THE VORTEX

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Past ACS President, and CalACS Chair (3) Attila Pavlath, Past ACS President and CalACS Past Chair Marinda WU presenting Greti Sequin with her Past Chair pin at the December 2018 Holiday Social.

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Chair's Message

Happy New Year to the members of the California ACS section! As we celebrate the New Year, I would like to reflect on the past year and say "Thank You" to our Past Chair, Greti Séquin.

During Greti's tenure as Chair, the California Section Executive Committee organized many successful events. Events included section meetings with talks on variety of topics, such as "Cryo-EM to visualize the molecular machinery involved in the regulation of gene expression", "Conversion of biomass to biobased para-xylene", "Emission of volatile organic compounds by bacteria and fungi", "Criminalistics - A Look Back and a Look Ahead", and we also successfully hosted two performances of ***No Belles*** (WCC).

We continued our participation of the Chemistry Olympiad (Eileen Nottoli), Chemistry Celebrates Earth Week (Sheila Kanodia), as well as additional public outreach events geared towards introducing elementary school to high school students to careers in science (Alex Madonik). We continued our Project SEED participation (Elaine Yamaguchi) and continue to update our members with the Vortex (Lou Rigali). These are just some of the events we organized/participated in the past year and we look forward to our continued participation in these events in 2019.

Also, special thanks to our treasurer Paul Vartanian, Secretary Michael Cheng, and Julie Mason, as their continued service is critical for the success of our local section. We are also happy to welcome back Jim Postma as he has been elected to the Chair-Elect position for 2019.

2019 is also a year of transition as we complete the roll-out of our second generation website which incorporates an updated calendar, visualization of our tweets (@CaliforniaACS), and ability to sign-up and pay for events directly on the webpage. We are also organizing events focused on future of chemistry so stay tuned. 2019 is also the International Year of the Periodic Table of Chemical Elements so stay tuned for events consistent with this theme.

Patrick S. Lee

Celebrate the start of 2019 "International Year of the Period Table" by attending the art exhibit "Elements", presented by the Marin Society of Artists. at the Marin Society of Artists, 1515 Third Street, San Rafael, CA 94901. January 10 to February 2, 2019, Wed-Sun noon to 4 p.m. Reception & awards presentation: Friday, January 11, 2019, 5 to 8 p.m. No cost, contact: 415-454-9561

More on GMO from GM Watch, December 2018

The row over whether genome-editing techniques should be regulated as genetic modification has given rise to a new terminology of "SDNs". What it mean and why we need to know?

In July 2018 the European Court of Justice ruled that new forms of genetic engineering, known as genome editing techniques, are just as much genetic modification procedures as the old forms, and that the products of these techniques must be regulated as GMOs. In the wake of that ruling a row has developed about which types of genome editing and other genetic modification procedures should fall under EU GMO legislation and thus be subjected to safety checks and GMO labelling.

GMWatch has always argued that genome editing techniques (sometimes called GM 2.0) should be regulated at least as strictly as older-style genetic modification techniques using transgenesis – moving genes between organisms or species. In our articles we've always used the terms "genome editing" and "genetic modification".

But there are now whole groups of people who use a new terminology of "SDNs" – short for site-directed nucleases – to distinguish between old and new GM techniques and to argue that the products of certain genome-editing techniques should not be regulated as GMOs. For those who are unfamiliar with SDN terminology, we've compiled this short guide to the language and arguments, with the help of molecular geneticist Dr Michael Antoniou.

What exactly are SDNs and why do they matter? All genetic modifications brought about by genome editing techniques use enzymes called site-directed nucleases (SDNs). A nuclease is an enzyme that is capable of cutting nucleic acid (DNA, RNA). In the case of SDNs they are able to cut across both strands of the DNA double helix bringing about a double-strand break. The "site-directed" nature of SDNs refers to the fact that these enzymes are designed to bring about a DNA double-strand break at a pre-determined location within the genome of the cells or organism being targeted.

The main SDN that is being used to generate gene edited crops is the CRISPR (clustered regularly interspaced short palindromic repeats) system. Other SDNs that can be employed are zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs).

