

## PEER REVIEW HISTORY

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### ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	Industrially produced trans fat in popular foods in 15 countries of the former Soviet Union from 2015-2016: a market basket investigation
<b>AUTHORS</b>	Stender, Steen

### VERSION 1 – REVIEW

<b>REVIEWER</b>	Vesna Vucic Institute for Medical Research, Serbia
<b>REVIEW RETURNED</b>	22-Aug-2018

<b>GENERAL COMMENTS</b>	<p>The manuscript "Industrially produced trans fat in popular foods in 2015-2016 in 15 countries of the former Soviet Union: a market basket investigation", measured industrially trans fats (ITF) in pre-packed biscuits/wafers/cakes purchased in large supermarkets in 15 countries of former Soviet Union. The paper is clear and well-written with important data on ITF content in almost 1000 products. The author should explain why sometimes g ITF is measured in 100g of wt and sometimes as % of fats. It would make manuscript easier to follow. Also, some countries such Latvia and Lithuania introduced a legislative ban, so it could be discussed if the study was performed before or after the legislation in these countries and in this light the differences between these countries and those with no legislation.</p>
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<b>REVIEWER</b>	Dr Leo Stevenson Liverpool John Moores University, UK
<b>REVIEW RETURNED</b>	23-Aug-2018

<b>GENERAL COMMENTS</b>	<p>Abstract - perhaps 'industrially produced trans fat' is better (at the start of the abstract). Would it be better to be a bit more specific about the 'declined significantly' comments at the end of the abstract.</p> <p>Introduction - the first paragraph has some vague statements that could be clarified - e.g. what constitutes a 'high' amount of trans fat?, some better clarification about foods that might be 'high' in industrially produced trans fats and foods that might be high in non-industrially produced trans fats (e.g. ruminant-based foods) and any possible difference/or not between these sources (this might be important later, since you mention not being able to discriminate between these sources later in the paper).</p> <p>p4 - line 6 - could the 'fewer CHD events' comment be more specific?</p> <p>Methods - use of 'first person' ('I') could be avoided (also appears in</p>
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	<p>Results section as well)</p> <p>p6 - some of the criteria used for sample purchase assume reliability in labelling of products - would it have been worth testing some sample products (perhaps as a control) that might not appear (from labelling information) to contain I-TFs to see if this was the case (or what levels were detected in a sample of these products)?</p> <p>Analysis of TF - although you refer to an AOAC method - you mention this was modified - but no details of how/in what way? Could you have a bit more specific detail about the method - which TFAs were being detected and measured - any controls, standardisation (e.g. standard reference materials) or anything further to confirm the quality of the measurements?</p> <p>You mention that any butter in any of these products might contribute ruminant TF - is there anything you could add to suggest how likely/unlikely this might be (was it possible to correct for this - would any labelling information or any information from elsewhere be able to suggest if this likely to be of any significance).</p> <p>Discussion - do you need to clarify what you mean by 'high concentration' at start of first paragraph?</p> <p>p11 - you assume that labelling information is reliable in your comments towards the top of this page - perhaps you could be more cautious about this (analysis of food products sometimes shows that labelling and ingredient information isn't always as good as we often assume).</p> <p>Towards the end of the Discussion perhaps you need to mention further that there may also be other factors other than I-TF (diet and other factors) that may well be very different between the countries in the study and Western Europe (which contribute to the higher CHD mortality).</p>
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### VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

1) The paper is clear and well-written with important data on ITF content in almost 1000 products.

Response  
Thank you very much

2) The author should explain why sometimes g ITF is measured in 100g of wt and sometimes as % of fats. It would make manuscript easier to follow.

Response  
The two different values have often given rise to some confusion and a more thorough explanation may – as suggested by the reviewer- make the manuscript easier to follow.

In line 4 in the result section of the original manuscript, it says:  
“The different products in each country were ranked according to I-TF level, expressed as grams per 100 g of the product (figure 1). This latter value tells how much TF the consumer gets when 100

grams of the product is consumed.”

