Institution: University of East Anglia		
Unit of Assessment: 3 - Allied Health Professions, Dentistry, Nursing and Pharmacy		
Title of case study: Accelerating commercialisation of polysulfide-containing biopesticides		
Period when the underpinning research was undertaken: 2009 – 2020		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g., job title):**	**Period(s) employed by submitting HEI:**
Chris Hamilton	Reader	2007– to present
Period when the claimed impact occurred: 2013 – 2020		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact

With increasing pressure to withdraw environmentally damaging synthetic pesticides there is a pressing need to accelerate the commercialisation of resistance-resilient biopesticides formulated using natural compounds. Towards this end, **Hamilton** has been in a research collaboration with Ecospray, a UK biopesticide company, since 2010. This collaboration has established the mode of action of bioactive garlic-derived polysulfide compounds. The detailed scientific insights, regarding the mode of action of thiol cofactors, established by **Hamilton** have: i) ***underpinned international regulatory approval*** of polysulfide-containing biopesticides and ii) ***greatly increased consumer confidence*** in these products. Stemming from product registration and increased consumer confidence, global distribution deals for Ecospray's Nemguard® product [with Certis (UK/EU), CBC Srl. (Southern Europe), Dudutech (Kenya), Savana (Senegal) and Global Agro (S. Korea)] have ***established global reach for polysulfide-containing biopesticides***. Through their global distribution, sales of Ecospray products have increased to >GBP3,000,000 since 2014.

2. Underpinning research

The research, that ultimately led to the impact detailed herein, was originally directed towards detoxification of and resistance to antibiotics in bacterial pathogens. In this arena, **Hamilton's** research discovered a novel low molecular weight thiol-sulphur cofactor called bacillithiol, which occurs amongst numerous pathogenic bacteria (e.g., *S. aureus, B. anthracis*) **[3a].** He published the first total synthesis of bacillithiol **[3b]** and characterised its role in the detoxification of and resistance to the antibiotic fosfomycin **[3c]**. **Hamilton's** research elucidated the critical role played by low molecular weight thiol cofactors (e.g., glutathione, bacillithiol) in maintaining an intracellular reducing environment in microbial pathogens, to protect against the oxidative stress they experience when engulfed by macrophage cells during infection of a human host **[3g]**.

Hamilton's 17-year track record in thiol cofactor metabolism/biochemistry was salient to establishing (in 2010) an ongoing research collaboration with a UK biopesticide company (Ecospray Ltd) who sought mechanistic insights regarding the mode of action of their polysulfide-based biopesticide products.

Working in collaboration with Ecospray, **Hamilton** unravelled the biochemical modes of action of Ecospray's garlic-derived compounds. This was achieved through a combination of different analytical and biochemical approaches directed at bacteria, nematodes and insects **[3d-f]. Hamilton's** research findings showed that the fundamental mechanisms of action of these pesticides are specifically:

1) Garlic-derived polysulfides enter the organism's cells and react with cellular thiol antioxidants (e.g., cysteine, glutathione).
2) This creates oxidative stress, which weakens the organism's ability to survive.
3) The polysulfides react with, and modify, thiol groups on multiple cellular proteins.

4) These polysulfide modifications impair protein function, which can weaken or kill the organism.

These findings are significant as most synthetic chemical pesticides have a single mode of action or cellular target that may be subject to genetic mutation and subsequent development of resistance. In contrast, **Hamilton's** research demonstrates that polysulfides have multiple modes of action, **[3d-e]**, which suggests that the development of resistance to polysulfide formulations would be extremely unlikely.