The arguments

Companies, researchers, and some scientific and regulatory organizations are arguing that genome editing outcomes known as SDN1 and SDN2 mimic what may happen in nature, do not give rise to GMOs, and should not be regulated as GM procedures. They argue that only large genetic changes, designated as SDN3, which like older-style transgenic techniques involve the addition of whole genes or other DNA elements, give rise to genetically modified organisms that should be assessed for risk.[1]

The language

The enzymes that bring about gene editing-type genetic modifications by cutting both strands of the DNA double helix are called site-directed nucleases or SDN for short. The application of SDNs brings about three possible outcomes, designated as SDN1, SDN2, and SDN3 (Figure 1). All these SDN outcomes start with a targeted double-strand DNA break. However, the events that follow the break are different, depending on the design of the SDN procedure.

Figure 1. Double-strand break repair in SDN1, SDN2, and SDN3. Definitions: NHEJ (non-homologous end-joining): The cut DNA is rejoined, but while doing this some base pairs may be eaten away or added, resulting in random deletions or additions of nucleotides, or rearrangement of DNA at the cut site. HDR (homology-directed repair): A donor DNA that carries the desired change and is similar in nucleotide sequence to the target site is used to introduce this change at the cut site. In this way you can, in principle, introduce intentional insertions, which can be whole genes, or

changes or deletions. The image is adapted from one published by the biotech industry lobby group PRRI.

The three types of SDN outcomes are defined as follows.

SDN1 refers to a double-strand break of the DNA helix, which is repaired without the addition of foreign DNA. The repair of the double-strand DNA break is mediated by a process known as non-homologous end joining (NHEJ). The repair mediated by NHEJ results in the re-joining of the broken strands of DNA. However, this re-joining invariably results in either a deletion of DNA base units (nucleotides) or insertion of additional nucleotides at the SDN1-mediated DNA break site. These insertion-deletion events are collectively referred to as "indels". Thus SDN1 always results in the disruption of the normal nucleotide sequence of the gene being targeted and therefore results in the destruction of its function in part or in whole (technically known as a gene knock-out). (See Figure 1, left side panel).

SDN2 also starts with a double-strand break of the DNA helix. However, unlike in the case of SDN1, repair of the DNA break does not take place via NHEJ-mediated indel formation. Instead, a short nucleotide template, which has a very similar nucleotide sequence to the area of the break, is introduced into the cells at the same time as the SDN. The cell then uses this short nucleotide template to repair the DNA break, through a process known as homology-directed repair (HDR). The end result is insertion either in part or in full of the short nucleotide template at the site of the SDN-mediated double-strand DNA break.

If the DNA repair template differs slightly in its nucleotide sequence from the site of the SDN-induced double-strand break, when it is inserted to repair the break, then a modification in the nucleotide sequence of the gene being targeted will have been brought about (see Figure 1, middle panel).

In this way, genome editors can bring about small but significant alterations in the function of a gene and its products, such as alterations in the activity of an enzyme, conferring new biochemical properties on the organism (e.g., tolerance to a herbicide).

SDN3 also initially results in a double-strand break in the DNA. However, similarly to SDN2, at the time when the SDN is introduced into cells, an additional foreign DNA fragment (which is very large compared to the template in SDN2 procedures) is also introduced. This large DNA fragment can contain a complete gene or other genetic material and acts as a template for repair of the double-strand DNA break brought about by the SDN.

As in the case of SDN2, the repair is by insertion of the new genetic material at the site of the double-strand DNA break mediated by the SDN. This takes place as follows. The new genetic material to be inserted is flanked at both ends by extensive nucleotide sequence regions that are the same as (homologous to) those found at the site of the SDN-induced double-strand DNA break. The cell uses these flanking regions of homologous DNA sequence to insert the foreign DNA fragment at the site of the double-strand DNA break, through the HDR process. As a result, a new gene or other genetic material is incorporated into the genome of the organism, imparting upon it novel functions and characteristics (see Figure 1, right side panel).