In line 10 in the same section, it says:

“The mean values of the percentages of fat that were I-TF in each of the countries, together with the SD and maximum values are shown. These percentages are of interest for food producers and for food authorities because they describe the fat that has been used in the food.”

A more thorough explanation has now been added to the Method section in the revised manuscript:

From the peaks in the chromatogram TF is calculated as a fraction (percent) of total fat in the product. This value is primarily of interest for food producers, food chemists and food legislators. The total amount of fat in the product is multiplied by the TF-fraction and the amounts of TF in gram per 100 grams of the product appear. This value is primarily of interest for the customers and for investigators of the health effect of intake of TF.

3) Also, some countries such as Latvia and Lithuania introduced a legislative ban, so it could be discussed if the study was performed before or after the legislation in these countries.

Response

In line 3-4 in the subsection Legislation and industrial trans fat in foods of the original manuscript it says:

“In 2009, Austria and Switzerland introduced a legislative ban similar to the Danish ban from 2004, followed by Iceland in 2011, Hungary and Norway in 2014, and Latvia, Georgia, Lithuania, and Slovenia between 2016 and 2018. Other Eastern European countries including Russia may be on their way to I-TF legislation.13,25,26 “

This has now been changed in the revised manuscript, and it has been explicitly mentioned that the samples were collected before fully implemented legislation about restriction of I-TF in foods in the various countries.

It has been possible to find a reference to the legislative restriction of I-TF also in Russia. This reference has been added the list of references (Ref 27) in the revised manuscript

Russia. Solnechnye Produkty ready to meet new trans-fat content standards

<http://www.blackseagrains.net/novosti/russia-solnechnye-produkty-ready-to-meet-new-trans-fat-content-standards> Accessed Sep 2018

The below sentences has been added in the revised manuscript

“In 2009, Austria and Switzerland introduced a legislative ban similar to the Danish ban from 2004, followed by Iceland in 2011, Hungary and Norway in 2014, and initiated by Latvia, Georgia, Lithuania, Slovenia and other Eastern European countries including Russia between 2016 and 2018.

13,25,26,27 The samples for the present study were all collected in the various capitals in 2015 or 2016 (supplementary table A) before any of the legislative restrictions of I-TF in food were fully implemented in these countries. However, some of their food producers were most likely aware of future legislation. They may have commenced a gradual removal of I-TF from some of their products already in 2015 and 2016. This may be the explanation for the relatively few products with TF in Lithuania and in Latvia. The explanation is apparently not valid for Estonia that only had 8 products with more than 2% of the fat as TF and has no internationally published plan for a legislation like the two other Baltic countries.(figure 1 and table 1)”

4) In this light the differences between these countries and those with no legislation.

Response

In line 16-21 in the subsection Legislation and industrial trans fat in foods of the original manuscript it says:

“If the trend of using fat with lower amounts of I-TF continues as observed (figure 4) or even accelerates among food factories in Russia and Ukraine that produce biscuits/cakes/wafers, the intake of I-TF in the 2 large countries may decrease, but in addition also the intake of I-TF among millions of inhabitants in the adjacent countries.18 “

The implication of legislative trans fat restriction in Russia has now been discussed more explicitly in the revised manuscript. The sentence given above has been replaced by the sentence given below

“The trend over time of using fat with lower amounts of I-TF (figure 4) will probably accelerate in 2018 among food factories in Russia due to the Russian legislation 27 and among factories in Ukraine due to competition with Russian factories. The intake of I-TF in the two large countries may decrease, but in addition also the intake of I-TF among millions of inhabitants in the adjacent countries due to the large export of biscuits/cakes/wafers from Russia and Ukraine to these countries.(table 1).18”

Reviewer: 2

1) Abstract - perhaps 'industrially produced trans fat' is better (at the start of the abstract).

Response

“Industrial trans fat” has been replaced by “industrially produced trans fat” in the entire manuscript, but not in the references

2) Would it be better to be a bit more specific about the 'declined significantly' comments at the end of the abstract.

