

Sustainably sourced Squalene for vaccines

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Abstract

Squalene is a key component in all major emulsion adjuvants currently used in licensed vaccines for humans, such as those used for treating seasonal and pandemic influenza. Moreover, squalene-based emulsion adjuvants are being used in many new lead vaccine candidates under development for protection against eg Tuberculosis, Herpes zoster, Malaria and HIV. Squalene is currently harvested from deep-sea sharks, posing an ecological threat to shark populations, which play a key role in marine ecosystems. To address this issue, we have partnered with Amyris Inc., a leading company in synthetic biology, to harness advances in synthetic biology with our own purification capabilities, to develop sustainably produced squalene with the required purity and performance characteristics to meet both quality and supply chain requirements.

This new source of squalene is sustainably produced via cutting-edge, proprietary technology, resulting in a product that is molecularly identical to shark squalene and that complies with the specifications of the Ph. Eur. monograph 2805, readily meeting the stringent requirements of vaccine manufacturers in being at least 99% pure. This poster also features stability data for this source of squalene, both as neat material and in a model application, whereby MF59- or AS03-like formulations were demonstrated to have identical stability when prepared using either shark or sustainably produced squalene. All this data further supports the use of this new source of squalene for vaccine applications.

Introduction: Squalene in vaccine formulations

Squalene is an essential component in emulsion adjuvants, enhancing the innate immune response. They currently play a major role in seasonal and pandemic influenza vaccines and have been demonstrated to be efficacious to other pandemic viruses such as SARS-CoV-2.

Adjuvant system	Originator/ Owner	Composition	Pathogen	Marketed/ registered product	Clinical development
MF59	Seqirus	Squalene oil, Sorbitan trioleate, Polysorbate 80	Viruses	Fluad Fluad Quadrivalent Celtura (Seasonal and pandemic influenza)	
AS03	GSK	Squalene oil, α -tocopherol ¹ , Polysorbate 80	Viruses	Pandemrix Prepandrix (pandemic influenza)	Phase III for Covid-19 virus
SE (Stable Emulsion)	AAHI ²	Squalene oil, Poloxamer 188, Synthetic POPC ³	Viruses		Phase II influenza
GLA-SE / (Glucopyranosyl Lipid Adjuvant) SLA-SE (Second generation Lipid Adjuvant)	AAHI	GLA & SE SLA & SE	Bacteria, Viruses, Eukaryotic pathogens		In various phases: Tuberculosis, Schistosomiasis, Herpes zoster, Malaria, HIV, pandemic influenza and others

¹ α -tocopherol is used due to its immuno-potentiating effects <https://doi.org/10.1038/s41541-023-00601-5>
²AAHI – The Access to Advanced Health Institute / Seattle, US
³POPC – 1-palmitoyl-2-oleoyl-glycerol-3-phosphocholine

The challenge: A significant need for sustainable and highly purified squalene

Challenge



Squalene is a key component in emulsion adjuvants for seasonal and pandemic influenza vaccines.

Solution



Advances in synthetic biology and biotechnology enabled the development of a sustainably produced squalene. Its characteristics meet both quality and supply chain requirements.



Currently used squalene is harvested from deep-sea sharks. This is an ecological threat and has the potential to further devastate shark populations which play a key role in marine ecosystems.



Sustainably sourced Squalene

Our solution: New, sustainable, highly purified, Squalene

- Croda has entered a partnership with **Amyris Inc.**, a leading company in synthetic biology
- Amyris has invented a **proprietary method** for sustainable production of squalene.
- Combined with our **outstanding expertise in purification techniques** we can offer a highly purified product

