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OPP Docket
Environmental Protection Agency Docket Center
(EPA/DC), (28221T)
1200 Pennsylvania Ave. NW
Washington, DC 20460–0001

Re: Proposed exemptions under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and the Federal Food, Drug and Cosmetic Act (FFDCA) for certain Plant Incorporated Protectants (PIPs) created through biotechnology [EPA-HQ-OPP-2019-0508]

Dear Sir/Madam,

These comments are submitted on behalf of Beyond Pesticides. Founded in 1981 as a national, grassroots, membership organization that represents community-based organizations and a range of people seeking to bridge the interests of consumers, farmers and farmworkers, Beyond Pesticides advances improved protections from pesticides and alternative pest management strategies that reduce or eliminate a reliance on pesticides. Our membership and network span the 50 states and the world.

We are writing in response to the call for comments on the proposed exemptions for certain plant incorporated protectants (PIPs) created through cisgenic biotechnology. Existing exemptions for PIPs are limited to those developed through conventional breeding. The proposed rule changes would allow cisgenic PIPs created through biotechnology to also be exempt under these existing regulations, in cases where those PIPs 1) pose no greater risk than PIPs that meet EPA safety requirements, and 2) could have otherwise been created through conventional breeding. The proposed rule also includes a process through which developers of PIPs based on sexually compatible plants created through biotechnology submit either a self-determination letter or request for EPA confirmation that their PIP meets the criteria for exemption. For increased flexibility in bringing PIPs to market, a developer can also submit both. Although cisgenic gene editing in crop plants generally pose less risk than transgenic PIPs, there are risks from cisgenic biotechnology greater than exist in those developed through conventional breeding alone. These potential risks are not inconsequential and, therefore,

blanket exemptions from existing regulations should not be granted. Rather than allowing developers to simply submit a self-determination letter or request from EPA for confirmation that their PIP meets the criteria for exemption, a developer should instead request a formal exemption and submit supporting documentation demonstrating safety requirements are met.

With the advent of new gene editing technologies, PIPs are being developed by several innovative approaches. Transgenic gene editing results in a genetically modified organism (GMO) based on the transfer and random insertion into the host plant genomes of genes isolated from other plant species or from other organisms. In cisgenic and intragenic gene editing methods, the transfer of genes and regulatory sequences are derived only from other genotypes of the same (intragenic) or sexually compatible species (cisgenic). In case of cisgenesis, the entire gene with its own regulatory sequences and in-sense orientation is transferred. Intragenesis uses different coding and regulatory sequences assembled either in sense or in antisense orientation, the latter if the aim is to reduce gene expression by activating the RNA interference (RNAi) pathway. Because PIPs created with cisgenic gene transfers are deemed consistent with conventional breeding methods, these are naively considered to have minimal risks and warrant exclusion from regulatory scrutiny. While, at least in principle, cisgenic products can be achieved by conventional breeding, the same is not true for intragenic ones.[1] Also, even though a cisgenic PIP may in principle be produced by conventional breeding, the complexity and time required to do so makes such an endeavor in many cases impractical.

In fact, cisgenenic PIPs pose many of the same risks as transgenic PIPs. The gene cassette developed to transfer a cisgene like a transgene will also include DNA sequences from at least one other species, and therefore the gene cassette as a whole will be transgenic. In addition, cisgenesis involves tissue culture, a highly mutagenic process. The only difference between cisgenic and transgenic crops is the choice of organism from which the main gene of interest is taken. It has been argued that this proposed cisgenic/transgenic distinction arises more from political than scientific considerations.[2] Experiments confirm that cisgenesis can result in significant unanticipated changes to a plant. The results of these experiments showed that a trait introduced via a cisgene can result in plants that differ in unanticipated and dramatic ways from their conventionally bred counterparts.[3] The differences observed would have important implications relevant to health and ecological risk assessments.

Cisgenic plants are created using the same highly mutagenic plant transformation techniques used to create other transgenic plants.[4] Cisgenes, like transgenes, may include anti-sense sequences, sequence changes to elude feedback inhibition and combinations of gene

[1] Cardi, T., 2016. Cisgenesis and genome editing: Combining concepts and efforts for a smarter use of genetic resources in crop breeding. *Plant Breeding*, *135*(2), pp.139-147.

[2] Schubert D and Williams D. 2006. 'Cisgenic' as a product designation. Letter to Nature Biotechnology 24(11): 1327-1329.

[3] Wilson A, Latham J. 2007. Cisgenic plants: Just Schouten from the hip? Indep Sci News. Available at: http://www.independentsciencenews.org/health/cisgenic-plants/.

[4] Wilson, A.K., Latham, J.R. and Steinbrecher, R.A., 2006. Transformation-induced mutations in transgenic plants: analysis and biosafety implications. *Biotechnology and Genetic Engineering Reviews*, *23*(1), pp.209-238.

coding and regulatory components from different genes and species.[5] It has been demonstrated under field conditions, that both the gene edited and conventional herbicide-resistant plants show decreased total seed numbers as compared to the herbicide-sensitive wild-type parents. However, when nutrients are added to field-grown plants, only the cisgenic plants still showed a fitness decrease.[6,7] Levels of outcrossing have also been reported to be higher in all engineered lines carrying the cisgene as compared to conventionally bred plants.[8]

The predominant gene editing systems are based on specific enzymes, including: (a) meganucleases, (b) zinc finger nucleases (ZFN), (c) transcription activator-like effector nucleases (TALEN) and (d) clustered regular interspaced short palindromic repeats/CRISPR-associated nucleases (CRISPR/Cas). These innovative gene editing methods make genetic engineering faster, easier, and more efficient. The CRISPR system is perhaps the most prevalent in the current production of cisgenic PIPs. The CRISPR approach involves expressing an RNA-guided endonuclease such as Cas9 along with guide RNAs (gRNA) directing it to a particular sequence to be edited. When the endonuclease cuts the target sequence, the cell repairs the damage by replacing the original sequence with homologous DNA. By introducing an additional template with appropriate homologues, an endonuclease can be used to delete, add or modify genes in a precise and simple manner.[9]

