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Mode-of-action evaluation for the effect of *trans* fatty acids on low-density lipoprotein cholesterol

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ABSTRACT

The purpose of this work is to systematically consider the data relating to the mode of action (MOA) for the effects of *industrially produced trans* fatty acid (iTFA) on plasma low-density lipoprotein (LDL) levels. The hypothesized MOA is composed of two key events: increased LDL production and decreased LDL clearance. A substantial database supports this MOA, although the key events are likely to be interdependent, rather than sequential. Both key events are functions of nonlinear biological processes including rate-limited clearance, receptor-mediated transcription, and both positive and negative feedback regulation. Each key event was evaluated based on weight-of-evidence analysis and for human relevance. We conclude that the data are inadequate for a detailed dose-response analysis in the context of the evolved Bradford Hill considerations; however, the weight of evidence is strong and the overall shape of the dose-response curves for markers of the key events and the key determinants of those relationships is well understood in many cases and is nonlinear. Feedback controls are responsible for maintaining homeostasis of cholesterol and triglyceride levels and underlie both of the key events, resulting in a less-than-linear or thresholded relationship between TFA and LDL-C. The inconsistencies and gaps in the database are discussed.

1. Introduction

This paper provides an evaluation of the mode of action (MOA) for the effect(s) of *industrially produced trans* fatty acid (iTFA)[2] intake on plasma low-density lipoprotein cholesterol (LDL-C) concentrations observed in clinical and epidemiological studies. Consideration of MOA is a key aspect of conducting any risk assessment of a chemical compound or nutrient, and it is particularly important when there is a question of how to extrapolate below the data and whether a threshold exists (in this case, whether there is a threshold for changes in LDL-C at relevant human doses of iTFA). Although mathematical approaches have been used to evaluate whether a threshold exists for a particular endpoint, the existence of a threshold is ultimately a judgment based on the underlying biology. It is essentially impossible to mathematically distinguish between dose-response patterns that represent a threshold and those that do not (Crump and Allen,

Abbreviations: %en, percent energy; apo, apolipoprotein; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; EPA, U.S. Environmental Protection Agency; ER, endoplasmic reticulum; FDA, U.S. Food and Drug Administration; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; HMG-CoAR, 3-hydroxy-3-methyl-glutaryl-CoA reductase; IDL, intermediate-density lipoprotein; ILSI, International Life Sciences Institute; IPCS, International Programme on Chemical Safety; iTFA, industrially produced *trans* fatty acid; KEDRF, Key Events Dose-Response Framework; KO, knockout; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; MOA, mode of action; MTTP, microsomal triglyceride transfer protein; PCSK9, proprotein convertase subtilisin/kexin type 9; PHO, partially hydrogenated oil; PUFA, polyunsaturated fatty acid; rTFA, ruminant *trans* fatty acid; SFA, saturated fatty acid; SCAP, SREBF cleavage-activating protein; SOAT, acyl-CoA:cholesterol acyltransferase; SREBF, sterol regulatory element-binding transcription factor; TERA, Toxicology Excellence For Risk Assessment; TFA, *trans* fatty acid; TG, triglyceride; VLDL, very low-density lipoprotein.

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[2] In this paper, the term *industrially produced trans fatty acid* (iTFA) is used to refer to the human intake and *trans fatty acid* (TFA) is used when talking about the biology, when there is not a clear distinction between the biological effects of iTFA and rTFA.

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2010). Any observations that are consistent with no dose-related change in response are also consistent with a slight, nonzero dose-related change. This is the reason why current risk assessment guidance states that the approach for low-dose extrapolation for cancer endpoints should be chosen based on the understanding of the chemical's MOA, not based on any modeling method, mathematical analysis, or inspection of the dose-response curve (EPA, 2005a); the same principle applies for noncancer endpoints. In other words, evaluation of whether a threshold exists for the endpoints of interest needs to be resolved based on an understanding of the biology of the effect, the MOA for how the exposure causes that effect, and basic biological principles. In particular, the risk assessment presumption is that dose-response curves for effects that occur via an MOA other than interacting with DNA are expected to have a threshold, even if testing has not been conducted down to doses low enough to identify a threshold (EPA, 2002).

Here, our focus is on the MOA by which iTFA intake elevates plasma low-density lipoprotein (LDL) levels (and also very low-density lipoprotein [VLDL] levels), because these increases represent one of the earliest and most important events associated with iTFA intake (FDA, 2013). In light of the complexity of atherogenesis and the development of coronary heart disease (CHD), it is appropriate to focus on the effect of iTFA on this early key event (increased LDL-C). It is noted, however, that even this early key event is not fully explained by iTFA intake, since there are multiple genetic and dietary factors that affect both LDL-C and CHD. The U.S. Environmental Protection Agency (EPA) makes a clear distinction between "mode of action" and "mechanism of action" in risk assessment (EPA, 2005a). MOA refers to a less detailed description of a sequence of events relative to the more detailed and often molecular understanding represented by mechanism of action. Although MOA is not as detailed, it provides sufficient evidence to draw a reasonable conclusion concerning an agent's apical effects (adverse effects typically seen in *in vivo* studies) and to permit information on precursor events to be incorporated into the risk assessment process. Thus, the focus of the analysis is on identifying key events leading to an apical effect, rather than developing a detailed molecular understanding, although mechanistic details can be used to support the MOA description.

