ANIMAL VACCINE ADJUVANTS: A new use for gas-to-liquids oils

Market trends have indicated an opportunity for Shell to expand into the animal vaccine adjuvant[1] market. The company is introducing Shell Ondina X gas-to-liquids (GTL) oils as alternatives to the white mineral oils commonly used in adjuvant emulsions. The purity and hydrocarbon structures of GTL medicinal grade white oils can provide considerable benefits. A Shell team is working with consultancy firm Sustainable Chemistry Consult to introduce Shell Ondina X oils and is seeking approval from formulators for their use as adjuvants in animal vaccines. The initial results are promising.

The rising global population means increased demand for farming and meat products and thus more animal vaccination and with fewer side effects.

The challenge

Vaccination is the principal strategy for the prevention and control of diseases in animals, and adjuvant use is an effective tool to enhance a vaccine's efficacy. By using an adjuvant, a smaller dose of antigen is necessary to stimulate the immune response. Adjuvants have two objectives: they slow the release of antigens (the vaccine's active substance) from the injection site and they can strengthen the stimulation of the immune system. Emulsions are often used for the former.

Emulsions are the most frequently used vaccine adjuvant formulations for veterinary vaccines. For this, the antigen is dissolved in water and then dispersed in mineral oil. This dispersion in the emulsion must be stable and fluid. Mineral oils are very efficient dispersers but can sometimes induce local reactions when carrying reactive antigens. In addition, adjuvants are mostly stored and injected at a low temperature (typically 4°C). Consequently, the emulsion should have a low viscosity for enhanced and fast injectability.

Oil adjuvants (or so-called emulsion adjuvants: oil in water, water in oil or water in oil in water) facilitate slow release of the antigen by forming a reserve at the injection site. The antigen is trapped in the aqueous phase and is passively released as the emulsion degrades within the living organism. Commonly used emulsion adjuvants for veterinary and research purposes are based on white mineral oils. White mineral oil for general pharmaceutical use is obtained by refining paraffinic crude oils and removing polycyclic aromatic structures; other unsaturated reactive hydrocarbons; sulphur compounds; nitrogen derivatives; and volatiles. Thus, a mineral oil of pharmaceutical quality is a complex substance made up of alkanes (linear, branched and cyclic) with different carbon chains. The length of these carbon chains is crucial for vaccine safety and efficacy.

Safety

Studies have shown that shorter carbon chains (less than or equal to C_{14}) induce more local

[1]Adjuvant: a substance that increases the humoral or cellular immune response to an antigen

reactions that lead to significant and undesirable side effects, and that the most appropriate hydrocarbon chain length in white mineral oil is between C_{15} and C_{32}. The oil should also have no aromatic compounds. Some of the key properties reported for white oils for use in adjuvants include a normal paraffin content of 10–25 wt%, a pour point between –3°C and –12°C, and a kinematic viscosity at 40°C of 3.5–8.0 cSt. These mineral oils correspond to the European Pharmacopoeia purity requirements for "light mineral (paraffinic) oil". Therefore, mineral oil adjuvants must be of low viscosity and high purity, and include nonpolar constituents to improve the efficacy of the final formulation when used in vaccines.

Oil adjuvanted vaccines formulated as water-in-oil emulsions have been used to enhance immune responses in poultry. However, such vaccines containing liquid paraffins often cause granulomas (a type of inflammatory response) at the injection site in chickens. Literature data on guinea pigs have observed that both the immune response and the side effects of water-in-oil emulsions vary with the type of liquid paraffin used. The studies reported that these variations were due to the differences in the hydrocarbon structural composition of the liquid paraffins. Studies also have indicated that it is imperative to study the action of the various saturated hydrocarbons, for example, alkanes, derived from petroleum when selecting a liquid paraffinic oil for adjuvant use in vaccine development.