Response

Yes

The end of the abstract says

“The % TF in the fat of products produced in Russia and in Ukraine in relation to the date of production declined significantly during the 2 years collection period.”

Based on the slopes of the regression lines in figure 4 that depicts TF as % of total fat in relation to the production dates for products from Ukraine and from Russia, the decline for products from Ukraine was 12.4 % point and for products from Russia 8.4 % points during the 2 years period. The products from Ukraine had a higher TF % in the start of the period than the products from Russia.

The sentence in the abstract has been changed in the revised manuscript

“The % TF in the fat of products produced in Russia and in Ukraine in relation to the date of production both declined approximately 10 % points during the 2 years collection period.”

The last sentence in the result section of the original manuscript says

“The TF concentrations decrease significantly during the 2 years in Russia ( $p < 0.0001$ ) as well as in Ukraine ( $p < 0.0001$ ), but the decrease did not differ significantly between the two countries ( $p = 0.12$ ) (figure 4).”

This sentence has been extended with a new sentence in the revised version

“The products from Ukraine had a higher TF % in the start of the period than the products from Russia. Based on the slopes of the regression lines in figure 4, the decline for products from Ukraine was 12.4 % points and for products from Russia 8.4 % points during the 2 years period.”

3) Introduction - the first paragraph has some vague statements that could be clarified - e.g. what constitutes a 'high' amount of trans fat?,

Response

It is arbitrarily set to more than 5 % of total fat being TF

In the revised version the sentence has been changed to “High amounts of trans fat (TF) in food i.e. more than 5% TF of total fat in the food”

4) some better clarification about foods that might be 'high' in industrially produced trans fats and foods that might be high in non-industrially produced trans fats (e.g. ruminant-based foods) and any possible difference/or not between these sources (this might be important later, since you mention not being able to discriminate between these sources later in the paper).

Response

In line 6-7 in the Introduction sections it says

“Fat from ruminants, for instance in dairy products, contains up to 5 % TF, which is considerably lower than the up to 50% TF that may be present in partially hydrogenated fat for human food.”

The reviewer now suggest some comments about the difference between the effect of dietary R-TF and I-TF in humans. I have decided to mention only one of the most important findings concerning that issue and to quote the paper

Gebauer SK, Destailats F, Dionisi F, et al. Vaccenic acid and trans fatty acid isomers from partially hydrogenated oil both adversely affect LDL cholesterol: a double-blind, randomized controlled trial. *Am J Clin Nutr* 2015;102(6):1339-46.

The paper has now been included in the list of references as ref 1 and a more clinical paper has been removed: ref. 4 Nagasawa Y, Shinke T, Toh R, et al. The impact of serum trans fatty acids concentration on plaque vulnerability in patients with coronary artery disease: Assessment via optical coherence tomography. *Atherosclerosis* 2017 <http://dx.doi.org/10.1016/j.atherosclerosis.2017.06.922>.

The following sentences have been added in the revised manuscript

“In a double blind, randomised controlled trial in humans R-TF resulted in the same increase in LDL-cholesterol in blood compared with I-TF when the two different types of fat were given in the diet in same amounts.<sup>1</sup> It suggests that R-TF and I-TF have similar harmful effect on plasma lipoproteins.”

5) p4 - line 6 - could the 'fewer CHD events' comment be more specific?

Response

p4-lines 6-10 say

“Based on the available evidence, Denmark in 2004 and some counties in New York State, USA in 2007, legally restricted the use of I-TF in the diet. After the restrictions, the lower mortality due to CHD in Denmark and the fewer CHD events, in counties in New York State beyond temporal trends,

strongly suggest that I-TF in the diet promotes CHD and a restriction in its use reduces CHD.<sup>7–9</sup>

It has now been changed to the following sentence in the revised manuscript and “fewer CHD events” have been specified