Synthetic biology and gene editing with use of gene drives offer advanced tools to control viral, bacterial, and fungal pathogens, parasitic weeds, and insect vectors of plant pathogens. Use of a gene drive could also be used to reverse herbicide resistance in weeds. Perhaps the biggest risks of the cisgenic approach with CRISPR are in a gene drive. A gene drive is a genetic engineering technology that is capable of spreading engineered traits through wild populations and even an entire species. What that means is that because genes are manipulated and those genes are incorporated into the genome, potentially these genes can then be transferred on to other organisms. Once transferred to other organisms they can become part of a nontargeted population and eventually those genes are in the environment at large. Using gene drives in agriculture can result in potential hazards at different scales, from molecular to ecosystem levels. A drive construct can therefore be considered as a mutagen, whose off-target effects will depend on the specificity of the gRNA in the CRISPR transfer cassette and on its susceptibility to retargeting mutations.[10] A mutation can happen mid-drive,

[5] Rommens, C.M., 2004. All-native DNA transformation: a new approach to plant genetic engineering. Trends in Plant Science, 9(9), pp.457-464.

[6] Bergelson J, Purrington CB, Palm CJ, Lopez-Gutierrez JC. 1996. Costs of resistance: A test using transgenic Arabidopsis thaliana. Proc Biol Sci. 263:1659-63.

[7] Purrington CB, Bergelson J. 1997. Fitness consequences of genetically engineered herbicide and antibiotic resistance in Arabidopsis thaliana. Genetics. 145(3):807-814.

[8] Bergelson J, Purrington CB, Wichmann G. Promiscuity in transgenic plants. Nature. 1998;395:25.

[9] Boglioli, E. and Richard, M., 2015. Rewriting the book of life: a new era in precision gene editing. *Boston Consulting Group (BCG)*.

[10] Rode, N.O., Estoup, A., Bourguet, D., Courtier-Orgogozo, V. and Débarre, F., 2019. Population management using gene drive: molecular design, models of spread dynamics and assessment of ecological risks. *Conservation Genetics*, pp.1-20.

which has the potential to allow unwanted traits to proliferate. Outcrossing or cross-breeding can potentially allow a drive to move beyond its target population. Additionally, even when the direct impact of a new trait(s) on a target is understood, the drive may have unpredicted side effects on the surroundings.

With the simplicity that new gene editing tools like CRISPR provide, it can be expected and likely that genetically modified crops whether cisgenic or transgenic will likely have multiple genes (traits) incorporated. This necessarily complicates assessment of potential risks as scenario permutations can increase exponentially. Further complicating these confounding assessments is the occurrence of pleiotropic gene action. Pleiotropy can involve a single gene having effects on two or more traits via independent biological pathways, for instance due to effects in different tissues, or because the effect of the variant on one trait is causally related to variation in another trait. Pleiotropy is the phenomenon in genetics whereby a DNA variant influences multiple traits. Pleiotropy is widespread because in plant and animal breeding, and in laboratory selection experiments, when selection is applied to one trait, the mean of other traits also changes from generation to generation.[11] The response to selection reflects the genetic correlation between traits, which summarizes the genome-wide average effects of pleiotropy at shared loci. Pleiotropic gene action can limit the rate of multivariate evolution when natural selection, sexual selection or artificial selection on one trait favors one allele, while selection on other traits favors a different allele. With the real possibility of outcrossing of alleles to crop relatives in surrounding areas, such induced gene evolution would pose troubling unintended and uncontrolled consequences in the environment.

Adding to this already confused morass of problematic risks is the potential for an introduced cisgenic gene to be a transposon. A transposon or transposable element is a gene that can change its position within a genome, sometimes creating or reversing mutations and altering the cell's genetic identity and genome size. Transposition often results in duplication of the same genetic material.[12,13] The risk of unwittingly introducing such genes into food supplies by cisgenic gene editing is amplified through efforts to untap the genetic diversity of distant relatives that have not been used before for food consumption[14,15]

[11] Gratten J, Visscher PM. 2016. Genetic pleiotropy in complex traits and diseases: implications for genomic medicine. Genome Med. 8(1):78.

[12] Bourque, G., Burns, K.H., Gehring, M., Gorbunova, V., Seluanov, A., Hammell, M., Imbeault, M., Izsvák, Z., Levin, H.L., Macfarlan, T.S. and Mager, D.L., 2018. Ten things you should know about transposable elements. Genome biol., 19(1), pp.1-12.

[13]McClintock, B., 1950. The origin and behavior of mutable loci in maize. Proceedings of the National Academy of Sciences, 36(6), pp.344-355.

[14] Hirsch CD, Springer NM. 2017. Transposable element influences on gene expression in plants. Biochim Biophys Acta Gene Regul Mech. 1860(1):157-165.

[15] Hammer, K. et al. 2003. Agrobiodiversity with emphasis on plant genetic resources. Naturwissenschaften 90, 241–250

In summary, we oppose blanket exemptions for cisgenic developed PIPs. Due to the variety and complexity of risks associated with these PIPs, the developers should not be allowed to self-assess their PIP crops ability to meet EPA safety requirements. The potential for the gene edited crop to possibly be created through conventional breeding is not adequate to allay all risk concerns as the plant was not developed by conventional breeding and may still pose novel and significant risks as outlined above. A developer should instead request a formal exemption and submit supporting documentation demonstrating safety requirements for the agency to comprehensively assess.

Respectfully,

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