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). In our assessment of animal health, we considered a wide range of hazards, ranging from macroscopic to biochemical changes (*e.g.,* changes in gene expression, differences in enzyme activity) that might affect the well-being of animal clones. For food consumption risks, animal clones bearing gross anomalies were excluded from the analysis, and emphasis was placed on identifying unique subtle hazards that could have arisen as the result of the SCNT process. The rationale for this approach is found in Chapter IV, and provides the molecular evidence for the role of epigenetic reprogramming as the source of these subtle hazards. Because of the assumption that hazards would be subtle, datasets 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). In this risk assessment, the most detailed level of resolution used for evaluating animal health has been physiological and biochemical measures of individual animals. It is likely, as technologies mature, that molecular techniques such as genomics, proteomics, and their integrated metabolomic measures will assist in such determinations (NAS 2004).

Compositional Analysis: To reach conclusions about the risks of consuming food produced by animal clones, findings regarding animal health (derived from the CBSA) were considered in conjunction with results of the Compositional Analysis approach. In an attempt to find potential subtle hazards, the data considered in this part of the risk assessment included measurements of gross composition (*e.g.,* carcass composition, percent fat and protein) as well as detailed analyses of vitamins and minerals, fatty acid profiles, and protein characterization of meat and milk produced by clones. The composition of foods produced by clones was compared to the composition of foods produced by comparator animals, and also to published reference ranges for meat and milk. These comparisons formed the basis of our determination of whether meat or milk from clones differs materially from meat or milk from conventional animals, and thus contributed to the overall conclusions regarding food consumption risks.

Weight of evidence: Weight of evidence evaluations do not rely on a single study or even a subset of studies. Instead, they are based on expert judgments on all of the information gathered in the course of a risk assessment. This allows for variability in the amount of information on any particular aspect of the evaluation, as well as inconsistency in endpoints evaluated. Chapters IV, V, and VI contain detailed descriptions of studies that were considered relevant to the hazard identification and characterization, and subsequent risk evaluation. For each adverse outcome identified, the empirical evidence for the causal association of cloning with that outcome was weighed against the empirical evidence indicating that there were associations with other causal agents or processes.

D. The Implications of Epigenetic Reprogramming for Clones and their Progeny (Chapter IV)

Epigenetics has been defined as the study of stable alterations in gene expression potentials that arise during development and cell proliferation. In sexual reproduction, a new diploid genome is created by the fusion of two haploid genomes. The subsequent expression of that genome into a functional organism is governed by a "program." There are several examples of epigenetic control of gene expression, of which DNA methylation is likely the best characterized.

Mammalian embryos experience major epigenetic reprogramming primarily at two times in their development, both of which have significant implications for cloning. One of these takes place soon after fertilization, and is referred to as preimplantation reprogramming; the other occurs during gametogenesis (the development of cells that ultimately become the sperm and egg). Because preimplantation reprogramming occurs after fertilization, and in the case of nuclear transfer, after fusion of the donor nucleus with the oöplast, it is the most immediately affected by the cloning process, and may be most directly implicated in the development of clones with defects. Gametogenic reprogramming may also be involved in the abnormalities noted in clones, but it likely has more far-reaching implications for progeny, because it generates the gametes used for the sexual reproduction of clones.

The efficiency of producing clones (*i.e.,* the number of live offspring born compared to the number of embryos transferred) by SCNT is very low. The reasons for this low efficiency may be related to inappropriate epigenetic reprogramming. When cloning, the donor nucleus must be coaxed to direct embryonic development as if it were a fertilization-derived zygote. Most of the time, this is not successful. Anomalous epigenetic reprogramming is observed at the global genomic and individual gene level in clone embryos and fetuses, and in similar developmental stages of animals produced using ARTs with significant *in vitro* culturing components. Many of these are lethal, as demonstrated by the low success rate of IVF and the even lower success rate of SCNT. In the small number of successful cases that ultimately result in clones that appear normal and healthy, reprogramming in SCNT-derived embryos appears to be as successful as reprogramming in fertilization-derived embryos. Live and apparently healthy clones may exhibit some level of epigenetic differences relative to fertilization-derived animals, but these differences do not appear to have adverse effects on their well-being or ability to grow and develop normally.

The Center assumes that if clones were to pose food consumption risks, the only mechanism by which those risks could arise would be from inappropriate epigenetic reprogramming, similar to those observed for other ARTs. It is important to note that the genes that are being dysregulated are the "normal," naturally present genes that comprise the animal's genome, and have not been

introduced via recombinant DNA techniques from other sources (*i.e.*, clones are not transgenic or genetically engineered animals).

