STUDY ON

WILDLIFE PRODUCTS PRODUCED FROM SYNTHETIC OR CULTURED DNA

(draft as at 2 August 2018)

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I BACKGROUND

1. This study was commissioned by the CITES Secretariat in implementing Decision 17.89-17.91 on *Specimens produced from synthetic or cultured DNA*, and to respond to the comment made by the CITES Animals and Plants Committees[1] that the definitions of synthetic or cultured DNA were not self-evident and would need to be clarified.

2. This study follows the detailed terms of reference that was finalized by the Standing Committee after its 69th meeting in late 2017, with the exception that the ordering of the sections was altered to address the scientific issues first, and the legal/policy considerations afterwards[2].

3. The study uses terms that are commonly used by molecular biologists, but are not defined in CITES; furthermore, some terms are difficult to define using the vocabulary of the Convention. While effort has been made to bring the language of this document closer to that used in CITES, some terms have been left deliberately vague in order to serve the purpose of the study. For example, the definition of the term "organism" used in this document is derived from the definition for "living organism" defined in the Cartagena Protocol on Biosafety[3], which means "any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids". This could denote a whole population of a given taxon or an individual animal/plant, depending on the context which has been used.

4. It is, or will soon become, possible to synthesize tissue from any organism, including animals and plants covered by CITES, using the techniques of modern biotechnology. This may include modifying the DNA in the tissue and/or using cell culture technology. In some circumstances, the culture of whole cells will be sufficient to provide a product that is identical (or essentially equivalent) to that found in the living organism – an example could be a powder which is derived from rhinoceros horn. In others, the tissue requires some structure, which will require multiple variants of cells derived from the original organisms and the use of a scaffold or the use of a 3D printing technique to grow the cells in an appropriate three-dimensional structure. The technologies available to sequence and modify the genomes of organisms also provide an opportunity to replicate organisms that are endangered.

[1] At the joint session of the 29th meeting of the Animals Committee and the 23rd meeting of the Plants Committee (Geneva, July 2017)
[2] For details on the terms of reference and the background to this study, see AC30 Doc.14/PC24 Doc.14.
[3] http://bch.cbd.int/protocol/

"Is the new technology to be welcomed because it holds the possibility of novel and radical solutions to global problems such as the perfect storm of shortages in food, water and energy resources or is it to be feared, for the impact of novel organisms and associated new economic arrangements on ecosystems and rural societies?"[4]

5. The technologies allow biological systems to be used in commerce and industry, that *may* have a significant impact on conservation.

    a. It is possible to clone individual organisms and therefore to maintain endangered populations in containment.

    b. The 'poaching', illegal or unsustainable use or destruction of organisms to feed an insatiable quest for their tissues (or derived products) could (arguably – see later) be assuaged through synthetic production of these tissues or products.

    c. The production of organisms, their parts or derivatives for trade is likely to be primarily in industrial or commercial premises (laboratories) rather than in the wild.

6. On the other hand, and using the same arguments, these technologies may be used for *laundering* of listed products where synthesised versions hide illegitimate usage. In addition, the presence on the market of manufactured artefacts could increase the market for the wild species. Caution is needed as the introduction of engineered products could have an unwanted impact on the ecosystem in which the wild organism occurs.

7. The precautionary approach defined in principle 15 of the Rio Declaration on Environment and Development (1992) [5] remains an important basis for ensuring that the use of modern biotechnological techniques that may impact on the environment are conducted in a way that minimises risks -

    > "In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation."

    CITES resolution 9.24 (paragraph 2) required that, "by virtue of the precautionary approach and in case of uncertainty regarding the status of a species or the impact of trade on the conservation of a species, the Parties shall act in the best interest of the conservation of the species concerned and, when considering proposals to amend Appendix I or II, adopt measures that are proportionate to the anticipated risks to the species"

8. This study is aimed at highlighting the potential scientific/technological and legal/regulatory aspects of the potential application of these technologies in accordance with the terms of reference as outlined in AC30 Doc. 14/PC24 Doc. 14 (Rev. 1) Annex 1. The study may quote external statements on the potential application of the technologies but does not make any value judgements on their potential benefits or risks of using them. The study also does not consider the socio-economic issues, including the realistic potential of commercialization, costs, and public perception of the products.

[4] Adapted from Redford KH, Adams W, Carlson R, Mace GM and Ceccarelli B (2014) "Synthetic Biology and the conservation of Biodiversity" Fauna & Flora International, Oryx **(48)3** 330-336

[5] http://www.unesco.org/education/pdf/RIO_E.PDF

II DESCRIPTION OF DIFFERENT WAYS THAT DNA CAN BE SYNTHESIZED, CULTURED OR OTHERWISE PRODUCED ARTIFICIALLY, AND HOW WILDLIFE PRODUCTS CAN BE PRODUCED FROM SYNTHETIC OR CULTURED DNA IN THE CONTEXT OF CITES

Introduction

9. This section attempts to look at the science which is used to derive products from synthetic tissues – which may include modification of the DNA that is found in all tissues. It embraces a vast field, and therefore provides footnotes pointing to many original articles to provide context.

10. The **genetic material (DNA)** in cells is primarily that which determines inheritance from generation to generation. The majority of this genetic material is found in the nucleus of eukaryotic cells with a small but important amount in the mitochondria and chloroplasts. The sequence of the DNA is highly conserved from generation to generation, and cells contain systems to both conserve and repair DNA.

11. **Cell culture** is the process by which cells are grown under controlled conditions, generally outside their natural environment. Cultures of plant cells can, and are used, to generate whole plants. Cultures of animal and human cells are now significant tools**:** Different variants of cell culture are used in modelling disease, IVF technology, stem cell and cancer research, monoclonal antibody production, regenerative medicine and therapeutic protein production[6].

12. **Tissue culture** is different, as it may involve a number of different cell types which form the tissue. The early part of twentieth century was the time when the basic principles for plant and animal cell cultures *in vitro* were developed. An understanding both of the techniques to be used to obtain stable cell cultures and of the changes in the cells during multiple cell-cycles was achieved in the latter half of the twentieth century. Tissue culture or the culture of organs is different from cell culture which in essence provides either a layer of cells on gel or suspension of the cells in liquid culture. Tissues require some form of supporting structure in order to develop the characteristic structures in three dimensions.

Scientific Background

13. During the life-cycle of an individual higher organism changes occur within the DNA molecules. These include epigenetic changes which alter the expression of genes, and changes to the ends of chromosomes (telomeres) which limit the number of cell divisions that can occur (termed senescence)[7].

    a. Non-genetic factors contribute to many cellular functions, traits and phenotypes[8]. "Epigenetic[9] change is a regular and natural occurrence but can also be influenced by several factors including age, the environment/lifestyle, and disease state. Epigenetic modifications can manifest as commonly as the manner in which cells differentiate to

[6] Jedrzejczak-Silicka M (2017) "History of Cell Culture" in "New Insights into Cell Culture Technology" Downloaded from: http://www.intechopen.com/books/new-insights-into-cell-culture- technology Chapter 1 http://dx.doi.org/10.5772/66905

[7] Telomeres are most commonly composed of non-coding tandemly repeated sequences. For humans, the telomeric sequence is TTAGGG, which extends in a 5' to 3' direction from the double-stranded DNA to a single stranded region.

[8] Stricke SH , Köferle A and Beck S, From profiles to function in epigenomics, NATURE Reviews Genetics **18** (January 2017) 51- 66

[9] Epigenetics is the study of heritable changes in gene expression (active versus inactive genes) that do not involve changes to the underlying DNA sequence

end up as a variety of cell types, from specialist brain cells to skin cells, liver cells, etc. Epigenetic change can have more damaging effects that can result in diseases like cancer. At least three systems including DNA methylation, histone modification and non-coding RNA (ncRNA)-associated gene silencing are currently considered to initiate and sustain epigenetic change"[10]

b. "Telomeres and their associated proteins serve to camouflage the chromosome ends from the DNA repair machinery that would otherwise identify them as double strand breaks, and from exonucleases that might recognize them as substrates."[11] They therefore protect the chromosome, but there is an attrition of telomere length with each cell division which results in cell death after a number of divisions. (i) telomeres shorten at each cell division due to incomplete replication of their ends; (ii) they are shortened by oxidative damage; and (iii) when telomeres. reach a critical length, cells enter a senescent state and cell division ceases.[12] This implies that normal mammalian cells (in particular) can only undergo a finite number of cell divisions before ceasing to divide. There are also likely to be changes in the cells during replication.

14. The term *'bioengineering'* has been used by many to refer to the deliberate modification of the DNA in organisms, and in this document is used as a generic term for the many different ways to modify organisms. In some countries genetic modification has been variously described as genetic engineering or genetic manipulation. Gene editing, described later as a significant advance in modifying genetic material would also fall within this definition.

15. In the last decade of the 20th century a huge project was undertaken, an international collaborative project whose goal was the complete sequencing of the human genome. In 2003 the full sequence of the human genome was published. It was noted that this genome could be thought of as a book with many uses – a history book, a technical manual providing the detailed blueprint for building ever cell in the body and it would prove to be a transformative moment in the history of medicine. In the last decade the speed at which sequencing of DNA molecules can be achieved has increased beyond recognition[13] – since 1982 the information in GenBank has doubled approximately every 18 months[14]. As of July 2018 there is information on the sequences of nearly 400000 organisms (eukaryotes comprising some 6000, prokaryotes 150000 and viruses nearly 18000). DNA sequencing and the ability to compare sequences from different (possibly related) organisms provides information about living systems and what makes them different.

16. With this information, it is possible to synthesise parts of the genome of any organism for which a sequence has been published and use that as a template for commercial products. This could mean that a product arising from a CITES listed species could contain nothing directly derived from that species other than the information stored in a database.

[10] https://www.whatisepigenetics.com/fundamentals/

[11] M Shawi, C Autexier, (2008) "Telomerase, senescence and ageing" Mechanisms of Ageing and Development **129** 3–10

[12] M J.P. Simons (2015) "Questioning causal involvement of telomeres in aging", Ageing Research Reviews 24 (2015) 191–196

[13] Heather, JM and Chain, B (2016) "The sequence of sequencer: the history of sequencing DNA" Genomics 107 1-8 – https://ac.els-cdn.com/S0888754315300410/1-s2.0-S0888754315300410-main.pdf?_tid=725e9586-bce5-4b8d-a2ca-1f50da64624f&acdnat=1532935312_1c9014f68d17eaba5d473b3cef625c89

[14] see https://www.ncbi.nlm.nih.gov/genbank/statistics/ for detailed information

Technology[15] (i): DNA Modification

17. During the last few decades it has become possible to modify the DNA[16] of both eukaryotic and prokaryotic organisms using a variety of techniques. The modification of the DNA of bacterial cells (prokaryotic organisms) has been extensively studied and has become commonplace in the laboratory and in commerce.

