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From Palm Refinery Residues to Vaccine Adjuvants: Qualifying PFAD-Derived Squalene Through Quality-by-Design and Immunological Comparability

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ABSTRACT

Squalene-based oil-in-water emulsions underpin established vaccine-adjuvant platforms, while palm fatty acid distillate (PFAD) provides a potentially recoverable non-animal source of squalene. This qualitative literature review critically integrates research on PFAD separation, pharmaceutical impurities, squalene-emulsion mechanisms, formulation stability, clinical vaccine evidence, and sustainable manufacturing. A 2025 pilot-scale PFAD-related separation study demonstrates substantial squalene enrichment, but reported chemical purity and recovery do not establish suitability for injectable use. Research on MF59, AS03, and experimental emulsions demonstrates formulation-specific immune effects and clinical utility; none directly validates PFAD-derived squalene as an interchangeable input. The synthesis identifies six connected gaps: feedstock representativeness, impurity-specific qualification, oxidative stability, scaled manufacturing reproducibility, formulation-matched biological comparability, and supply-chain lifecycle performance. We propose a five-gate translational pathway from feedstock characterization to material qualification, emulsion control, nonclinical testing, and context-specific regulatory and clinical evaluation. Alternative microbial and semisynthetic sourcing routes provide critical benchmarks. The review concludes that PFAD-derived squalene is a credible research candidate for diversifying vaccine-manufacturing inputs, but claims of pharmaceutical equivalence, superior immunogenicity, or sustainability must remain conditional on direct comparative evidence. The resulting source-to-vaccine framework establishes testable priorities for multidisciplinary research and risk-based industrial development.

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Introduction

Vaccine Adjuvants as Complex Pharmaceutical Systems

Adjuvants are central to modern vaccine design because immune protection is not determined by antigen identity alone. The duration, breadth, quality, and location of the response can depend upon the formulation in which antigen is presented and the early innate signals generated after administration. Oil-in-water emulsions containing squalene have helped establish this principle in licensed influenza vaccines and in recombinant-protein vaccine development. Their clinical record creates a promising reference platform for materials innovation, but it also imposes stringent demands on composition, manufacturing reproducibility, and safety. A change in the biological origin of one ingredient must therefore be assessed as a pharmaceutical change, rather than presumed innocuous because the nominal ingredient name remains constant [1-3].

The diversity of adjuvant formulations further complicates comparisons that appear straightforward on a chemical label. MF59 and AS03 both contain squalene, yet differ in auxiliary ingredients and cannot be treated as interchangeable delivery vehicles. Moreover, an emulsion is a structured multiphase product whose biological behavior is influenced by droplet characteristics, surfactant organization, antigen compatibility, and injection-site interactions. The clinical effectiveness of a licensed squalene emulsion reflects the performance of that complete product under defined manufacturing and clinical conditions. This distinction provides the interpretive foundation for evaluating palm-derived squalene: evidence that the molecule can be extracted does not demonstrate that a replacement material can reproduce the full quality profile of an established adjuvant [4-6].

The Opportunity and the Missing Translational Bridge

Palm refining generates a heterogeneous stream of fatty acids and minor constituents, including unsaponifiable molecules that may be recovered for higher-value applications. Palm fatty acid distillate, or PFAD, is attractive in this respect because using selected side streams could create additional value from existing refining infrastructure. A pilot-scale separation investigation reported substantial enrichment of squalene from a PFAD-derived liquid residue, with a highest reported purity of approximately

98% and recovery approaching 90.95%. These outcomes support engineering feasibility under the studied conditions, not clinical readiness. In particular, an assay reporting overall purity does not identify the remaining molecular species or establish that their concentrations are suitable for an injectable preparation [7-9].

The distinction matters because highly purified vegetable-derived oil can still contain feedstock-associated minor components, process-derived residuals, or new degradation products. A manufacturing train intended for food or cosmetic use may have a different set of acceptance criteria from a train intended to supply a pharmaceutical starting material. Squalene itself is unsaturated and vulnerable to oxidative reactions, making oxygen exposure, processing temperature, light, packaging, and hold time consequential. The central scientific problem is not merely whether PFAD can yield a high squalene percentage; it is whether the resulting material can be characterized, controlled, and shown to perform reproducibly when formulated as part of a vaccine adjuvant[10-12].

Research Aims, Boundaries, and Original Contribution

This review asks seven connected questions: what is known about PFAD squalene recovery; which source and process variables matter for product quality; what biological mechanisms characterize squalene emulsions; which physicochemical attributes determine formulation performance; what comparative experiments are necessary; how regulatory and manufacturing qualification should be sequenced; and when alternative sourcing would yield a verifiable supply-chain advantage. The main objective is to develop a source-to-vaccine translational framework grounded in chemical engineering, formulation science, immunology, and clinical evidence. Rather than inferring efficacy from molecular identity, the analysis identifies each evidence boundary that must be crossed before a palm-derived material can reasonably be regarded as suitable for pharmaceutical development [13-15].

The article is deliberately neither a promotional claim about palm commodities nor a quantitative estimate of clinical benefit. It treats established vaccine-adjuvant efficacy as evidence for particular tested formulations, PFAD separation studies as evidence for technological recovery, and alternative microbial or semisynthetic supply chains as comparative industrial options. The novelty rests on explaining why these evidence streams cannot be conflated and on organizing them into a staged qualification pathway. A useful outcome is a research agenda that identifies experiments capable of invalidating the central development hypothesis as well as experiments that might support it. This makes the proposed roadmap relevant to vaccine developers, separation engineers, quality specialists, and researchers in palm-producing economies [16-19].

Literature Review

Squalene Identity, Stability, and Sourcing

Squalene is an acyclic triterpene hydrocarbon with multiple carbon–carbon double bonds, and its physicochemical properties make it useful as the oil phase of selected emulsions. Its hydrophobicity and unsaturation are consequential rather than incidental: they affect dispersion behavior, analytical method selection, and susceptibility to chemical transformation. Squalane, a hydrogenated related molecule, should not be substituted conceptually for squalene because saturation changes chemical behavior and pharmaceutical history. Likewise, a product described as containing squalene does not establish its purity, residual-solvent profile, or oxidation state. A robust characterization strategy must establish chemical identity with orthogonal analytical methods and separately address related substances, volatile residuals, and storage-related changes [20,21].

Pharmaceutical relevance depends on impurity-specific material qualification, not on the largest value reported by a bulk purity assay described a renewable semisynthetic route meeting relevant pharmacopeial expectations and reported both physicochemical emulsion comparisons and an experimental immunological assessment against an established squalene source. Their work supplies a closer benchmark for an intended vaccine ingredient than recovery percentage alone, although its findings do not demonstrate the acceptability of PFAD-derived material [22].