3. References to the research

The underpinning research outputs have all been published in competitive, international, peer-reviewed journals and form part of a larger body of such published work. Citation numbers are from Google Scholar (12/2/21) (UEA authors in **bold**, EcoSpray authors <u>underlined</u>)

[3a] Bacillithiol is an antioxidant thiol produced in *Bacilli*.
G. L. Newton, M. Rawat, J. J. La Clair, **V. K. Jothivasan**, T. Budiarto, **C. J. Hamilton**, Al Claiborne, J. D. Helmann, R. C. Fahey,
Nature Chemical Biology (**2009**), 625-627.
DOI:10.1038/nchembio.189. Citations: 251

[3b] Chemical and chemoenzymatic syntheses of bacillithiol, a unique low molecular weight thiol amongst low G + C Gram positive bacteria.
S. V. Sharma, V. K. Jothivasan, G. L. Newton, H. Upton, J. I. Wakabayashi, M. G. Kane, **A. A. Roberts,** M. Rawat, J. J. La Clair, **C. J. Hamilton**
Angew. Chem. Int. Ed. (**2011**), *50*, 7101-7104.
DOI:10.1002/ange.201100196. Citations: 52

[3c] Mechanistic studies of FosB: a divalent metal-dependent bacillithiol-*S*-transferase that mediates fosfomycin resistance in *Staphylococcus aureus.*
A.A. Roberts, S.V. Sharma, A.W. Strankman, S.R. Duran, M. Rawat, **C.J. Hamilton**
Biochemical Journal. (**2013**), *451,* 69-79.
DOI:10.1042/BJ20121541. Citations: 66

[3d] Antimicrobial garlic-derived diallyl polysulfanes: Interactions with biological thiols in *Bacillus subtilis.*
M. Arbach, T.M. Santana, H. Moxham, R. Tinson, <u>A. Anwar</u>, <u>M. Groom</u>, and **C.J. Hamilton.**
Biochimica Biophysica Acta General Subjects, (**2019**), *1863*, 1050-1058.
DOI:10.1016/j.bbagen.2019.03.012. Citations: 6

[3e] The disulfide stress response and protein-S-thioallylation caused by allicin and diallyl polysulfanes in *Bacillus subtilis* as revealed by transcriptomics and proteomics.
B.K.Chi, N.T.T Huyen, V.V. Loi, M. Gruhlke, M. Schaffer, U. Mäder, S. Maaß, D. Becher, J. Bernhardt, **M. Arbach, C.J. Hamilton,** A.J. Slusarenko, & H. Antelmann,
Antioxidants, (**2019**), *8*, 605.
DOI:10.3390/antiox8120605. Citations: 7

[3f] Diallylpolysulfides from Garlic, Modes of Action and Applications in Agriculture
Student: **Miriam Arbach,** Supervisor: **Chris Hamilton**, *PhD thesis, UEA (funded by Ecospray Ltd),* (**2014**)
Available from: https://ueaeprints.uea.ac.uk/id/eprint/50025/

[3g] Importance of bacillithiol in the oxidative stress response of Staphylococcus aureus.
A.C. Posada, S.L. Kolar, **R.G. Dusi**, P. Francois, **A.A. Roberts**, **C.J. Hamilton**, G.Y. Liu, A. Cheung
Infection and Immunity. (**2014**) Jan;82(1):316-32.
DOI: 10.1128/IAI.01074-13. Citations: 51

Key Research Funding Includes:

2014-2018: Garlic Derived polysulfides: Modes of Action and Applications for Green Pesticides in Integrated Crop Management
BBSRC iCASE project with Ecospray industrial partner
Student: Emma Gould, Supervisor: Chris Hamilton

2015-2018: Side ridge injection (SRI)
Innovate UK Agri-tech catalyst. Co-funded by BBSRC (UEA element) and Innovate UK (Industrial component)
PIs = Ecospray Ltd, TargetSet Engineering Ltd, Chris Hamilton
Value: GBP191,915

4. Details of the impact

Plant parasitic roundworms (nematodes) cause approximately 12% of global food crop loss. These losses equate to an annual cost of USD85,000,000,000. The threat to food security is further exacerbated by increases in pest nematode populations (e.g., there has been a 300% increase in the UK since 1990) and increased resistance to chemical nematicides. With increasing pressure to withdraw environmentally-damaging synthetic pesticides, and very little by way of alternatives being developed in commercial R&D pipelines, there is a pressing need to accelerate the commercialisation of resistance-resilient biopesticides formulated using natural compounds. Polysulfide-based products, such as those developed and marketed by Ecospray Ltd are now addressing this global need.