Inappropriate epigenetic reprogramming is not expected in the sexually reproduced progeny of animal clones at levels that exceed those observed in other ARTs or natural reproduction. Unlike their clone parent(s), the progeny of clones are produced by the union of male and female gametes. Production of these gametes *de novo* by the clone parents appears to reset any residual epigenetic reprogramming errors associated with nuclear transfer. Therefore, anomalies present in clones do not appear to be transmitted to the next generation, and the offspring that are produced are normal and healthy. Progeny of clones are thus not anticipated to pose any additional food safety concerns compared with other animals produced via sexual reproduction.

E. Risks to Animals Involved in Cloning (Chapter V)

To identify the potential hazards and assess any resulting risks to animals associated with cloning, Chapter V focuses on the health of clones at all five developmental nodes (pregnancy and parturition, perinatal, juvenile, reproductive, post-pubertal). Health risks to surrogate dams carrying clone fetuses are also considered, and the health outcomes of SCNT are compared with the outcomes of other ARTs. The overall conclusion of Chapter V is that animals involved in the cloning process (*i.e.*, cattle and sheep surrogate dams, and clones) are at increased risk of adverse health outcomes. The increased risks in cattle and sheep clones appear to be limited to the early stages of the life cycle. Although none of the adverse outcomes is unique to cloning, the incidence of these abnormalities observed in animals produced by SCNT is increased compared to animals produced by other ARTs.

Cows and ewes used as surrogate dams for SCNT-derived pregnancies are at increased risk of health problems during pregnancy and parturition. These problems include abnormal placental development and function and complications during late gestation such as hydrops (hydroallantois)[4] and dystocia (difficult birth) due to excessive fetal size. Overgrowth of the fetus and complications during late pregnancy are collectively referred to as large offspring syndrome (LOS). These conditions also occur with other ARTs that have a significant *in vitro* culturing component, but at a lower frequency. In contrast to cattle and sheep, surrogate swine and goat dams bearing clones do not appear to be at increased risk of complications during pregnancy.

Once clones are born, there are distinct differences between the species with respect to health

[4]The bovine fetus develops in a fluid-filled membrane called the amniotic sac. Surrounding the amniotic sac is a second fluid-filled membrane, the allantoic sac. Wastes from the fetus accumulate in the fluid contained in the allantoic sac. Hydroallantois, also referred to as hydrops, is excessive accumulation of fluid within the allantoic sac during pregnancy.

risks. In swine and goat clones, morbidity and mortality do not appear to be increased during the perinatal period. In calf and lamb clones, however, the incidence of both morbidity and mortality are increased during the perinatal period compared to calves and lambs produced using other ARTs. Clinical signs in perinatal clones associated with LOS include respiratory problems, prolonged recumbency,[5] enlarged umbilical cord, hyper/hypothermia, contracted flexor tendons, and symptoms associated with abnormal development of the major organs. Survival of these clones appears to be a function of both the severity of the clinical signs and appropriate post-natal management.

Similar to the perinatal period, the risk of morbidity and mortality in clones during the juvenile period varies among species. Compared with animals produced by natural service or ARTs, bovine clones continue to be at an increased risk of morbidity or mortality up to approximately six months of age. These risks appear to be sequellae of the abnormalities first noted in earlier stages of development that persist beyond the perinatal period. In contrast, swine and goat clones do not appear to be at increased risk of morbidity or mortality during the juvenile period. Swine and goat clones, as well as clone calves that are not adversely affected by congenital abnormalities, appear healthy throughout the juvenile period and exhibit normal patterns of growth and development.

As clones approach puberty, no increased risk of adverse health effects have been reported in any of the species evaluated. Clones of both sexes appear to have normal reproductive function, are fertile, and can produce normal offspring via sexual reproduction. Finally, the available information indicates that mature clones are normal and healthy, and there are no increased health risks at this developmental node relative to conventional animals.

Currently, it is not possible to draw any conclusions regarding the longevity of livestock clones or possible long-term health consequences associated with cloning due to the relatively short time that the technology has existed.

Sexually derived progeny of animal clones appear to be normal and healthy. As described in Chapter IV, any residual epigenetic reprogramming errors in clones are expected to be reset during gametogenesis, resulting in production of normal offspring by sexual reproduction. Consistent with these predictions, the data on the health status of clone progeny indicate that there is no increased risk of health problems in these animals compared with conventional animals.

[5] Respiratory problems and prolonged recumbency appear to be the most common problems associated with perinatal death in clone calves.

F. Food Consumption Risks (Chapter VI)

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

In order to determine whether epigenetically-caused subtle hazards pose food consumption risks, CVM has developed a two-pronged approach. The first component, the *Critical Biological Systems Approach* (CBSA), incorporates a systematic review of the health of the animal clone or its progeny. Its role in the evaluation of food consumption risks is premised on the hypothesis that a healthy animal is likely to produce safe food products. 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 CBSA 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 edible products that pose no food consumption risks; the latter implies that clones with anomalies are likely to be considered unsuitable for food. The second component, 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 pose no additional risks. It relies on the comparison of individual components of edible products, and the identification of appropriate comparators.