Sourcing has evolved from reliance on shark-liver-derived materials toward plant, microbial, and semisynthetic alternatives. The environmental attraction of avoiding marine animal feedstocks is substantial, but a credible comparison must examine the full sequence from raw materials to the final qualified oil. A renewable starting material does not automatically mean that purification has a favorable energy balance or that the final product can satisfy good manufacturing practice. Fisher and colleagues described kilo-scale GMP synthesis of renewable semisynthetic vaccine-grade squalene, providing a particularly important counterexample to the assumption that only natural extraction can diversify supply. For PFAD, the meaningful benchmark is thus the quality and scalability of all feasible routes, not botanical provenance alone [23-25].

PFAD as an Industrially Variable Feedstock

PFAD is obtained during physical refining and is not a chemically uniform isolated compound. Its exact composition reflects crude-oil origin, upstream storage, deodorization conditions, and possible subsequent fractionation. Free fatty acids typically dominate, while unsaponifiable constituents occur at lower and variable concentrations. Recovery studies involving vitamin E, phytosterols, or squalene show the technological value of these minor fractions, but they also demonstrate why broad references to ‘PFAD composition’ obscure the actual starting matrix. A squalene-enriched residue obtained after previous extraction steps may differ considerably from unprocessed PFAD in both desired-component concentration and impurity burden [26-28].

Pretreatment choices can alter the material available to downstream separation. Esterification, saponification, supercritical-fluid extraction, adsorption, and distillation each influence the balance between recovery, throughput, reagent consumption, and chemical stress. Studies on palm and other vegetable-oil distillates illuminate feasible separation principles, although performance from olive or mixed-oil distillates cannot be transferred to PFAD without a matrix-specific test. For medicinal development, that transferability issue is more serious: a process optimized for recovery may co-concentrate minor impurities or introduce process residuals. Consequently, separation efficiency should be reported alongside full material balance and a species-resolved impurity profile, including samples from representative feedstock batches [29-31].

Alternative Squalene Technologies and their Implications

Microbial biosynthesis is an increasingly developed alternative to extraction from plants or animals. Work in Yarrowia lipolytica has demonstrated metabolic-engineering strategies that increase squalene generation by modifying precursor supply, compartmentalization, lipid metabolism, and secretion. Other investigations using waste cooking oil demonstrate how a waste-

derived carbon source may be linked to microbial production rather than direct recovery of a pre-existing squalene fraction. Such findings enlarge the strategic field in which PFAD must compete. They do not, however, mean that fermentation-derived squalene is intrinsically vaccine-qualified: microbial impurities, purification burden, and process reproducibility also require rigorous demonstration [31-35].

Engineering yeast cell-wall integrity and optimizing microbial pathways are relevant because recoverable titer, purification complexity, and mass transfer together determine industrial feasibility. Reviews of fermentation strategies describe the promise of cell-factory development, but the scientific literature often emphasizes production metrics without equal attention to injectable-material impurity limits or a defined vaccine-adjuvant comparability standard. Industrial route selection therefore requires more than comparing the highest published purity or titer. A transparent assessment should distinguish demonstration scale, manufacturing controls, quality data, dependence on feedstock markets, and the availability of an appropriate regulatory evidence package. Plant extraction, yeast fermentation, and semisynthetic methods may ultimately supply different segments of the market rather than one universally preferred pathway [36-40].

Immunological Rationale of Squalene Emulsions

Oil-in-water emulsions can modify how vaccination is perceived by innate immune cells and how immune activation develops at the injection site and draining lymph nodes. Their activity is not adequately explained by describing squalene as an independent receptor agonist. Emulsion formulation can create tissue-level interactions involving cell recruitment, antigen transport, inflammatory mediators, and coordination between dendritic cells and responding lymphocytes. Research on MF59 and related systems highlights complex effects on the magnitude and quality of adaptive responses, including antigen-specific B-cell processes. The relevant scientific unit is therefore the engineered emulsion in its antigenic context; a chemically identical squalene phase may behave differently when combined with different surfactants or formulated at different droplet sizes [41-43].

Mechanistic findings also warn against portraying all emulsion adjuvants as if they signal through identical pathways. Experimental comparisons have identified differences in dendritic-cell function and dependence on innate signaling components between particular squalene-emulsion formulations. Other studies have shown contributions from RIPK3-associated pathways to selected CD8 T-cell responses without corresponding effects on antibody readouts in the examined model. These divergent outcomes underscore the need for multiple measures of biological activity, including cellular and humoral endpoints, instead of relying solely on a single antibody concentration. Such differences are particularly consequential when benchmarking a novel source of squalene against an established complete adjuvant [44,45].

Recent mechanistic research makes the requirement for endpoint specificity even clearer. reported that perturbing uric-acid signaling in mouse studies of squalene-based emulsions reduced selected dendritic-cell, B-cell and T-cell responses, implicating damage-associated signaling in the adjuvant effect. This observation is relevant to mechanism-oriented assay selection, but it neither identifies a universal pathway for all emulsions nor demonstrates any advantage for a palm-derived squalene source [46].

Clinical Performance is Formulation-Specific

Experimental studies of chemically related oil phases provide a cautionary comparator for material substitutions. found that different semisynthetic terpenoids produced different adjuvant properties when incorporated into emulsion systems, underlining the importance of the complete formulation and not merely the renewable nature of a hydrocarbon component. For PFAD-derived squalene the central hypothesis remains narrower: a chemically characterized replacement material may support comparable product quality if matched manufacturing and biological investigations confirm it [47].

The clearest translational evidence for squalene-containing adjuvants derives from studies of finished vaccines rather than isolated oils. Investigations of MF59-adjuvanted influenza vaccines in older adults and AS03-adjuvanted protein vaccines have generated evidence concerning immunogenicity, efficacy, and safety under defined conditions. These data establish a credible clinical rationale for the adjuvant class, while leaving the source of pharmaceutical squalene in any proposed PFAD product untested. Clinical results can therefore inform target product profiles and benchmark endpoints, but cannot substitute for source-specific chemical and formulation qualification. Failure to respect this distinction would amount to transferring a clinical claim between materially different supply chains without demonstrating comparability [48-51].

Studies comparing enhanced influenza vaccines require especially careful interpretation. Performance varies by season, circulating strains, patient characteristics, antigen production platform, outcome definition, and choice of comparator. Evidence syntheses have not consistently shown superiority of one enhanced platform over every other enhanced platform, even where advantages relative to standard-dose vaccines were observed. This should discourage simplistic claims that adding squalene produces a universal or fixed benefit. In a development program for palm-derived squalene, a meaningful question is whether a carefully matched formulation retains the predefined performance of a reference emulsion, not whether the palm feedstock itself increases vaccine efficacy beyond that of licensed products [52-55].