18. The first published account of the directed chemical synthesis of an oligonucleotide (a polymer of a small number of nucleotides) was in 1955 when Michelson and Todd reported the preparation of a dithymidinyl nucleotide (Michelson and Todd, 1955). In the 1970s it became possible to synthesise oligonucleotides almost at will with the first reported chemical synthesis of a DNA molecule coding for a protein reported in 1970[17]. These techniques have evolved, and nowadays, not only can a single strand of DNA be synthesized, but also complete genes, chromosomes and bacterial genomes can be produced from scratch in *in vitro* conditions. Almost all applications of modern biotechnology require the synthesis of a piece of DNA which can then be used as a probe for a gene, a pointer to the site of modification of a genome, or for insertion into the genome in addition to the gene already present or replacing a gene or part of a gene. This permits the structured modification of genetic elements providing the mechanism for tailored design of desired products.

19. Modification of the DNA of dicotyledonous **plants** was, until recently primarily achieved through the use of a bacterium, *agrobacterium tumifaciens*, that inserts a DNA sequence (synthesised from known sequences that occur in other organisms or extracted from other organisms) into that of a plant using a piece of circular DNA (Ti plasmid).[18] The position of insertion is imprecise. The use of a marker that enables modified cells to be distinguished from those either unaffected or modified in a deleterious fashion (because of the insertion in an inappropriate position in the DNA of the plant) is important as the plant can often be regenerated from a single cell. Further (and possibly multiple) sexual crossing of the modified plant with unmodified relatives ensures that any unwanted modifications can be identified and excluded before commercialisation (see a publication of the Australian Office of gene technology regulator[19])

20. DNA modification of animals has always been challenging. The technology was initiated in the 1980s; a detailed examination of genetically modified animals is addressed in a publication of the Netherlands Commission on Genetic Modification (COGEM):[20]

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[15] 'Technology' is defined as "the application of scientific knowledge to the practical aims of human life or, as it is sometimes phrased, to the change and manipulation of the human environment" and is sometimes referred in this report as "techniques of biotechnology", or simply as "techniques". They are used interchangeably.

[16] DNA – "The DNA molecule consists of two polynucleotide chains in the form of a double helix, containing phosphate and the sugar deoxyribose and linked by hydrogen bonds between the complementary bases adenine and thymine or cytosine and guanine. DNA is self-replicating, plays a central role in protein synthesis, and is responsible for the transmission of hereditary characteristics from parents to offspring"(https://www.collinsdictionary.com/dictionary/english/dna)

[17] Agarwal KL, Buchi H, et al. (1970) Total synthesis of the gene for an alanine transfer ribonucleic acid from yeast. Nature, 227(5253): 27 34

[18] Gelvin B. S. (2003) "Agrobacterium-Mediated Plant Transformation: the Biology behind the "Gene-Jockeying" Tool". Microbiology and Molecular Biology Reviews 67(1): 16–37 -

A. tumefaciens Ti plasmids have been produced that lack the genes responsible for gall formation (disarmed plasmids). Genes to be inserted into the plant are put into the T-DNA section of these disarmed plasmids. A. tumefaciens cells carrying such plasmids cannot produce a gall in an infected plant but will transfer the T-DNA sequence carrying the genes of interest into the plant cell where they stably integrate into the plant genome

[19] http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/plant-modifications-ref-1-htm

[20] Genetically Modified Animals: A Wanted and Unwanted Reality. COGEM topic report (2012) CGM/120111-01

"The most common route for producing a GM genetically modified (GM) animal is to inject foreign DNA into a fertilised egg, also known as 'microinjection'. For mammals, injected eggs are placed into a 'foster' mother where they develop to term and offspring are born normally, carrying the extra, foreign DNA. This DNA is now part of a chromosome, so when the GM animal mates and produces offspring, the transgene is inherited in the same way as any other DNA and a line of GM animals is bred that carries the extra DNA. The first GM animal, a mouse, was made in the early 1980s, and this technology has been successfully applied to most mammals, including cattle, pigs and sheep, poultry, fish, and also Drosophila and other insects."

21. The ability to edit genomic DNA inside cells has been limited by the dearth of effective tools which break the chains of DNA at specific sites[21]. However, this has changed during the last few years, resulting in a revolution due to the ability to determine the sequence of the DNA precisely and easily, to manufacture DNA sequences on demand, and modify DNA in organisms in a *precise* manner. "Since 1975, reading and writing platforms have exhibited increases in throughput of three-million-fold and one-billion-fold" and a million-fold reduction in cost in the past 10 years in both reading and writing"[22]. Genome editing involves "targeted interventions at the molecular level of DNA or RNA function at the molecular level of DNA or RNA function, deliberately to alter the structural or functional characteristics of biological entities. These entities include complex living organisms, such as humans and animals, tissues and cells in culture, and plants, bacteria and viruses. Characteristics of many kinds, from the colour or number of blooms in flowering plants, to some disease traits in animals and plants, can be altered, though the extent to which, and ease with which, such alterations can be made is highly variable"[23]

22. The major advance in the editing of DNA to modify the DNA expression is that of **CRISPR/Cas9[24]** technologies. The advent of targetable nucleases (enzymes that can break the DNA chains) has given researchers the ability to induce specific double-strand breaks in the DNA.[25] It is now possible to "directly edit or modulate the function of DNA sequences in their endogenous context in virtually any organism of choice, enabling them to elucidate the functional organization of the genome at the systems level, as well as identify causal genetic variations."[26] Cas9 is one of a group of RNA-guided endonucleases. A short strand of RNA complementary to the double stranded DNA directs the cleavage of the phosphodiester bond within both strands of the polynucleotide chain. As the DNA targets are recognized via RNA-DNA base pairing, changing the sequence of the guide RNA easily alters DNA specificity and therefore, specific sites within the genome can be targeted.[27] "Researchers create a small piece of RNA with a short guide sequence that attaches (binds) to a specific target sequence of DNA in a genome. The RNA also binds to the Cas9 enzyme. As in bacteria, the modified RNA is used to recognize the DNA sequence, and the Cas9 enzyme cuts the DNA at the targeted location. Although Cas9 is the enzyme that is used most often, other enzymes (for example Cpf1) can also be used. Once the DNA is cut, researchers use the cell's own DNA repair machinery to add or delete pieces of

[21] Carroll, D. (2014). Genome engineering with targetable nucleases. Annual Reviews. Biochem. 83, 409–439.

[22] Chari, R., and Church, G.M. (2017). Beyond editing to writing large genomes. Nature Reviews Genetics volume 18, pages 749–760

[23] Nuffield Council on Bioethics (2016) "Genome Editing: An Ethical Review" http://nuffieldbioethics.org/wp-content/uploads/Genome-editing-an-ethical-review.pdf

[24] CRISPR is an abbreviation of Clustered Regularly Interspaced Short Palindromic Repeats. CRISPR – Cas9 is an abbreviation for CRISPR-associated protein 9.

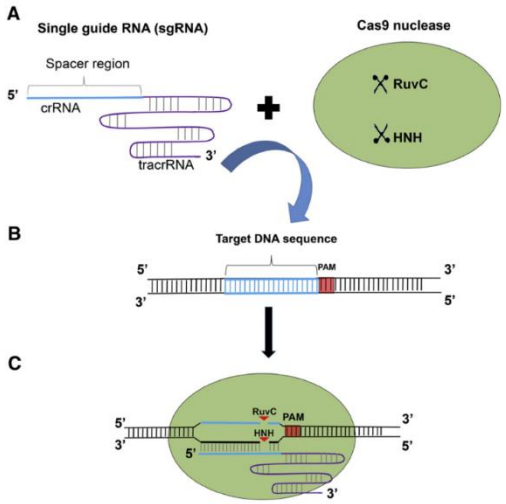
[25] Carroll D (2013) "Staying on target with CRISPR-Cas" Nature Biotechnology **31**(9) 807-809

[26] Hsu P, Lander ES, and Zhang F (2014) Development and Applications of CRISPR-Cas9 for Genome Engineering , Cell 157, June 5, 1262

[27] Sternberg SH and Doudna JA (2015) "Expanding the Biologist's Toolkit with CRISPR-Cas9" Molecular Cell **58**, 568-574. http://dx.doi.org/10.1016/j.molcel.2015.02.032

genetic material, or to make changes to the DNA by replacing an existing segment with a customized DNA sequence."[28],[29] The discovery of the CRISPR system has led to modifications of the Cas9 protein and the discovery of similar molecules that increase the precision of the cut in the double stranded DNA significantly.

23. It is the specificity of this technology that is seen to be important if it is to be used for conservation, cloning, therapy in animals and humans, industrial manufacturing or even for food. Much work has been going on to improve efficiency of the process and ensure that modifications to the DNA are as specific as possible and at the desired site in the DNA.[30] A detailed review of the technology, and its ethical aspects was published by the Nuffield Council on Bioethics in 2016[31] (a newer publication on the implications for the editing of the human genome was published in July 2018[32])



Schematic representation of the CRISPR/Cas9 complex. (A) The CRISPR/Cas9 system requires a single guide RNA (sgRNA) and CRISPR-associated protein 9 (Cas9) containing two nuclease domains, RuvC and HNH. sgRNA consists of CRISPR RNA (crRNA) (indicated by the blue bold line) and a trans-activating crRNA (tracrRNA) (indicated by the purple bold line). The crRNA region is also called the spacer region. (B) The spacer region in the crRNA is complementary to a 20 bp-long target DNA sequence, which is known as the protospacer (indicated by the blue bold line). The target DNA sequence is immediately followed by a short sequence known as the protospacer adjacent motif (PAM) (indicated by the red box). (C) sgRNA targets the complementary target DNA sequence, and Cas9 nuclease follows to generate a double stranded break at the target site.[33]

[28] https://ghr.nlm.nih.gov/primer/genomicresearch/genomeediting

[29] A detailed article on the modern methods of editing DNA, beyond the scope of this report can be found in Gupta RM, Musunuru K (2014). Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. The Journal of Clinical Investigation. 2014;124(10):4154-4161. doi:10.1172/JCI72992.