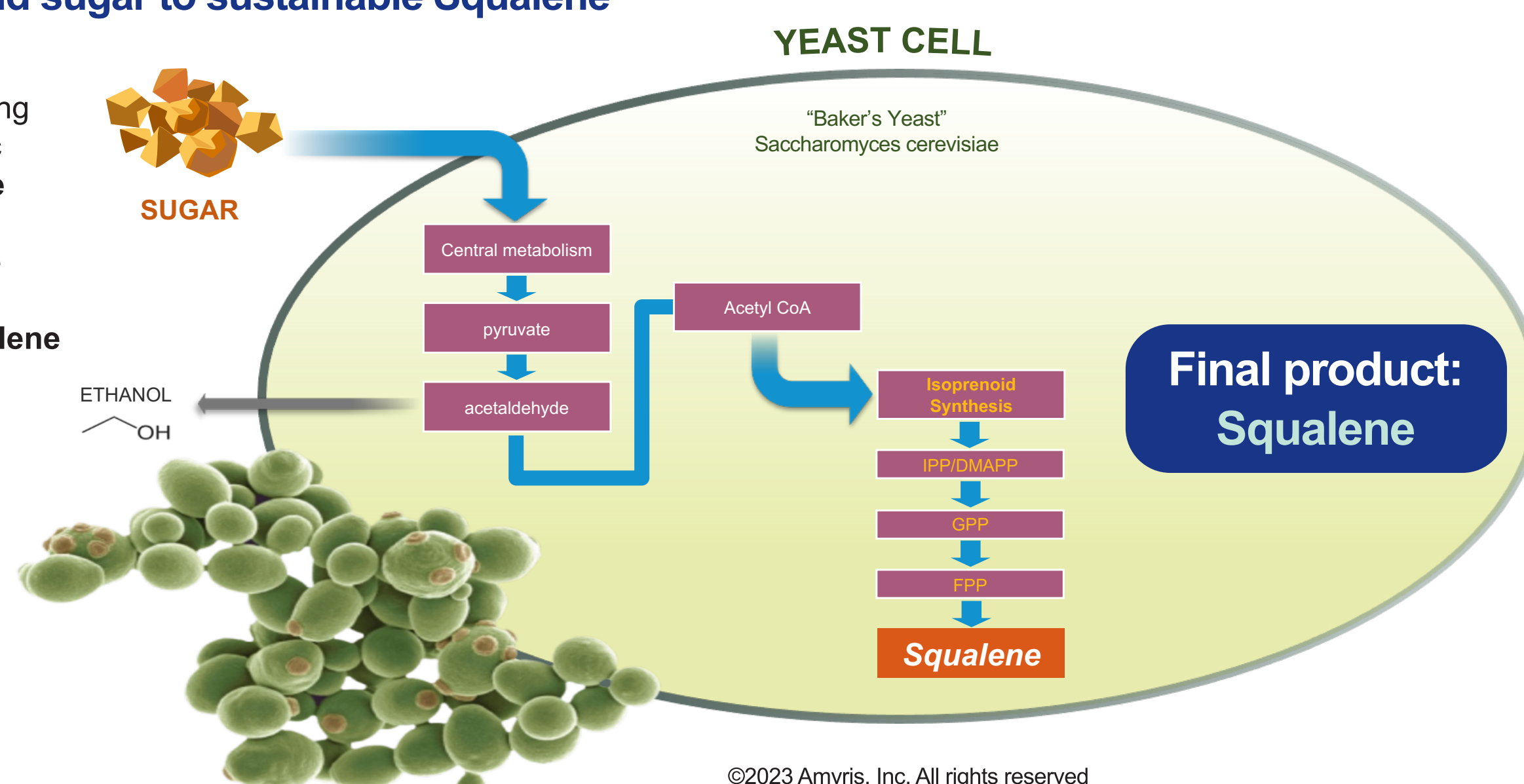
Sustainably sourced Squalene offers:

- Superior purity of at least 99% in line with stringent requirements of vaccine manufacturers
- Molecularly identical with shark squalene
- Equivalent stability in emulsion adjuvants
- Sustainable production via cutting-edge, proprietary technology
- Protecting shark populations and thus marine ecosystems
- Consistent and secure supply