“Based on the available evidence, Denmark in 2004 and some counties in New York State, USA in 2007 legally restricted the use of I-TF in the diet. After the restrictions in Denmark, there was a 4.3 % lower yearly mortality rate due to CHD beyond temporal trends.<sup>7</sup> A similar decline of 4.5 % in CHD mortality rate was found in the counties with restrictions in New York State compared with similar counties without restrictions. <sup>8</sup> Three and more years after the restriction in the counties, a hospital admission rate for myocardial infarction and stroke events combined was 6.2 % lower than in similar counties without restrictions.<sup>9</sup> These findings from 2 different groups of researchers, strongly suggest that I-TF in the diet promotes CHD and a restriction in its use reduces CHD.”

6) Methods - use of 'first person' ('I') could be avoided (also appears in Results section as well)

Response

“I” has been removed in the first line of Methods and in the second last sentence in Results. The two sentences have been reformulated accordingly in the revised manuscript.

7) p6 - some of the criteria used for sample purchase assume reliability in labelling of products - would it have been worth testing some sample products (perhaps as a control) that might not appear (from labelling information) to contain I-TFs to see if this was the case (or what levels were detected in a sample of these products)?

Response

The coloured area in the various panels in figure 1 are proxies for the intake of I-TF in the subpopulation that buys and consume these products. All products that were purchased fulfilled the 2 criteria obtained from the labelling and from the list of ingredients: 1) More than 15 gram of fat in 100 gram of the product and 2) terms that suggest a content of trans fatty acids. As the reviewer, rightly mention the purchase assume reliability in labelling of the products. After purchase, the products are analysed for total lipids and for trans fat. While the total lipid nearly always are above 15 grams per 100 gram of products, about 25% of the products contain less than 2% of the fat as trans fat. We are thus dealing with an “over labelling” of trans fat content. The extent appears from the values given for each country in the panels in figure 1. It has previously been explained in ref 21 partly by an effort by the producer to empty stores of old package material after a change of the recipe to fat without I-TF even if the recipe is permanently changed.

The reviewer now suggest that this may also go the opposite way: products may contain TF although the list of ingredients does not have the inclusion terms. This possibility was not investigated in this or in any of the other similar studies by our group. It would require many trans fat determinations to get a reliable value. The costs are 100\$ per determination in the US –lab. But of course this “under labelling” of trans fat definitely occurs but the frequency is presently unknown. It means that the values reported in this investigation for the number of different pre-packaged products with more than 2 % TF are minimum values because some products are “under labelled”. This weakness of the method has now been added in the number of weaknesses of using this method as a proxy for intake of I-TF. It has also to be taken into account when results from different countries are compared.

The following sentence has been included in the subsection Strengths and weaknesses of the study in the revised manuscript

“It is also assumed that if the inclusion words are not present in the list of ingredients, the product contains less than 2% of the total fat as TF.”

In the section Strength and limitation of this study the same sentence has been included in the revised manuscript

A limitation is the assumption that if the inclusion words are not present in the list of ingredients, the product contains less than 2% of the total fat as TF.

8) (or what levels were detected in a sample of these products)?

Response

Many of the products that have less than 2% of the trans fat has less than 0.1 % of total fat as TF

9) Analysis of TF - although you refer to an AOAC method - you mention this was modified - but no details of how/in what way? Could you have a bit more specific detail about the method - which TFAs were being detected and measured - any controls, standardisation (e.g. standard reference materials) or anything further to confirm the quality of the measurements?

Response

Line 8-12 in subsection Analysis of TF

For analysis the foods were homogenized, and the fatty acid content was analysed using gas chromatography on a 66-m highly polar capillary column, using a modification of the AOAC 996-06 method. All analytical work on samples was conducted by Microbac Laboratories in Warrendale, Pennsylvania, USA, an ISO-17025-certified laboratory.