Methodology: Qualitative Literature Review

Review Design and Analytical Strategy

The study uses a qualitative literature review with critical interpretive synthesis. The objective is to connect research domains that use different primary methods and report different outcome types: chemical separation yield, impurity characterization, emulsion physicochemistry, molecular immunology, clinical trial outcomes, and health-system manufacturing capacity. This approach is appropriate because the review seeks to define a translational qualification problem rather than estimate a pooled treatment effect. It is expressly not a systematic literature review or meta-analysis, and it does not claim exhaustive retrieval, registered eligibility criteria, risk-of-bias scoring for all records, or PRISMA compliance. The analytical focus is on the coherence of claims across domains and the limitations of moving from one level of evidence to the next [50-55].

Purposive Identification and Interpretive Coding

The literature was assembled purposively around searches pairing squalene with PFAD, palm refining, molecular distillation, unsaponifiables, yeast biosynthesis, renewable sourcing, oil-in-water adjuvant, MF59, AS03, microfluidic emulsion, quality-by-design, and vaccine manufacturing. Priority was given to

peer-reviewed work published during 2020–2026, including experimental process studies, mechanistic research, clinical investigations, and scholarly syntheses. Selected older foundational concepts informed interpretation but were not needed as formal references in this contemporary bibliography. Sources were grouped by five analytic dimensions: source and recovery; identity and impurity control; emulsion product attributes; biological and clinical evidence; and economic, sustainability, and regulatory readiness [1-10].

Quality Appraisal without Systematic-Review Claims

Critical appraisal considered whether each study directly examined PFAD-derived squalene, a squalene emulsion using another source, a different plant processing stream, or an adjacent technology such as microbial production. Attention was given to study scale, reported analytical methods, comparator appropriateness, endpoint specificity, and the limits of inference. A clinical trial of an AS03-containing vaccine was treated as evidence for that particular vaccine, not as evidence for PFAD chemical purity. Similarly, a laboratory study reporting squalene enrichment was treated as source-processing evidence, not as confirmation of biological safety. Divergent or negative findings were deliberately retained where they constrained generalization. This transparent separation of evidentiary levels constitutes the principal rigor mechanism of the qualitative method [23,11,7].

Scope and Limitations of the Review

No new analytical assays, toxicological experiments, pharmacokinetic measurements, or vaccine trials were conducted for this article. The proposed acceptance criteria, decision gates, and experimental comparisons are research recommendations and should not be confused with official numeric regulatory specifications. Manufacturing quality requirements also depend on the intended market, development status, dosage form, and the sponsor's agreed regulatory strategy. The literature base is dominated by distinct studies of squalene recovery and established vaccine adjuvants, with an important gap in direct studies linking both. Accordingly, conclusions are strongest on the existence of separate technology platforms and least certain on claims of their interchangeability [18,28,2].

Results: Thematic Findings

Theme I - Recovery from PFAD is Technically Plausible but Matrix-Dependent

The pilot-scale PFAD research provides a tangible technological starting point. Jalani and colleagues reported major enrichment of squalene in a residue derived from PFAD processing, with a maximum purity approaching 98% and recovery approaching 91% under reported experimental conditions. Such figures are relevant to deciding whether separation is worth pursuing at larger scale, but they are conditional on the composition of the particular upstream residue and the processing sequence used to generate it. Neither figure describes the identity or toxicological significance of the remaining material. Nor can either be generalized automatically to every palm refinery, whose refining settings and side-stream management may differ [28].

Adjacent PFAD investigations show that minor components can be concentrated or formulated using varied downstream technologies. Work on unsaponifiable fractions, separation of tocols and phytosterols, and supercritical carbon dioxide extraction provides transferable process insights about fractionation, solubility, and feedstock pretreatment. However, these studies frequently optimize nutritional or other noninjectable product characteristics. Their usefulness in this analysis is as evidence of separation feasibility and process complexity, not as a shortcut to medicinal qualification. A pharmaceutical development program would need a defined feedstock acceptance specification, traceable batches from multiple refining cycles, and a documented account of each chemical treatment before purification [1,21,40].

The distinction between engineering recovery and injectable material qualification is mapped in Figure 1. Its sequential evidence requirements make clear why a reported separation yield cannot substitute for compositional and impurity-based material qualification [18,28].

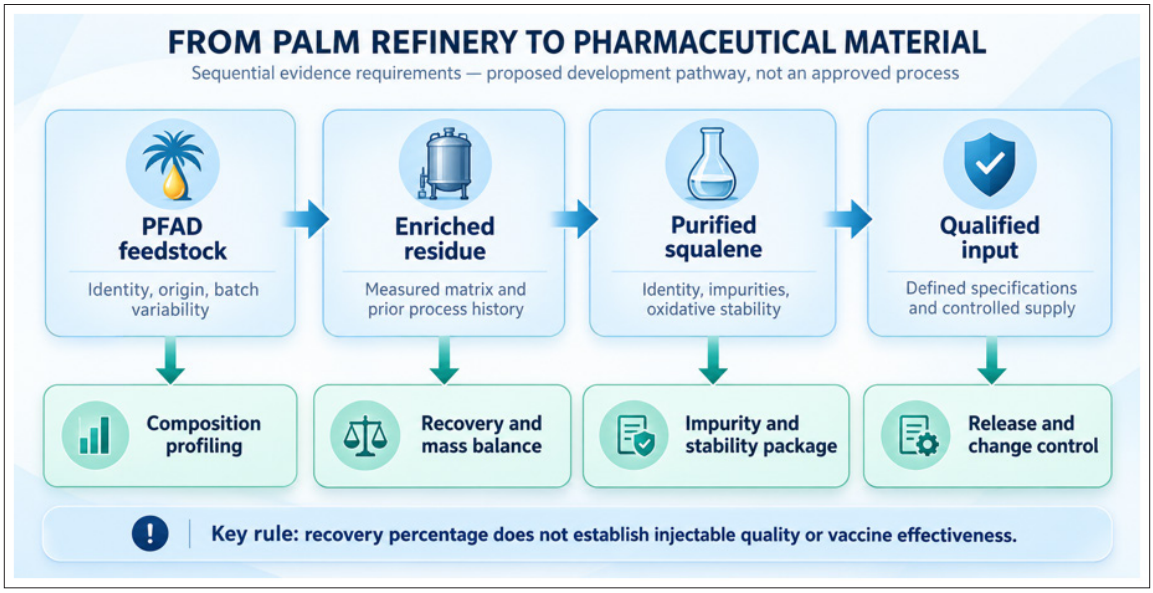


Figure 1: Proposed source-to-material qualification pathway for recovery of squalene from PFAD-Associated Refining Residues. Each Downward Arrow Denotes An Independent Evidence Requirement

Figure 1 organizes the upstream and downstream operations that a PFAD-based material would traverse. Each transition is associated with a different kind of evidence: compositional analysis for feedstock intake, process capability for purification, impurity qualification for pharmaceutical release, and biological comparability for vaccine development. The figure is deliberately not a depiction of an already validated manufacturing process. Rather, it shows where data would be required and where the manufacturer should reject or reprocess a batch that cannot meet predefined criteria. A major implication is that the development program must identify at which point traceable palm refining material becomes a controlled pharmaceutical starting material, with a defensible and auditable chain of custody [18,28].

