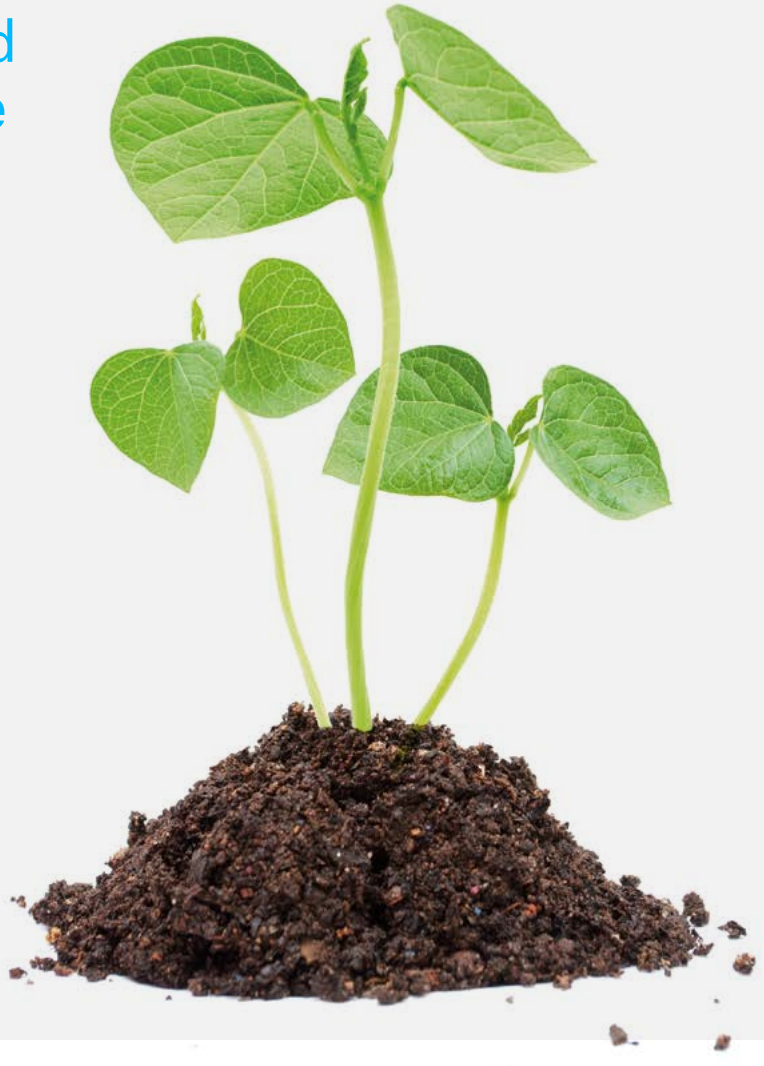
# in focus

# Microbial pest control agents

## Regulatory perspectives of product identity and characterization in the US and EU



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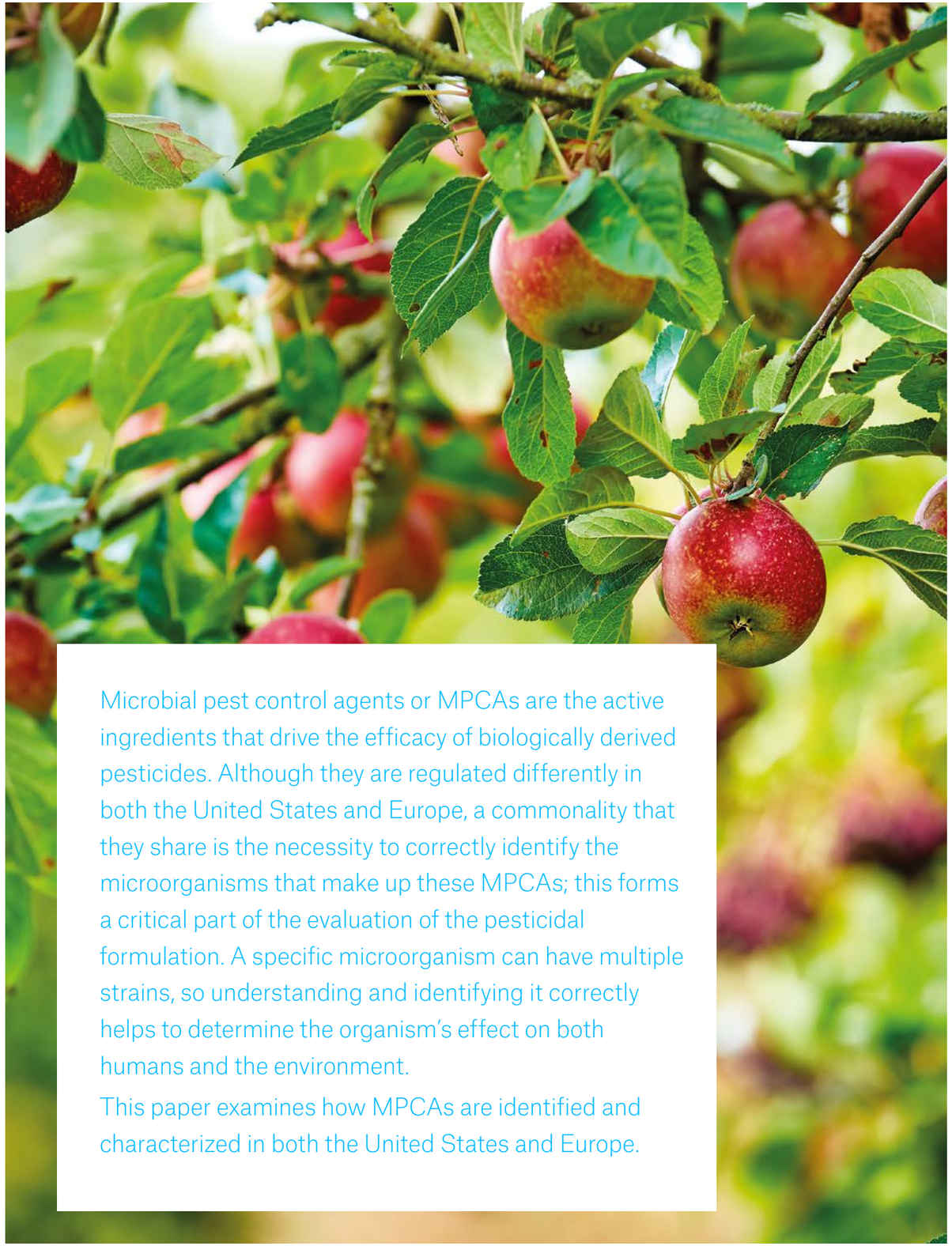


Microbial pest control agents or MPCAs are the active ingredients that drive the efficacy of biologically derived pesticides. Although they are regulated differently in both the United States and Europe, a commonality that they share is the necessity to correctly identify the microorganisms that make up these MPCAs; this forms a critical part of the evaluation of the pesticidal formulation. A specific microorganism can have multiple strains, so understanding and identifying it correctly helps to determine the organism's effect on both humans and the environment.

This paper examines how MPCAs are identified and characterized in both the United States and Europe.

Biologically derived pesticides or "biopesticides" are typically naturally occurring and often specific to the target species, with unique and less toxic modes of action as compared to conventional pesticides. The active ingredients[1] (AI) of the biopesticides referred to as microbial pest control agents (MPCAs) include (but are not limited to) bacteria, algae, fungi, viruses, and protozoa, both naturally occurring and those improved by genetic manipulation or natural selection. Examples of commonly used biopesticides in the agricultural sector include biofungicides (*Trichoderma, Pseudomonas, Bacillus*), bioherbicides (*Phytophthora*), and bioinsecticides (*Bacillus thuringiensis* or "Bt").