Assessing the safety of food products from animal clones and their progeny[6] 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. No adverse outcomes have been noted in clones that have not also been observed in animals derived via other ARTs or natural mating that enter the food supply unimpeded.

2. Conclusions Regarding Potential Food Consumption Risks

Based on this review of the body of data on the health of animal clones, the composition of meat and milk from those animals and corresponding information on clone progeny, CVM has drawn the following conclusions:

a. Cattle Clones

[6] Although milk from clones might be marketed for human consumption, CVM anticipates that relatively few animal clones will enter the food supply as meat (*e.g.*, if culled from the herd due to injury or senescence). Relative to clones, it is more likely the progeny of clones will be used to produce meat and milk for human consumption.

Edible products from healthy juvenile bovine clones pose no additional risk(s) relative to corresponding products from contemporary conventional comparators.
The underlying biological assumption for this developmental node is that if anomalies were found in the youngest clones, the juvenile developmental node would be a period of equilibration and normalization as those animals proceeded toward adulthood. Animals experiencing severe developmental abnormalities are not expected to survive. The data are consistent with such a hypothesis.

Juvenile bovine clones that survive the perinatal period are largely healthy and normal. Although some younger clones in this developmental node may be more physiologically unstable than their conventional counterparts, most are able to equilibrate their physiological status and go on to exhibit normal patterns of growth and development. This normalization has been observed consistently in juvenile bovine clones except for those experiencing the sequellae of the developmental abnormalities present at birth. In some cases, these adverse outcomes can persist beyond the perinatal period, resulting in an increased risk to the health of these clones during the first six months of life. Animals bearing these problems are not expected to pass inspection and would not be allowed into the food supply, and therefore are not expected to contribute to food consumption risks. However, no additional subtle hazards that could pose food consumption risks were identified during the juvenile period, as demonstrated by the analysis of clinical chemistry and hematology data, demonstrating that healthy juvenile clones exhibit appropriate physiological responses to developmental signals.

Edible products derived from adult bovine clones pose no additional risk(s) relative to corresponding products from contemporary conventional comparators.
This conclusion is based on application of both prongs (CBSA and Compositional Analysis) of the risk assessment approach. The body of data comprising the CBSA approach is consistent with the biological prediction that there are no underlying biological reasons to suspect that healthy animal clones pose more of a food safety concern than conventional animals of similar age and species.

The data show that healthy adult clones are virtually indistinguishable from their comparators even at the level of clinical chemistry and hematology. These data also confirm the observation that physiological instabilities noted earlier in the lives of the clones are resolved in the juvenile developmental node (see previous conclusions regarding other developmental nodes), and do not reappear as the clones age. There are some reports of early deaths of clones; as these animals would be prohibited from entering the food supply, they do not pose a food consumption risk. Data on reproductive function in cows or bulls of this age cohort indicates that healthy bovine

clones surviving to reproductive maturity function normally and produce healthy offspring. These observations are consistent across studies. Given that reproduction is the most difficult "biological hurdle" placed on an organism, the observation of normal reproductive function provides an additional degree of confidence in the conclusion regarding the appropriate development of these animals.

All of the reports on the compositional analysis of meat or milk from bovine clones show that there are no biologically significant differences in the composition of milk derived from clone and non-clone cattle. Additionally, data from one report show no difference in allergenic potential for meat or milk derived from clone cattle compared to meat or milk from non-clone comparators, and neither meat nor milk from clone or non-clone cattle induced mutations *in vitro*. Finally, none of the reports identified an endpoint that would pose a hazard for human consumption.

b. Swine Clones

Edible products from adult swine clones pose no additional risk(s) relative to corresponding products from contemporary conventional comparators.

This conclusion is based on the same underlying biological assumption as cited for adult bovine clones. Because the data are more heavily weighted towards adult, market sized animals, judgments regarding the safety of food products from swine clones are provided in one aggregate set of comments.

Once piglet clones are born, they appear to be healthy. The most compelling argument for the normal health status of swine clones results from 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 conventional pigs. No significant differences were observed in either behavior, epigenetic, or physiological measurements, indicating that these animals were not materially different from the comparators. Another small dataset on swine clones reared in very unusual settings (i.e., deprivation of colostrums, initial husbandry in pathogen-free conditions, switching to commercial settings) is confounded with respect to outcome. Nonetheless, these clones were able to respond appropriately to this stress, and their carcass characteristics, reproductive performance, including semen quality, farrowing rates and litter sizes were within normal reference ranges for conventional swine. No biologically relevant differences were observed in the composition of meat from these clones or their comparators.