Enabling Ecospray Regulatory Approval

Since 2000, Ecospray have developed, patented and distributed nematicides (Nemguard®, and Eagle GreenCare), based on food-grade garlic oil formulations. These products contain a collection of sulfur-rich polysulfides as their active ingredients and engage a market for environmentally sustainable alternatives to current synthetic chemical pest control products. However, botanical extracts claiming to display beneficial biological activity are commonly viewed with suspicion. Since circa 2009, EU regulatory authorities (European Food Safety Authority (EFSA) and the Commission) began placing greater expectations regarding evidence of botanical extracts' mode of action being included in new applications for pesticide regulatory approval.

In 2010, to satisfy the increasingly stringent requirements for regulatory approval of product use, Ecospray approached **Hamilton** to identify the diallyl polysulfide modes of biological activity in their developmental products. In 2014, using **Hamilton's** research findings, Ecospray secured product approval for Nemguard® as a biopesticide for application in food crops **[5.1]**. The importance of the mechanistic data to the regulatory approval was confirmed by the Ecospray Technical Director.

> *"It was during this period that Ecospray was compelled to generate dossiers to secure access to the EU annexe 1 data base under directive 91/414 and the first questions from regulators on the mode of action answered. Understanding of the mode of action for any crop protection product is a particularly important regulatory matter as it relates to potential toxicity and development of resistance [....]* **[5.1]**

Hamilton's mechanistic data were a key part of Ecospray's successful Annex I pesticide product registration application and have since been used to support the successful approval of additional product registrations **[5.2, page 6].** This is the first time that these multiple modes of action have been demonstrated in studies of polysulfide-treated organisms at the biochemical level.

Hamilton's findings have been incorporated in numerous dossiers used to secure regulatory approval of Ecospray products in crop protection settings in the UK, Europe, Africa and Asia **[5.2].** For example, in 2016, EU zonal registration was secured for Nemguard® use and this proved to be a key driver to further commercial exploitation and to secure regulatory approval for applications in a much broader spectrum of crops across Europe, UK, Africa and New Zealand. For example, in Kenya it has helped secure approval for use in flower production. **[5.3]**

Expanding the Ecospray Marketshare

Initially, Ecospray's main garlic-derived nematicide (Nemguard®, marketed as Eagle GreenCare) was distributed on a small scale to protect elite sports turf (e.g., golf courses and professional football pitches) from nematode damage. Looking to expand their market, Ecospray increased the applications and market base to include products targeted at combatting other plant pests (nematodes, insects & fungi) in food crops.

To expand their market Ecospray required better explanations for the mechanism by which their products worked. Hamilton's research reported multiple modes of antimicrobial action in bacteria, and subsequently demonstrated analogous effects in nematodes, insects and fungi. This enhanced knowledge base enabled Ecospray to support the successful outputs of their field trial activities with scientific explanations of Nemguard® mechanisms of action against a range of different pests that compromise yields of a wide range of food crops. These include major UK crops (carrots, parsnips, potatoes) and EU (tomatoes and melon) as well as international horticulture crops (e.g., roses in Africa).

Since 2013, Hamilton has served as an expert advisor on the mechanisms of action of Nemguard® through the presentation of his research findings to investors, distributors and end-users around the world in the form of presentations and product literature **[5.4-5.7].** This led to Ecospray signing global distribution deals with Certis (UK/EU), CBC Srl. (Southern Europe), Dudutech (Kenya), Savana (Senegal) and Global Agro (S. Korea), thereby greatly increasing the global availability of Nemguard®.

The distribution deal with Certis in 2014 was particularly timely because an incident at the DuPont pesticide manufacturing plant (La Porte, Texas) stalled the supply chain of the mainstream nematicide used in crop protection, Vydate®.