[30] Carroll, D (2013). "Staying on target with CRISPR-Cas: four independent studies shed light on the specificity of RNA-guided genome editing tools based on the Streptococcus pyogenes Cas9 protein." Nature Biotechnology, vol. 31, no. 9, 2013, p. 807 Academic OneFile, http://link.galegroup.com/apps/doc/A344207979/AONE?u=unict&sid=AONE&xid=64ddcd3c. Accessed 24 Mar. 2018.,

[31] Nuffield Council on Bioethics (2016) "Genome Editing: An Ethical Review" http://nuffieldbioethics.org/wp-content/uploads/Genome-editing-an-ethical-review.pdf

[32] Nuffield Council on Bioethics (2018) Genome Editing and Human Reproduction: social and ethical issues (London: Nuffield Council on Bioethics)

[33] Young-Il, Bharathi Suresh, Hyongbum Kim, Suresh Ramakrishna (2015) "CRISPR/Cas9 system as an innovative genetic engineering tool: Enhancements in sequence specificity and delivery methods" Biochimica et Biophysica Acta 1856 (2015) 234–243

24. These technologies are thought to allow replacement of earlier methods for modifying the DNA (or recently RNA[34]) in organisms. It is possible to introduce genes into specific sites in the genome which alter the characteristics of an organism. The precautionary approach becomes an important consideration particularly when handling viable organisms that could be introduced into the environment.

    a) This could be used to 'resurrect' organisms through hybridisation with related organisms.

    b) Organisms that are close to extinction could be modified by the addition of a variety of genes or by their deletion to adapt them to changed environments and in theory, increase fecundity

    c) Genes can be extracted from organisms that need conservation and added to less threatened related organisms so that pressure on the former is relieved through production of specific products without the need to harvest regulated organisms

    d) There may be (rare) unwanted insertions or deletions in the edited genome. Care therefore needs to be taken to ensure that only the desired outcome occurs in the modified organism; once again this can be achieved in general – at least in plants - through back-crossing with unmodified specimens.

25. **Gene drive** is an expansion of these technologies that allows for a trait to be propagated throughout a population. "Gene drives are systems of biased inheritance in which the ability of a genetic element to pass from a parent to its offspring through sexual reproduction is enhanced. Thus, the result of a gene drive is the preferential increase of a specific genotype, the genetic makeup of an organism that determines a specific phenotype (trait), from one generation to the next, and potentially throughout the population.[35] – Indeed, a wide variety of gene drives occur in nature that can cause genetic elements to spread throughout populations to varying degrees. Researchers are studying how to harness such natural mechanisms (e.g., transposable elements, homing endonucleases, and meiotic drive) to develop gene-drive modified organisms. Preliminary evidence suggests that gene drives developed in the laboratory with CRISPR/Cas9 could spread a targeted gene through nearly 100% of a given population of yeast, fruit flies, or mosquitoes.[35]

26. DNA is not only altered through mutations in the sequence. Changes to the DNA or it associated structures can occur, termed epigenetic changes. "Epigenetics is the study of a group of mechanisms that affects how genes are 'read' by cells. It's the term used to explain how a gene expresses an organism's characteristics (active versus inactive genes) and to what degree. Epigenetics is akin to "directing"—it orchestrates how genes work, which shapes the behaviour of all organisms. It also describes heritable changes in gene expression that do not involve changes to the underlying DNA—a change in phenotype without a change in genotype—which in turn affects how cells read the genes. At least four systems—methylation, non-coding RNA (ncRNA)-associated gene silencing, histone modifications and chromatin remodelling of DNA—are currently considered to initiate and sustain epigenetic change." [36] It is likely that an understanding of epigenetics will play a role in the survival or endangered species in the laboratory, and ultimately in the wild.

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[34] See https://phys.org/news/2018-03-crispr-cas9-rna.html

[35] National Academies of Sciences, Engineering, and Medicine. 2016. Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values. Washington, DC: The National Academies Press. doi: 10.17226/23405.

[36] https://geneticliteracyproject.org/category/epigenetics/?mc_cid=615b71adba&mc_eid=cc680d1eb4 (accessed 6 May 2018)

27. Whilst the set of technologies already described are used for altering the genetic material (possibly modifying the germline and hence becoming heritable), **synthetic biology** involves many different technologies. "As well as molecular biology, synthetic biology interfaces with engineering, chemistry, physics, computer science and systems biology and is focused on developing more rapid and simple methods to produce genetically modified organisms (GMOs) by adding or removing genes, or creating genetic elements from scratch"[37] Amongst the many definitions of synthetic biology, in 2016 the Royal Society defined synthetic biology as "The design and construction of novel artificial biological pathways, organisms and devices or the redesign of existing natural biological systems"[38] The European Group on Ethics in Science and new technologies defined it as "A new research field within which scientists and engineers seek to modify existing organisms by designing and synthesising artificial genes or proteins, metabolic or developmental pathways and complete biological systems in order to understand the basic molecular mechanisms of biological organisms and to perform new and useful functions".[37] These new developments could have positive or negative impacts on the long-term survival of the species in the wild.

Technology (ii): Cell culture

28. It is not necessarily only the modification of DNA that could drive the production of desired tissues. It may be advances in the way cells can be isolated, grown and exploited that is already being used for multiple purposes

29. The selection, isolation and culturing of particular cell types is crucial where tissues from organisms are to be grown; in some instances, cells may be isolated and grown in culture and assembly of a tissue might be induced. In other cases, assembly of a specific tissue may involve numerous steps. The first step would be to isolate specific cell types. Tissues involve multiple different cells, and the *creation* of a tissue may require culturing of multiple cell types in isolation from one another and their combination using some form of scaffolding to simulate the tissue.

30. There has been a long history of culturing the cells of living organisms. A culture is a method of multiplying living cells by letting them multiplicate in predetermined culture media under controlled laboratory conditions. Cell culture refers to the removal of cells from an animal or plant and their subsequent growth in a favourable artificial environment. A culture may originate from a single cell, in which case all of the cells are likely to be the same as one another (clones). If the starting material is heterogenous, many different cell types (or even organisms) may be present in the cultured medium.

31. There are many different cells in a multicellular organism. For example, it is estimated that there are 37.2 trillion cells in the human body. Attempts at identifying these indicate that there are at least 300 variants amongst these myriads in the human body[39]

32. For a unicellular organism, the culturing and growth of the organisms over many 'generations' has been studied for over a century, and for many of these organisms, the conditions required to allow replication in the laboratory have been described and are well understood. The **bacteria**

[37] Science for Environment Policy (2016) Synthetic biology and biodiversity. Future Brief 15. Produced for the European Commission DG Environment by the Science Communication Unit, UWE, Bristol. Available at: http://ec.europa.eu/science-environment-policy

[38] Science for Environment Policy (2016) Synthetic biology and biodiversity. Future Brief 15. Produced for the European Commission DG Environment by the Science Communication Unit, UWE, Bristol. Available at: http://ec.europa.eu/science-environment-policy, page 7

[39] S Connor (2017) in "The Cell Atlas" MIT Technology Review, **120(2)** p59-61

that can be grown in the laboratory are only a small fraction of the total diversity of organisms that exists in nature[40] There are many unculturable organisms where the conditions for growing them in the laboratory have not, as yet, been identified – "the culturing efforts of the last 2 centuries had managed to replicate permissive growth conditions for only a small subset of the total bacterial diversity."[32] An understanding of the techniques necessary for growing unicellular organisms, including those considered *unculturable* could provide the information necessary for conservation in the future.

Plants

33. For many **plants**, it is possible to regenerate a complete plant from a single cell. "Many somatic plant cells, including some fully differentiated types (e.g. leaf mesophyll), provided they contain intact nuclear, plastid and mitochondrial genomes, have the capacity to regenerate into whole plants. This phenomenon is totipotency, an amazing developmental plasticity that sets plant cells apart from most of their animal counterparts and was first demonstrated by Steward and Reinert in the 1950s."[41] Most plants at most stages of the life cycle have some populations of cells that are totipotent.

34. In order for plant cells to grow in culture, many of the chemicals normally available within the plant must be made available to the cells in the medium. The selection of chemicals is made so as to promote the growth of particular cells. Pieces of plant tissue will slowly divide and grow into a colourless mass of cells if they are kept in special conditions. While the regeneration of a whole plant from a single cell is possible, the process requires specific plant hormones to stimulate **totipotency**.[42] Loss of totipotency in plant cells is probably due to genetic changes, including physical changes to chromosomes, for example loss of DNA, nucleotide substitution, endopolyploidy) or epigenetic changes, which are changes in gene expression as a consequence of development, for example DNA methylation).[41]

35. In addition, vegetative or clonal propagation is a technique used in agriculture independently of cell cultures. Plant tissue (such as roots, stem or leaves) that is able to replicate is used. Many plants are produced using this method (e.g. potato and rose) whilst it is the only method that can be used for propagating banana. This type of propagation occurs through apomixis (seed development without meiosis and fertilization) and/or vegetative reproduction (regeneration of new plants from vegetative parts). Hence, vegetative propagation involves the multiplication of genetically identical copies of a cultivar by asexual reproduction called "clonal propagation," and a plant population derived from a single individual by asexual reproduction constitutes a clone.[43]

Animals

36. The stable introduction of new genetic material into breeding programs for **animals** is now possible through some form of germ line modification. Genetic modification of embryonic stem

[40] EJ Stewart (2012) "Growing unculturable bacteria" J Bacteriol. 2012 Aug;194(16):4151-60. doi: 10.1128/JB.00345-12. Epub 2012 Jun 1.

