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Review

# Oral exposure to mineral oils: Is there an association with immune perturbation and autoimmunity?

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ABSTRACT

Mineral oils is a generic term that describes a category of petroleum products, that may include lubricating base oils and highly refined base oils. Parenteral exposure of rodents to certain mineral oil hydrocarbons has been reported to induce immune perturbation associated with the development of autoimmune responses. Consumers are exposed to a variety of mineral oil hydrocarbons via food and food contaminants, and in particular via food packaging. It is relevant, therefore, to consider whether dietary exposure to mineral oils results in similar effects; and that is the purpose of this article. There is no evidence that oral or dietary exposure of experimental animals to mineral oils will induce autoimmune responses, and the information that is available indicates that dietary exposure does not provoke such responses. There are epidemiological reports that suggest an association between mineral oils and autoimmunity in humans. However, the presumption in such instances is of high levels of exposure by inhalation or via the skin, and by reference to the data available from animal studies it is probable that dietary exposure would be ineffective.



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## 1. Introduction

In 2012 the European Food Standards Agency (EFSA) published a Scientific Opinion on mineral oil hydrocarbons in food (EFSA, 2012). That report revealed that consumers are exposed to a variety of mineral oil hydrocarbons (MOH) via food and food contaminants, and in particular via food packaging (Castle et al., 1993; Grob et al., 1997; Heimbach et al., 2002; Biedermann-Brem and Grob, 2011). It was concluded that the adverse health effect of particular concern was inflammation associated with the induction of hepatic microgranulomas (EFSA, 2012). However, the Opinion also summarised as follows the potential of mineral oil saturated hydrocarbons (MOSH) to impact on the immune system such as to provoke autoimmune responses: *"In arthritis-prone rodent models, intradermal and intraperitoneal injection of high doses of certain MOSH can alter immune function or induce autoimmune responses. Weaker effects were observed following short term exposure through abraded skin. Whether long term oral exposure would have similar consequences is unknown although one short term study suggests this might not be the case"* (EFSA, 2012). That conclusion has prompted a consideration of whether oral exposure to mineral oils might represent a potential human health risk with

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regard to immune perturbation and the stimulation of autoimmune responses. That is the issue addressed here.

## 2. Mineral oils—a complex substance

Crude Oil is distilled in order to manufacture a variety of petroleum products that include fuels, heating and mineral oils. These can be grouped into categories according to composition and refinery history. For regulatory purposes these are recognized as *UVCB* substances (***U**nknown or **V**ariable composition, **C**omplex reaction products and **B**iological materials)* (Rasmussen et al., 1999). This is because by being derived from crude oil, petroleum-derived substances are by nature complex in hydrocarbon composition and therefore difficult to describe using molecular formulae based on exact chemical composition. Moreover, petroleum complex substances are defined by their refinery history.

Although fuels are less refined petroleum substances and are often only straight-run (distilled from crude oil without any further treatment), other substances such as mineral oil are further refined to meet specific technical (e.g. viscosity) and toxicological specifications (Mackerer et al., 2003).

Mineral oils is a generic name used to refer to a category of petroleum products, called base oils, that include lubricating base oils and highly refined base oils (i.e. white oils). The latter undergoes severe hydrotreatment to remove aromatics in order to meet the required specifications for food and pharmacopeia uses (such as, for example, in laxatives or in food contact applications). Compositionally, highly refined mineral oils are complex substances of hydrocarbon constituents that include normal, iso and cyclo alkanes (also called normal and iso paraffins and naphthenics, respectively). Because of technical requirements lubricating base oils have controlled levels of aromatics (Mackerer et al., 2003) resulting in different proportions of paraffin and naphthenic constituents when compared with white oils which are essentially free of aromatics (which may be present, but only at trace levels). Therefore, the levels of alkane, naphthenic and aromatic constituents vary according to crude oil source, refinement history, and technical and safety specifications. With regard to the carbon ranges of hydrocarbon constituents of 'mineral oils'. Anderson et al. (2003) have reported an analysis of a naphthenic lubricating base oil indicating that 90% of carbon numbers are between C16 and C29, with maxima at C21 for naphthenics and C23 for aromatics. The conversion of aromatics to naphthenics by hydrotreatment shifts the naphthenic maxima, but will not alter the carbon number range. However, considering variations in mineral oil manufacturing history to meet specific product requirements, it can be generally anticipated that a 'mineral oil' can have carbon numbers within a wide range of between approximately C15 and C40 (Kuroda et al., 2004a).