Theme II-Impurities and Oxidation Define a more Demanding Quality Problem

A reported purity percentage is a useful screening metric but is not equivalent to a pharmaceutical impurity specification. The identity, toxicological relevance, and batch variability of minor constituents are more informative than an undifferentiated two-percent residual fraction. The analytical package should examine structurally related hydrocarbons, oxidized squalene species, traces of solvents or separation media, potential metal contamination from equipment, and other matrix-related components justified by process knowledge. The actual target list should be derived from measured feedstock and process hazards rather than copied uncritically from food processing or cosmetic ingredient standards. The methodology must also establish that the squalene assay accurately quantifies the intended compound in the presence of close analogues and oxidation products [18,5,10].

Chemical oxidation deserves specific control because squalene contains multiple reactive double bonds. External research on squalene chemistry demonstrates its capacity to form oxidation-related products, although studies of atmospheric squalene–ozone reactions do not directly establish which products arise in sealed pharmaceutical manufacturing. They are evidence of chemical susceptibility, not a substitute for process-specific forced-degradation experiments. Development studies should therefore include appropriately validated stability-indicating methods, defined exposure to heat and oxygen, container-closure compatibility, and time-dependent impurity mapping. Requirements for antioxidant addition would need separate evaluation, as antioxidants may alter emulsion composition and biological performance [10,35].

This analytical concern has been strengthened by a recent method-oriented study. presented a carbonyl-content assay using derivatization and chromatographic analysis to monitor oxidation-related products in squalene-in-water vaccine adjuvants, illustrating how stability testing can target chemically meaningful degradation products rather than relying on visual appearance or total squalene assay alone. The exact panel of degradation markers would still need validation for the proposed PFAD-derived input and its intended emulsion; no existing method should be assumed to identify every possible process-specific impurity [42].

Palm refining also requires broader process-hazard awareness. Research on food oils documents the formation and mitigation of certain processing contaminants, including glycidyl esters and 3-MCPD-related compounds. That literature is relevant for developing upstream hazard hypotheses, but it does not demonstrate that such compounds persist in squalene purified through a proposed pharmaceutical separation train. Detection and transfer must be empirically investigated before hazard narratives become product specifications. A defensible strategy begins with targeted and untargeted analytical screening and proceeds to risk-based control of substances actually detected or credibly introduced during processing. This avoids both ignoring potentially harmful impurities and wrongly treating every known refined-oil contaminant as a demonstrated contaminant of purified squalene [53,21].

Theme III - Alternative Routes Sharpen the Benchmark for PFAD Development

The literature on microbial squalene and semisynthetic vaccine-grade production shows that route diversification is already technologically active. Fermentation studies in engineered yeasts have increased production capability through modifications to precursor pathways, subcellular metabolism, and secretion, while selected work has explored waste-based substrates. These developments create an alternative path in which carbon feedstock becomes squalene biosynthetically rather than through direct extraction. By contrast, the reported kilo-scale renewable semisynthetic GMP work provides a closer pharmaceutical comparator because manufacturing standards and intended vaccine-grade use are explicit. None of these studies alone establishes a universal cost or sustainability advantage, but together they make the commercial development threshold for PFAD-based squalene more realistic [34,49,39,9,18].

The decision to pursue PFAD may be justified by access to palm refining infrastructure and potential value recovery from existing process streams. However, that justification weakens if numerous solvent-intensive separation steps, unusually high heat inputs, or extensive waste treatment are required for pharmaceutical purity. Fermentation also faces purification and infrastructure challenges, and semisynthetic approaches require appropriate intermediates and process controls. The pertinent comparison is therefore between fully specified pathways delivering equivalent material quality at the required scale. Indicators should include yield per unit feedstock, energy demand, effective batch throughput, solvent reuse, waste treatment, lot-to-lot variance, and the cost of a validated pharmaceutical quality system [46,48,44,24].

Theme IV- Emulsion Behavior Depends on more than Squalene Origin

Formulation studies demonstrate that squalene can be incorporated into adjuvant emulsions using different manufacturing approaches. Microfluidic methods offer controlled droplet generation and may improve the study of formulation parameters, while self-emulsifying systems and variations in surfactant stabilization provide alternative development platforms. Animal studies have reported immunological activity for particular experimental squalene emulsions, but those results are tied to the studied antigen, dose, excipient mixture, and route of administration. A future PFAD comparison should not introduce multiple simultaneous formulation changes because doing so would make any difference in biological response impossible to attribute to the source of squalene [6,8,51].

Manufacturing scale also deserves treatment as an independent dimension of product comparability. described process changes that supported scale-up from thousands to millions of doses in a squalene-emulsion manufacturing context, while maintaining attention to droplet characteristics and manufacturing efficiency. This result concerns an established emulsion manufacturing process, rather than the substitution of a new palm-derived oil phase; nevertheless, it shows why process robustness and in-process measurements should enter the development plan before

claims about vaccine supply capacity [43].

Droplet diameter and distribution, oil content, free surfactant concentration, viscosity, pH, ionic composition, and aggregation behavior are relevant product attributes. Some may be affected by trace components carried with the oil phase, while others are governed primarily by equipment and process settings. Research on oil-phase selection in different emulsion systems reinforces the importance of formulation-specific optimization. The key translational question is whether independently manufactured batches using qualified palm-derived squalene fall within predefined physicochemical comparability ranges and remain stable throughout the intended storage period. Meeting numerical targets at initial manufacture is necessary but insufficient if the emulsion changes during cold-chain transport or repeated handling [52,25,6].

Quality-by-Design offers a useful organizing principle, though experience with liposomal products or dried vaccines should be treated as an analogy rather than direct validation of PFAD-based squalene. A development team can define a target product profile and map the relationships among material attributes, process parameters, and critical quality attributes. Structured experiments may then establish acceptable operating ranges and the sensitivity of the product to plausible raw-material variation. This risk-based approach is more informative than simply reproducing a nominal recipe with a different oil source. It also makes it easier to detect whether chemical purity, droplet performance, or biological activity becomes the limiting constraint at successive scales [2,20,52].