[41] http://plantsinaction.science.uq.edu.au/edition1/?q=content/10-2-1-concept-totipotency

[42] totipotency (Lat. *totipotentia*, "ability for all [things]") is the ability of a single cell to divide and produce all of the differentiated cells in an organism. Spores and zygotes are examples of totipotent cells. In the spectrum of cell potency, totipotency represents the cell with the greatest differentiation potential. (https://en.wikipedia.org/wiki/Cell_potency)

[43] For further information see "Plant Tissue Culture. Theory and Practice, a Revised Edition. Edited by S.S. Bhojwani, M.K. Razdan. Volume 5, Pages 1-767 (1996) https://www.sciencedirect.com/bookseries/studies-in-plant-science/vol/5/suppl/C

cells in culture followed by the production of chimeric animals using blastocyst injection is a tool for this modification.[44] Nuclear transfer enabled the production of clones (Dolly the sheep) and could be used to increase the number of animals within an endangered species. The addition of DNA material can be achieved through the injection of desired genes into the pronucleus of a zygote. It does not always work, as integration may not occur during the first cell cycle resulting in mosaic embryos. The position of insertion in the genome is essentially random, which can both disrupt other genetic processes or make the expression of the desired trait variable. The advent of Crispr/Cas9 technologies is expected to hugely increase the scope of possible modifications of particular cells within an organism.

37. It is possible to isolate animal cells and culture them. Normal animal cells usually divide only a limited number of times (approximately 30 times) before losing their ability to proliferate, which is a genetically determined event known as senescence. Almost 60 years ago Hayflick was able to show that cultured human cells have limited capacity to divide – the limitation in number of cell divisions has become known as the Hayflick limit. Some cell lines become immortal through a process called transformation, which can occur spontaneously or can be chemically or virally induced. When a finite cell line undergoes transformation, and acquires the ability to divide indefinitely, it becomes a continuous cell line. Cell immortalisation is a very complicated cellular process and the exact biological mechanisms are still largely not well understood.[45] It remains difficult to immortalise cells.

38. The animal cells are difficult to grow *in vitro* as different cells within a tissue would require specific nutrients and in general, some kind of matrix is required (solid support). Generally, cells with rapid growth and high regenerating capacity are used; these are extracted from a section of organ or tissue (eg. liver, kidney, tumours). If attempts are made to culture a primary cell population past the Hayflick limit, the cells would have to be modified in some way in order to circumvent senescence[46]

    a. Cancer cells are spontaneous immortalised cells that have undergone genetic changes that make them *immortal.* Hence the expression of an immortalising oncogene in the normal cell could enable a continuous cell line

    b. Many viral genes affect the cell cycle and can be used to avert senescence through repression of some enzymes involved in the cell cycle. The most effective way of inducing immortality in cell lines is through viral infection.

    c. Express an immortalizing oncogene in normal cells.

    d. Shortening of telomeres (in mammals, thousands of repeats of TTAGGG) would appear to lead to senescence. The most common immortality gene is telomerase which is able to extend the telomeres and hence (probably) enable the cells in culture to undergo infinite cell divisions. "Normal human cells stably expressing transfected telomerase can maintain the length of their telomeres, and exceed their maximum lifespan by more than 5-fold."[47]

[44] Campbell KHS, Wilmut I, (1996) "Totipotency or multipotentiality of cultured cells: Applications and progress" Theriogenology **47**, Issue 1, 1 January 1997, Pages 63-72

[45] Applied Biological Material Inc, "General Guidelines for Cell Immortalization" see http:''www.abmgood.com

[46] McLean J.S. (1999) Immortalization Strategies for Mammalian Cells. In: Jenkins N. (eds) Animal Cell Biotechnology. Methods in Biotechnology™, vol 8. Humana Pressi

[47] Shay JW and Wright WE (2000) "Hayflick his limit and cellular ageing" Nature Reviews – Molecular Cell Biology , **1,** 72-76

39. A cell line is a permanently established cell culture that will proliferate indefinitely given that appropriate fresh medium and space is provided. Cell lines differ from cell strains in that they become immortalised, this can result by inducing the cells to virus or chemical agents. [48]

Example application of cell culture (rhino horn and elephant ivory)[49]

40. The horns of most animals have a bony core covered by a thin sheath of keratin, the same substance as hair and nails. Rhino horns are unique, however, because they are composed entirely of keratin, with areas of melanin and calcium deposits that help maintain the structure.

41. The rhinoceros horn is not, however, simply a clump of modified hair; it most closely resembles the structure of horses' hoofs, turtle beaks and cockatoo bills. If the cells forming this horn are isolated, immortalised and grown in culture, arguably a powder could easily be derived that might be used as authentic rhino horn. An elephant tusk, on the other hand, resembles a tooth, enormously enlarged maxillary incisors, the growth of the tusk is continuous throughout life and its size at any age is dependent on the sex of the animal, the rate of attrition and breakage of the tooth, as well as genetic and environmental factors.

42. The implication of the above example is that simple cell culture to produce either rhino horn or the basics of elephant tusk could not provide anything resembling the products sought after by collectors of ivory or of rhino horn unless the desired product is a powder derived from a specific tissue.

Conversion of somatic cells into stem cells

43. Stem cells are undifferentiated cells that have the potential to develop into many different cell types in the organism during early life and growth. In addition, in many tissues they serve as a sort of internal repair system, dividing essentially without limit to replenish other cells. Only fertilised embryos and blastomeres up to the 8-cell stage are considered to be totipotent cells in humans[50]

44. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell.[51] These are unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity. They can be induced to become tissue- or organ-specific cells with special functions. Embryonic stem cells were first identified in 1981; by 1998 a method had been devised to isolate stem cells from human embryos and culture them in the laboratory. "In 2006 researchers made another breakthrough by identifying conditions that would allow some specialized adult cells to be "reprogrammed" genetically to assume a stem cell-like state. These are termed induced pluripotent stem cells (iPSCs)." Embryonic stem cells are

[48] Establishing a Cell Line. In: Introduction to Cell and Tissue Culture. Introductory Cell and Molecular Biology Techniques. Springer, Boston, MA(1998) https://link.springer.com/chapter/10.1007/978-0-585-27571-0_10

[49] Sources:

Ryder ML (§962) "Structure of Rhinoceros Horn" Nature volume 193, 1199–1201

Hieronymus TL, Witmer, LM and Ridgely, Ryan C. (2006) "Structure of white rhinoceros (Ceratotherium simum) horn investigated by X-ray computed tomography and histology with implications for growth and external form" Journal of Morphology, October 2006, 267(10), 1172-1176

Raubenheimer E, Bosman M, Vorster R and Noffke C (1998) "Histogenesis of the chequered pattern of ivory of the African elephant (Loxodonta Africana)" Archives of Oral Biology 43 (1998) 969-977

[50] Jong Soo Kim, Hyun Woo Choi, Sol Choi, Jeong Tae Do (2011) "Reprogrammed Pluripotent Stem Cells from Somatic Cells" International Journal of Stem Cells **4** ,1-8.

[51] https://stemcells.nih.gov/info/basics/1.htm

pluripotent. Adult stem cells are thought to be limited to differentiating into the different cell types of their tissue of origin.[52]

45. If somatic cells, found in normal tissue, could be converted into stem cells, it would be possible to grow any organ, or even arguably, be used as the basis for embryos which could regenerate the whole animal. For example, Takahashi *et al* argue that "Successful reprogramming of differentiated human somatic cells into a pluripotent state would allow creation of patient- and disease-specific stem cells."[53] They were able to show that "Human iPS cells were similar to human embryonic stem (ES) cells in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity. Furthermore, these cells could differentiate into cell types of the three germ layers in vitro and in teratomas. These findings demonstrate that iPS cells can be generated from adult human fibroblasts."

46. The ability to isolate pluripotent stem cells, even where modification of the cells has been used to induce pluripotency is an important step in the regeneration of organs or tissues which may then be used in industrial or commercial contexts

Technology (iii): Tissue (Organ) culture

47. Since its emergence in the mid-1980s, tissue engineering has exploded as an exciting and multidisciplinary field aiming to develop biological substitutes to structure, and even restore, replace or regenerate defective tissues. [55]

48. Tissues and organs contain a multiplicity of different cell types. There is interaction between these cells which is important for their function. Tissues and organs are also three dimensional. The absence of the normal tissue microenvironment can alter cellular responses. Three-dimensional cultures that position cells on synthetic matrices, or organoid or organ-on-a-chip cultures, in which different cell spontaneously organize contacts with other cells and natural matrix only partly overcome this limitation.[54] The media used for culture are the same as for cell and tissue culture, yet trying to keep organs or parts of organs in culture requires the provision of nutrients and oxygen, possibly deep within the tissue.[55] Stimulation of cells in culture to produce 'structures' similar to those produced *in vivo'* remains difficult.

49. While cell cultures provide a monolayer of cells in a petri dish/plastic or glass support, an extension of the culturing technique provides for a gel matrix that can allow tridimensional growth of the cells, in such a way that their confluence can achieve some depth with a few millimetres of height, mimicking tissue-like structures.

50. In order to maintain the relationships between cells in culture they need to be grown in some kind of three-dimensional matrix. In embryonic tissue there is self-assembly, where the cells grow in such a way as to form an organ – involving self-assembly. This is not easily achieved starting from cells in culture. Scaffolds represent important components for tissue engineering. "Apart

[52] NIH Stem Cell Information Home Page. In *Stem Cell Information* [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2016 [cited March 27, 2018] Available at < //stemcells.nih.gov/info/basics/5.htm>

[53] Takahashi et al (2007) "Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors" Cell 131, 861–872, November 30, 2007 861-872

[54] Al-Lamki RS, Bradley JR and Pober JS (2017) "Human Organ Culture: Updating the Approach to Bridge the Gap from In Vitro to In Vivo in Inflammation, Cancer, and Stem Cell Biology." Front. Med. **4**:148. doi: 10.3389/fmed.2017.00148

[55] Chan BP and Leong KW (2008) "Scaffolding in tissue engineering: general approaches and tissue-specific considerations" Eur Spine J **17** (Suppl 4):S467–S479 DOI 10.1007/s00586-008-0745-3

from blood cells, most, if not all other, normal cells in human tissues are anchorage-dependent residing in a solid matrix called extracellular matrix (ECM). There are numerous types of ECM in human tissues, which usually have multiple components and tissue-specific composition."[55] The different cells that form a tissue are necessarily put together in a ratio and spatial arrangement similar to that found in the original tissue. There are many problems with organ culture, including

a. Organ cultures, in general, cannot be propagated, hence for each 'experiment' there is a need for a fresh organ from a donor.

b. Variations are high, and reproducibility is low.

c. Difficult to prepare, besides being expensive

51. Any artificial scaffold needs to fill the tissue void, to provide structural support and to deliver growth factors and/or cells that have the ability to form tissues[56] The table below indicates that which is observed in vivo, and that which is required of an extracellular matrix on which the cells can proliferate.