Some hydrocarbon constituents such as *n*- hexadecane (n-C16) and pristane (iso-C19) have been shown to induce autoimmunity in mice (Kuroda et al., 2004a,b; Reeves et al., 2009). They may be present in a mineral oil at varying (low) levels, but always as part of the complex environment, such that their presence in the oil will not dictate the inherent toxicological profile of the complex substance as a whole. That is, the toxicological properties of a 'mineral oil' must be based on assessment of the whole substance, rather than on the hazard profile of individual components.

Thus, it is necessary to draw a distinction between alkanes as specific hydrocarbon substances, and as constituents of a complex substance (such as mineral oil). This is important when addressing the question posed here of whether oral exposure to mineral oil has the potential to cause modulation of the immune system or autoimmunity.

## 3. The concept of adjuvants and mineral oils

A working definition of an adjuvant is a material that is able to enhance immune responses without itself necessarily providing any specific antigenic stimulus. That is, adjuvants have the potential to promote immune responsiveness to an unrelated antigen without themselves initiating an immune response (Kimber and Dearman, 2010). A more formal definition of an adjuvant is that of any substance that is able to accelerate, prolong or augment an immune response, and it is because of those properties that adjuvants find important clinical application in vaccinology (Petrovsky and Aguilar, 2004; Reed et al., 2008). However, in addition to enhancing protective immune responses, adjuvants may also have some potential to induce or promote inappropriate responses (allergic or autoimmune responses) that will result in adverse health effects (Gupta, 1993; Jaakkola and Knight, 2008; Yoo and Perzanowski, 2014; Shaw et al., 2014; Bagavant et al., 2014).

Among substances known to have adjuvant properties are the following: aluminium salts (alum), oil-based materials (including Freund's incomplete and complete adjuvants), cholera toxin, unmethylated CpG dinucleotide- containing DNA, and various Toll-like receptor (TLR) ligands (Schijns, 2000; Klinman, 2003; Lindblad, 2004; Reed et al., 2008; Warshakoon et al., 2009). The mechanisms through which adjuvants can influence the initiation and development of adaptive immune responses vary and are complex. However, the relevant mechanisms normally embrace one or more of the following events or processes, many of which are interrelated: enhanced delivery or deposition/localisation of antigen, provision of danger signals that serve as co-activators of the immune system, activation of antigen-presenting dendritic cells (DC), and engaging the innate immune system through ligation of TLR and other receptors (Cox and Coulter, 1997; Schijns, 2000; Kool et al., 2008; Warshakoon et al., 2009; Monie et al., 2009; Flach et al., 2011).

Finally, it is relevant to acknowledge also that, in addition to augmenting immune responses, some adjuvants can exert more selective effects on the immune system and influence the quality and characteristics of developing responses (Freytag and Clements, 2005; De Gregorio et al., 2008; Kool et al., 2008; Korsholm et al., 2010).

It is apparent that the adjuvant properties of materials will be affected by a number of variables, including, among others, the dose, timing of administration relative to antigen, and the route of exposure. In considering the potential toxicity of adjuvants it is important, therefore, to establish the conditions of exposure under which inherent immunomodulatory properties will translate into adverse health effects.

In this article the adjuvant properties of mineral oils and mineral constituents are considered. There is no doubt that some mineral oil hydrocarbons can display adjuvant activity. Thus, for instance, Freund's adjuvants (based upon an 85:15 mix of paraffin oil: mannide monooleate with [complete] or without [incomplete] killed mycobacteria) have been used for decades to potentiate immune responses in experimental animals (Whitehouse et al., 1974).

However, the particular focus here is to explore whether any inherent potential to induce or exacerbate autoimmune responses is likely to be expressed following oral exposure.

## 4. Mineral oils and autoimmunity in experimental models

Most of the data available on the ability of mineral oils or mineral oil components to initiate or exacerbate autoimmune responses in experimental animals derive from studies in which exposure has been via parenteral administration.