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In response to the Letter to the Editor from Xiao regarding our article, "FEMA GRAS assessment of natural flavor complexes: Asafetida oil, garlic oil and onion oil"

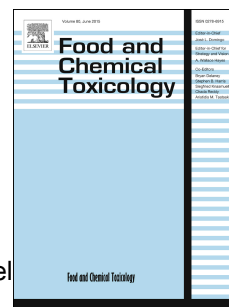
Jeanne M. Davidsen, Samuel M. Cohen, Gerhard Eisenbrand, Shoji Fukushima, Nigel J. Gooderham, F. Peter Guengerich, Stephen S. Hecht, Ivonne M.C.M. Rietjens, Thomas J. Rosol, Christie L. Harman, Danarubini Ramanan, Sean V. Taylor

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**In response to the Letter to the Editor from Xiao regarding our article,
“FEMA GRAS assessment of natural flavor complexes: Asafetida oil,
garlic oil and onion oil”**

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In response to the Letter to the Editor from Xiao regarding our article, “FEMA GRAS assessment of natural flavor complexes: Asafetida oil, garlic oil and onion oil,” (Ref), we would like to provide the following perspectives.

First, we would like to thank the letter’s author for their comments on the rigor of our safety review of this group of flavorings, and we thank them for the kind words regarding the value of this study for food safety and the food industry.

In response to the author’s assertion that previous publications have reported that garlic oil is genotoxic, we would like to draw specific attention to the numerous *in vitro* and *in vivo* studies on garlic oil that are described in Table 6 of our publication. As one example, we would highlight the results from Lin et al., 2022, which indicates no evidence of mutagenic or clastogenic potential in a series of studies. Similarly, we cite a publication from Kaur and Singh (2007) that showed no evidence of DNA damage from garlic oil in a comet assay in mice. These studies and the others reported in our publication for garlic oil and its identified constituents allow for a conclusion that garlic oil is not genotoxic.

We would also note that, based upon our review, the publications that are cited by the letter’s author do not provide significant information regarding the genotoxic potential of garlic oil. Joseph et al. (1989) is an acute toxicity assessment, albeit only at a single dose in fasted rats. Additionally, the toxic effects were not observed with fasted rats that were “adapted” to garlic oil feeding in advance of the study. Similarly Dutta et al., 2021 report an acute toxicity study focusing on diallyl sulfide. Appendix A of our article notes the identified constituents of garlic oil as reported by the flavor industry, and diallyl sulfide was not reported at any significant level within garlic oil used as a flavor ingredient. Dutta et al., 2021 does report the results of chromosomal aberration analysis for diallyl sulfide, but utilizes a non-standard, non-guideline methodology. They report some evidence of chromosomal aberrations, albeit only at the top concentration administered to mice (1920 mg/kg bw) and not at the lower concentration of 160 mg/kg bw. The 1920 mg/kg bw dose was above the maximum tolerated dose (MTD) reported by Dutta et al for both males and females. Exceeding the MTD is not recommended in OECD Test Guidelines for genotoxicity studies. Toxic effects can be detected in some genotoxicity studies as false-positive outcomes (see, for instance, the comet assay results for perillaldehyde, described in Hobbs et al., 2016).

Finally, we note that the re-evaluations of Generally Recognized as Safe (GRAS) status that are conducted by the FEMA Expert Panel assess the safety only under the conditions of intended use as flavoring ingredients in food. We appreciate the comments of the letter’s author, and hope that our response provides helpful clarity.

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Table 6: Summary of genotoxicity assay data for NFCs and Group 26 constituents of NFCs

Test Substance	Test Type (System)	Doses Tested	Results	Reference
Sulfur derivative constituents				
<i>In vitro</i>				
Allyl disulfide	Bacterial reverse mutation assay <i>Salmonella typhimurium</i> TA100 ^a	1.5 – 150 µg/mL ^c	Negative ^a	(Eder et al., 1980)
Allyl disulfide	<i>In vitro</i> chromosome aberration assay - Chinese Hamster Ovary (CHO) cells ^a	2-25 µg/mL	Positive ^{a,b}	(Musk et al., 1997)
Allyl disulfide	Sister Chromatid Exchange CHO Cells ^a	2-10 µg/mL	Positive ^{a,b}	(Musk et al., 1997)
Allyl sulfide	Bacterial reverse mutation assay <i>Salmonella typhimurium</i> TA100 ^a	4 – 450 µg/mL ^c	Negative ^a	(Eder et al., 1980)
Allyl sulfide	<i>In vitro</i> chromosome aberration assay CHO cells ^a	200-600 µg/mL	Positive ^{a,b}	(Musk et al., 1997)
Allyl sulfide	Sister Chromatid Exchange CHO Cells ^a	200-600 µg/mL	Positive ^{a,b}	(Musk et al., 1997)
37% Allyl propyl disulfide, 31% propyl disulfide and 32% allyl disulfide	Bacterial reverse mutation assay <i>S. typhimurium</i> TA100 ^a	1.5 – 150 µg/mL ^c	Negative ^a	(Eder et al., 1980)
Allyl propyl disulfide	Bacterial reverse mutation assay <i>S. typhimurium</i> TA97, TA98, TA100, TA1535 and TA1537 ^a	0-333 µg/plate	Negative ^a	(Zeiger et al., 1988)