The extra-cellular matrix in tissue *in vivo*	**Analogous functions of scaffolds in engineered tissues**
Provides structural support for cells to reside	Provides structural support for exogenously applied cells to attach, grow, migrate and differentiate *in vitro* and *in vivo*
Contributes to the mechanical properties of tissues	Provides the shape and mechanical stability to the tissue defect and gives the rigidity and stiffness to the engineered tissues
Provides bioactive cues for cells to respond to their microenvironment	Interacts with cells actively to facilitate activities such as proliferation and differentiation
Acts as the reservoirs of growth factors and potentiates their actions	Serves as delivery vehicle and reservoir for exogenously applied growth-stimulating factors
Provides a flexible physical environment to allow remodelling in response to tissue dynamic processes such as wound healing	Provides a void volume for vascularization and new tissue formation during remodelling[55]

52. It is possible to derive artificial organs which mimic the layout of organs found in living systems using three-dimensional printing techniques where the organ is built up layer by layer using ink-jet printing technologies. It allows for using different cell types to be placed in anatomically correct positions relative to others (equivalent to different colour inks).

Example of organ printing technologies[57,58]

53. The custom designed Integrated Tissue and Organ Printing (ITOP) system consists of a sophisticated nozzle system with a resolution of 2-50 μm that deposits cell-laden hydrogels together with biodegradable polymers. The cell matrix - consisting of gelatine, fibrinogen, hyaluronic acid and glycerol – is optimized to promote cell survival and differentiation whilst providing support and uniform dispersion. Mechanical shape and strength is provided by poly(r -

[56] Howard D, Buttery LD, Shakesheff KM and Roberts SJ (2008) "Tissue engineering: strategies, stem cells and scaffolds" J. Anat. **213** 66–72 doi: 10.1111/j.1469-7580.2008.00878.x

[57] Nature Reviews Rheumatology I Published online 3 Mar 2016; corrected on line 9 March 2016; doi:10.1038/n rrheum.2016.29. - Kao,g, H.W. et al.(2016) A 3D bioprinting system to produce human scale tissue constructs with structural integrity. Nat. Biotech. http;//dx.doi.ocg/10.1038/nbL3413

[58] Graham AD *et al* (2017) "High-Resolution Patterned Cellular Constructs by Droplet-Based 3D Printing" *Scientific Reports* **7**, Article number: 7004(2017) doi:10.1038/s41598-017-06358-x

caprolactone) polymers, which were chosen for their low melting temperature. After printing, the addition of a thrombin solution induces crosslinking of the fibrinogen and the unlinked components are then washed away.

Example application of tissue culture (beef)[59]

54. "Made with some breadcrumbs, egg, and 20,000 lab-grown cow muscle cells, the world's first lab-grown burger made its debut last year. It was a proof of concept, evidence that you can make meat in lab. The technology is too difficult and expensive to show up at grocery stores any time soon. In the future, however, proponents hope so-called cultured meat will get cheaper. If it does, making beef from stem cells could be an environmentally friendly alternative to, you know, killing animals for food."[1] "Producing cultured meat for processed meat products, such as sausages, burgers and nuggets should be comparatively simple, whereas cultured meat which should be more highly structured, such as for an *in-vitro* steak is considerably more of a challenge".

Example application of tissue culture (rhino horn)[60]

55. There is much hype about producing rhino horn in order to produce rhino horn equivalents that my impact on the disastrous poaching and killing of rhinoceros in the wild. A number of companies are (or have been) attempting to print horns using 3D printing technology. The cells are directly derived from the rhinoceros. In addition, powders which are identical to those that can be produced from rhino-horn can be produced from cell in culture.

Example application of tissue culture (elephant ivory)[61]

56. In theory, ivory could also be produced – although the characteristic patters in the structure would be difficult to mimic. Another group of scientists have been examining the cellular and molecular structure of ivory in order to examine the possibility of creating synthetic ivory in the laboratory which would, to all intents and purposes mimic or even be indistinguishable from the real thing. Where there is a need to produce a material which to all intents and purposes mimics the real three-dimensional object, a detailed understanding of the structure would be needed.

III RELEVANT SCIENTIFIC AND TECHNOLOGICAL ELEMENTS TO BE CONSIDERED IN THE CONTEXT OF CITES

57. Where products are manufactured synthetically or derived from CITES-listed species, Regulation will depend on the nature of the product in comparison to that derived from the listed species as to whether they:

    e) are indistinguishable. These would primarily be chemicals, whether high value or simple. For these products, there would be no way in which the synthetic product could be distinguished from that derived from the wild through laboratory testing.

[59] Sources:
Diep F (2014) "What Does It Take To Make Meat From Stem Cells?" Popular Science May 21, 2014.
http://www.futurefood.org/in-vitro-meat/index_en.php
https://www.futuremeat.org/resources

[60] Sources:
Buhr S (2015) Biotech Startup Pembient Is Making Rhino Horns, Sans Rhino"
https://techcrunch.com/2015/04/27/cuzscience/
https://africageographic.com/blog/biologist-aims-to-grow-synthetic-rhino-horns/
https://www.savetherhino.org/latest_news/news/1508_synthetic_rhino_horn_will_it_save_the_rhino

[61] Smithsonian Institute "Appalled by the Illegal Trade in Elephant Ivory, a Biologist Decided to Make His Own"
https://www.smithsonianmag.com/science-nature/faking-elephant-ivory-180963226/

f) could have some sort of ‘’kite-mark’ or biological barcode inserted so that they can easily be distinguished, preferably by simple tests so that border controls can be effective. This could be, for example, slight changes to protein sequence that do not occur naturally and do not have functional impact but are readily observed e.g. colour change

g) have inherent characteristics that enable differentiation e.g. epigenetic differences that are easily detected or sequences in the DNA for which probes can be made which easily distinguish the synthetic from the natural. Once again, the insertion of a ‘kite-mark’ would make differentiation relatively simple.

h) the regularity of the cell structure may allow the distinction between synthetic and wild-based products and could be used as a marker

Chemicals

58. Technologies could be used to produce chemicals extracted from CITES-listed specimens. They are purified ingredients/compounds that could take on the form of e.g., oils, acids, salts, alkaloids, antibiotics, and so on. They may be used in different sectors, including cosmetics, pharmaceuticals, fragrance, and food processing.

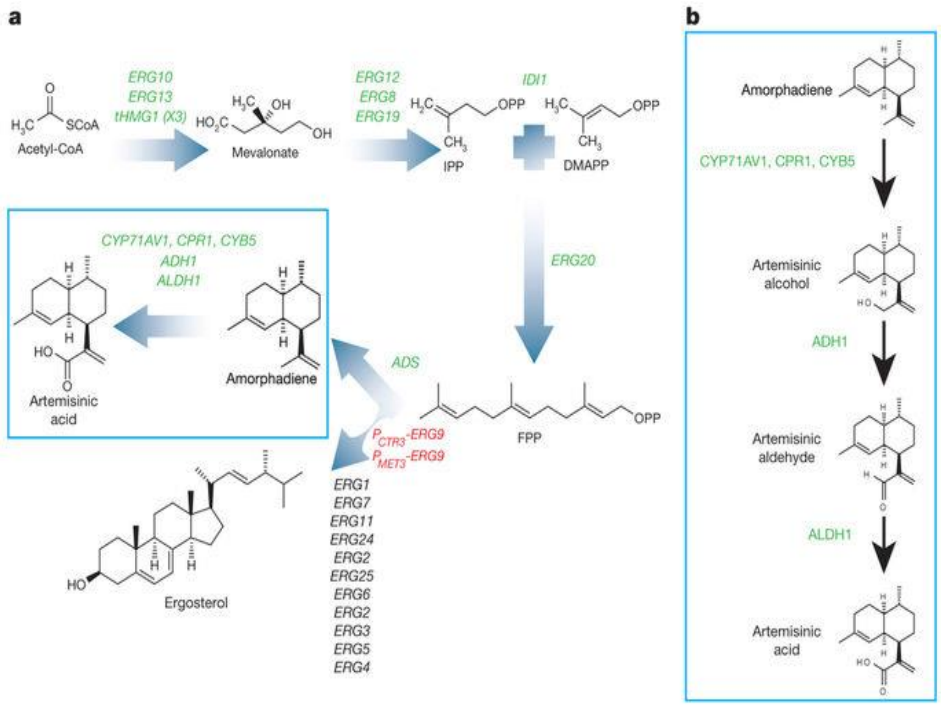
59. As the chemicals are purified, markers cannot be used to indicate the origin of the product. In these instances, there may be no difference between the product produced artificially and that derived from the organism. The chemicals could be synthesised in the laboratory or produced using cell culture of tissues derived from the original organism or from microbes engineered to produce the desired compound. The chemicals could themselves then be modified ensuring greater selectivity for a specific purpose:

a) “Microorganisms have become an increasingly important platform for the production of drugs, chemicals, and biofuels from renewable resources. Advances in protein engineering, metabolic engineering, and synthetic biology enable redesigning microbial cellular networks and fine-tuning physiological capabilities, thus generating industrially viable strains for the production of natural and unnatural value-added compounds.” These could include “synthesis of valued-added products including alkaloids, terpenoids, flavonoids, polyketides, non-ribosomal peptides, biofuels, and chemicals.” [62]

b) An example would be the synthetic production of arteminisin produced by the plant *Artemisia annu*a [63] The authors “determined the full artemisinic acid biosynthetic pathway and developed a process for the production of the antimalarial drug artemisinin by fermentation of simple inexpensive carbon substrates using engineered *S. cerevisiae* to produce artemisinic acid, followed by extraction and chemical conversion to artemisinin.[63]

[62] Jing Du and Zengyi Shao (2011) “Engineering microbial factories for synthesis of value-added products” J Ind Microbiol Biotechnol. 2011 Aug; 38(8): 873–890

[63] Paddon C J *et al* (2013) “High-level semi-synthetic production of the potent antimalarial artemisinin” Nature **496**, 528–532 (25 April 2013) doi:10.1038/nature12051



c) A second example of high value chemicals that are now produced synthetically are musks. "Synthetic musks, known as white musks in the perfume industry, are a class of synthetic aroma compounds to emulate the scent of deer musk and other animal musks (ambergris, castoreumand civet). Synthetic musks have a clean, smooth and sweet scent lacking the faecal notes of animal musks. They are used as flavourings and fixatives in cosmetics, detergents, perfumes and foods, supplying the base note of many perfume formulas. Most musk fragrance used in perfumery today is synthetic".[64]

d) Technologies could be used to produce herbicides or pesticides that impact as minimally as possible on non-target organisms through increased selectivity and better understanding of their mode of action. For example, the information derived from the DNA of the CITES protected species could arguably be used to design chemicals used in agriculture which specifically have little or no effect on particular species.