Croda **exclusively** commercialises this Squalene as a component or in formulated products for use in **human and veterinary vaccines, vaccine adjuvants, drug delivery systems, nucleic acid delivery systems, RNA carriers, and excipients**

From yeast and sugar to sustainable Squalene

Yeast isoprenoid pathway engineering based on synthetic biology – the core technology for commercial scale production of sustainable Squalene



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Sustainably sourced Squalene: Product characteristics

1. Monograph compliance

European Pharmacopoeia

The Ph. Eur. monograph 2805 defines minimum specification for inclusion of squalene as an adjuvant in a vaccine formulation.

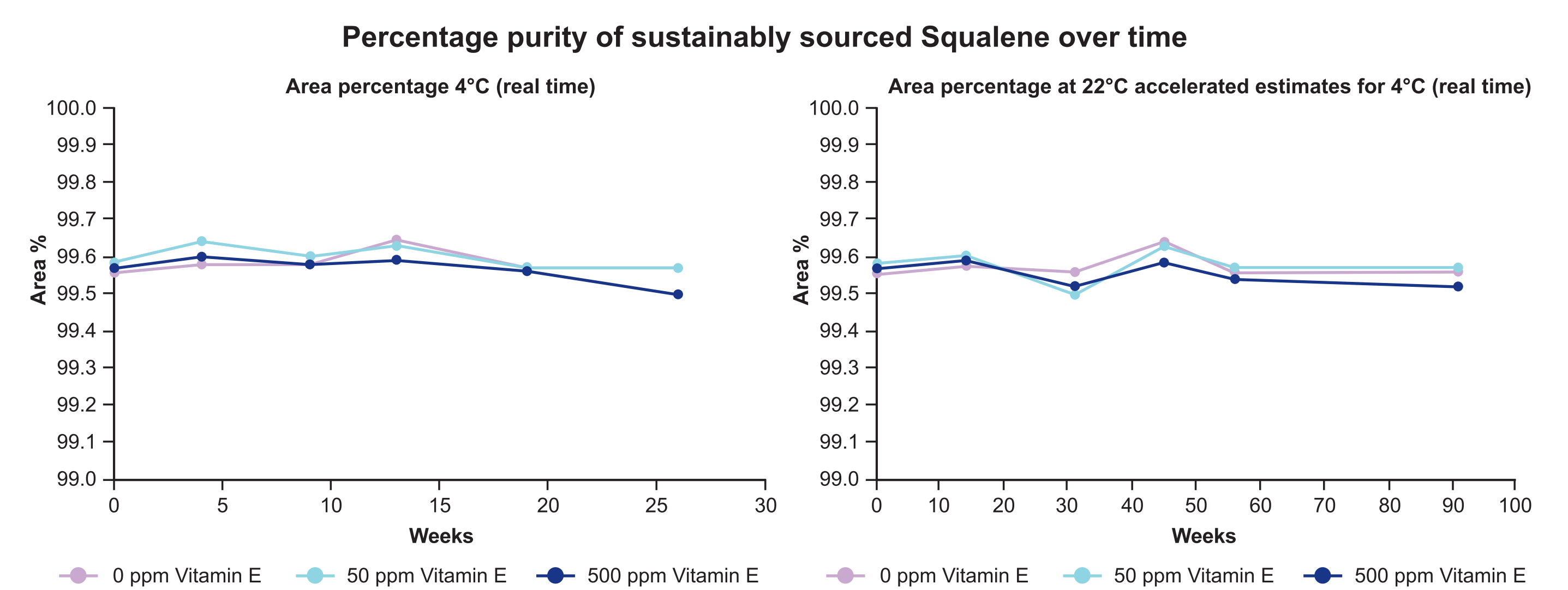
Our sustainably sourced Squalene complies with the specifications of the Ph. Eur. monograph 2805.