It is important to note that the final SDN1/2/3 outcomes take place after the SDN has made the DNA double-strand break and are thus independent of the SDN being used. The DNA repair takes place via the cell's natural DNA repair pathways (NHEJ, HDR), which takes place totally independently of the SDN being used. Thus the final SDN1/2/3 outcome is at the mercy of the efficiency and precision of NHEJ and HDR – and there is plenty of evidence that these processes are not precise or predictable.[2,3] Claims of the "precise" nature of gene editing need to be put into this context. Small changes can have large effects.

The use of all types of SDNs and their outcomes (SDN1/2/3) are genetic modification procedures with unintended outcomes, at

(Continued on page 7)

PFAS: PFOA, and PFOS (Part 2)

Bill Motzer

Polyfluoroalkyl substances (PFAS) and related synthetic/manufactured perfluorinated compounds or chemicals (PFC) have been identified as chemicals of emerging concern (CEC) (see A Chemist's Conundrum, June and September-October 2010 Vortex). PFC are organo-fluorine (fluorocarbon) compounds containing only carbon to fluorine and carbon to carbon bonds and the parent functionalized organic species. Common PFC functional groups are hydroxyl, carbolic acid, chlorine, perfluoro ethers, and sulfonic acid. Within these functional groups are many PFAS chemicals; perhaps one-thousand or more. Typically, the chemical names are abbreviated (Table 1).

The PFC polytetrafluoroethylene (PTFE or Teflon) was discovered in 1938 by Dr. Roy Plunkett, a research chemist with E.I. du Pont de Nemours and Company at their Jackson Laboratory in Deepwater, New Jersey. By the early 1940s, these almost entirely synthetic chemicals were manufactured in industrial quantities. PFC and PFAS have been used in fluoropolymer manufacturing because these chemicals impart valuable properties such as fire resistance and oil and water repellency. Fluoropolymer compounds were used in non-stick cookware surfaces such as Teflon and clothing waterproof membranes such as Gore-Tex. Additional uses include floor waxes, carpet stain guards, fire-fighting foams, paints, sealant, cleaning products, paper and engineering coatings for industrial manufacturing, and as emulsifiers coating varieties of food packages such as microwave popcorn bags. Fluoropoly-

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Table 1: Some Common PFAS and Their Abbreviations

Abbreviation	Common Chemical Name	IUPAC Name	Chemical/ Molecular Formula	CAS No.
PFOA (C7)	Perfluorooctanoic acid	pentadecafluorooctanoic acid	$C_8HF_{15}O_2$	335-67-1
PFOS (C8)	Perfluorooctane sulfonic acid	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid	$C_8HF_{17}O_3S$	1763-23-1
PFNA	Perfluorononanoic acid	heptadecafluorononanoic acid	$C_9HF_{17}O_2$	375-95-1
PFDA	Perfluorodecanoic acid	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluoro decanoic acid	$C_{10}HF_{19}O_2$	335-76-2
PFOSA (or FOSA)	Perfluorooctane sulfonaminde	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctane-1-sulfonamide	$C_8H_2F_{17}NO_2S$	754-91-6
MeFOSAA (aka Me-PFOSA-AcOH)	2-(N-Methyl-perfluoro octane sulfonamido) acetic acid	2-[1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctyl sulfonyl (methyl) amino] acetic acid	$C_{11}H_6F_{17}NO_4S$	2355-31-9
Et-FOSAA (aka Et-PFOSA-AcOH)	2-(N-Ethyl-perfluoro octane sulfonamido) acetic acid	2-[ethyl(1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro octylsulfonyl) amino] acetic acid	$C_{12}H_8F_{17}NO_4S$	2991-50-6
PFHxS	Perfluorohexane sulfonic acid	1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluorohexane-1-sulfonic acid	$C_6HF_{13}O_3S$	355-46-4

mer products may therefore contain trace amounts of PFOA which can be imparted to foods packaged by these materials. PFOA may also result from degradation byproduct from other types of PFC.