In the United States, the Biopesticides and Pollution Prevention Division (BPPD) in the Office of Pesticide Programs (OPP) at the Environmental Protection Agency (EPA or Agency) is responsible for the regulation of biopesticides, which includes microbial and biochemical pesticides. The EPA recognizes biopesticides as distinct from conventional chemical pesticides and has developed specific data requirements and testing guidelines for this category of pesticide. Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), MPCAs and all other pesticides must be evaluated for their risks and benefits to humans and the environment by the EPA. Testing is required in the areas of product analysis, toxicology, residue analysis on food crops, ecological effects, and environmental expression.[2]

Biopesticides are different from conventional chemical pesticides because the MPCAs driving their efficacy are less toxic in comparison to conventional chemicals.

[1] The term active substance is also used in Europe
[2] The pesticide data requirements are found in Title 40 of the Code of Federal Regulations, part 158 (40 CFR 158)

Unlike the USA, the European Union (EU) does not recognize “biopesticides” as a regulatory category; instead they are regulated as plant protection products (PPP) under Regulation (EC) No. 1107/2009. Directive 2009/128/EC established a framework to achieve sustainable use of pesticides, Integrated Pest Management (IPM) and the use of non-chemical alternatives to pesticides, which includes the development and use of biological pesticides. A limited risk-based categorization was included in 1107/2009 introducing the categories “basic substances” and “low risk substances” for active substances, and “low risk products” for plant protection products. Biopesticides should generally qualify as low-risk active substances, provided they meet the criteria outlined in Article 4 of Regulation 1107/2009. Basic substances are approved in accordance with paragraphs 2 to 6 and by way of derogation from Article 5 of Regulation 1107/2009.

Various supporting EU guidance documents have been developed to facilitate the evaluation of the specific circumstances around biopesticides, specifically microbial, botanical and semiochemical active substances and their end use products. Regulation 283/2013 Part B and Regulation 284/2013 Part B set out the data requirements for microorganisms including viruses as an active substance[3] and as a plant protection product in accordance with Regulation 1107/2009 concerning the placing of PPPs on the market. For safe use of microorganisms as an active ingredient, the regulations require that each microorganism must be identified, characterized and named up to the strain level.

**To qualify as a low-risk active substance in the EU, a biopesticide must meet the criteria listed in Article 4 of Regulation 1107/2009 including:**

1. Product residue must not have an unacceptable effect on the environment
2. Product residue may not have an immediate or delayed effect on human health
3. Product shall be sufficiently effective

# US MARKET

## What is product identity?

Thorough identification and characterization of the microbial AI is critical to the success of the MPCA approval. Regulators expect the most accurate and current taxonomic information to verify the identity of the microbial agent.

Applicants must provide the following information, as required by the regulations[4]:

**Product name**

The product name must be identifiable and traceable with the microorganism’s original name and self-identified strain identification number. For example: *Bacillus subtilis*, strain OVSTSG001.

**Trade name**

The unique brand name of each product and any alternative brand names of the product must be identified to enable easy tracking in the market.

**List of AIs**

- The list of AIs in the product should include the biological (and common name, if available) for each microorganism, its nominal concentration (expressed as activity, such as cfu/g, spores/ml, etc.), and its guaranteed minimum activity
- A seed culture of the microorganism must be deposited with an internationally recognized culture collection agency, such as American Type Culture Collection (ATCC) or Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). Proof of the deposit and its appropriate accession number must be provided
- The applicant for registering an MPCA product must specify the current regulatory status of the proposed microorganism and whether the microorganism is a new isolate of a currently registered species since a new isolate is a new AI. For example, each of the many strains of *Bacillus subtilis* are separate AIs, as are separate isolates of a given strain
- The applicant should identify the geographical occurrence of the microorganism, such as “ubiquitous in North America” or “worldwide”
- The identity of any inert ingredients in an MPCA must also be provided to EPA