The mineral-oil-based emulsion residue at the injection site can be minimised by increasing the dispersibility of the emulsion through decreasing its viscosity or adding a hydrophilic emulsifier (surfactant). Unfortunately, these adaptations sometimes negatively affect the emulsion's stability or the immunological response. However, some studies have shown that highly purified nonmineral based oils such as GTL oils are well tolerated, as they are rapidly metabolised and eliminated from the injection site, which means less local inflammation. (A mineral oil stays longer at the injection site and it is eliminated by macrophages and partially metabolised into fatty acids.)

An opportunity

The major medicinal white oils used in animal vaccines include products such as Marcol 52, Eolane 130, 150 and 170, and Drakeol 6 VR, and represent more than 25,000 t of the white oils currently being used in adjuvant vaccine applications worldwide. There are also some nonmineral-oil-based adjuvants that are used to avoid or reduce the side effects associated with mineral white oils. In addition, trials with plant-based oils have had only limited success. However, global population increases mean rising demand for farming and meat products and thus more animal vaccination.

In addition, adjuvant manufacturers are now looking on the market for hydrocarbons that are free of substances such as pristane that can cause an undesirable autoimmune response and that also comply with the purity requirements of EU and US pharmacopeia regulations, as do Shell Ondina X oils (their registration as low-viscosity, synthetic paraffin oils in the German Pharmacopoeia is ongoing).

These global market trends indicated an opportunity for Shell to expand in this market ▸▸▸

TABLE 1
Properties of various oils used in adjuvants for animal vaccine compared with GTL oils.

Product	White oil	Hydrocracked oil	Shell Ondina X 415	Shell Ondina X 411	Shell Ondina X 409	Squalane	Squalene
Chemical class	Saturated hydrocarbon	Saturated hydrocarbon	Saturated hydrocarbon	Saturated hydrocarbon	Saturated hydrocarbon	Saturated hydrocarbon	Unsaturated hydrocarbon
Origin	Mineral oil	Mineral oil	Synthetic	Synthetic	Synthetic	Shark liver	Shark liver
Boiling range (°C)	280–400	280–320	320–380	305–355	270–295	285	285
Kinematic viscosity (cSt)	7.2 at 40°C	4.1 at 40°C	9.3 at 40°C	6.0 at 40°C	3.5 at 40°C	34 at 20°C	14 at 25°C
Pour point (C°)	–6	–18	–39	–15	–18	–38	–75
Density (kg/m^3)	840 at 15°C	813 at 15°C	806 at 15°C	800 at 15°C	785 at 15°C	805 at 20°C	858 at 20°C
Oxidation stability	++	++	++	++	++	++	0
Isomer types	Iso-, n- and alkylated cycloparaffins (>50%)	Iso, n- and alkylated cycloparaffins	Linear and low-branched hydrocarbons; n-paraffins <10%	Linear and low-branched hydrocarbons; n-paraffins <1%	Linear and low-branched hydrocarbons; n-paraffins <15%	Highly branched, saturated hydrocarbon	Highly branched, unsaturated hydrocarbon–iso-olefin
Carbon number range	C_{15}–C_{30}	C_{15}–C_{19}	C_{17}–C_{31}	C_{18}–C_{24}	C_{15}–C_{19}	C_{30}	C_{30}