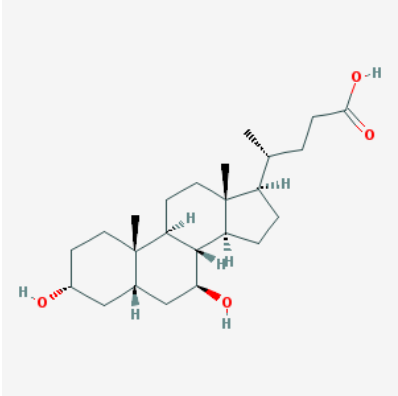
e) Microbial cells can be altered to produce specific chemicals – a simple example is the production of vitamin C (ascorbic acid) using yeasts[65] There is a huge diversity in microbial cell types that could be used as 'cell factories' - bacteria, archae, yeast and filamentous fungi. These 'factories' are used in structural biology, food microbiology, natural products, biomining, nanotechnology and biosensing, for example. [66] Although microbial cells are not subject to the requirements of the Convention, their products could either alleviate the stress on endangered organisms through their no longer being required or even increase the commercial stress on the *real* products

f) "Plant sources of most high-value natural products (NPs) are not domesticated and therefore their production cannot be undertaken on an agricultural scale. Further, these plant species are often slow growing, their populations limiting, the concentration of the target molecule highly

[64] https://en.wikipedia.org/wiki/Synthetic_musk (accessed 6 May 2018)
[65] Sauer M *et al (2004)* "Production of l-Ascorbic Acid by Metabolically Engineered *Saccharomyces cerevisiae and Zygosaccharomyces bailii"* Appl Environ Microbiol. 2004 Oct; 70(10): 6086–6091. doi: 10.1128/AEM.70.10.6086-6091.2004
[66] Maurilio De Felice *et al* (2008) "The scientific impact of microbial cell factories" Microb Cell Fact. 2008; 7: 33. Published online 2008 Dec 1. doi: 10.1186/1475-2859-7-33

variable and routinely present at extremely low concentrations". Techniques for culturing the cells of such plants and for modifying, either through the techniques described in the previous sections or through identifying the conditions that enable efficient production of the desired products are now well-understood. Modifying the expression of genes in such plants could permit their commercial exploitation, ensuring the survival of the original plants in the wild. The identification of plants that are endangered, but which produce NPs, and for which exploitation could lead to extinction is important so that the techniques described in this paper could be used to alleviate the environmental pressure.

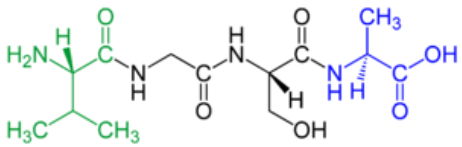
g) Bear bile has been a well-known Chinese medicine for thousands of years[67]. One of its constituents, Ursodeoxycholic acid, is a bile acid which is produced naturally in mammals which was first found in bears. It works both by reducing the amount of cholesterol released by the liver and by dispersing cholesterol. It can be produced synthetically, and it is used to break up or solubilise gallstones. Synthetic ursodiol was first studied as a pharmaceutical treatment for gallstones in the 1970s and for primary biliary cirrhosis in the 1980s. The synthetic procedure often includes the use of microorganisms (or moulds) to convert a chemical into that which is similar or identical to the original bile acid. It is a relatively simple chemical.



60. Impurities in the chemical extracts of natural-sourced products may permit differentiation from synthetic products, which may only contain the active (target) chemical compound.

61. Oligopeptides (sometimes simply called peptides) are short chains of amino acids linked as found in proteins and consisting of between 2 and 20 amino acids. They are important as they are often capable of entering a cell where a larger molecule could not. Many will have physiological function, and some are extremely toxic to humans and animals (an example is alpha amanitin – the main toxin from the species Amanita phalloides). They are produced by a wide variety of organisms and have many uses in research and therapeutics. Different organisms will produce a variety of different oligopeptides, often as a defence against predators – snake venom, for example, contains a range of oligopeptides. If a particular peptide is identified in a CITES listed species, it can easily be synthesised from the basic building blocks using chemical synthesis or through reverse engineering to produce a gene which will make the required peptide and introduce the gene into a micro-organism.

[67] Sha Li, Hor Yue Tan, Ning Wang, et al., "Substitutes for Bear Bile for the Treatment of Liver Diseases: Research Progress and Future Perspective," Evidence-Based Complementary and Alternative Medicine, vol. 2016, Article ID 4305074, 10 pages, 2016. https://doi.org/10.1155/2016/4305074



A tetrapeptide (Val-Gly-Ser-Ala)

Proteins

62. Proteins could be synthesised and be available commercially:

    a) The proteins could be indistinguishable from those derived from nature but in some instances it may be desirable, in order to protect the native species, to require minor changes to be made to the protein sequence of the synthetically produced protein such that the modification does not impact in a negative manner on its efficacy (whatever the purpose of production) but could be used for detection purposes.

    b) The production of proteins within organisms is likely to be influenced by the environment, and **gene drive** could be utilised in order to minimise the impact of altered environments (such as that induced by climate change). Caution would have to be exercised, as the modification could result in invasion of non-indigenous habitats. An example of an analysis taking this into account is an examination of the impact of climate change on the proteins and gene expression of some freshwater fish.[68]

    c) It is not only simple chemicals that have been identified in living organisms that can be produced synthetically, or through the use of microorganisms. Biologics[69] are a diverse range of products which may be derived synthetically or may be extracted from tissues or organs. Some are highly purified, and can be precisely identified – short strings of amino acids, or even some proteins. The production of others depends on their source – monoclonal antibodies might differ when produced in different animals even though they have the same function. Factor C, for example, is a serine protease proenzyme which is involved in clotting (it is implicated in anti-coagulation, inflammation, cell death and maintaining the permeability of blood vessels). Recombinant Factor C is a non-animal derived reagent used to detect bacterial endotoxins in pharmaceutical products. [70]

Cells, tissues, and higher (up to whole organisms)

63. The technologies enable the production of specific cells or tissues, modified or otherwise, which could be used to produce products that impact on CITES protected species. As identified in a previous section, the technologies could be used to immortalise these cells, allowing the culture and production of vast quantities of desired cells. Genetic markers could be inserted into the genome of the cultured products which could allow for simple tests as to whether a product has been synthesised or derived from the endangered organism. For some complex multi-cellular products, the absence of contaminants and the regularity of the cell structure may allow the

[68] Jesus TF *et al* (2017) "Protein analysis and gene expression indicate differential vulnerability of Iberian fish species under a climate change scenario" PLoS One. 2017 Jul 18;12(7):e0181325. doi: 10.1371/journal.pone.0181325. eCollection 2017.

[69] https://www.fda.gov/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cber/ucm133077.htm

[70] Bolden J and Smith K (2017) "Application of Recombinant Factor C Reagent for the Detection of Bacterial Endotoxins in Pharmaceutical Products", J Pharm Sci Technol. Sep-Oct;71(5):405-412. doi: 10.5731/pdajpst.2017.007849. Epub 2017 Jul 20.

distinction between synthetic and wild-based products. 3D printing technology could be used to mimic irregularities and contaminants in the synthetic product.

64. It would theoretically be possible to replicate specific tissues, such as skin, wood, ornamental plants, fur, corals for jewellery and even meat:

    a. The culturing of ornamental plants such as orchids (which require a particular micro-environment) has advanced significantly in recent years.[71]

    b. Reptile skin is covered with scutes or scales which, along with many other characteristics, distinguish reptiles from animals of other classes. Scales are made of alpha and beta keratin and are formed from the epidermis (contrary to fish, in which the scales are formed from the dermis) [72].

    c. In order to produce muscle tissue myo-satellite cells or those from embryonic or adult stem cells which have the capacity to differentiate into other, specialised cells could be used[73]. "Cells must be cultured in a suitable growth medium, but muscle cells alone cannot produce a muscle. A scaffold is required to provide a structure for cell attachment, and to support cell differentiation and proliferation and permit ingress of blood and other nutrients into the mass of cells. Collagen and fat are also needed in the structure created [74]

    d. Examples include that of Atlantic bluefin tuna which is heading towards extinction, but synthetic, cultured fish muscle could replace wild-caught fish and safeguard the species.[75]

IV RELEVANT ELEMENTS THAT MAY BE CONSIDERED FROM A LEGAL AND REGULATORY PERSPECTIVE

65. This section of the study explores the following issues from the terms of reference of the study:
    i) The pertinence and relevance of including an operational definition of the term 'part or derivative' in Resolution Conf. 9.6 (Rev. CoP16) in the context of the discussion on products produced by technologies described in Section I above; and

    ii) the pertinence and usefulness of creating a new source code for "bioengineered" wildlife products derived from synthetic or cultured DNA as a separate category of specimens.

66. There would appear to be three possible scenarios for products (including whole organisms) that are produced using new biological technique. This description of a product applies to *specimens*, *parts* and *derivatives*:

    a. The manufactured product is treated as if it is the listed species, with all requirements already identified for the product from which it is derived applying in exactly the same manner,

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[71] For a review, see *Recent Advances in Orchid Tissue Culture*: Semiarti, Endang & ROLES OF CLASS-1 KNOX GENE IN SHOOT DEVELOPMENT A REVIEW. Journal of Biological Researches. 20. Or Recent Advances in Orchid Tissue Culture - https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=5&ved=0ahUKEwi-we_fyrLbAhXjPZoKHRoYBk4QFghYMAQ&url=https%3A%2F%2Fwww.researchgate.net%2Ffile.PostFileLoader.html%3Fid%3D515568bfd11b8bd22d00000c%26assetKey%3DAS%253A272106741272579%2540144188671116 4&usg=AOvVaw3jt7vtZIEL4pgePn2Masyu

[72] See https://en.wikipedia.org/wiki/Reptile_scale

[73] Myosatellite cells are the precursors of skeletal muscle cells and differentiate in order to form muscle fibres

[74] Ralph Early (2018) https://www.foodethicscouncil.org/blog/177/19/Is-clean-meat-the-meat-of-the-future/ Accessed 24/05/18

[75] Finless Foods: https://finlessfoods.com/

b. The manufactured product is treated as a totally new substance which is not related (for regulatory purposes) to that from which it is derived. If manufactured, it can be assumed to not be endangered in any way, and hence requires no action when traded across international borders from member states, and

c. The product is sufficiently related to the parent organism, and a regulatory system would have to be provided by the country in which it is manufactured to demonstrate that cross-border trade could be permitted for the product.