[3] Defined in Article 2(2) of Regulation (EC) No. 1107/2009

[4] 40 CFR 158.740

## Characterizing MPCA active ingredients

### Identification of naturally occurring MPCAs

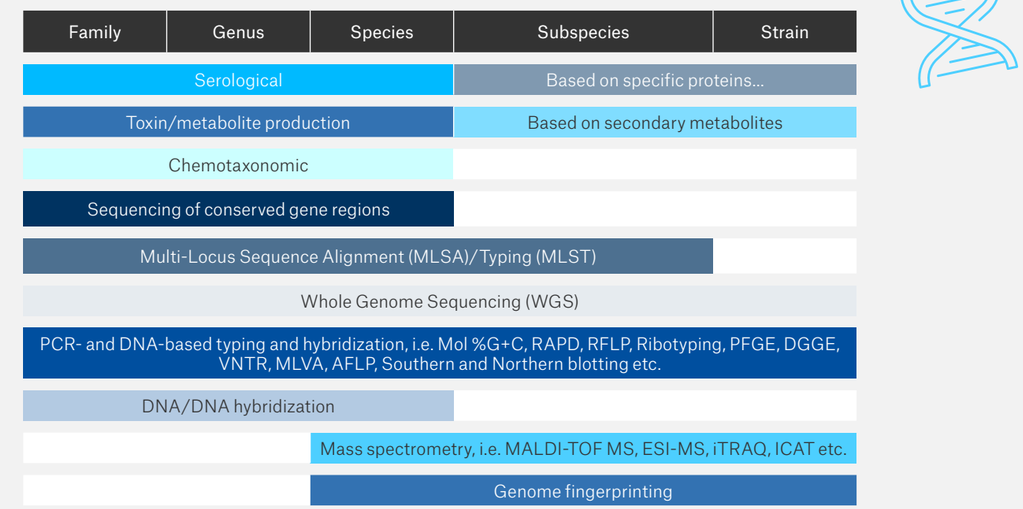
The identification of the proposed microorganism is crucial because its many traits will be considered in the EPA's assessment of its potential to cause adverse effects in humans and/or the environment.

Taxonomy is a means of organizing organisms and showing their relationships to each other based on three major components: classification, nomenclature, and identification, or the determination that an unknown microorganism belongs in a recognized group of organisms.

Traditional phenotypic and biochemical analyses may be the best ways to determine similarities and differences between a given isolate and closely related taxa. However, these methodologies are not always reliable for common genera.

Modern classification techniques largely rely on nucleic acid analysis, which shows greater reliability between common genera. The genetic methodologies, such as genome sequencing, rRNA sequencing, Mol % G+C, DNA-DNA hybridization, multi-locus sequence typing, whole cell protein profiling, and genome fingerprinting, have been successfully applied to some otherwise difficult-to-identify taxa. Figure 1 shows the relative taxonomic resolution of various molecular techniques to identify microorganisms up to the strain level. However, expert advice may be necessary to select an appropriate genetic method and perform data analysis toward the identification of the proposed microorganism. While the choice of microbial identification method is up to the applicant, the data interpretation of the identified analyte must be executed by experts in the field to ensure that it is reproducible.

*Figure 1 - Relative taxonomic resolution of various microbial identification techniques*




For each proposed microorganism, the taxonomic designation must include the genus, species, and strain. The most current name should be used, referring to the *Approved Lists of Bacterial Names* (1989) approved by the international bacteriological community or subsequent lists published by the *International Journal of Systematic Bacteriology*. All other names for a bacterium are considered synonyms. For guidance on naming viruses, consult the *International Code of Virus Classification* and *Nomenclature* (1990) and the *Sixth Report of the International Committee on Taxonomy of Viruses* (1995). For fungi, list the most common current names with a reference to a readily available source that cross-references synonyms, such as USDA's U.S. National Fungus Collections Fungal Database. Where applicable, both teleomorphic and anamorphic forms should be named.

The EPA requires applicants to submit information substantiating the taxonomy of each proposed microorganism. The types of data should be appropriate to the method of choice, i.e., morphological, biochemical, immunological, or physiological characteristics, with a description of the method(s) used. Similarly, when genetic methods are used, the relevant method should be explained in detail with the interpretation of data. All the relevant gene sequences from the genome or the sequences from conserved regions used to identify the microorganism should be provided in totality with their appropriate match. It is prudent to specify the number of replicates, and the number of isolates that were used for identification purposes. If the sequences are in a publicly accessible database such as NCBI, the accession numbers are sufficient in lieu of the sequence data. The phylogenetic analysis of results (% of phylogenetic proximity) is required for the true identity of the natural isolate.