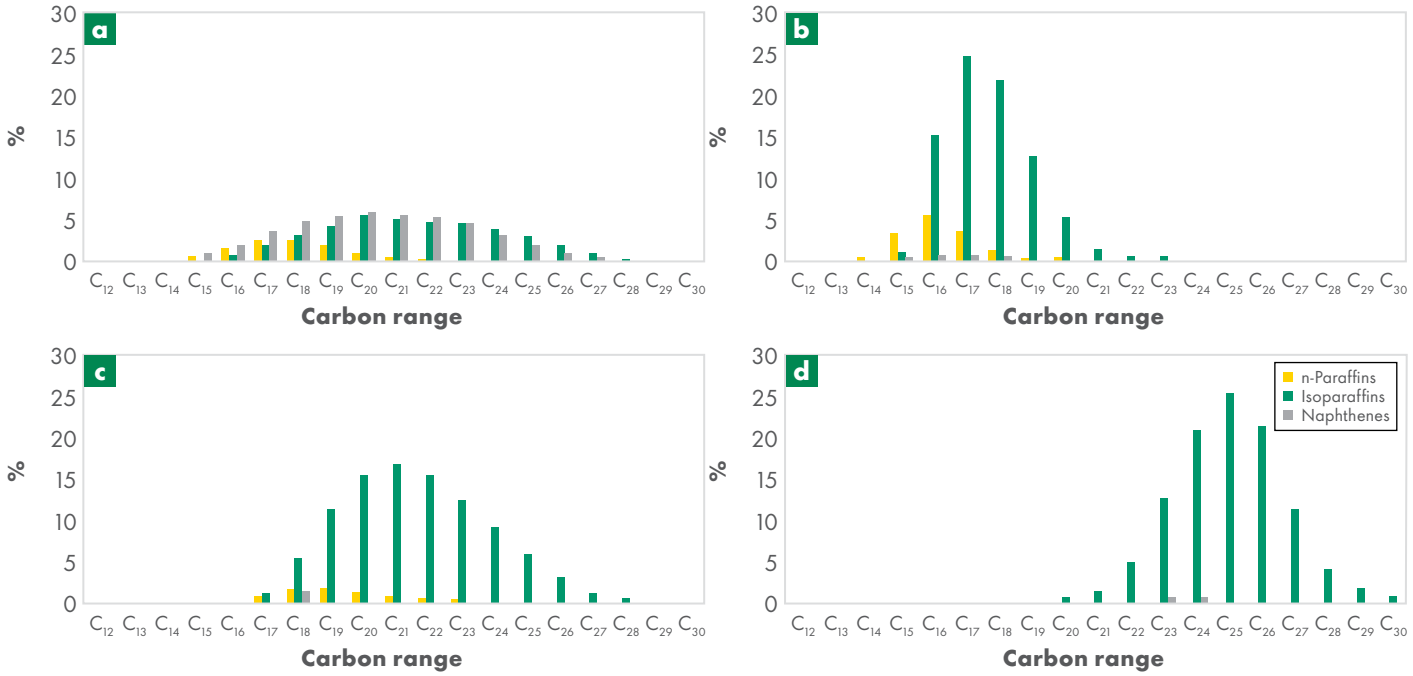

FIGURE 1
Carbon number distribution of **(a)** a typical white medical oil and Shell Ondina X GTL oils; **(b)** X 409; **(c)** X 411; and **(d)** X 415.

by introducing Shell Ondina X GTL oils for adjuvant use. A Shell team is working with consultancy firm Sustainable Chemistry Consult on introducing Shell Ondina X oils. The team is seeking approvals from animal vaccine formulators, so Sustainable Chemistry Consult's experience with detailed procedures and expertise on formulation trials and immunity tests are crucial to better understanding of vaccine efficacy and safety.

Shell Ondina X oils for adjuvants

Shell GTL oils are based on Fisher–Tropsch synthesis technology, which converts a very clean feedstock, natural gas, into high-quality, low-aromatic liquid products that offer alternatives to crude-oil-derived mineral oils. Shell Ondina X GTL oils predominantly contain isoparaffinic hydrocarbon structures with long, linear-chain backbones and have been purified by hydrotreatment. The resulting medical grade oils are very pure, virtually free of aromatic, sulphur and nitrogen compounds, and significantly more stable than traditional white oils.

For the adjuvant market, the Shell Ondina X products under consideration include the X 409, X 411 and X 415 grades, which have the following key features:

- The degree of hydrocarbon branching is relatively low.
- The level of cyclic saturated paraffins is very low (<2%).
- The oils are virtually free of aromatics and polycyclic aromatic hydrocarbons.
- The oils meet EU pharmaceutical and FDA purity requirements.

Table 1 compares the properties of Shell Ondina X oils with other oils currently used in vaccine adjuvants (data collected from web sources).