Squalene		EUROPEAN PHARMACOPOEIA 10.0	
	01/2020:2805	Temperature:	
		Time (min)	280
	Column	0-12	275
	Injection port		300
	Detection		
	Direction: flame ionisation.		
	Injection: 1 μ l.		
	Relative retention with reference to squalene (retention time = about 8.1 min): methyl lignocerate = about 0.9.		
	System suitability: reference solution (S):		
	- resolution: minimum 5.0 between the peaks due to methyl lignocerate and squalene.		
	Calculate the percentage content of $C_{30}H_{50}$ taking into account the assigned content of squalene CRS.		
	STORAGE:		
	In an airtight container, protected from light.		
	LABELLING:		
	The label states the origin of squalene (vegetable or animal).		
	This monograph applies to squalene used as an adjuvant in vaccines.		
			01/2017:1266

2. Purity

- Our sustainably sourced Squalene offers superior purity of $\geq 99\%$
- The specification includes two purity test methods:
 - EP 2.2.28 GC weight assay
 - An additional validated test method to determine the higher purity in line with stringent requirements of vaccine manufacturers
- Experience shows that for vaccine formulations, a purity of at least 99% is required
- Vaccine-grade squalene obtained from shark liver oil reaches a purity of $>99\%$ (<https://doi.org/10.1155/2015/367202>)

3. Sustainable Squalene stability*

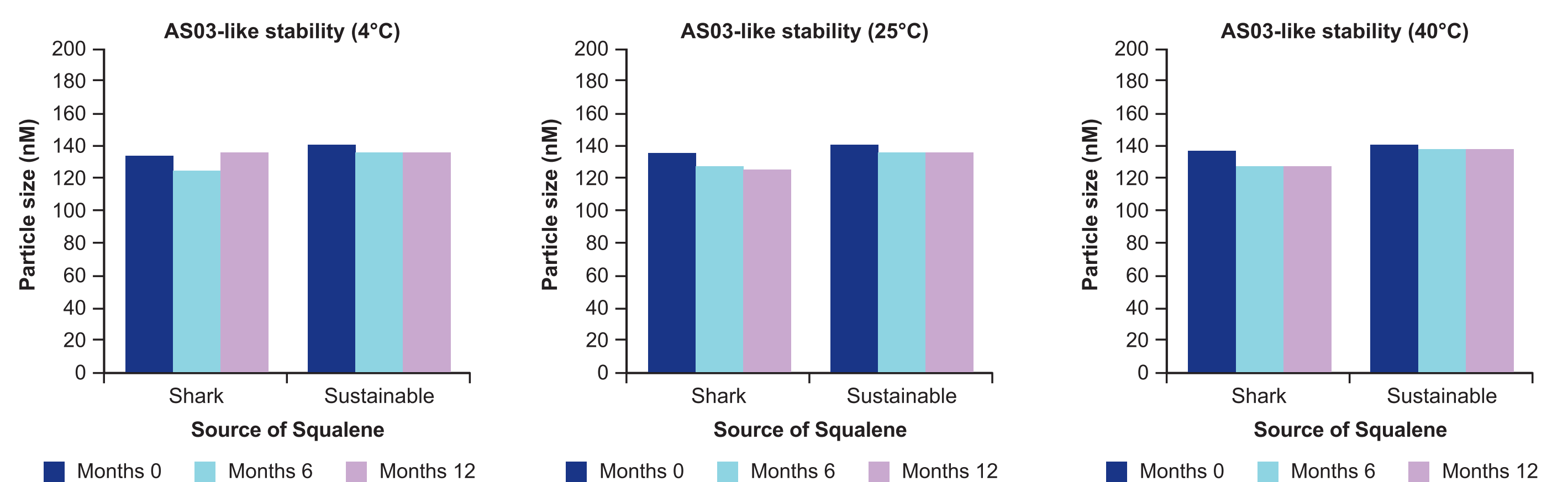


- Sustainable Squalene from Amyris is stable for at least 1 year based on stability studies (under nitrogen).
- It is likely stable for longer than 1 year: stability studies are ongoing

*Data generated by Amyris. Croda will run stability studies over 3 years in accordance with ICH guidelines.