Currently, the two most studied PFAS are PFOA and PFOS because their environmental distribution is widespread and global. They are extremely persistent, having been detected in low concentrations in the environment and in blood of much of the general U.S. population. Average PFOA blood levels in persons age 12 or older are 2.1 parts per billion (ppb), with 95% of the general population at or below 5.7 ppb. Average PFOS blood levels are 6.3 ppb, with 95% of the general population at or below 21.7 ppb. They are also detected at much lower concentrations in urine, breast milk, and umbilical cord blood. Additionally, PFOA and PFOS remain in people for prolonged periods (e.g., PFOA has a half-life in human tissue of 2 to 4 years; PFOS for 5 to 6 years).

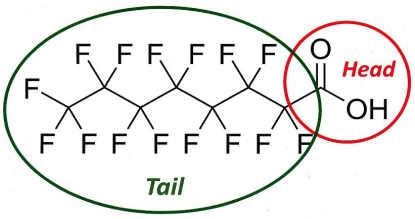
Subsequent testing in laboratory animals has indicated possible developmental and other adverse effects. Therefore, PFOA and PFOS may pose potential human health risks given their potential toxicity, mobility, and bioaccumulation potential.

The likelihood of adverse health effects depends on the amount and concentration of ingested PFAS and exposure time span. Accordingly, in 1999, the U.S. Environmental Protection Agency (U.S. EPA) began investigating PFC after receiving data on PFOS toxicity and its global distribution. PFOS was the key ingredient in the fabric repellant Scotchgard manufactured by 3M. In May 2000, under U.S. EPA pressure, 3M, announced the phaseout of PFOA, PFOS, and PFOS-related product production. However, PFOS and PFOS-related chemicals are currently produced in China.

PFOA Chemistry and Properties

PFOA, the conjugate base of perfluorooctanoate, is a perfluorinated carboxylic acid. PFOA's chemical structure consists of a perfluorinated n-octyl tail group and a carboxylate head group (Figure 1). For PFOA, the functional group is to the right of the structure, or at the head of the molecule (the carbon and oxygen known as the carboxylate group) and the long chain of carbons to the left of the functional group is the alkyl tail, fully saturated with fluorine. For these chemicals, the carbon-fluorine bond is one of the shortest and strongest known bonds, making these chemicals so environmentally persistent. Additionally, the head group is hydrophilic and reactive, strongly interacting with polar groups, specifically water. The tail is both hydrophobic, lipophobic, and essentially inert not interacting with polar or non-polar chemical moieties. Because of the hydrophobic tail these fluorocarbons are less susceptible to the London dispersion force than hydrocarbons. PFOA was used as a surfactant because it can lower the surface tension of water more than other hydrocarbon surfactants with exceptional stability because of the perfluoroalkyl tail group. The stability of PFOA is desired industrially but is a cause of concern environmentally.

Figure 1: Perfluorooctanoic acid (PFOA) structure



unexpected toxicity or allergenicity, or undesirable environmental impacts.

On-target or off-target effects can result from modifications that are described as small; that is, where one or a few nucleotides of a gene have been altered. However, even changing a single nucleotide within a gene sequence can induce drastic changes in the gene's function and/or its expression. Such changes can be brought about in SDN1, SDN2, and SDN3 procedures. For example, a change in the function of an enzyme through alteration of its active site can lead to its being able to carry out unintended biochemical reactions.

In addition, genes and their RNA or protein products work in networks. Thus an apparently small change in one gene can affect the function of other members of the network in which the edited gene functions.

Thus even the "small" genetic modifications brought about by genome editing, which are claimed to mimic what may occur in nature and thus not to require regulation, can affect the function of the targeted gene and other gene functions in addition.

For the above reasons, all organisms resulting from SDN1, SDN2, and SDN3 procedures should be subjected to a process-specific GMO-specific risk assessment and regulated as GMOs.

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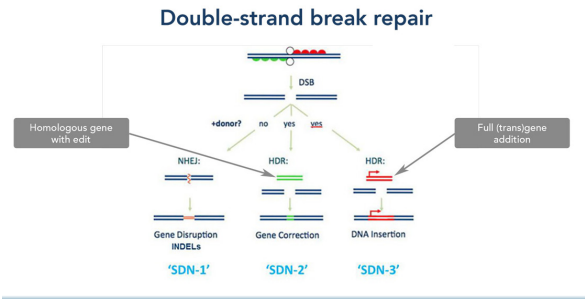

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