As summarized in Figure 2, the evidence chain connecting oil chemistry with vaccine responses contains several independently testable levels. Each level must be characterized before a full-system comparison can support a claim of functional similarity [37,33].

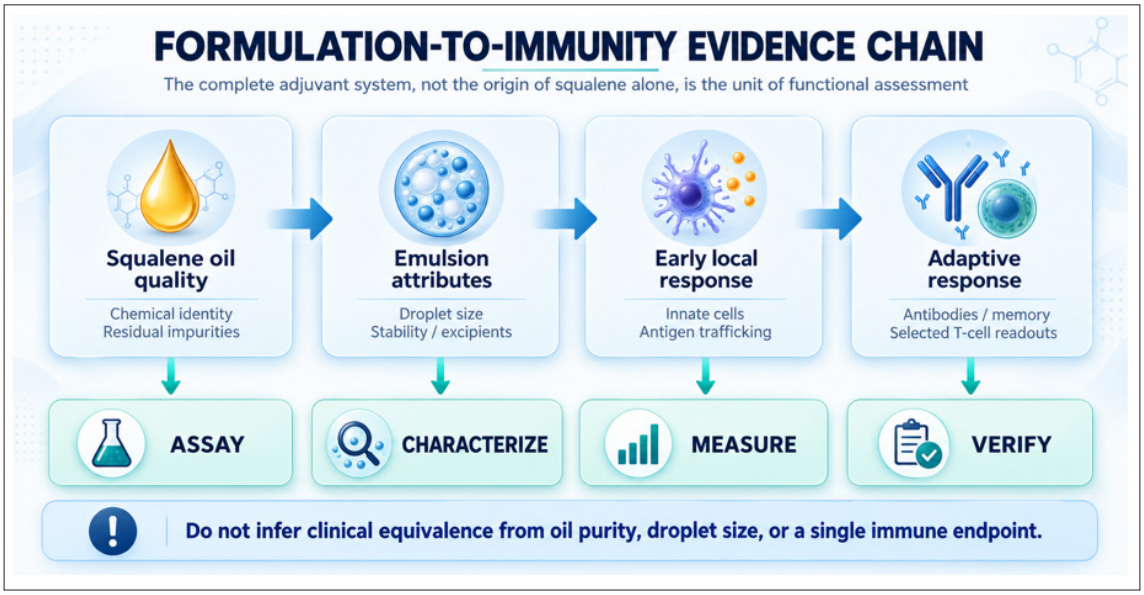


Figure 2: Conceptual Separation of Material Identity, Emulsion Physicochemistry, Local Innate Responses, and Antigen-Specific Adaptive Immunity. Formulation-Specific Measurements are Required at every Level

Figure 2 separates the sequence from formulation properties to innate and adaptive responses. This separation matters because assays for oil purity, particle size, local inflammation, antibody generation, and safety answer distinct questions. A visually similar emulsion is not necessarily immunologically equivalent; conversely, a transiently comparable antibody titer does not rule out important differences in tissue exposure or inflammatory effects. The preferred evaluation strategy therefore uses a tiered panel of measurements rather than a single surrogate, with a reference product manufactured using a well-characterized existing squalene supply. Different readouts are interpreted collectively and in the context of the exact antigen and experimental model [37,29,54].

Theme V-Mechanistic and Clinical Vaccine Evidence Sets Boundaries

Research on adjuvants has established that early innate responses can influence later B-cell and T-cell outcomes, but the direction and size of effects depend on the specific formulation. In mechanistic investigations, selected emulsion adjuvants differ in signaling dependence and dendritic-cell responses; work on RIPK3-associated processes further demonstrates that cellular and antibody outcomes need not move together. For pharmaceutical comparability, these findings mean that the evidence package should encompass the biological axes most relevant to the reference formulation, including innate-cell recruitment, antigen transport, antigen-specific antibody quality, and selected cellular responses. A fixed checklist applied indiscriminately across every antigen would be less informative than a justified, product-specific set of assays [37,29,55].

Human investigations add additional dimensions of biological relevance. Studies of AS03-adjuvanted vaccines report patterns of memory B-cell magnitude and persistence, while analyses of innate signatures explore predictors of antibody response quality. Preclinical macaque studies provide examples of the breadth of antigen-specific responses that may be achieved in a tested vaccine system. Collectively these findings demonstrate that adjuvants can influence response quality beyond peak antibody quantity. They do not establish that a squalene feedstock substitution would preserve those signatures; that issue remains for direct comparative testing

using otherwise matched formulations [22,47,16].

A 2024 mouse study combining recombinant SARS-CoV-2 spike with squalene-containing emulsions and defined Toll-like-receptor agonists further illustrates the influence of co-formulated immune stimulants. Changes in immune polarization and cross-protective responses in that model cannot be attributed to squalene independently of antigen identity and the accompanying agonists. Such experiments are useful for selecting discriminating immune endpoints, but direct clinical or material-source equivalence cannot be inferred from them [32].

Clinical studies of recombinant protein and influenza vaccines further confirm that final-product outcomes can be measured and compared under controlled conditions. Phase 3 evidence for AS03-containing recombinant vaccines, phase 3 assessment of MF59-containing influenza vaccination, and phase 2 evaluation of an MF59-adjuvanted cell-derived influenza candidate all provide examples of clinically meaningful endpoints and study designs. Yet their findings are influenced by antigen selection, population, production technology, and comparator choice. They should not be pooled conceptually into a claim that every squalene emulsion has the same efficacy, much less that PFAD-derived squalene has already been clinically validated [23,12,11,4,15,3].

Real-world comparative studies can complement clinical trials, but they also illustrate interpretive complications. Influenza vaccination effectiveness may vary with season, circulating strain, patient frailty, comorbidity, and ascertainment of hospital or outpatient outcomes. Observational relative-effectiveness estimates from a particular influenza season should not be transplanted into a material-qualification assessment. More importantly, comparative evidence among adjuvanted, high-dose, and other enhanced influenza platforms does not isolate the causal contribution of squalene or of feedstock provenance. The PFAD research agenda is better served by controlled analytical and preclinical comparability initially, with human studies contemplated only if a defined regulatory and scientific rationale warrants them [26,27,13,17].

Theme VI -Global Supply Resilience is A Potential Benefit, Not A Proven Outcome

The literature on vaccine manufacturing consistently identifies geographically concentrated technical capacity, financing, skills, and supply chains as obstacles to equitable access. Manufacturing resilience is therefore a plausible public-health benefit of developing additional qualified suppliers of critical ingredients. However, a new squalene source will improve resilience only if it produces compliant lots reliably, integrates with established vaccine production, avoids creating new single points of failure, and can compete on total delivered cost. The existence of palm refining capacity in a country does not imply the existence of qualified vaccine ingredient manufacture, sterile product formulation capability, or regulatory readiness [30,36,14,31].