Shell Ondina X oils are currently being evaluated by formulators for use in adjuvants for vaccines for poultry, pigs, cattle and fish. Leading pharmaceutical companies in the veterinary vaccine field have been convinced of the oils' benefits, so testing is under way on these medical grade oils.

Figure 1 shows the carbon number distribution for a traditional white oil compared with the Shell Ondina X grades X 409, X 411 and X 415. The data show that Shell Ondina X grades have a narrower carbon number distribution. Thus, they are likely to have improved oxidation stability resulting in optimal molecular weights compared with a traditional white oil with its much broader carbon number distribution. Consequently, it is likely that Shell Ondina X oils will show better and safer immune responses when used in adjuvants for vaccines.

Figure 2 shows 3D gas chromatography plots comparing the chemical composition of a traditional white oil with Shell Ondina X 411. The plots clearly show that the mineral-oil-based product contains isoprenoid substances of toxicological concern, including pristane. Pristane is known to induce autoimmune reactions when injected into mice (a classical model for immunology studies). Even though both types of oils comply with the high-purity requirements in accordance with the EU and US pharmacopoeia, Shell Ondina X oils do not contain substances such as pristane, which indicates that adjuvants using such oils would have a higher safety profile and would be unlikely to induce chronic inflammatory responses when used in animal vaccines.

Key benefits of Shell Ondina X GTL oils for adjuvants

Following promising initial trials carried out by leading pharmaceutical companies, Shell is expecting the first major tests on large herds and granting of the required regulatory approvals, depending on the outcome of the field trials and the speed of regulatory evaluation. The physical properties of the Shell Ondina X grades clearly indicate that such oils can be used effectively in adjuvants and would be advantageous in a market currently involving traditional white oils, squalene and squalane. However, it is vital that the formulators make adjuvants from the higher purity Shell Ondina X grades to ensure optimum vaccine efficacy and safety (World Health Organization requirements).

By selecting defined chain length ranges for the Shell Ondina X oils, different permeation properties can be achieved. Because of their chemical structure, these hydrocarbons have a low viscosity in relation to their molecular mass. The different average molecular mass of the Shell Ondina X oils enables control of efficacy and slow-release reserve properties. Their chemical structure also means Shell Ondina X oils cannot form haptens (products from reactions with body proteins), so should not cause allergenic reactions or complement activation-related pseudoallergies.

The Shell Ondina X GTL oils are characterised by their homogeneous chemical structure. This factor would also provide the adjuvant formulators with advantages with respect to the physical and chemical properties that are beneficial in tissue distribution and metabolism. The saturated and low-branched hydrocarbons in Shell Ondina X oils are metabolised to organic fatty acids that can be reused by the body or degraded by beta oxidation. However, the highly branched hydrocarbons found in mineral oil products often lead to dead-end metabolites that are difficult to eliminate from the body.

Shell Ondina X oil grades show excellent oxidation stability under chemical conditions and can protect hydrolytically sensitive molecules until they are absorbed by antigen-presenting or other cells that initiate the immunological response.

In addition, as the highly pure, saturated hydrocarbons found in Shell Ondina X oils are nonconductive, they could possibly be used for a specific adjustment of liposome properties.

A good outlook

Through the initial promising trial outcomes provided by several pharmaceutical companies, the perception of Shell Ondina X oils in the adjuvant market and worldwide is expected to improve and lead to further testing. The following benefits are supported by proprietary data or published literature (references are available).