4. Comparative testing with shark Squalene (1)*

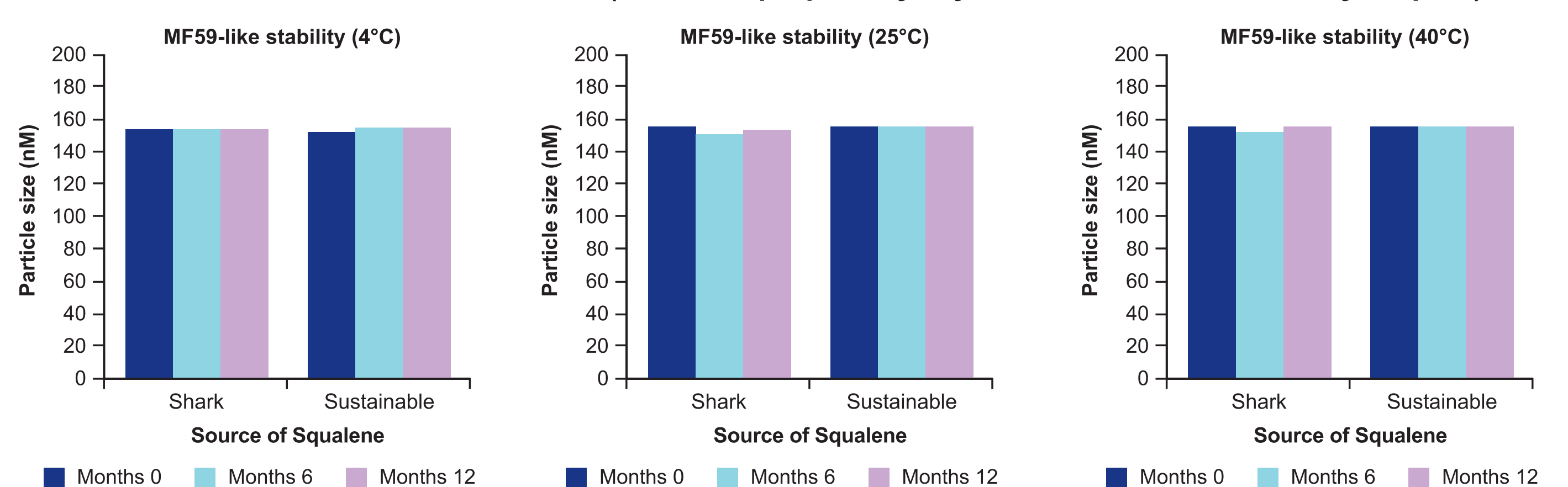
Particle size in AS03-like formulations (AS03 is a proprietary adjuvant formulation owned by GSK)



- AS03-like has identical stability when formulated with either shark (SAFC[®], GMP) or sustainable Squalene (Amyris, non-GMP).
- Accelerated stability (40°C) results indicate real time (4°C) stability of over 4 years

5. Comparative testing with shark Squalene (2)*

Particle size in MF59-like formulations (MF59 is a proprietary adjuvant formulation owned by Seqirus)



- MF59-like has identical stability when formulated with either shark (SAFC[®], GMP) or sustainable Squalene (Amyris, non-GMP)
- Accelerated stability (40°C) results indicate real time (4°C) stability of over 4 years

*Data generated by Amyris. The extrapolated stability of over 4 years is based on the Arrhenius equation.

Conclusion

Sustainably sourced and highly purified Squalene – the solution for vaccine formulations. For more information, please contact your local sales representative or visit our website www.crodapharma.com. References to supporting scientific papers and articles available upon request.

With data from amyris

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