67. The product derived using modern biotechnological techniques could range from a whole organism which could be produced from one or more cells from the parent organism (examples currently regulated include the production of orchids in culture) to derivatives or parts of the original organism (see definition of these below) or could be derived from cells isolated from 'waste products' (e.g. strands of hair/fur, faeces or even urine). Each of these products could fall within any of the three possibilities listed in paragraph 66. In addition, it is possible that material could be synthesised *de novo* using sequences listed in databases and introduced into unrelated organisms for the manufacture of products that are to all intents and purposes identical to that derived from listed species but which have no direct link to that species.

68. Three-dimensional printing, or similar techniques, provides for competent organs or tissues that contain a variety of different cells organised in a manner which mimics that found in similar tissues found in the natural organism. It is already almost possible to produce working organs using these techniques that not only mimic the look and feel of the original, but also are capable of performing the same function.

69. There are many uses of the new technologies described in the above section that may have an impact on the implementation of CITES, directly or otherwise. On one hand, they may be used to replace products that are currently derived from species regulated by CITES, which may result in reducing pressure on the species in the wild, while continuing to fulfil the market with the desired product. On the other hand, the commercial availability of the synthetic product may lead to an increased demand of the product, driving some suppliers to mix wild-sourced products into the market.

70. Tissues or artefacts that are manufactured synthetically are likely to impact on the 'original' product – whether this would increase the likelihood of greater or lesser exploitation of the endangered species may depend on the ability to distinguish the two, among other factors, so that different regulatory measures may need to be applied.

71. Where it is impossible to differentiate between products derived from CITES regulated species and synthetic products, the synthetic product should be considered as regulated in the same way as the original product. Where they can be differentiated, some form of regulation is likely still to be necessary.

72. Where there is no direct link between the product and the CITES regulated species, but where the product is chemically identical (or almost identical) to that derived from a listed species a certification or permit system differentiating that product becomes essential

Definition of the term 'part or derivative'

73. Before considering the term 'part or derivative' it is necessary to recall that the Convention requires permits for any "specimen" of a species included in the three appendices. A specimen is defined in Article 1(b) of the Convention:

> "Specimen" means:
>
> (i) any animal or plant, whether alive or dead;
>
> (ii) in the case of an animal: for species included in Appendices I and II, any readily recognizable part or derivative thereof; and for species included in Appendix III, any readily recognizable part or derivative thereof specified in Appendix III in relation to the species; and
>
> (iii) in the case of a plant: for species included in Appendix I, any *readily recognizable part or derivative* thereof; and for species included in Appendices II and III, any readily recognizable part or derivative thereof specified in Appendices II and III in relation to the species

74. The definitions for "part or derivative" in the context of bioengineered products may be considered on the basis of the following:[76]

    a. **Part** is defined in the CITES glossary of terms as "Any part of an animal or plant (e.g. skin, shell, root) whether raw or processed in a simple way (e.g. preserved, polished, etc.). Parts are usually readily identifiable. On the other hand,

    b. **Derivative** is identified in the glossary as "any processed part of an animal or plant (e.g. medicine, perfume, watch strap)."[77]

75. It is assumed for the purposes of this paper that a synthetic tissue or organ produced using the new technologies could be a **part** as defined above, as it could resemble in most particulars a part directly obtained from the animal or plant. The production of derivatives using cells, tissues or DNA obtained from an animal or plant could then be addressed through the same procedures as currently used.

76. The new technologies could provide mechanisms identifying the genes that make a particular organism different from others, extracting those genes and inserting them into other species. In addition, organisms can be used to produce products normally associated with other species. It is this that permits the manufacture of chemicals, biologics, tissues or organs normally associated with other species. If a common species is used to manufacture a product which would normally be associated with a CITES listed species, could this be considered to be a derivative? Even though the DNA used to make the chemical, it forms an almost insignificant part of the genome of the original species, or could even be a totally synthetic gene modified in the recipient organism which has genes that would normally perform the same function as that from the original. It may therefore be prudent not to regulate these products.

77. Resolution Conf. 9.6 (Rev. CoP16) limits the definition of parts and derivatives in paragraph 3(b) by providing that "urine, faeces and ambergris that has been naturally excreted are waste products and are therefore not covered by the provisions of the Convention". Cells that might be used for

[76] see https://cites.org/eng/resources/terms/glossary.php#part as well as Resolution Conf. 10.13 (Rev. CoP15)
[77] https://cites.org/eng/resources/terms/glossary.php#part

propagation in culture, and eventually leading to products or tissues associated with a listed species could be derived from such waste products. It is not absolutely clear that, having been designated as not being covered by the convention could, and perhaps should, once artificially propagated, fall within the definition of part or derivative.

78. It is useful in this context to consider the discussion on the definition of the term 'artificially propagated' (Decision 17.175-17.177, 16.156 (Rev. CoP17)) as it has been possible to regenerate plants from cuttings or even single cells for some considerable time. The definition in Resolution Conf. 11.11 for trade in plants provides a definition of *artificially propagated* for plants as those grown from "seeds, cuttings, cuttings, divisions, callus tissues or other plant tissues, spores or other propagules that either are exempt from the provisions of the Convention or have been derived from cultivated parental stock". Plants would then be artificially propagated if the traded specimens do not contain any material collected from the wild.[78]

79. If the definition of artificially propagated is extended to all 'organisms' from which cells or DNA has been extracted and propagated in some way that could not have been achieved naturally, an additional regulatory system could be instigated to permit trade in these derivatives.

80. There are other types of "part or derivative" that may result from bioengineering.[79] In the context of CITES, an *extract* is defined as "[A]ny substance obtained directly from plant material by physical or chemical means regardless of the manufacturing process. An extract may be solid (e.g. crystals, resin, fine or coarse particles), semi-solid (e.g. gums, waxes) or liquid (e.g. solutions, tinctures, oil and essential oils)."[80] This definition is specific to plants, but could be extended to address artificially propagated tissues, biologics or chemicals from any species through the use of modern biotechnology.

81. In contrast to chemicals whose structure is known, most parts and derivatives have a complex composition that are not easily identified or characterized. In some instances, these products could have a marker inserted during the manufacturing process. A problem remains, as they tend to be complex mixtures or structures that have been deliberately engineered to involve many different cell types and DNA and or proteins. .

82. Clarification provided in Resolution Conf 9.6 (Rev CoP16) that 'readily recognisable part or derivative' is defined as "any specimen which appears from an accompanying document, the packaging or a mark or label, or from any other circumstances, to be a part or derivative of an animal or plant of a species included in the Appendices, unless such part or derivative is specifically exempted from the provisions of the Convention.

83. The previous sections of this report have indicated that it may be extremely difficult to identify products produced by modern biotechnology and derived from wild specimens of the species identified in CITES Appendices I, II and III by looking at the specimen or by analysing the product itself. In some instances, biological markers incorporated in the synthetic material may provide a mechanism for differentiation, although this will not apply to chemicals (extracts), which cannot in general be identified using any form of marker, and arguably directly replace that which would have been derived from listed species.

84. The most effective procedure to make them "readily recognisable" would therefore be to require a regulatory system to prove that the products are produced synthetically, while biological

[79] https://cites.org/eng/resources/terms/glossary.php#extract and document CoP16 Doc. 75 (Rev. 1)
[80] Taken from document CoP16 Doc. 75 (Rev. 1). See https://cites.org/eng/resources/terms/glossary.php#e

markers would be available where concerns required further tests to assure that the products were indeed synthetic.

85. Products that are derived from tissue or cell culture of cells or DNA that has been extracted from listed species may pose real problems for those entrusted to ensure that trade in listed species does not occur without certification. In many cases, the products (parts, derivatives or extracts) are virtually indistinguishable from those directly derived. It may be that unscrupulous dealers would attempt to pass-off or launder 'the original product as one that has been synthesised using modern techniques. Once again, an effective regulatory system may be the only way in which this can be discouraged. The equivalent of phytosanitary certificates could be utilised as certificate of artificial propagation[81].

86. Those entrusted at borders with ensuring that trade in endangered species is within the law may well be unable to enforce the requirements as they will not have to technologies in place to distinguish *real* from *synthetic*. Although probes for specific biological barcodes could be available relatively cheaply, the vast range of different probes needed makes this non-viable. Where the product is relatively simple (protein or tissue powder) detection systems at borders may be readily available, but fraud will be possible through adulteration.

87. A consideration will have to be undertaken to identify changes to law (both criminal and environmental) to address fraud where synthetic products are virtually indistinguishable from that derived from the wild. It is likely that synthetic derivatives of listed species will be produced, whether as chemicals, biologics, tissues or structures. A system will have to be instituted to regulate these parts and derivatives which may arise from a single cell of one individual animal/plant from the wild.

Existing procedures within CITES

88. In many cases the products may draw comparisons to how 'look-alike' species[82] are being treated in the Convention. There are difficulties in introducing differential regulatory controls. Similar to that indicated in the paper cited in footnote 82 an additional regulatory mechanism could be instigated in order to differentiate and regulate commercial trade in some of the derivative products which cannot be simply identified. More complex derivatives or parts may have to be identified as listed as if they were directly derived from the original listed species.

89. "Look-alike" products should be treated as derivatives under the Convention unless systems are put into place that enable differentiation through a regulatory system where appropriate. This would particularly be true where tissue culture and organ culture have been used to produce products that closely resemble three-dimensional structures.

90. It has been suggested that certain biological samples should not be subject to the provisions of the Convention, including i) synthetically derived DNA that does not contain any part of the original template; ii) urine and faeces; and iii) synthetically produced medicines and other pharmaceutical products such as vaccines that do not contain any part of the original genetic material from which they are derived (proposal CoP12 Prop.1). It remains important that there are simplified procedures to expedite the issuance of permits and certificates where trade would have a negligible impact (or none) on the conservation of listed species. The inclusion of some synthetic products in such a scheme (where appropriate) may be an effective way in which the trade in those products could be allowed.