At this moment of crisis, the availability of robust mechanistic data relating to the mode of action of polysulfides was critical to the fast-tracked emergency approval of Nemguard® for treatment of potato cyst and root knot nematode infestations in UK potatoes and carrots **[5.8].** The importance of a recognised mechanism of action has given confidence to farmers to treat their crops earlier than normally permitted by traditional pesticides. An extract from the Certis website below exemplifies how Mr Guy Poskitt used Nemguard® in order to safeguard the supply of 90,000t of UK carrots, equivalent to *ca.* 13% of the total UK production in 2015:

> *"As a grower of 1,400 acres of carrots and parsnips, Mr Poskitt supplies fresh produce to retailers for 48 weeks of the year. Carrots were recently identified as a crop where production is in danger of becoming unsustainable due to the impact of pesticide loss on food production, in a report commissioned by the NFU, AIC and CPA and produced by independent farm business consultants Andersons.... NEMguard seems to work as a nematicide at a similar level to Vydate (oxamyl) for nematode control but has the major bonus of not having a MRL (maximum residue level) or harvest interval which means we can use NEMguard on early crops without a worry."* **[5.4]**

Hamilton's data have been included in promotional product information brochures and farm operator technical documents **[5.4-5.7]** that support the usage of Ecospray products**.** There is strong evidence that the availability of mechanistic data for Nemguard encourages the use of the products at a large scale in food production sites – see testimonial from *Spearhead/Greens of Soham:*

> *"Throughout the period 2013-2020, Spearhead/Greens of Soham were introduced to the chemistry behind the obvious nematicidal effect and the scope and depth of biochemical research on Nemguard SC being supported by Dr Chris Hamilton at UEA. The mode of action of crop protection products is something that modern farming must be familiar with as this knowledge impacts on many aspects of crop protection throughout the life of a crop and the future planning of rotations to*

resistance management and minimisation of negative environmental impact and sustaining soil health." **[5.9]**

Driven by the ability to secure an expanding portfolio of regulatory approvals in different crops and geographic regions since 2017, the scope and uptake of polysulfide pesticides in several countries has greatly enhanced commercial sales, distribution and applications in sustainable arable and horticultural crop protection. The Technical director of Ecospray summarises some *"examples of the commercial value of the research completed between UEA and Ecospray"* **[5.1]** including:

- GBP1,800,000 of Nemguard® (liquid and granule) sales to distributors in Southern Europe (CBC) used in the protection of >7500 Hectares of arable crops (e.g., tomato, pepper, aubergine and cucumber).
- GBP500,000 of Nemguard sales of Eagle GreenCare to UK distributors of sports turf maintenance products (Rigby Taylor) **[5.10].** Used for nematode control on 850ha of elite sports turf (golf courses, premier league football pitches and Wembley stadium).
- >GBP200,000 of Nemguard® sales to distributors in Morocco, Kenya, Zimbabwe, Senegal and Gabon (Dudutek & Savana) used in green bean and ornamental rose production.

The most recent extensions of registered product use mean that these crop pest control treatments are scheduled to emerge in Australian, New Zealand and South Korean markets.

5. Sources to corroborate the impact

[5.1] Testimonial letter from Technical Director of ECOSpray Ltd. (November 2020)

[5.2] Extracts from example product Registration applications, which quote mode of action (highlighted)

[5.3] Nemguard registration in Kenya for rose and French bean production (2019) from khusoko.com (Downloaded November 2020)

[5.4] Certis webpages (2014) (*detailed quote of Hamilton's mode of action comments*) from certiseurope.co.uk. (Downloaded November 2020)

[5.5] Certis Nemguard Factsheet (2014) (*describing mode of action*) from certiseurope.co.uk. (Downloaded November 2020)

[5.6] Nemguard brochure from Ecospray (*describing mode of action on pages 11-12*)

[5.7] Senegal product users information leaflet, for NEMguard (*describing mode of action, in French*)

[5.8] Emergency product registration announcement for NEMguard DE in April 2015, from certiseurope.co.uk (Downloaded November 2020)

[5.9] Testimonial letter from Greens of Soham (a major UK potato producer) (November 2020)

[5.10] Testimonial letter from the Chemicals Product Manager at Rigby Taylor (November 2020)