corresponding products from non-clone goats. The consistency of reproductive function, even in a small cohort of animals, adds to the confidence that can be placed in the judgment that these animals are as normal and healthy as their conventional counterparts.

e. Progeny of Clones

The underlying biological assumption for progeny animals is that generation of the cells that ultimately become ova and sperm naturally resets epigenetic signals for gene expression. This

process is thought to effectively "clear" the genome of incomplete or inappropriate signals. The data to confirm this underlying assumption are limited but consistent across species. Cursory reports of normal reproductive function of clone progeny add to the empirical demonstration that clone progeny are as healthy and normal as their conventional counterparts. The one instance in which milk from a daughter of a bull clone has been evaluated seems to indicate that the milk of clone progeny is similar in composition to non-clone contemporaries. Likewise, the one available report of the reproductive development of a goat clone offspring indicates that the animal functions appropriately for its breed. Additional detailed data on the health status of clone progeny would further increase the degree of confidence that could be applied to the safety of edible products derived from healthy clone progeny. In the absence of detailed information on the health of the progeny of livestock clones, the mouse model has offered convincing evidence that even anomalous phenotypes in clones such as prepubertal obesity are not passed on to the offspring of the obese clones.

Edible products from the progeny of healthy clones are likely to be as safe to eat as the corresponding products of progeny from conventional animals. It is likely that clone progeny (from the first sexual breeding of a clone through subsequent generations) will provide the overwhelming majority of clone-derived cattle, swine, sheep, and goats in the US. The weight of evidence evaluation for clone progeny is dominated by the biological assumption of epigenetic resetting via the natural passage through the germ line, and supported by the consistent lack of detection of adverse outcomes in progeny of clones among and across species (including the mouse model), and limited reports on the health of individual clone progeny animals. Additional detailed data on the health status of clone progeny would increase the empirical support for the assumption underlying this conclusion. Nonetheless, we do not believe that consumption of edible products from clone progeny would pose any additional risk relative to consumption of similar products from non-clone progeny.

f. Summary of Risk Statements for Food Safety

The current weight of evidence suggests that there are no biological reasons, either based on underlying scientific assumptions or empirical studies, to indicate that consumption of edible products from clones of cattle, pigs, sheep or goats poses a greater risk than consumption of those products from their non-clone counterparts. The level of certainty is highest for bovine clones, followed in decreasing order of certainty, by porcine, caprine, and ovine clones. Edible products from the progeny of healthy clones are likely as safe to eat as similar products from the progeny of non-clone animals, based on underlying biological assumptions, compelling evidence from the mouse model system, and limited data in the species evaluated. The one study of the composition of milk from bovine clones does not indicate any food safety concerns. The level of confidence that may be placed in these conclusions could be increased by additional data, particularly with respect to composition of edible products.

E. Concluding Statements

Animal Health: SCNT can pose an increased frequency of health risks to animals involved in the cloning process, but these do not differ qualitatively from those observed in other ARTs or natural breeding. In particular, the frequency of live normal births appears to be low, although the situation appears to be improving as the technology matures. In particular, cattle and sheep appear to be subject to a set of syndromes referred to as LOS that do not appear to be present in swine or goats. Surrogate dams are at risk of complications from birth if the fetus suffers from LOS. Clones exhibiting LOS may require additional supportive care at birth, but can recover and mature into normal, healthy animals. Most clones that survive the perinatal period are normal and healthy as determined by physiological measurements, behavior, and veterinary examinations.

Food Consumption Risks: Edible products from normal, healthy clones or their progeny do not appear to pose increased food consumption risks relative to comparable products from conventional animals. Confidence in this conclusion is relatively high due to empirical evidence from bovine clones, and the consistency of empirical observations among the other species. Progeny of clones are likely to be as safe to eat as their non-clone counterparts based on underlying biological assumptions, evidence from model systems, and limited, but consistent empirical observations in the species evaluated. Additional data on the health status of progeny, and composition of milk and meat from clones and their progeny would serve to further increase the confidence in these conclusions.