The modifications consist of separate compounds for use as surrogate and internal standard and a slightly different dilution scheme than is suggested in the method. Individual standard components are used. The method makes it possible to get total fat as triglycerides, which are then broken down into mono, poly, saturated and total trans fat. The modified method went through a validation study and was approved by A2LA. Calibration is verified using standards at the beginning and after every ten samples. A control sample (canola oil) is analysed with every extraction. The laboratory determines a range of trans fatty acids:

C14:1T Methyl Myristelaidate

C16:1T Methyl Palmitelaidate

C18:1T Methyl Petroselaidate

C18:1T Methyl Transvaccenate

C18:1T Methyl Elaidate

C18:2TT Methyl Linoelaidate

C20:1T Methyl 11-Trans Eicosenoate

C22:1T Methyl Brassidate

Conjugated linoleic acid (CLA) if it occurs in the samples is not added to the total trans fat of the sample.

The expanded uncertainty for the method is about +/- 0.25 %. The laboratory undergo annual proficiency testing and are audited every other year by A2LA to ensure compliance of this method to ISO 17025

The reviewer ask for a bit more specific details about the method. This is given above. The description in the submitted version is only slightly expanded. The important information about regularly proficiency testing was added

“For analysis the foods were homogenized, and the fatty acid content was analysed using gas chromatography on a 66-m highly polar capillary column, using a modification of the AOAC 996-06 method. The method makes it possible to get total fat as triglycerides, which are then broken down into mono-, poly-, saturated- and total TF. Total TF may consist of up to eight different trans fatty acids. All analytical work on samples was conducted by Microbac Laboratories in Warrendale, Pennsylvania, USA, The laboratory undergo annual proficiency testing and are audited every other year to ensure compliance of this method to ISO 17025.”

10) You mention that any butter in any of these products might contribute ruminant TF - is there anything you could add to suggest how likely/unlikely this might be (was it possible to correct for this - would any labelling information or any information from elsewhere be able to suggest if this likely to be of any significance).

Response

The last 2 sentences in the Method section of the submitted manuscript

“If butter as a ruminant fat has been used in the product in addition to partially hydrogenated vegetable oil, some of the TF in the product may be derived from butter that on average contains a few percent of the fat as TF. In this paper, the term I-TF is used even though a minor portion in certain products may be TF derived from ruminant fat.”

The following sentences were added in the revised manuscript

If butter is present in a product which have been analysed for TF in the present study and the value is higher than 5% TF of total fat, butter has diluted the I-TF in the product, because butter adds more non-TF than it adds TF. Values larger than 5% TF in the product are consequently minimum values for I-TF as percent of total fat. In products with less than 5% TF as percent of total fat that contain butter, the I-TF will be lower than the TF value. Such products are because of their low amounts of TF per 100 gram of product of less importance for the health of the consumer than products with much higher TF values and of less interest for health authorities and food scientists.

11) Discussion - do you need to clarify what you mean by 'high concentration' at start of first paragraph?

Response

Yes, as also explained in response to the reviewer's comment 3.

It is arbitrarily set to more than 5 % of total fat being TF

The first sentence of the Discussion in the submitted manuscript

“The findings of this study clearly demonstrate that in 2015-2017 I-TF was present in high concentrations in many different brands of biscuits/cakes/wafers in the countries of the former Soviet Union, except for the three Baltic countries.”

The sentence has been changed to the following sentence in the revised manuscript

“The findings of this study clearly demonstrate that in 2015-2017 I-TF was present in high concentrations i.e. more than 5 % I-TF in the total fat in many different brands of biscuits/cakes/wafers in the countries of the former Soviet Union, except for the three Baltic



countries”

12) p11 - you assume that labelling information is reliable in your comments towards the top of this page - perhaps you could be more cautious about this (analysis of food products sometimes shows that labelling and ingredient information isn't always as good as we often assume).

**Response**

This issue has been dealt with in the response to the reviewers comment 7 but beside the changes described in response to comment 7, the sentence from page 11 line 7-10 in the submitted manuscript says

“This suggests that the inclusion criteria are rather broad in relation to the presence of TF and/or that the terms on the list of ingredients do not always reflect the composition of the fat in the product.”