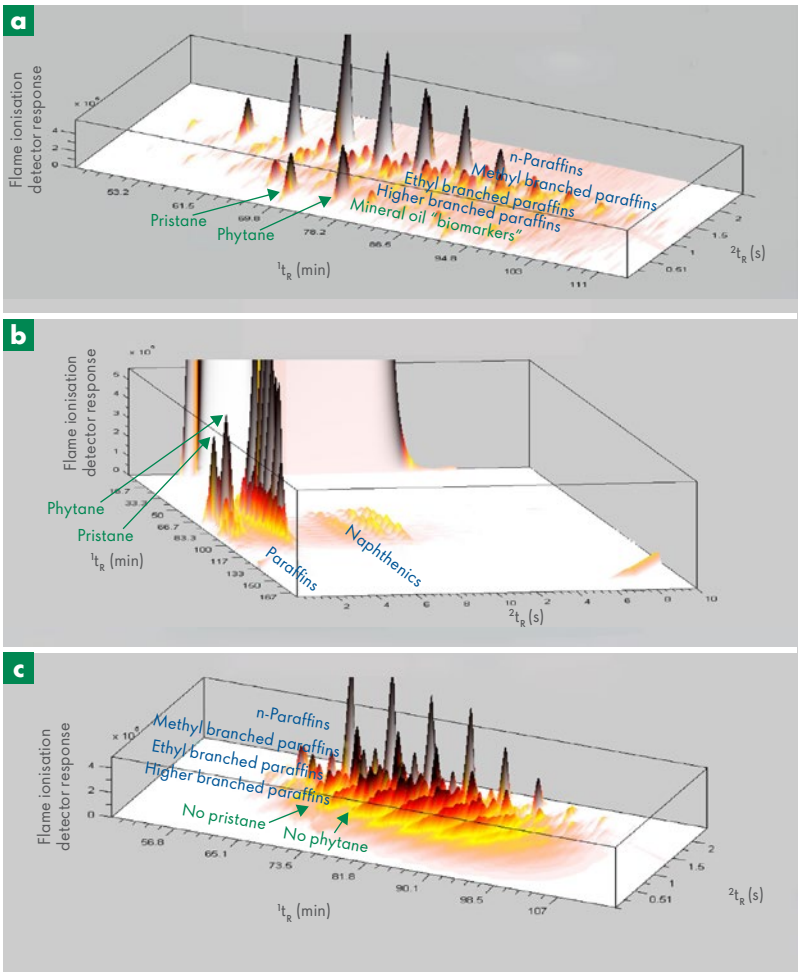

FIGURE 2
3D gas chromatography plot comparison between a white oil (**a** and **b**) and Shell Ondina X 411 (**c**).

When injected as part of a vaccine formulation, Shell Ondina X oils are:

- expected to show low tissue accumulation and rapid elimination when compared with mineral oils (based on feeding studies in rats);
- not expected to cause autoimmune reactions triggered by isoprenoid hydrocarbons such as pristane, as they do not contain these molecules;
- not expected to have adverse effects on fertility or developing embryos, as they are virtually free of polycyclic aromatic hydrocarbons;
- not expected to affect the delicate adjuvant emulsion stability and thus should cause little tissue damage, as such high-purity medical grade GTL oils have a low viscosity owing to the presence of low-branched hydrocarbons.

Shell Ondina X oils could be used as a more sustainable alternative to squalene-based adjuvants for influenza and coronavirus vaccines. Squalene oil is obtained from shark liver and it is estimated that the total annual shark catch is 100 million, including endangered species. Additionally, squalene oil can contain pristane. GTL oils might thus be a suitable alternative to squalene and, thus, indirectly alleviate the pressure on shark populations. ▸▸▸

The results of 28-d OECD toxicity testing indicate that Shell Ondina X 409 and X 411 oils are classified as readily biodegradable, whereas the Shell Ondina X 415 grade is classified as inherently biodegradable. These excellent biodegradability characteristics and the very low systemic toxicological risks for the high-purity medical grade Shell Ondina X GTL oils mean that they offer a high level of safety for use in adjuvants in animal vaccines.

Customers wanting to use Shell Ondina X oils in adjuvants can be assured of high purity grades (free of aromatics and heavy metals) and microbe-free products through the high temperature of the hydrotreatment process and subsequent appropriate storage and handling practices, and specific packaging of products (when required).

Apart from promising advantages when used as vaccine adjuvants, Shell Ondina X oils are suitable for other life science applications such as personal care, food processing and agricultural spray oils.

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