Environmental claims require the same causal discipline. A PFAD-to-squalene pathway may reduce reliance on marine animal supply and extract more value from refining streams, but its impacts depend on coproduct allocation, alternative PFAD uses, solvent demand, energy sources, and transportation. Life-cycle modeling of PFAD-derived fuel illustrates how assumptions regarding the treatment of PFAD influence environmental results in another application; it is not a lifecycle assessment of vaccine-grade squalene. For the proposed pharmaceutical pathway, sustainability should be demonstrated through a boundary-defined comparative assessment of equivalent functional outputs rather than asserted from the label 'waste-derived.' The potential benefits are meaningful enough to test rigorously, not strong enough to accept without measurement [50,24,35].

Table 1: Summarizes the Main Evidence Domains and the Specific Uncertainty that Remains at each Interface. The table is a critical interpretive synthesis: it does not represent pooled findings or a formal assessment of study certainty, and the strength of each inference depends upon which material and formulation were actually examined [28,18,33].

Table 1: Critical Synthesis of Evidence Streams and Unresolved Requirements for PFAD-Derived Squalene Translation.

Evidence domain	Established findings	Unresolved qualification need
PFAD recovery	Pilot-scale squalene enrichment from a specified PFAD-derived residue.	Multi-refinery representativeness and traceable lot-to-lot input variation.
Pharmaceutical material	Renewable semisynthetic vaccine-grade squalene has been manufactured under GMP.	PFAD-specific assay validation, oxidation profile, impurity limits and batch consistency.
Formulation engineering	Emulsion scale-up and microfluidic formulation methods have been reported.	Matched palm/reference squalene emulsions and stability across production lots.
Mechanistic immunity	Specific emulsions influence innate recruitment, antigen handling and adaptive immunity.	Comparative immune readouts for the same antigen, dose, excipients and route.
Clinical evidence	Selected MF59- and AS03-containing finished vaccines have clinical trial evidence.	No direct clinical validation of a vaccine formulated with PFAD-derived squalene.
Sustainability	Alternative sources could diversify supply chains.	Quality-equivalent comparative lifecycle and techno-economic assessments.

The evidence domains show that no single class of measurement establishes the entire translation pathway. Progression requires convergent findings across source chemistry, robust manufacturing, emulsion quality and product-specific safety; observed clinical efficacy of established vaccines is informative but not transferable as direct evidence for untested PFAD-derived squalene [18,43,23].

Discussion and Analysis

Reconciling Source Chemistry with Pharmaceutical Identity

The combined evidence supports a conditional, rather than affirmative, answer to the central feasibility question. Palm-derived squalene can be enriched substantially from selected PFAD-related residues, and squalene is a documented component of successful vaccine adjuvant systems. However, no logical operation turns these two established observations into proof that the recovered PFAD product meets injectable pharmaceutical requirements. Pharmaceutical identity consists of more than confirming the dominant molecular structure: it includes impurity control, handling history, reproducibility, and compatibility with a defined formulation. Consequently, route-specific contamination and degradation profiles must be compared with an appropriate qualified reference material before any claim of technical interchangeability is justified [28,18,35].

The most efficient research strategy would define an explicit reference standard from the outset. A sponsor could select an existing well-characterized squalene material used for adjuvant development and manufacture both control and test emulsions with otherwise matched ingredients. The squalene oils should be characterized independently before emulsification, and the resulting emulsions evaluated using the same validated physical and biological methods. This design prevents chemical similarity from being confused with experimental equivalence and identifies whether any observed difference arises before or after formulation. If a material repeatedly fails pre-specified limits, it should be revised or rejected rather than allowed to progress on the strength of its renewable origin [18,6,2].

An Evidence Hierarchy for Quality and Function

A useful hierarchy begins with compositional feasibility, proceeds to pharmaceutical material quality, then to emulsion manufacturability, biological comparability, toxicological qualification, and finally clinical relevance. These levels are nested, not interchangeable. Analytical purity establishes the amount of the main component under a specified assay. Formulation comparability establishes that the oil can be processed into a product with controlled critical quality attributes. Biological comparability assesses immunological and safety responses in appropriate models. Clinical benefit, when it is necessary to demonstrate, concerns a complete vaccine and an indicated population, not merely a raw-material procurement decision. The hierarchy thus makes visible where confidence is warranted and where major inference gaps remain [28,25,55,23].

A common problem in emerging bioactive ingredient development is the tendency to privilege a conspicuously high purity value while giving less attention to what remains after purification. For injectable materials, this is precisely reversed: a small amount of an unidentified reactive or biologically active substance can be more consequential than a larger amount of an innocuous, well-characterized related molecule. Orthogonal chromatography and spectrometry, oxidative stability studies, elemental analysis where appropriate, and process-specific evaluation of possible residuals can define that uncertainty. The final analytical program should be proportionate to a toxicological risk assessment and the intended route of administration. In this sense, 'pharmaceutical grade' is an outcome of a demonstrated quality system, not a label that follows from percent purity alone [18,10,53].

Formulation Comparability and Immunogenicity

The biological mechanism literature suggests that a future PFAD evaluation should avoid a narrow focus on antibody titer. Emulsion-associated cell recruitment and responses in draining lymphoid tissues may influence later antibody magnitude, affinity maturation, memory development, or cellular immunity. A comparative package could therefore combine injection-site histology or validated imaging with innate cytokine measurements, antigen-specific antibody assays, functional neutralization where relevant, and assessment of memory-related outcomes. These measures should be selected in advance for the chosen vaccine platform and interpreted together, rather than using favorable isolated endpoints after the experiment [38,22,47,54].

Experimental comparisons require high-quality controls. A palm-squalene emulsion should be compared with a formulation made from an appropriate qualified reference squalene lot, keeping antigen identity, oil content, surfactants, manufacturing settings, dose, and route constant. Where differences occur, chemical characterization should help determine whether the cause is a trace component, oxidation, or an unintended change in emulsion structure. Experiments should include more than one independent oil lot because one successful batch cannot demonstrate process capability. Investigators should also use predefined equivalence or comparability criteria suited to the assay and its variability, avoiding unsupported claims of equivalence when a conventional significance test simply finds no difference [37,6,51].

A useful positive comparator exists outside the palm-refining pathway. In a semisynthetic squalene investigation, comparative emulsions produced with renewable and shark-derived materials had similar physicochemical stability over the reported storage interval and similar measured immune activity in the selected mouse model. That study demonstrates the type of matched evaluation that a new source could emulate, but not that comparable results will automatically follow for a PFAD-derived batch with a different impurity and thermal history (Fisher et al., 2023a; Fisher et al., 2023b).