A new sentence has been added in the submitted manuscript

“ If the list of ingredients does not have the inclusion words the product may still have high amounts of TF and are not bought and analysed for TF.”

13) Towards the end of the Discussion perhaps you need to mention further that there may also be other factors other than I-TF (diet and other factors) that may well be very different between the countries in the study and Western Europe (which contribute to the higher CHD mortality).

The following sentence has now been added in the revised manuscript

Based on data from 2009 to 2011, there is a fivefold difference in age-standardized mortality rates of CHD between some countries in Western Europe and countries in Central and Eastern Europe. Central Asia has the highest rates.<sup>10</sup> Central- Eastern Europe and Central Asia were the world regions with the highest current age-standardized cardiovascular mortality rates, which are more than twice those of Latin America and the Caribbean.<sup>29</sup> These differences cannot be explained only by differences between the countries in the intake of I-TF.

Line 12-15 in the same section –the last sentence in the original manuscript says

“Restriction of the use of I-TF in foods, either voluntarily by food producers or more efficiently by legislation, may be an easily implemented first strategy for the reduction of cardiovascular diseases in these countries.”

The reduction has been replaced with “some reduction”

The sentence in the revised manuscript is now

“Restriction of the use of I-TF in foods, either voluntarily by food producers or more efficiently by legislation, may be an easily implemented first strategy for some reduction of cardiovascular diseases in these countries.”

**VERSION 2 – REVIEW**

<b>REVIEWER</b>	Vesna Vucic Institute for Medical Research, Belgrade, Serbia
<b>REVIEW RETURNED</b>	01-Oct-2018

<b>GENERAL COMMENTS</b>	The author has addressed my concerns sufficiently to recommend publication of the manuscript in its current form.
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<b>REVIEWER</b>	Dr Leo Stevenson Senior Lecturer, Food Science & Nutrition, Liverpool John Moores University, Liverpool, UK
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<b>REVIEW RETURNED</b>	09-Oct-2018
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<b>GENERAL COMMENTS</b>	<p>The amended manuscript is much clearer, and limitations of the study are clearly discussed. Some interesting and important work is discussed.</p> <p>I only have two minor issues with the current version:</p> <p>In the 'Analysis of TF' section the paper mentions that the AOAC 996-06 method was modified - but it could be clearer in what way this AOAC method was changed.</p> <p>Towards the end of page 9, I found the comments about 'best before by' dates and 'shelf life' a little confusing. If you are not using the 'best before by date' as an indicator of shelf-life, how is this being determined (could this be a little clearer?).</p>
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## VERSION 2 – AUTHOR RESPONSE

Reviewer 2 writes:

In the 'Analysis of TF' section the paper mentions that the AOAC 996-06 method was modified - but it could be clearer in what way this AOAC method was changed. Response: I have now described in details in what way the AOAC method was changed by adding the following sentences to the method section.

Two procedures were modified in minor ways. The final step of the extraction/methylation calls for 1 mL of hexane to be added in accordance with AOAC.996.06. In the modified method, 4 mL were added. This is done to alleviate the dilution that needs done at the instrument. The levels of the instrument are low and sample results are over range. This procedure cuts down on any possible error by only having one dilution. The method also calls for two forms of C11:0 undecanoic acid to be used as surrogate and internal standard. In the modified method C11:0 triundecanoin was used as an internal standard as per method and calculate it throughout the analysis alleviating the need for another form to be used as surrogate.

The reviewer also found the comments about 'best before by' dates and 'shelf life' a little confusing. If you are not using the 'best before by date' as an indicator of shelf-life, how is this being determined (could this be a little clearer?).

I have now added towards the end of page 9 that the length of shelf life in months or days appeared on the package together with the date of production.

I have replaced ref 27 with an official statement from Russian authorities. The previous ref 27 was an advertisement from a private Ukrainian company.

Furthermore I have accessed the various homepages mentioned in the list of references . One FDA

homepage did not exist anymore. It has now been replaced with another homepage with a similar message. The other homepages were still available.