## Identification of genetically engineered MPCAs

Genetically engineered (GE) MPCAs have essentially the same data requirements as naturally occurring MPCAs. However, per the EPA guidance document "Microbial pesticide OPPTS 885.1100 product identity," additional data may be required concerning the GE process and the results of that process:

- Identity and characterization of recipient and donor microorganisms
- Identity of inserted or deleted genetic material, i.e., source, nature, size, base sequence data, and/or restriction endonuclease map, etc.
- Information on the gene control regions
- Descriptions of the phenotypic traits acquired and lost due to genetic alteration
- Gene stability (reversion tendency or rate of horizontal and/or vertical gene transfer with other organisms) of the altered chromosomal regions, or extrachromosomal entity reversion tendency or rate of gene transfer (horizontal and/or vertical) with other organisms
- Characterization of genetic material adjacent to intentionally inserted gene

EPA requires notification prior to small-scale field testing of GE microorganisms to determine if an Experimental Use Permit is needed. In any case, under FIFRA, microbial pesticides, like all other pesticides, must be evaluated for their risks and benefits. Before a product registration is granted, the following issues need to be resolved:

- Potential adverse effects on non-target organisms
- Environmental fate of the GE microorganisms
- Potential pathogenicity and infectivity of the microorganism to humans

Expert advice from professionals in the field will be required to meet the EPA's requirements for field testing of a GE MPCA, which may fall under FIFRA and the Federal Food Drug and Cosmetic Act (FFDCA).

### Alternative nomenclature

The applicant must specify any alternatives, synonyms, or superseded names associated with the proposed microorganism.

### Origin and natural occurrence:

The agency recommends providing information on the geographical location and specific environmental conditions of the site of microbial isolation. Based on the identification, the applicant can provide a geographical distribution of isolates, such as "ubiquitous soil/water/air microorganism."

### Biological properties

The MPCA's pesticidal properties must be identified with respect to the target species, pest host range, life cycle, and mode of action. The mode of action can be described in relation to the MPCA's intended use and how the microorganism functions by altering the physical, chemical, or biological environment. This could involve a description of changes in microbial activity under changes in pH, biogeochemical cycling, or ecological interaction with other organisms. The microorganism's optimal growth conditions must be given based on *Bergey's Manual of Systemic Bacteriology*, Vol. 3.

### History of use

The Agency recommends providing a history of use to confirm the existing safety profile of a new isolate. Experts in the field may recommend providing a literature-based safety profile of the isolate, or if available, a qualified presumption of safety status from a regulatory agency such as the EPA or European Food Safety Authority (EFSA). The applicant can also discuss the approval status of similar species for other applications in the field.

## EU MARKET

Most biopesticides in the EU market are based on microbial plant protection products. Currently, *Bacillus* sp. based microbial pesticide products are among the most common form of microbial agents because of their relatively easier and cheaper production process in comparison to other microbial strains including fungal agents. Due to tight assemblage of closely-related species or strains in the microbial community, it is important to clearly identify and characterize the subject organism so that the data acquired from its release in the environment could be interpreted for its effectiveness.

Like the US, in the EU per Regulation 283/2013 (part B), the active substance in a microbial pesticide must be identified, characterized and named at the strain level. The identification and characterization of active substances up to strain level are necessary to distinguish them from their closely-related pathogenic variants. Very limited guidance is available on the characterization of microorganisms for plant protection use. The only guidance released caters to the characterization of microorganisms used as feed additives.

Other than biochemical and physiological methods of identification, a handful of genetic methods for strain identification, as described in Figure 1, exist. These identification methods also provide implications for widespread use in the industry and contract research laboratories to identify and characterize the novel bacterial strain as an active substance in biological control. The method of choice for unequivocal strain-level identification could include PFGE, MLST, multi-locus variable number tandem repeats analysis, repetitive sequence-based polymerase chain reaction (rep-PCR), and PCR-ribotyping. Even though these methods are unequivocal, they suffer from bias based on the choice of primers or restriction enzymes due to the nature of PCR amplification. Therefore, whole genome sequencing could be an ideal way to definitively determine the microbial strain. False positives could be avoided by cross-examination using combination methods, such as those identifying gene versus protein listed in Figure 1.