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 sheep clones to draw conclusions with respect to potential risks that could be posed from the consumption of food products.

With the exception of reports on Dolly, CVM was unable to find any publicly available reports on the health status of live sheep clones. There are several studies addressing methodological issues for optimizing the generation of clones, but these do not address post-natal health. There are reports of anomalies noted in fetal sheep clones that have died or been terminated, and reports on the pathology associated with animals that do not survive. Although these are instructive for understanding the molecular and developmental pathways that may be perturbed during the process of SCNT, these studies have limited relevance to addressing food safety because the deceased animals would not have been allowed to enter the food supply. CVM was not able to find any reports on the composition of milk or meat from sheep clones.

d. Goat Clones

Edible products from goat clones pose no additional food consumption risk(s) relative to corresponding products from contemporary conventional comparators.

This conclusion is based on the same underlying biological assumption cited for the other livestock species, and a relatively small but compelling dataset. Once clone embryos are transferred to surrogate dams and pregnancies are confirmed, the "success rate" for live births is quite high. The animals appear to develop normally through reproductive age, and the available data indicate their physiological responses are appropriate for age and breed. The reproductive development and function of male Nigerian Dwarf goat clones demonstrate that those animals 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. No meat or milk composition data were identified for goat clones.

e. Clone Progeny

Edible products derived from the progeny of clones pose no additional food consumption risk(s) relative to corresponding products from other animals.

Relative to the amounts of meat and milk derived directly from clones in the U.S., it is likely that more edible products (both meat and dairy) will be produced by the progeny of clones. These progeny, unlike their clone parents, are produced by normal sexual reproduction. The underlying biological assumption for health of progeny animals (explained in Chapter IV) is that passage through the process of creating the cells that ultimately become ova and sperm naturally resets epigenetic signals for gene expression, and effectively "clears" the genome of incomplete or

inappropriate signals. This assumption has been supported by empirical[7] evidence in the mouse model system, which clearly indicates that phenotypic alterations noted in the parent clones are not passed to their sexually-derived progeny. Detailed observations of the progeny of bovine and swine clones demonstrate that these progeny are born healthy, develop normally, and do not exhibit any of the anomalies observed in clones. One extensive dataset on the progeny of swine clones providing direct data on the composition of their meat indicates that these animals are essentially indistinguishable from the comparable progeny of non-clone animals. These empirical data, together with our underlying biological assumption, support the conclusion that edible products from clone progeny pose no additional food consumption risk(s) relative to edible products from any other sexually reproduced animals.

We therefore concur with the high degree of confidence that the outside scientific community (NAS 2002 a,b) places in the underlying biological assumption, and conclude that consumption of edible products from clone progeny would not pose any additional food consumption risk(s) relative to consumption of similar products from sexually-derived animals.

G. Concluding Statements (Chapter VII)

For Animal Health: SCNT results in 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. At this time, the overall efficiency of SCNT is low. Cattle and sheep exhibit a set of clinical signs collectively 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, or from accumulation of fluid in the cavities of the placenta (hydrops). Risks to clones associated with LOS include increased incidence of fetal and neonatal death, and abnormalities that may require additional supportive care during the perinatal period. Clones affected by LOS can recover and mature into normal, healthy animals, but many succumb to complications of LOS during the juvenile period. The risk of morbidity and mortality appears to decrease with age, and after approximately six months of age most bovine clones are normal and healthy as determined by physiological measurements, behavior, and veterinary examinations. Progeny of animal clones also have been reported as normal and healthy.

For Food Consumption Risks: Extensive evaluation of the available data has not identified any subtle hazards that might indicate food consumption risks in healthy clones of cattle, swine, or goats. Thus, edible products from healthy clones that meet existing requirements for meat and milk in commerce pose no increased food consumption risk(s) relative to comparable products

[7] Empirical refers to that which can be seen or observed alone, often without reliance on theory. In the context of this risk assessment, conclusions drawn on empirical evidence are those that are drawn strictly based on the data. These conclusions may later be put in the context of underlying biological assumptions.

from sexually-derived animals. The uncertainties associated with this judgment are a function of the empirical observations and underlying biological processes contributing to the production of clones. There is less uncertainty about the health of clones as they age and have more time to exhibit the full range of functionality expected of breeding stock.

Edible products derived from the progeny of clones pose no additional food consumption risk(s) relative to corresponding products from other animals based on underlying biological assumptions, evidence from model systems, and consistent empirical observations.

The results of this comprehensive risk assessment agree with the preliminary findings of the NAS (2002a) conclusions that "The products of offspring of clone[s] ... were regarded as posing no food safety concern because they are the result of natural matings," and "In summary there is no current evidence that food products derived from adult somatic cell clones or their progeny present a food safety concern."