It is especially important not to collapse AS03 and MF59 into a generic 'squalene vaccine' model. Their complete compositions are different, and studies of one cannot automatically serve as evidence for the other. A pragmatic early development program might begin with a research-scale MF59-like formulation if scientifically justified, while clearly avoiding claims of exact identity to a proprietary approved system. Expansion to AS03-like platforms would require attention to their additional components and distinct mechanisms. Clinical trial literature can help specify desired vaccine-response outcomes, but a sponsor should not claim clinical efficacy by borrowing efficacy estimates from trials that never used the palm-derived material [41,38,12,11].

Quality-By-Design and the Practical Test Matrix

A structured Quality-by-Design program should identify critical material attributes of the squalene input, critical process parameters of its purification and emulsification, and critical quality attributes of the final emulsion. A preliminary risk assessment might flag raw-material composition, residence time at elevated temperature, exposure to oxygen, solvent stripping, adsorbent carryover, and storage conditions. Experimental work can then test which variables meaningfully affect impurity formation, droplet characteristics, and stability. Although QbD applications in liposomal formulations and vaccine drying are not direct evidence for squalene sourcing, they show how systematic relationships between material and process variables can support robust product development [2,20,6].

The test matrix should include an explicit baseline period when process and analytical variability are characterized independently. Before comparing biological outcomes, the laboratory must show that squalene assays, particle-sizing methods, and relevant stability tests are fit for purpose. For each oil lot, the certificate of analysis should be reconciled against independent confirmatory measurements where feasible. Batch trends rather than only pass/fail outcomes can expose emerging drift in impurity or oxidation profiles. Stress testing should include the storage and transport conditions that the product is likely to encounter. The ultimate objective is not the largest possible volume of test data but a proportionate and interpretable package that explains why the source can be controlled [18,53,5].

Stability-indicating analysis should be performed alongside conventional droplet metrics throughout the proposed manufacturing and storage program. Payne et al. (2026) provide an example of targeted carbonyl detection for squalene-adjuvant oxidation, and Phan et al. (2020) show the utility of complementary particle-sizing and chromatographic measurements during manufacturing scale-up. Combining orthogonal measurements with predefined trends would help identify chemical change before it manifests as visible emulsion instability, although the acceptable limits must be defined for the particular candidate formulation [42,43].

Figure 3 Presents the Proposed Five Decision Gates Linking Raw-Material Assessment to Context-Specific Clinical Development. The Graphic is a Research Planning model and Intentionally does not Establish Official Regulatory Thresholds or Claim Authorization [18,42].

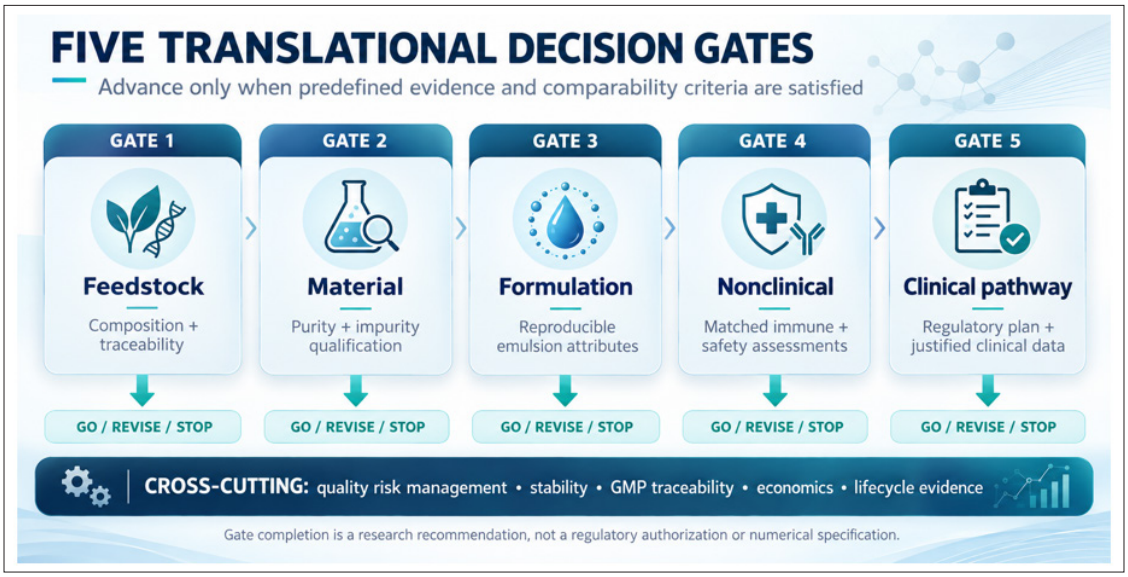


Figure: Five Proposed Go/Revise/Stop Decisions for Moving PFAD-derived Squalene from Process Feasibility Toward a Justified Vaccine-Development Context

Figure 3 depicts a proposed sequence of translational decision gates. The first two gates concern feedstock consistency and material identity; the next two concern repeatable emulsion manufacture and biologically meaningful nonclinical comparability; and the final gate concerns the relevance of clinical investigation and a justified regulatory pathway. Progression is conditional at every transition. If product quality cannot be controlled at the earlier stages, conducting more elaborate vaccine animal experiments is inefficient and ethically difficult to defend. Conversely, once chemical and physical comparability are established, the experimental agenda should become increasingly biological, rather than repeatedly demonstrating extraction yield [28,18,37].

Safety, Clinical Extrapolation and Regulatory Expectations

A source substitution can be low risk when its impurity and process profiles are well understood, but 'chemically identical' does not in itself mean 'regulatorily interchangeable.' The intended regulatory pathway will depend upon the developmental stage and legal status of the complete vaccine, the magnitude of material changes, and the evidence agreed with the relevant authority. For a novel product, the developer should qualify raw-material suppliers, establish GMP-relevant controls, characterize substances that could enter the patient, and define formulation acceptance criteria before making a clinical claim. These recommendations are principles of risk management; they are not presented here as a substitute for jurisdiction-specific guidance or a binding regulatory decision [18,41,20].

Toxicological assessment should likewise focus on realistic exposures. A trace compound observed in a concentrated intermediate is not necessarily present in the purified squalene, and a compound present in purified oil may be further diluted within the final emulsion. Nevertheless, the route of administration makes uncertainty about certain impurities consequential. Investigators should determine which components are detectable in the clinical-intended material, whether they degrade during storage, and whether relevant systemic or local exposure is plausible. Test systems must be chosen to answer these product-specific questions rather than to generate a generic catalogue of animal toxicity endpoints. The most defensible result is a documented risk-based assessment in which remaining uncertainty is explicit [53,10,18].