It is important to recognize that the underlying risk assessment presumptions are different for cancer and noncancer endpoints. Because a predominant MOA for carcinogens is via interaction with DNA, and further because regulatory guidance assumes that one mutation theoretically could cause cancer, the default approach for cancer risk assessment is a linear extrapolation from experimental doses to low doses relevant to human exposure. The burden of proof is then to show that some other MOA applies and results in a nonlinear dose response. In contrast, noncancer endpoints are presumed to act via nonlinear or threshold MOAs and have been shown to do so in the vast majority of cases. Although it is possible that some agents may produce effects other than cancer through MOAs with a linear dose response, this requires a burden of proof similar in extent to that needed to show a nongenotoxic or threshold MOA for cancer endpoints.

TFA are isomers of unsaturated fatty acids with at least one double bond in the *trans* configuration. iTFA are formed during heat processing and when vegetable oils undergo the process of partial hydrogenation that converts these liquid oils into semisolid fats. During the hydrogenation process, some *cis* double bonds are converted to *trans* bonds, while other double bonds become saturated (Lichtenstein et al., 2001). Although the hydrogenation process creates a variety of geometric and positional fatty acid isomers, the major *trans* isomer formed in the production of iTFA is elaidic acid (18:1 t9). Accordingly, elaidic acid is the iTFA isomer most frequently tested in *in vitro* and animal studies. In addition to iTFA, dairy and meat products derived from ruminant animals are also a source of dietary TFA; however, these ruminant *trans* fatty acids (rTFA) appear to be functionally distinct from iTFA (Turpeinen et al., 2002) and may have a beneficial effect (Bassett et al., 2010; Mozaffarian et al., 2006). Thus, this analysis focuses on the MOA for iTFA.

Among the health effects of iTFA, one of the earliest and most important events is increased serum LDL levels (FDA, 2013). Compared to *cis* unsaturated fats, dietary consumption of a calorically equivalent amount of iTFA increases plasma LDL-C levels, reduces levels of beneficial high-density lipoprotein cholesterol (HDL-C), and increases the ratio of total cholesterol to HDL-C, a reliable marker of increased CHD risk (Brouwer et al., 2010; Mozaffarian et al., 2006). Since the U.S. Food and Drug Administration (FDA) 2003 rule on nutrition labeling regulations to require declaration of the TFA content of food in the nutrition label of conventional foods and dietary supplements, food products have been reformulated to eliminate or to substantially reduce the amount of iTFA (Doell et al., 2012). As a result of this reformulation and public awareness, the FDA estimates that iTFA consumption has decreased from a mean adult (age $\geq$20 years) intake of *trans* fat from products containing polyhydrogenated oils (PHO) of 2%en (percent energy) based on a 2000-calorie diet in 2003, to a mean *trans* fat intake for the U.S. population aged $\geq$2 years[3] who consumed one or more of the processed foods identified as containing PHO of 0.6%en (FDA, 2013).

These estimates by the FDA in 2010 were based on food consumption data from the 2003–2006 National Health and Nutrition Examination Survey (NHANES), market share information, and *trans* fat levels based on label declaration data and analytical data for products that were identified as containing PHO. The FDA estimates of TFA intake are substantially lower than the estimates by Honors et al. (2014) based on a survey of people in the Minnesota Heart Study. Honors et al. (2014) found that intake of total TFA was decreasing with time, but that the mean total TFA intake was 1.9%en in the 2007–2009 timeframe. The reason for the difference between the FDA data and those of Honors et al. is not clear but may be related to the difference in the study population (national vs. Minneapolis–St. Paul region), differences in definitions (TFA from products containing PHO vs. age-adjusted mean intake), or other factors. This paper hypothesizes that the MOA for increased serum LDL-C resulting from iTFA consumption consists of two key events: (1) an increased rate of LDL particle production and (2) a decreased rate of LDL particle clearance. A novel aspect of the analysis presented here is the application of a systematic risk assessment approach to a dietary macronutrient. The application of this approach is useful in providing a structure for evaluating all existing relevant data and identifying data gaps. However, our analysis is limited by the fact that the applicable research (both clinical and nonclinical) was not designed with the relevant risk assessment questions in mind. For example, there are very few data on dose response or temporality for key events or markers of key events. Instead, much of the research on TFA has focused on simply elucidating the relevant biology and regulatory pathways, but not necessarily the effects specific to iTFA. Furthermore, although key events are usually considered to follow a specific sequential pathway, interactions among cellular processes controlling lipid

[3] The FDA noted that: "While we did not calculate a mean intake for ages 20 years or more, based on the similarity in the intakes calculated for children aged 2–5 years, teenage boys, and persons aged 2 years or more, we believe there would not be a significant difference between the intake estimated for persons ages 2 years or more and that for persons ages 20 years or more."