[81] see https://cites.org/eng/imp/Exemptions_and_special_procedures
[82] https://cites.org/sites/default/files/eng/cop/07/doc/E07-30.pdf

Relevant discussions outside of CITES

91. It has been argued, for example, that cell-cultured foods need regulations on food safety, processes and procedures. Indeed, the US Food and Drug Administration has organised a meeting (12th July 2018) to "gather comments and research on foods produced using animal cell culture technology," The FDA Commissioner has stated that ""We expect that most or all starter cells for food applications will come from living animals for the foreseeable future for commercial and marketing reasons. While currently animal cells can be produced from the starter cells in bioreactors, businesses are also working to commercialize processes by which cells can be cultured using biocompatible scaffolding or other techniques to permit the formation of complex tissues." This is in line with that discussed in this paper.

Convention on Biological Diversity (CBD)

92. The CBD has recently convened an open-ended online forum on the impact of synthetic biology. It had already indicated to parties that the precautionary approach should be used when assessing the use of synthetic biology in relation to the aims of the Convention. including an evaluation on the availability of tools to detect and monitor the organisms, components and products. The most recent discussion on this topic took place at the last meeting of the Subsidiary Body on Scientific, Technical and Technological Advice (SBSTTA-22) in July 2018.[83] The is attached to this paper.

93. The current discussion on synthetic biology under CBD, including recommendation adopted at the meeting at SBSTTA-22[84] are summarized below:

    a. Due to rapid developments in the field of synthetic biology (which is broadly defined in these papers) the CBD will have to monitor and assess developments including gene editing in order to review "new information regarding the potential positive and potential negative impacts of synthetic biology vis-à-vis the three objectives of the Convention and those of its Protocols. (UNEP/CBD/COP/12/INF/11)[85]

> The objectives of this Convention, to be pursued in accordance with its relevant provisions, are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to technologies, and by appropriate funding.

    b. The products are derived synthetically, in most cases are not whole organisms and most of the techniques used in their production will be *contained use*[86] in industrial facilities, the *precautionary approach* may not be as important as that for organisms released into the environment (in addition, note article 6 of the Protocol). Where synthetic organisms are capable of replication within the environment the precautionary approach as defined in the Protocol would apply.

[83] https://www.cbd.int/doc/c/6e0d/b361/a877d43db3665160cce5d96e/sbstta-22-04-en.pdf
[84] https://www.cbd.int/meetings/SBSTTA-22
[85] https://bch.cbd.int/synbio/
[86] The Cartagena Protocol on Biosafety to The Convention on Biological Diversity defines "Contained use" as "any operation, undertaken within a facility, installation or other physical structure, which involves living modified organisms that are controlled by specific measures that effectively limit their contact with, and their impact on, the external environment" https://www.cbd.int/doc/legal/cartagena-protocol-en.pdf

c. The conservation and sustainable use of biodiversity, and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources may be affected, both positively and negatively, by living organisms resulting from synthetic biology, as well as by non-living products or components." [87]. In many instances the synthetic products may be derived from cells collected from organisms that are protected within their country of origin, and the requirements of national legislation as to the removal from or commercial exploitation of these resources must be respected

d. "Given the current uncertainties regarding engineered gene drives, the free, prior and informed consent of indigenous peoples and local communities might be warranted when considering the possible release of organisms containing engineered gene drives that may impact their traditional knowledge, innovation, practices, livelihood and use of land and water"

e. "Furthermore, the relationship between synthetic biology and its ethical implications for societal views towards nature, as well as the relationship between mankind and ecosystems, were noted as cross-cutting issues with respect to all three objectives of the Convention."

94. There are significant differences between the objectives of the CBD (including the protocol and supplementary-protocols) and that of CITES. For the Cartagena Protocol the issue is primarily that of any impact on the environment due to the release of modified organisms into the environment. Its objective is:

> "In accordance with the precautionary approach contained in Principle 15 of the Rio Declaration on Environment and Development, the objective of this Protocol is to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements."

95. If an organism is to be released into the environment and could have an adverse impact on that environment a detailed risk assessment is required (Article 11) and risk management procedures are identified in Article 12. Where the organism is to be transferred across an international border the exporting country must arrange for notification to the importing country prior to the intentional trans-boundary movement. The risk assessment requirements are set out in annex III to the Protocol specifying that objective of the assessment is to identify and evaluate the potential adverse effects of the LMOs on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account risks to human health. These requirements would apply to any viable organisms, including live specimens of CITES-listed species as well. They would not apply to

a) Parts or derivatives that are not viable organisms which might be released into the environment

b) Products that are intended for use within containment even where they are viable organisms

96. The Nagoya – Kuala Lumpur supplementary protocol on liability and redress to the Cartagena Protocol on Biosafety (Supplementary Protocol), addresses the need for rules and procedures in the field of liability and redress related to living modified organisms. The Supplementary Protocol

[87] https://www.cbd.int/doc/c/0bc5/ef82/a4da41e530a897de6abc3ca7/sbstta-22-inf-17-en.pdf

requires that response measures are taken in the event of damage resulting from living modified organisms, or where there is sufficient likelihood that damage will result if timely response measures are not taken. The Supplementary Protocol also includes provisions in relation to civil liability. Its provisions would apply only to viable organisms that cause damage in the environment following a trans-boundary movement

97. The Nagoya Protocol may have greater impact for transboundary movement of products from endangered species, regardless of whether they are produced using modern biotechnology. It aims at the sharing of the benefits arising from the utilization of genetic resources in a fair and equitable way. Its objective is

> The objective of this Protocol is the fair and equitable sharing of the benefits arising from the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to technologies, and by appropriate funding, thereby contributing to the conservation of biological diversity and the sustainable use of its components.

98. There are three issues for CITES when considering the production of viable organisms which are derived from CITES listed species and produced using modern biotechnology if they are deliberately or inadvertently released (see Resolution Conf. 13.10 (Rev. COP 14):

a. within the country in which they are made, and which could impact on the biodiversity and on CITES listed species in that country

b. within a second country where the CITES listed species exists and therefore there may be a potential risk of harm to the listed species, including sexual recombination between the released species and the native species

c. within a country in which the CITES listed species does not occur, but in which the released organism has the potential to grow and spread. The impact on the natural environment, but also on trade in products derived from that species which could impact trade.

United Nations Food and Agriculture Organisation (FAO)

99. FAO has recently published a document on "*Potential implications of new synthetic biology and genomic research trajectories on the International Treaty for Plant Genetic Resources for Food and Agriculture*"[88] (October 2017). It is envisaged that techniques of modern biotechnology would impact on "(1) mining plant genomic information for gene editing purposes in agriculture; (2) mining for use outside of agriculture; and (3) using the plant as a 'workhorse' to produce other products.

100. The paper clarifies that the use of sequence information of species in order to produce new products may not result in transparency in the manner in which the original material has been obtained. The implications of the use of genetic information from for which benefit sharing should be adduced is also considered in the paper.

101. The production of whole organisms, derivatives or parts may impact adversely (or positively) on those who depend on the exploitation of the natural organisms. This would be taken into

[88] http://www.fao.org/fileadmin/user_upload/faoweb/plant-treaty/GB7/gb7_90.pdf

account through the mechanisms of CBD, but could impact on the manner in which licencing for the production of these parts or derivatives is addressed under CITES.

102. The products of modern genetic techniques are likely to be similar (if not identical to) those derived from their 'natural' equivalents, but the manner in which they are traded may be different. There are positive and negative factors in the deployment of these new techniques. It is unlikely that use could be stopped. The products would either have to be treated as if they impacted on the wild species, and hence subject to the same regulatory regime as that listed in the appendices or in some circumstances the products could be regulated separately from that which pertains to products derived from the wild.

Possible options for a new regulatory system/mechanism to address parts and derivatives produced from biotechnology

103. Simplified procedures could be used for the issuance of permits and certificates, if there is a need to facilitate and expedite trade that will have a negligible impact[89] on the conservation of the species concerned in the wild. In particular where the artificial manufacture of products from listed species where they are sufficiently related to the parent organism yet are totally synthetic. A regulatory system would have to be provided by the country in which it is manufactured to demonstrate that cross-border trade could be permitted under the following conditions:[90]

a. There would be a need to maintain a register of persons and bodies that may benefit from simplified procedures, as well as the species that they may trade under the simplified procedures. Parties would have the responsibility to ensure that the registration is effectively maintained. In these circumstances it could be industrial laboratories and factories that are identified as producing these products.

b. The procedures would have to provide for permits and certificates that fully identify the synthetic product and the reasons for such permits or certificates. This may be possible using a new source code to explicitly provide that the products may have been derived from DNA or tissue derived from the wild or synthesised from a known DNA sequence, that they are not "taken from the wild" and will not negatively impact on the conservation or preservation of the wild species directly.

c. The products could be viable organisms which are capable of propagation in the wild (an example would be orchids), viable organisms that are unlikely to survive in the wild (could be deliberately disabled to ensure that they could not survive), derivatives and parts of organisms or completely synthetic samples derived from information about the listed species (published DNA sequences, for example). They could also be indirectly derived from listed species using published DNA sequences rather than DNA, cells or tissues from the listed species. Permits and certificates would have to identify which of these possibilities apply.

d. CITES documents would have to show that the products are not ranched specimens, not animals bred in captivity, or only plants that are *artificially propagated* in that genetic material or cells have been subjected to biotechnological propagation. They may also need to be required to indicate the manner in which the precautionary approach has been addressed where the resulting product is a viable organism or where it could impact on organisms in the wild.

[89] Or even a positive impact
[90] Adapted from document 51 for COP12

e. A mechanism would have to be introduced allowing for the authorisation of registered persons or bodies to enter specific information on the face of any CITES document in order to fully characterise the synthesized product and provide the reasons for exemption from the requirements for listed species. It may be done in a manner similar to the current CITES registers[91] for operations that breed Appendix-I animal species for commercial purposes, operations that artificially propagate specimens of Appendix-I species for commercial purposes, and Scientific institutions entitled to the exemption provided by Article VII, paragraph 6, of the Convention.

f. Provision for any other special conditions that would be applied for that particular product could be provided.

[91] https://cites.org/eng/resources/registers.php