Clinical evidence is important but must be positioned appropriately. Large AS03-containing and MF59-containing vaccine studies demonstrate that defined emulsion formulations can be evaluated through immunogenicity, reactogenicity, safety monitoring, and disease outcomes. A manufacturer contemplating use of palm-

derived squalene should use those study designs as contextual benchmarks rather than as evidence that the palm-derived ingredient is clinically equivalent. In some developmental circumstances a validated comparability package may allow a change to be managed without starting a new complete efficacy program; in others, additional studies could be necessary. The distinction depends on the product, its history, analytical findings, and regulatory agreement [23,4,15,17].

Sustainability, Economics and Global Vaccine Access

The potential public-health value of PFAD sourcing extends beyond chemical manufacturing. Qualified, distributed ingredient suppliers could reduce a narrow supply dependency and possibly support regional participation in vaccine value chains. Yet refining capacity, pharmaceutical purification, finished-dose manufacture, and independent quality oversight are distinct capabilities. The global manufacturing literature highlights the need for skilled workforces, technology transfer, sustainable demand, regulation, and investment. A proposal that adds palm squalene production without addressing these complementary capacities may produce an economically fragile ingredient business rather than meaningful vaccine resilience. Any strategic plan should therefore define the expected market, buyers' qualification requirements, and continuity arrangements under supply disruption [14,31,36,24].

The environmental comparison also needs a carefully chosen functional unit, such as a kilogram of fully qualified, specification-compliant squalene delivered to a vaccine manufacturer. A comparison expressed solely per kilogram of PFAD processed might reward a low-yield route even when additional upstream material or energy is required. Analyses should report coproduct allocation assumptions, the counterfactual use of PFAD, solvent recycling, electricity mix, and the geographic distance between refinery and pharmaceutical finishing facilities. Because PFAD already has markets, treating it universally as a zero-burden waste risks misstating the net benefit. The most persuasive sustainability case would report transparent sensitivity analyses and acknowledge trade-offs with other available sourcing routes [50,44,35].

Prioritized Future Research Agenda

The first research priority is a multi-batch, multi-refinery characterization study establishing realistic compositional ranges for the PFAD-related stream intended for squalene production. The second is a reproducible process-development study that produces material with analytically characterized impurities, stability behavior, and traceable mass balance. The third is a head-to-head comparison with qualified squalene from another source, employing orthogonal chemical methods and more than one independently produced lot. Fourth, the candidate material should be incorporated into formulation-matched emulsions with measurement of process capability, particle attributes, and stability. These stages supply stronger information than a sequence of unrelated single-batch demonstrations [28,7,5,18].

The fifth priority is a biologically focused comparability program, with immune activation and local tolerance assessed using justified assays and controls. It should distinguish genuine equivalence claims from non-significant differences in underpowered experiments and should evaluate whether formulation changes persist through storage. Sixth, lifecycle and techno-economic assessments should compare the PFAD route with microbial and semisynthetic approaches on equivalent pharmaceutical quality grounds. Seventh, developers should seek early scientific advice on the evidence needed for their intended vaccine-development context. A measured positive result at each stage would reduce uncertainty; a negative result would reveal where technology redesign or abandonment is warranted [6,37,51,34,18].

Implications for Indonesia and Other Palm-Producing Settings

Palm-producing countries have potential comparative advantages in feedstock access, industrial process expertise, and proximity to refining facilities. These advantages should be interpreted as the beginning of a manufacturing-development opportunity rather than proof of a pharmaceutical value chain. A credible initiative would require partnerships among refiners, analytical laboratories, vaccine manufacturers, universities, and relevant regulatory authorities. The first investment decision should favor demonstrable analytical competence and process control over premature construction of a large vaccine ingredient plant. Internationally credible quality documentation could eventually support technology transfer and qualified procurement, but both demand continuing compliance and commercial reliability, not merely a successful university-scale extraction experiment [24,31,30].

A sensible national research portfolio could combine a modest PFAD purification pilot with analytical-method validation, a controlled emulsification platform, and comparative research involving another qualified squalene supply. Such integration would make it possible to identify early whether the principal bottleneck is feedstock variability, oxidation, residual impurities, emulsion behavior, or cost. Public funding might support shared testing capabilities and translational collaboration, while manufacturing partnerships would require explicit intellectual-property and supplier-quality arrangements. By making failure criteria visible from the beginning, this strategy could reduce sunk-cost risks and limit pressure to overinterpret early technical successes [36,14,24].

Conclusion

The available literature supports PFAD-derived squalene as an interesting recovery and valorization opportunity, not as an already qualified vaccine-adjuvant ingredient. Engineering research has demonstrated substantial enrichment from selected palm refining residues, while separate mechanistic and clinical research supports the utility of particular squalene-containing vaccine formulations. The two evidence streams have not yet been joined by a sufficient source-specific package of impurity characterization, formulation comparability, nonclinical evaluation, and regulatory qualification. The principal contribution of this qualitative review is to clarify that gap and make it experimentally testable, rather than to infer clinical efficacy from feedstock origin or headline chemical purity.

Pharmaceutical translation should follow sequential, risk-based decision gates. Representative feedstock characterization must precede robust purification; material identity and impurity qualification must precede comparable emulsification; stable product attributes must precede definitive biological comparisons; and clinically relevant claims must remain linked to tested vaccines and appropriate regulatory decisions. Cross-functional collaboration is essential because chemical engineering, analytical chemistry, formulation science, immunology, toxicology, and manufacturing quality each address different parts of the qualification problem. These principles also reduce the likelihood that a promising circular-economy technology will be advanced beyond the maturity of its evidence.

Future research should prioritize replicated separation across refinery sources, stability-indicating analytical methods, process-

related impurity qualification, formulation-matched head-to-head testing against appropriate reference squalene, and proportionate nonclinical evaluation. A comparative lifecycle and economic assessment should be conducted using functionally equivalent quality outputs rather than simplistic labels such as renewable or waste-derived. Regulatory engagement should define the scope of necessary supporting data for the specific intended application. Demonstrating material comparability would represent a major milestone, but it would not by itself establish clinical benefit for any vaccine product.

The longer-term opportunity is a diversified supply of reliable vaccine-manufacturing inputs linked to qualified industrial capabilities in palm-producing regions. Whether PFAD can contribute meaningfully depends on demonstrating not only purity but repeatable, documented pharmaceutical quality, satisfactory formulation function, acceptable safety, and competitive lifecycle performance. A positive assessment under those conditions would provide a defensible route toward industrial translation. Until such evidence is available, palm-derived squalene should be described as a candidate pharmaceutical feedstock whose promise is substantial but conditional.

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