Using a combination of these different strain typing techniques can provide a valid and robust way to identify and characterize novel microbial agents and distinguish them from pathogenic variants. Nevertheless, expert opinion on the choice of methods for unambiguous strain-level characterization of novel biological control strains is highly recommended. The biopesticide sector requires a similar level of expertise from competent authorities to evaluate and interpret the outcomes from the technologies applied for strain identification to allow the regulatory process to function and deliver these products to the EU market.

In summary, regulatory guidance has not kept pace with scientific progress in the characterization of bacterial strains used as biocontrol agents. It is however arguable that regulatory guidance is frequently produced in other areas of plant protection regulation in the absence of adequate scientific validation. The limited progress for microbial plant protection product characterization is therefore hampering the regulatory process and consequently the rate of appearance of new products on to the EU market.

### How TSG Consulting can help

TSG has many years of experience registering new microbial active ingredients as well as new products containing currently registered active ingredients. We help clients navigate the current regulatory framework in the US and EU. Our services include, but are not limited to:

- Strategic guidance on the regulatory requirements to help our clients make commercial decisions
- Review of the related evidence to identify and characterize microorganisms including acceptable claims for your product before agency evaluation
- Assistance in gathering scientific evidence to meet the federal and state data evaluation criteria to ensure compliance
- Preparation and submission of applications to register microbial pesticides with the EPA and each state in the US, as well as in the EU

In addition, through TSG's active participation in various trade industries, we continuously monitor regulatory changes related to MPCAs at both federal and state levels in the US, and in the EU. This enables us to keep our clients informed of the latest developments and any upcoming changes in the regulatory landscape that may affect their products.

To gain more insight on MPCAs and how TSG can help to navigate the current regulatory landscape for biopesticides, contact us at:

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**About the Authors**

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Dr. Singh has 20 years' experience in microbiology and a deep understanding of regulatory approval pathways for biopesticides, medical devices and antimicrobial products. Hands-on experience with laboratory testing allows Om to guide companies on the standards needed to demonstrate the safety and efficacy of their end use products. He previously worked at the US Food and Drug Administration's (FDA) Center for Devices and Radiological Health (CDRH).

Iain D. Watt, MSc, Head of Plant Protection

Iain has over 30 years' experience in the crop protection sector, working in research, advisory and industry roles in the UK and overseas. He has extensive experience of plant protection product authorization processes for many global markets, with a particular focus on the EU and the US. Iain joined TSG from an international SME role, where he spent 14 years as Regulatory Affairs Manager.

## About TSG Consulting

TSG Consulting provides companies with high-quality regulatory and scientific consulting services. We help clients worldwide address the technical and regulatory issues in taking their products to market in multiple jurisdictions. Our scientific expertise, regulatory knowledge and understanding of local nuances enable our clients to navigate the complex and ever-changing regulatory landscape across the globe.

We serve a number of key markets and industry sectors including agricultural, industrial, consumer, food and beverage, animal health, and medical. Our teams comprise scientists and regulatory experts – many of whom have previously held positions at regulatory agencies, departments, and in industry. This combination of science, regulatory expertise and knowledge of how institutions and industry operate provides our clients with superior and well-rounded guidance.

TSG Consulting has offices in the USA, Canada, France, Germany, Spain and UK. TSG is a Science Group (London listed) company.

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## About Science Group plc

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Science Group's services fall into four broad categories: Applied Science, Product Development, Technology Advisory and Regulatory. These services are combined with vertical market expertise in the Medical, Consumer, Food & Beverage, Industrial, Chemical and Energy sectors. With offices throughout Europe, North America and China/Hong Kong and with over 30 languages written and spoken, Science Group supports a global client base.

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