Test Substance	Test Type (System)	Doses Tested	Results	Reference
<i>In vivo</i>				
Diallyl thioethers(68.1% diallyl disulfide; 19.7% diallyl sulfide; 12.2% diallyl trisulfide)	<i>In vivo</i> micronucleus assay Male ICR/C3H mice	32.8, 66.7 mmol/kg bw	Negative	(Marks et al., 1992)
Natural flavor complexes				
<i>In vitro</i>				
Alcoholic extract of Asafetida	Bacterial reverse mutation assay – Streptomycin-dependent <i>S. typhimurium</i> TA98	5000-50,000 µg/plate	Weakly positive ^d	(Shashikanth and Hosono, 1986)
Asafetida Extract	Bacterial reverse mutation assay – <i>S. typhimurium</i> TA100 and TA1535	25,000, 50,000 µg/plate	Negative ^d	(Soudamini et al., 1995)
Garlic oil	Bacterial reverse mutation assay <i>S. typhimurium</i> TA98, TA 100, TA1535, TA1537 and TA1538 ^a	5 – 2000 µg/plate	Negative ^a	(Hachiya et al., 1983)
Garlic oil	Bacterial reverse mutation assay - <i>S. typhimurium</i> TA100 ^a	62.5 - 500 µg/plate	Negative ^a	(Park, 2002)
Garlic oil	Bacterial reverse mutation assay <i>S. typhimurium</i> TA98, TA 100, TA1535 and TA1537 ^a	0.5 – 1000 µg/plate	Negative ^a	(Lin et al., 2022)
Garlic oil	Rec assay – <i>B. subtilis</i> H17 Rec ⁺ , M45 Rec ^{-a}	1,3 and 5 mg/disk	Negative ^a	(Ueno et al., 1983)
Garlic oil	Rec assay – <i>B. subtilis</i> H17 Rec ⁺ , M45 Rec ^{- a}	5 mg/disk	Negative ^a	(Hachiya et al., 1983)

Test Substance	Test Type (System)	Doses Tested	Results	Reference
Garlic oil	<i>In vitro</i> chromosome aberration assay in Chinese hamster ovary cells (CHO-K1) ^a	0, 1, 1.5 2.5, 5 and 10 µg/mL	Negative ^{a, e}	(Lin et al., 2022)
Onion oil	Bacterial reverse mutation assay – <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538 ^a	1 - 50,000 µg/plate	Negative ^a	(Hachiya et al., 1983)
95% ethanol extract of powdered onion	Bacterial reverse mutation assay – <i>S. typhimurium</i> TA98 and TA102 ^a	10,000 µg/plate	Negative ^a	(Mahmoud et al., 1992)
Aqueous and 99% ethanol extract of sliced and homogenized onion	Bacterial reverse mutation assay – <i>S. typhimurium</i> TA98 and TA100 ^a	0-10 mg/ml	Negative ^a	(Martínez et al., 1999)
Onion oil	Rec assay – <i>B. subtilis</i> H17 Rec+, M45 Rec- ^a	Max. 10 mg/disk	Equivocal ^a	(Hachiya et al., 1983)
Onion oil	Rec assay – <i>B. subtilis</i> H17 Rec+, M45 Rec- ^a	10 mg/disk	Equivocal ^a	(Ueno et al., 1984)
Onion oil	<i>In vitro</i> chromosome aberration assay in Chinese hamster fibroblasts	0.04 mg/ml	Equivocal	(Ishidate et al., 1984)
<i>In vivo</i>				
Asafetida powder suspended in saline solution	<i>In vivo</i> Sister chromatid exchange assay – C57Bl/6 mice	0, 500 and 1000 mg/kg bw	Positive	(Abraham and Kesavan, 1984)
Aqueous extract of garlic	<i>In vivo</i> chromosome aberration assay – Swiss albino mice	25, 50, 100 mg/kg bw	Positive	(Das et al., 1996)
Aqueous extract of garlic	<i>In vivo</i> chromosome aberration assay – Swiss albino mice	5%	Negative	(Shukla and Taneja, 2002)

Test Substance	Test Type (System)	Doses Tested	Results	Reference
Garlic oil	<i>In vivo</i> comet assay – Male mice (5/dose)	0.25, 0.5 ml	Negative	(Kaur and Singh, 2007)
Garlic oil	<i>In vivo</i> micronucleus assay – male ICR mice	0, 15, 25, 50 mg/kg bw	Negative	(Lin et al., 2022)
Onion oil	<i>In vivo</i> micronucleus assay – male ddY mice	0, 250, 500, 1000 mg/kg bw	Negative	(Hayashi et al., 1988)

^a With and without S9 metabolic activation system

^b Positive result correlated to cytotoxicity

^c Modified suspension assay used

^d Without S9 metabolic activation

^e Same concentrations used in both 3 h and 20 h experiments